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Impact of Fibroscan in the management of liver tumors

Muthukumarassamy Rajakannu

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Muthukumarassamy Rajakannu. Impact of Fibroscan in the management of liver tumors. Surgery. Université Paris Saclay (COMUE), 2017. English. NNT : 2017SACLS365 . tel-01827252

HAL Id: tel-01827252

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Impact de l'utilisation du FibroScan® dans la prise en charge des tumeurs du foie

«Thèse de doctorat de l'Université Paris-Saclay,
préparée à l'Université Paris-Sud »

École doctorale n°569 : Innovation thérapeutique : du fondamental à l'appliqué (ITFA)

Spécialité de doctorat : Sciences Chirurgicales

Thèse présentée et soutenue à Villejuif, le 15 novembre 2017

Dr Muthukumarassamy RAJAKANNU

Composition du Jury :

Pr Daniel CHERQUI

PUPH, Université Paris-Sud (UMR S1193) Président

Pr Pierre NAHON

PUPH, Université Paris-Seine-Saint-Denis (UMR S1162) Rapporteur

Pr François-René PRUVOT

PUPH, Université Lille (UMR S1172) Rapporteur

Pr Pierre BEDOSSA

PUPH, Université Paris Diderot (UMR 1149) Examineur

Pr Maite LEWIN-ZEITOUN

PUPH, Université Paris-Sud (UMR S1193) Examineur

Pr Eric VIBERT

PUPH, Université Paris-Sud (UMR S1193) Directeur de thèse

Pr Christian POÛS

PUPH, Université Paris Saclay (UMR S1193) Co-Directeur de thèse

“All of this demonstrates why few research scientists are in policy-making positions of public trust. Their training for details produces tunnel vision, and men of broader perspective are required for useful application of scientific progress”

Michael Shimkin

Acknowledgments

At first, I should thank all the patients who participated in this research project without any hesitation. I am deeply indebted to their kindness and willingness.

Next, I would like to thank all the members of the jury, Prof Daniel Cherqui, Prof Pierre Bedossa, Prof Maite Lewin, Prof Pierre Nahon, and Prof François-René Pruvot, for kindly agreeing to participate and critically review my thesis amidst their busy schedule. My special gratitude to Prof Eric Vibert, Director of my thesis, Prof Christian POÛS, Co-director of my thesis, and Prof Didier Samuel, Dean of the faculty of medicine, Paris-Sud university and Director of Inserm UMR S1193, for having confidence in me and providing me with the opportunity to work in their research team. I am grateful for their individualized heuristic teaching, their support and critical inputs during the three years of my research.

I am grateful to Dr Véronique Miette and Dr Laurent Sandrin, Echosens[™], Paris for accepting me in their CIFRE program and providing me with necessary financial and material support, which ensured successful conduct of this research. I would also like to thank all the members of research and development for accepting me in their midst and their patient answers to my questions about the intricacies of transient elastography. My special thanks to Mr Hecham Azrak for his valuable help in statistical analysis.

I would like to convey my thanks to Prof René Adam, Prof Denis Castaing, Prof Daniel Cherqui, and Prof Antonio Sa Cunha for permitting me to include their patients in my study and their critical review of my manuscripts for publication. My special thanks to Prof Catherine Guettier, Prof Mylène Sebahg and Prof François Le Naour for participating in my PhD research. I thank the entire Centre Hépatobiliaire team of consultants, fellows, residents, nurses, coordinators and secretaries for their support to my work.

I take this opportunity to thank all my colleagues and collaborators at Hôpital Paul Brousse especially Dr Coilly A, Dr Kaščáková S, Ms Frezouls W, Ms Dumont S, and Ms Blandin F. This study would not have been possible without their help and encouragement.

Finally, I am very grateful to my family and my friends for being my pillars of strength. As always their understanding, support, and strength helped me to survive these lonely years and complete my work. I dedicate this work to them.

R. Muthukumarassamy

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List of abbreviations

2D-SWE – Two-dimensional shear wave elastography
ACR – Acute cellular rejection
AFP – α -fetoprotein
AGA – American gastroenterological association
ALD – Alcoholic liver disease
ALT – Alanine aminotransferase
APRI – Aspartate platelet ratio index
ARFI – Acoustic radiation force impulse imaging
AST – Aspartate aminotransferase
AUROC – Area under receiver operating characteristic curve
BCLC – Barcelona clinic liver cancer
BMI – Body mass index
cACLD – compensated advanced chronic liver disease
CAP – Controlled attenuation parameter
CECT – Contrast enhanced computed tomography
CI – Confidence interval
CLD – chronic liver disease
CRP – C-reactive protein
CSPH – Clinically significant portal hypertension
CTx – Chemotherapy
dB/m – Decibels/meter
DUS – Doppler ultrasonography
EASL – European association for the study of liver
EFSUMB – European federation of societies for ultrasound in medicine and biology
eLIFT – Easy live fibrosis test
ELF – European liver fibrosis score
FHVP – Free hepatic venous pressure
FIB-4 – Fibrosis-4 score
FLR – Future liver remnant
FM – FibroMeter™
FTIR – Fourier transform Infrared spectroscopy
GDA – Gastroduodenal artery
HA – Hepatic artery
HBV – Hepatitis B virus
HCC – Hepatocellular carcinoma

HCV – Hepatitis C virus
H & E – Hematoxylin and eosin
HIV – Human immunodeficiency virus
HR – Hazard ratio
HVPG – Hepatic venous pressure gradient
ICG-K – Indocyanine green clearance rate
ICG-R15 – Indocyanine green retention at 15 min
INR – International normalized ratio
IQR/M – Inter-quartile range/median
IR – Infrared spectroscopy
kPa – KiloPascals
LB – Liver biopsy
LDLT – Living donor liver transplantation
LR – Liver resection
LS – Liver stiffness
LSM – Liver stiffness measurement
LT – Liver transplantation
METAVIR – Meta-analysis of histological data in viral hepatitis
MRE – Magnetic resonance elastography
NAS – Non-alcoholic fatty liver disease activity score
NAFLD – Non-alcoholic fatty liver disease
NASH – Non-alcoholic steatohepatitis
NPV – Negative predictive value
OR – Odds ratio
PAN – Probability of active NASH score
PH – Portal hypertension
PHLF – Post-hepatectomy liver failure
PPV – Positive predictive value
pSWE – Point shear wave elastography
PVE – Portal vein embolization
PVP – Portal venous pressure
RFA – Radiofrequency ablation
ROC – Receiver operating characteristic
SCD – Skin capsule distance
SLV – Standard liver volume
SSI – Supersonic shear wave imaging

Sens – Sensitivity

Spec – Specificity

SWV – Shear wave velocity

TE – Transient elastography

TG – Triglyceride

UGIE – Upper gastrointestinal endoscopy

US – Ultrasound

VCTE – Vibration Controlled Transient Elastography™

WC – Waist circumference

WHVP – Wedged hepatic venous pressure

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Introduction

Surgery is the only curative treatment option for the patients with either primary or metastatic liver tumors. Post-operative outcomes after hepatectomy depends on the volume and the quality of the non-tumoral liver remnant, called functional liver remnant (FLR). Moreover, the extent of hepatectomy is limited by the amount of hepatic reserve to be left behind to sustain vital functions. In patients with liver metastasis, either colorectal or non-colorectal in origin, peri-operative chemotherapy is the standard of care. The hepatotoxicity of these regimens vary and various studies have shown that prolonged pre-operative chemotherapy has a negative impact on the post-operative outcomes. In patients with hepatocellular carcinoma (HCC), underlying liver disease (hepatic fibrosis, steatosis and portal hypertension) not only determines the post-operative outcomes but also predicts the risk of long-term recurrence. Further, clinically significant portal hypertension is a risk factor for tumor progression and poor response to transarterial chemoembolization in patients with HCC listed for liver transplantation (LT). Therefore, evaluation of the quality of FLR and degree of portal hypertension is an important aspect in the management of patients undergoing hepatectomy for various indications. Recently, some authors have demonstrated that transient elastography by FibroScan[®] be utilized to predict post-hepatectomy complications like post-hepatectomy liver failure. FibroScan[®] is a non-invasive method of assessing the degree of hepatic fibrosis and steatosis by estimating liver stiffness (LS) and controlled attenuation parameter (CAP), respectively. However, the cut-off values vary between these studies and no predictive model has yet been validated. Therefore, we planned to study these aspects prospectively at Centre Hépatobiliaire in Hôpital Paul Brousse with the aim of proposing a LS-based statistical model to predict post-hepatectomy complications and risk of dropout in HCC patients listed for LT. In addition, we planned to evaluate a new prototype of FibroScan[®] capable of performing transient elastography directly on the surface of liver.

My PhD work consisted of three main areas of research: 1. Patients undergoing liver resection for various hepatobiliary diseases, 2. Patients with hepatocellular carcinoma planned for LT, and 3. Liver graft during machine perfusion or in animal model. Patients planned for hepatectomy and LT were included prospectively in the study after informed consent, and I evaluated pre-operatively LS and CAP of non-tumoral liver in these patients. The prototype of FibroScan *in situ*[®] was tested on a liver graft during normothermic perfusion and on a pig liver. All patients were followed regularly after hepatectomy up to three months and in patients listed for LT until transplantation or dropout from the waiting list. I did various analyses to validate the performance LS and CAP to estimate hepatic fibrosis and steatosis, respectively. In a subset of patients, hepatic fat content and quantity of liver fibrosis were evaluated by infrared spectroscopy and digital morphometry and compared with CAP and LS, respectively. A new LS-based score was developed and validated to predict clinically significant portal hypertension. Several nomograms were developed to predict various post-operative complications after hepatectomy by combining LS with other clinical and laboratory parameters and these results are presented in my thesis manuscript.

I begin my manuscript with a brief summary of various ultrasound elastography techniques, and then give a detailed literature review about transient elastography by FibroScan[®] and its applications in hepatology and liver surgery. The results are then presented in the form of articles under the following categories, 1. Hepatic fibrosis and hepatic steatosis, 2. Portal hypertension, 3. Post-operative outcomes after hepatectomy, 4. Oncological outcomes after hepatectomy for HCC. I end the manuscript with a brief discussion, some important conclusions from this study, and the list of references.

Review of Literature

Ultrasound Elastography

Ultrasonography is a widely utilized medical imaging technique in clinical practice for the last 40 years. It is based on the propagation of mechanical high frequency (≥ 2 MHz) compressional waves called ultrasound (US). It allows the reconstruction of morphological images of organs, but lacks quantitative information about tissue elastic properties; in fact, the bulk modulus that governs the propagation of US is almost homogenous in different biological tissues and does not depend on tissue elasticity. Elasticity is the property of a material to return to its original form after the stress applied to it is removed. When subjected to stress, an object undergoes deformation to its original size and shape; the amount of deformation is called strain. Elastography aims at quantitative imaging of tissue stiffness (Young's E modulus) and provides additional clinical relevant information by mapping the stiffness, estimated either from analysis of the strain in the tissue under a stress (quasi-static methods), or by imaging shear waves whose propagation is governed by tissue stiffness rather than by its bulk modulus. Young's E modulus exhibits important variations between different biological tissues, which makes it ideal for the characterization of different tissues with an excellent contrast. It also quantifies tissue stiffness which is exact quantitative reproduction of a clinician's palpation.^{1,2,3}

Based on the stress applied to the tissue, US elastography is classified in to two types:

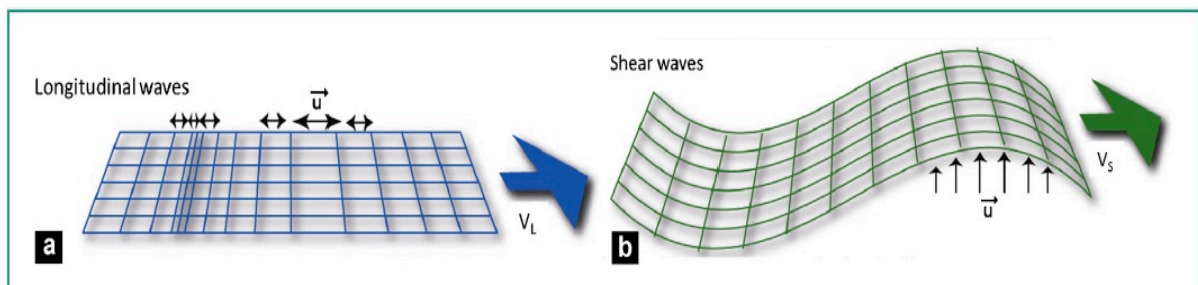


Figure 1: Types of stress applied in ultrasound elastography: a-ultrasound longitudinal wave (P) spreads by successive volume variations of the medium, the displacement of the medium u is parallel to its propagation direction with a speed of V_L ; b-the shear wave by successive movements that are perpendicular to the direction of propagation with a speed of V_S .¹

- ***Quasi-static method Elastography***

The displacement and the generated strain by a constant stress are estimated using 2D correlation of US images. Most often, the displacement, which is relatively large, is calculated by 2D correlation of conventional B-mode ultrasound images. Strains are then calculated by spatial derivation following one or possibly two directions for the most evolved approaches (Figure 2). This technique is simple to implement and is widely spread in the world of radiology, especially for breast lesions classification. The main limitations of this technique are operator dependent variations in the stress applied, and the absence of a specific quantification. In addition, the use of a stress applied by the operator limits the technique to superficial organs, mainly the breast or the thyroid. Some of the commercially available systems include:

- “Real-time Tissue Elastography” by Hitachi™ is based on a quasi-static elastography method and it allows to qualitatively show the stiffness of tissue in a color image superimposed on the standard ultrasound B-mode image
- “eSie Touch Elastography Imaging” from Siemens™

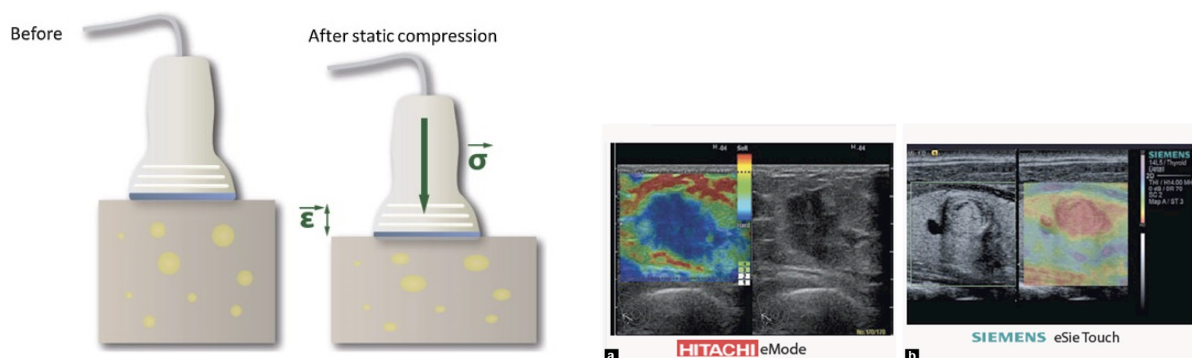


Figure 2: Static elastography reconstructs as “elastogram” or “strain image” by calculating the deformations related to a static compression imposed by the operator via the probe. Examples: a-strain image of a carcinoma; b-strain image of the thyroid.¹

- ***Dynamic Elastography***

In dynamic methods, a time-varying force is applied to the tissue, which can either be a short transient mechanical force or an oscillatory force with a fixed frequency. A time-varying

mechanical perturbation will propagate as mechanical waves which in a solid body can be compressional waves or shear waves (Figure 3). The compressional waves that propagate at high frequencies in the human body (~ 1500 m/s), can be used to image the body. Shear waves, which are only generated at low frequencies (10-2000 Hz) propagate more slowly, and their speed ($\sim 1-50$ m/s) is directly related to the medium shear modulus ($\mu = \rho V_s^2$), where ρ is the density of the area (~ 1000 kg/m³).

In biological tissues, which are almost incompressible, the Young's modulus can be approximated as three times the shear modulus ($E = 3\mu$). The shear wave propagation speed can thus be used to map the Young's modulus quantitatively. Dynamic elastography techniques, which rely on shear waves propagation, can produce quantitative and higher resolution Young's modulus map compared to quasi-static methods.

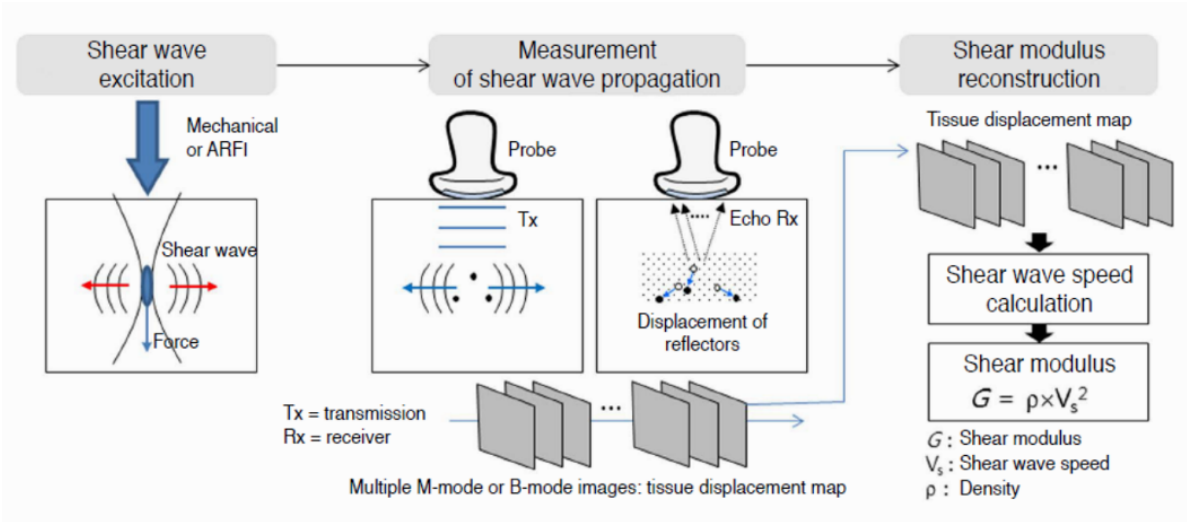


Figure 3: Schematic diagram depicting the process of shear wave-based ultrasound elastography where a perpendicular stress force to a target organ in order to induce shear on the tissue. The information on the propagating shear wave including the velocity of the shear wave could be measured by obtaining radiofrequency images with a high frame rate, which is used to generate a tissue displacement map. Then, the elastic property for quantitative estimation is calculated by the propagating velocity of the shear wave.²

The most commonly utilized dynamic liver US elastography techniques such as transient elastography, acoustic radiation force impulse (ARFI), and supersonic shear wave imaging are compared with examples in the Table 1 and Figure 4 below:

Table 1: Characteristics of various liver ultrasound elastography techniques²

Technique	Transient elastography	ARFI imaging	Supersonic shear-wave imaging	Real-time tissue elastography
Type of force	Dynamic	Dynamic	Dynamic	Quasi-static
Applied force	Mechanical impulse	US-induced radiation force impulse	US-induced radiation force impulse	Intrinsic (heartbeat)
Measurement of strain	Single measure, beam-line average	Single image within a box	Image within a color box	Full area image
Estimated parameter	Elastic modulus converted from shear wave velocity (kPa)	Velocity of shear wave (cm/sec)	Elastic modulus converted from shear wave velocity (kPa)	Strain ratio
Qualitative or quantitative	Quantitative	Qualitative/quantitative	Quantitative	Qualitative
Clinical evidence	Very much	Much	Little	Scanty

ARFI, acoustic radiation force impulse.

Figure 4a: Transient Elastography (TE) by FibroScan[®]

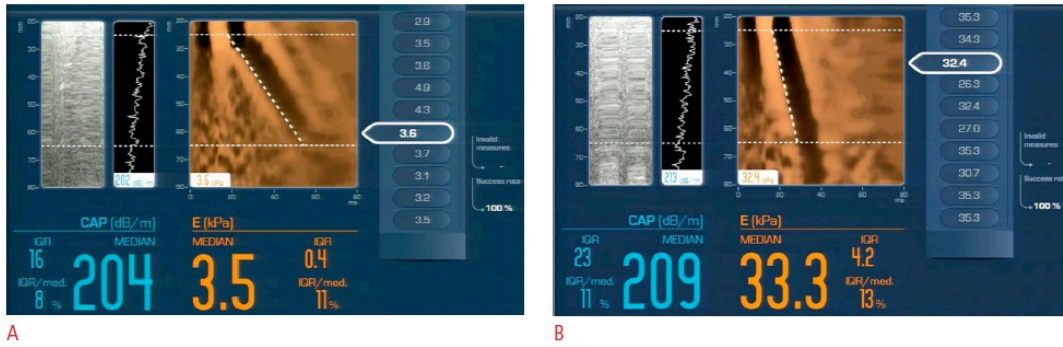


Figure 4b: Acoustic Radiation Force Impulse imaging:

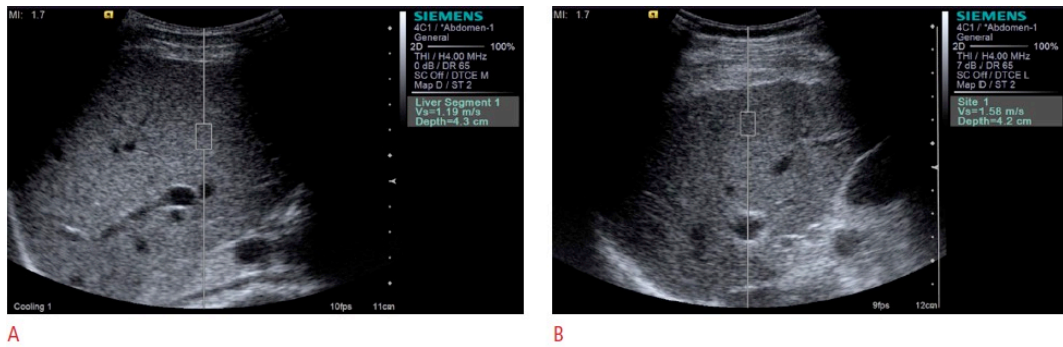


Figure 4c: Supersonic Shear-wave Elastography:

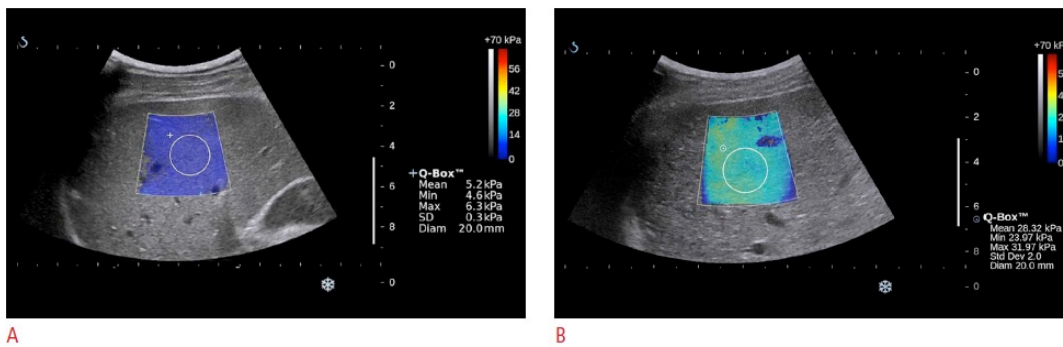


Figure 4: Ultrasound elastography in normal liver (A) compared with cirrhotic liver (B)²

Figure 5: Classification various liver elastography systems²

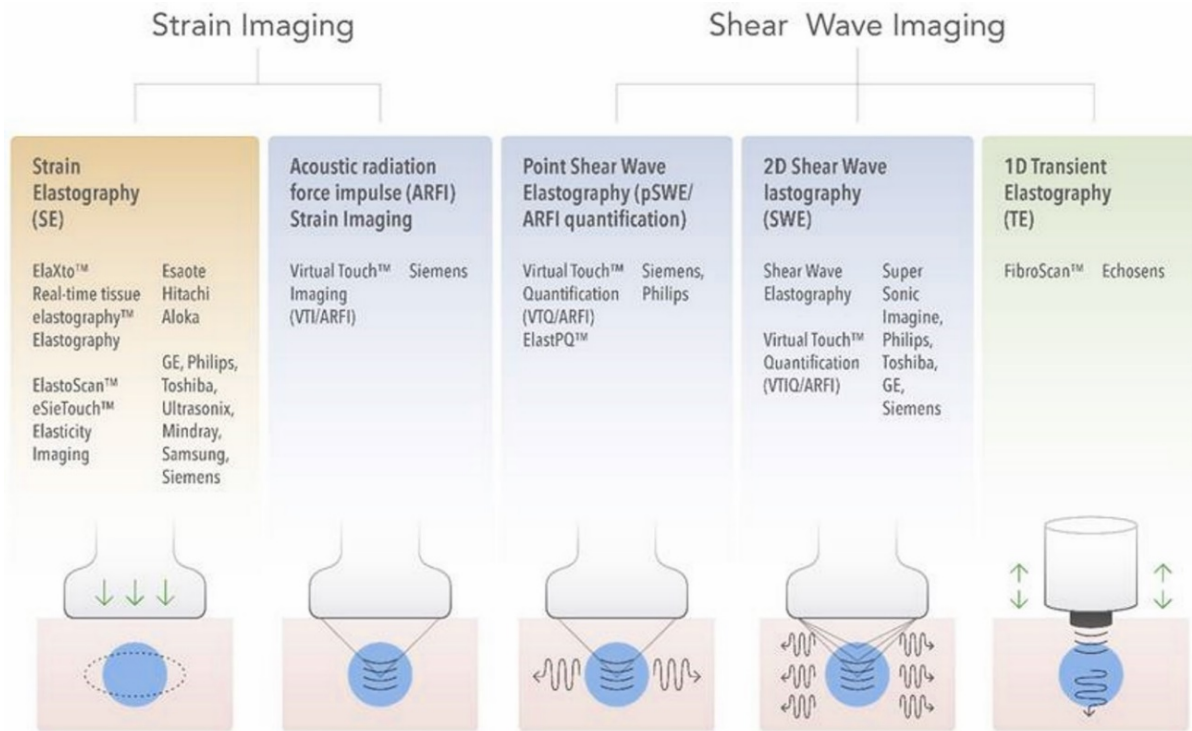


Table 2: Comparison of different liver elastography methods⁴

Method	Selected examples	Advantages	Disadvantages
Transient elastography	• FibroScan (Echosens, France)	<ul style="list-style-type: none"> • User friendly, rapid learning curve, bedside examination • Well-defined quality criteria • Good reproducibility • Most extensively evaluated • Good diagnostic accuracy for liver fibrosis staging • Excellent diagnostic accuracy for excluding liver cirrhosis • Prognostic value in cirrhosis 	<ul style="list-style-type: none"> • Dedicated device is required • ROI cannot be selected • ROI is rather small • No B-mode orientation • Limited applicability (ascites, severe obesity) • Operator-related and patient-related variability
Point SWE	<ul style="list-style-type: none"> • VTIQ using ARFI imaging (Siemens Healthcare, Germany) • ElastPQ (Philips Healthcare, Netherlands) • Shear Wave Measurement (Hitachi Aloka Medical, Japan) 	<ul style="list-style-type: none"> • Integrated into a conventional ultrasound machine • ROI localization can be chosen under B-mode visualization • Good applicability (not limited by ascites or obesity) • Comparable diagnostic accuracy to transient elastography for the staging of liver fibrosis and diagnosis of cirrhosis 	<ul style="list-style-type: none"> • Units in m/s only in some machines with narrow range of values • Quality criteria not well defined • Smaller ROI size than transient elastography • Size of ROI cannot be modified • Less well evaluated • No prognostic studies published
2D SWE	<ul style="list-style-type: none"> • ShearWave Elastography (SuperSonic Imagine, France) • Virtual Touch IQ (Siemens Healthcare, Germany) • Logiq E9 (GE Healthcare, UK) • Aplio 500 (Toshiba Medical Systems, UK) 	<ul style="list-style-type: none"> • Integrated into a conventional ultrasound machine • ROI size larger than transient elastography and point SWE • ROI size can be modified • Real-time measurement possible • High range of values (2–150 kPa) • Good applicability (not limited by ascites or obesity) • Comparable diagnostic accuracy to transient elastography for the staging of liver fibrosis and diagnosis of cirrhosis 	<ul style="list-style-type: none"> • Quality criteria not well defined • Less well evaluated • No prognostic studies published
MRE	<ul style="list-style-type: none"> • MR Touch (GE Healthcare, UK) • MRE (Philips Healthcare, Netherlands, Siemens Healthcare, Germany) 	<ul style="list-style-type: none"> • Can be integrated into conventional magnetic resonance machines • Examination of a whole liver lobe possible • Good applicability • Excellent diagnostic accuracy for the staging of liver fibrosis and cirrhosis 	<ul style="list-style-type: none"> • Requires magnetic resonance unit • More costly than transient elastography • Less well evaluated than transient elastography • Failure due to claustrophobia and iron overload

ARFI, acoustic radiation force impulse; MRE, magnetic resonance elastography; ROI, region of interest; SWE, shear wave elastography; VTIQ, Virtual Touch tissue quantification.

Transient Elastography by FibroScan® 502 Touch

FibroScan® is a non-implantable active medical device belonging to European class IIa that uses ultrasound to measure the stiffness of the tissues or organs by dynamic elastography called transient elastography (TE). It enables rapid non-invasive and painless quantification of liver fibrosis and steatosis. FibroScan® 502 Touch and its probes (S/M/XL) are manufactured by Echosens™, 30 Place d'Italie, 75013 Paris, France and each device is numbered to enable its easy identification and traceability (Figure 6). The device consists of a probe, a computer and its dedicated electronic system. The probe is made up of an electrodynamic vibrator, and an ultrasound transducer fixed to the end of vibration axis, which is then connected to the computer. The main function of this medical device to quantify hepatic fibrosis and steatosis by estimating Liver Stiffness (LS) and Controlled Attenuation Parameter (CAP) respectively.

Figure 6: FibroScan® machine and its probes⁵



Table 3: Characteristics of the acquisitions by different FibroScan® probes⁵

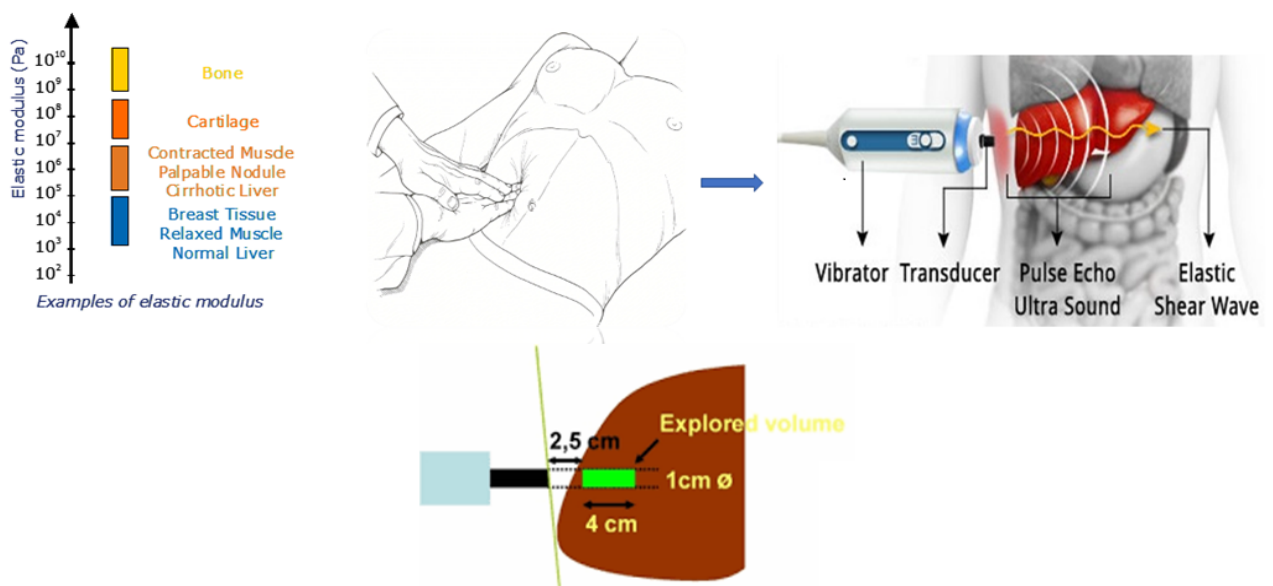
Characteristics	S Probe	M Probe	XL
Size	158x52 mm	158x52 mm	158x52 mm
Weight	0.5 Kg	0.5 Kg	0.5 Kg
Transducer	5 mm	7 mm	10 mm
Diameter			
Frequency	5 MHz	3.5 MHz	2.5 MHz
Measurement depth	S1: TP* <45 cm S2: 45 cm < TP <75 cm	25 – 65 mm TP* >75 cm SCD* <2.5 cm	35 – 75 mm SCD* <2.5 cm

*TP-thoracic perimeter, SCD-skin capsule distance

Mechanism of measurement of liver stiffness:

The measurement of LS is based on Young's elastic modulus and the technique named as "Vibration Controlled Transient Elastography™" (VCTE). This technology improves upon the traditional palpation in evaluating the hardness/ elasticity of a diseased organ and enables an objective quantification of its stiffness (quantified palpation) as illustrated in Figure 7.

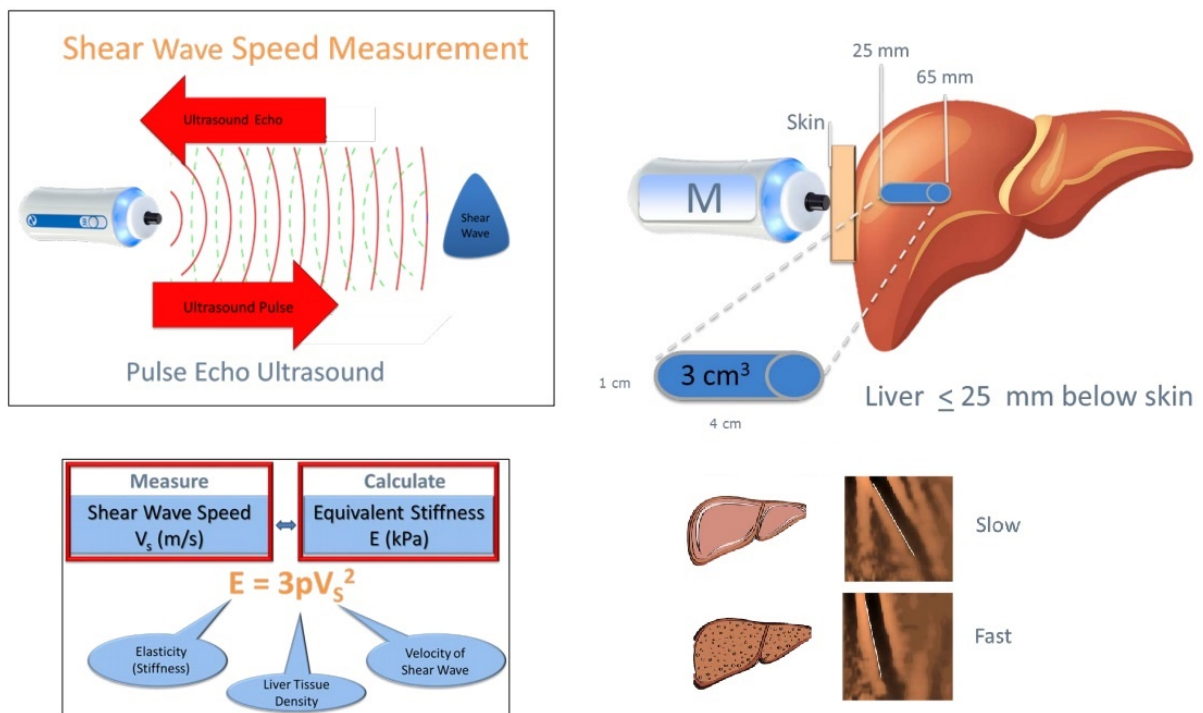
Figure 7: Quantified palpation with transient elastography⁵



- FibroScan[®] measures LS on the right liver through the right intercostal spaces while the patient is lying supine on the examination table and the examination.
- The ultrasonic transducer probe is mounted on the axis of an electro-mechanic piston vibrator
- The vibrator generates a low amplitude impulsion which in turn creates a low frequency plastic shear wave (50Hz) that propagates through the skin, subcutaneous tissue and then in the liver as shown in the Figure 8.
- Synchronized with the mechanical impulse, ultra-rapid pulse-echo ultrasound (6000 Hz) acquisition is utilized to follow the propagation of the shear wave and calculate its velocity, which is proportional to tissue stiffness.

- A proprietary algorithm is utilized to determine the propagation speed of the mechanical shear wave from the ultrasound signals.
- Stiffer the tissue, the faster the shear wave propagates.
- TE measures LS in a volume that approximates a cylinder 1 m in diameter and 4 cm in length at a depth of 2.5 – 6.5 cm with M probe or 3.5 – 7.5 cm with XL probe. This volume is atleast 100 times bigger than a biopsy sample.
- LS is expressed in kilopascal (kPa) and corresponds to atleast 10 validated measurements, with a range of 0.5 – 75. LS of normal liver is 5.49 ± 1.59 kPa.

Figure 8: Principle of liver stiffness measurement⁵



VCTE™ technology assures LS estimated by FibroScan® is:

- Operator and device independent.
- Reliable and reproducible.
- Not affected by the respiratory movements and cardiac pulsation.
- LS were considered valid only if ≥ 10 measurements, with $\geq 60\%$ success rate and interquartile range/median ratio (IQR/M) $\leq 30\%$.

Table 4: Features of Vibration Controlled Transient Elastography™ technology⁵

Parameters controlled by VCTE™	Benefits
Shear wave frequency (50 Hz)	Measured LS depends on the frequency
Shear wave amplitude	Obtain sufficient strain in the rigid medium
Probe Pressure on patient's skin	Ensures proper transmission of shear wave from vibrator to liver
Shear wave propagation quality	Validity of the measurement

The availability of two types of probes – M and XL for adults allows accurate assessment of LS and CAP adapted to the anthropometry of the patient in particular abdominal wall thickness. M probe measures LS from the depth of 2.5 cm to the depth of 6.5 cm whereas XL probe measures from 3.5 to 7.5 cm depth. The region of interest is 3 cm² of liver parenchyma on the right lobe and is same when either M or XL probes are used (Table 3).^{5,6,7}

Mechanism of measurement of Controlled Attenuation Parameter:

The ultrasound signals utilized to measure the propagation of the mechanical shear wave in the liver parenchyma are also utilized to determine CAP according to a proprietary algorithm. This CAP is measuring ultrasound attenuation (go and return path) at 3.5 MHz using the signal acquired by the probe (Figure 9). Ultrasound attenuation is a physical property of the medium of propagation, which corresponds to the loss of energy as ultrasound travels through the medium. Therefore, in an organ CAP depends the depth of measurement, and the tissue properties like its constituents, homogeneity, etc. As CAP is estimated simultaneously in the same region of interest where LS is measured and on the same signals, it is available only when LS measurements are validated according to the same criteria used for LS. FibroScan® thus enables simultaneously non-invasive assessment of both hepatic fibrosis and steatosis. CAP is expressed in decibel/meter (dB/m) with a range of 100 – 400. The range of normal CAP values is 156.0 – 287.8 dB/m.^{8,9}

Figure 9: Principle of controlled attenuation parameter measurement⁵

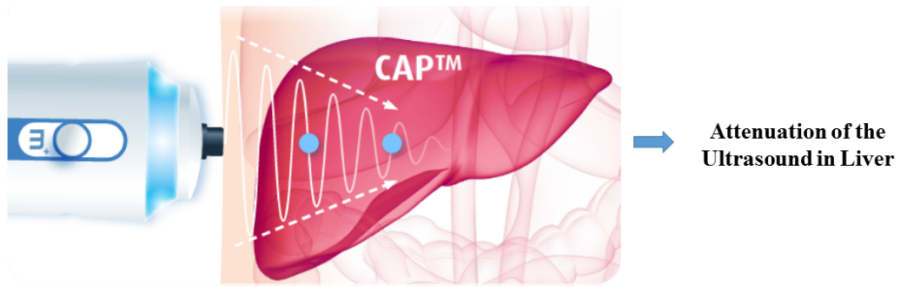
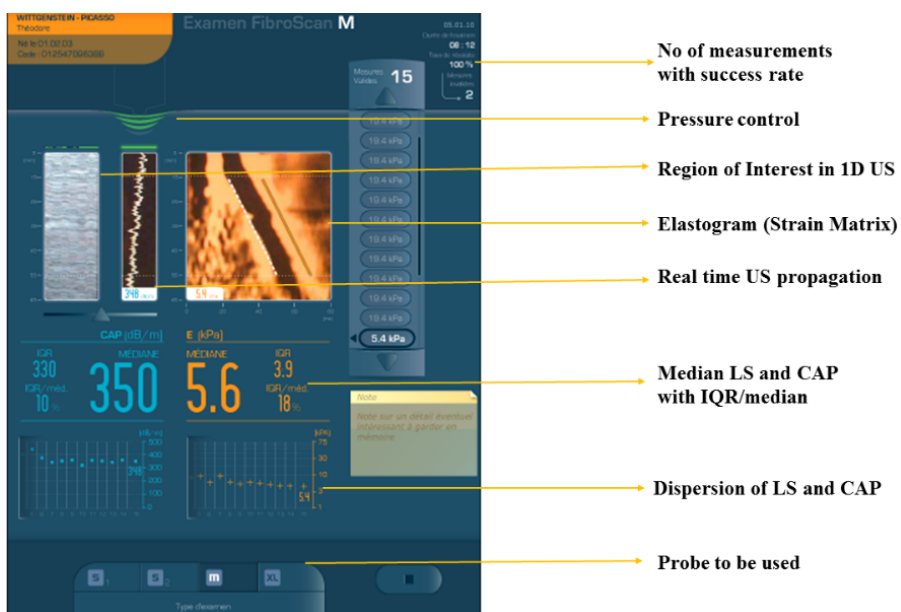
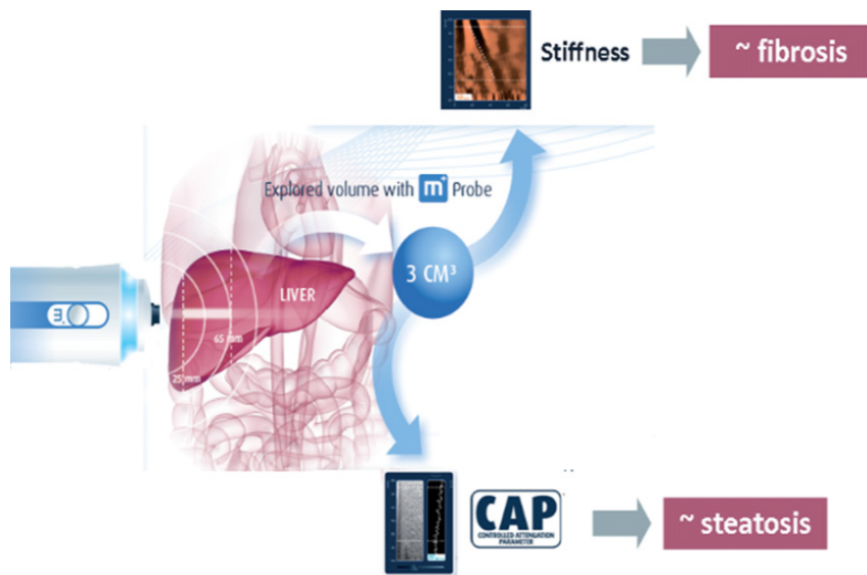


Figure 10: Liver stiffness and controlled attenuation parameter estimation by transient elastography (A) and display of the results in FibroScan® (B)⁵



Transient elastography has many potential applications in hepatology as diseased liver loses its elasticity and becomes rigid that can be quantified by TE. Various pathologies affecting LS are summarized in the Figure 11.

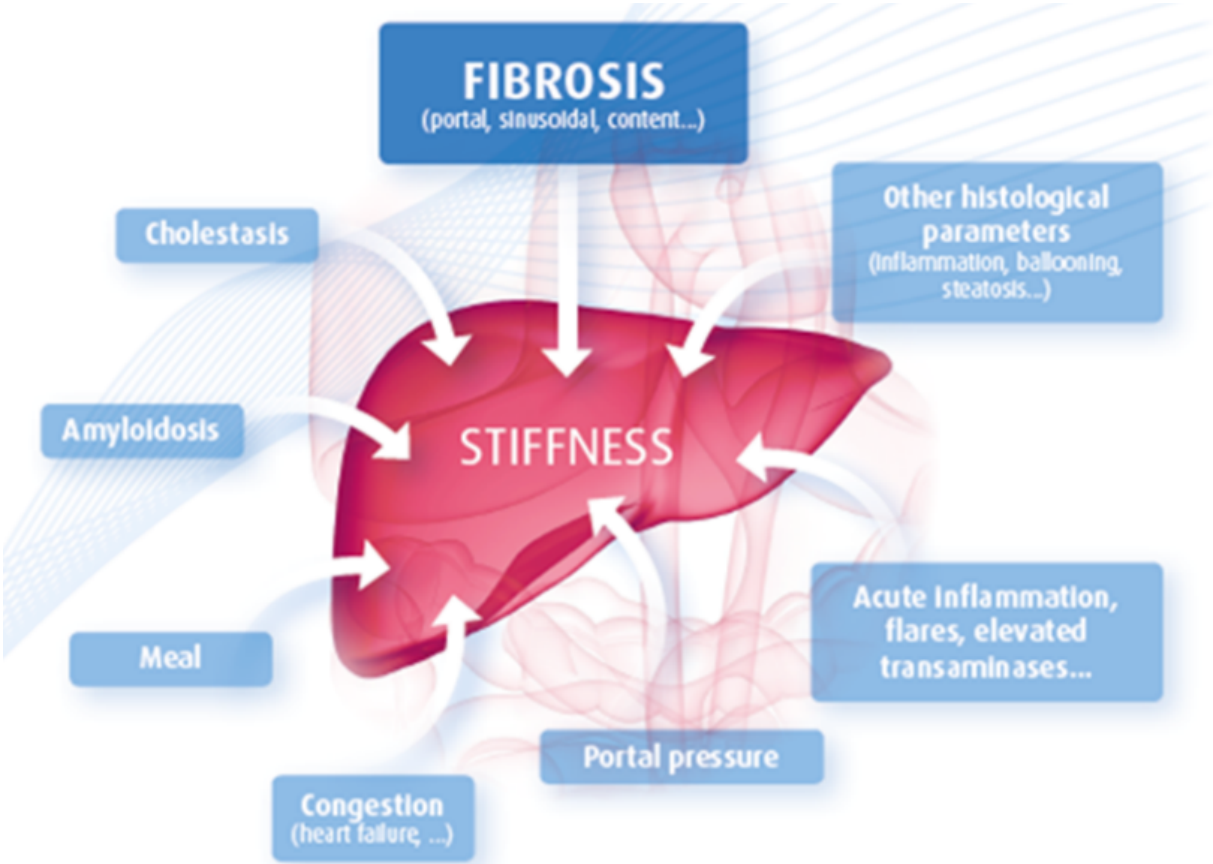


Figure 11: Potential clinical applications of transient elastography⁵

Hepatic Fibrosis

Liver fibrosis is part of the structural and functional alterations in most chronic liver diseases. It is one of the main prognostic factors as the amount of fibrosis is correlated with the risk of developing cirrhosis and liver-related complications in viral and non-viral chronic liver diseases^{10,11}. Liver biopsy (LB) has traditionally been considered the reference method for evaluation of tissue damage such as hepatic fibrosis in patients with chronic liver disease. Pathologists have proposed robust scoring system for staging liver fibrosis such as the semi-quantitative meta-analysis of histological data in viral hepatitis (METAVIR) score¹²⁻¹⁶. In addition computer-aided morphometric measurement of collagen proportional area, a partly automated technique, provides an accurate and linear evaluation of the amount of fibrosis^{17,18}. Histology gives a snapshot and not an insight into the dynamic changes during the process of fibrogenesis (progression, static or regression). However, immune-histochemical evaluation of cellular markers such as smooth muscle actin expression for hepatic stellate cell activation, cytokeratin 7 for labeling ductular proliferation or CD34 for visualization of sinusoidal endothelial capillarization or the use of two-photon and second harmonic generation fluorescence microscopy techniques for spatial assessment of fibrillar collagen, can provide additional “functional” information.¹⁹ All these approaches are valid provided that the biopsy is of sufficient size to represent the whole liver. Indeed, LB provides only a very small part of the whole organ and there is a risk that this part might not be representative for the amount of hepatic fibrosis in the whole liver due to heterogeneity in its distribution. Extensive literature has shown that increasing the length of LB decreases the risk of sampling error. Except for cirrhosis, for which micro-fragments may be sufficient, a 25 mm long biopsy is considered an optimal specimen for accurate evaluation, though 15 mm is considered sufficient in most studies. Not only the length but also the caliber of the biopsy needle is important in order to obtain a piece of liver of adequate size for histological evaluation, with a 16 gauge needle

being considered as the most appropriate to use for percutaneous LB. Inter-observer variation is another potential limitation of biopsy, which is related to the discordance between pathologists in biopsy interpretation, although it seems to be less pronounced when biopsy assessment is done by specialized liver pathologists. Beside technical problems, LB remains a costly and invasive procedure that requires physicians and pathologists to be sufficiently trained in order to obtain adequate and representative results – this again limits the use of LB for mass screening. Last but not least, biopsy is an invasive procedure, carrying a risk of rare but potentially life-threatening complications.¹²⁻¹⁶ These limitations have led to the development of non-invasive methods for assessment of liver fibrosis.^{6,7} Although some of these methods are now commonly utilized in patients for first-line assessment, biopsy remains within the armamentarium of hepatologists when assessing the etiology of complex diseases or when there are discordances between clinical symptoms and the extent of fibrosis assessed by non-invasive approaches.^{15,16} Because no reliable scoring system is yet available, a semi-quantitative estimation, either visual or automated, of fibrotic deposits in the four main sites — centrilobular vein, portal tract and perisinusoidal space, together with width and number of septa when present (Table 5 and Figure 12).

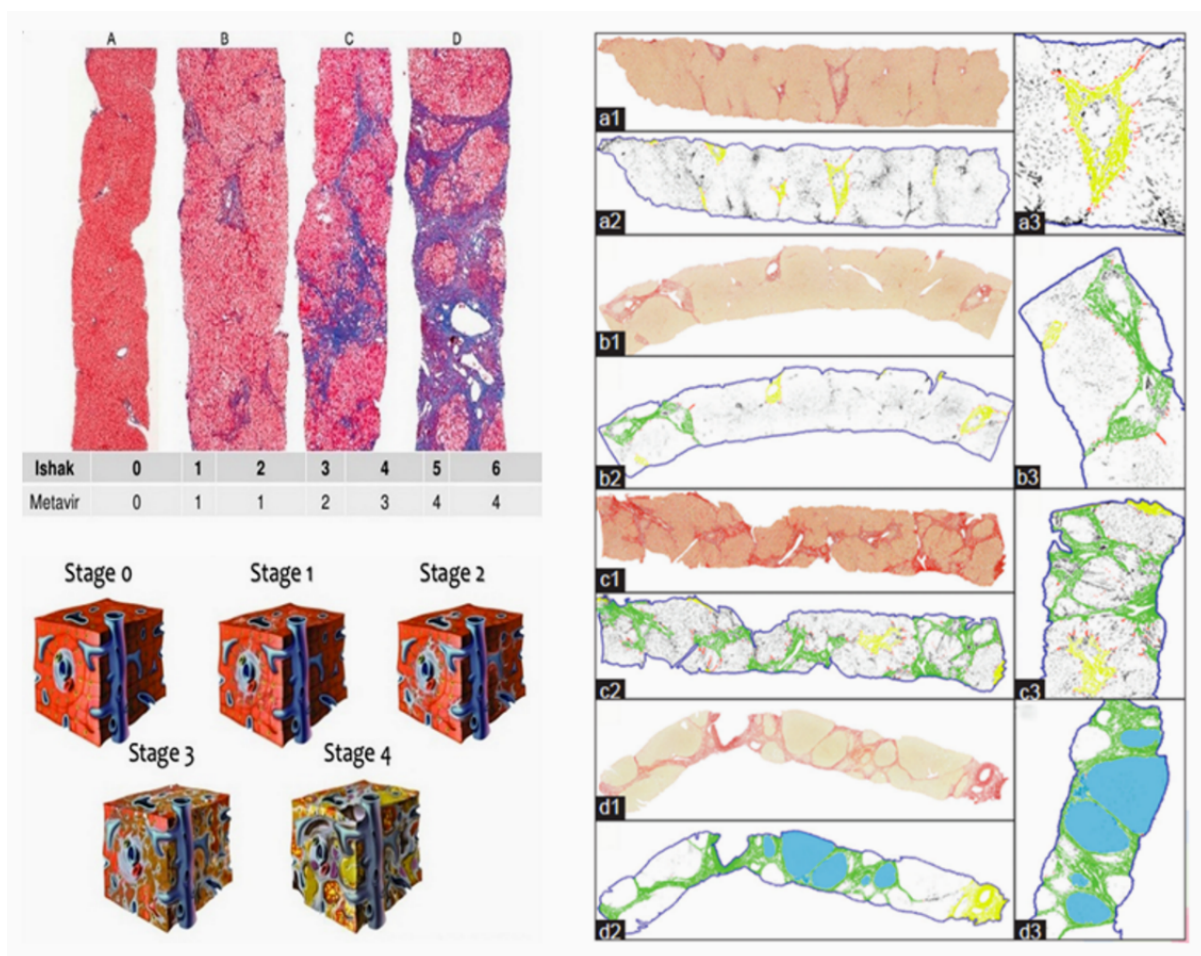
Table 5: METAVIR scoring system for grading hepatic fibrosis¹⁶

Stage	Description
METAVIR	
F0	No fibrosis
F1	Stellate enlargement of portal tract without septa
F2	Enlargement of portal tract with rare septa
F3	Numerous septa
F4	Cirrhosis
Ishak	
0	No fibrosis
1	Fibrous expansion of some portal areas with or without short fibrous septa
2	Fibrous expansion of most portal areas with or without short fibrous septa
3	Fibrous expansion of most portal areas with occasional portal-to-portal bridging
4	Fibrous expansion of portal areas with marked portal-to-portal bridging and portal-to-central bridging
5	Marked bridging (portal-to-portal and/or portal-to-central), with occasional nodules (incomplete cirrhosis)
6	Cirrhosis (probable or definite)

METAVIR = Meta-analysis of Histologic Data in Viral Hepatitis.

* Clinically significant fibrosis is usually defined as METAVIR stages F2 to F4 or Ishak stages 3 to 6. Cirrhosis is typically defined as METAVIR stage F4 or Ishak stages 5 or 6; however, METAVIR stage F3 includes patients with early or developing cirrhosis.

Figure 12: Quantification of hepatic fibrosis by METAVIR scoring system¹⁶ (A) and digital morphometry¹⁷ (B)



Non-invasive tests could be divided into serological tests and imaging tests. Although standard “liver function” tests, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are inaccurate when used alone, several models have been developed that use them in combination with other markers of advanced liver disease, such as platelet count. Of those models that utilize routine, readily available tests, the AST-Platelet Ratio Index (APRI) and Fibrosis-4 score (FIB-4) have gained the most attention. Both APRI and FIB-4 have high specificity and negative predictive values (NPV) for advanced fibrosis or cirrhosis. However, both have only moderate positive predictive values (PPV) and many patients fall in-between the upper and lower cut-off values, giving an indeterminate result. More complex serum panels have been developed including FibroSure[®]/FibroTest[®] and FibroMeter (FM)[™], which may offer additional accuracy compared to APRI or FIB-4 but

have extra costs. The alternative non-invasive imaging tests, such as FibroScan® or magnetic resonance elastography (MRE). While MRE is available in few specialized centers, TE is easy to use and is widely available in all hepatobiliary centers.²⁰⁻²⁵

Table 6: Characteristics and diagnostic performance of various serum liver fibrosis markers in clinical practice³

Tests	Variables	Disease	Diagnostic objective	Performance (AUROC)
APRI	AST, platelets	HCV	F2 F4	0.76/0.80 0.82/0.89
Fib4	Age, AST, ALT, platelets	HIV-HCV	F2	0.76–0.85
Fibrotest	A2M, GGT, haptoglobin, apoA1, total bilirubin	HCV	F2 F4	0.78/0.85 0.89–0.92
Hépascore	Age, sex, HA, A2M, GGT, bilirubin	HCV	F2 F4	0.82/0.85 0.89/0.94
Fibromètre V virus	Age, HA, A2M, PT, platelets, urea, AST	HCV, HBV	F2 F4	0.89 0.9
Fibromètre A alcohol	Age, HA, A2M, PT	Alcohol	F2	0.96
Fibromètre S fatty liver disease	AST, ALT, platelets, ferritine, blood glucose, weight, age	Fatty liver disease	F2	0.96

A2M: alpha-2 macroglobulin; PT: prothrombin time; HA: hyaluronic acid; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

In 2003, Sandrin *et al* published the first report of the use of this VCTE™ technology for assessment of hepatic fibrosis. The intra- and inter-operator reproducibility of the technique, as well as its ability to quantify liver fibrosis, were evaluated in 106 patients with chronic hepatitis C (HCV).⁶

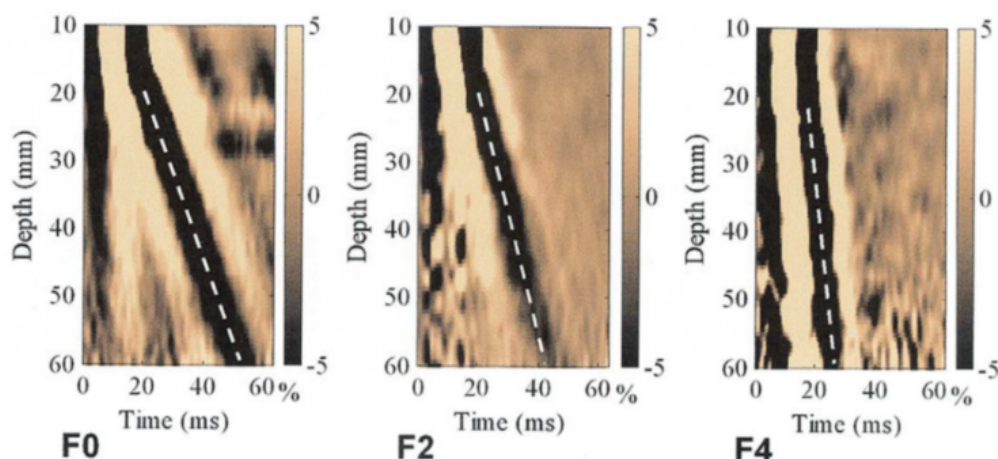


Figure 13: Transient elastography by VCTE™ technology⁶

Liver elasticity measurements were reproducible (standardized coefficient of variation=3%), operator-independent and well correlated (partial correlation coefficient=0.7, $p<0.001$) to METAVIR fibrosis grade. The areas under the receiver operating characteristic

(ROC) curves were 0.88 and 0.99 for the diagnosis of patients with significant fibrosis ($>F2$) and with cirrhosis (F4) respectively.^{6,7}

In chronic liver disease due to hepatitis C, many publications have demonstrated excellent diagnostic performance of LS to histological stage of fibrosis according to METAVIR scoring system. FibroScan[®] has an excellent area under receiver operating curve (AUROC) to diagnose cirrhosis and a good performance to diagnose patients with significant hepatic fibrosis ($F\geq 2$). Similarly, in patients with chronic hepatitis B infection, LS had similar performance to hepatitis C patients. AUROC for patients with significant fibrosis ($F\geq 2$) and cirrhosis were about 0.8 and 0.9 respectively (Tables 7 and 8). In a recent publication, Nakamura *et al* compared liver fibrosis staging by FibroScan[®] to pathological findings of liver resection specimen in HCV infection. In the training set, AUROC for diagnosis $F\geq 2$ was 0.97, $F\geq 3$ was 0.92, and for F4 was 0.92. In the validation set, at a cut-off value of 5.9 kPa, sensitivity (Sens), specificity (Spec), positive predictive value (PPV) and negative predictive value (NPV) for $F\geq 2$ were 95.7%, 0.0%, 97.8% and 0.0%, respectively, of 9.8 for $F\geq 3$ were 86.2%, 52.6%, 73.5% and 71.4%, and of 15.5 for F4 were 100%, 71.8%, 45%, and 100%.²⁶ While analyzing patients with viral hepatitis co-infected with human immunodeficiency virus (HIV), it was found that FibroScan[®] accuracy to diagnose cirrhosis in HCV patients was as good in HIV negative as in HIV positive patients, and was not impaired by anti HIV treatment. LS was proficient in discriminating between absence or mild fibrosis and moderate to severe fibrosis, with up to 80% of patients being classified correctly.²⁷ Further, various studies have proposed LS to monitor treatment response in viral hepatitis because LS decreased below the baseline in patients who have achieved sustained virological response to antiviral treatment in both HBV and HCV patients.²⁸⁻³⁰

Table 7: Diagnostic performance of liver stiffness by transient elastography in hepatitis C infection

Publication ³¹⁻³⁴	N	AUROC	Cut-off	Sens	Spec	AUROC	Cut-off	Sens	Spec
		F ≥2	F2 (kPa)	(%)	(%)	F4	F4 (kPa)	(%)	(%)
Ziol <i>et al</i> , 2005	251	0.79	8.8	56	84	0.97	14.6	86	96
Shaheen <i>et al</i> , 2007	546	0.83	8	64	87	0.90	12.6	86	91
Lupsor <i>et al</i> , 2013	1202	0.89	7.4	80	84	0.97	13.2	94	93
Afdhal <i>et al</i> , 2015	188	0.89	8.4	82	79	0.92	12.8	84	86

Table 8: Diagnostic performance of liver stiffness by transient elastography in hepatitis B infection

Publication ³⁵⁻³⁹	N	AUROC	Cut-off	Sens	Spec	AUROC	Cut-off	Sens	Spec
		F ≥2	F2 (kPa)	(%)	(%)	F4	F4 (kPa)	(%)	(%)
Marcellin <i>et al</i> , 2009	202	0.81	7.2	70	83	0.93	11	93	87
Cardoso <i>et al</i> , 2012	202	0.87	7.2	74	77	0.94	11	89	75
Verveer <i>et al</i> , 2012	125	0.85	6	-	-	0.90	13	-	-
Zhu <i>et al</i> , 2011	175	0.95	7.9	88	91	0.98	13.8	93	91
Chon <i>et al</i> , 2012	2722	0.86	7.9	74	78	0.93	11.7	85	82

Table 9: Diagnostic performance of liver stiffness by transient elastography in mixed aetiologies

Publication ⁴⁰⁻⁴⁴	N	AUROC	Cut-off	Sens	Spec	AUROC	Cut-off	Sens	Spec
		F ≥2	F2 (kPa)	(%)	(%)	F4	F4 (kPa)	(%)	(%)
Foucher <i>et al</i> , 2006	711	0.80	7.2	64	85	0.96	17.6	77	97
Vergara <i>et al</i> , 2007	123	0.87	7.2	88	86	0.95	14.6	91	88
Miaillhes <i>et al</i> , 2011	59	0.85	5.9	81	87	0.96	9.4	92	94
Bota <i>et al</i> , 2013	1131	0.87	-	78	84	0.93	-	89	87
Ferraioli <i>et al</i> , 2013	252	0.86	6.9	71	86	0.96	9.6	95	90

Table 10: Liver stiffness cut-off values according to METAVIR fibrosis grades

METAVIR Scoring System		Fibroscan Cut-off values
F0	No fibrosis	--
F1	Mild fibrosis – portal fibrosis without septa	< 7.1 kPa
F2	Moderate fibrosis – portal fibrosis and few septa	7.1 to 8.8 kPa
F3	Severe fibrosis – numerous septa without cirrhosis	9.5 to 9.6 kPa
F4	Cirrhosis	12.5 to 14.6 kPa

Figure 14: Liver stiffness scoring card illustrating cut-off values in various liver diseases⁵

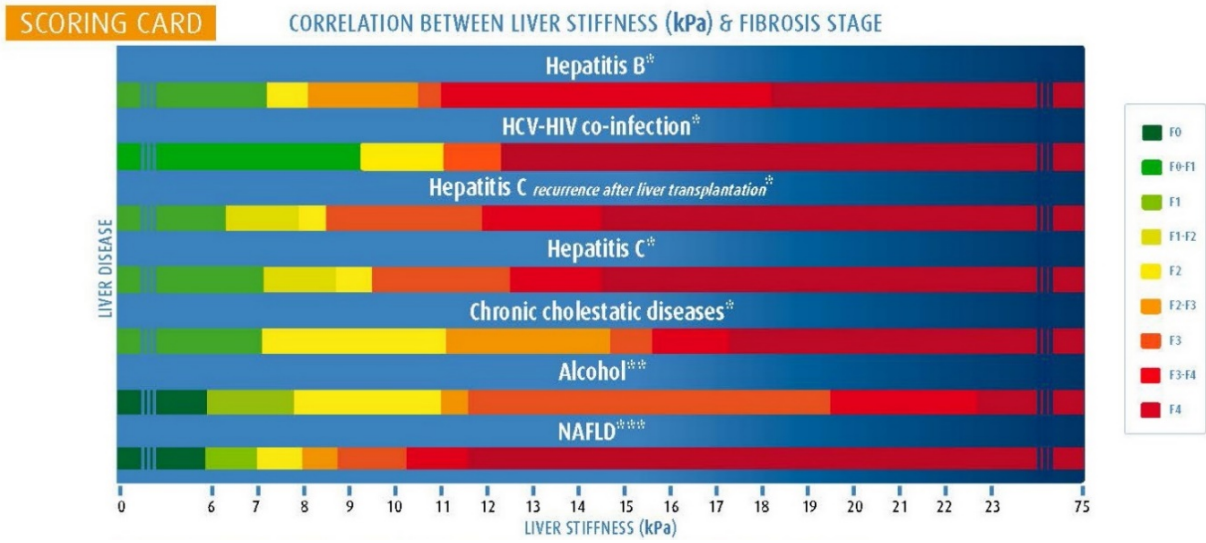
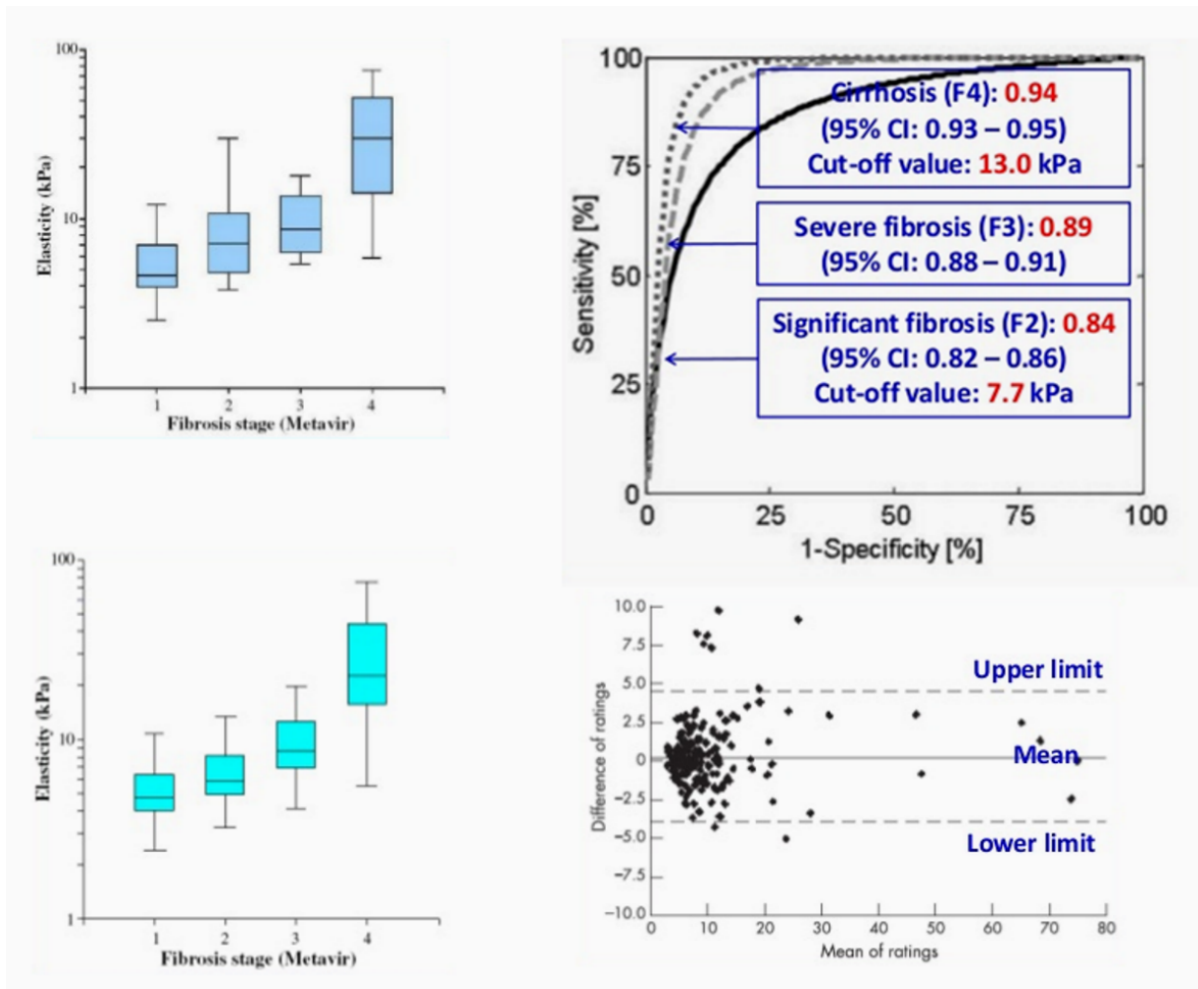


Figure 15: Liver stiffness to stage liver fibrosis: Box and Whiskers plots^{28,31} (A and B), area under ROC curve⁴⁶ (C) and Bland-Altman plot⁴⁷ for reproducibility of measurements



Other specific categories of patients where FibroScan[®] has demonstrated good diagnostic performance include those with alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD). In a recent meta-analysis by Pavlov *et al* , LS was compared with liver biopsy in patients with alcoholic liver disease and a proposed cut-offs of 7.5 for F_{≥2}, 9.5 for F_{≥3} and 12.5 for F4.⁴⁸ However, LS would be influenced by inflammation associated with alcohol consumption and abstinence from alcohol is advised when a patient would undergo TE.

Table 11: Meta-analysis of diagnostic performance of liver stiffness in alcoholic liver disease⁴⁸

METAVIR	N	Cut-off (kPa)	Sens (%)	Spec (%)
F _{≥2}	338	7.5	94	89
F _{≥3}	564	9.5	92	70
F4	330	12.5	95	71

Nonalcoholic fatty liver disease is the leading cause of chronic liver disease in developed countries, with an estimated prevalence of 20-30% and increasing to 70% in diabetics and as high as 90% in obese individuals.⁴⁹⁻⁵³ Given the pathophysiological link with metabolic syndrome, rates of NAFLD are likely to continue to rise with the obesity pandemic.⁵⁴ Within a community cohort, prevalence of patients with NAFLD who have nonalcoholic steatohepatitis (NASH) has been estimated at 3-4% and rising as high as 12.2% in middle-aged patients.^{55,56} Patients with NASH are six times more likely to develop liver-related mortality over 20 years than those with non-NASH NAFLD while those with advanced fibrosis has the highest risk of mortality. These individuals with NASH and fibrosis have the greatest risk of progression to advanced disease, cirrhosis, hepatocellular carcinoma, and death.⁵⁶⁻⁵⁹ Therefore, it is critical to distinguish between patients with fibrosis, NASH, and non-NASH NAFLD. However, noninvasive distinction of NASH from non-NASH NAFLD using imaging remains elusive because the diagnosis of NASH historically relies on

the observation of ballooning degeneration of hepatocytes as well as steatosis and inflammation — not simply steatosis and fibrosis. The current gold standard for diagnosis and histological assessment of NAFLD is a biopsy.⁵⁶ Unfortunately, LB is invasive, subject to complications, costly, and limited by sampling errors and intra-observer variability.^{13,14} Furthermore, given the high prevalence of NAFLD, it is neither practical nor cost effective to perform liver biopsies in all patients who are at risk for NASH and fibrosis. Recently, several noninvasive imaging modalities based on elastography have been developed. To date, these modalities have proven promising in the assessment of liver fibrosis in chronic liver disease, though rigorous comparative studies in patients with NAFLD are lacking.^{56,60-63} The most validated among the imaging modalities is LS measured by TE.^{56,60,64,65}

FibroScan[®] was able to differentiate accurately patients with significant ($F \geq 2$) fibrosis and rule out advanced liver fibrosis both in adult and paediatric NAFLD patients. In a meta-analysis by Musso and his colleagues, NAS fibrosis score and TE were the only independent validated techniques to detect significant fibrosis when compared with serum fibrosis markers such as FibroTest[®], BARD score, European liver fibrosis (ELF) test, FM[™]. In 19% of NAFLD patients with increased waist circumference and body mass index, the conventional M probe gives uninterpretable results for liver stiffness. Therefore, XL probe that produces lower width shear waves for obese patients is useful and 60% of the patients not measured by M probe would be reliably evaluated by XL probe. The XL probe has similar overall diagnostic accuracy when compared to M probe with an AUROC of 0.85 for $F \geq 3$ and 0.9 for cirrhosis with overall sensitivity of 88%, specificity of 95% and a cut-off value of 7.8 kPa in all stages. In total, 78 – 84% of patients will have reliable LS measurement, upon using first-line M probe followed by XL probe use in the event of failing M probe.⁶⁰

In a recent report, Cassinotto *et al* compared non-invasive assessment of liver fibrosis three impulse elastography techniques in 349 consecutive patients with chronic liver diseases

who underwent LB. Both point-quantification shear wave elastography (pSWE) and two-dimensional shear wave elastography (2D-SWE) use ARFI technology to generate shear waves in the liver, and the shear wave speed is calculated in meters per second. pSWE is most commonly referred to as ARFI in the hepatology literature, whereas 2-D SWE is referred to as supersonic shear imaging (SSI) based on one of the four systems available clinically. SSI, FibroScan[®], and ARFI correlated significantly with histological fibrosis score ($r=0.79$, $p<0.001$; $r=0.70$, $p<0.001$; $r=0.64$, $p<0.001$, respectively). AUROCs of SSI, FibroScan[®], and ARFI were 0.89, 0.86, and 0.84 for the diagnosis of mild fibrosis; 0.88, 0.84, and 0.81 for the diagnosis of significant fibrosis; 0.93, 0.87, and 0.89, for the diagnosis of severe fibrosis; 0.93, 0.90, and 0.90 for the diagnosis of cirrhosis, respectively. SSI had a higher accuracy than TE for the diagnosis of severe fibrosis ($F\geq 3$) ($p=0.001$), and a higher accuracy than ARFI for the diagnosis of significant fibrosis ($F\geq 2$) ($p<0.001$). No significant difference was observed for the diagnosis of mild fibrosis and cirrhosis.⁶⁶

Table 12: Comparison of FibroScan (VCTE) with acoustic radiation force impulse (ARFI) and Supersonic shear imaging (SSI)⁶⁶

Fibrosis Stage	VCTE-Kwok	VCTE-Cassinotto	ARFI-Fierbinteanu Braticevici	ARFI-Cassinotto	SSI-Cassinotto
F ≥ 2	6.7-7.7 kPa	6.2-9.8 kPa	1.165 m/s	0.95 - 1.32 m/s	6.3 - 8.7 kPa
AUROC	0.79-0.87	0.76-0.87	0.891-0.997	0.70-0.83	0.79-0.90
Sensitivity, %	67-94	60-90	84.8	56-90	71-90
Specificity, %	61-84	45-90	90.3	36-91	50-90
F ≥ 3	8.0-10.4 kPa	8.2-12.5 kPa	1.48 m/s	1.15-1.53 m/s	8.3-10.7 kPa
AUROC	0.76-0.98	0.80-0.90	0.956-1.000	0.78-0.89	0.83-0.92
Sensitivity, %	65-100	57-90	86.4	59-90	71-91
Specificity, %	75-97	61-90	95.2	63-90	71-90
F4	10.3-17.5 kPa	9.5-16.1 kPa	1.635 m/s	1.3-2.04 m/s	10.5-14.4 kPa
AUROC	0.91-0.99	0.79-0.92	0.958-1.000	0.78-0.89	0.82-0.92
Sensitivity, %	78-100	65-92	91.7	44-90	58-90
Specificity, %	82-98	62-90	92.3	67-90	72-90

Abbreviations: kPa, kilopascals; m/s, meters per second.

Castéra *et al* investigated various factors that influence the reliability of LS, the frequency and determinants of LS failure and unreliable results over a 5-year period, based on 13,369 examinations (134,239 shots). LS failure was defined as zero valid shots, and unreliable examinations were defined as fewer than 10 valid shots, an interquartile range (IQR)/M $\geq 30\%$, or a success rate $<60\%$. LS failure occurred in 3.1% of all examinations (4%

at first examination [n=7261]) and was independently associated at first examination with BMI >30 kg/m² (odds ratio (OR), 7.5; 95% confidence interval (CI), 5.6-10.2; P=0.0001), operator experience fewer than 500 examinations (OR 2.5 [1.6-4.0]; P<0.0001); age greater than 52 years (OR 2.3 [1.6-3.2]; P=0.0001), and type 2 diabetes (OR 1.6 [1.1-2.2]; P=0.009). Unreliable results were obtained in a further 15.8% of cases (17% at first examination) and were independently associated at first examination with BMI>30 kg/m² (OR 3.3 [2.8-4.0]; P<0.0001), operator experience <500 examinations (OR 3.1 [2.4-3.9]; P=0.0001), age greater than 52 years (OR 1.8 [1.6-2.1]; P=0.0001), female sex (OR 1.4 [1.2-1.6], P=0.0001), hypertension (OR 1.3 [1.1-1.5]; P=0.003), and type 2 diabetes (OR 1.2 [1.0-1.5]; P=0.05). When metabolic syndrome and waist circumference were taken into account in a subgroup of 2835 patients, waist circumference was the most important determinant of LS failure and unreliable results. Thus, in their cohort, liver stiffness measurements were uninterpretable in nearly one in five cases.

The principal reasons were obesity, particularly increased waist circumference, and limited operator experience. Different conditions that affect the reliability of LS in patients including inflammation, increased central venous pressure, and cholestasis are summarized in Figure 16.⁶⁷

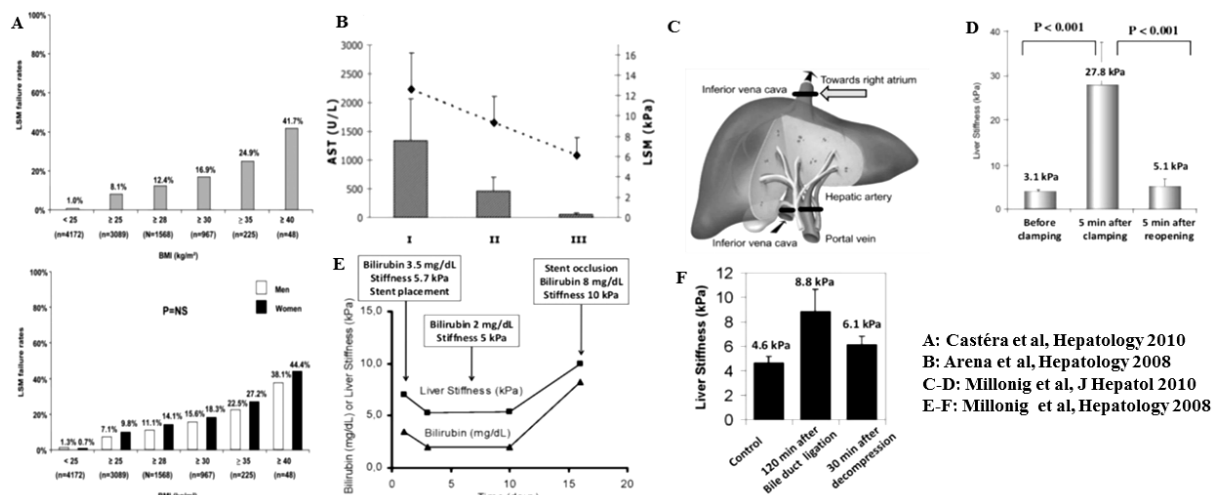
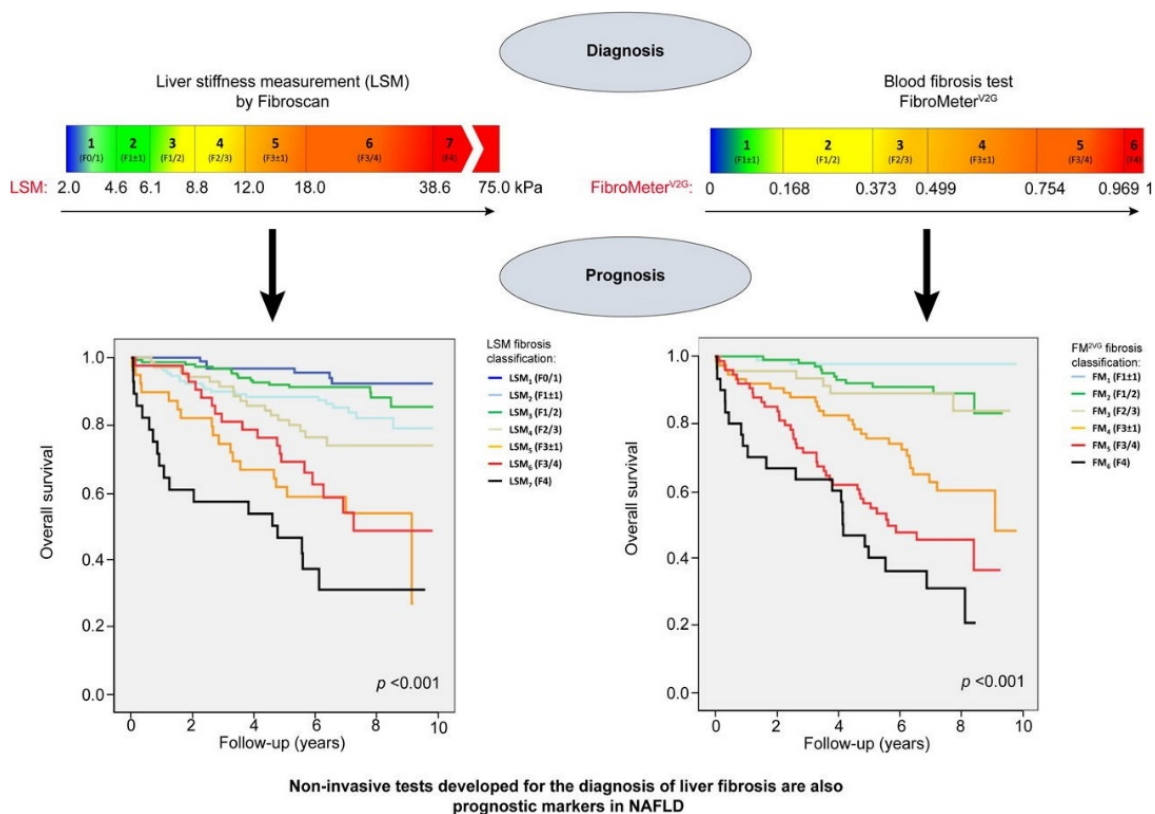


Figure 16: Factors affecting liver stiffness measurement: A-body mass index⁶⁷, B-inflammation⁶⁸, CD-increased central venous pressure⁶⁹, EF-cholestasis⁷⁰

Similarly, Chan *et al* demonstrated that the patients with the same fibrosis staging but higher ALT levels tended to have higher LS and the diagnostic performance for low stage fibrosis was more seriously affected when ALT was elevated. They developed a new algorithm with different LS cut-off values for normal and elevated ALT levels. Based on this algorithm, LB could be avoided in 62% and 58% patients with normal and elevated ALT level.⁷¹

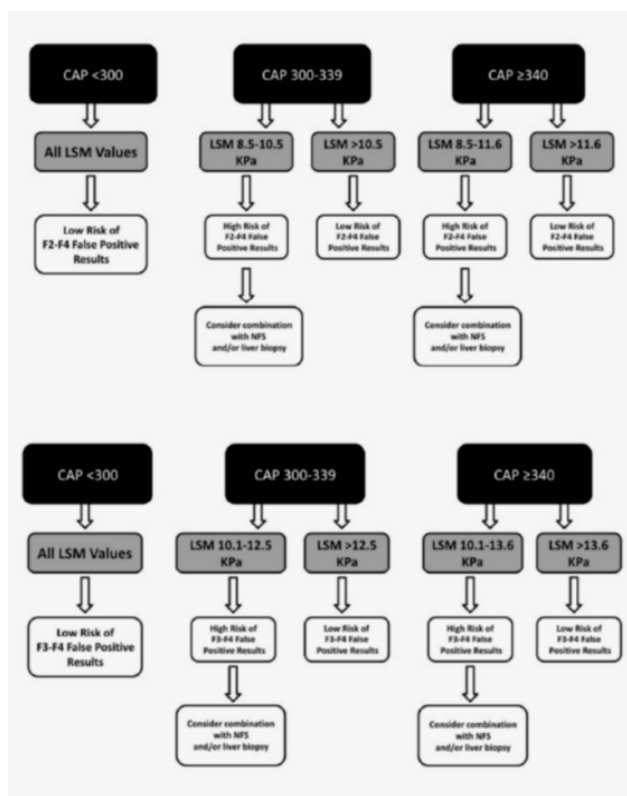
Boursier and his colleagues recently reported that FibroScan[®] and FibroMeter^{V2G} were the most accurate tests for non-invasive evaluation of liver fibrosis in NAFLD when compared with seven blood tests. AUROCs for advanced fibrosis were, respectively, 0.83±0.02 and 0.82±0.02 ($p \leq 0.04$ vs other tests). Two fibrosis classifications were developed to precisely estimate the histological fibrosis stage and patients' prognosis from LS or FM^{V2G} without biopsy (diagnostic accuracy, respectively: 80.8% vs 77.4%, $p=0.19$).⁷²

Figure 17: Fibrosis stage classification by FibroScan and FibroMeter^{V2G} with their corresponding overall survival according to the stage of fibrosis⁷²



The severity of hepatic steatosis influences the LS measurement in patients with NAFLD as reported by Petta *et al.* In their recent publication, they highlighted that the presence of severe steatosis (>66%), detected by histology or by US, resulted in higher LS values (false-positive) for subjects without significant fibrosis (F0-2). They proposed a decisional flow-chart predicting fibrosis was suggested by combining both LS and CAP values.⁶⁵

Figure 18: Decisional tree to predict hepatic fibrosis with liver stiffness and controlled attenuation parameter⁶⁵



Puigvehi *et al* published recently a study evaluating the impact of anthropometric features on the applicability and accuracy of FibroScan[®] in 1,084 overweight and obese patients. The applicability of M and XL probe was 88.8% and 98%, respectively. Waist circumference (WC) (OR: 0.97; 95%CI: 0.94-0.99; $p < 0.001$) and skin-capsule distance (SCD) (0.83; 0.79-0.87; $p < 0.001$) were independently related to unreliable LS (M probe). The SCD was >25 mm in 5.5% of individuals with a BMI ≤ 35 kg/m² and a WC ≤ 117 cm, with LSM (M probe) applicability rising to 94.3%. In contrast, 36.9% of patients with a

BMI>35 kg/m² and/or a WC>117 cm presented a SCD>25 mm, with M probe applicability being 73.1%.⁷³ The diagnostic accuracy (AUROC) using the M probe to identify significant steatosis (0.76), fibrosis (0.89) and cirrhosis (0.96) was very high in patients with a BMI≤35 kg/m² and a WC≤117 cm. Therefore, the applicability and accuracy of the FibroScan® M probe to identify fibrosis and steatosis was excellent in overweight and obesity grade I (BMI≤35 kg/m²) with a WC≤117 cm. The XL probe increased the applicability of TE in obesity grade II-III (BMI>35 kg/m²).⁷³

Boursier and his colleagues established the current reliability criteria for LS evaluation by FibroScan® in 2013. They evaluated the usual criteria for reliability of LS measurement in a large multicentric cohort of 1165 patients suffering from chronic liver disease and had a liver biopsy and LS evaluation. In multivariate analyses with different diagnostic targets, LS median and IQR/M were independent predictors of fibrosis staging, with no significant influence of <10 valid measurements or LS success rate. These two reliability criteria determined three LS groups: “very reliable” (IQR/M<0.10), “reliable” (0.10<IQR/M<0.30 or IQR/M>0.30 with LS median <7.1 kPa), and “poorly reliable” (IQR/M>0.30 with LS median >7.1 kPa). The rates of well-classified patients for the diagnosis of cirrhosis were, respectively: 90.4%, 85.8%, and 69.5% (*p*<0.001). LS reliability depends on IQR/M according to liver stiffness median level, defining thus three reliability categories: very reliable, reliable, and poorly reliable LS as shown in the Table 13.⁷⁴

Table 13: Reliability criteria for liver stiffness measurement by FibroScan®: very reliable (white), reliable (light grey) and poorly reliable (dark grey)⁷⁴

LSE Diagnosis *:		LSE Median			Patient Rate (%)
		<7.1 F _{FS} 0/1	7.1 ≤ and <12.5 F _{FS} 2/3	≥12.5 F _{FS} 4	
IQR/M	≤0.10		Very reliable LSE		16.6
	0.10 < and ≤0.30		Reliable LSE		74.3 †
	>0.30		Poorly reliable LSE		9.1

Because it is quick and easy in most cases, LSE should include 10 valid measurements whatever the LSE success rate.

*LSE diagnosis after categorization of LSE median into estimated Metavir fibrosis stages (F_{FS}) according to the diagnostic cutoffs of Castera et al.: 7.1 kPa for significant fibrosis and 12.5 kPa for cirrhosis (12).

†Including the subgroup with IQR/M >0.30 and LSE median <7.1.

Table 14: Diagnostic performance of liver stiffness to detect significant fibrosis in non-alcoholic fatty liver disease

Publication ^{60,75-79}	N	AUROC	Cut-off	Sens	Spec	PPV	NPV
		F \geq2	F2 (kPa)	(%)	(%)	(%)	(%)
Yoneda <i>et al</i> , 2008	97	0.87	6.7	88.2	73.9	78.9	85.0
Nobili <i>et al</i> , 2008	50	0.99	7.4	100	92	80	100
Wong <i>et al</i> , 2010	309	0.84	7.0	79.2	75.9	69.6	84.0
Lupsor <i>et al</i> , 2010	65	0.79	6.8	66.7	84.3	60.0	87.8
Gaia <i>et al</i> , 2010	72	0.80	7.0	76	80	75	78
Musso <i>et al</i> , 2011	563	0.84	7.0	79	76	-	-

In 2015, European association for the study of the liver (EASL) published its recommendations as practice guidelines on the utilization of non-invasive tests for the evaluation of liver disease severity and prognosis and they are as follows:⁸⁰

- Non-invasive tests should always be interpreted by specialists in liver disease, according to the clinical context, considering the results of other tests (biochemical, radiological and endoscopic) and taking into account the recommended quality criteria for each test and its possible pitfalls (A1)
- Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good inter-laboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer’s recommendations for the patented tests (A1)
 - TE is a fast, simple, safe and easy to learn procedure that is widely available. Its main limitation is the impossibility of obtaining results in case of ascites or morbid obesity and its limited applicability in case of obesity and limited operator experience (A1)
 - TE should be performed by an experienced operator (>100 examinations) following a standardized protocol with the patient, fasting for at least 2 hours, in the supine

position, right arm in full abduction, on the mid axillary line with the probe-tip placed in the 9th to 11th intercostal space with a minimum of 10 shots (A1)

- Correct interpretation of TE results in clinical practice must consider the following parameters: (A1)
 - IQR/M value (<30%),
 - Serum aminotransferases levels (<5 x upper limit of normal range)
 - BMI (use XL probe >30 kg/m² or if skin-to-capsule distance is >25 mm)
 - Absence of extra-hepatic cholestasis, ongoing excessive alcohol intake
 - Absence of right heart failure, or other causes of congestive liver
- Although alternative techniques, such as pSWE/ARFI or two-dimensional shear wave elastography (2D-SWE) seem to overcome limitations of TE, their quality criteria for correct interpretation are not yet well-defined (A1)
- At present correct interpretation of pSWE/ARFI results in clinical practice should systematically take into account the potentially confounding parameter: (B1)
 - Fasting for at least 2 hours, transaminases levels (<5 x ULN), absence of extra-hepatic cholestasis and absence of right heart failure
- MR elastography is currently too costly and time consuming for routine clinical practice use and seems more suited for research purposes (A1)

In 2015, Baveno VI consensus workshop proposed the following recommendations in their position paper published in 2015 under the category ‘Screening and surveillance: Invasive and non-invasive methods’:⁸¹

A. Definition of compensated advanced chronic liver disease (cALD)

- The introduction of TE in clinical has allowed the early identification of patients with chronic liver disease (CLD) at risk of developing clinically significant portal hypertension (CSPH) (1b:A)
- For these patients, the alternative term ‘advanced chronic liver disease (cALD)’ has been proposed to better reflect that the spectrum of severe fibrosis and cirrhosis is a continuum in asymptomatic patients , and that distinguishing between the two is often not possible on clinical grounds (5:D)
- Currently, both terms cALD and CSPH are acceptable (5:D)
- Patients with suspicion of cALD should be referred to a liver disease specialist for confirmation, follow-up and treatment (5:D)

B. Criteria to suspect cALD

- Liver stiffness by TE is sufficient to suspect cALD in asymptomatic subjects with known causes of CLD
- TE often has false positive results; hence two measurements on different days are recommended in fasting conditions (5:D)
- TE values <10 kPa in the absence of other known clinical signs rule out cALD; values between 10 and 15 kPa are suggestive of cALD but need further test for confirmation; values >15 kPa are highly suggestive of cALD (1b:A)

C. Criteria to confirm cALD

- Invasive methods are employed in referral centers in stepwise approach when the diagnosis is in doubt or as confirmatory tests
- Methods and findings that confirm the diagnosis of cALD are:
 1. Liver biopsy showing severe fibrosis or established cirrhosis (1a:A)

2. Collagen proportionate area measurement on histology provides quantitative data on the amount of fibrosis and holds prognostic value (2b:B) and its assessment is recommended (5:D)
3. Upper GI endoscopy showing gastroesophageal varices (1b:A)
4. Hepatic venous pressure gradient measurement; values >5 mmHg indicate sinusoidal portal hypertension (1b:A)

EASL also recommended that performing TE plus a blood test to diagnose significant fibrosis and the test concordance would confirm the diagnosis. The FibroMeter^{VCTE} (FM^{VCTE}) is a new formula combining the serum test FMTM and LS measured by TE. A stepwise algorithm called the easy liver fibrosis test (eLIFT) was developed as a first-line procedure that selects at-risk patients who need further evaluation with FM^{VCTE}, an accurate fibrosis test combining blood markers and TE. The diagnostic study group consisted of 3754 CLD patients with liver biopsy who were 2:1 randomized into derivation and validation sets. In the prognostic study group, longitudinal follow-up of 1275 CLD patients with baseline fibrosis tests. Diagnostic study: eLIFT, an “at-a-glance” sum of points attributed to age, gender, gammaglutamyl transferase, AST, platelets and prothrombin time, was developed for the diagnosis of advanced fibrosis. In the validation set, eLIFT and fibrosis-4 (FIB4) had the same sensitivity (78.0% vs. 76.6%, $p = 0.470$) but eLIFT gave fewer false positive results, especially in patients ≥ 60 years old (53.8% vs. 82.0%, $p < 0.001$), and was thus more suitable as screening test. FMTM with VCTETM was the most accurate among the eight tests for fibrosis evaluated. The sensitivity of the eLIFT-FM^{VCTE} algorithm (first-line eLIFT, second-line FM^{VCTE}) was 76.1% for advanced fibrosis and 92.1% for cirrhosis. In the prognostic study group, patients diagnosed as having “no/mild fibrosis” by the algorithm had excellent liver-related prognosis with thus no need for referral to a hepatologist. This new algorithm, called

the eLIFT-FM^{VCTE}, accurately identifies the patients with advanced chronic liver disease who need referral to a specialist, and those with no or mild liver lesions who can remain under the care of their usual physician.⁸²

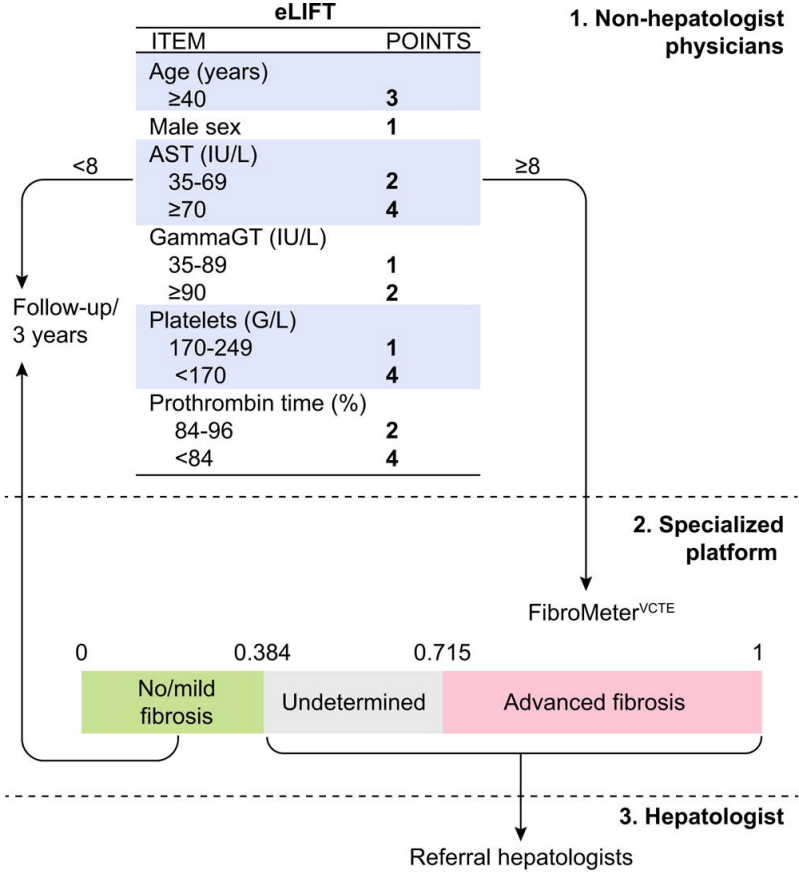


Figure 19: eLIFT-FM^{VCTE} algorithm⁸²

Calès *et al* validated the EASL recommendations for a combined test. Further, they demonstrated that FM^{VCTE} expressed in classification instead of score improved the performance and would avoid 99% of liver biopsies (LB) by offering precise staging in chronic hepatitis C.⁸³ Loong *et al* compared the performance of LS, FMTM-NAFLD, FM^{VCTE}, and other serum tests (APRI, Fib-4 index, BARD score, NAFLD fibrosis score, and AST-to-ALT ratio) in 215 NAFLD to diagnose hepatic fibrosis. They found that LS alone could exclude significant

and advanced fibrosis in NAFLD patients. Using FM^{VCTE} in patients with high liver stiffness increased PPV to rule in F2-4 and F3-4.⁸⁴

More recently, American gastroenterological association (AGA) published their recommendations on the use of TE to diagnose hepatic fibrosis:⁸⁵

A. Management of patients with Chronic Viral Hepatitis C:

Recommendation: In patients with chronic hepatitis C, the AGA recommends VCTETM, if available, rather than other nonproprietary, noninvasive serum tests (APRI, FIB-4) to detect cirrhosis.

Recommendation: In patients with chronic hepatitis C, the AGA suggests a VCTETM cutoff of 12.5 kPa to detect cirrhosis

Recommendation: In non-cirrhotic patients with HCV who have achieved SVR after anti-viral therapy, the AGA suggests a post-treatment vibration controlled transient elastography cutoff of 9.5 kPa to rule out advanced liver fibrosis.

Recommendation: In adult patients with chronic hepatitis C, the AGA suggests using VCTE rather than MRE for detection of cirrhosis. Management of patients with chronic hepatitis B.

Recommendation: In patients with chronic hepatitis B, the AGA suggests VCTETM rather than other nonproprietary noninvasive serum tests (ie, APRI and FIB-4) to detect cirrhosis.

Recommendation: In patients with chronic hepatitis B, the AGA suggests a VCTETM cutoff of 11.0 kPa to detect cirrhosis.

B. Management of patients with Alcoholic Liver Disease:

Recommendation: In patients with chronic alcoholic liver disease, the AGA suggests a VCTETM cutoff of 12.5 kPa to detect cirrhosis and prediction of esophageal varices.

Recommendation: In patients with suspected compensated cirrhosis, the AGA suggests a vibration controlled transient elastography cutoff of 19.5 kPa to assess the need for esophagogastroduodenoscopy to identify high-risk esophageal varices.

European federation of societies for ultrasound in medicine and biology (EFSUMB) recently proposed the following recommendations:⁸⁶

A. Fibrosis staging in chronic hepatitis C:

- TE can be used as the first-line assessment for the severity of liver fibrosis in patients with chronic viral hepatitis C. It performs best with regard to the ruling out of cirrhosis (1b:A). Broad consensus (17/0/1, 94%).
- LS changes after successful anti-HCV treatment should not affect the management strategy (e. g. surveillance for HCC occurrence in patients at risk) (3:D). Broad consensus (16/0/1, 94%).

B. Fibrosis staging in chronic hepatitis B:

- TE is useful in patients with CHB to identify those with cirrhosis. Concomitant assessment of transaminases is required to exclude flare up (elevation >5 times upper limit of normal). (1b:A). Broad consensus (17/1/0, 94%).
- TE is useful in inactive HBV carriers to rule out fibrosis (2:B). Strong consensus (18/0/0, 100%).
- LS changes under HBV treatment should not affect the management strategy (e.g. surveillance for HCC occurrence in patients at risk) (2b:B). Strong consensus (16/0/0, 100%).

C. Fibrosis staging in NAFLD:

- TE can be used to exclude cirrhosis in NAFLD patients (2a:B). Broad consensus (13/0/3, 81%).

D. Fibrosis staging in alcoholic liver disease (ALD):

- TE can be used to exclude cirrhosis in patients with ALD, provided that acute alcoholic hepatitis is not present (2b:B). Strong consensus (15/0/0, 100%).

Table 15: Advantages and disadvantages of various non-invasive tests to estimate hepatic fibrosis⁸⁰

Serum biomarkers	Measurement of liver stiffness			
	Transient elastography	ARFI (pSWE)	2D-SWE	MR elastography
Advantages				
<ul style="list-style-type: none"> • Good reproducibility • High applicability (95%) • No cost and wide availability (non-patented) • Well validated • Can be performed in the outpatient clinic 	<ul style="list-style-type: none"> • Most widely used and validated technique: standard to be beaten • User-friendly (performed at bedside; rapid, easy to learn) • High range of values (2-75 kPa) • Quality criteria well defined • Good reproducibility • High performance for cirrhosis (AUROC >0.9) • Prognostic value in cirrhosis 	<ul style="list-style-type: none"> • Can be implemented on a regular US machine • ROI smaller than TE but location chosen by the operator • Higher applicability than TE (ascites and obesity) • Performance equivalent to that of TE for significant fibrosis and cirrhosis 	<ul style="list-style-type: none"> • Can be implemented on a regular US machine • ROI can be adjusted in size and location and chosen by the operator • Measures liver stiffness in real-time • High range of values (2-150 kPa) • Good applicability • High performance for cirrhosis 	<ul style="list-style-type: none"> • Can be implemented on a regular MRI machine • Examination of the whole liver • Higher applicability than TE (ascites and obesity) • High performance for cirrhosis
Disadvantages				
<ul style="list-style-type: none"> • Non-specific of the liver • Unable to discriminate between intermediate stages of fibrosis • Performance not as good as TE for cirrhosis • Cost and limited availability (proprietary) • Limitations (hemolysis, Gilbert syndrome, inflammation...) 	<ul style="list-style-type: none"> • Requires a dedicated device • ROI cannot be chosen • Unable to discriminate between intermediate stages of fibrosis • Applicability (80%) lower than serum biomarker: (obesity, ascites, operator experience) • False positive in case of acute hepatitis, extra-hepatic cholestasis, liver congestion, food intake and excessive alcohol intake 	<ul style="list-style-type: none"> • Unable to discriminate between intermediate stages of fibrosis • Units (m/sec) different from that of TE (kPa) • Narrow range of values (0.5-4.4 m/sec) • Quality criteria not well defined • Prognostic value in cirrhosis? 	<ul style="list-style-type: none"> • Further validation warranted • Unable to discriminate between intermediate stages of fibrosis • Quality criteria not well defined • Learning curve? • Influence of inflammation? 	<ul style="list-style-type: none"> • Further validation warranted especially in comparison with TE • Not applicable in case of iron overload • Requires a MRI facility • Time-consuming • Costly

ROI, region of interest.

Hepatic Steatosis

Due to pandemic of over-nutrition, and its related metabolic risks including central obesity, glucose intolerance, dyslipidemia, hypertension, NAFLD has become a global public health issue. It is the leading cause of chronic liver disease in developed countries, with an estimated prevalence of 20-30% and increasing to 70% in diabetics and as high as 90% in obese individuals.^{49-53,87-89} In Asia, similar prevalence of NAFLD has been found in the range of 15-30% in the general population and over 50% in patients with diabetes and metabolic syndrome.⁸⁹ Given the pathophysiological link with metabolic syndrome, rates of NAFLD are likely to continue to rise with the obesity pandemic. The spectrum of disorder included in NAFLD are benign micro/macrovesicular hepatic steatosis, NASH, hepatic fibrosis, cirrhosis, and hepatocellular carcinoma. Although the disease remains asymptomatic most of the time, it can slowly progress to end-stage liver disease.^{87,88} In United States, NAFLD-related liver disease is the most common indication for LT.⁸⁷ Hepatic steatosis is the core pathological change of NAFLD and represents the cytoplasmic accumulation of fat droplets in cytoplasmic vesicles. The amount of 5% is utilized as the cut-off differentiating between physiological and pathological steatosis according to lipid content or imaging.⁹⁰⁻⁹² Microvesicular and macrovesicular steatosis are two morphological forms of hepatic steatosis. Macrovesicular steatosis is the accumulation of a single large fat droplet in a hepatocyte pushing the nucleus to the periphery. However, it is not unusual to observe hepatocytes with multiple small to medium-sized fat droplets. Hence, the term “macrovesicular steatosis” is broadened to include those hepatocytes with small to medium-sized fat droplets. On the other hand, microvesicular steatosis is characterized by the accumulation of much smaller uniform minute fat droplets in a hepatocyte with central nucleus. Diffuse microvesicular steatosis is not a pathological feature of NAFLD, whereas focal microvesicular steatosis could be found in 10% of liver biopsies in NAFLD. The presence of focal microvesicular steatosis in the background of

conventional macrovesicular steatosis is associated with higher disease severity in NAFLD.⁹⁰ Simple steatosis is believed to be a benign and non-progressive condition with no impact on the long-term survival, while steatohepatitis is with increased liver-related mortality.⁹⁰⁻⁹³ Steatohepatitis is a characteristic pathological pattern featured by steatosis >5%, inflammation and hepatocellular ballooning and Mallory-Denk bodies. Hepatocellular ballooning is the hallmark of steatohepatitis and is characterized by cellular swelling, rarefaction of the hepatocytic cytoplasm, and clumped strands of intermediate filaments.⁹⁰ Fibrosis is a histological feature signifying chronicity and disease progression. Centrizonal fibrosis and pericellular/perisinusoidal fibrosis are the characteristic features of fatty liver disease, either NAFLD or ALD.⁹⁰ Periportal fibrosis and bridging fibrosis develops as the disease progresses eventually establishing cirrhosis after repetitive hepatic injury, fibrosis, parenchymal extinction and hepatocellular regeneration (Figure 20).⁹⁰ Therefore, it is critical to identify the patients who have simple steatosis early and distinguish them from those with patients with steatohepatitis, fibrosis, and cirrhosis. The current standard to make the distinction of NASH from non-NASH NAFLD relies on the observation of ballooning degeneration of hepatocytes as well as steatosis and inflammation by biopsy.⁵⁶ The pathological committee of the NASH clinical research network designed and validated a NAFLD activity score (NAS) for use in clinical trials. The scoring system comprised 14 histological features evaluated semi-quantitatively and grouped under four categories: *steatosis* (0-3), *lobular inflammation* (0-2), *hepatocellular ballooning* (0-2), and *fibrosis* (0-4). The NAS score is the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning. In both adults and children, $NAS \geq 5$ correlated with a diagnosis NASH and those with score < 3 were diagnosed as non-NASH on liver biopsies (Table 16).⁹² However, Brunt *et al* have further elaborated on the clinicopathological meanings of NAS score and histopathological diagnosis of NASH. They concluded that the diagnosis of definite steatohepatitis should based on presence and pattern

of specific histological abnormalities on liver biopsies, which does not always correlate with threshold values of the semiquantitative NAS. Clinical trials and observational studies should take these different performance characteristics into account.⁹⁴

Figure 20: Spectrum of non-alcoholic fatty liver disease with three histological grades of hepatic steatosis^{90,92}

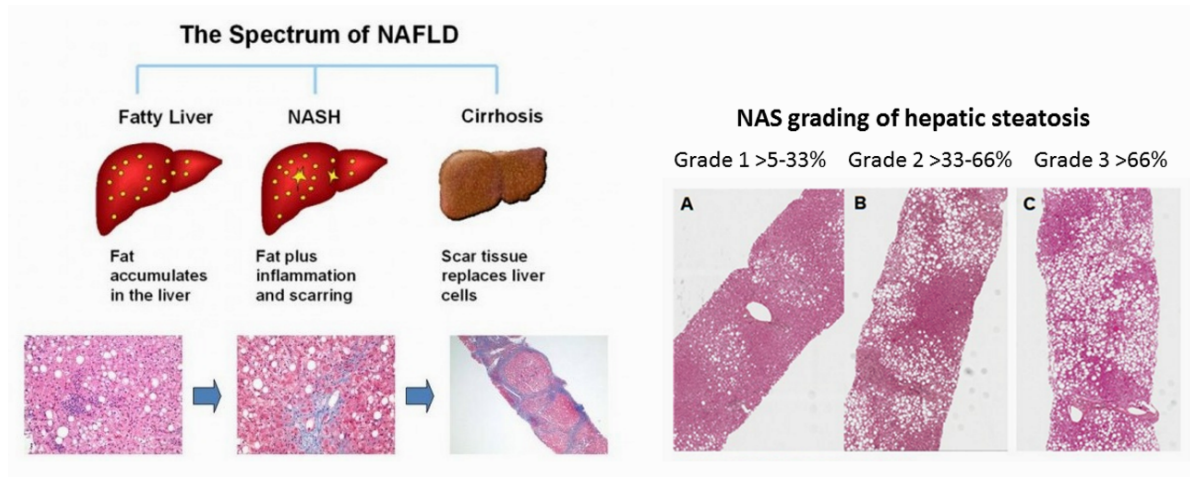


Table 16: Non-alcoholic fatty liver disease activity (NAS) score⁹²

NASH activity grade: grade = total score: S + L + B (range 0–8)					
Steatosis	S score	Lobular inflammation	L score	Hepatocyte ballooning	B score
< 5%	0	None	0	None	0
5–33%	1	< 2	1	Few ballooned cells	1
34–66%	2	2–4	2	Many ballooned cells	2
> 66%	3	> 4	3		
NASH fibrosis stage			Stage		
None			0		
Mild, zone 3 perisinusoidal fibrosis			1a		
Moderate, zone 3 perisinusoidal fibrosis			1b		
Portal/perportal fibrosis only			1c		
Zone 3 perisinusoidal and portal/perportal fibrosis			2		
Bridging fibrosis			3		
Cirrhosis			4		

Another quantifying hepatic steatosis is by estimating lipid content on tissue sections by infrared (IR) spectroscopy. Le Naour and his colleagues performed IR micro-spectroscopic analysis on frozen liver samples harvested from 27 human liver surgical specimens exhibiting various levels of steatosis (0–90%) based on histological examination. Acquisition of IR spectra on unstained frozen sections was achieved first using a commercially available laboratory IR microscope that allows scanning a whole tissue section ranking between 1 to 5 mm² (up to 10 000 spectra with 50 x 50 μm² aperture size) in less than 5 minutes. Important variations in intensity of bands attributed to lipids (3000–2800 cm⁻¹) were observed even in the non-steatotic part of fatty livers despite normal histological aspect thus strengthening that IR microspectroscopy detected subtle chemical changes occurring in the liver tissue. In contrast, the intensity of bands attributed to proteins (Amide I and Amide II: 1485–1710 cm⁻¹) was not significantly affected by steatosis. They further demonstrated that an average IR spectrum from an area 500x500 μm² was representative of the whole tissue section. The quantification of the lipid content was addressed from the IR spectra by calculating the ratio of integrated intensity of bands attributed to lipids (2800–3000 cm⁻¹) related to proteins (Amide II: 1485-1595 cm⁻¹) (Figure 21). This ratio allowed normalizing the intensity variation of the bands due to variations in thickness of the tissue section.^{95,96}

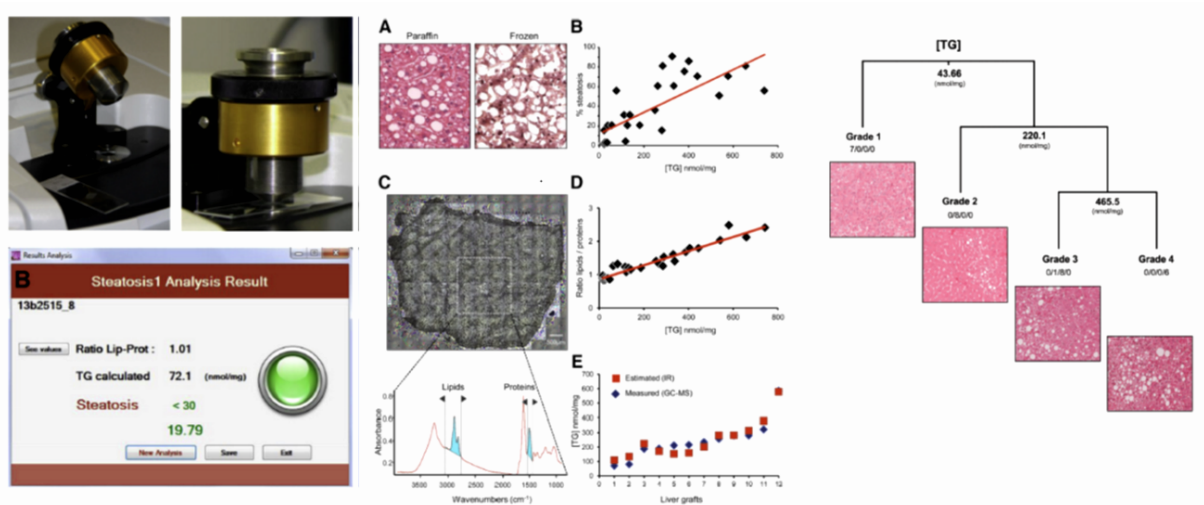


Figure 21: Infrared spectroscopic quantification and classification of hepatic steatosis⁹⁵

For each patient, the average ratio lipids/proteins measured by IR microspectroscopy was plotted as a function of the concentration of TG leading to exhibit a marked linearity (Fig). A standard curve was further established to quantify the TG content in nmol/mg and thus the level of steatosis. The measured amount of hepatic TG has been proven to accurately reflect the hepatic TG content assessed by gas phase chromatography coupled to mass spectroscopy (Figure 21A-E). Further, they were able to classify hepatic steatosis into four grades according to TG content.^{95,96}

Unfortunately, LB is invasive, subject to complications, costly, and limited by sampling errors and intra-observer variability.^{13,14} Further, with the increasing prevalence of NAFLD worldwide, there is a continuing need to develop noninvasive methods to assess hepatic steatosis, steatohepatitis and fibrosis, because it is neither practical nor cost effective to perform liver biopsies in all patients who are at risk for steatosis, steatohepatitis, and fibrosis.

Recently, several noninvasive imaging modalities based on elastography have been developed.^{97,98} The most validated, and commonly utilized is transient elastography, which simultaneously evaluates both hepatic fibrosis (LS) and steatosis (CAP). In 2010, Sasso *et al* reported a new non-invasive evaluation of hepatic steatosis using VCTE™ technology of FibroScan® called controlled attenuation parameter or CAP.⁸ Ultrasonic attenuation was assessed using a novel proprietary algorithm named CAP™. This ultrasonic attenuation coefficient is an estimate of the total ultrasonic attenuation (go-and-return path) at 3.5 MHz and is expressed in dB/m. CAP is evaluated using the same radio-frequency data and in the same region of interest than the ones used for LS and is only appraised if the acquisition is “valid”. CAP is therefore VCTE™ guided, ensuring the operator obtains an ultrasonic attenuation value of the liver only (Figure 22).⁸

CAP was first validated as an estimate of ultrasonic attenuation at 3.5 MHz using Field II simulations and tissue-mimicking phantoms. Performance of the CAP was then appraised on 115 patients, taking the histological grade of steatosis as reference. CAP was significantly correlated to steatosis (Spearman $r=0.81$, $p<0.001$). AUROC was equal to 0.91 and 0.95 for the detection of more than 10% and 33% of steatosis, respectively. Furthermore, results show that CAP can efficiently separate several steatosis grades (Figure 23). These promising results encouraged further studies on CAP as a noninvasive, immediate, objective and efficient method to detect and quantify steatosis.^{8,9,99,100}

Figure 22: Block diagram of liver stiffness and controlled attenuation parameter measurement⁸

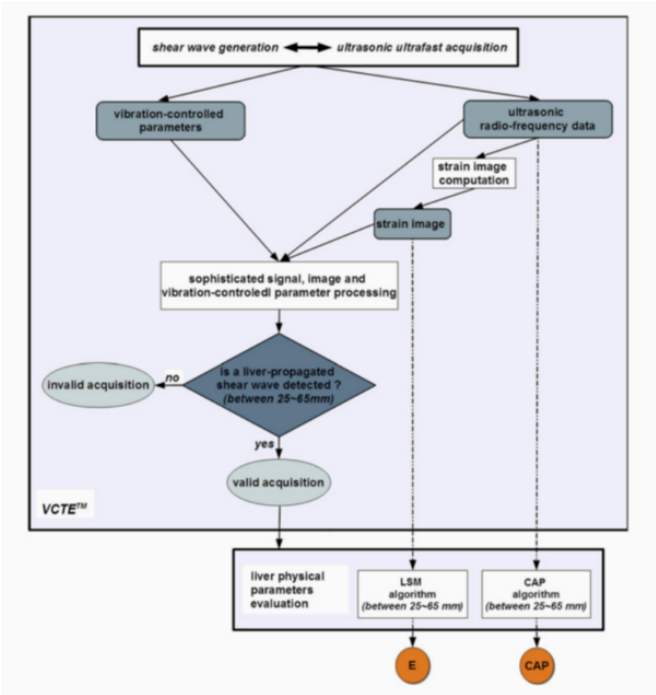
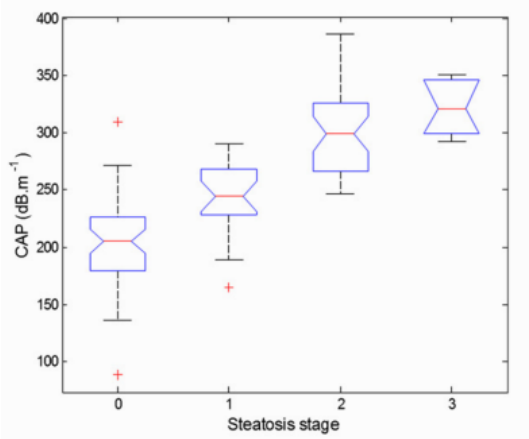
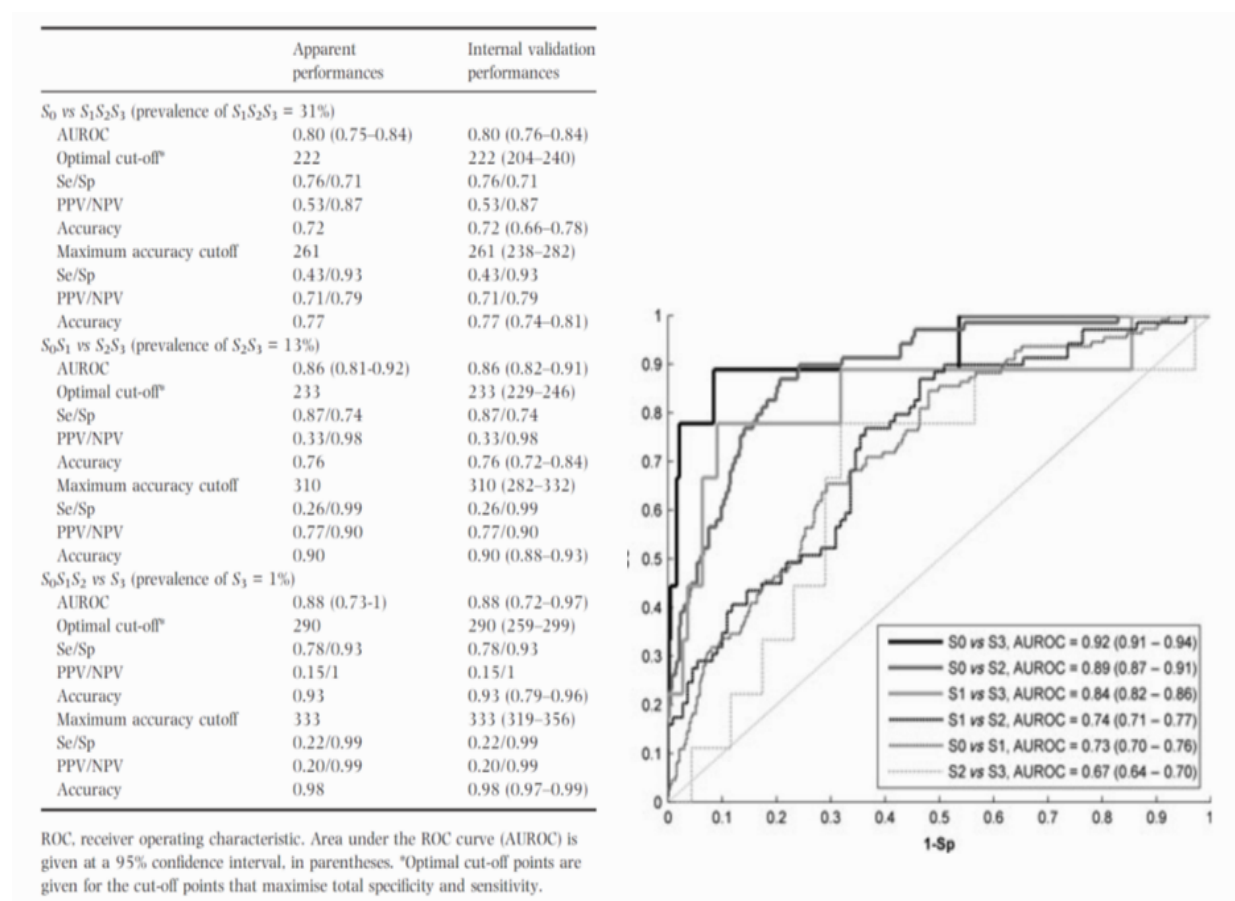


Figure 23: Controlled attenuation parameter values in dB/m for each steatosis grade in chronic hepatitis C⁸



Sasso and her colleagues further evaluated the performance of CAP in a large cohort of 615 HCV-infected patients who had underwent FibroScan[®] and liver biopsy. Fibrosis was evaluated with METAVIR score and steatosis was categorized by visual assessment as S0: steatosis <10% of hepatocytes, S1: 11-33% steatosis, S2: 34-66% and S3: 67-100%. In multivariate analysis, CAP was related to steatosis grade ($p<0.001$) independent of fibrosis stage. AUROCs for CAP to detect different grades steatosis were 0.80 for $S\geq 1$, 0.96 for $S\geq 2$ and 0.88 for S3 (Fig). LS and CAP exhibited a good ability to differentiate fibrosis stages and steatosis grades, respectively.¹⁰⁰

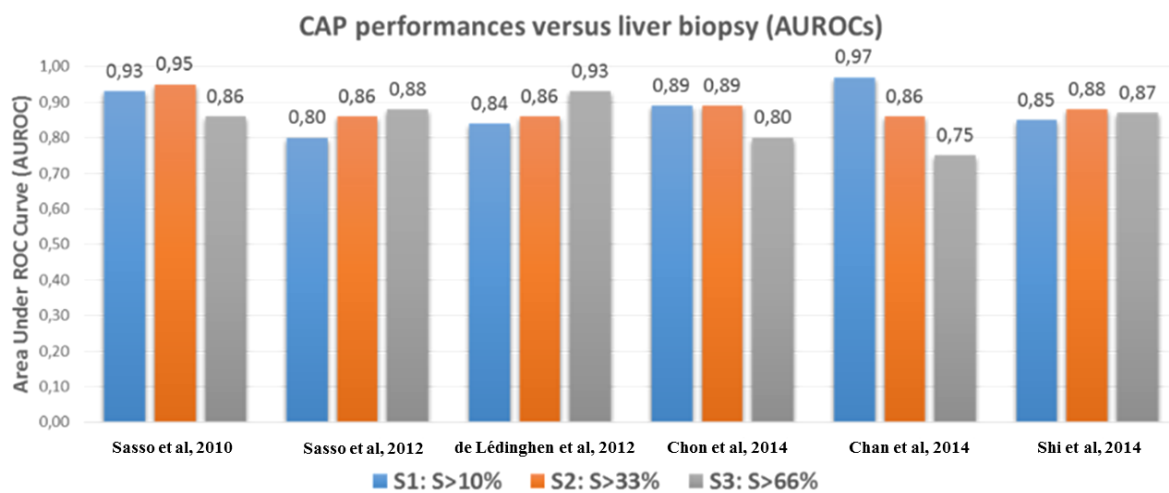
Table 17: Apparent and internal validation performances in terms of area under ROC curves for the optimal and maximum accuracy cut-off points to determine $S\geq 1$, $S\geq 2$ and $S=3$ using controlled attenuation parameter.¹⁰⁰



Chon *et al* defined the normal range of CAP values in 264 healthy subjects and evaluated the factors associated with CAP. The mean CAP was 224.8 ± 38.7 dB/m (range, 100–308), and the range of normal CAP (5th–9th percentiles) was 156–287.8 dB/m. The mean CAP was significantly higher in the healthy subjects when compared with potential liver donors

(227.5±42 vs 218.2±28.3 dB/m). CAP did not differ significantly according to age and gender in either groups. Body mass index ($p=0.03$) and triglyceride levels ($p<0.001$) were independently associated with CAP values in healthy subjects.¹⁰³ Many other publications have validated the diagnostic performance of CAP compared with LB as illustrated in the Figure 24.¹⁰¹

Figure 24: Summary of controlled attenuation parameter performances in various studies^{8,100,102-104}



The studies by de Lédinghen *et al* and Myers *et al* compared CAP to three blood steatosis tests: Steatotest, Fatty liver index and Hepatic steatosis index.^{102,105} CAP provided higher AUROCs than the blood tests for the diagnosis of steatosis grades $S \geq 1$ (>10% hepatocytes containing lipid vesicles), $S \geq 2$ (>33%) and S3 (>66%). CAP had certain advantages when compared to the blood tests: the test results are immediately available and less sensitive to the influence of antidiabetic or lipid-lowering drugs. The CAP cut-off for diagnosis of >10% steatosis in these two studies and were 266 (AUROC: 0.84) and 283 dB/m (AUROC: 0.81), respectively. Fujimori *et al* compared non-invasive CAP assessment with M probe of hepatic steatosis with actual hepatic fat content determined morphometrically using computerized optical image analyzing system in 82 NAFLD patients. CAP showed an excellent correlation with actual hepatic fat content in patients with BMI <28 kg/m² ($r=0.6$, $p<0.001$) but not in patients with BMI ≥ 28 kg/m². CAP quantitiveness was affected by

presence of stage 2-4 fibrosis, but not the presence of hepatocyte ballooning and severity of lobular inflammation.¹⁰⁶ Another study of 155 patients with CLD from Japan by Masaki *et al*, CAP was significantly correlated with steatosis grade, and there were significant differences between CAP value of those with S0 ($\leq 5\%$ steatosis) and S1-3 ($>5\%$ steatosis) grades ($p < 0.001$). The optimal cut-off defined to $>5\%$ steatosis was 232.5 dB/m² (AUROC: 0.88).¹⁰⁷

With the introduction of XL probe, Sasso *et al* assessed the performance of CAP measured using XL probe with a center frequency of 2.5 MHz that was different from M probe whose frequency was 3.5 MHz, without modifying the range of values (100–400 dB/m). CAP validation was successfully performed on Field II simulations and on tissue-mimicking phantoms. *In vivo* performance was assessed in a cohort of 59 patients spanning the range of steatosis. *In vivo* reproducibility was good and similar with both probes. The area under receiver operative characteristic curve was equal to 0.83/0.84 and 0.92/ 0.91 for the M/XL probes to detect .2% and .16% liver fat, respectively, as assessed by magnetic resonance imaging. They confirmed the validity of XL probe to be similar to M probe to evaluate hepatic steatosis and inferred that the patients can be assessed simultaneously for steatosis and fibrosis using the FibroScan[®], regardless of their morphology.⁹

Table 18: Diagnostic performance of controlled attenuation parameter (CAP) with M and XL probes⁹

	CAP with the M probe	CAP with the XL probe	<i>p</i> Value (Delong test)
S $\geq 2\%$ (Prevalence = 69%)	AUROC = 0.83 [0.71–0.95] Cutoff = 251 dB/m Se = 0.78/Sp = 0.78 Acc = 0.80	AUROC = 0.84 [0.73–0.95] Cutoff = 254 dB/m Se = 0.83/Sp = 0.78 Acc = 0.83	0.76
S $\geq 8\%$ (Prevalence = 51%)	AUROC = 0.87 [0.78–0.97] Cutoff = 267 dB/m Se = 0.80/Sp = 0.79 Acc = 0.81	AUROC = 0.90 [0.82–0.99] Cutoff = 270 dB/m Se = 0.88/Sp = 0.79 Acc = 0.85	0.50
S $\geq 16\%$ (Prevalence = 22%)	AUROC = 0.92 [0.85–0.99] Cutoff = 299 dB/m Se = 0.92/Sp = 0.88 Acc = 0.90	AUROC = 0.91 [0.83–0.99] Cutoff = 301 dB/m Se = 0.92/Sp = 0.81 Acc = 0.85	0.78

S = steatosis; AUROC = area under the receiver operating characteristic curve; Se = sensitivity; Sp = specificity; Acc = accuracy.

Lupsor-Platon and his colleagues validated the diagnostic accuracy (DA) of CAP measured by TE for the non-invasive assessment of hepatic steatosis by a prospective study on a group of 201 consecutive CLD biopsied patients. In multivariate analysis, hepatic

steatosis was only independent histological factor associated with CAP. Maximal DA was obtained for the prediction of S1 (76.1%), S2 (82.1%) and S3 (81.2%) grades. AUROCs (cut-offs) for $S \geq 1$, $S \geq 2$ and S3 were 0.81 (260), 0.82 (285), and 0.84 (294).¹⁰⁸

Ahn *et al* studied the relationship between CAP and hepatic steatosis in ALD and NAFLD. A mixed cohort patients with either ALD (80) or NAFLD (106) who were diagnosed with fatty liver by ultrasound and who had CAP measured were analyzed retrospectively. CAP score was significantly correlated with US ($r=0.58$, $p<0.001$) and there was no significant difference between ALD ($r=0.52$, $p=0.64$) and NAFLD ($r=0.57$, $p<0.001$). AUROCs in all patients for $S \geq 2$ and S3 grades were 0.79 and 0.84, respectively. For sensitivity $\geq 90\%$, CAP cut-offs for the detection of $S \geq 2$ and S3 were, respectively, 245 and 279.5 dB/m in ALD versus 255.5 and 290.5 dB/m in NAFLD.¹⁰⁹

Another interesting study by Lee *et al* investigated whether TE could discriminate patients with NASH from those with NAFLD in a study with 89 (48.5%) non-NASH NAFLD participants, and 94 (51.4%) NASH patients. CAP and LS were significantly correlated with steatosis grades ($r=0.66$, $p<0.001$) and fibrosis ($r=0.71$, $p<0.001$), respectively. They developed a CLA model with CAP, LS and alanine aminotransferase and validated its performance to diagnose NASH by NAS score on liver biopsy (AUROC: 0.83).¹¹⁰ Another study by Kwok *et al* assessed CAP and LS prospectively in 1918 type 2 diabetes patients using either M or XL probes. By multivariable analysis, female gender, higher body mass index, triglycerides, fasting plasma glucose and ALT and non-insulin use were associated with increased CAP. Longer duration of diabetes, higher body mass index, increased ALT and spot urine albumin:creatinine ratio and lower high-density lipoprotein-cholesterol were associated with increased LS. Ninety-four patients (80% had increased LS) underwent LB: 56% had steatohepatitis and 50% had F3-4 disease. They concluded that diabetic patients have a high prevalence of NAFLD and advanced fibrosis. Those with obesity and

dyslipidemia are at particularly high risk and may be the target for liver assessment by FibroScan[®] as a screening test.¹¹¹

In a recent individual patient data meta-analysis, Karlas *et al* established optimal cut-offs from 21 studies containing histology-verified CAP data (M probe, FibroScan[®]) for grading of steatosis (S0–S3). ROC analysis after correcting for center effects was used as well as mixed models to test the impact of covariates on CAP. The primary outcome of this meta-analysis was establishing CAP cut-offs for distinguishing steatosis grades.¹¹²

Table 19: Population characteristics in the meta-analysis by Karlas and his colleagues¹¹²

	All patients (n = 2735)	S0 (n = 1391)	S1 (n = 754)	S2 (n = 427)	S3 (n = 163)
Female, n (%)	1030 (37.7)	606 (43.6)	232 (30.8)	135 (31.6)	57 (35.0)
Age, yr, mean ± SD	45.4 ± 13.5	43.6 ± 13.4	47.5 ± 13.5	47.4 ± 12.6	45.3 ± 13.8
BMI, kg/m ² , mean ± SD	25.0 ± 3.9	23.6 ± 3.6	25.7 ± 3.6	27.2 ± 3.8	27.6 ± 3.5
<25	1448 (52.9)	961 (69.1)	331 (43.9)	120 (28.1)	36 (22.1)
25–30	967 (35.4)	355 (25.5)	330 (43.8)	200 (46.8)	82 (50.3)
>30	320 (11.7)	75 (5.4)	93 (12.3)	107 (25.1)	45 (27.6)
Etiology, n (%)					
NAFLD/NASH	537 (19.6)	48 (3.5)	178 (23.6)	216 (50.6)	95 (58.3)
Hepatitis B	1003 (36.7)	612 (44.0)	285 (37.8)	81 (19.0)	25 (15.3)
Hepatitis C ^a	997 (36.5)	612 (44.0)	250 (33.2)	103 (24.1)	32 (19.6)
Other	198 (7.2)	119 (8.6)	41 (5.4)	27 (6.3)	11 (6.7)
Diabetes ^b , n (%)					
NAFLD/NASH	195 (37.4)	16 (34.0)	73 (42.0)	75 (36.1)	31 (33.3)
Hepatitis B	51 (5.2)	21 (3.5)	16 (5.8)	12 (15.6)	2 (8.3)
Hepatitis C	47 (10.7)	15 (6.8)	20 (13.4)	4 (8.9)	8 (33.3)
Other	22 (13.4)	16 (14.5)	4 (11.8)	1 (7.7)	1 (14.3)
ALT ^c , U/L, median [IQR]					
Female	47 [27, 83]	42 [24, 79]	47 [31, 81]	59 [35, 91]	58 [38, 98]
Male	56 [34, 94]	53 [31, 93]	49 [33, 79]	67 [45, 101]	87 [61, 135]
AST ^d , U/L, median [IQR]					
Female	42 [27, 69]	37 [25, 62]	44 [29, 75]	47 [31, 76]	51 [37, 78]
Male	40 [29, 62]	39 [28, 65]	38 [27, 56]	43 [33, 61]	51 [38, 71]
Days between biopsy and CAP, median [IQR]	1.0 [0.0, 3.5]	1.0 [0.0, 3.5]	1.0 [0.0, 5.0]	1.0 [0.0, 3.5]	1.0 [0.0, 3.5]
Liver stiffness, kPa, median [IQR]	7.4 [5.4, 11.8]	6.8 [5.1, 10.2]	7.8 [5.6, 12.7]	8.3 [6.1, 14.0]	8.6 [6.1, 12.0]
Fibrosis staging ^e , n (%)					
F0	304 (11.3)	152 (11.2)	85 (11.4)	54 (12.7)	13 (8.0)
F1	970 (36.2)	527 (39.0)	248 (33.4)	145 (34.1)	50 (30.7)
F2	725 (27.0)	387 (28.6)	194 (26.1)	96 (22.6)	48 (29.4)
F3	334 (12.4)	139 (10.3)	97 (13.1)	66 (15.5)	32 (19.6)
F4	350 (13.0)	147 (10.9)	119 (16.0)	64 (15.1)	20 (12.3)

ALT, alanine transaminase; AST, Aspartate transaminase; BMI, body mass index; CAP, controlled attenuation parameter; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.
^a Includes co-infections.
^b Data available from n = 2098 patients.
^c Data available from n = 2468 patients.
^d Data available from n = 2370 patients.
^e Data available from n = 2683 patients.

Data from 19/21 eligible papers were provided, comprising 3830/3968 (97%) of patients. Considering data overlap and exclusion criteria, 2735 patients were included in the final analysis (37% hepatitis B, 36% hepatitis C, 20% NAFLD/NASH, 7% other). Steatosis distribution was 51%/27%/16%/6% for S0/S1/S2/S3. CAP values in dB/m (95%CI) were influenced by several covariates with an estimated shift of 10 (4.5–17) for NAFLD/NASH

patients, 10 (3.5–16) for diabetics and 4.4 (3.8–5.0) per BMI unit. AUROCs were 0.82 (0.809–0.837) and 0.86 (0.850–0.880) respectively. Optimal cut-offs were 248 (237–261) and 268 (257–284) for those above S0 and S1 respectively.¹¹²

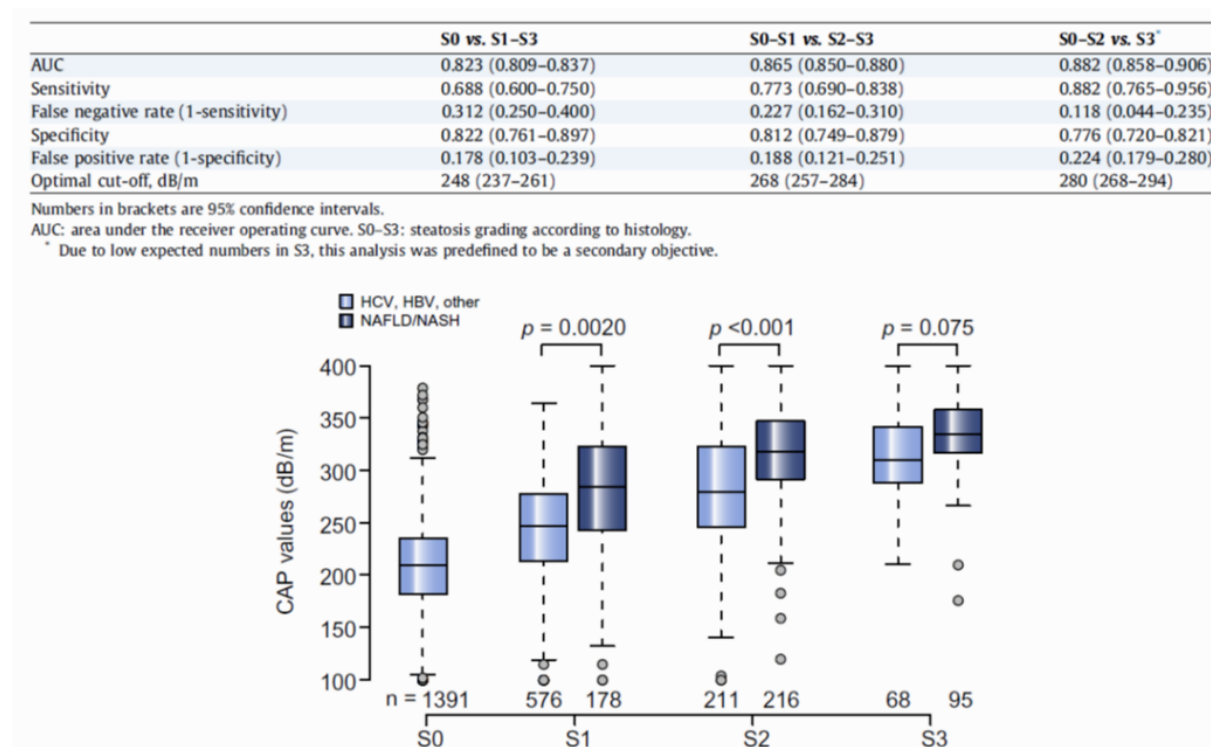


Table 20: Optimal controlled attenuation parameter cut-offs determined by bootstrapped receiver operating characteristic analysis along with box plots¹¹²

Another prospective study of 5323 CAP examination by de Lédinghen *et al* analyzed the relationship between and various clinico-biological parameters: age, gender, BMI, waist circumference, hypertension, diabetes, metabolic syndrome, alcohol use, LS, indication, and different biological parameter. By multivariate analysis, factors significantly associated with elevated CAP were BMI >25-30 kg/m², BMI >30 kg/m², metabolic syndrome, alcohol use >14 drinks/week and LS >6 kPa. The factors associated with CAP measurement failure were female gender, BMI and metabolic syndrome. In the 440 patients with LB, for the diagnosis of steatosis >10%, steatosis >33%, and steatosis >66%, AUROCs of CAP were 0.79 (95% CI: 0.74–0.84, $p < 0.001$), 0.84 (95% CI: 0.80–0.88, $p < 0.001$), 0.84 (95% CI: 0.80–0.88, $p < 0.001$), respectively. They concluded that CAP provides an immediate assessment of steatosis simultaneously with LS and the strong association of CAP with the metabolic

syndrome and alcohol use could be of interest for the follow-up of NAFLD or alcoholic patients.¹¹³

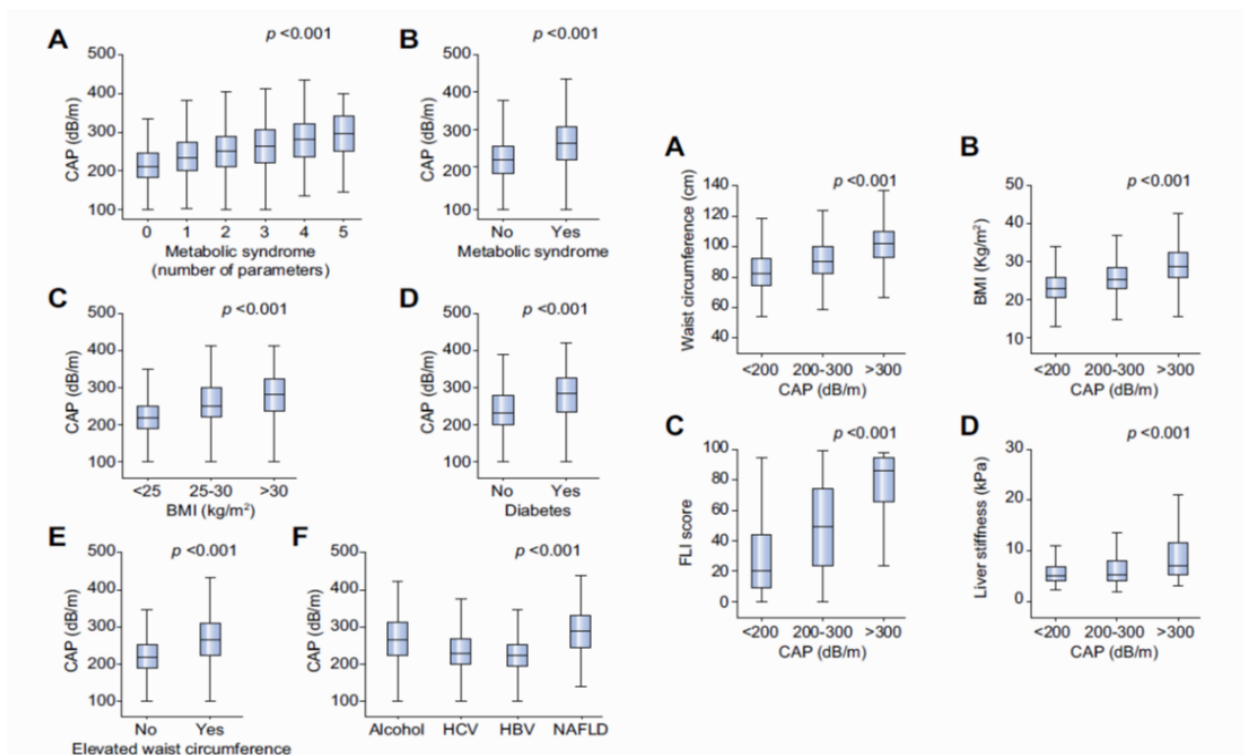
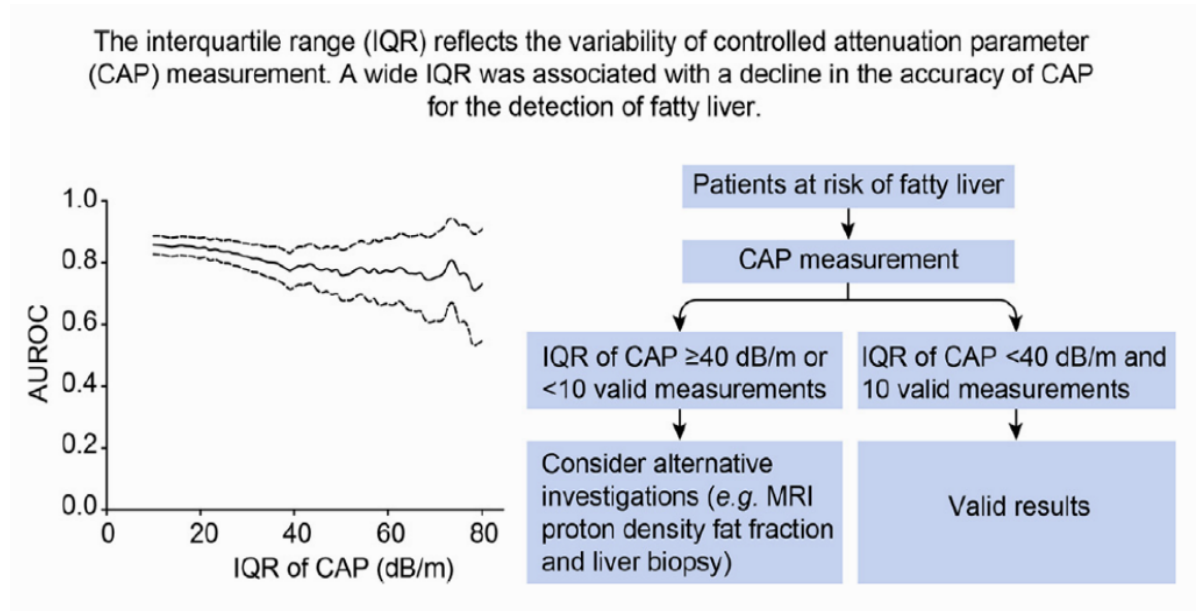


Figure 25: Categories of controlled attenuation parameter according to different parameters¹¹³

On the other hand, Wong *et al* evaluated CAP using the M probe prior to LB in 754 consecutive patients with different liver diseases (101 HBV, 154 HCV, 349 NAFLD, 37 autoimmune hepatitis, 49 cholestatic liver disease, 64 others) at three centers in Europe and Hong Kong with the aim of defining the validity criteria for CAP. AUROC for CAP diagnosis of fatty liver (>5% steatosis) was 0.85 (95% CI: 0.82–0.88). The interquartile range of CAP had a negative correlation with CAP ($r=-0.32$, $p<0.001$), suggesting IQR/M ratio of CAP would be an inappropriate validity parameter. In the derivation cohort, the IQR of CAP was associated with the accuracy of CAP (AUROC: 0.86, 0.89 and 0.76 in patients with IQR of CAP <20 [15% of patients], 20–39 [51%], and ≥ 40 dB/m [33%], respectively). Likewise, AUROC of CAP in the validation cohort was 0.90 and 0.77 in patients with IQR of CAP <40 and ≥ 40 dB/m, respectively ($p=0.004$). The accuracy of CAP in detecting grade 2 and 3

steatosis was lower among patients with body mass index ≥ 30 kg/m² and F3-4 fibrosis. The validity of CAP for the diagnosis of fatty liver is lower if the IQR of CAP is ≥ 40 dB/m.¹¹⁴

Figure 26: Algorithm for reliable controlled attenuation parameter (CAP) measurement¹¹⁴



A novel Fibroscan[®]-based score was developed by Echosens[™] and available as web-based application (Probability of Active NASH score) to differentiate between fatty liver and NASH from a single examination. In this multicentric study, 174 patients suspected of NAFLD prospectively underwent TE within two weeks of a standard of care liver biopsy. NASH was diagnosed using fatty liver inhibition of progression algorithm by two expert pathologist and NASH severity was graded according to the NAS score. This new Probability of Active NASH (PAN) score is calculated from a single measurement of LS and CAP and it was able to correctly classify 79% of patients with/without NASH (NAS score ≥ 3) as well as correctly staging severity in 86%.¹¹⁵

Finally, Crossan *et al* evaluated the cost-effectiveness of various non-invasive tests in patients with chronic HBV infection and elevated transaminases and /or viral load who would normally undergo LB to make treatment decisions. FibroScan[®] was the most cost-effective option with an incremental cost-effectiveness ratio of £23,345 particularly to identify and treat in HBeAg-positive patients with F ≥ 2 fibrosis.¹¹⁶ LB costs were reported to range from €703

to €1,566 in European centers, and \$2,745 in United States, depending on whether complications occurred.¹¹⁷ FibroScan[®] cost would include equipment cost (€71,760), annual maintenance contract cost (€5,400), and negligible costs for consumables. The cost per examination would be €1,000 and €100 at 20 and 150 examinations per year, respectively.¹¹⁸

Another unique feature of transient elastography by FibroScan[®] is that it requires minimal training (≥ 10 observed valid measurements on patients) and once valid LS is obtained it is very accurate to exclude advanced hepatic fibrosis. Armstrong *et al* further evaluated the learning curve of LS compared with modified Ishak fibrosis stage on LB. Learning curve analysis highlighted that the greatest improvement in validity of LS rates occurred in the operator’s first ten Fibroscans, reaching 64.7% validity by the 50th Fibroscan.¹¹⁹

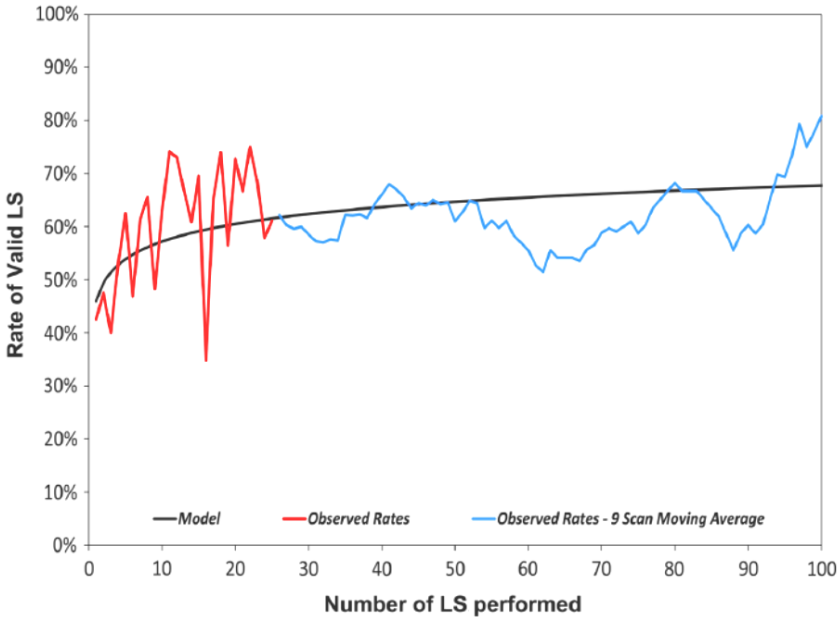


Figure 27: Learning curve analysis of liver stiffness measurement by FibroScan^{®24}

Portal Hypertension

Portal hypertension (PH) is unavoidable serious complication of progressive liver disease and it is responsible for the main complications of cirrhosis. The main causes of PH in Western countries are CLD and cirrhosis of the liver, caused by viral hepatitis, ALD or NAFLD, and represents the third to fifth leading cause of death in adults. CLDs is characterized by progressive liver tissue fibrogenesis and extensive vascular changes occurring within the liver and in the splanchnic vasculature that results in PH (\uparrow PVP).¹²⁰ It is well established that PH is not only a mechanical consequence of altered hepatic architecture with increased hepatic vascular resistance but also a dynamic phenomenon characterized by net predominance of vasoconstrictors in hepatic microenvironment and increased portal perfusion through splanchnic arteriolar vasodilatation caused by endogenous vasodilators.^{121,122} PH is a clinical syndrome characterized by a pathological increase in portal venous pressure. This increase in pressure causes the pressure gradient between the portal vein and inferior vena cava, called hepatic venous pressure gradient (HVPG), to raise above the normal range (1-5 mmHg).^{123,124}

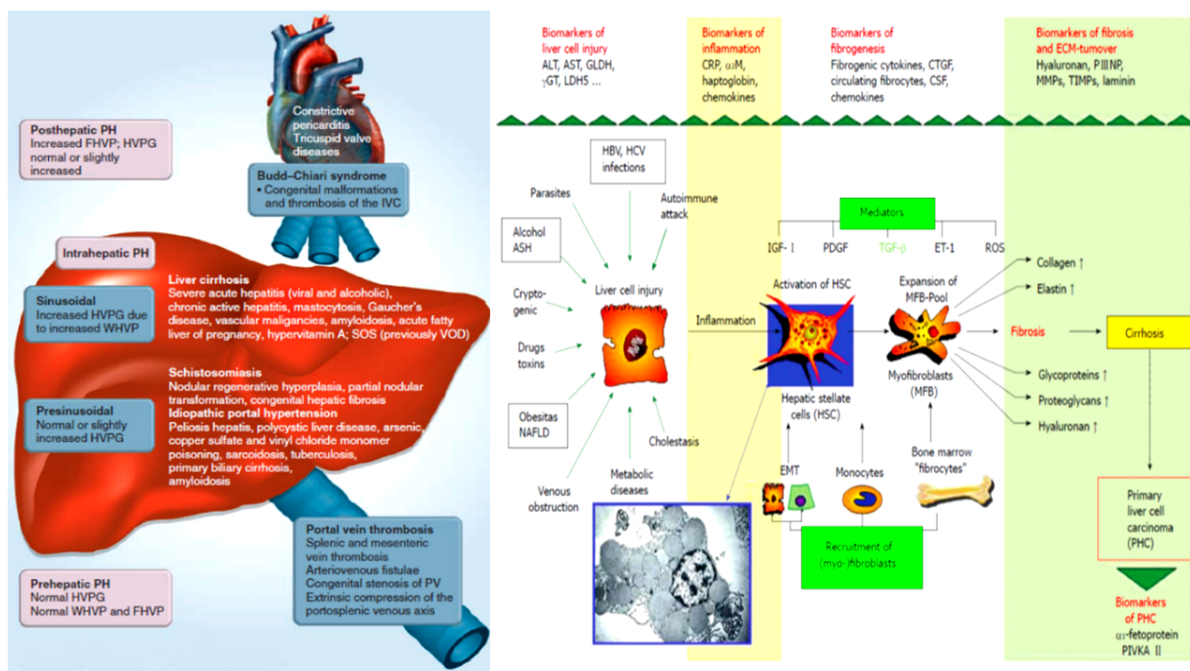
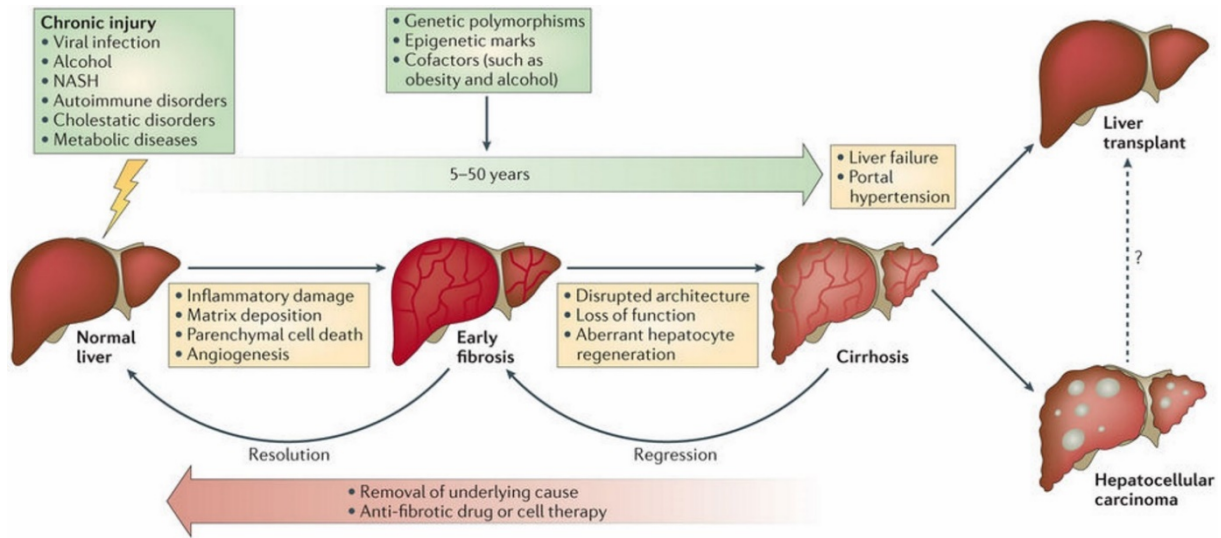


Figure 28: Types of portal hypertension and pathogenesis of cirrhosis [Gressner et al, Comp Hepatol 2007]

Figure 29: Natural history of chronic liver disease [Pellicoro et al, Nat Rev Immunol, 2014]



When HVPG increases to ≥ 10 mmHg life-threatening complications of PH can arise. These complications include formation of portosystemic collaterals and varices, upper gastrointestinal bleeding resulting from ruptured gastroesophageal varices and portal hypertensive gastropathy, ascites, renal dysfunction, hepatic encephalopathy, arterial hypoxemia, disorders in the metabolism of drugs or endogenous substances that are normally eliminated by the liver, bacteremia from intestinal translocation, and hypersplenism.¹²⁴⁻¹³⁰

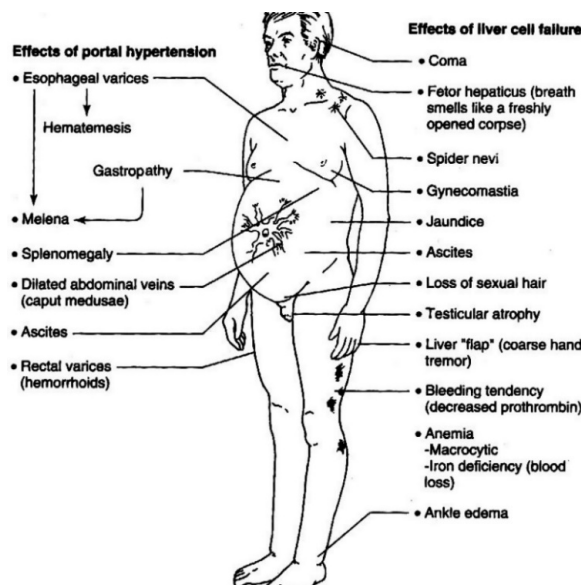


Figure 30: Complications of cirrhosis and portal hypertension

Assessment of Portal hypertension

Hepatic venous pressure gradient:

HVPG is an accurate, reproducible and safe method of measuring portal pressure in patients with CLD or cirrhosis of any etiology with the exception of primary biliary cirrhosis. The direct measurement of portal pressure by invasive techniques such as transhepatic or tranvenous catheterization/puncture of hepatic vein, portal vein and/or splenic pulp puncture are no longer favored.^{123,129,130} The direct measurements are now mostly utilized in specific case of presinusoidal PH and in cholestatic disorders. The most commonly utilized method to estimate HVPG is percutaneous catheterization of the hepatic vein and measuring free hepatic venous pressure (FHVP) and wedged hepatic venous pressure (WHVP).¹²³⁻¹³⁰ The difference between these WHVP and FHVP is the portocaval pressure gradient, HVPG. The FHVP is a measure of the pressure of unoccluded hepatic vein. The WHVP is measured by occluding the hepatic vein and stopping the blood flow that causes the static column of blood so formed to equalize in pressure with preceding vascular territory, which is in this case, the hepatic sinusoids (Figure 31).

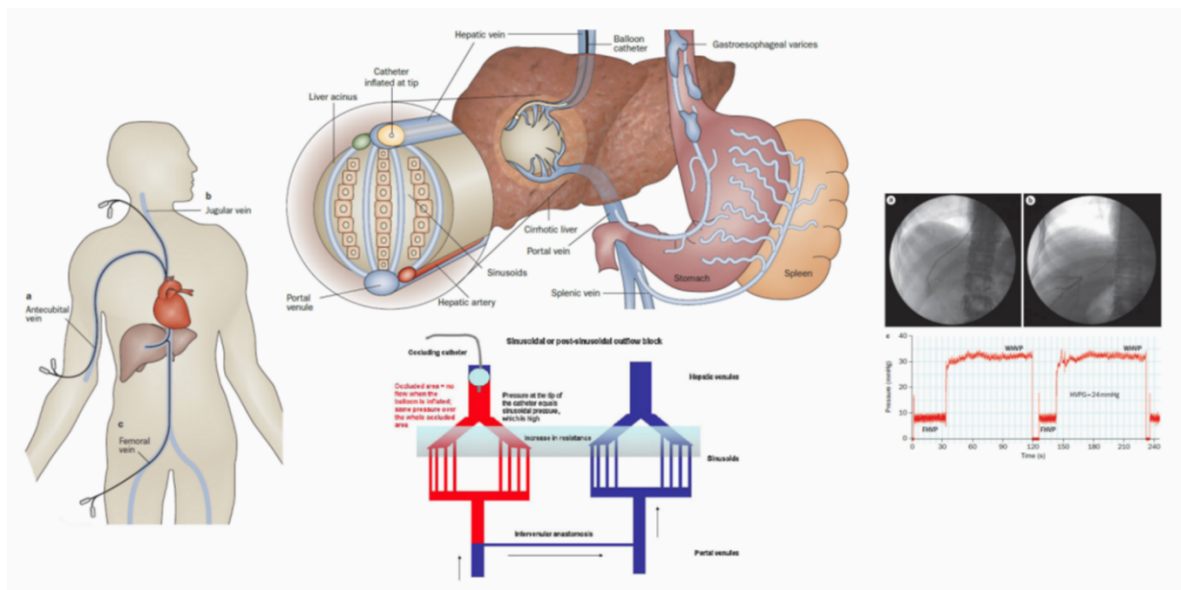


Figure 31: Measuring hepatic venous pressure gradient in a cirrhotic patient^{123,130}

Thus, it is a measure of hepatic sinusoidal pressure, not the portal pressure. In the normal liver, WHVP is slightly lower (by~1mmHg) than portal pressure, owing to pressure equilibrium through the interconnected sinusoids.^{123,130} In liver cirrhosis, however, the static column of blood created by occluding the hepatic vein cannot be decompressed at the sinusoidal level because the connections between sinusoids are disrupted because of the presence of fibrous septa and nodule formation. Therefore, WHVP gives an accurate estimate of the portal pressure in patients with advanced liver disease and cirrhosis due to either alcohol or viral hepatitis.¹²³⁻¹²⁵ HVPG reflects portal pressure changes that occurs when there is a modification in either resistance caused by mechanical (fibrosis, regenerative nodules, or thrombosis) or functional abnormalities (increased vascular tone) and by changes in portal or collateral blood flow ().in healthy adults, HVPG values are normally within the range 1–5 mmHg. HVPG value of 6–9 mmHg corresponds to pre-clinical sinusoidal portal hypertension, whereas clinically-significant portal hypertension is diagnosed when HVPG is ≥ 10 mmHg, at which point clinical manifestations of portal hypertensive syndrome, such as varices, bleeding, gastropathy, and ascites, might appear.^{123,124,131-133}

Portal pressure as measured by HVPG has been validated as a surrogate outcome measure in hepatology to assess disease severity and prognosis in chronic viral hepatitis, acute alcoholic hepatitis, risk of HCC and to diagnose the recurrence of HCC after hepatectomy, hepatitis C recurrence after liver transplantation. In patients with compensated cirrhosis, CSPH is a major determinant of patients' survival, as it indicates an increased risk of developing gastroesophageal varices, first decompensation, and HCC on follow-up.^{123,125} It has also been proven to be an effective end-point in clinical trials to assess a new drug therapy and to monitor treatment response in PH (Table 21).

Clinical decompensation is defined by the onset of ascites, variceal bleeding, jaundice or hepatic encephalopathy, and marks the symptomatic phase of cirrhosis. PH is present 100%

of patients with decompensated disease and the prevalence of varices needing treatment is much more frequent in decompensated patients so upper gastrointestinal endoscopy (UGIE) is always needed in these patients.^{81,134,135}

Table 21: Prognostic value of hepatic venous pressure gradient in chronic liver diseases¹²³

Single HVPG measurements
≥6 mmHg: risk of disease progression in patients with HCV recurrence after OLT
≥10 mmHg: defines clinically significant portal hypertension, with the risk of developing varices, ascites, clinical decompensation, and HCC; risk of decompensation after liver resection for HCC
≥12 mmHg: rupture of varices
≥16 mmHg: increased risk of mortality
≥20 mmHg: treatment failure and mortality in acute variceal bleeding
≥22 mmHg: mortality in acute severe alcoholic hepatitis
Changes in HVPG in repeat measurements
Reduction to ≤12 mmHg: abolition of risk of first variceal bleeding and rebleeding
Reduction of ≥10% from baseline: reduced risk of development of varices
Reduction of ≥12% from baseline: reduced risk of spontaneous bacterial peritonitis
Reduction of ≥20% from baseline: reduced risk of first variceal bleeding, rebleeding, ascites, and mortality
Reduction of ≥10–12% from baseline after acute intravenous propranolol administration: reduced risk of first variceal bleeding, rebleeding, and mortality
Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OLT, orthotopic liver transplantation.

Upper GI endoscopy:

Another ‘gold standard’ method in the evaluation of PH is the use of UGIE for the detection of varices. By far, UGIE is the best way of assessing the presence of esophageal varices, where its accuracy is much greater than CT angiography. The other advantages with endoscopy are its ability to assess the severity by detecting the indicators of increased bleeding (cherry red spots and wale marks) and to treat high-risk varices. Therefore, the current recommendations for any patient with cirrhosis should undergo screening UGIE at diagnosis, and repeat follow-up examinations if no varices are found or if no preventive treatment is initiated in patients with low-risk varices (Grade 1 varices without red color signs in Child A patient).^{134,135}

Both HVPG and UGIE are minimally invasive, safe with low rate of complications, but they cause patient discomfort, require hospitalization, and therefore, increase the burden for medical providers and increased cost of medical care. Because of these issues, there is a clinical need to find effective non-invasive ways of assessing PH that could substitute hemodynamic measurements and endoscopy.^{25,81,135}

Various non-invasive methods of assessing PH include EASL criteria (presence of ascites or encephalopathy or esophageal varices or splenomegaly and thrombocytopenia $<100,000/\text{mm}^3$), splenomegaly >10 cm on CT scan, biochemical markers such as FibroTest[®], Doppler US evaluation portal vein blood flow, and elastography of liver and spleen.¹³⁶⁻¹⁴¹

Liver stiffness by transient elastography (FibroScan[®]):

Numerous studies have proven that TE is a safe, reproducible, and rapid (it can be performed in <10 min) tool to detect advanced fibrosis or cirrhosis. TE has been shown to be related to the degree of portal and such a correlation is somewhat expected as hepatic fibrosis is the main determinant of both tissue stiffness and of intrahepatic resistance to portal blood flow. LS can increase independently of fibrosis due to food ingestion, inflammation, cholestasis and liver congestion.^{7,24,32} Despite the limitations cited above, several publications have demonstrated that LS not only estimates hepatic fibrosis but also evaluates HVPG.

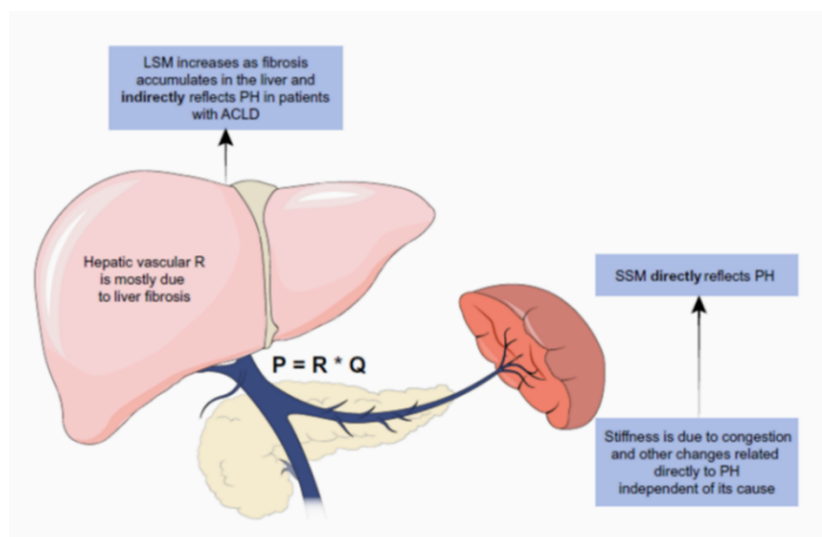
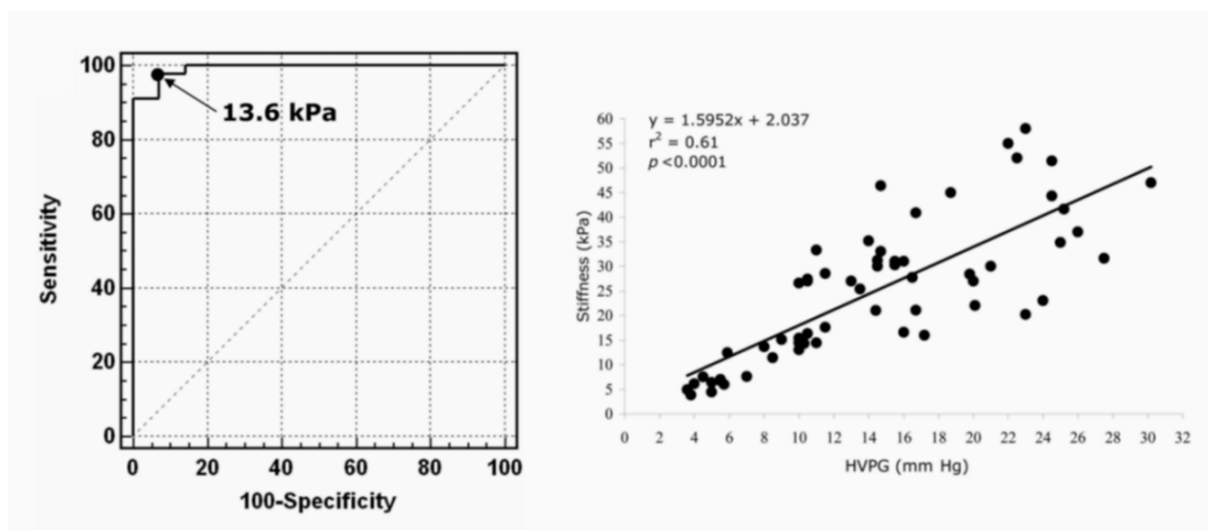


Figure 32: Principle of liver and splenic stiffness measurement by transient elastography¹³⁵

Vizzuti and his colleagues evaluated for the first time the ability of LS to predict severe portal hypertension compared with that of HVPG in 61 consecutive patients with HCV-related chronic liver disease. A strong relationship between LS and HVPG measurements was found in the overall population ($r=0.81$, $p<0.0001$). However, although the correlation was excellent for HVPG values less than 10 or 12mmHg ($r=0.81$, $p=0.0003$ and $r=0.91$, $p<0.0001$, respectively), linear regression analysis was not optimal for HVPG values >10 mm Hg ($r^2=0.35$, $p<0.0001$) or >12 mm Hg ($r^2=0.17$, $p=0.02$). The AUROC for the prediction of HVPG >10 and >12 mm Hg were 0.99 and 0.92, respectively and at LS cutoff values of 13.6 kPa and 17.6 kPa, sensitivity was 97% and 94%, respectively. In patients with cirrhosis, LS positively correlated with the presence of esophageal varices ($p=0.002$), although no correlation between LS and esophageal varices size was detected. AUROC for the prediction of esophageal varices was 0.76 and at a LS cutoff value of 17.6 kPa sensitivity was 90%. They concluded that LS was a promising non-invasive tool for the identification of chronic liver disease patients with CSPH or severe PH and could be employed for screening patients to be subjected to standard investigations including UGIE and hemodynamic studies.¹⁴²

Figure 33: ROC analysis of liver stiffness (LS) to predict clinically significant portal hypertension and linear regression analysis of LS to predict hepatic venous pressure gradient (HVPG)¹⁴²



Another study by Bureau *et al* reported similar performance of LS in predicting CSPH in a study of 150 patients (89 cirrhotic) who underwent TE, LB, and hemodynamic assessment. HVPG was found to be correlated with LS ($r=0.858$; $p<0.001$) and inversely correlated with prothrombin index ($r=-0.718$; $p<0.001$). Regarding significant PHT, AUROC for LS and prothrombin index were 0.95 [0.90–0.99] and 0.89 [0.84–0.95] respectively. The cut-off value of 21 kPa accurately predicted significant PH in 92% of the 144 patients for whom LS was successful. However, the correlation was excellent with HVPG values between 5-10 or 12 mmHg and less strong in patients with an HVPG >12 mmHg. In some patients with variceal bleeding, LS did not diagnose HVPG >20 mmHg. This finding would be explained by the fact that in the early stages of the disease the main factor determining PH is hepatic fibrosis, therefore it would be well related to HVPG. Once CSPH is established, the progression of PH depends not only on hepatic fibrosis, but also on other factors, especially those related to the hemodynamic circulation, splanchnic vasodilatation and the resistance in portosystemic collaterals.¹⁴³

Table 22: Correlation of hepatic venous pressure gradient (HVPG) with different parameters¹⁴⁴

	HVPG	LS	Prothrombin index	Age	Platelets	γ GT	Serum albumin	Serum bilirubin	AST
LS	0.858 <i>P</i> < 0.001	1							
Prothrombin index	-0.718 <i>P</i> < 0.001	-0.699 <i>P</i> < 0.001	1						
Age	0.335 <i>P</i> < 0.001	0.280 <i>P</i> = 0.001	-0.213 <i>P</i> = 0.010	1					
Platelets	-0.353 <i>P</i> < 0.001	-0.292 <i>P</i> < 0.001	0.452 <i>P</i> < 0.001	-0.025 <i>P</i> = 0.762	1				
γ GT	0.340 <i>P</i> < 0.01	0.405 <i>P</i> < 0.001	-0.225 <i>P</i> = 0.006	0.440 <i>P</i> = 0.593	-0.097 <i>P</i> = 0.241	1			
Serum albumin	-0.647 <i>P</i> < 0.001	-0.705 <i>P</i> < 0.001	0.727 <i>P</i> < 0.001	-0.296 <i>P</i> < 0.001	0.347 <i>P</i> < 0.001	-0.274 <i>P</i> = 0.001	1		
Serum bilirubin	0.600 <i>P</i> < 0.001	0.577 <i>P</i> < 0.001	-0.683 <i>P</i> < 0.001	0.117 <i>P</i> = 0.161	-0.278 <i>P</i> = 0.001	0.293 <i>P</i> < 0.001	-0.604 <i>P</i> < 0.001	1	
AST	0.288 <i>P</i> < 0.01	0.389 <i>P</i> < 0.001	-0.276 <i>P</i> = 0.001	-0.127 <i>P</i> = 0.122	-0.095 <i>P</i> = 0.249	0.502 <i>P</i> < 0.001	-0.358 <i>P</i> < 0.001	0.482 <i>P</i> < 0.001	1
ALT	-0.178 <i>P</i> = 0.030	-0.125 <i>P</i> = 0.137	0.165 <i>P</i> = 0.047	-0.297 <i>P</i> < 0.001	0.112 <i>P</i> = 0.176	0.293 <i>P</i> < 0.001	0.130 <i>P</i> = 0.176	0.046 <i>P</i> = 0.587	0.709 <i>P</i> < 0.001

The overall correlation between quantitative variables was determined using the Spearman Rho's pair-wise coefficient matrix analysis. The highest correlation coefficients were found between HVPG on one hand, and LS and prothrombin index, on the other (bold characters). All the *P*-values are given uncorrected.

Another interesting study by Robic *et al* followed up 100 patients with CLD of varying etiologies who underwent LS and HVPG measurement on the same day over a period of two years. Within the two-year follow-up, 41 patients developed, at least, one liver disease-related complication. The performances of HVPG and LS for predicting the occurrence of these complications were not significantly different: AUROCs of 0.815 and 0.837, respectively. When considering only complications related to PH, both methods were found to be similarly accurate: AUROCs of 0.830 and 0.845, for HVPG and LS, respectively. When patients were divided in two groups according to LS value below or above 21.1 kPa, actuarial rates of remaining free of any complication at two years were 85.4% vs. 29.5%, respectively. When only PH-related complications were considered, these rates were 100% vs 47.5%, respectively. The performances of LS and HVPG were also similar in the subgroup of 65 patients with cirrhosis. LS proved as effective as HVPG in predicting clinical decompensation and PH-related complications in patients with CLD.¹⁴⁴

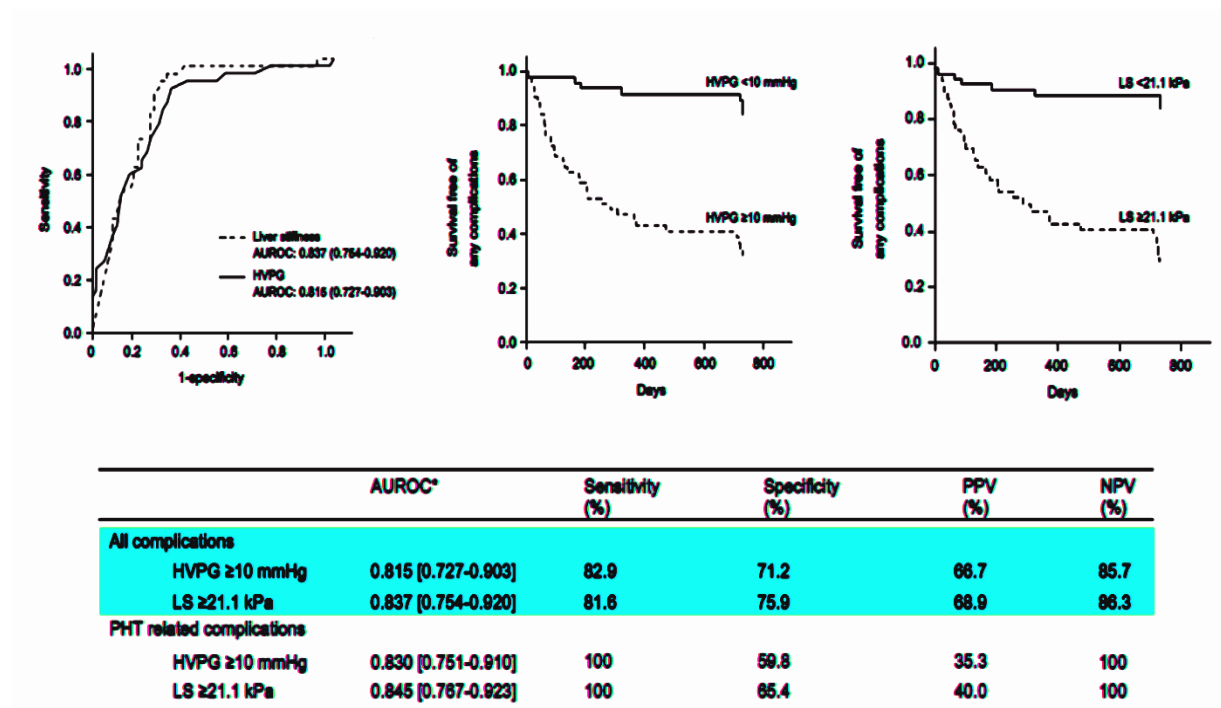


Figure 34: ROC analysis of liver stiffness (LS) and hepatic venous pressure gradient (HVPG) for development of complications and Kaplan Meier analysis for complication-free survival according to LS and HVPG¹⁴⁴

Similar study from Australia by Kitson *et al* confirmed that CSPH and LS >34.5 kPa predicted PH-related complications with 100% and 75.0% sensitivity, 40.3% and 69.4% specificity, 43.1% and 52.5% PPV, and 100% and 86.2% NPV, respectively.¹⁴⁵ According to a collective data published by Berzigotti *et al*, TE could be helpful in ruling out or ruling in CSPH but it was not accurate enough to replace HVPG in quantifying the exact severity of PH and therefore TE was unlikely to be useful in monitoring hemodynamic response to drug therapy.¹³⁵

A study of 711 patients with chronic liver diseases due to HCV or HBV infection, alcohol, NASH, other, or a combination of the above aetiologies. LS was significantly correlated with fibrosis stage ($r=0.73$, $p<0.0001$). AUROC (95%CI) were 0.80 (0.75–0.84) for patients with significant fibrosis ($F>2$), 0.90 (0.86–0.93) for patients with severe fibrosis ($F3$), and 0.96 (0.94–0.98) for patients with cirrhosis. Using a cut off value of 17.6 kPa, patients with cirrhosis were detected with PPV and NPV of 90%. LS was significantly correlated with clinical, biological, and morphological parameters of liver disease. With an NPV >90%, the cut off values for the presence of oesophageal varices stage 2/3, cirrhosis Child-Pugh B or C, past history of ascites, hepatocellular carcinoma, and esophageal bleeding were 27.5, 37.5, 49.1, 53.7, and 62.7 kPa, respectively.⁴⁰

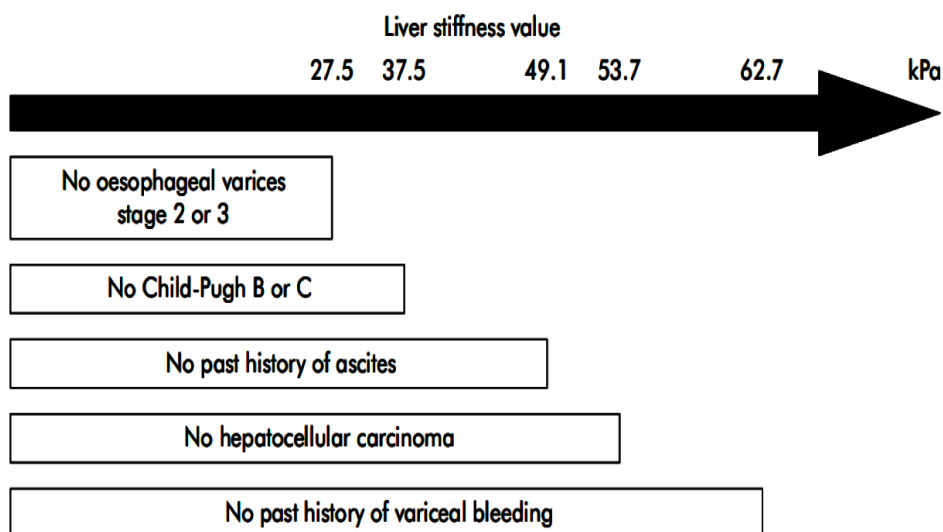


Figure 35: Significance of the wide range in liver stiffness measured by transient elastography⁴⁰

Castera *et al* reviewed the performance of TE in published literature and found that TE had similar performance to some of the biochemical markers in predicting the progression of PH toward the levels of clinically significant (i.e. HVPG ≥ 10 mmHg) and severe (HVPG ≥ 12 mmHg) as well as the presence and the size of esophageal varices (Tables 24 and 25).¹⁴⁶

Table 23: Publications evaluating performance liver stiffness to diagnose oesophageal varices¹⁴⁶

Authors, [Ref.]	Patients (n)	Etiologies	Study design	Child-Pugh A (%)	End point	Prevalence OV (%)	Cut-offs (kPa)	AUC	Se (%)	Sp (%)	PPV (%)	NPV (%)	+LR	-LR	Saved endoscopy (%)
Kazemi <i>et al.</i> ,	165	CLD	Retro. mono.	n.a.	OV LOV	45 28	13.9 19.0	0.83 0.84	95 91	43 60	57 48	91 95	1.7 2.3	0.13 0.14	66 69
Vizzutti <i>et al.</i> ,	47	HCV	Pro. mono.	60	OV	66	17.6	0.76	90	43	77	66	1.6	0.23	74
Pritchett <i>et al.</i> ,	211	CLD	Retro. mono.	n.a.	OV LOV	n.a. 37	19.5 19.8	0.74 0.76	76 91	66 56	56 91	82 55	2.2 2.1	0.36 0.16	n.a. 69
Bureau <i>et al.</i> ,	89	CLD	Pro. mono.	34	OV LOV	72 48	21.1 29.3	0.85 0.76	84 81	71 61			2.9 2.1	0.22 0.31	81 71
Castera <i>et al.</i> ,	70	HCV	Retro. mono.	100	OV LOV	36 19	21.5 30.5	0.84 0.87	76 77	78 85	68 56	84 94	3.5 5.1	0.31 0.27	73 79
Pineda, <i>et al.</i> ,	102	HIV-HCV	Pro. multi.	76	CROV*	13	21.0	0.71	100	32	25	100	1.5	0.0	44
Nguyen <i>et al.</i> ,	183	CLD	Retro. mono.	63	LOV	22	48.0	0.76	73	73	44	90	2.7	0.37	73
	58	HCV/HBV	mono.			17	19.8	0.73	89	55	27	97	2.0	0.20	60
	103	Alcohol				25	47.2	0.77	85	64	44	93	2.4	0.23	69
Malik <i>et al.</i> ,	124	CLD	Retro. mono.	n.a.	OV	51	20.0	0.85	n.a.	n.a.	80	75	n.a.	n.a.	n.a.

*CROV: clinically relevant OV requiring primary prophylaxis of bleeding, i.e. patients carrying LOV or OV with red signs or Child-Pugh class C.

CLD, chronic liver diseases; HCV, chronic hepatitis C; HIV-HCV, co-infection with human deficiency virus and hepatitis C virus; HBV, hepatitis B virus; AUC, area under ROC curve; Se, sensitivity; Sp, specificity; PPV & NPV, positive and negative predictive values; +LR & -LR, positive and negative likelihood ratios; Pro. mono., prospective monocentric; Pro. multi., prospective multicentric; Retro. mono., retrospective monocentric.

The percentage of saved endoscopy was calculated as the percentage of correctly classified patients by pooling true negative and true positive.

Table 24: Publications evaluating performance of liver stiffness to diagnose clinically significant portal hypertension (HVPG ≥ 10 mmHg)¹⁴⁶

Authors, [Ref.]	Patients (n)	Etiologies	Study design	Prevalence of clinically significant portal hypertension (%)	Cut-offs HVPG ≥ 10 mmHg (kPa)	AUC	Se (%)	Sp (%)	PPV (%)	NPV (%)	+LR	-LR
Carrion <i>et al.</i> ,	124	HCV-LT	Pro. mono.	21	8.7* -	0.92	90	81	81	90	4.7	0.12
Vizzutti <i>et al.</i> ,	61	HCV	Pro. mono.	77	13.6	0.99	97	92	97	92	13.7	0.02
					17.6**	0.92	94	81	86	91	4.9	0.08
Sanchez-Condé <i>et al.</i> ,	38	HIV-HCV	Pro. mono.	74	14.0	0.80	93	50	84	71	3.5	0.62
					23.0**	0.80	83	67	79	71	2.5	0.49
Lemoine <i>et al.</i> ,	44	HCV	Retro. mono.	77	20.5	0.76	63	70	88	35	2.1	0.53
	48	Alcohol	mono.	83	34.9	0.94	90	88	97	64	7.5	0.13
Bureau <i>et al.</i> ,	150	CLD	Pro. mono.	51	21.0	0.94	90	93	93	91	12.8	0.10

*Hepatic venous pressure gradient (HVPG) ≥ 6 mm Hg; **severe portal hypertension HVPG ≥ 12 mm Hg.

AUC, area under ROC curve; Se, sensitivity; Sp, specificity; +LR, positive likelihood ratio; -LR, negative likelihood ratio; HCV, chronic hepatitis C; HCV-LT, liver transplant for hepatitis C; CLD, chronic liver diseases; Pro. mono., prospective monocentric; Retro. mono., retrospective monocentric.

In a report from the “Anticipate study”, Abraldes *et al* evaluated the patients with cALD and developed a non-invasive tool, called Liver Stiffness to Spleen/Platelet score (LSPS), to rule in or rule out the presence of CSPH and varices needing treatment (VNT). All non-invasive tests (LS, LS+Platelet count, Platelet-Spleen ratio) reliably identified patients

with high-risk of CSPH, and LSPS had the highest discrimination. LSPS values above 2.65 were associated with risks of CSPH above 80%. None of the tests identified patients with very low risk of all-size varices, but both LSPS and a model combining TE and platelet count identified patients with very low risk (<5%) risk of VNT, suggesting that they could be used to triage patients requiring screening endoscopy. LSPS values of <1.33 were associated with a <5 % risk of VNT, and 26% of patients had values below this threshold. LSM combined with Platelet count predicted a risk <5% of VNT in 30% of the patients. Nomograms were developed to facilitate point-of-care risk assessment.¹⁴⁷

Figure 36: ROC analysis of liver stiffness to spleen-platelet score (LSPS) to diagnose varices and varices needing treatment (VNT)¹⁴⁷

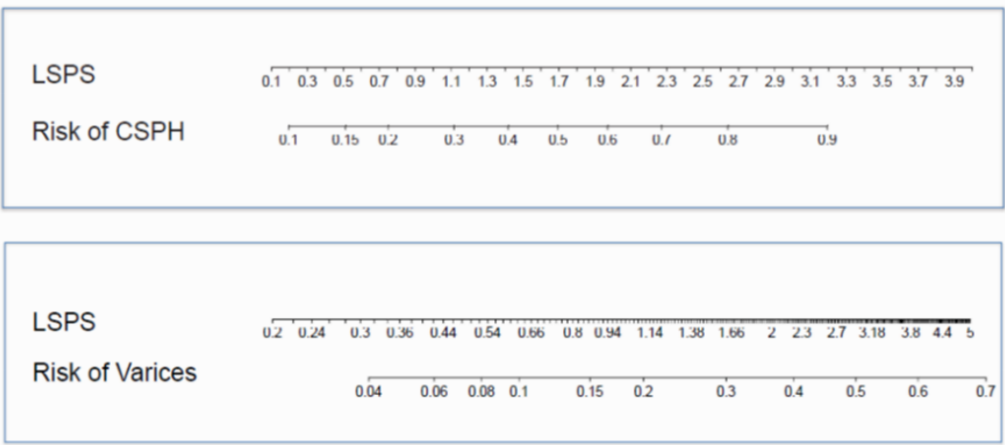
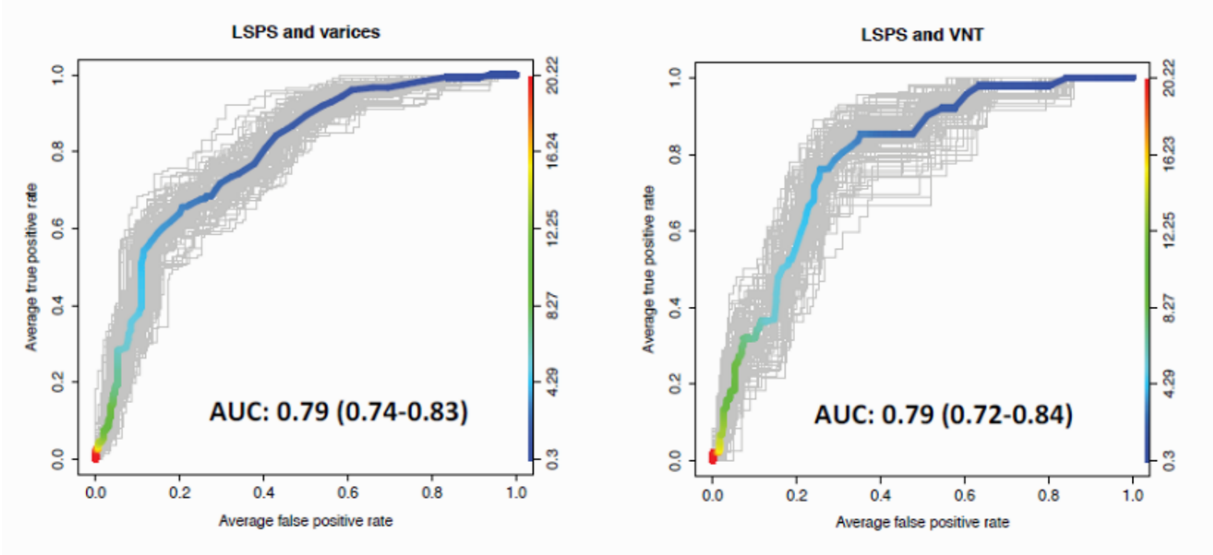


Figure 37: Nomogram with liver stiffness to spleen-platelet score (LSPS) to predict the risk of clinically significant portal hypertension (CSPH) and varices needing treatment (VNT)¹⁴⁷

In a unique study by Procopet *et al*, the diagnostic performance of six biochemical scores, LS alone, and artificial neural network models with and without LS were analyzed. The best non-invasive method for diagnosing cirrhosis, CSPH and esophageal varices was LS with C-statistics of 0.93, 0.94, and 0.9, respectively. Artificial neural networks integrating different serological tests and LS did not increase the increase the diagnostic accuracy of LS alone. Among the serum tests or scores Fibrosis-4 and Lok score had the best performance.¹³³

Table 25: Diagnostic performance of liver stiffness (LS), artificial neural network (ANN) and serological biomarkers¹³³

Test Cut off value	Cirrhosis		CSPH		Esophageal varices	
	Accuracy	p	Accuracy	p	Accuracy	p
ANN + LS	86.3%	<0.001	84%	<0.001	81.8%	<0.001
ANN – LS	77.3%	<0.001	75%	0.007	77.3%	0.04
LS (13.01 kPa)	84%	<0.001	-	-	-	-
LS (13.6 kPa)	-	-	81.8%	<0.001	84%	<0.001
LS (21.1 kPa)	-	-	90%	<0.001	84%	<0.001
Lok > 0.5	75.6%	0.001	-	-	-	-
Lok > 0.73	-	-	80.5%	0.006	73.1%	0.01
FIB 4 > 3.25	70.4%	0.007	72.7%	0.01	75%	0.006
Risk > –1	-	-	72.7%	0.01	70.5%	0.02
HVPG > 10 mmHg	-	-	-	-	84%	<0.001

ANN + LS, artificial neural network including liver stiffness; ANN – LS, artificial neural network without liver stiffness; LS, liver stiffness; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient.

A prospective study by Augustin *et al* aimed to detect PH before the development of complications in 250 patients with asymptomatic CLD using routine data and LS. After screening TE, patients with LS ≥ 13.6 kPa were further evaluated by UGIE and HVPG measurement and were divided in to three groups–Group A: Platelets $\geq 150,000/\text{mm}^3$ and normal abdominal US, Group B: Platelets $< 150,000/\text{mm}^3$ and normal abdominal US and Group C: $< 150,000/\text{mm}^3$ and abnormal US (splenomegaly and nodular liver surface). All patients in Groups B and C had PH. No patients in Group A had varices. This simple strategy was proposed to detect PH among asymptomatic patients.¹⁴⁸

A meta-analysis by You and his colleagues included 11 studies from literature and evaluated the correlation between LS and HVPG and the diagnostic performance of LS in assessing CSPH. The summary correlation coefficient was 0.783 (95%CI: 0.74–0.82). Summary sensitivity, specificity and area under the hierarchical summary AUROCs were 87.5% (95%CI: 75.8–93.9%), 85.3% (95%CI: 76.9–90.9%) and 0.9, respectively. The

subgroup with low cut-off values of 13.6–18 kPa had better summary estimates (sensitivity 91.2%, specificity 81.3% and partial AUROC 0.921) than the subgroup with high cut-off values of 21–25 kPa (sensitivity 71.2%, specificity 90.9% and partial AUROC 0.769). In summary, LS correlated well with HVPG and represented good diagnostic performance in diagnosing clinically significant portal hypertension.¹⁴⁹ To use as a sensitive screening tool, they proposed a low LS cut-off values of 13.6–18 kPa. In another recent study from India, Kumar *et al* found TE had a significant but modest correlation with HVPG ($r=0.36$, $p<0.001$) in 326 patients with cirrhosis (45% cryptogenic, 34% alcoholic, 15% viral). AUROC for TE in predicting CSPH was 0.74 (95%CI: 0.66-0.82) with a cut-off value of 21.6 kPa.¹⁵⁰ In a systematic review and meta-analysis published Kim *et al*, eight studies were analyzed and TE was demonstrated to detect PH (HVPG ≥ 6 mmHg) with a summary sensitivity and specificity of 0.88% and 0.74%, respectively. Regarding CSPH, the summary sensitivity and specificity were 0.85% and 0.71%, respectively. The overall correlation estimate of TE and HVPG was 0.75 (95%CI: 0.65-0.82, $p<0.0001$).¹⁵¹

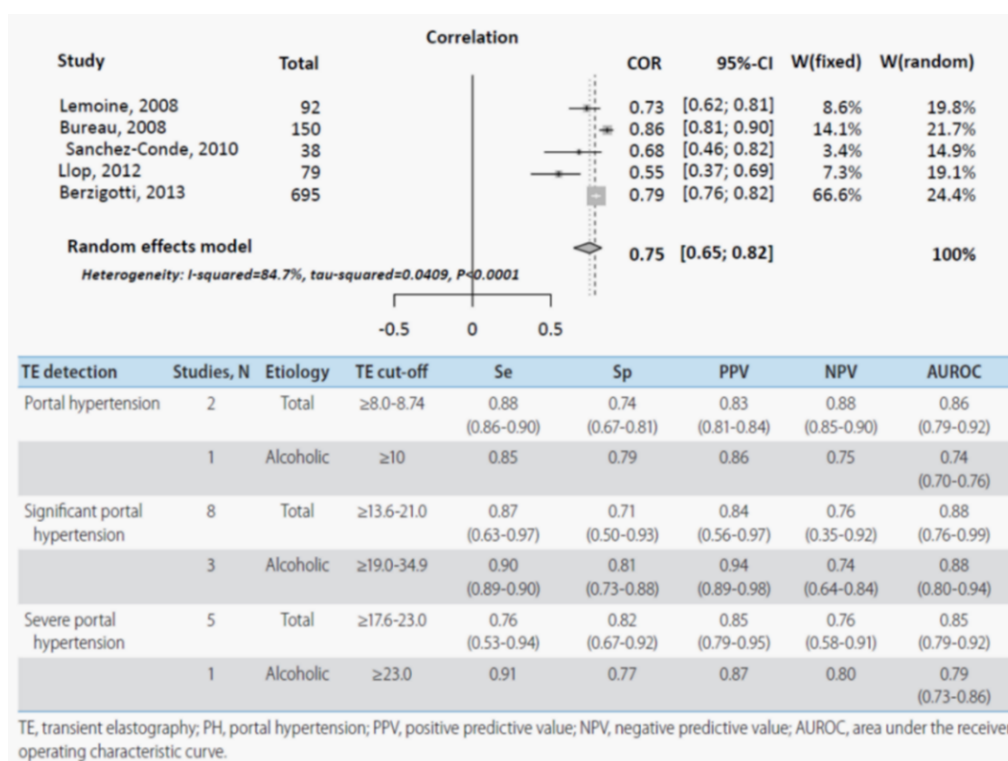


Figure 38: Pooled correlation between transient elastography and hepatic venous pressure gradient¹¹⁵

Various practice guidelines and recommendations about the utilization of TE in the management of patients with PH are as follows:

Baveno VI consensus workshop recommendations:⁸¹

a. Diagnosis of CSPH in patients with cACLD:

- HVPG measurement is the gold-standard method to assess the presence of CSPH, which is defined as HVPG ≥ 10 mmHg (1b;A)
- By definition, patients without CSPH have no gastroesophageal varices, and have a low five year risk of developing them (1b;A)
- In patients with virus related cACLD non-invasive methods are sufficient to rule-in CSPH, defining the group of patients at risk of having endoscopic signs of PH. The following can be used (2b;B)
- Liver stiffness by TE (≥ 20 – 25 kPa; at least two measurements on different days in fasting condition; caution should be paid to flares of ALT; refer to EASL guidelines for correct interpretation criteria), alone or combined to platelets and spleen size
- The diagnostic value of TE for CSPH in other aetiologies remains to be ascertained (5;D)
- Imaging showing collateral circulation is sufficient to rule-in CSPH in patients with cACLD of all aetiologies (2b;B)

b. Identification of patients with cACLD who can safely avoid screening endoscopy:

- Patients with a liver stiffness < 20 kPa and with a platelet count $> 150,000$ have a very low risk of having varices requiring treatment, and can avoid screening endoscopy (1b;A)
- These patients can be followed up by yearly repetition of TE and platelet count (5;D)
- If liver stiffness increases or platelet count declines, these patients should undergo screening esophagogastroduodenoscopy (5;D)

AGA Recommendations:⁸⁵

- In patients with suspected chronic liver disease undergoing elective non-hepatic surgery, a VCTE™ cutoff of 17.0 kPa to detect CSPH to inform preoperative care.

EFSUMB Recommendations:⁸⁶

- LS with TE is useful to identify patients with a high likelihood of having clinically significant portal hypertension (HVPG ≥ 10 mmHg) (2b:B). Strong consensus (15/0/0, 100%)
- Liver stiffness using TE combined with platelet count is useful to rule out varices requiring treatment (2b:B) [194]. Although preliminary results are encouraging, there is insufficient evidence to recommend pSWE and 2D-SWE in this setting. Broad consensus (13/0/1, 93%)

Role of Transient Elastography in Liver Surgery

A) Liver Resection

Post-operative complications:

The era of hepatic surgery began with a left lateral lobectomy performed successfully by Langenbuch *et al* in 1887. Since then, liver resections (LR) have been performed widely for the treatment of various hepatobiliary diseases, such as benign and malignant tumors, intrahepatic lithiasis, parasitic diseases and abscesses. Operations on the liver are especially challenging because of its unique anatomic architecture and its vital functions.¹⁵²⁻¹⁵⁵ Despite technical advances and high experience of LR of specialized centers, it is still burdened by relatively high rates of post-operative morbidity (4.1-47.7%) and mortality (0.2-9.7%).^{156,157} Common post-hepatectomy complications include fever and infections, hemorrhage, ascites, bile leakage, pleural effusion, and liver failure.¹⁵⁵ Improvements in patient selection, peri-operative management, cross-sectional imaging, our understanding of liver anatomy, and surgical techniques have led to both increase in surgical indications, complex LR, and number of operations performed in high-risk patients. Once as high as 20%, mortality rates after major hepatectomy have dropped to $\leq 2\%$ in most specialized centers. It is critical to identify those at a high and low-risk of post-operative complications and mortality in order to provide accurate and informed pre-operative counselling, adapting the surgical strategy, better optimization of the patient for surgery, and choosing an alternative treatment option in patients with a formidable risk of morbidity and mortality.¹⁵⁴⁻¹⁶⁴

The short and long-term prognosis of patient undergoing LR depends on the quantity and quality of the future liver remnant (FLR) as demonstrated by Shinodh *et al*. They proposed FLR of $\geq 20\%$ standard liver volume in normal liver, $\geq 30\%$ in injured liver due to prolonged chemotherapy or hepatitis, and $\geq 40\%$ in cirrhotic liver.^{165,166} Hemodynamic

assessment of HCC patients who are planned for LR has also proven to predict early and late post-operative outcomes especially in patients with advanced liver disease and cirrhosis. Bruix *et al* and Boleslawski *et al* have demonstrated that HVPG was an independent risk factor for postoperative hepatic decompensation and 90-day mortality in cirrhotic patients undergoing LR for Barcelona Clinic Liver Cancer (BCLC) stage 0/A HCC. Further, 5-year survival after LR in patients with and without CSPH were >70% and 50-60% respectively. The evaluation of HVPG is considered as a key step in the pre-operative assessment of HCC patients planned for hepatectomy.¹⁶⁷⁻¹⁷⁰ Similarly, Llovet *et al* have advocated HVPG measurement in cirrhotic patients with resectable HCC to make informed treatment decisions – LR or liver transplantation (LT).¹⁷¹ Therefore, it is of paramount importance to pre-operatively know the quality of FLR and the degree PH. Transient elastography by FibroScan[®] is a non-invasive method of accurately quantifying hepatic fibrosis, predicting CSPH and long-term prognosis in patients with CLD and cirrhosis.^{40,80,81,142,146} Thus, LS, which estimates both hepatic fibrosis and HVPG, could be used pre-operatively to predict post-hepatectomy outcomes. Moreover, McCormack *et al* have reported that patients undergoing major hepatectomy with hepatic steatosis had significantly higher post-operative serum transaminase and bilirubin levels, and lower prothrombin time. Blood loss ($p=0.04$) and transfusions ($p=0.03$), and ICU stay ($p=0.001$) were increased in steatotic patients. Complications were higher in steatotic patients when considered either overall (50% vs 25%, $p=0.007$) or major (27.5% vs 6.9%, $p=0.001$) complications. Preoperative cholestasis was a highly significant risk factor for mortality in patients with hepatic steatosis.¹⁷² Therefore, CAP measured simultaneously by TE could be of additional benefit in predicting post-operative outcomes by assessing hepatic steatosis.

The first study that extended the utilization of TE in the surgical field investigated prospectively the performance of LS to predict post-hepatectomy liver insufficiency (PHLF)

defined by 50-50 criteria or death in 91 consecutive patients undergoing hepatectomy for HCC. Kim *et al* demonstrated that LS was a better predictor than indocyanine green retention at 15 min (ICG-R15) in predicting PHLF with AUROC for LS and ICG-R15 of 0.82 vs 0.62, respectively. In multivariate analysis, LS >25.6 kPa was the sole predictor of PHLF.¹⁷³

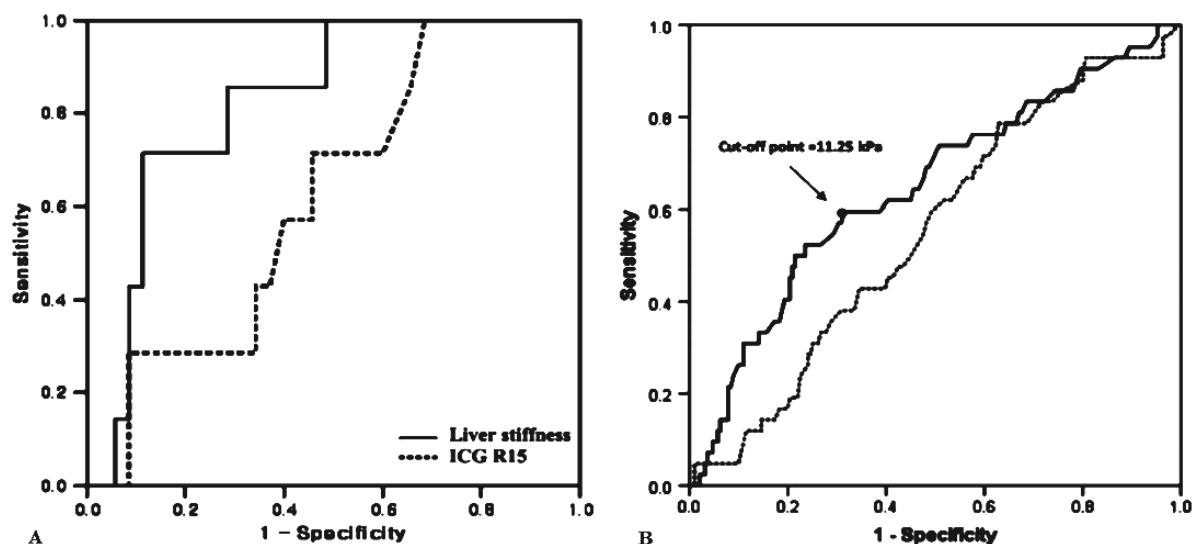


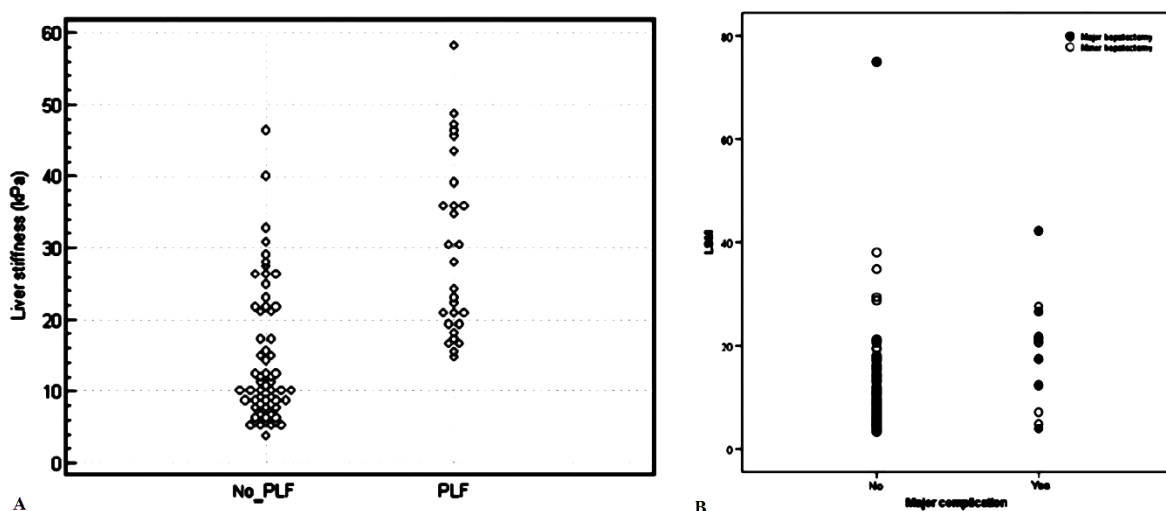
Figure 39: ROC analysis of the diagnostic performance of liver stiffness and indocyanine green retention at 15 min to predict post-hepatectomy liver failure in the publications by Kim *et al*¹⁷³ (A) and Chong *et al*¹⁷⁴ (B)

A recent work by Chong *et al* proved pre-operative LS to be an accurate predictor high-grade PHLF defined by International Study Group of Liver Surgery (ISGLS) in 255 HCC patients and compared with ICG-R15. They observed that LS had better performance when compared to ICG-R15. LS >12 kPa was an independent predictor of high-grade PHLF but also major post-operative complications according to Dindo-Clavien classification of surgical complications. Further, the diagnostic performance of LS was better in patients with left lobe tumors compared to those with right lobe tumors (AUROC: 0.83 vs 0.62).¹⁷⁴

Cescon and his colleagues published a pioneering study evaluating FibroScan[®] in predicting the outcomes of LR for HCC in 2013. The accuracy of LS measurement in predicting PHLF, the presence of cirrhosis, and the presence of clinical signs of PH were assessed in 92 patients undergoing hepatectomy for HCC who were prospectively evaluated with preoperative LS. Patients with LS \geq 15.7 kPa had significantly higher risk of PHLF with

AUROC of 0.86, 96.1% sensitivity, 68.7% specificity, 55.6% PPV and 97.8% NPV. Patients with cirrhosis and PH had LS higher than 12.6 kPa ($p<0.001$) and 19.6 kPa ($p<0.001$), respectively.¹⁷⁵ Similarly, Wong *et al* compared the performance of LS and ICG-R15 as a predictor of post-hepatectomy outcomes in a mixed cohort of 105 patients undergoing hepatectomy for various indications. For post-hepatectomy outcomes, only LS but not ICG showed significant correlation with major postoperative complications on receiver operating characteristic curves, with AUROC=0.79 ($p<0.001$). Using the calculated cutoff at 12.0 kPa, LS had sensitivity of 85.7% and specificity of 71.8% in the prediction of major postoperative complications. It was also an independent prognostic factor for major postoperative complications by multivariate analysis. The operative blood loss and transfusion rate were also significantly higher in patients with LS >12.0 kPa.¹⁷⁶

Figure 40: Distribution of liver stiffness with respect to development of post-hepatectomy liver failure¹⁷⁵, (A) and major complications¹⁷⁶ (B)



Recently, Donadon *et al* reported that LS ≥ 9.7 kPa and CAP ≥ 250 dB/m as predictors of post-operative complications with AUROC of 0.73 and 0.66, respectively. However, in multivariate analysis, elevated LS, presence esophageal varices, major resections, and blood loss >500 mL, were the independent predictors of complications.¹⁷⁷ Another recent study from China found that there was a significant correlation between LS and ICG-R15. In

patients with LS ≥ 11 kPa vs < 11 kPa, there was significantly higher ICG-R15 (17% vs 10%, $p=0.02$). Similarly, for patients with ICG-R15 $\geq 10\%$ compared to those with $< 10\%$, there was significantly higher LS (12 kPa vs 7.6 kPa, $p=0.01$). There was a significant correlation between LS and post-operative peak International normalized ratio (INR) ($r=0.43$) whereas ICG-R15 had significant correlation with post-operative peak serum aminotransferases ($r=0.41$ for AST and $r=0.57$ for ALT).¹⁷⁸

Table 26: Correlation of liver stiffness, indocyanine green retention at 15 min and other laboratory parameters¹⁷⁸

Parameters	Liver stiffness r co-efficient	Liver stiffness P value	ICG R15 r co-efficient	ICG R15 P value
Liver stiffness	–	–	0.342	0.023
ICG R15	0.342	0.023	–	–
Age	–0.032	0.836	0.100	0.517
Bilirubin	0.136	0.378	0.349	0.020
ALT	0.228	0.137	–.080	0.605
AST	0.455	0.002	0.095	0.541
Albumin	–.0710	<0.001	–0.525	<0.001
Creatinine	0.071	0.649	0.141	0.361
INR	0.682	<0.001	0.548	<0.001
AFP	0.140	0.369	0.081	0.605
Platelet count	–0.176	0.253	–0.322	0.033
MELD score	0.431	0.003	0.519	<0.001
HCC size	0.248	0.108	–0.211	0.174

Two recent studies revisited the need for HVPG assessment in patients with resectable HCC. The first study by Cucchetti *et al* evaluated HVPG in 70 consecutive patients prospectively as a predictor of grade B or C PHLF. HVPG was useful to stratify the risk of PHLF but they concluded that the cut-off of 10 mmHg was restrictive and would exclude one-quarter of the patients who would benefit from surgery without short or mid-term post-operative sequelae.¹³¹ Llop *et al* on the other hand compared pre-operative LS and HVPG in 97 Child Pugh A HCC patients and assessed the ability of LS to non-invasively predict CSPH. LS was effective in only half of the patients to correctly classify as having or not CSPH. In the remaining half, LS was either not applicable or inaccurate. Thus, HVPG was still a non-replaceable method to detect CSPH.¹⁷⁰ Kim *et al* on the other hand developed and validated a predictive model called calculated HVPG (cHVPG) using ICG-R15, serum albumin, international normalized ratio, and platelet count. In the validation cohort, patients with

cHVPG <10 mmHg underwent LR while those with cHVPG \geq 10 mmHg were not resected. Among those patients who underwent LR, there was no difference in patient and tumor characteristics, operative outcome, and survival rates between patients with ICG-R15 < or \geq 20%. Thus, a simple equation using serological tests could replace invasive HVPG measurement in patients with resectable HCC.¹⁷⁹

Magnetic resonance elastography (MRE) and ARFI are emerging as an attractive and effective alternative to TE in assessing LS. Abe *et al* prospectively investigated whether the stiffness measurement by MRE correlated with hepatic fibrosis and post-operative outcomes. The median MRE-LS in 175 patients was 3.4 (range: 1.5–11.3) kPa, and the pathologic grade of fibrosis was significantly correlated with LS ($r=0.68$, $p<0.001$). The median blood loss during transection per unit area was 4.1 mL/cm² (range: 0.1–37.0 mL/cm²), and the frequency of major complications was 16.0%. MRE-LS was the only independent prognostic factor for both blood loss (regression coefficient: 1.14, 95% CI: 0.45–1.83, $p=0.001$) and major complications (OR: 2.14, $p<0.001$). ROC analysis indicated a significant correlation between MRE-LS and major complications with calculated AUROC of 0.81 ($p<0.001$), and the sensitivity and specificity for prediction of major complications with a cut-off value of 5.3 kPa were 64.3% and 87.8%, respectively. On the other hand, the amount of blood loss was significantly correlated with the frequency of major complications ($p=0.003$).¹⁸⁰ By contrast, Shen *et al* investigated elastography by ARFI and presented the results of their study comparing LS measured in shear wave velocity (SWV) estimated by ARFI, ICG clearance rate (ICG-K) and biochemical markers. AUROCs for grade B or C PHLF were 0.78 for SWV, 0.75 for hyaluronic acid and 0.68 for ICG-K. They proposed a risk model with SWV with a performance of 79.9% for all grades and 83.5% for grade B or C PHLF.¹⁸¹

Another test commonly utilized in Eastern countries to measure hepatic functional reserve is ICG clearance test and it is being routinely used to predict post-operative outcomes.¹⁸²⁻¹⁸⁵ After intravenous injection, the vital dye ICG almost completely binds to plasma protein and is distributed in the serum alone with no extracellular distribution. ICG is exclusively removed by the liver via a carrier-mediated mechanism, and is excreted unchanged into the bile and does not undergo any enterohepatic circulation. The disappearance curve of ICG has two distinct linear components, initial rapid fall represents the uptake of ICG from plasma into liver and the second slow decay represents the elimination from liver. The ratio of the ICG plasma concentration at 15 min to its initial concentration is ICG-R15. In a patient with an ideal initial plasma concentration of 100 mg/ml after a bolus of 0.5 mg/Kg, ICG-R15 is pharmacologically equivalent to ICG-K. An important study by Imamura *et al* established the criteria for LR incorporating ascites, serum total bilirubin level, and ICG-R15 in a decision-tree. In patients with decompensated liver disease (presence of ascites or elevated bilirubin level), LR was contraindicated. In corporation of ICG-R15 in the model enabled the patients to be classified in to several groups in which various types of hepatectomies would be safe.^{183,185}

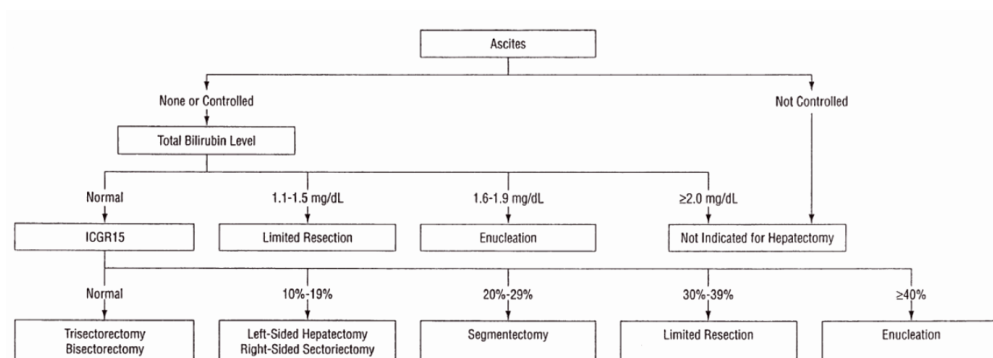


Figure 41: Decision tree for selecting an operative procedure in patients with impaired hepatic functional reserve¹⁸³

Lau *et al* demonstrated that ICG-R15 was the single best predictor of post-hepatectomy mortality in 127 non-cirrhotic and cirrhotic patients with HCC. Peri-operative

monitoring of ICG-K by pulse spectrophotometer evaluated FLR before, during and after LR. Age (65 yr) and ICG-K (0.009/min) were independent predictors of PHLF.¹⁸² In another study of 28 LRs for HCC, LS was demonstrated to have a moderate correlation with ICG-R15 but both of these tests failed to predict early post-operative complications.¹⁷⁸ A recent study confirmed the predictive value of ICG-K in FLR in patients undergoing complex hepatectomies with extrahepatic bile duct resection. ICG-K was an important independent risk factor for PHLF and post-operative mortality. Yokoyama *et al* further highlighted that the systematic use of ICG-K as a criterion to determine the operative indication resulted in an acceptable mortality rate of 2% with these challenging resections that included combined vascular resections and panceatoduodenectomy.¹⁸⁶ In an interesting analysis by Kim *et al*, actual FLR (aFLR) was compared to standardized FLR (sFLR) to determine criteria for safe hepatectomy using CT volumetry and ICG-R15. In patients without cirrhosis, PHLF developed in patients with sFLR <25% irrespective of ICG-R15. In patients with cirrhosis, aFLR and sFLR had no correlation with postoperative total bilirubin. A sFLR : ICG-R15 ratio of <1.9 was an independent predictor of PHLF.¹⁸⁷ In a similar study, Hwang *et al* proposed ICG-K fraction of FLR to total liver volume ratios of 0.04 and 0.05 with 70% and 65% hepatic parenchymal resections, respectively in normal and diseased livers for safe hepatectomy.¹⁸⁸ Some of the other reported risk models include albumin-bilirubin score, simplified frailty index, LiMAX algorithm, prothrombin activity, serum hyaluronic acid, and Tc-99^m galactosyl human serum albumin liver scintigraphy.¹⁸⁹⁻¹⁹³

Oncologic outcomes:

Hepatocellular carcinoma is the most common primary liver cancer ($\geq 90\%$) and it is second leading cause of cancer-related death worldwide.^{194,195} Various risk factors for HCC development such as cirrhosis (chronic liver damage from inflammation and fibrosis) from viral hepatitis, alcohol abuse and/or metabolic syndrome are well defined. Other cofactors

include tobacco smoke inhalation, and intake of aflatoxin B. Thus, hepatic fibrosis is present in virtually all patients with chronic liver injury, regardless of etiology.¹⁹⁵ While the ability of the liver to regenerate may attenuate some of the injury and associated deposition of extracellular matrix, the majority of patients with CLD will ultimately progress over decades to advanced fibrosis and cirrhosis. Progressive fibrosis is a common pathway for all forms of CLD and closely linked epidemiologically to HCC risk. Although the molecular and genetic events that predispose a fibrotic liver to cancer development remain unclear, a permissive hepatic microenvironment provides fertile soil for transition of damaged hepatocytes into HCC. An altered biomechanical environment with increased matrix stiffness, which are characteristic of inflammation and fibrosis, was a strong predictor of HCC development.¹⁹⁵⁻¹⁹⁷ Schrader *et al* demonstrated *in vitro* that increasing matrix stiffness promoted HCC cell proliferation and chemotherapeutic resistance, whereas a soft environment induced reversible cellular dormancy and stem cell characteristics in HCC.¹⁹⁸ Similarly, overall survival and recurrence-free survival in patients who underwent LR for HCC depends on the tumor stage (size, number), tumor biology (serum α -fetoprotein (AFP), vascular invasion), quality of non-tumoral liver (cirrhosis), degree of PH, patient's performance status, and interaction among these factors.^{167,168,199-205} EASL-EORTC recommends BCLC staging to be used to determine the choice of treatment according to the patient and HCC characteristics to achieve optimal short and long-term results (Figure 42).^{167,200}

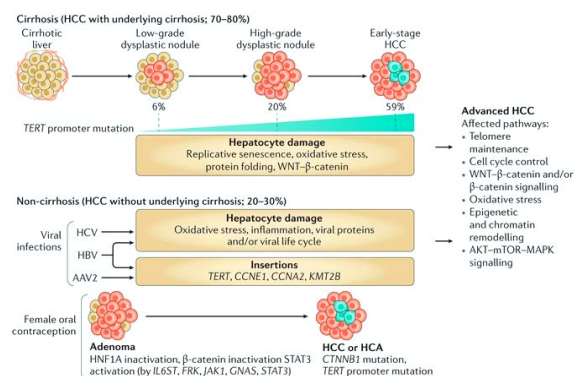
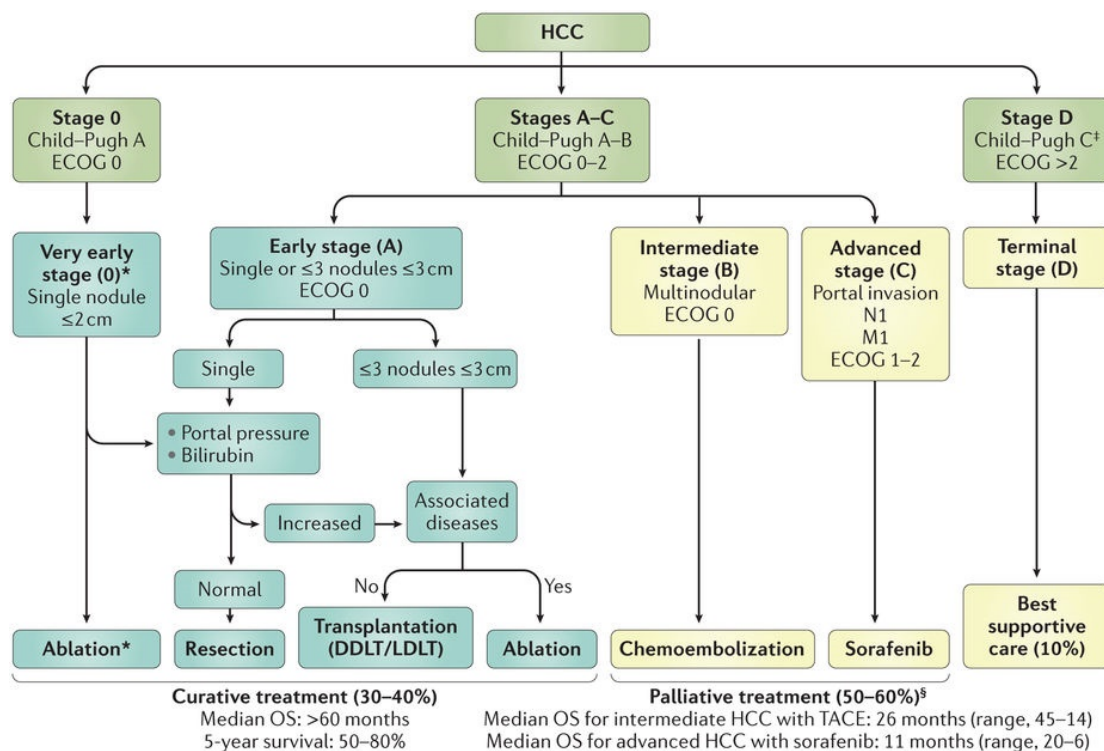


Figure 42: Molecular oncogenesis of hepatocellular carcinoma¹⁹⁵

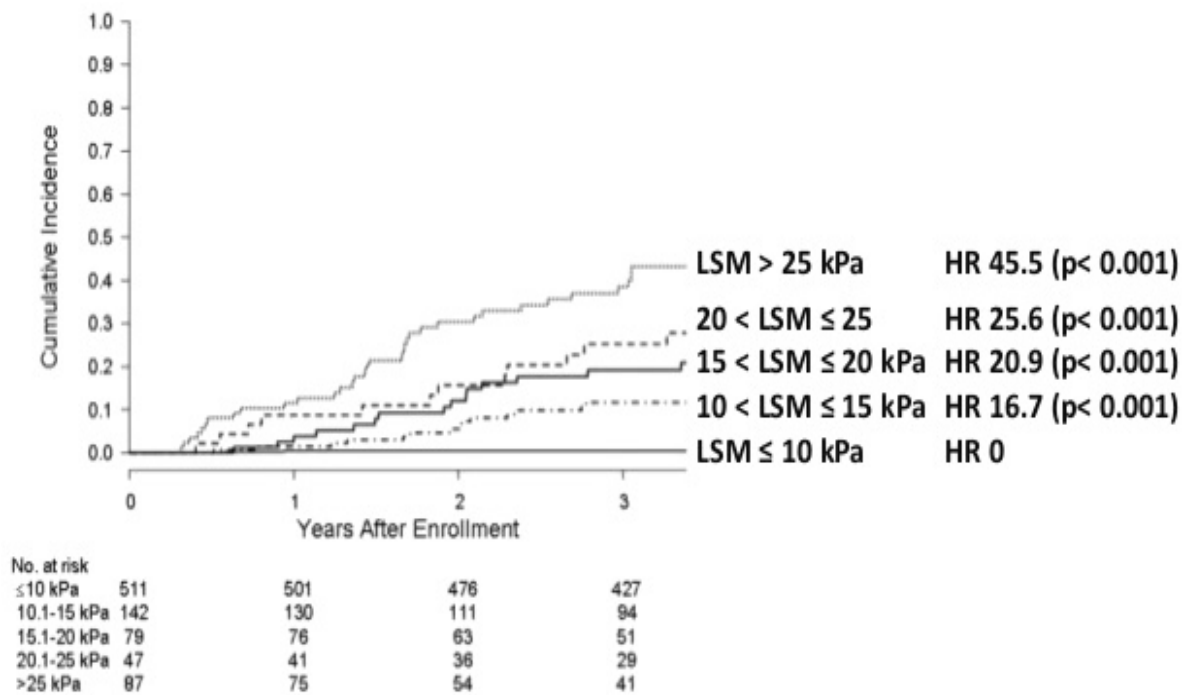
Bruix and his colleagues have demonstrated that serum total bilirubin and absence of CSPH were the best predictors of an excellent outcome after LR with 5-year survival rate of >70%. By contrast, patients with CSPH were at higher risk of decompensation after LR and their survival was significantly reduced to 50-60%.^{3,168-170} In a systematic review and meta-analysis, Berzigotti *et al* demonstrated that CSPH evaluated by any method increased the risk of 3- and 5-year mortality by 2.1 times and risk of clinical decompensation after surgery by 3 times. Therefore, their team advocates systematic HVPG measurement to make treatment choice.²⁰² Another recent publication by Kluger *et al* proved that tumor biology (poorly differentiated tumor, satellite lesions, AFP, and microvascular invasion), intra-operative blood transfusion, and cirrhosis were the independent predictors of poor survival.²⁰⁴ Therefore, TE would be a valuable surrogate to estimate the risk of HCC development and recurrence of HCC after resection in patients with CLD by evaluating the severity of underlying liver disease: degree of fibrosis, PH and aggressivity of HCC.

Figure 43: BCLC staging and treatment strategy in hepatocellular carcinoma³



Masuzaki *et al* evaluated the risk HCC development in 866 patients with CLD during the follow-up period (mean, 3.0 years) and stratified the risk according LS at inclusion. HCC developed in 77 patients (2.9% per 1 person-year). The cumulative incidence rates of HCC at one, two, and three years were 2.4%, 6.0%, and 8.9%, respectively. Adjusting for other significant confounding factors, patients with higher LS were revealed to be at a significantly higher risk, with a hazard ratio (HR), as compared to LS <10 kPa, of 16.7 (95% CI: 3.71-75.2; $p<0.001$) when LS was 10.1-15 kPa, 20.9 (95% CI: 4.43-98.8; $p<0.001$) when LS was 5.1-20 kPa, 25.6 (95%CI: 5.21-126.1; $p<0.001$) when LS 20.1-25 kPa, and 45.5 (95% CI: 9.75-212.3; $p<0.001$) when LS >25 kPa (Figure 44).²⁰⁶

Figure 44: Cumulative risk of hepatocellular carcinoma development according to liver stiffness²⁰⁶



Liu and his colleagues classified 156 untreated patients with HBV-related cirrhosis into two groups - without HCC group and the HCC group. Comparative analyses of LS and serum CRP level were conducted between these two groups. LS values and serum C reactive protein (CRP) levels were found to be significantly higher in the HCC group compared with

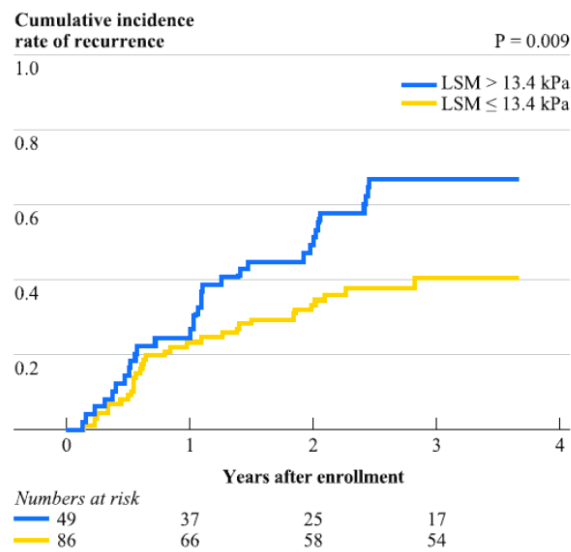
those without HCC ($p<0.01$). The LS values and serum CRP levels were not significantly different between AFP-positive and -negative patients. A high LS value was a high-risk factor for HCC in patients with chronic hepatitis B. The CRP-positive rate was significantly higher in the HCC group compared with that in LC group in a subset of patients with high LS values ($p<0.01$). They concluded that the combined detection of LS and serum CRP might complement the measurement of AFP in the diagnosis of HBV-related HCC, and improve the identification of patients with AFP-negative HCC.²⁰⁷ A prospective study by Jung *et al* assessed pre-operative LS in 133 patients who underwent curative LR. HCC recurred in 62 (46.6%) patients during follow-up (median: 25 months). In multivariate analysis, together with satellite nodule and Edmonson-Steiner grade III–IV, LS was an independent predictor of late (≥ 1 year) recurrence ($p<0.05$; HR: 1.0) as in Table 27. When the study population was stratified into two groups using the optimal cut-off value (13.4 kPa) that maximized the sum of sensitivity (64.7%) and specificity (76.1%) from time-dependent ROC curves (AUROC=0.68). Patients with LS >13.4 kPa had 1.9 times greater risk for recurrence ($p=0.01$) compared with those with LS ≤ 13.4 kPa (Figure 44).^{208,209}

Table 27: Independent predictors of hepatocellular carcinoma recurrence after curative resection²⁰⁸

Variable	Univariate	Multivariate	
	<i>P</i> value	<i>P</i> value	Hazard ratio (95 % CI)
Early recurrence (<i>n</i> = 133)			
Aspartate aminotransferase	0.042	0.07	–
Albumin	0.024	0.557	–
Edmonson-Steiner grade III-IV	<0.001	<0.001	3.161 (1.668–5.992)
Liver stiffness measurement value	0.015	0.103	–
Late recurrence (<i>n</i> = 85)			
Platelet count	0.002	0.386	–
Portal vein invasion	0.031	0.136	–
Satellite nodule	0.007	0.004	12.028 (2.197–65.839)
Liver cirrhosis	0.045	0.087	–
Liver stiffness measurement value	0.008	0.025	1.065 (1.008–1.126)

CI confidence interval

Figure 45: Cumulative incidence of recurrence stratified by liver stiffness cut-off of 13.4 kPa²⁰⁸



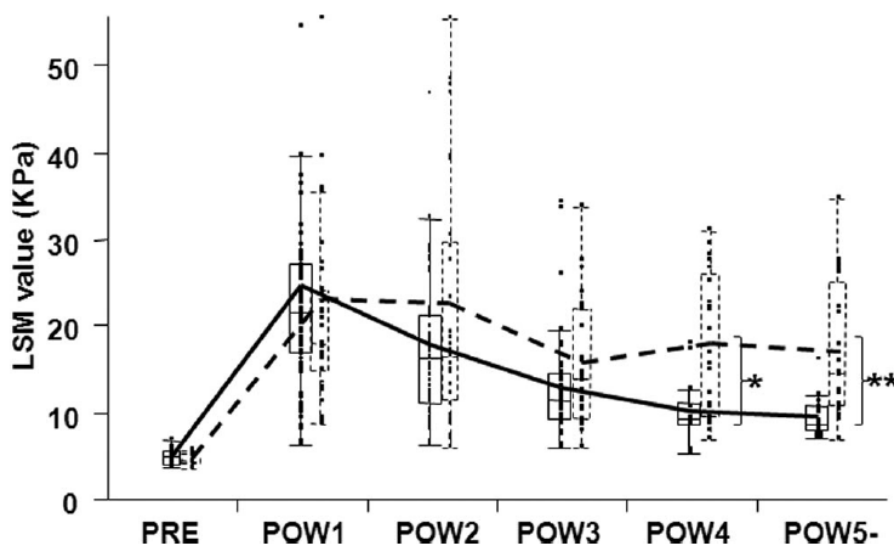
A recent report by Lee *et al* of 228 patients undergoing radiofrequency ablation of HCC established that LS was an important prognostic factor for recurrence. Liver cirrhosis, platelet count, multiple tumors, and LS were the independent predictors of HCC recurrence. When the study population was stratified into early (<1 year) and late (\geq 1 year) recurrence groups, LS was an independent predictor of late recurrence, along with liver cirrhosis and spleen diameter. The risk of late recurrence was higher in patients with LS values of \geq 13 kPa than in those with LS < 13 kPa (adjusted HR=4.51, 95%CI: 2.131-7.724, $p<0.001$). Recurrence was the only predictor of overall survival (HR=18.58, 95%CI: 2.424-142.486, $p=0.005$).²⁰⁹

Role of transient elastography in liver transplantation

Monitoring of graft function and complications in the peri-transplantation period:

To date, there has been little evidence that supports TE usefulness in monitoring liver grafts in the peri-transplantation period.²¹⁰ However, some studies indicated that LS in patients with acute liver damage might increase substantially regardless of chronic structural changes.^{68,211} Thus, TE was hypothesized to be a non-invasive monitoring tool to trace dynamic LS changes in post-transplant liver graft. Inoue *et al* measured 678 LS measurements from 24 living donor liver transplantation (LDLT) recipients, 24 corresponding donors (preoperatively only), another five donors with a remnant right liver, and three deceased donor liver transplantation recipients. TE was performed pre-operatively once, post-operatively every morning until post-operative day 14, and three times a week after that until the patient was discharged from the hospital (Figure 46). They reported that LS values were greatest in the first post-operative week (mean 24.8 kPa) and declined thereafter. Furthermore, recipients who had experienced complications such as acute cellular rejection (ACR), hepatic arterial thrombosis, and sepsis had significantly higher LS values than those without complications beyond the fourth ($p=0.006$) and fifth postoperative week ($p=0.003$).²¹²

Figure 46: Longitudinal evolution of liver stiffness values after liver transplantation²¹²



Another study in 48 LDLT recipients, Lee *et al* reported higher LS values were associated with lower graft-to-recipient weight ratios and higher serum bilirubin levels in the first post-LT week. These phenomena were postulated to be due to intra-hepatic cholestasis, persistent portal hypercirculation and/or regeneration after transplant irrespective of hepatic fibrosis, outflow obstruction, or biliary obstruction.²¹³

Recently, a Spanish multicentric study explored the relationship between LS and severity of ACR after LT and changes in LS values after treatment of rejection in a cohort that consisted of 27 liver recipients with biopsy-proven ACR, 30 stable recipients with normal liver tests, and 30 HCV-infected LT recipients with histologically proven HCV recurrence. The median baseline LS was 5.9 kPa in the mild rejection group, 11 kPa in the moderate/severe group ($p=0.001$), 4.2 kPa in stable recipients ($p=0.02$ versus mild rejection), and 13.6 kPa in patients with recurrent HCV ($p=0.17$ versus moderate/severe rejection). AUROC of LS to discriminate mild versus moderate/severe ACR was 0.92, and a LS cut-off of 8.5 kPa yielded a PPV of 100% to diagnose moderate/severe rejection. LS improved in 7%, 21%, and 64% of patients with moderate/severe rejection at 7, 30, and 90 days (Figure 47).²¹⁵

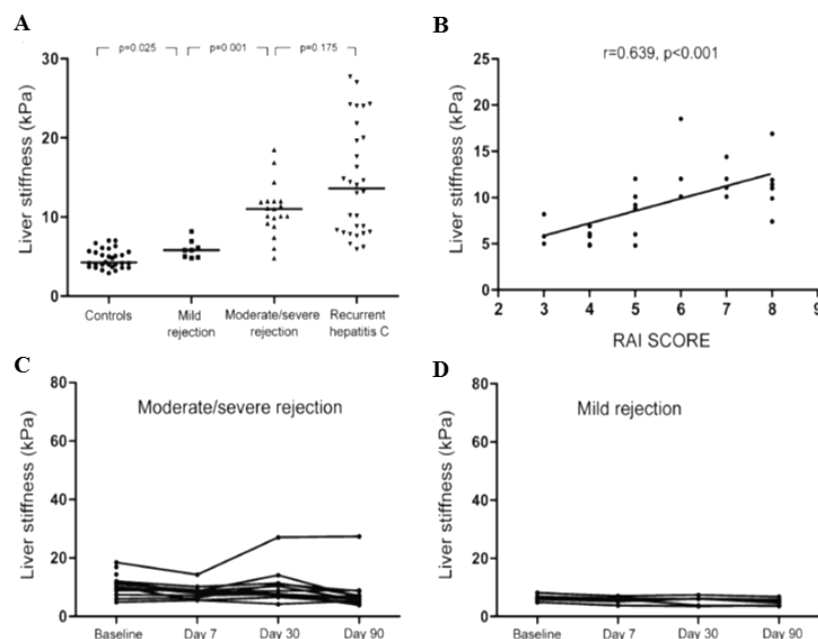


Figure 47: Liver stiffness values in patients without and with acute cellular rejection (A), correlation of liver stiffness with RAI Banff score (B), longitudinal assessment of liver stiffness in patients with rejection (C, D)²¹⁵

Predicting graft disease in liver recipients after LT:

In LT, liver biopsies have been proven to be an indispensable diagnostic and prognostic tool for managing recipients. The studies of long-term LT patients have shown a high prevalence of histological abnormalities in protocol LBs even in the absence of abnormal liver function tests.²¹⁵⁻²¹⁷ In most LT patients, several risk factors are presumably involved in allograft fibrosis progression.²¹⁸⁻²²⁰ For example, in HCV patients, viral re-infection is a major factor contributing to hepatocyte injury. Fibrosis progression in non-HCV patients was attributed to risk factors such as metabolic syndrome, NASH, and history of biliary obstruction, as well as to surgical factors. Additionally, profibrogenic effects of calcineurin inhibitors have been demonstrated both in vitro and in vivo, and may have contributed to fibrosis progression.^{221,222} Many transplant centers perform protocol LBs to assess fibrosis progression to determine the need for specific intervention and for accurate prognostication. However, because LB has several inherent drawbacks owing to its invasiveness, especially when repeated examinations are required, TE might be a good non-invasive but accurate alternative to LB. The clinical utility of TE has been investigated to assess the severity of recurrent HCV infection.²²³⁻²²⁶ HCV recurrence in post-LT patients is nearly universal and has an unpredictable and often accelerated course of disease progression to PH and liver cell failure, vs HCV infection of a native liver.^{227,228} This might explain the more rapid liver fibrosis progression in HCV patients when compared with patients transplanted for other indications. Hepatitis and fibrosis occur in 75–80 and 10–30% of recipients, respectively, at 5 years.^{229,230} Furthermore, cholestatic hepatitis occurs in approximately 10% of LT patients and leads to graft failure and death.²³¹ The presence of significant liver fibrosis at 1 year after transplantation identifies patients at high risk of graft loss.^{232,233} However, antiviral therapy initiated too early has no mortality benefit but often substantial side effects, whereas therapy initiated too late may decrease efficacy and clinical benefits.^{234,235} Therefore, determining the

appropriate timing for commencing antiviral therapy is very important. In this respect, TE results are well correlated with hepatic fibrosis histological scores, although some influence of hepatitis-associated necro-inflammatory activity is observed.^{223,224}

The study by Carrion *et al* reported that AUROC was 0.90 for significant fibrosis and 0.98 for cirrhosis in 124 LT recipients with recurrent HCV infection. Using a cutoff value of 8.5 kPa, the sensitivity, specificity, NPV, and PPV of TE for diagnosing significant liver fibrosis were 90, 81, 79, and 92%, respectively. When using a cutoff value of 12.5 kPa, the sensitivity, specificity, NPV, and PPV for cirrhosis diagnosis were 100, 87, 50, and 100%, respectively.²²⁴ These findings were supported by subsequent studies.^{236,237} In a prospective, longitudinal study of sequential paired examinations using TE and LB in liver graft recipients with recurrent HCV, TE changes over time were dynamically correlated with not only changes in liver fibrosis stage but also with changes in necro-inflammatory activity and the occurrence of complications such as cellular rejection, cholestasis, and *de novo* autoimmune hepatitis.²³⁴ These observations indicate that TE is a reliable predictor of liver graft damage independent of HCV recurrence. However, there remain only limited data about the clinical application of TE in patients undergoing LT for end-stage liver diseases other than HCV.

Beckebaum and his colleagues prospectively assessed the efficacy of TE, biochemical tests, and more complex scores in determining fibrosis stage in 157 patients transplanted for HCV infection or non-HCV-related liver diseases. Although TE performed better in HCV patients than in non-HCV patients, it is still a reliable method of assessing severe fibrosis in HCV patients: the optimal LS cutoffs were 4.7 and 5.0 kPa for $F \geq 1$, 7.1 and 7.3 kPa for $F \geq 2$, 10.9 and 9.9 kPa for $F \geq 3$, and 17.3 and 12.6 kPa for $F = 4$, respectively, in HCV versus non-HCV patients. The corresponding AUROCs for $F \geq 1$, $F \geq 2$, $F \geq 3$, and $F = 4$ were 0.95 and 0.86, 0.89 and 0.85, 0.97 and 0.88, and 0.99 and 0.97, respectively, for HCV versus non-HCV patients.²³⁸ In another prospective study by Rigamonti *et al*, TE proved to be an accurate and

independent predictor of graft damage regardless of the etiology, which was not an unexpected finding because LS was previously shown to correlate not only with hepatic fibrosis but also with necro-inflammatory activity, cholestasis, steatosis, and cellular rejection. They identified two LS cutoffs for diagnosing graft damage: 5.3 kPa with 100% sensitivity and 7.4 kPa with 100% specificity. In patients with LS values ≤ 5.3 kPa, the post-test probability of graft damage fell to 0%, but in patients with LS >7.4 kPa, the post-test probability increased to 100%.²³⁹ Because histological changes are potentially clinically significant (e.g. progressive graft fibrosis), monitoring patients with TE to identify the presence of graft damage may be valuable for early identification of LT patients that require further histological assessment of the graft or modifications of their immunosuppressive therapy regimen.^{240,241}

Crespo *et al* studied 144 HCV–infected and 48 non–HCV)-infected LT recipients and evaluated the prognostic value of TE 1 year after transplantation to predict clinical decompensations and graft and patient survival. In HCV patients, cumulative probabilities of liver decompensation five years after LT were 8% for patients with LSM <8.7 kPa versus 47% for patients with LS ≥ 8.7 kPa ($p<0.001$). Five-year graft and patient cumulative survival were 90% and 92% in patients with LS <8.7 kPa ($p<0.001$) and 63% and 64% in patients with LS ≥ 8.7 kPa ($p<0.001$), respectively (Figure 48). Patients with low LS one year after LT had excellent outcomes independently from receiving antiviral treatment or achieving sustained virological response. In contrast, graft survival significantly improved in patients with LS ≥ 8.7 kPa who achieved sustained response. No association between outcomes and LS at 12 months was observed in non-HCV patients. In conclusion, LS one year after LT is a valuable tool to predict hepatitis C-related outcomes in recurrent hepatitis C and can be used in clinical practice to identify the best candidates for antiviral therapy (Figure 49).²³² Crespo *et al* reported recently that combination of TE with biomarkers (APRI, ELF, and FIB-4) improved

the diagnostic accuracy to detect the patients at higher risk of severe HCV recurrence can be identified early, 6 months after LT.²⁴³

Figure 48: Cumulative probability of clinical decompensation (A) and graft survival (B) according to the presence of a liver stiffness measurement (LSM) cut-off of 8.7 kPa²⁴³

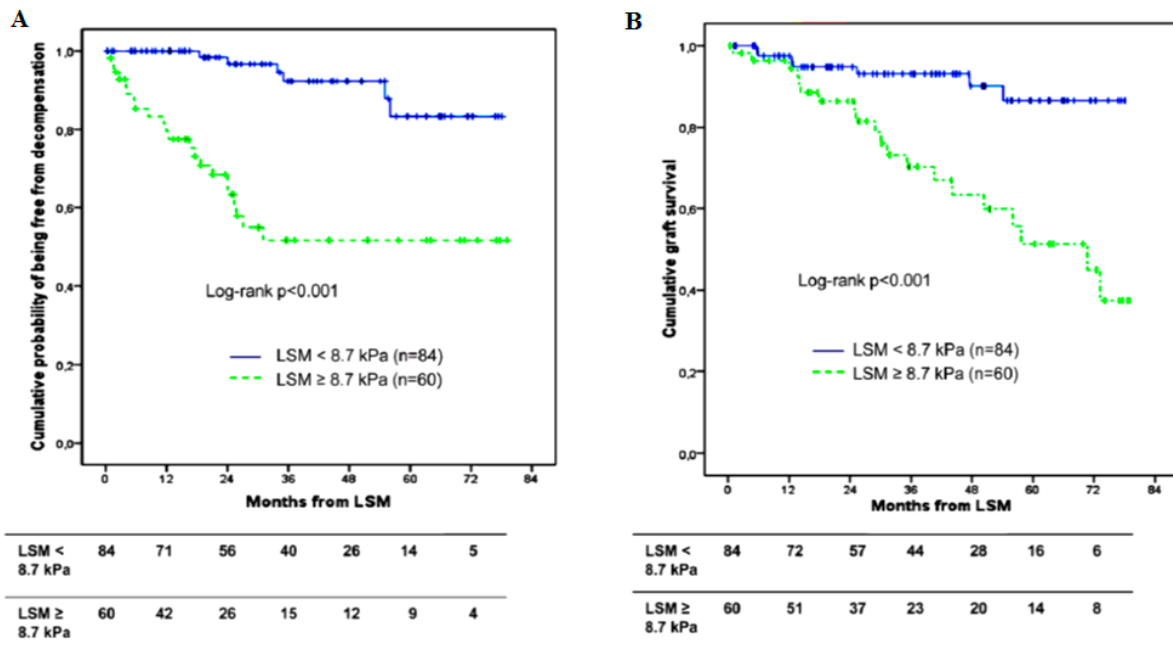
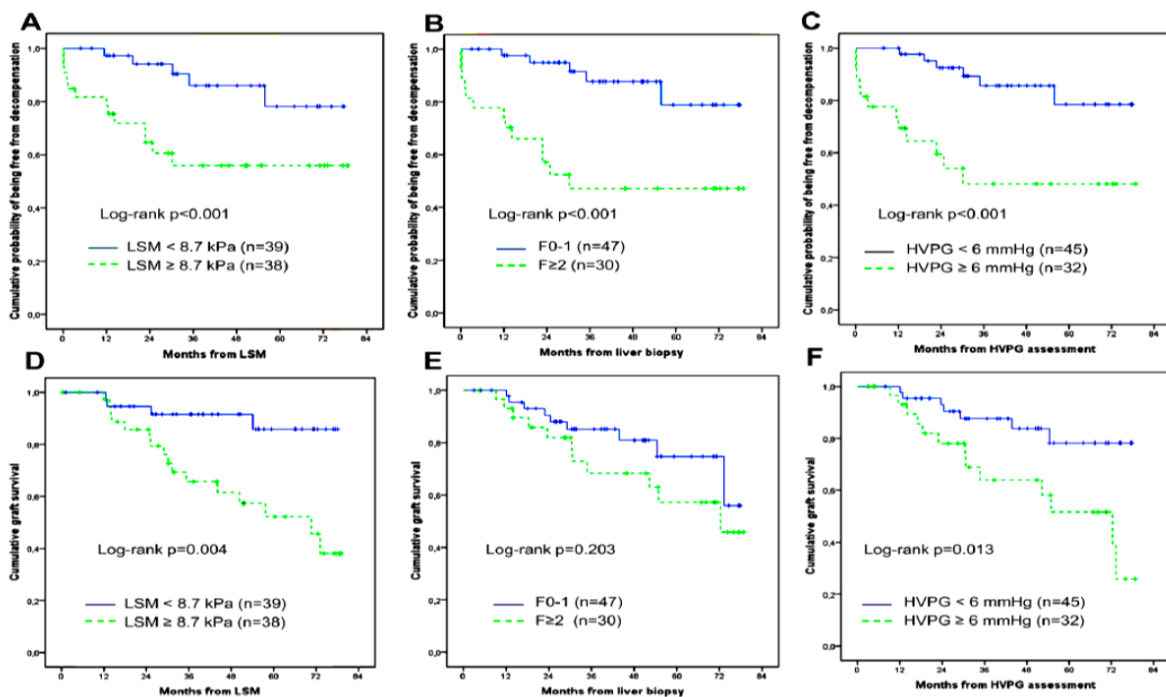


Figure 49: Cumulative probability of clinical decompensation (A–C) and graft survival (D–F) according to the presence of a liver stiffness measurement (LSM) cut-off of 8.7 kPa one year after transplantation (A and D), the presence or absence of significant fibrosis (B and E) and the presence or absence of portal hypertension (C and F).²⁴³



Aims and Objectives

Study Hypothesis:

Surgical resection is the only curative treatment for the patients with either primary or metastatic liver tumors. The volume and the quality of the non-tumoral liver remnant, called functional liver remnant (FLR), limits the extent of hepatectomy. In patients with liver metastasis, either colorectal or non-colorectal in origin, peri-operative chemotherapy is the standard of care. The hepatotoxicity of these regimens vary and various studies have shown that prolonged pre-operative chemotherapy has a negative impact on the post-operative outcomes. Shinodh *et al* proposed that at least 30% FLR to be must be ensured in patients with colorectal liver metastasis and receiving >12 weeks of chemotherapy to avoid post-hepatectomy liver failure (PHLF).¹⁶⁵ In the case of primary liver malignancy, underlying liver disease (hepatic fibrosis, steatosis and portal hypertension) not only determines the post-operative outcomes but also predicts the risk of recurrence at long-term. Further, Faitot *et al* have demonstrated that clinically significant portal hypertension was a factor for progression of hepatocellular carcinoma and poor response to transarterial chemoembolization in patients listed for liver transplantation.¹³² Therefore, evaluation of the quality of FLR and degree of portal hypertension is an important aspect in the management of patients undergoing hepatectomy for various indications. FibroScan[®] is a non-invasive method of assessing the degree of hepatic fibrosis and steatosis by estimating liver stiffness (LS) and controlled attenuation parameter (CAP), respectively. Some studies have demonstrated that LS could predict PHLF with Kim *et al* even suggesting that LS performed better than ICG clearance test.^{173,210} However, the cut-off values vary between these studies and no predictive model has yet been validated. Our hypothesis was that transient elastography-based model would enable us to predict post-operative outcomes especially 90-day morbi-mortality in patients undergoing hepatectomy for various indications.

Primary Objective:

- To develop and validate a statistical model using LS and/or CAP along with other pre-operative parameters to predict 90-day severe morbidity in patients undergoing hepatectomy for various hepatobiliary diseases.

Secondary Objectives:

- To validate the diagnostic performance of LS to accurately classify the degree of fibrosis
- To evaluate the ability of CAP to diagnose different grades of hepatic steatosis
- Quantification of hepatic steatosis and fibrosis by infrared spectrometry and morphometry, respectively
- Correlation of LS with portal pressure and hepatic venous pressure gradient measured intra-operatively
- To test the ability of LS to predict the risk of dropout due to HCC progression while waiting in the list for liver transplantation
- To develop and test a prototype of FibroScan[®] in situ to measure LS and CAP directly on the surface of liver

Study Endpoints:

- Dindo Clavien classification of surgical complications and comprehensive complication index would be utilized to grade and classify post-operative complications. 90-day severe morbidity, defined as >Grade 2 complications according Dindo Clavien classification was the primary endpoint
- PHLF was defined according to 50-50 criteria and/or peak total bilirubin >120 $\mu\text{mol/L}$
- Presence of ascites, jaundice, and/or encephalopathy would constitute hepatic decompensation and persistence of hepatic decompensation beyond 90-day post-operative period would be called persistent hepatic decompensation
- METAVIR scoring system and NAS score were utilized to grade hepatic fibrosis and steatosis, respectively.
- Clinically significant portal hypertension was defined by hepatic venous pressure gradient ≥ 10 mmHg
- Overall survival and disease-free survival after hepatectomy for HCC
- Dropout rate from the waiting list for LT
- Histological features of HCC

Patients and Methods

My PhD research consisted of three main fields of interest namely, liver resection, liver transplantation and liver machine perfusion.

Part 1: Hepatectomy for liver tumors

All patients who were planned to undergo hepatectomy were recruited prospectively from October 2014 until August 2016 after informed consent. Transient elastography was performed pre-operatively within two weeks of planned hepatectomy under fasting condition using FibroScan[®] Touch 502 standard device with M or XL probes. Similarly, all patients underwent indocyanine green clearance test before hepatectomy to assess the functional reserve of liver. A systematic and exhaustive collection of all pre-operative patient's clinical data including tumor characteristics, LS, CAP, routine blood tests, intra-operative parameters, and post-operative outcomes up to three months were prospectively registered into an online database (https://hpbchir.iplesp.upmc.fr/ehpbchir/gestion_site/).

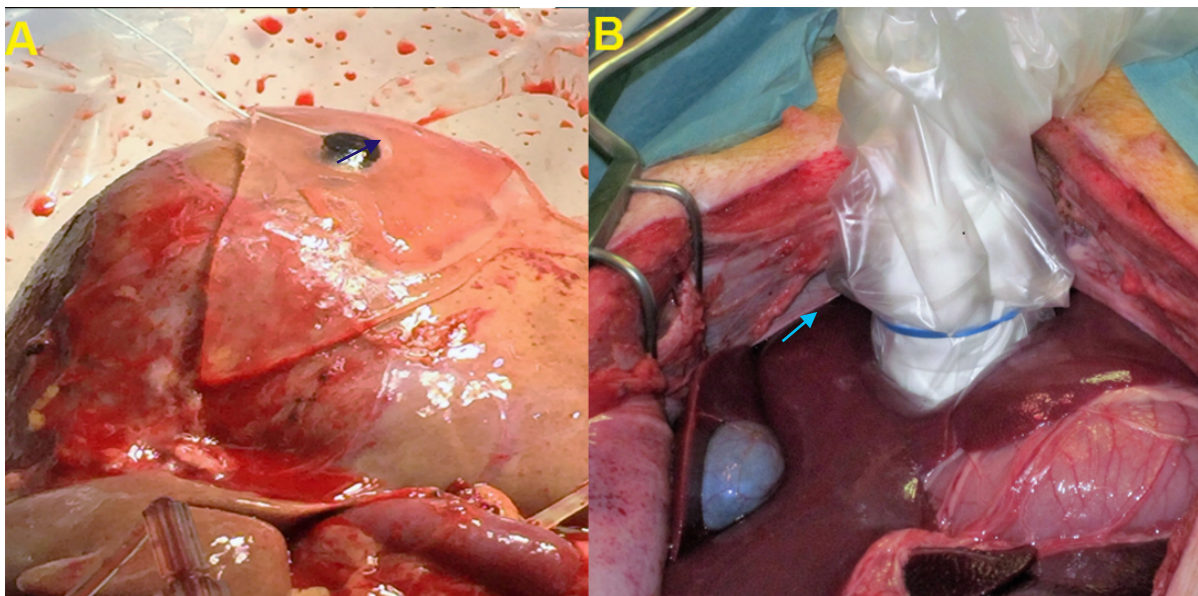
Part 2: Liver transplantation

All patients who were planned to undergo LT were recruited prospectively from January 2015 until December 2016 after informed consent. At the time of inscription into the LT waiting list, all patients underwent TE using FibroScan[®] Touch 502 standard device with M or XL probes under fasting condition. A prospective systematic collection of all pre-operative patient's clinical data including tumor characteristics, LS, CAP, routine blood tests was performed and the patients were followed up until transplantation or dropout from the waiting list due tumor progression or death. A web-based database (https://hpbchir.iplesp.upmc.fr/transpchir/gestion_site/) was utilized to collect data.

Part 3: FibroScan in situ

Echosens™ is developing a new prototype of FibroScan® capable of measuring LS and CAP directly on the liver surface. This prototype was tested during normothermic machine perfusion of steatotic liver graft that was not utilized for LT and CAP was measured continuously in real time. The second trial of the prototype was performed in an animal model (white swine) and both LS and CAP were assessed directly on the surface of the liver.

Figure 50: Transient elastography by FibroScan® *in situ* directly on the surface of liver



A-Liver machine perfusion; B-Porcine liver *in situ* (Courtesy of Echosens™, Paris, France)

Transient Elastography:

In all patients, TE was performed pre-operatively in fasting condition using FibroScan® Touch 502 with M or XL probes. A single operator blinded to the patient's clinical details performed all the TE. Liver stiffness was measured on the right lobe of the liver through intercostal spaces by placing the probe on the skin between the ribs while the patient was lying supine with the right arm in maximal abduction. A liver portion of at least 6 cm thick, free of large vascular structures and tumor, was located by an ultrasonic time-motion image and at least ten successful measurements were performed on each patient.

Median of ≥ 10 valid measurements was considered as LS expressed in kilopascal (kPa). The validity criteria are summarized in the Table 28.

Table 28: Criteria for liver stiffness measurement by transient elastography^{67,74}

Liver Stiffness Evaluation and Definitions
What is LS? LS = Median of ≥ 10 successful stiffness measurements with a range of 1.5-75 kPa
What is TE failure? When no LS measurements are obtained with 10 attempts
How is the success rate calculated? Success rate = number of successful measurements/total number of attempts and expressed in %
What are LS validity criteria? A valid LS must have the following three criteria: <ul style="list-style-type: none"> ▪ ≥ 10 successful measurements at a single site ▪ Success rate of $\geq 60\%$ ▪ LS < 7.1 irrespective of IQR/M ; LS ≥ 7.1 kPa if IQR/M < 0.30 An invalid LS is when one or more of the criteria are not fulfilled ≥ 10 valid LS measurements under supervision is needed to practice TE independently

Indocyanine green clearance test:

An intravenous bolus of 0.5 mg/kg of patient’s body weight was injected into a large vein in an arm and the blood samples were collected from the other arm every four minutes from time 0 until 16 minutes. ICG-R15 was the ratio between ICG concentration 15 min after injection and initial concentration (normal range, 0%-10%). ICG clearance was the volume of plasma entirely cleared off ICG per unit time with a normal range of 6-15 mL/min/kg. ICG plasma disappearance rate was the percentage change over time of the reduction of ICG blood concentration starting from a concentration of 100% (normal range, >18-24% per minute).¹⁸³

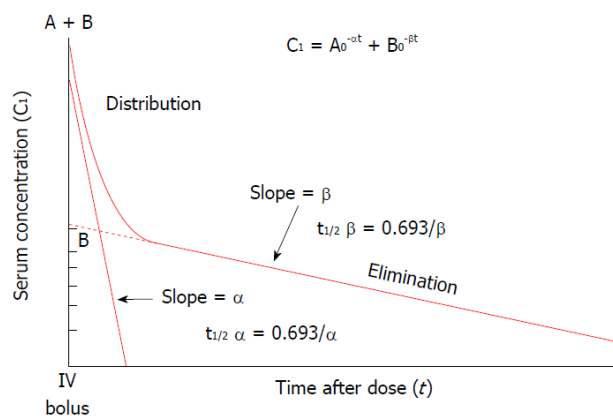
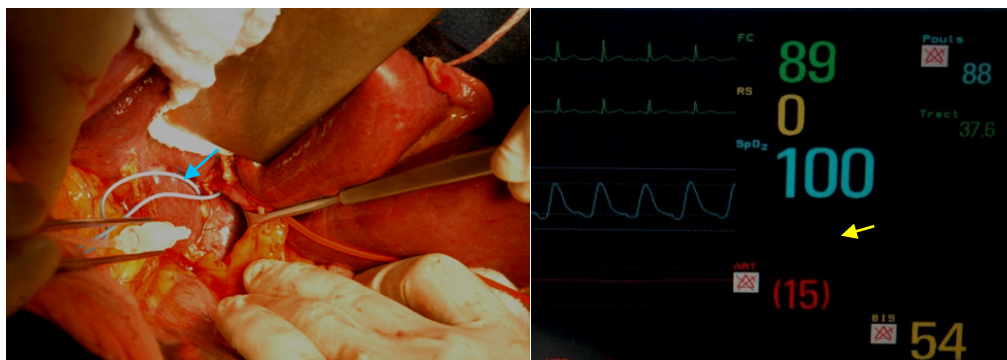


Figure 51: Schematic representation of indocyanine green kinetics¹⁸³

Hepatic Venous Pressure Gradient:

Intra-operative measurement of HVPG was performed by direct puncture of portal vein in the hepatic pedicle and infra-hepatic inferior vena cava using a 25-gauge needle connected to a manometer with the patient in supine neutral position and after zeroing to the atmospheric pressure at the level of heart. HVPG was obtained by calculating the difference between the measured portal and hepatic venous pressures. This technique was feasible only during laparotomy and was performed at the discretion of individual surgeon before the commencement of hepatectomy/transplantation. In our center, there was no protocol to measure HVPG in all patients.²⁴⁴

Figure 52: Intra-operative measurement of portal and vena caval pressure by direct puncture of portal vein and inferior vena cava



Liver Resection:

The objective of hepatectomy was to resect all detectable lesions with tumor-free margins except in case of benign tumors.

Colorectal liver metastasis:

Once the radiological diagnosis of liver metastasis was confirmed in the tumor board by a multidisciplinary team consisting of surgeons, oncologists, and radiologists, our policy was to propose potentially curative resections of all metastatic sites with combination chemotherapy (CTx) ± biologic agents. Response to CTx was evaluated every 2-3 months

according to response evaluation criteria in solid tumors criteria and with serum tumor markers: carcinoembryonic antigen and carbohydrate antigen 19-9. Surgery was considered when the overall strategy could eradicate both hepatic and extra-hepatic disease. Disease progression during CTx was considered as a relative contra-indication to curative surgery. Staged hepatic and EHD resections were performed with CTx during this interval between two operations to prevent disease progression.²⁴⁵

When downsizing by CTx would not be sufficient to allow curative resection owing to contact with major vessel or bile duct, three specific techniques were practiced to increase resectability. Portal vein embolization (PVE) was performed when estimated FLR was <30%.²⁴⁶ For bilateral metastases, hemi-hepatectomy would be combined with the use of radiofrequency ablation for few contralateral, unresectable, deeply located lesions ≤ 3 cm in diameter. When multinodular bi-lobar metastases could neither be completely resected by a single procedure nor treated with hepatectomy combined with ablation, two-stage hepatectomy was considered.²⁴⁷ Liver-first strategy was used in certain cases of synchronous presentation when colorectal primary tumor was not complicated and hepatic tumor burden was significant.²⁴⁸ Post-operative follow-up was at one month and then every four to six months with physical examination, serum tumor markers, and imaging. Chemotherapy was offered post-operatively for 6-8 cycles to decrease the risk of recurrence. In case of local or distant recurrence, resection was performed if it could be curative.²⁴⁹

Hepatocellular carcinoma:

Pre-operative evaluation consisted of laboratory tests including liver function tests, serum tumor markers (AFP), imaging studies, ICG-R15, and LS but it did not include HVPG measurement. Usually hepatectomy was performed when model for end-stage liver disease (MELD) ≤ 12 , platelet count $\geq 80,000$ and ICG-R15 $\leq 20\%$. When the FLR would not be sufficient, PVE was performed to ensure FLR $\geq 0.5\%$ of body weight or $\geq 20\%$ of standard

liver volume in non-cirrhotic liver.¹⁶⁵ Transarterial chemoembolization followed by PVE was the treatment strategy when planning major hepatectomy in patients with cirrhosis.²⁵⁰ The technique of liver resection for HCC has been standardized in our unit since 1982.^{251,252} The objective of hepatectomy was to resect all detectable lesions with tumor-free margins of ≥ 1 cm while leaving behind adequate liver parenchyma to ensure normal liver function. At surgery, exploration of the abdominal cavity is performed to detect extrahepatic spread. Systematic intra-operative liver ultrasound is carried out. Enlarged hepatic lymph nodes and any suspicious nodules are excised for frozen section histology. In general, anatomic resections were preferred to wedge resections. Parenchymal dissection is done using the ultrasonic dissector (Cavitron Ultrasonic Aspirator, Valley Lab Inc., Boulder, USA), and resections are usually performed under intermittent clamping of the portal triad. After LR, serial follow-up of patients were provided to monitor the post-operative complications and HCC recurrence. A contrast-enhanced abdominal computer tomography (CECT) was performed at post-operative 1-week and 1-month and then alternating abdominal ultrasonography and CECT were performed every four months along with serum AFP and liver function tests.

Liver transplantation:

Donor liver procurement and transplantation were done using our standard techniques.²⁵³⁻²⁵⁶ Preservation of the donor livers was done using the IGL1[®] (Waters medical systems, USA), Celsior[®] (Genzyme Polyclonais S.A.S., France) or Scot15[®] (Macopharma, France) solutions. The site of anastomosis depended on the number, size and quality of the donor and recipient arteries. The arterial anastomosis was fashioned under a surgical loop with a running 7-0 or 8-0 polypropylene suture between the common hepatic artery (CHA) of the graft (using a patch created with the origin of the gastroduodenal artery (GDA) or splenic artery) and the CHA of the recipient at the origin of the GDA. When the recipient's HA was

not suitable, the arterial anastomosis was fashioned on the splenic artery or the aorta. If the graft artery was too short, a donor iliac artery graft was used as an inter-positional conduit on the supra- or infra-renal aorta. In the absence of an iliac artery graft, 8-mm Gore-Tex[®] prosthesis was used. Right accessory branches to the graft arising from the superior mesenteric artery were reconstructed with an end-to-end anastomosis between the proximal stump of the coeliac artery and the proximal stump of the superior mesenteric artery of the graft. In this situation, the distal stump of the superior mesenteric artery was anastomosed onto the recipient artery. Arterial anastomosis was done using a separated 8-0 polypropylene suture under a surgical microscope if the diameter of the arterial graft was smaller than 3 mm, and notably in the case of living donors or split transplants. Flows in the arterial, portal and hepatic veins were systematically checked by DUS during the surgical procedure.²⁵⁷

All patients were transferred to the intensive care unit after LT. HA flow was assessed daily DUS and resistive index was between 0.4 and 0.8 was considered normal.^{258,259} All patients received triple immunosuppressive regimen including corticosteroids, mycophenolate mofetil and tacrolimus or basiliximab in patients with renal dysfunction. Peri-operative prophylactic antibiotics were administered in all patients. CT angiography was performed between post-operative days (POD) 7-10 or when HA flow abnormalities was present in DUS. In the absence of any haemorrhage and/or low platelet count <50,000/ml, all patients received heparin from POD 1 to maintain an activated partial thromboplastin time at 1.5-2.0 fold the baseline value and later substituted with prophylactic low-molecular weight heparin. Acetylsalicylic acid (250 mg /day) was introduced as early as possible in case of a small artery (<5 mm).²⁶⁰

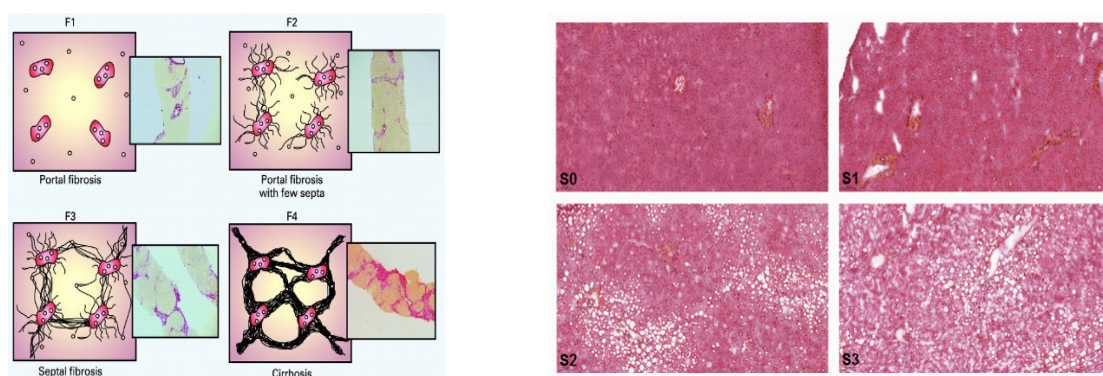
After discharge from the hospital, the patients were followed up every 15 days during the first two months, and then every 3 months. Follow-up consisted of a physical examination, liver function tests and DUS. CT was performed in the event of a drop in the arterial resistive

index and/or biological abnormalities, in order to assess HA patency. Immunosuppression was maintained on prednisolone, tacrolimus along with mycophenolate mofetil. Serum tacrolimus level was kept at 10-15 ng/mL for the first 2 weeks then maintained at 5-10 ng/ml. Serum cyclosporine level was kept at 200-300 ng/mL for the first 3 months then maintained at 100-200 ng/mL.²⁶⁰

Histopathological evaluation of surgical specimen:

Two expert pathologist, blinded to patients' clinical data, evaluated hematoxylin and eosin (H&E) stained sections of the tumor and non-tumoral explant liver from hepatectomy and LT. Macroscopic and microscopic tumor characteristics studied included number, size, percentage of necrosis, tumor-free margin, differentiation, presence of satellite nodules, macro/microscopic vascular invasion, and capsule around the tumor. Non-tumoral liver distant from the resected tumor was assessed for fibrosis and steatosis. Fibrosis was graded according to METAVIR scoring system as shown in the Figure 53A.¹⁵ Similarly, hepatic steatosis was classified by NAS steatosis grades (Figure 53B).⁹²

Figure 53: METAVIR scoring system for hepatic fibrosis and NAS grades of hepatic steatosis

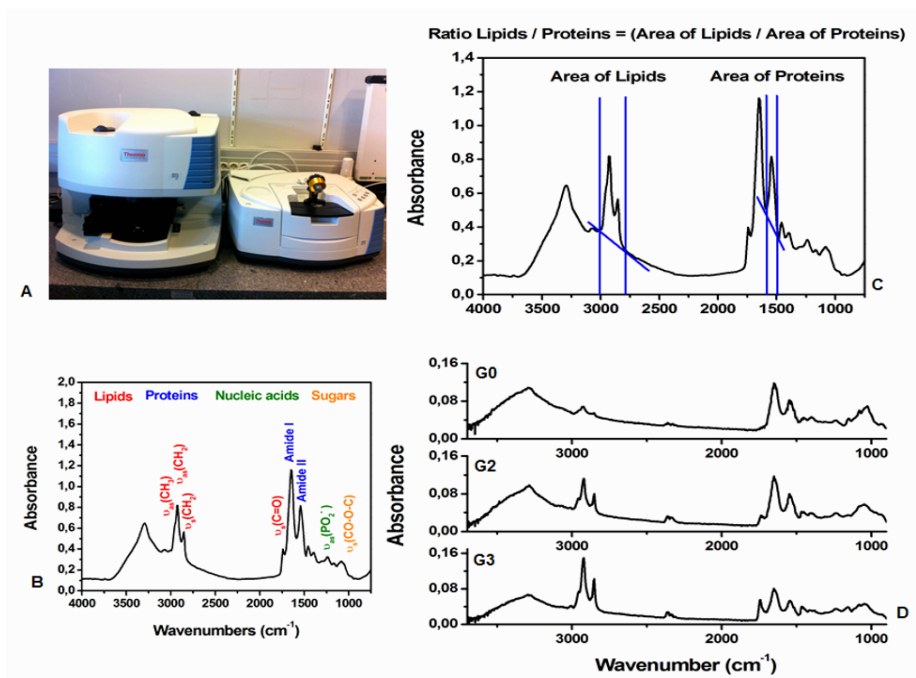


Fourier transform infrared spectroscopy:

Fourier transform infrared spectroscopy (FTIR) is based on the determination of absorption of infrared light due to resonance with vibrational motions of functional molecular

groups. A frequency will be strongly absorbed if its photon energy coincides with the vibrational energy levels of the molecule. Therefore, FTIR is a very powerful technique that provides fingerprint information on the chemical composition of the tissue sample and all the absorptions bands of lipids, proteins and sugars are seen within the range of IR spectrum (Figure 54B and C). A frozen tissue section was deposited on a regular glass slide and the tissue's IR spectrum was recorded using A-ATR- FTIR spectrometer connected to Nicolet™ iN10 infrared microscope (Figure 54A). The acquired IR spectrum thus obtained would correspond to an average spectrum of an area 2 x 2 mm² of the tissue section. The quantification of the triglyceride (TG) content was then addressed from the acquired spectrum by calculating the ratio of integrated intensity of bands attributed to lipids (2800-3100 cm⁻¹) relative to proteins (Amide II: 1485-1595 cm⁻¹) as illustrated in Figure 54 B and C and expressed in nmol/mg.

Figure 54: Infrared spectroscopic grading of hepatic triglyceride content



As shown in Figure 54D, with the increasing level of steatosis, the intensity of CH₂ and CH₃ vibrational modes attributed to lipids significantly increased (3000-2800 cm⁻¹),

whereas the vibrational modes attributed to proteins (Amide I and Amide II: 1485-1710 cm^{-1}) were not significantly affected by steatosis. Although not significant, a small decrease of absorption bands attributed to proteins was however evident. Additionally the noticeable increase of the absorption band corresponding to ester was detected when higher levels of steatosis were detectable within the tissue.

Morphometry:

Computerized morphometric analysis was used for the quantitative assessment of fibrosis in the liver biopsies by an independent investigator who was blinded to the histologic score of the biopsies. Liver sections stained with 0.1% Picrosirius red for collagen were scanned to obtain high quality images using a computerized image analysis system composed of a photomicroscope and digital camera (Figure 55). We determined the areas of collagen in different zones of the liver by morphometric analysis. The areas of fibrosis or collagen were quantified in multiple random fields on the captured digitalized images. Artifacts (folds, dust) were then manually removed. Lumens, sinusoids, and any other parts of the liver tissue that contained no collagen were excluded from the measurement. The mean areas of global fibrosis were calculated in percentage for further correlation with METAVIR fibrosis score and LS.

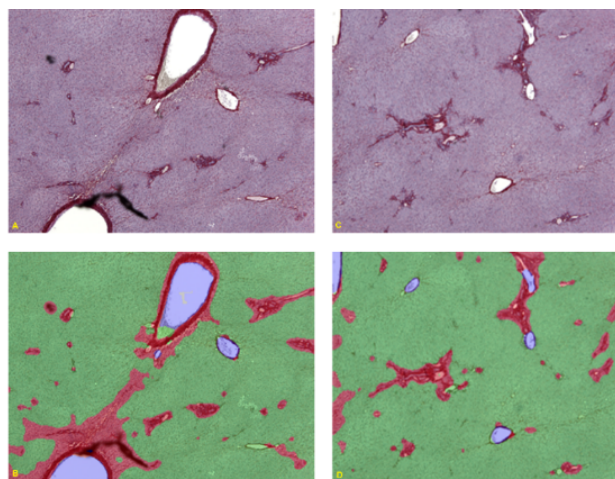


Figure 55: Digital morphometric quantification of hepatic fibrosis

Statistical analysis:

Categorical variables are reported as counts (percentage) and continuous variables as median (IQR). Chi square, Fischer exact, Wilcoxon or Mann Whitney U tests were utilized to compare patient demographics, tumor characteristics, and peri-operative outcomes between two groups. Bivariate correlation were performed with Spearman and Pearson tests. Potential predictors for a binary outcome were identified by Wilcoxon test, Mann Whitney U test, or univariate logistic regression and those variables that were significant at 5-20% in the univariate analysis were entered into multivariate binary logistic regression (Backward elimination method of Wald) to determine independent predictors. The discriminative performances of LS, CAP, and predictive models were analyzed by receiver operating characteristic (ROC) with the area under the ROC curve (AUROC) as the main readout. Calibration plot and Heat map fit of the predicted probabilities were utilized to assess and compare the Goodness of fit for a predictive model (Hosmer–Lemeshow test). The ideal fit was a 45-degree line and the shading of the plotted line indicated the degree to which fit deviations are larger than expected due to sampling variation. Cox proportional hazard model was performed to identify independent predictors of overall and disease-free survival. SPSS 21.0 package (SPSS Inc., Chicago, IL) and R version 3.2.5 (<https://www.r-project.org>) were utilized to perform the analysis. p value <0.05 was considered significant.

Results

Hepatic Fibrosis

Hepatic Steatosis

Article 1:

Title: Prospective validation of transient elastography for staging liver fibrosis in patients undergoing hepatectomy and liver transplantation

Authors :

1. Muthukumarassamy Rajakannu^{1,2,3}
2. Audrey Coilly^{1,2,3,4}
3. René Adam^{1,3,5}
4. Didier Samuel^{1,2,3,4}
5. Eric Vibert^{1,2,3,4}

Affiliations :

¹Inserm Unité UMR-S1193, Villejuif, F-94800, France

²Université Paris-Sud, Villejuif, F-94800, France

³AP-HP, Hôpital Paul-Brousse, Centre Hépatobiliaire, Villejuif, F-94800, France

⁴DHU Hepatinov, Villejuif, F-94800, France

⁵Inserm Unité UMR-S776, Villejuif, F-94800, France

Author's Contributions :

- Muthukumarassamy Rajakannu – Design of the study, data collection, data analysis and interpretation and drafting the article.
- Audrey Coilly – Drafting and critical revision of the manuscript.
- René Adam – Critical revision and final approval of the manuscript for submission.
- Didier Samuel – Critical revision and final approval of the manuscript for submission.
- Eric Vibert – Conception of the study, critical revision and final approval of the manuscript for submission.

Financial support:

Muthukumarassamy Rajakannu is a PhD student at Université Paris Sud. L'Association Nationale de la Recherche et de la Technologie, Paris, France and EchosensTM, Paris, France, funded his work.

Corresponding Author:

Pr. Eric Vibert MD, PhD

Centre Hépatobiliaire,

AP-HP Hôpital Paul Brousse,

12 Avenue Paul Vaillant Couturier,
Villejuif,
France.
Tel : +33 1 45 59 30 36,
Fax : +33 1 45 59 38 57,
Email : eric.vibert.pbr@gmail.com

Letter to the Editor:

Prospective validation of transient elastography for staging liver fibrosis in patients undergoing hepatectomy and liver transplantation

To the Editor,

We read with great interest the recent review by Berzigotti in which she confirms the validity of liver stiffness measurement (LSM) by transient elastography (TE) to diagnose accurately compensated advanced chronic liver disease (cACLD) and predict clinically significant portal hypertension [1]. Recently, the Baveno VI consensus and European association for the study of the liver guidelines have proposed LSM cutoff of 10 kPa to rule in cACLD [2,3]. LSM <10 kPa would exclude cACLD, and LSM >15 kPa would be highly indicative of cirrhosis; for results between these two points, additional work-up would be needed [4,5]. The performance of TE has been well validated in viral hepatitis but less so in other chronic liver diseases such as non-alcoholic fatty liver disease [1-5]. Proposed cut-offs for a given endpoint vary (for instance, 10.3 - 22.3 kPa in cirrhosis) among different studies due to differences in the study design, histological scoring system used, and heterogeneous nature of the study population [1,2,5,6]. The other determinants of LSM were the presence of space-occupying lesions, cholestasis, inflammation and obesity [1].

We performed a prospective monocentric study in order to assess the utility of TE in the management of patients undergoing hepatectomy or liver transplantation. The secondary

endpoints were to evaluate the discriminatory performance of LSM and to test robustness of Baveno VI criteria of 10 kPa to rule in cACLD in a mixed cohort, including various aetiologies especially alcoholic and non-alcoholic fatty liver diseases cohort with varying degrees of liver fibrosis. TE of non-tumoral liver was performed using FibroScan[®] Touch 502 Standard device with M or XL probes in all patients prospectively before surgery after obtaining informed consent. Four hundred and ten patients (63.7% men) with varying degrees of underlying liver disease [liver tumors without liver disease (53.7%), viral (14.9%), alcohol (12.9%), non-alcoholic fatty liver disease (7.3%), and others (11.2%)] were enrolled with valid LSM. All TE measurements were compared with the histological assessment of the resected liver specimen for fibrosis stage evaluated by two expert pathologists using METAVIR scoring system.[7] An optimal cut-off to differentiate the different stages of fibrosis was determined by receiver operating characteristic (ROC) analysis of LSM and METAVIR stages of fibrosis. TE was a reliable method for diagnosing different stage of fibrosis with area under ROC curve (AUROC) varying from 0.85 for F1 stage to 0.97 for cirrhosis [Table 1]. The performance of TE improved as the stage of fibrosis increased. In accordance with Baveno VI criteria, a cut-off of 10 kPa would be an accurate cut-off to rule in cACLD [AUROC: 0.95; 95%CI:0.923-0.973; sensitivity: 86.1%; specificity: 90.1%]. LSM ≥ 15 kPa would be an accurate cut-off to rule in and rule out cirrhosis with 94.5% positive and 91.4% negative predictive values. In earlier stages of fibrosis, TE seemed to have a better capacity to rule in with a cut-off of 6 kPa for $F \geq 1$ and 8 kPa for $F \geq 2$ stages with about 90% positive predictive value.

The reliability of TE was further analyzed in patients with histology-proven steatosis $\geq 34\%$ [N=34] and in obese patients with body mass index (BMI) ≥ 30 [N=73]. Although there was a trend towards better performance of TE in patients without significant steatosis, the difference was not statistically significant. AUROC for patients without and with steatosis $\geq 34\%$

respectively were 0.95[95% CI: 0.922-0.975] and 0.95[95% CI: 0.867-1.0] in $F \geq 3$ and 0.97[95% CI: 0.943-0.991] and 0.95[95% CI: 0.890-1.0] in $F=4$. The cut-off of 10 kPa would diagnose cACLD with >90% specificity in these two groups. With respect to obese patients, the use of both M and XL probes ensured similar performance of TE when compared to non-obese patients. AUROC in patients with BMI <30 and BMI ≥ 30 respectively were 0.95[95% CI: 0.927-0.979] and 0.93[95% CI: 0.836-0.998] in $F \geq 3$ and 0.96 [95%CI: 0.926-0.985] and 0.99[95% CI: 0.985-1.0] in $F=4$.

This study prospectively validated, in explant liver after surgery, the Baveno VI criteria for non-invasive diagnosis of cACLD and cirrhosis using LSM not only in virus-related liver disease but also in other aetiologies. The performance of TE in obese patients and in steatotic liver was also confirmed in this study. Our experience is unique as patients with varying aetiologies and varying degrees of liver fibrosis were included and the cut-offs determined could be extrapolated in wide range of clinical situations.

Acknowledgements : L'Association Nationale de la Recherche et de la Technologie, Paris, France and EchosensTM, Paris, France, funded this research.

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Muthukumarassamy Rajakannu^{1,2,3}

Audrey Coilly^{1,2,3,4}

René Adam^{1,3,5}

Didier Samuel^{1,2,3,4}

Eric Vibert^{1,2,3,4*}

¹Inserm Unité UMR-S1193, Villejuif, F-94800, France

²Université Paris-Sud, Villejuif, F-94800, France

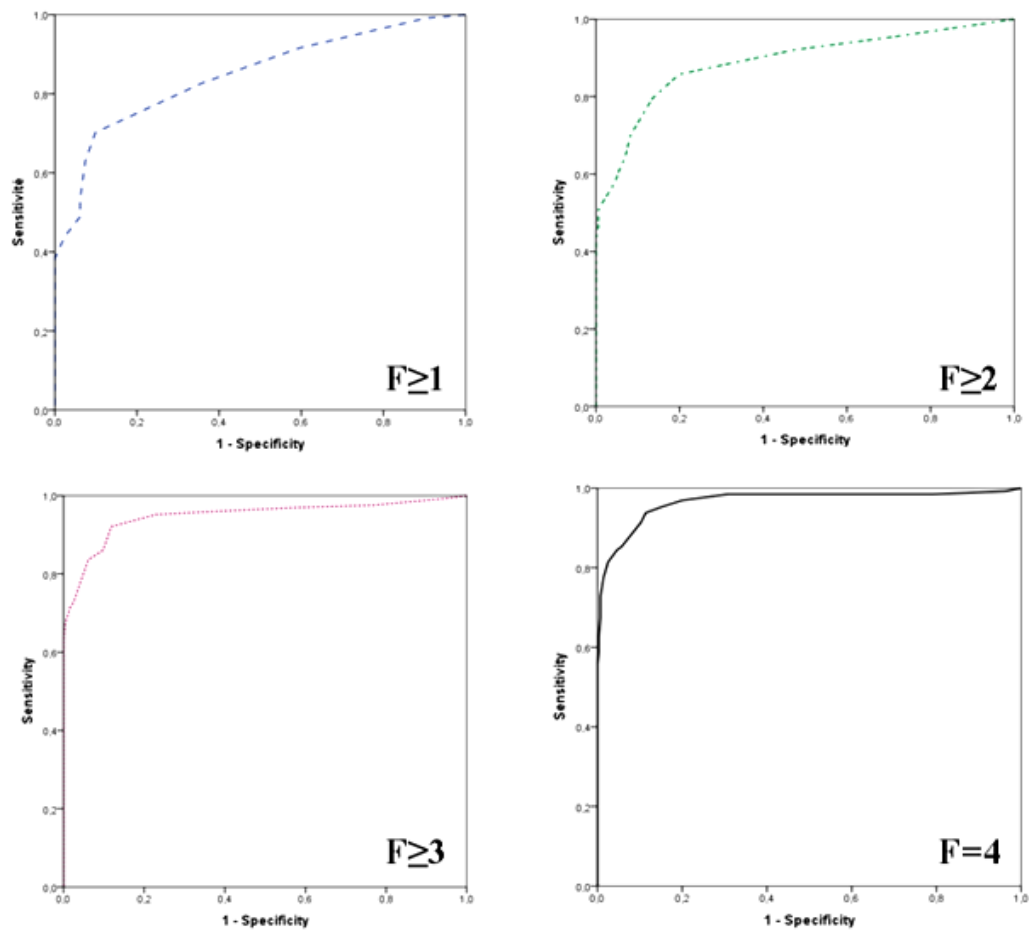
³AP-HP, Hôpital Paul-Brousse, Centre Hépatobiliaire, Villejuif, F-94800, France

⁴DHU Hépatinov, Villejuif, F-94800, France

⁵Inserm Unité UMR-S776, Villejuif, F-94800, France

*Corresponding Author: Centre Hépatobiliaire, AP-HP Hôpital Paul Brousse, 12 Avenue Paul Vaillant Couturier, Villejuif, France. Tel : +33 1 45 59 30 36, Fax : +33 1 45 59 38 57, Email : eric.vibert.pbr@gmail.com

Figure: Diagnostic performance of liver stiffness measured by transient elastography according to the METAVIR fibrosis grades in 410 patients



Metavir Score	Prevalence N (%)	Area Under ROC	95% CI	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	LR+ %	LR- %
F ≥ 1	329 (80.2)	0.85	0.812 - 0.891	6*	82.6	65.0	92.7	45.5	2.4	0.3
F ≥ 2	239 (58.3)	0.89	0.858 - 0.921	8*	79.5	86.4	90.8	71.5	5.9	0.2
F ≥ 3	165 (40.2)	0.95	0.923 - 0.973	10*	86.1	90.1	86.9	89.6	8.7	0.2
F = 4	129 (31.5)	0.97	0.944 - 0.988	15*	81.4	97.5	94.5	91.4	32.6	0.2

p < 0.0001 for all fibrosis stages; ROC-receiver operating characteristic curve; CI-confidence interval; PPV-positive predictive value; NPV-negative predictive value; LR-likelihood ratio; *Cut-off at 80% sensitivity; †Bayesian VI criteria

Article 2: Quantification of hepatic steatosis in patients undergoing liver resection: A comparative study of preoperative controlled attenuation parameter versus Fourier transform-infrared spectroscopy and histopathological estimation on surgical specimens

Authors:

Muthukumarassamy Rajakannu^{#1,2,3} MBBS, MS, DNB, MRCS, Slavka Kaščáková^{#2,3} PhD, Sarah Goodband², Astrid Laurent-Bellue^{1,2,3} MD, René Adam^{1,3,4} MD, PhD, Denis Castaing^{1,2,3} MD, Daniel Cherqui,^{1,3} MD, Antonio Sa Cunha^{1,3} MD, Didier Samuel,^{1,2,3} MD, PhD, François Le Naour^{2,3} PhD, Catherine Guettier,^{1,2,3} MD, PhD, Eric Vibert,^{1,2,3} MD, PhD

Affiliations :

¹Centre Hépato-Biliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

Running title:

Keywords:

Correspondance :

Prof. Eric Vibert

Centre Hépato-Biliaire,

AH-HP Hôpital Paul Brousse,

12 Avenue Paul Vaillant Couturier,

94800 Villejuif, France

[#]Authors Muthukumarassamy Rajakannu and Slavka Kaščáková have contributed equally to the study

Abstract

Background: Controlled attenuation parameter (CAP) measured by transient elastography (TE) is a non-invasive method for hepatic steatosis (HS) assessment. Fourier transform infrared spectroscopy (FTIR) for hepatic triglyceride (TG) content is a new standard for tissue quantification of HS as it avoids the variations associated with conventional diagnosis of non-alcoholic fatty liver disease activity (NAS) steatosis grades by histopathological examination.

Methods: Patients undergoing hepatectomy or liver transplantation were enrolled prospectively to undergo TE by FibroScan™ M/XL probes. Histological NAS grading by an expert pathologist and measurement of TG content using Nicolet™ iN10 FTIR spectrometer were performed on the frozen samples of resected non-tumoral liver.

Results: Eighty-three patients [56.6% men] with median age of 60 years were included. Indications for hepatectomy were 54.2% liver metastasis, 39.8% primary liver cancer and 6% others. CAP had a significant correlation with both reference methods, CAP vs FTIR [$r=0.43$] and CAP vs HPE [$r=0.36$]. FTIR had an excellent correlation with HPE [$r=0.7$]. Area under ROC curve (AUROC) for CAP vs NAS grades of steatosis were 0.65[95%CI:0.5-0.8] for $S \geq 1$, 0.70[95%CI:0.6-0.8] for $S \geq 2$ and 0.77[95%CI:0.7-0.9] for $S3$. Similarly, AUROC for CAP vs FTIR grades were 0.70[95%CI:0.6-0.8] for $G \geq 1$, 0.75[95%CI:0.6-0.9] for $G \geq 2$ and 0.79 [95%CI:0.6-0.9] for $G3$. When FTIR grades was reference, CAP demonstrated better a discriminatory performance for diagnosing HS and significant steatosis.

Conclusion: CAP is a promising tool for noninvasive screening of HS. FTIR is an objective quantitative measurement of HS on tissue sections compared to HPE. Therefore, FTIR grades should be considered as a reference standard for HS quantification by CAP in clinical studies.

Introduction

Diagnosis and quantification of hepatic steatosis (HS) has gained considerable importance in the last decade because of the exponential increase in the incidence of obesity, metabolic syndrome and associated non-alcoholic fatty liver disease (NAFLD).¹ In developed countries, NAFLD *per se* has become the most common cause of chronic liver disease (CLD) and its complications such as steatohepatitis, hepatocellular carcinoma and end-stage liver disease with an estimated prevalence of 20-30% in general population, up to 70% and 90% in diabetic patients and obese individuals, respectively.²⁻⁴ Moreover, diagnosis of HS is an important aspect in the management of patients with CLD as it is an important co-factor for disease progression and non-response to antiviral treatment.^{5,6} Further, HS is an important risk factor post-hepatectomy morbidity and graft dysfunction or failure after liver transplantation.^{7,8} Liver biopsy is the current gold standard method of diagnosis of HS and its associated histological lesions.^{9,10} Due to its invasive nature and inherent drawbacks such as sampling error, imperfect reproducibility of the semi-quantitative histological grading, various non-invasive methods have been developed to accurately quantify and grade HS. Non-invasive methods of HS assessment include ultrasound-based techniques like liver ultrasonography, controlled attenuation parameter, computed tomography-based liver-spleen attenuation ratio and magnetic resonance imaging (MRI)-based techniques such as proton based fat fraction, spectroscopy.¹¹⁻¹⁴ Of these techniques, controlled attenuation parameter (CAP), which measures ultrasonic attenuation in the liver using signals acquired by transient elastography (TE), is widely utilized to diagnose and quantify HS.¹⁵⁻¹⁸ The aim of the study was to validate CAP as a screening tool to detect significant HS and propose optimal cutoff values by comparing with histological non-alcoholic fatty liver disease activity (NAS) steatosis grades and hepatic lipid content measured by Fourier transform infrared spectroscopy (FTIR).¹⁹⁻²¹

Patients and methods

Consecutive patients planned for hepatectomy or transplantation at our center were recruited prospectively from October 2014 to August 2016, after obtaining an informed consent in accordance to the principles of the Declaration of Helsinki (Edinburgh revision 2000). All clinical data of the patients including epidemiological data, liver function tests were recorded at inclusion. The exclusion criteria were presence of significant ascites, non-valid liver stiffness measurement (LSM), pregnancy, non-availability of frozen liver tissue for analysis, and a liver biopsy unsuitable for grading steatosis with <6 portal triads. The Ethics committee of our university approved the study protocol.

CAP measurement by transient elastography:

Simultaneous acquisition of LSM and CAP in the non-tumoral liver was performed by TE using FibroScan[®] 502 Touch Standard device with M or XL probes (Echosens[™], Paris, France). The attenuation of ultrasound signals utilized to measure the propagation of the mechanical shear wave in the liver parenchyma during TE is quantified as CAP using a proprietary algorithm based on Vibration Controlled Transient Elastography[™] technology. Median of ten individual measurements at a single site in a patient irrespective of success rate and interquartile range/median (IQR/M) was considered as CAP and it is expressed within the range 100-400 dB/m. All measurements were performed through the intercostal space on the right liver free of any large vascular structures with the patient in decubitus position with the right arm in maximum abduction and at least ten consecutive measurements were acquired at a single site under TM and A-mode control. An experienced operator was blinded to the patient's clinical data. All patients were operated within two weeks of TE. Only measurements with a success rate $\geq 60\%$ and an IQR/M $< 30\%$ if LSM ≥ 7.1 kPa were considered as valid TE.^{22,23}

Histopathological assessment of steatosis (HPE):

Tissue sections were prepared from the frozen samples of non-tumoral explant liver and utilized HS assessment by histology and FTIR. Semi-quantitative visual assessment of percentage of the hepatocytes with lipid accumulations in cytoplasmic vesicles on hematoxylin and eosin stained tissue sections was performed by an experienced pathologist blinded to clinical data and CAP values and graded as: S0-steatosis <5%, S1- 5-33%, S2-34-66%, and S3-67-100% of hepatocytes (Figure 1).¹⁹

Fourier transform Infrared spectroscopy:

The principle of FTIR is based on the determination of absorption of infrared (IR) light due to resonance with vibrational motions of functional molecular groups. A frequency will be strongly absorbed if its photon energy coincides with the vibrational energy levels of the molecule sample and all the absorptions bands of lipids, proteins and sugars are seen within the range of IR spectrum. Therefore, FTIR is a very powerful technique that provides fingerprint information on the chemical composition of the tissue, particularly lipid content in this study. A frozen tissue section was deposited on a regular glass slide and the tissue's IR spectrum was recorded using A-ATR-FTIR spectrometer connected to Nicolet™ iN10 infrared microscope (Figure 3A). The acquired IR spectrum thus obtained would correspond to an average spectrum of an area $2 \times 2 \text{ mm}^2$ of the tissue section. The quantification of the triglyceride (TG) content was then addressed from the acquired spectrum by calculating the ratio of integrated intensity of bands attributed to lipids ($2800\text{-}3100 \text{ cm}^{-1}$) relative to proteins (Amide II: $1485\text{-}1595 \text{ cm}^{-1}$) and expressed in nmol/mg (Figures 3B and 3C). As illustrated in Figure 3D, with the increasing level of steatosis, the intensity of CH_2 and CH_3 vibrational modes attributed to lipids significantly increased ($3000\text{-}2800 \text{ cm}^{-1}$), whereas the vibrational modes attributed to proteins (Amide I and Amide II: $1485\text{-}1710 \text{ cm}^{-1}$) were not significantly affected by steatosis.²⁰ An experienced researcher measured TG content and mean of three measurements per tissue section was considered as TG content of a patient. Then, the patients

were grouped into four grades according to TG content as G0: <43.66 nmol/mg, G1: 43.66-220.1 nmol/mg, G2: >220.1-465.5 nmol/mg, and G3: >465.5 nmol/mg.²¹

Definitions:

Accumulation of fat droplets in 5% of hepatocytes (S1+) was considered as pathological steatosis and defined hepatic steatosis by visual histological assessment by pathologist. Triglyceride content ≥ 43.66 nmol/mg (G1+) was the definition of HS by FTIR. Significant steatosis was defined as $\geq 34\%$ hepatocytes by histology (S2+) and >220.1 nmol/mg by FTIR (G2+). Finally, severe steatosis was defined as $\geq 67\%$ hepatocytes by histology (S3) and >465.5 nmol/mg by FTIR (G3).

Statistical analysis:

Discrete variables are reported as counts (percentage) and parametric variables as median (range). Receiver operating characteristic (ROC) analysis with area under ROC curve (AUROC) as a main readout was performed to compare CAP with FTIR grades of steatosis and CAP cut-off values for each grade of steatosis was determined by maximizing sensitivity and specificity (Youden index). Sensitivity, specificity, diagnostic accuracy (DA), positive predictive value (PPV) and negative predictive value (NPV), positive and negative likelihood ratios (LR) were calculated for CAP cut-offs in the study and overall cohorts. Bivariate correlation of CAP, HPE and FTIR was tested by Spearman rank correlation. Kruskal-Wallis H test and Mann Whitney U test were utilized to test the CAP differences between different grades of steatosis. SPSS 21.0 package (SPSS Inc., Chicago, IL) was utilized to perform the analysis. *P* value <0.05 was considered significant.

Results

A total of 410 out of 665 patients who underwent hepatectomy and liver transplantation had a valid TE and 83 patients among them with CAP measurement, HPE steatosis grade, and TG

content measured by FTIR constituted the study cohort (Figure 1). Median age of patients (47 men and 36 women) was 60 years (range, 25-87). The indications for hepatectomy were liver metastasis (54.2%), primary liver malignancy (39.8%), and other liver diseases (6%). Median (range) of CAP, HPE steatosis, and TG content were 238 (118-351) dB/m, 20 (0-90) %, and 131 (0-6188) nmol/mg, respectively. Table 1 summarizes the patient characteristics, liver function tests, LSM, CAP, HPE and TG content of the study population.

NAS grades vs FTIR grades of steatosis

There was excellent correlation between percentage of steatosis measured by HPE and TG content by FTIR ($r=0.71$, $p<0.0001$). CAP had a significant but moderate correlation with both HPE ($r=0.36$, $p=0.001$) and TG content ($r=0.43$, $p<0.0001$). There was progressive increase in the discriminatory performance of CAP as the FTIR grade of steatosis with AUROCs of 0.7 for $G\geq 1$, 0.75 for $G\geq 2$, and 0.79 for $G=3$ (Figure 4). The optimal cut-off values (DA) determined were 225 dB/m (68.7%) for $G\geq 1$, 255 dB/m (75.9%) for $G\geq 2$, and 268 dB/m (81.9%) for $G=3$. Sensitivity, specificity, NPV, positive LR, and DA of the cut-offs determined with TG content was better than the similar cut-offs determined with HPE (Figure 4). The median (range) CAP values in dB/m according to the FTIR steatosis grades were 211 (118-316) in G_0 , 226 (136-330) in G_1 , 260 (118-326) in G_2 , and 276 (118-351) in G_3 steatosis. The FTIR steatosis grades were able to better differentiate CAP values between the grades (Figure 5). There was a significant difference in CAP values between G_3 compared with G_0 and G_1 steatosis. Further, CAP values had better discriminatory performance for diagnosing HS, significant steatosis, and severe steatosis if FTIR grades were utilized as reference criteria compared to NAS grades for steatosis (Figure 6).

Discussion

Controlled attenuation parameter by FibroScan[®] is a promising screening tool for assessing HS pre-operatively in patients programmed for hepatectomy or transplantation. In the present study, the diagnostic performance of CAP was good in all three grades of steatosis (AUROC ≥ 0.7) and it improved as the grade of steatosis increased. CAP has high NPV to rule out significant and severe steatosis. With respect to the reference of evaluating CAP, actual hepatic fat content measured objectively on tissue sections by FTIR should be utilized in the place of semi-quantitative visual assessment of macrovesicular fat by pathologist. When FTIR steatosis grades were utilized as reference, CAP demonstrated better discriminatory performance to diagnose HS, significant steatosis and severe steatosis as illustrated in Figures 4, 5, and 6. Moreover, in the present study CAP had better correlation with TG content than percentage of HS. This is probably explained by the fact CAP estimates all the fatty infiltration, not only vesicular fat, in the region of interest and future clinical studies evaluating the performance of CAP should utilize actual hepatic lipid content as reference instead of NAS grades.

The most sensitive and accurate method of assessing intrahepatic fat is magnetic resonance spectroscopy but it is limited by its high cost and non-availability in most centers. Liver ultrasound is utilized as first-line imaging method of assessing HS in real time given its low cost, safety, and wide availability. However, it is operator dependent and evaluation is at best semi-quantitative with good accuracy in $>20\%$ steatosis.¹¹⁻¹⁴ CAP has recently emerged as first-line and point-of-care technique to assess HS. It measures the degree of ultrasound attenuation due to hepatic fat at the standardized frequency of 3.5MHz by Vibration-Controlled Transient Elastography[™].^{16,17} Many authors have evaluated the diagnostic performance of CAP and found that it had correlated well with NAS grades of steatosis in liver biopsy. Further, CAP values were not influenced by the presence of fibrosis or cirrhosis. In most studies, CAP >215 dB/m was the cut-off to diagnose $\geq 10\%$ fatty infiltration of

hepatocytes and >250 dB/m correlated with significant steatosis of $\geq 34\%$.^{14,16,17,24-28} A recent study by de Lédighen and his colleagues, AUROCs for $\geq 10\%$, $\geq 34\%$, and $\geq 67\%$ grades of steatosis were 0.79, 0.84, and 0.84, respectively. Factors associated with high CAP values were obesity, metabolic syndrome, alcoholism, and increased liver stiffness.²⁶ Another prospective study of 201 patients with chronic liver disease, AUROC of CAP was >0.8 for all grades of HS but it could not differentiate between S2 and S3 grades similar to the present study.²⁷ On the other hand, Ahn *et al* compared CAP with ultrasound assessment of HS in NAFLD and alcoholic liver disease. They demonstrated that CAP correlated ultrasound grades of steatosis ($r=0.58$) and there was no difference in alcoholic and non-alcoholic fatty liver diseases.²⁸ A recent meta-analysis by Karlas *et al* demonstrated the excellent performance of CAP in diagnosing HS and established optimal CAP cut-offs in dB/m of 248 for $S\geq 1$, 268 for $S\geq 2$, and 280 for S3.²⁹ Wong and his colleagues demonstrated in a large cohort with different liver diseases that variability of CAP could be reduced by applying a new validity criteria – IQR $<40\%$. When IQR $<40\%$, AUROC of CAP to diagnose $>5\%$ HS was 0.90 whereas it was 0.76 when IQR $\geq 40\%$.³⁰ In the present study, diagnostic performance (AUROC) of CAP was lower compared to recent literature probably to the following reasons: 1. Lower prevalence of grade 3 steatosis ($<5\%$), 2. Site of biopsy was different from the site of CAP measurement, and 3. Non-application of validity criteria. Despite these limitations, CAP demonstrated similar performance to published literature when FTIR grades of steatosis was the reference. High NPVs of 80.1% and 91.4% were observed for exclusion of G2 and G3 steatosis, respectively. Overall, sensitivity, specificity, PPV, NPV and positive LR were better for CAP when FTIR grades were reference values. In a unique study by Fujimori *et al*, CAP has similar correlation ($r=0.48$) to actual hepatic fat content evaluated by morphometry in 82 NAFLD patients.³¹

In this study, we compared for the first time CAP with two methods of evaluating HS on tissue sections, namely NAS grades and FTIR grades of steatosis. We have demonstrated TG content measured by FTIR to be a better reference as it correlated better with actual hepatic fat content than NAS grades evaluated by HPE. In this study, the patients with various liver diseases were analyzed and therefore, the results could be extrapolated to wide range of clinical scenarios. The main limitations of this mono-centric study were small study population and low prevalence of G3 steatosis, the main determinant of AUROC. These facts should be considered while interpreting the predictive values.

Conclusions

Non-invasive screening of hepatic steatosis by CAP looks promising and significant steatosis could be ruled out with NPV of >80% and DA of >75%. Being an objective and accurate quantitative measurement of hepatic fat content on tissue sections, FTIR steatosis grades should be utilized to assess the performance of CAP in clinical studies. Based on FTIR grades, we propose a CAP cut-off values in dB/m 225 for $G \geq 1$, 255 for $G \geq 2$, and 268 for G3 steatosis in patients with various underlying liver diseases.

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Table 1: Patients characteristics and noninvasive tests in the study cohort

Variables	N=83
Age in years, median (IQR)	60 (52-70)
Gender, Male : Female	47 (56.6):36 (43.4)
Body mass index in kg/m ² , median (IQR)	24 (21-27)
Diabetes mellitus	8 (9.6)
Arterial hypertension	27 (32.5)
Dyslipidemia	11 (13.3)
Hepatectomy patients	83 (100)
▪ Liver metastasis	45 (54.2)
▪ Primary liver malignancy	33 (39.8)
▪ Others	5 (6)
Model for end-stage liver disease score, median (IQR)	7 (6-8.5)
Blood hemoglobin in g/dL, median (IQR)	13.6 (12.1-14.7)
Platelet count in 10 ⁹ /L, median (IQR)	231 (182-283)
Serum fibrinogen in g/L, median (IQR)	4 (3-4)
International normalized ratio, median (IQR)	1.1 (1-1.2)
Blood urea nitrogen mmol/L, median (IQR)	5 (4-6)
Serum creatinine in μmol/L, median (IQR)	70 (63-84)
Serum total bilirubin in μmol/L, median (IQR)	10 (8-14)
Serum aspartate aminotransferase in IU/L, median (IQR)	36 (28-54)
Serum alanine aminotransferase in IU/L, median (IQR)	32 (20-48)
Serum γ-glutamyl transpeptidase in IU/L, median (IQR)	80 (39-199)
Serum alkaline phosphatase in IU/L, median (IQR)	103 (71-136)
Serum albumin in g/L, median (IQR)	37.4 (34.5-40.8)
Liver stiffness measurement in kPa, median (IQR)	3.3 (5.2-9.8)
Controlled attenuation parameter in dB/m, median (IQR)	238 (195-270)
Percentage of steatosis, median (IQR)	20 (4-60)
Triglyceride content in nmol/mg, median (IQR)	131 (20-383)
METAVIR fibrosis score in non-tumoral liver parenchyma:	17 (20.5)
▪ F0	28 (33.7)
▪ F1	13 (15.7)
▪ F2	9 (10.8)
▪ F3	16 (19.3)
▪ F4	
NAS grade of steatosis in non-tumoral liver parenchyma:	24 (28.9)
▪ S0	27 (32.5)
▪ S1	17 (20.5)
▪ S2	15 (18.1)
▪ S3	
FTIR grades of steatosis in non-tumoral liver parenchyma:	28 (33.7)
▪ G0	23 (27.7)
▪ G1	13 (15.7)
▪ G2	19 (22.9)
▪ G3	

*Data are expressed as counts (percentage) unless otherwise stated; IQR-interquartile range, METAVIR-meta-analysis of histological data in viral hepatitis, NAS-non-alcoholic fatty liver disease activity score, FTIR-Fourier transform infrared spectroscopy

Figure 1: Study flow chart

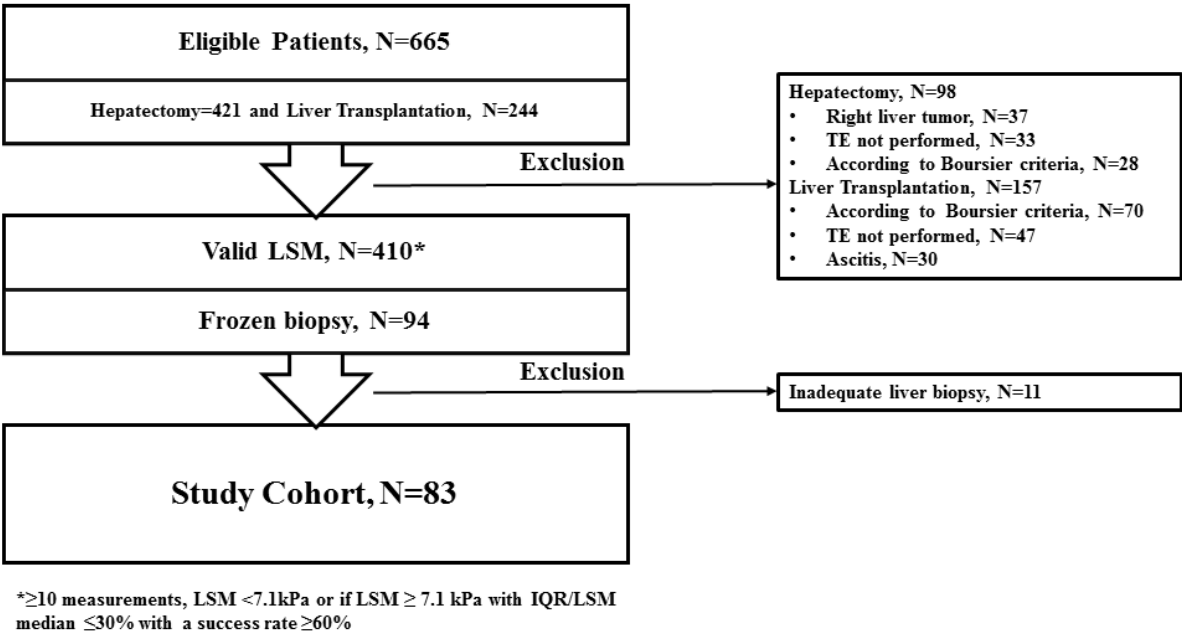


Figure 2: Non-alcoholic fatty liver disease steatosis grades on hematoxylin and eosin stained frozen tissue sections: A-S0 (≤5%), B-S1 (>5%), C-S2 (≥34%), and D-S3 (≥67%)

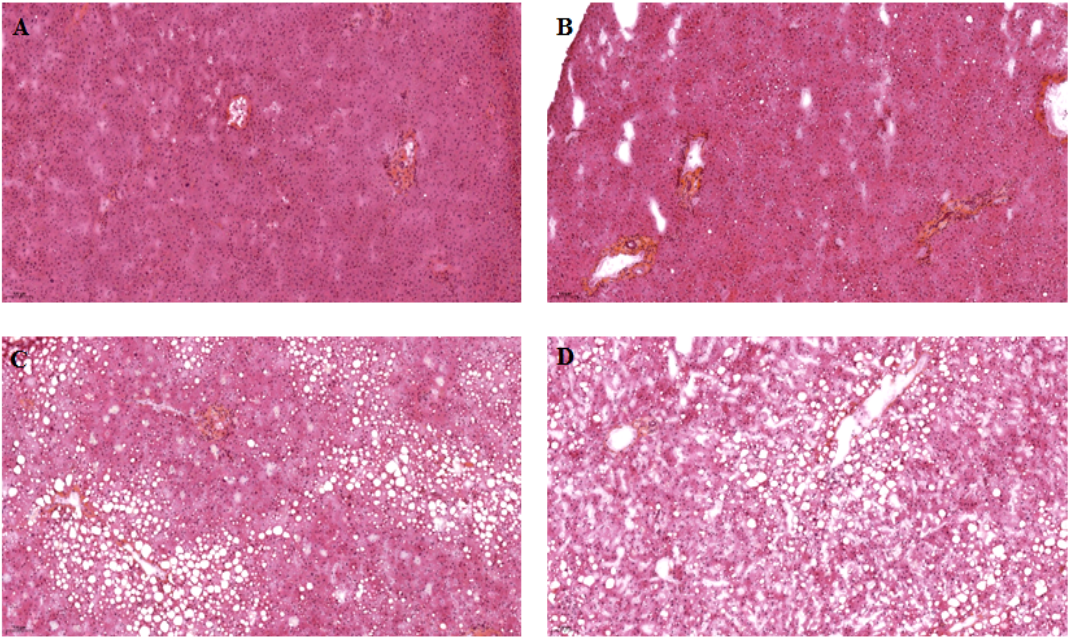


Figure 3: Fourier transform infrared spectroscopy: A-ATR-FTIR spectrometer connected to Nicolet™ iN10 infrared microscope, B-infrared spectrum, C-quantification triglyceride content as ratio of integrated intensity of bands attributed to lipids relative to proteins, D-infrared spectra of different grades of steatosis

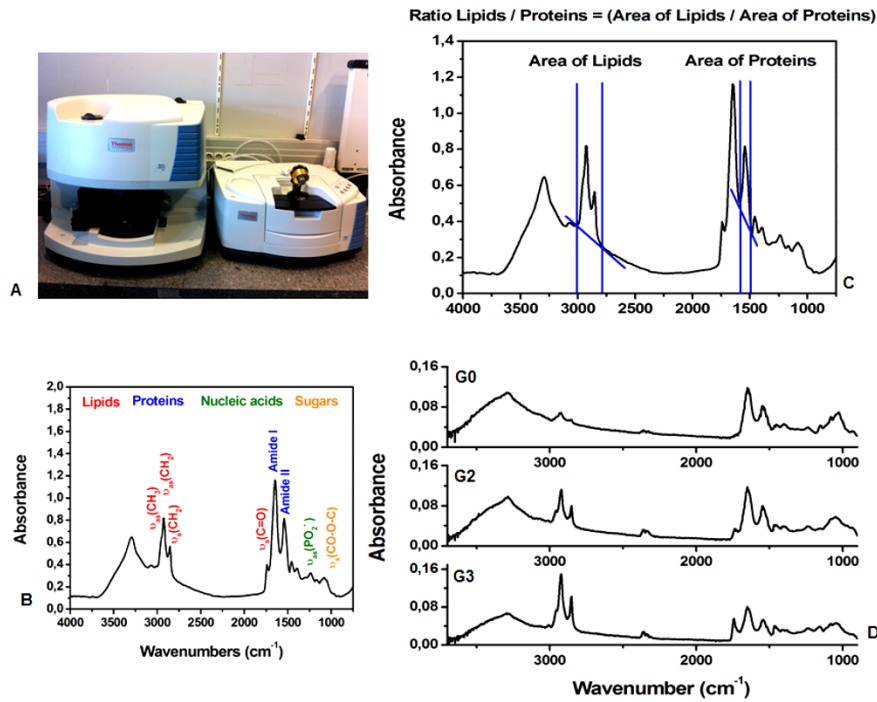
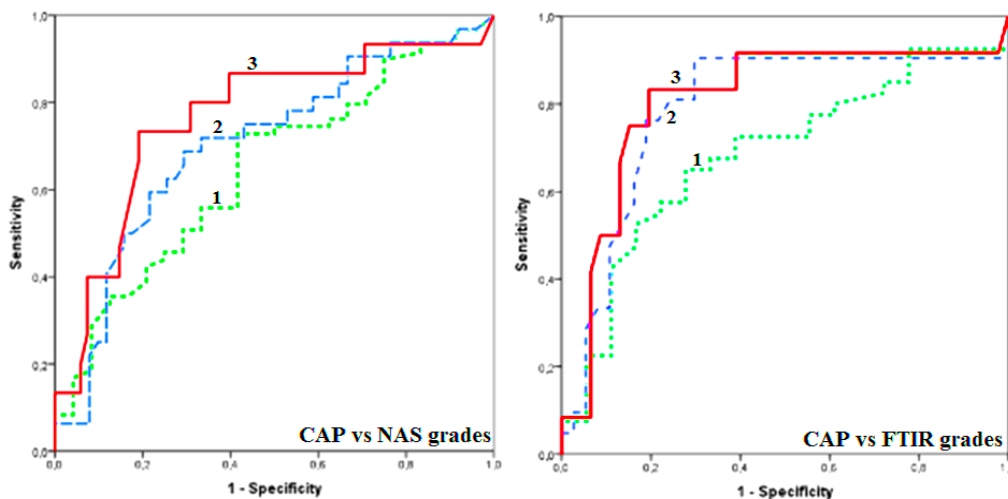


Figure 4: Receiver operating characteristic analysis of controlled attenuation parameter (CAP) with NAS grades (A) and FTIR grades of steatosis (B)



Curve	Steatosis Grades	Prevalence N (%)	Area Under ROC	95% Confidence Interval	P value [#]	Cut-off [*]	Sensitivity %	Specificity %	PPV %	NPV %	+LR	-LR	DA %
1	FTIR G≥1	55 (66.3)	0.700	0.59-0.82	0.6	225	70.9	64.3	70.9	64.3	1.9	0.5	68.7
1	NAS S≥1	59 (71.1)	0.648	0.52-0.78		216	72.9	58.3	81.1	46.7	1.7	0.5	68.7
2	FTIR G≥2	32 (38.6)	0.747	0.62-0.87	0.6	255	71.9	80.4	68.8	80.4	3.7	0.3	75.9
2	NAS S≥2	32 (38.6)	0.704	0.59-0.82		245	68.8	70.6	58.3	76.6	2.3	0.4	68.7
3	FTIR G=3	19 (22.9)	0.787	0.65-0.92	0.8	268	73.7	84.4	58.3	91.4	4.9	0.3	81.9
3	NAS S=3	15 (18.1)	0.766	0.62-0.91		268	73.3	80.9	45.8	66.3	3.8	0.3	79.5

[#]p<0.05 all steatosis stages; PPV-positive predictive value; NPV-negative predictive value; LR-likelihood ratio; ^{*}DeLong test; ^{*}Youden Index

Figure 5: Kruskal-Wallis test analysis of controlled attenuation parameter according to NAS grades (A) and FTIR grades of steatosis (B)

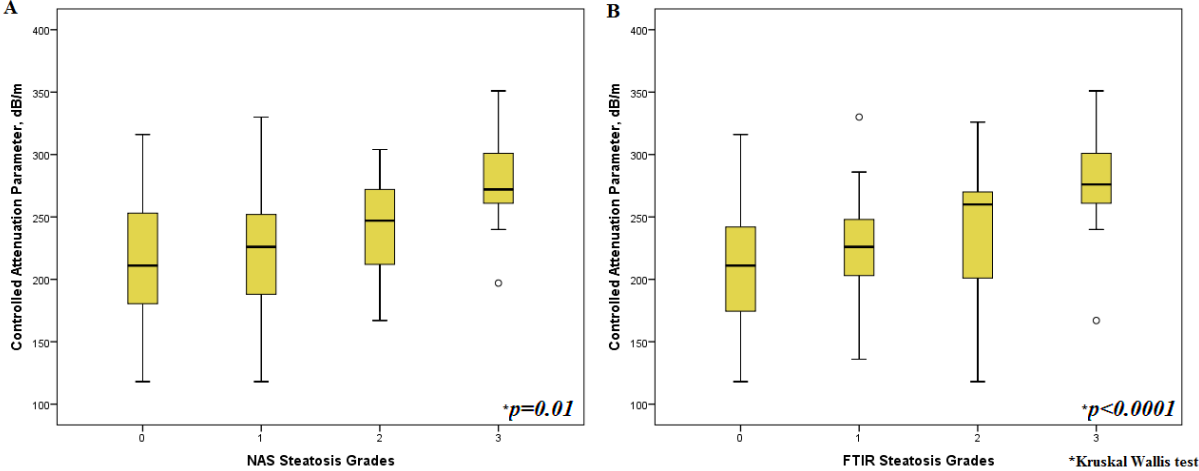
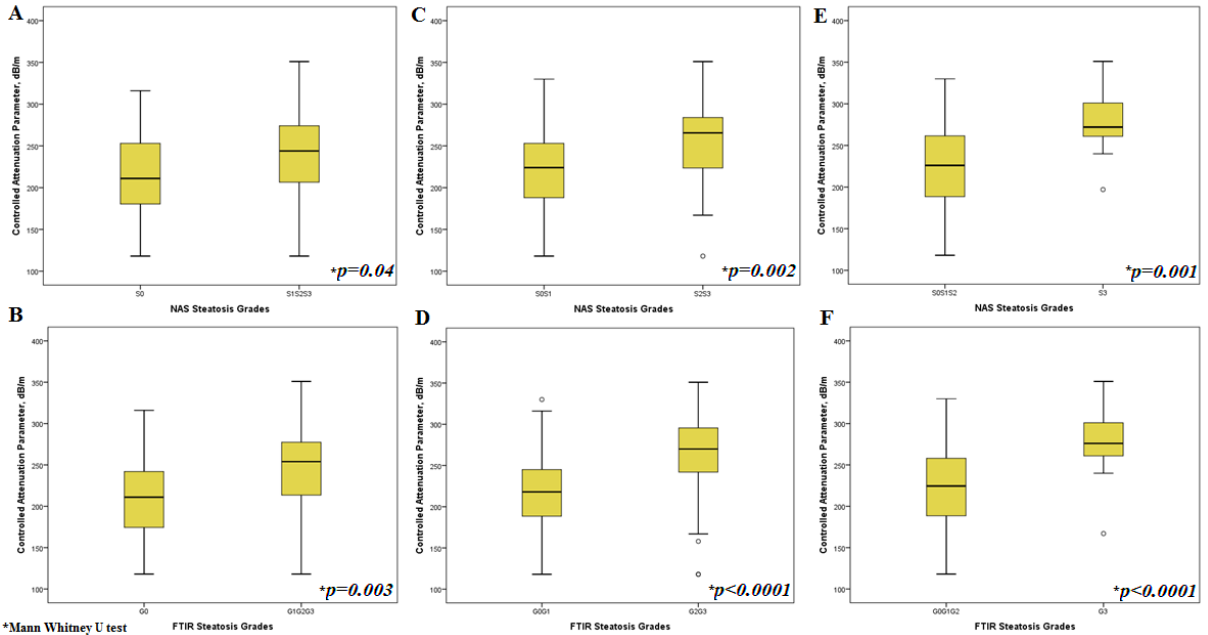


Figure 6: Discriminatory performance of controlled attenuation parameter to diagnose hepatic steatosis, significant steatosis, and severe steatosis according to NAS grades and FTIR grades of steatosis



Supplementary Results:

Figure 1: Diagnostic performance controlled attenuation parameter according to the NAS steatosis grades in 410 patients

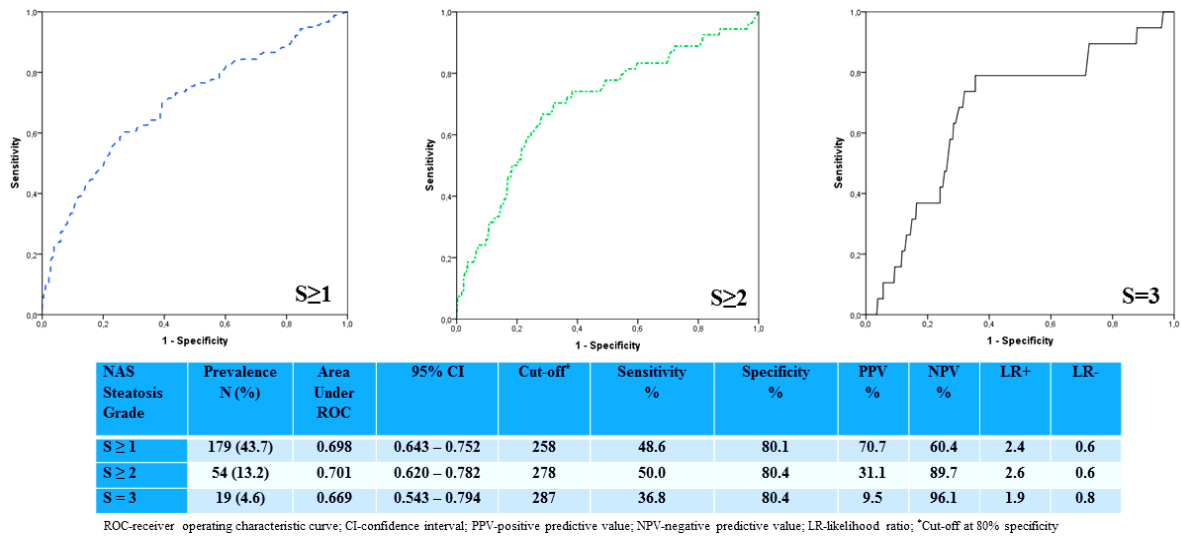


Figure 2: Box Whiskers plots demonstrating the performance of liver stiffness and controlled attenuation parameter according to METAVIR fibrosis grades and NAS steatosis grades, respectively in 410 patients

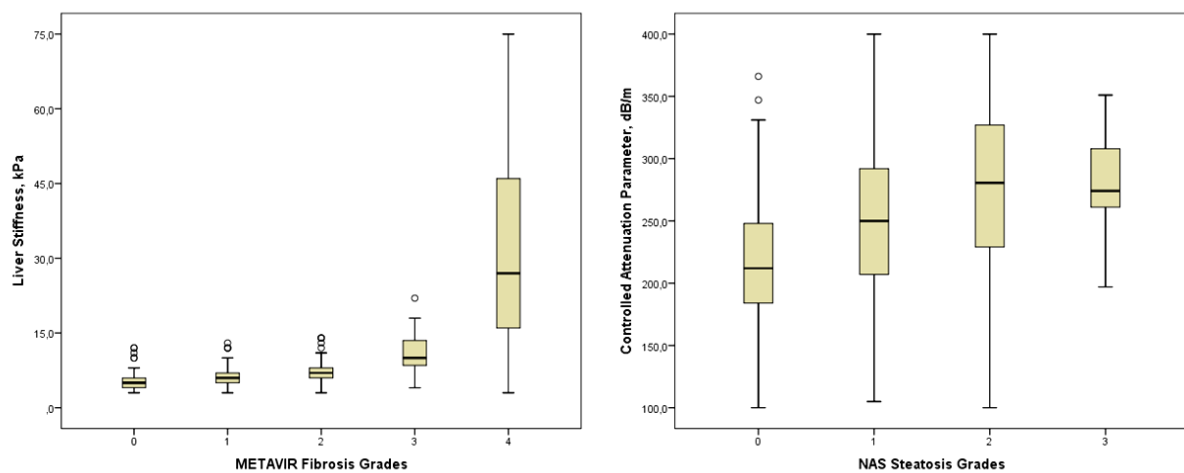


Table 1: Characteristics of patients undergoing liver resection and transplantation with valid transient elastography measurement by FibroScan®

Variables [#]	N=410 (100)
Age in years, median (range)	61(17-87)
Gender, Male:Female	261(63.7):149 (36.3)
Body mass index in kg/m ² , median (range)	25(21-28)
Hepatectomy patients	323(78.8)
▪ Primary liver malignancy	129(31.5)
▪ Liver metastasis	141(34.4)
▪ Benign tumors	43(10.5)
▪ Others	10(2.3)
Liver transplantation patients	87(21.2)
▪ Alcoholic cirrhosis	31(7.6)
▪ Virus-related cirrhosis	20(4.9)
▪ Others	36(8.8)
Blood hemoglobin in g/dL, median (range)	13(7-19)
Platelet count in 10 ⁹ /L, median (range)	213(17-811)
Serum fibrinogen in g/L, median (range)	3(1-8)
International normalized ratio, median (range)	1(1-5)
Prothrombin time in %, median (range)	88(19-100)
Blood urea nitrogen mmol/L, median (range)	5(1-48)
Serum creatinine in μmol/L, median (range)	71(44-719)
Serum total bilirubin in μmol/L, median (range)	10(2-681)
Serum aspartate aminotransferase in IU/L, median (range)	36(10-405)
Serum alanine aminotransferase in IU/L, median (range)	30(10-848)
Serum γ-glutamyl transpeptidase in IU/L, median (range)	82.5(82-2597)
Serum alkaline phosphatase in IU/L, median (range)	101(10-1020)
Serum albumin in g/L, median (range)	37(18-66)
Liver stiffness measurement in kPa, median (range)	8(3-75)
Hepatic venous pressure gradient in mmHg, median (range)	5(0-31)
Controlled attenuation parameter in dB/m, median (range)	232(100-400)
METAVIR fibrosis score of non-tumoral liver parenchyma: F0:F1:F2:F3:F4	81(19.8):90(22.0):74 (18.0):36 (8.8):129(31.5)
METAVIR fibrosis score of non-tumoral liver parenchyma: A0:A1:A2:A3:Missing data	131(32):131(32):82(20):17(4.1):49(12)
NAS steatosis grades of non-tumoral liver parenchyma: S0:S1:S2:S3:Missing data	190(46.3):131(32):41(10):16(3.9):32(7.8)

[#]Data expressed as counts(percentage) unless specified, METAVIR-meta-analysis of histological data in viral hepatitis, NAS-non-alcoholic fatty liver diseases activity score

Table 2: Factors influencing liver stiffness measurement by transient elastography

Variables [#]	Univariate Analysis		Multiple linear regression				Bootstrapped multiple linear regression		
	Beta	p	Beta	p	95% CI		p	95% CI	
					Lower	Upper		Lower	Upper
Age, years		0.76							
Female gender	-4.24	0.02							
Body mass index, kg/m ²	0.69	<0.001	0.59	<0.001	0.281	0.897	0.003	0.223	0.962
Viral aetiology	7.33	<0.001	6.13	0.001	2.478	9.773	0.011	1.987	10.885
Liver malignancy	-7.02	<0.001							
Serum total bilirubin, µmol/L	0.13	<0.001	0.10	<0.001	0.079	0.119	0.001	0.073	0.147
Serum aspartate aminotransferase, IU/L	0.13	<0.001	10.09	<0.001	7.043	13.143	0.001	6.332	13.944
Serum alanine aminotransferase, IU/L		0.24							
Serum γ-glutamyl transferase, IU/L	0.01	0.05							
Serum alkaline phosphatase, IU/L	0.02	<0.001							
Controlled attenuation parameter, dB/m		0.06	-0.03	0.02	-0.049	-0.003	0.08	-0.054	0.003
NAS steatosis grades		0.06							

[#]All unrelated variables with $p \leq 0.10\%$ in univariate analysis were entered into multivariate stepwise linear regression and the variables that remained in the equation were entered into multivariate linear regression bootstrapped at 1000

Table 3: Risk factors for false positive results in liver stiffness measurement ≥ 10 kPa by transient elastography

Variables [#]	Prev N(%)	Univariate Analysis		Multivariate Logistic Regression				Bootstrapped Multivariate Logistic Regression			
		Beta	p	Beta	p	O R	95% CI		p	95% CI	
							Lower	Upper		Lower	Upper
Age ≥ 60 years	223(54.8)	1.68	0.01	1.67	0.01	5.29	5.34	18.28	0.01	1.55	18.48
Female gender	137(36.2)		0.27								
Body mass index ≥ 30 kg/m ²	73(17.9)		0.89								
Viral aetiology	61(15)		0.48								
Liver malignancy	297(73)	1.31	0.08								
Serum total bilirubin ≥ 54 µmol/L	32(7.9)		0.58								
Serum aspartate aminotransferase ≥ 50 IU/L	117(28.7)	0.66	0.15								
Serum alkaline phosphatase ≥ 250 IU/L	47(11.5)	0.94	0.08								
Controlled attenuation parameter ≥ 250 dB/m	153(37.6)		0.68								
NAS steatosis grade ≥ 2	57(15.1)		0.22								
Constant					<0.001				0.001	0.65	18.85

[#]All unrelated variables with $p \leq 15\%$ in univariate analysis were entered into multivariate logistic regression by backward elimination (Wald) method and the variables that remained in the equation were entered into multivariate logistic regression bootstrapped at 1000

Table 4: Diagnostic performance of liver stiffness according to age of the patients**(age <60 years, N=187, age ≥60 years, N=223)**

Metavir Score	Age years	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
F≥1	<60	144	0.810	0.749 – 0.872	0.18
	≥60	185	0.868	0.812 – 0.924	
F≥2	<60	92	0.878	0.824 – 0.932	0.89
	≥60	147	0.883	0.838 – 0.927	
F≥3	<60	71	0.943	0.898 – 0.988	0.78
	≥60	94	0.951	0.922 – 0.979	
F=4	<60	57	0.973	0.939 – 1.000	1.49
	≥60	72	0.958	0.928 – 0.988	

Table 5: Diagnostic performance of LS according to the etiology of underlying**liver disease (viral disease, N=61; others, N=349)**

Metavir Score	Liver disease	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
F≥1	Viral	58	0.830	0.700 – 0.961	0.93
	Others	271	0.836	0.793 – 0.880	
F≥2	Viral	54	0.903	0.824 – 0.982	1.41
	Others	185	0.879	0.843 – 0.915	
F≥3	Viral	47	0.950	0.892 – 1.000	1.17
	Others	118	0.943	0.913 – 0.973	
F=4	Viral	44	0.937	0.875 – 0.999	0.27
	Others	85	0.974	0.952 – 0.996	

Table 6: Performance of LS according to the indication for liver resection and**transplantation (cancer, N=299; others, N=111)**

Metavir Score	Surgical Indication	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
F≥1	Cancer	245	0.817	0.762 – 0.872	0.04
	Others	82	0.898	0.842 – 0.955	
F≥2	Cancer	177	0.861	0.820 – 0.903	0.003
	Others	64	0.950	0.908 – 0.991	
F≥3	Cancer	111	0.929	0.893 – 0.965	0.01
	Others	54	0.984	0.961 – 1.000	
F=4	Cancer	82	0.947	0.913 – 0.982	0.01
	Others	47	0.996	0.989 – 1.000	

Table 7: Diagnostic performance of LS according to the NAS steatosis grades
(S01, N=329; S23, N=57)

Metavir Score	Indication	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
F \geq 1	S01	259	0.850	0.805 - 0.896	1.62
	S23	48	0.794	0.679 - 0.909	
F \geq 2	S01	189	0.881	0.843 - 0.918	0.44
	S23	36	0.914	0.840 - 0.989	
F \geq 3	S01	125	0.948	0.920 - 0.976	1.34
	S23	28	0.928	0.845 - 1.000	
F=4	S01	99	0.963	0.935 - 0.990	1.19
	S23	18	0.956	0.910 - 1.000	

Table 8: Diagnostic performance of LS according to body mass index
(BMI \geq 30, N=73; BMI<30, N= 337)

Metavir Score	BMI kg/m ²	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
F \geq 1	\geq 30	63	0.879	0.800 - 0.959	1.62
	<30	266	0.838	0.792 - 0.884	
F \geq 2	\geq 30	46	0.921	0.857 - 0.985	1.72
	<30	193	0.881	0.845 - 0.917	
F \geq 3	\geq 30	39	0.930	0.863 - 0.998	0.53
	<30	126	0.953	0.927 - 0.979	
F=4	\geq 30	33	0.995	0.985 - 1.000	1.98
	<30	96	0.956	0.926 - 0.985	

Table 9: Diagnostic performance of LS according to body mass index
(overweight, N=279; normal, N=191)

Metavir Score	BMI kg/m ²	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i> value
F \geq 1	\geq 25	185	0.854	0.798 - 0.911	1.40
	<25	144	0.832	0.773 - 0.892	
F \geq 2	\geq 25	137	0.895	0.852 - 0.937	1.35
	<25	102	0.880	0.832 - 0.929	
F \geq 3	\geq 25	100	0.952	0.919 - 0.985	1.29
	<25	65	0.942	0.902 - 0.982	
F=4	\geq 25	81	0.975	0.952 - 0.999	1.66
	<25	48	0.951	0.908 - 0.994	

Table 10: Factors influencing controlled attenuation parameter by transient elastography

Variables [#]	Univariate Analysis		Multiple linear regression				Bootstrapped multiple linear regression		
	Beta	p	Beta	p	95% CI		p	95% CI	
					Lower	Upper		Lower	Upper
Age, years	0.52	0.02							
Female gender	-22.28	0.001							
Body mass index, kg/m ²	6.2	<0.001	6.42	<0.001	5.22	7.63	0.001	5.53	7.89
Viral aetiology	-23.69	0.01							
Liver malignancy	13.91	0.06							
Serum total bilirubin, µmol/L		0.12							
Serum aspartate aminotransferase, IU/L	-0.12	0.08							
Serum alanine aminotransferase, IU/L		0.39							
Serum γ-glutamyl transferase, IU/L		0.49							
Serum alkaline phosphatase, IU/L	-0.04	0.06							
Liver stiffness, kPa	-0.37	0.06	-0.674	<0.001	-1.02	-0.32	0.001	-1.03	-0.27
METAVIR fibrosis grades		0.49							

[#]All unrelated variables with $p \leq 0.10$ in univariate analysis were entered into multivariate linear regression and the variables that remained in the equation were entered into multivariate linear regression bootstrapped at 1000

Table 11: Risk factors for false negative results in CAP<250 dB/m by transient elastography

Variables, N(%) [#]	Prevalence N(%)	Univariate Analysis		Multivariate Logistic Regression				Bootstrapped Multivariate Logistic Regression			
		Beta	p	Beta	p	OR	95% CI		p	95% CI	
							Lower	Upper		Lower	Upper
Age ≥60 years	223(54.8)		0.99								
Female gender	137(36.2)		0.53								
Body mass index ≥30 kg/m ²	73(17.9)	-1.36	0.01	-1.45	0.01	0.24	0.08	0.68	0.01	-3.28	-0.63
Viral aetiology	61(15)	0.64	0.05								
Liver malignancy	297(73)	0.63	0.07	0.71	0.04	2.03	1.02	4.02	0.05	0.07	1.66
Serum total bilirubin ≥54 µmol/L	32(7.9)		0.62								
Serum aspartate aminotransferase ≥50 IU/L	117(28.7)		0.59								
Serum alkaline phosphatase ≥150 IU/L	104(25.6)		0.44								
Liver stiffness ≥10 dB/m	159(38.8)	0.57	0.03	0.69	0.01	1.99	1.18	3.39	0.01	0.16	1.26
METAVIR fibrosis ≥3	163(40)		0.26								
Constant					<0.001				0.001	-3.02	-1.42

[#]All unrelated variables with $p \leq 15\%$ in univariate analysis were entered into multivariate regression and the variables that remained in the equation were entered into multivariate logistic regression bootstrapped at 1000

Table 12: CAP performance according to the etiology of underlying liver disease (viral disease, N=61; others, N=349)

Metavir Score	Liver disease	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
S ≥1	Viral disease	26	0.625	0.470 – 0.780	0.31
	Others	162	0.710	0.652 – 0.768	
S ≥2	Viral disease	10	0.830	0.646 – 1.000	1.83
	Others	47	0.683	0.593 – 0.773	
S ≥3	Viral disease	3	0.648	0.198 – 1.000	0.47
	Others	16	0.667	0.538 – 0.796	

Table 13: CAP performance according the indication for liver resection and transplantation (cancer, N=299; others, N=111)

Metavir Score	Surgical Indication	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
S ≥1	Cancer	156	0.691	0.628 – 0.753	0.79
	Others	32	0.709	0.589 – 0.829	
S ≥2	Cancer	47	0.699	0.611 – 0.787	1.16
	Others	10	0.684	0.481 – 0.886	
S ≥3	Cancer	16	0.663	0.523 – 0.802	1.03
	Others	3	0.657	0.376 – 0.937	

Table 14: CAP performance according to METAVIR fibrosis grades (F012, N=245; F34, N=165)

Metavir Score	METAVIR F stage	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
S ≥1	F012	145	0.712	0.643 – 0.780	1.47
	F34	72	0.676	0.586 – 0.765	
S ≥2	F012	29	0.699	0.597 – 0.803	0.88
	F34	28	0.711	0.589 – 0.834	
S ≥3	F012	11	0.728	0.578 – 0.877	1.67
	F34	8	0.606	0.412 – 0.801	

Table 15: CAP performance according to body mass index (overweight, N=219; normal, N=191)

Metavir Score	BMI kg/m ²	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i> value
S ≥1	≥25	120	0.710	0.635 – 0.784	1.83
	<25	68	0.630	0.543 – 0.716	
S ≥2	≥25	39	0.697	0.605 – 0.789	1.64
	<25	18	0.610	0.449 – 0.770	
S ≥3	≥25	11	0.586	0.437 – 0.734	0.35
	<25	8	0.724	0.474 – 0.974	

**Table 16: CAP performance according to body mass index
(BMI \geq 30, N=73; BMI<30, N=337)**

Metavir Score	BMI kg/m ²	Prevalence N (%)	Area Under ROC	95% CI	<i>p</i>
S \geq 1	\geq 30	43	0.760	0.623 – 0.898	1.81
	<30	145	0.660	0.598 – 0.722	
S \geq 2	\geq 30	14	0.626	0.459 – 0.793	0.54
	<30	43	0.686	0.587 – 0.786	
S \geq 3	\geq 30	2	0.685	0.453 – 0.918	0.93
	<30	17	0.697	0.556 – 0.837	

Portal Hypertension

Article 3:

Liver stiffness-based model predicts hepatic venous pressure gradient in patients undergoing hepatectomy and liver transplantation

Authors:

Muthukumarassamy Rajakannu,^{1,2,3} MBBS, MS, DNB, MRCS, Audrey Coilly,^{1,2} MD, Hecham Azrak,⁵ Nicolas Golse,¹MD, Marc Antoine Allard,¹MD, Daniel Cherqui,^{1,3}MD, Antonio Sa Cunha,^{1,3}MD, Denis Castaing,^{1,2,3}MD, Veronique Miette,⁵ René Adam,^{1,3,4}MD, PhD, Didier Samuel,^{1,2,3}MD, PhD, Eric Vibert,^{1,2,3}MD, PhD

Affiliations:

¹Centre Hépatobiliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

⁵Research and Development, Echosens, Paris, France

Correspondence:

Prof. Eric Vibert

Centre Hépatobiliaire,

AH-HP Hôpital Paul Brousse,

12 Avenue Paul Vaillant Couturier,

94804 Villejuif, France

Email: eric.vibert@aphp.fr

Electronic word count: 2743

Number of tables: 3

Number of figures: 7

List of abbreviations:

Alkp – Serum alkaline phosphatase

AST – Aspartate aminotransferase

AUROC – Area under receiver operating characteristic curve

Bili – Total bilirubin

CLD – Chronic liver disease

CT – Computed tomography

DA – Diagnostic accuracy

Fbg – Serum fibrinogen
Hb – Blood hemoglobin level
HCC – Hepatocellular carcinoma
HVPG – Hepatic venous pressure gradient
HVPG₁₀ – Hepatic venous pressure gradient ≥ 10 mm Hg
INR – International normalized ratio
IQR – Interquartile range
LFT – Liver function test
LSM – Liver stiffness measurement
LSPS – Liver stiffness to spleen size/platelet count ratio
MELD – Model for end-stage liver disease
NPV – Negative predictive value
PH – Portal hypertension
Plat – Total platelet count
PVP – Portal venous pressure
ROC – Receiver operating characteristic
TE – Transient elastography

Keywords: Portal hypertension, liver cirrhosis, esophageal varices, transient elastography, chronic liver disease

Financial support:

L'association nationale de la recherche et de la technologie (ANRT), Paris, France in collaboration with Echosens[™], Paris, France, funded this research.

Conflict of interest:

Muthukumarassamy Rajakannu and Eric Vibert have received research grants from Echosens[™], France. Hecham Azrak and Veronique Miette are employed by Echosens[™], France.

Author's contribution:

RM – Study design, data collection and manuscript preparation

AC – Manuscript preparation and revision

HA – Statistical analysis

NG, MAA – Data collection

DCH, ASC, DCA, RA – Study design and manuscript approval

EV, VM – Study design, analysis and manuscript approval

Abstract

Invasive hepatic venous pressure gradient (HVPG) measurement is the gold standard test to assess the degree of portal hypertension. The aim was to develop and validate a model predictive of $HVPG \geq 10 \text{ mmHg}$ ($HVPG_{10}$) using pre-operative noninvasive makers. A total of 161 patients [66% men, median age of 63 years] who have been programmed for liver resection/transplantation (LR/LT) and have had paired measurement of preoperative liver stiffness measurement (LSM)+liver function test (LFT), and intraoperative HVPG were enrolled prospectively. Primary liver malignancy [34.2%] and end-stage liver disease due to alcohol [11.2%] were the most common indications for LR and LT respectively. Median MELD score, LSM, and HVPG were 6, 9.5kPa, and 5mmHg respectively. No underlying liver disease (F0/1) was found in 32.9% patients, chronic liver disease (F2/3) and cirrhosis (F4) were found in 32.9% and 34.2% patients respectively. The study cohort was randomly divided into training [n=106] and validation [n=55] sets. Independent predictors of $HVPG_{10}$ in the training set, LSM [$P < 0.01$, OR=1.1], total bilirubin [$P = 0.04$, OR=0.9], alkaline phosphatase [$P = 0.02$, OR=1], and international normalized ratio [$P < 0.01$, OR=41.4], were used to develop a probability score model. Area under receiver operating curve in the training and validation sets were 0.91 [95%CI: 0.83-0.98] and 0.93 [95%CI: 0.86-0.99] respectively with a cutoff of 0.15. $HVPG_{10}$ score was calculated by multiplying the probability by 100. In the overall cohort, $HVPG_{10}$ score=15 would predict the individual risk of $HVPG_{10}$ with 83% accuracy, 90% sensitivity, 81% specificity and 96% negative predictive value. It is an easy-to-use noninvasive continuous scale tool which would accurately rule out the risk of decompensation and need for endoscopy in esophageal varices screening in >95% patients.

Introduction

Portal hypertension (PH) is a serious but unavoidable consequence of chronic liver disease (CLD) and cirrhosis. It is characterized by the increase in portal venous pressure (PVP) and this increase causes the pressure gradient between the portal vein and the inferior vena cava, called hepatic venous pressure gradient (HVPG), to rise above the normal range of 1-5 mmHg.^{1,2} When HVPG increases to ≥ 10 mmHg (HVPG₁₀), defined as clinically significant portal hypertension, these patients develop complications such as gastroesophageal variceal bleeding, ascites, hepatic encephalopathy, renal dysfunction, and hepatocellular carcinoma (HCC).²⁻⁸ Further, HVPG₁₀ increases the risk of morbidity and mortality in patients undergoing liver resection or transplantation for HCC.⁹⁻¹² Therefore the diagnosis of HVPG₁₀ is an important step in the management of patients with chronic liver disease. The direct measurement of portal pressure by invasive techniques such as transhepatic or tranvenous catheterization of portal vein and splenic pulp puncture are no longer favored.^{1,7,8} The most commonly utilized method to estimate HVPG is percutaneous catheterization of the hepatic vein and by calculating the difference between wedged and free hepatic venous pressure.¹⁻⁸ Recently several noninvasive techniques have been proposed to evaluate the presence and severity of PH. They were based on liver function tests, transient elastography (TE), hyperkinetic syndrome, intrahepatic vascular resistance, ultrasonography exploration of splanchnic vasculature, computed tomography based HVPG score, magnetic resonance imaging measurement of liver architecture and splenic artery blood flow, and by evaluating the clinical complication of PH.¹³⁻²⁶ European association for the study of the liver and Baveno VI consensus workshop have proposed that the measurement of HVPG₁₀ as the gold standard and liver stiffness measurement (LSM) by TE ≥ 20 -25 kPa as a criteria to rule-in HVPG₁₀.^{27,28} It must be pointed-out that LSM is a continuous numerical value, and the use of cut-off leads to loss of significant information in the risk stratification of the patients.¹⁶ Therefore, the aim of the present study was to develop and validate a continuous risk

prediction model based on LSM and liver function tests (LFT) to be used in an outpatient clinic to rule-in or rule-out HVPG₁₀.

Patients and Methods

In this monocentric cross-sectional study, the patients who have been programmed to undergo a liver resection or transplantation were recruited prospectively from November 2014 until August 2016. The aim of this study was to evaluate the discriminatory performance of LSM in predicting HVPG and to construct a HVPG₁₀ score model using LSM and other noninvasive markers in this study population with varying degrees of liver fibrosis – normal liver to decompensated cirrhosis. Clinical and laboratory data were obtained at inclusion and HVPG was measured intra-operatively. The inclusion criteria for recruiting the patients into the study cohort were: (1) Valid LSM with interquartile range (IQR)/median <30%, (2) Intra-operative measurement of HVPG, and (3) histological assessment of the resected liver specimen. The patients without valid TE measurement within three months of surgery and those with missing data were excluded. HVPG₁₀ (HVPG \geq 10 mmHg) was the primary endpoint of this study. The pretest probability of HVPG₁₀ in surgical patients was estimated to be 20-30%.¹⁵ Liver histology was performed by two expert pathologists and the degree of fibrosis in non-tumoral explant liver was graded using METAVIR scoring system on a five-point scale, from F0-no fibrosis to F4-cirrhosis.²⁹ The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institute ethics committee and an informed consent was obtained from each patient. Figure 1 demonstrates the study flowchart.

Transient Elastography

In all patients, TE was performed pre-operatively in fasting condition using FibroScan[®] Touch 502 with M/XL probes. A single operator blinded to the patient's clinical details

performed all the TE. Liver stiffness was measured on the right lobe of the liver through intercostal spaces by placing the probe on the skin between the ribs while the patient was lying supine with the right arm in maximal abduction. A liver portion of at least 6 cm thick, free of large vascular structures and tumor, was located by an ultrasonic time-motion image and ten successful measurements were performed on each patient. Median of ≥ 10 valid measurements was considered as LSM expressed in kilopascal (kPa) according to Boursier's criteria.³⁰

Hepatic Venous Pressure Gradient

HVPG was measured intra-operatively by direct puncture of portal vein in the hepatic pedicle and infra-hepatic inferior vena cava using a 25-gauge needle connected to a manometer with the patient in supine neutral position and after zeroing the atmospheric pressure. The pressure gradient was calculated by subtracting the hepatic venous pressure from the portal venous pressure. This technique was feasible only during laparotomy and was performed at the discretion of individual surgeon before the commencement of hepatectomy/transplantation.

Statistical analysis

Categorical variables are reported as counts (percentage) and continuous variables as median (IQR). The patients were randomized into training set (two-thirds) and validation set (one-third). In the training set, the pre-operative variables entered into analysis were age, gender, body mass index, hemoglobin (Hb), platelet count (Plat), fibrinogen (Fbg), international normalized ratio (INR), prothrombin activity, total bilirubin (Bili), aspartate aminotransferase (AST), alanine aminotransferase, γ -glutamyl transpeptidase, alkaline phosphatase (Alkp), and LSM. The study population was randomly divided into two-thirds training and one-thirds validation cohorts. In the training cohort, the potential predictors of HVPG₁₀ were identified by univariate Wilcoxon test and those variables that were significant at 15% in the univariate analysis were entered into multivariate binary logistic regression (Backward selection method

of Wald) to determine independent predictors of $HVPG_{10}$. A probability score model was constructed using the four independent predictors (Bili, INR, Alkp and LSM) and Youden's Index was determined to be the cut-off for this score. This model was then validated by applying the same cut-off value. The discriminative performances of the predictive model and LSM were analyzed by receiver operating characteristic (ROC) with the area under the ROC curve (AUROC) as the main readout. Heat map fit of the predicted probabilities and smoothed empirical probabilities of this score model was performed with a histogram indicating the location and frequency of observations. The ideal fit was a 45-degree line and the shading of the plotted line indicated the degree to which fit deviations are larger than expected due to sampling variation. Wilcoxon test was performed to test the ability of the score to classify patients into low and high-risk groups by Baveno VI criteria for esophageal varices. SPSS 21.0 package (SPSS Inc., Chicago, IL) and R version 3.2.5 were utilized to perform the analysis. P value <0.05 was considered significant.

Results

The study cohort consisted of 113 (70.2%) patients who underwent hepatectomy and 48 (29.8%) liver-transplanted patients with a median age of 63 years (range, 17-87). Primary liver malignancy (34.2%) was the most common indication for hepatectomy and end-stage liver disease due to alcohol (11.2%) and viral hepatitis (7.5%) were the principal indications for liver transplantation. This study cohort consisted of patients with no underlying liver disease (31.7%), those with chronic liver disease (32.9%), and cirrhotic patients (34.2%). Table 1 summarizes the baseline characteristics of the patients included in the study.

Predictive Model: $HVPG_{10}$ score

Univariate analysis of the pre-operative noninvasive tests in the training cohort of 106 patients identified eight probable predictive factors of $HVPG_{10}$ namely, Hb, Plt, Bili, AST, INR, Fbg, Alkp and LSM. Independent predictive factors identified by multivariate regression were Bili,

INR, Alkp, and LSM and a score model was developed with these four predictors [Table 2]. The AUROC of the model was 0.9 and a cut-off of 0.15, determined by maximizing the sensitivity and specificity, demonstrated a diagnostic accuracy (DA) of 85.8%. When this model was applied to the internal validation cohort of 55 patients, AUROC was 0.93 (95%CI: 0.86-0.99) with 92.3% sensitivity, 85.7% specificity, 87.3% DA and 97.3% negative predictive value (NPV) as shown in Figure 2. The comparison of patients' characteristics in the training and validation cohorts is shown in Table 3.

In the overall cohort of 161 patients, the present model had an excellent discriminatory performance with an AUROC of 0.91 with 95%CI of 0.86-0.96. Sensitivity, DA, and NPV for the cut-off of 0.15 were 89.5%, 82.6% and 96.1% respectively. The positive and negative likelihood ratios were 4.6 and 0.1 respectively. Although LSM alone had a good performance (AUROC=0.86), the discriminative capacity was improved by addition of Bili, INR and Alkp into the model [Figure 3A and 3B]. Further, the calibration of the model was tested by Hosmer-Lemeshow goodness of fit test ($P=0.72$) and is illustrated in the Heat Map plot [Figure 3C].

HVPG₁₀ score would be calculated by multiplying the probability by 100. For example, the probability of 0.15 would result in HVPG₁₀ score of 15. In this study population with a pretest probability for HVPG₁₀ of 23.9%, posttest probabilities of positive (≥ 15) and negative (< 15) test results would be 58.7% and 2.9% respectively [Supplement 2]. Thus, the patients with HVPG₁₀ score < 15 would have a very low probability ($\leq 3\%$) having of HVPG ≥ 10 mmHg. Further, HVPG₁₀ score could accurately differentiate low-risk (LSM < 20 kPa+platelet count $\geq 150 \times 10^9/L$) and high-risk (LSM ≥ 20 kPa+platelet count $< 150 \times 10^9/L$) patients according to Baveno VI criteria for esophageal varices screening [Figure 4].

Discussion

The present model, HVPG₁₀ score, is an easy-to-use continuous scale tool based on simple parameters LSM, Bili, INR, and Alkp. It has demonstrated an excellent discriminatory performance in training, validation and overall cohorts. In this study, the patients had a wide spectrum of liver disease and function from patients with normal liver to decompensated cirrhotics and therefore, this model was constructed on the wide spectrum of HVPG due to various degrees of liver fibrosis. A HVPG₁₀ score cut-off value of 15 demonstrated an excellent DA, sensitivity, specificity, negative predictive value, positive and negative likelihood ratios. Thus, HVPG₁₀ score would be a very effective to rule-out HVPG₁₀ in an outpatient clinic with posttest probability of negative test of $\leq 3\%$ and avoid further unnecessary invasive tests. Further, the present score demonstrated discriminatory performance similar to Baveno VI criteria for esophageal varices.

Chronic liver disease is characterized by progressive liver tissue fibrogenesis and extensive vascular changes occurring within the liver and in the splanchnic vasculature that results in PH (\uparrow PVP).³¹ It is well established that PH is not only a mechanical consequence of altered hepatic architecture with increased hepatic vascular resistance but also a dynamic phenomenon characterized by net predominance of vasoconstrictors in hepatic microenvironment and increased portal perfusion through splanchnic arteriolar vasodilatation caused by endogenous vasodilators.^{32,33} This increase in PVP is usually measured indirectly by HVPG measurement transjugular catheterization of hepatic veins. Esophageal varices develop when HVPG increases beyond the threshold of 10 mmHg and clinical decompensation in the form bleeding, ascites, hepatic encephalopathy and renal dysfunction develop when HVPG increase over 12 mmHg. Recently, Baveno VI consensus proposed HVPG measurement as the gold standard method for diagnosing HVPG₁₀.²⁷ Thus, HVPG measurement and gastroesophageal endoscopy play an important role in the assessment of PH.^{28,34} However, these are invasive methods which require a specific set-up and expertise

due to a small but definite risk of complications and thus limiting their universal applicability.^{1,35} In a recent review, Berzigotti highlighted the need of simple non-invasive methods that would accurately and rapidly identify patients with low-risk of HVPG₁₀ (who could safely avoid invasive tests) and patients with HVPG₁₀ (at high risk of complications and varices requiring further testing), would constitute a step in the right direction of personalized medicine.¹⁶ Liver stiffness measured by TE has been studied extensively in this regard and LSM provided an accurate discrimination between with and without HVPG₁₀.^{13,14,16-22,27,28} However, an exact HVPG could not be reliably estimated by LSM and the choice of cut-off value remains controversial.^{16,36} A cut-off of 12-13 kPa had a high sensitivity (>90%) but it lacked the high specificity (>90%) of 21-22 kPa whereas a cut-off of 21-22 kPa had a low sensitivity and had a specificity similar to HVPG. Llop *et al* concluded that LSM between 12-21 kPa as a gray area and advocated HVPG measurement in all patients with compensated cirrhosis undergoing hepatectomy for liver tumors.³⁶ Therefore, Baveno VI consensus workshop concluded that HVPG measurement as the gold-standard method to diagnose HVPG ≥ 10 mmHg and in patients with virus-related CLD, LSM by TE (>20-25 kPa) could be used to identify patients with risk of having endoscopic signs of PH.²⁷ Further, the use of cut-offs leads to loss of relevant information as LSM is measured as a numerical continuous value (3-75 kPa).¹⁶ Various authors have attempted to improve upon the diagnostic performance of TE with addition various clinical and serological parameters such spleen size, platelet count and splenic stiffness. In recent publication of the 'Anticipate' study, Abraldes *et al* compared the performances of LSM alone, LSM to spleen size/platelet count ratio (LSPS), and platelet count-spleen size ratio in prediction of various clinical endpoints. It had the highest discrimination in identifying patients with high-risk of HVPG₁₀ and those with a low-risk of varices needing treatment. None of these three models identified patients with low-risk of HVPG₁₀ and all size varices.²³ Moreover splenomegaly is not a reliable sign of PH and

indirect evaluation of HVPG using platelet count, splenic size \pm esophageal varices has fallen out of favor with the hepatologists due to lack of specificity.^{37,38} Therefore, LSPS score where splenic size and platelet count are important constituents is unlikely to be applied into routine clinical practice. Another method proposed by Kim *et al* utilized serological tests such as albumin, INR and platelet count and indocyanine green retention at 15 minutes to estimate HVPG₁₀ in patients undergoing liver resection.¹⁵ In western countries, indocyanine green clearance is not routinely practiced in surgical practice and it has several disadvantages such as (1) test results are not reliable in patients with cholestasis and when the perfusion of the liver is altered for example in portal vein thrombosis, and (2) repeated measurements would not be feasible. Imaging methods have shown promise in evaluating HVPG. Doppler Ultrasonography and computed tomography (CT) were limited by their poor sensitivity and reproducibility. In addition, the risk of ionizing radiation has hampered the wide usage of CT.^{17,25} Although magnetic resonance imaging has recently emerged as an accurate and reliable method as it provides anatomical, structural and quantitative information, it requires a dedicated radiologist and specialized sequences to calculate the stage of fibrosis and HVPG limiting its routine use in the clinic.²⁶

The present study has several strengths. First, HVPG₁₀ score was constructed in a population with wide spectrum of HVPG and enabling its application in various clinical situations from surgical patients to esophageal varices screening using Fagan's nomogram could be used to rule-in or rule-out HVPG₁₀ as illustrated in Supplement 1. HVPG₁₀ score would be able to identify the patients at risk of post-operative complication and HCC recurrence after hepatectomy as the study population included surgical patients as well. This non-invasive score could also help in monitoring the disease progression on a continuous scale, and diagnose the progression of PH beyond the threshold of HVPG ≥ 10 mmHg. Secondly, inter-observer variability, even when performed by non-specialists, is reduced with the use LSM by

TE and serological parameters in the model. Third, the robustness of the HVPG₁₀ score was demonstrated by validating in an internal cohort, bootstrapping the analysis and calibrating of the predicted probability. Fourth, the performance of TE, which constitutes the principal component of this model, was ascertained in this mixed cohort of various etiologies similar to previously published series [Supplement 2]. Finally, the model could be developed into an internet or device-based application that can be applied to a patient at any given point in time. The limitations of this study were the low prevalence of the primary endpoint (HVPG₁₀) and HVPG was not measured in all patients who had a valid LSM. These facts should be considered while interpreting the predictive values. Other models like LSPS score and splenic stiffness could not be compared with the present score due to lack of information about splenic size and stiffness in the study. Though we used robust modelling with internal validation, the performance of HVPG score should be validated in an external cohort.

In conclusion, in a well-characterized population, we have demonstrated that a combination of LSM and LFT would predict HVPG as binary outcome. In particular, HVPG₁₀ score is an easy-to-use and readily available tool that can predict the individual risk of HVPG ≥ 10 mmHg and monitor disease progression and response to treatment in a continuous scale.

Acknowledgements

L'Association Nationale de Recherche et de la Technologie, Paris, France and Echosens™, Paris, France, funded this research.

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Table 1: Patients characteristics and noninvasive tests in the study

Variables	N=161
Age in years, median (IQR)	63 (54-69)
Gender, Male:Female	106 (65.8):55 (34.2)
Body mass index in kg/m ² , median (IQR)	25 (21-28)
Hepatectomy patients	113 (70.2)
▪ Primary liver malignancy	55 (34.2)
▪ Liver metastasis	43 (26.7)
▪ Benign tumors	9 (5.6)
Liver transplantation patients	48 (29.8)
▪ Alcoholic cirrhosis	18 (11.2)
▪ Virus-related cirrhosis	12 (7.5)
▪ Others	18 (11.2)
Model for end-stage liver disease score, median (IQR)	6 (6-10.5)
Blood hemoglobin in g/dL, median (IQR)	13 (11.4-14.2)
Platelet count in 10 ⁹ /L, median (IQR)	210 (120-277.5)
Serum fibrinogen in g/L, median (IQR)	3.5 (2.8-4.5)
International normalized ratio in log, median (IQR)	0.1 (0-0.3)
Prothrombin time in %, median (IQR)	85 (67-97)
Blood urea nitrogen mmol/L, median (IQR)	5 (4.1-6.9)
Serum creatinine in μmol/L, median (IQR)	71 (63-85.3)
Serum total bilirubin in μmol/L, median (IQR)	11 (8-24)
Serum aspartate aminotransferase in IU/L, median (IQR)	39 (28-56)
Serum alanine aminotransferase in IU/L, median (IQR)	32 (20-50)
Serum γ-glutamyl transpeptidase in IU/L, median (IQR)	120 (48-246.5)
Serum alkaline phosphatase in IU/L, median (IQR)	113 (75-189)
Serum albumin in g/L, median (IQR)	37 (32.7-40.5)
Liver stiffness measurement in kPa, median (IQR)	9.5 (5.9-21.3)
Hepatic venous pressure gradient in mmHg, median (IQR)	5 (3-9)
METAVIR fibrosis score of non-tumoral liver parenchyma:	
▪ F0	26 (16.1)
▪ F1	27 (16.8)
▪ F2	37 (23.0)
▪ F3	16 (9.9)
▪ F4	55 (34.2)

#Data are expressed as counts (percentage) unless otherwise stated; IQR-interquartile range

Table 2: Univariate and multivariate analyses of the patients in the training cohort (n=106)

Variables	Univariate Analysis		Multivariate Analysis			
	<i>p</i> *	OR	<i>p</i>	OR	95% CI	
					Lower	Upper
Age in years, mean±SD	0.401	0.78				
Male gender, N (%)	0.313	0.36				
Body mass index in kg/m ² , mean±SD	0.231	0.08				
Blood hemoglobin in g/dL, mean±SD	<0.001	37.9				
Platelet count in 10 ⁹ /L, mean±SD	<0.001	1.73				
Serum fibrinogen in g/dL, mean±SD	0.002	2.56				
International normalized ratio in log, mean±SD	<0.001	0.08	0.007	41.36	3.94	857.92
Serum total bilirubin in μmol/L, mean±SD	0.036	0.26	0.048	0.99	0.99	1.00
Serum aspartate aminotransferase in IU/L, mean±SD	0.087	0.21				
Serum alanine aminotransferase in IU/L, mean±SD	0.141	0.46				
Serum γ-glutamyl transpeptidase in IU/L, mean±SD	0.368	0.36				
Serum alkaline phosphatases in IU/L, mean±SD	0.065	0.22	0.018	1.00	1.00	1.01
Liver stiffness measurement in kPa, mean±SD	<0.001	0.06	0.005	1.06	1.02	1.11
Hepatic venous pressure gradient in mmHg, mean±SD	<0.001					

*The variables with *P*<0.15 in the univariate analysis were entered into multivariate binary logistic regression by backward entry of Wald method; HVPg-hepatic venous pressure gradient; OR-odds ration; CI-confidence interval; SD-standard deviation

Table 3: Comparison of the patients' characteristics and serological tests between the training and internal validation set

Variables [#]	Training Set N=106 (65.8)	Validation Set N=55 (34.2)	<i>P</i> *
Age in years, mean±SD	61.2 ± 12.3	57.0 ± 16.6	0.17
Male gender	34 (32.1)	21 (38.2)	0.49
Body mass index in kg/m ² , mean±SD	24.9 ± 5.1	24.5 ± 3.9	0.76
Blood hemoglobin in g/dL, mean±SD	12.6 ± 2.1	13.1 ± 2.4	0.21
Platelet count in 10 ⁹ /L, mean±SD	212.2 ± 113.7	209.1 ± 124.3	0.74
Serum fibrinogen in g/L, mean±SD	3.7 ± 1.3	3.8 ± 1.6	0.69
International normalized ratio in log, mean±SD	0.2 ± 0.3	0.2 ± 0.3	0.68
Prothrombin time in % , mean±SD	78.7 ± 21.1	80.2 ± 21.6	0.54
Serum total bilirubin in μmol/L, mean±SD	44.1 ± 127.8	46.36 ± 114.0	0.63
Serum aspartate aminotransferase in IU/L, mean±SD	52.1 ± 48.8	45.8 ± 29.7	0.27
Serum alanine aminotransferase in IU/L, mean±SD	48.4 ± 67.9	34.4 ± 21.9	0.19
Serum γ-glutamyl transpeptidase in IU/L, mean±SD	259.9 ± 403.1	165.8 ± 205.7	0.12
Serum alkaline phosphatase in IU/L, mean±SD	186.6 ± 220.2	149.8 ± 116.6	0.77
Serum albumin in g/L, mean±SD	35.7 ± 6.4	37.5 ± 7.5	0.40
Liver stiffness measurement in kPa, mean±SD	19.3 ± 19.4	16.4 ± 19.9	0.05
Hepatic venous pressure gradient in mmHg, mean±SD	7.0 ± 5.8	6.7 ± 6.8	0.23

[#]Data expressed as counts (percentage) unless stated, *Wilcoxon test, SD-standard deviation

Figure 1: Study Flowchart

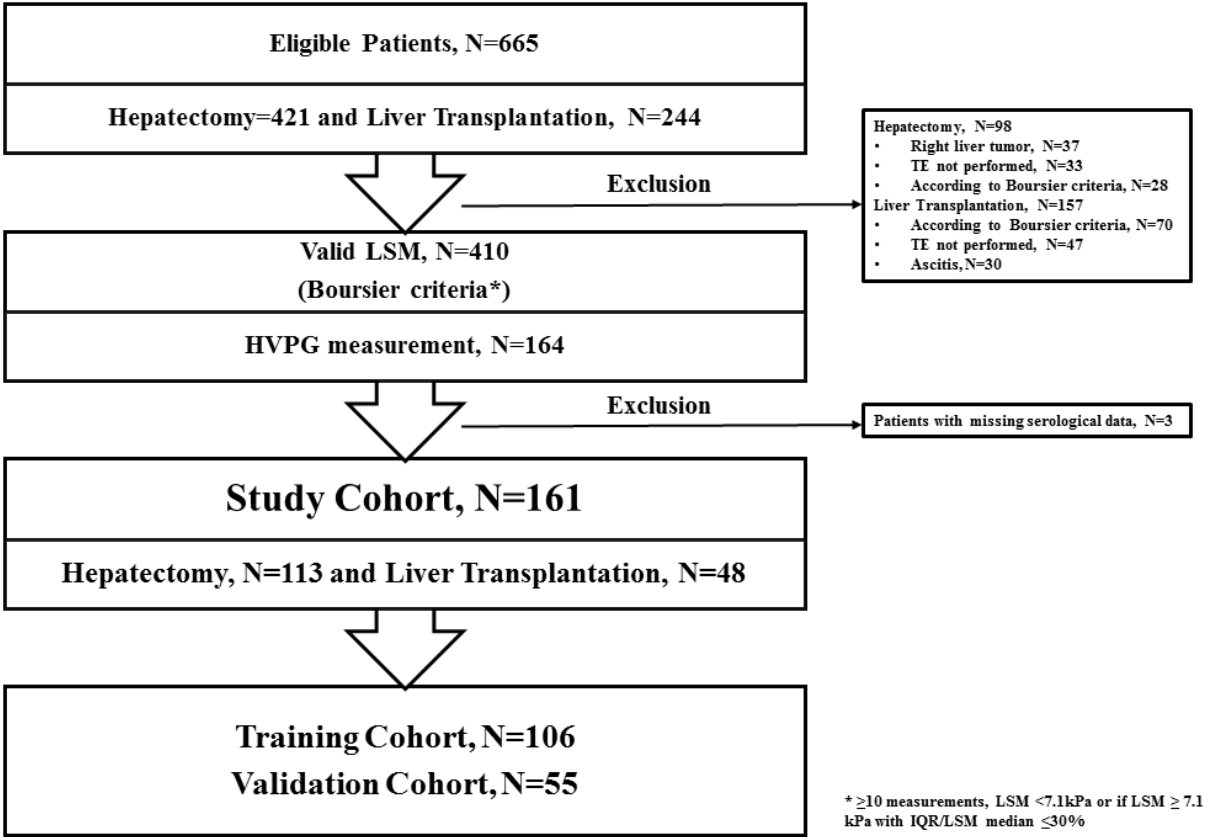


Figure 2: Receiver operating characteristic analysis of hepatic venous pressure gradient score in the training and validation cohorts

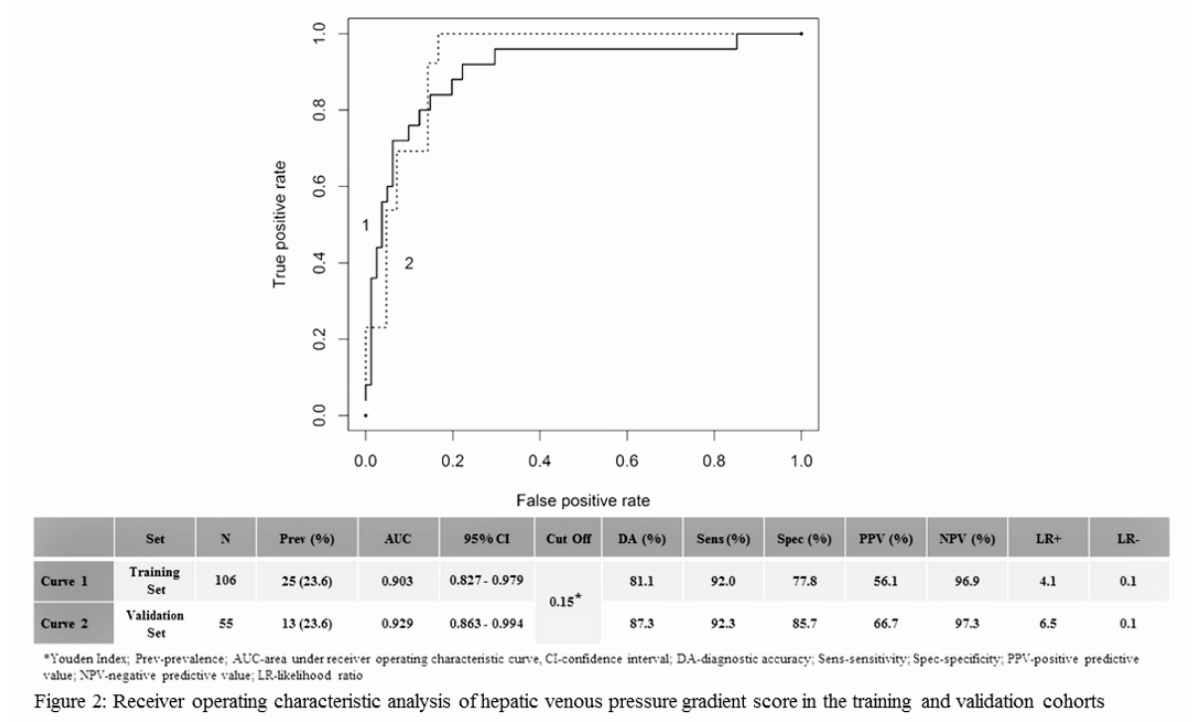


Figure 2: Receiver operating characteristic analysis of hepatic venous pressure gradient score in the training and validation cohorts

Figure 3: Performance of hepatic venous pressure gradient (HVPG₁₀) score bootstrapped at 500 in the overall cohort of 161 patients and Heat map plot of the score at 5000 re-samplings with histogram below illustrating the frequency and distribution of the observations

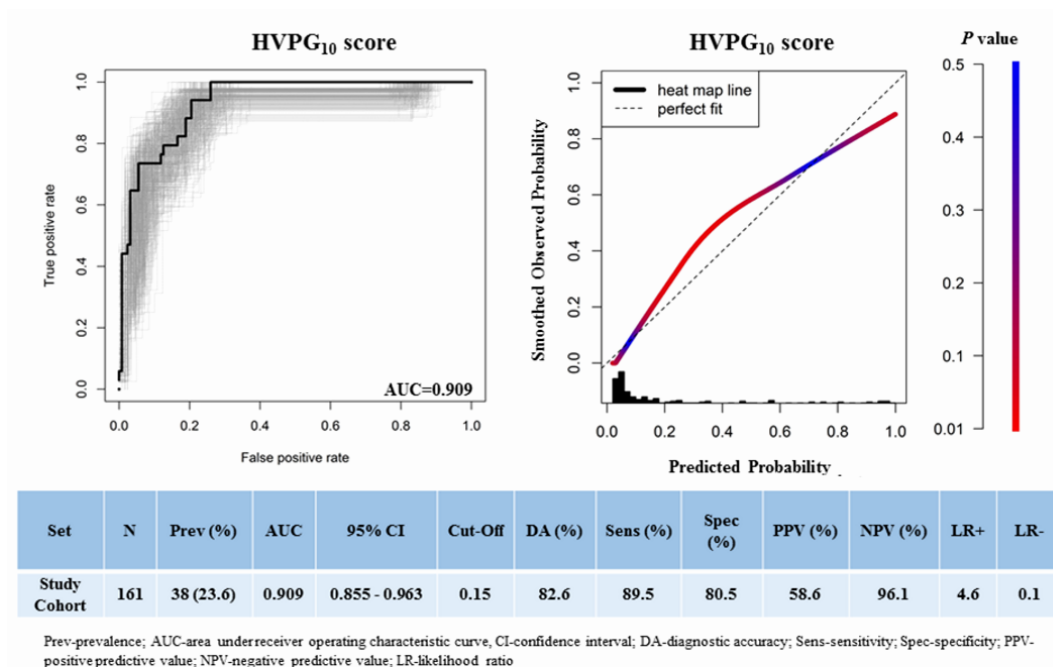


Figure 3: Performance of hepatic venous pressure gradient (HVPG₁₀) score bootstrapped at 500 in the overall cohort of 161 patients and Heat map plot of the score at 5000 re-samplings with histogram below illustrating the frequency and distribution of the observations.

Figure 4: Bar chart demonstrating the ability of hepatic venous pressure gradient (HVPG₁₀) score to classify patients with low and high-risk of esophageal varices by Baveno VI criteria

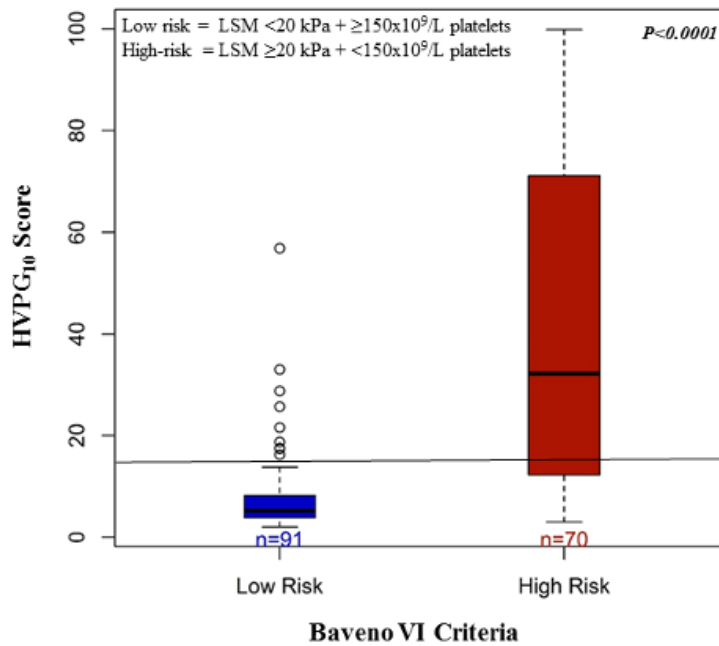
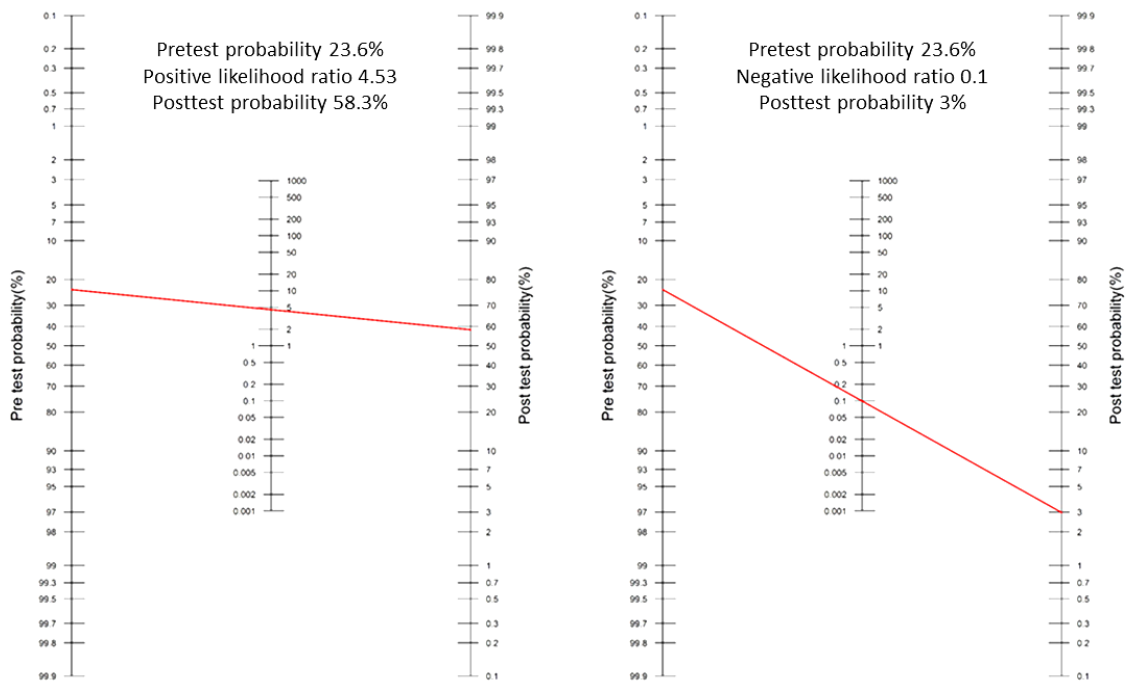


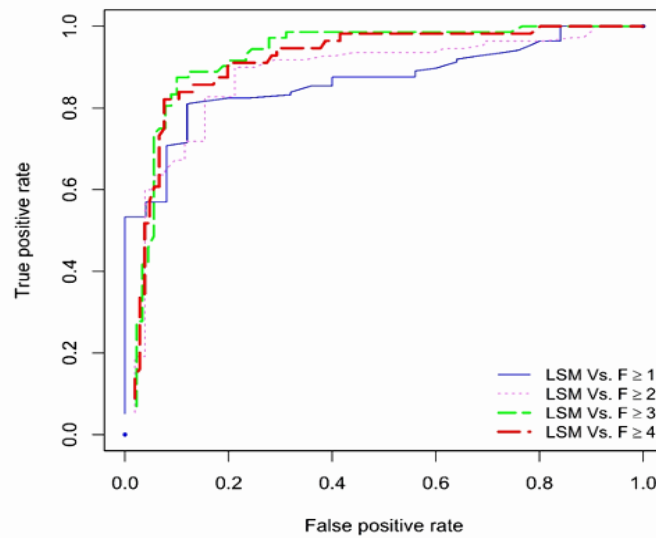
Figure 4: Bar chart demonstrating the ability of hepatic venous pressure gradient (HVPG₁₀) score to classify patients into low and high-risk of esophageal varices by Baveno VI criteria

Supplement 1: Fagan’s nomogram for pre-test and post-test probability of hepatic venous pressure gradient score



Supplement 1: Fagan’s nomogram for pre-test and post-test probability of hepatic venous pressure gradient score

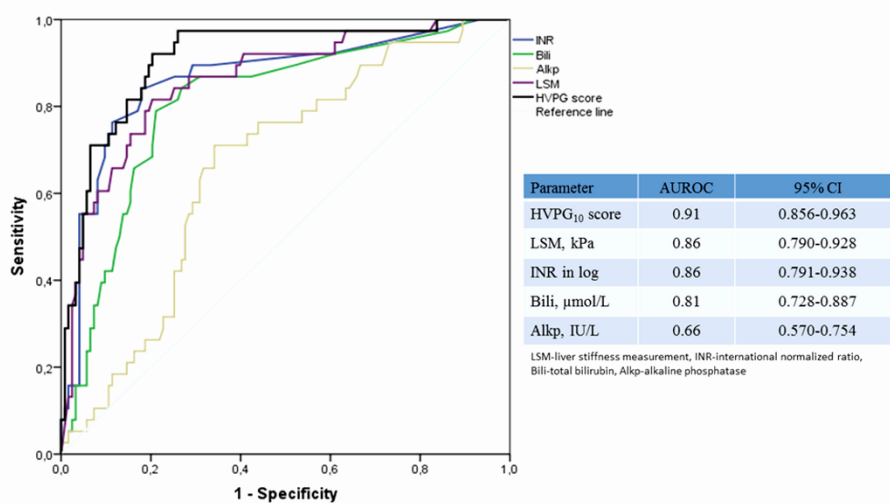
Supplement 2: Receiver operating characteristic analysis of liver stiffness measurement for the fibrosis grades according to METAVIR scoring system



METAVIR Fibrosis Stage	Prevalence,%	Area under ROC	95% Confidence Interval
F \geq 1	83.9	0.866	0.804-0.927
F \geq 2	67.1	0.876	0.815-0.938
F \geq 3	44.1	0.925	0.881-0.969
F=4	34.2	0.914	0.866-0.961

Supplement 2: Receiver operating characteristic analysis of liver stiffness measurement for the fibrosis grades according to METAVIR scoring system.

Supplement 3: Comparison of the discriminatory performances of hepatic venous pressure gradient (HVPG₁₀) score, liver stiffness measurement (LSM), international normalized ratio (INR), total bilirubin (Bili), and alkaline phosphatase (Alkp) by receiver operating characteristic analysis.



Supplement 3: Comparison of the discriminatory performances of hepatic venous pressure gradient (HVPG₁₀) score, international normalized ratio, total bilirubin, alkaline phosphatase and liver stiffness measurement by receiver operating characteristic analysis

Supplementary results:

Table 1: Patients characteristics and non-invasive tests in the external cohort

Variables*	N = 73(100)
Age in years, median(IQR)	60(51-70)
Gender, male : female	52(71) : 21(29)
Body mass index in kg/m ² , median(IQR)	26(23.7-28.1)
Etiology	
▪ Viral hepatitis	60(83)
▪ Alcoholic liver disease	12(16)
▪ Others	1(1)
Blood hemoglobin in g/dL, median(IQR)	NA
Platelet count in 10 ⁹ /L, median(IQR)	94(70-125)
Serum fibrinogen in g/L, median(IQR)	NA
International Normalized ratio in log, median(IQR)	0.20(0.13-0.28)
Prothrombin time in %, median(IQR)	54.3(48.6-56.5)
Blood urea nitrogen mmol/L, median(IQR)	6.7(4.3-9.4)
Serum creatinine in μmol/L, median(IQR)	65.1(55.4-75.0)
Serum total bilirubin in μmol/L, median(IQR)	16.3(12- 29)
Serum aspartate aminotransferase in IU/L, median(IQR)	71.5(39.5-101.5)
Serum alanine aminotransferase in IU/L, median(IQR)	60(34-95)
Serum γ-glutamyl transpeptidase in IU/L, median(IQR)	66(41-100)
Serum alkaline phosphatase in IU/L, median(IQR)	102(76-147)
Serum albumin in g/L, median(IQR)	38(35-42)
Liver stiffness measurement in kPa, median(IQR)	22.3(16.1-35.3)
Hepatic venous pressure gradient in mmHg, median(IQR)	13(11-16)
METAVIR fibrosis score	
▪ F0	
▪ F1	
▪ F2	NA
▪ F3	
▪ F4	

*Data expressed as counts (percentage) unless specified, IQR-interquartile range, NA-not available

Table 2: Comparison of the patients' characteristics and serological tests between the study cohort and external validation cohort

Variables#	Training Set N = 161	Validation Set N = 73	<i>P</i> *
Age in years, mean±SD	59.8±14.0	60.1±10.5	0.64
Male gender, N(%)	106(66)	52(71)	0.51
Body mass index in kg/m ² , mean±SD	24.8±4.7	26.1±3.3	0.02
Platelet count in 10 ⁹ /L, mean±SD	211±117	100±43	<0.001
International normalized ratio in log, mean±SD	0.22±0.32	0.19±0.22	0.01
Prothrombin time in %, mean±SD	79.2±21.2	49.8±23.0	<0.001
Blood urea nitrogen mmol/L, mean±SD	6.11±3.57	8.61±8.60	0.02
Serum creatinine in μmol/L, mean±SD	89.2±71.7	68.6±20.8	<0.001
Serum total bilirubin in μmol/L, mean±SD	44.9±122.9	32.6±66.5	<0.001
Serum aspartate aminotransferase in IU/L, mean±SD	49.9±43.3	76.7±42.3	<0.001
Serum alanine aminotransferase in IU/L, mean±SD	43.6±56.9	72.3±48.2	<0.001
Serum γ-glutamyl transpeptidase in IU/L, mean±SD	227.8±350.6	104.5±142.6	<0.001
Serum alkaline phosphatase in IU/L, mean±SD	174.1±191.6	126.3±76.2	0.3590
Serum albumin in g/L, mean±SD	36.3±6.8	38.0±4.9	0.09
Liver stiffness measurement in kPa, mean±SD	18.3±19.6	26.8±14.4	<0.001
Hepatic venous pressure gradient in mmHg, mean±SD	6.9±6.2	15.3±14.9	<0.001

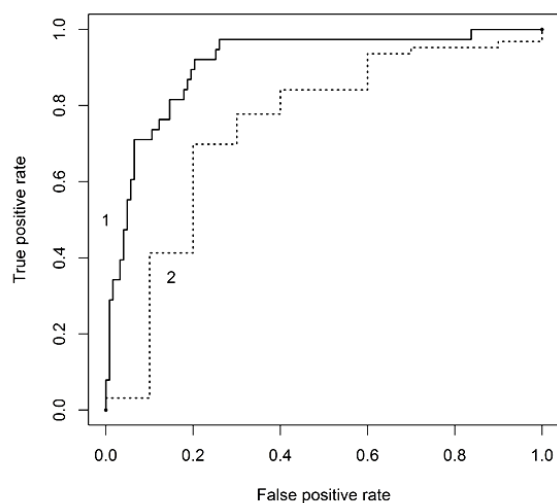
#Data expressed as counts (percentage) unless stated; *Wilcoxon test, SD-standard deviation

Table 3: Independent predictors of drop-out from the waiting list of liver transplantation in patients with MELD ≤ 15 (N=64)

Variables	Prevalence (%)	Drop-out					
		Uni-variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
			<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI
Recurrent HCC	No 44 (68.8) Yes 20 (31.3)	0.7					
Waiting time, months	<12 49 (76.6) ≥ 12 15 (23.4)	0.18					
Platelet count, $10^9/L$	<250 57 (89.1) ≥ 250 7 (10.9)	0.07	1.89	0.03	6.67 (1.18-37.78)	0.01	-0.1-22.78
α -fetoprotein, ng/mL	<100 59 (92.2) ≥ 100 5 (7.8)	0.9					
HVPG ₁₀ Score	<50 43(67.2) ≥ 50 9(14.1)	0.06	1.83	0.02	6.25 (1.29-30.35)	0.01	0.18-3.99
CAP, dB/m	≥ 240 22 (34.4) <240 20 (31.3)	0.9					
TACE/Ablation/Resection	Yes 83 (79.1) No 22 (20.9)	0.5					
Constant					<0.001	0.001	-2.86--0.86

*All unrelated variables significant at $\leq 20\%$ in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, CAP-controlled attenuation parameter, HVPG-hepatic venous pressure gradient, TACE-transarterial chemoembolization

Figure 1: Comparison of the performance of HVPG₁₀ score in the study and external validation cohorts



Curve	Set	N	Prev (%)	AUC	95% CI	Cut Off	DA (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR+	LR-
1	Study Cohort	161	38 (23.6)	0.909	0.855 - 0.964	0.15	82.0	86.8	80.5	57.9	95.2	4.5	0.2
2	External Cohort	73	63 (86.3)	0.741	0.546 - 0.936		63.0	60.3	80.0	95.0	24.2	3.0	0.5

Figure 2: Relationship between portal pressure and liver stiffness measured by transient elastography

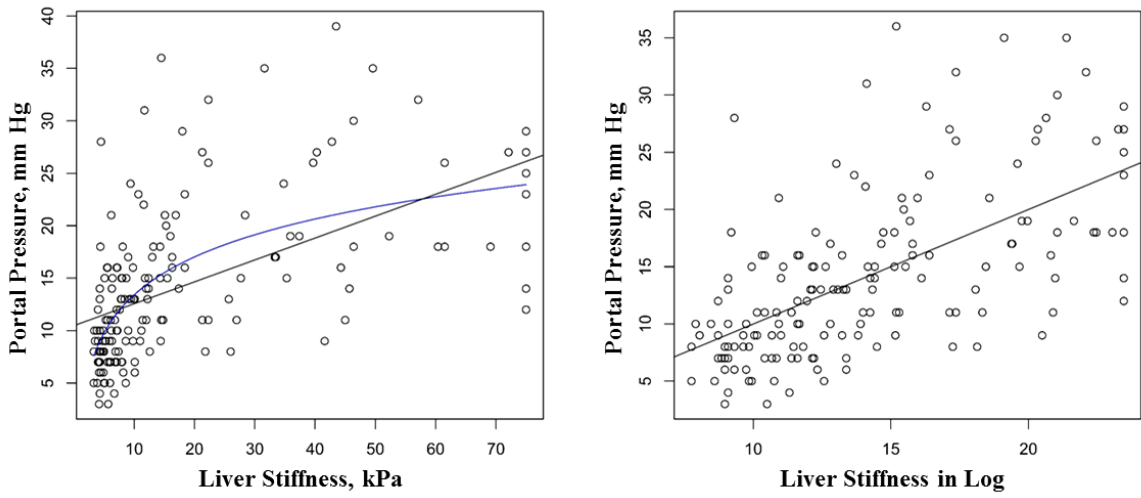
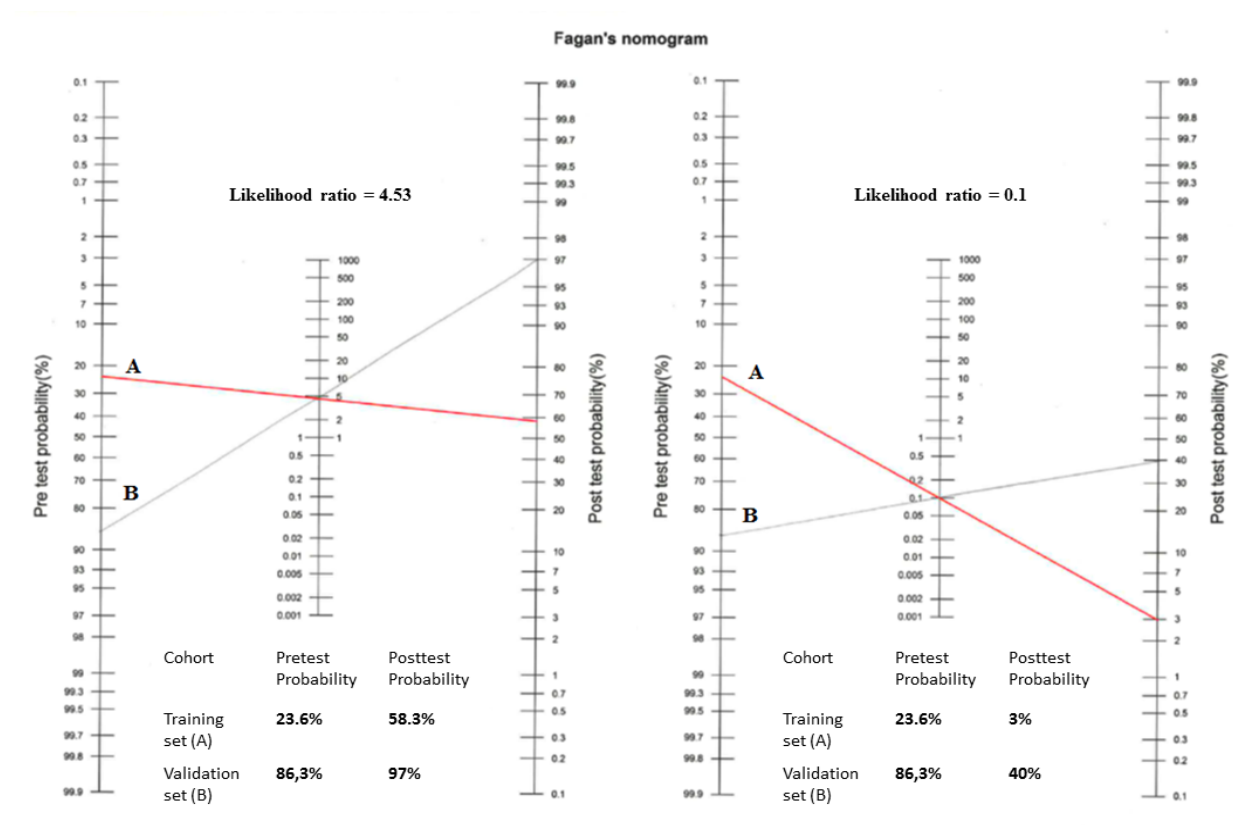


Figure 3: Fagan nomogram the performance of HVPG₁₀ score in the study and external cohorts



Post-operative Outcomes

Article 4:

Prospective study of patients undergoing hepatectomy for various indications to develop and validate a nomogram predictive of 90-day severe morbidity and 90-day mortality

Authors:

Muthukumarassamy Rajakannu,^{1,2,3} MBBS, MS, DNB, MRCS, Daniel Cherqui,^{1,3} MD, Nicolas Golse,¹ MD, Oriana Ciacio,¹ MD, Gabriella Pittau,¹ MD, Marc Antoine Allard,¹ MD, Antonio Sa Cunha,^{1,3} MD, Denis Castaing,^{1,2,3} MD, Didier Samuel,^{1,2,3} MD, PhD, Catherine Guettier,^{1,2,3} MD, PhD, René Adam,^{1,3,4} MD, PhD, Eric Vibert,^{1,2,3} MD, PhD

Affiliations:

¹Centre Hépato-Biliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

Running title: Nomograms to predict post-hepatectomy severe morbidity and mortality.

Keywords: Liver resection, liver stiffness, post-operative complication, transient elastography.

Financial support: L'Association Nationale de la Recherche et de la Technologie, Paris, France and EchosensTM, Paris, France funded this study.

Correspondance:

Prof. Eric Vibert

Centre Hépato-Biliaire

AH-HP Hôpital Paul Brousse

12 Avenue Paul Vaillant Couturier

94800 Villejuif

France

Abstract

Background: Post-operative complications affects the long-term survival and quality of life in patients undergoing liver resection (LR). No model has yet been validated to predict 90-day severe morbidity and mortality.

Methods: Prospective recruitment of patients undergoing LR for various indications was performed. Pre-operative clinical and laboratory data including liver stiffness (LS) and indocyanine green retention and intra-operative parameters were analyzed to develop predictive nomograms for post-operative severe complications and mortality. An external validation was performed for two nomograms by calibration plots.

Results: The most common indications in 418 LRs performed were colorectal metastases [35.6%], hepatocellular carcinoma [25.6%] and benign liver tumors [14.3%] with 39.2% of patients had ≥ 3 segments resected. Severe morbidity and mortality were observed in 20.8% and 2.2% patients, respectively during 90-day post-operative period. Post-hepatectomy liver failure was observed in 19 [4.5%] patients resulting in death in four. Independent predictors of 90-day severe morbidity were age [Odds ratio (OR):1.02, $p=0.06$], LS [OR:1.23, $p=0.04$], number of resected segments [OR:1.28, $p=0.004$], and operative time [OR:1.01, $p=0.01$]. Independent predictors of 90-day mortality were diabetes mellitus [OR:6.6, $p=0.04$], tumor size >50 mm [OR:4.8, $p=0.08$], LS ≥ 22 kPa [OR:7.0, $p=0.04$], and operative time 6 hours [OR:6.1, $p=0.05$]. Two nomograms were developed using these predictors and validated using an external cohort by testing the Goodness of fit in calibration plots [$p=0.7$ for severe morbidity, $p=0.6$ for mortality].

Conclusion: Proposed nomograms would enable personalized approach in surgical management of patients with liver tumors according to their clinical profile and individual center's expertise.

Introduction

Liver resection (LR) is the standard of care treatment in patients having primary and metastatic liver tumors. With improvements in the surgical technique and peri-operative managements of patients undergoing LR, there has been a considerable improvement in the safety and post-operative outcomes over the last decade.¹⁻³ Peri-operative morbidity and mortality rates has reduced to 10-20% and 0-2%, respectively in high volume hepatobiliary centers but a few recent population-based analyses have reported morbidity rates of 20-30% and mortality rates of 5-10% allowing room for considerable improvements.³⁻¹² Post-operative complications have a major impact on the quality of life and long-term survival of patients after LR in addition to increasing health care costs.^{6,7,13} A previous study from our center had demonstrated that post-hepatectomy liver failure (PHLF) in patients undergoing LR for colorectal liver metastasis was an important poor prognostic factor for 2-year survival.⁶ Another multicentric study from France reported similar results in patients operated for intrahepatic cholangiocarcinoma wherein 5-year overall survival significantly reduced in patients developing post-operative severe complications.⁷ Several publications have reported various risk factors for adverse post-operative outcomes and some authors have published risk models for various complications and mortality including one with >20 pre- and intra-operative factors to predict individual complications.^{4,6,7,11,12,14-19} Recently, liver stiffness (LS) measured by transient elastography (TE) has been demonstrated to be an reliable non-invasive predictor of post-operative complications.²⁰⁻²³ However, none of these models have been validated in an external cohort and universally utilized to risk-stratify patients planned for hepatectomy. The aim of the present study was to develop and validate easy-to-use LS-based nomograms predictive of 90-day post-operative severe morbidity and 90-day mortality to enable personalized approach in the managements of patients planned for LR.

Patients and Methods

Consecutive patients undergoing LR in our center from October 2014 to August 2016 were recruited prospectively. Clinical, laboratory and peri-operative data were collected at inclusion and at every post-operative follow-up visits using an online hepatectomy database (https://hpbchir.iplesp.upmc.fr/ehpbchir/gestion_site/). Final follow-up was on November 30, 2016. The Ethics Committee of the hospital approved the study protocol and from each patient gave an informed consent according to the principles of the Helsinki Declaration.

Liver resection

Pre-operative assessment: Pre-operative evaluation consisted of physical examination, LS measurement, laboratory tests including liver function tests, imaging studies like ultrasonography, contrast-enhanced abdominal computer tomography (CECT) ± volumetry, magnetic resonance imaging (MRI), LS, and indocyanine green retention at 15 minutes (ICG-R15). FibroScan[®] Touch 502 with M/XL probes (Echosens[™], Paris, France) was utilized to perform TE pre-operatively in patients programmed for hepatectomy. LS was measured on the right lobe of the liver through intercostal spaces under fasting conditions and median value of ≥ 10 valid measurements was kept as representative of LS in kilopascal (kPa).²¹⁻²³ The objective of LR was to resect all visible lesions with tumor-free margins while leaving behind adequate liver parenchyma to ensure normal post-operative liver function except in benign liver tumors where enucleation were preferred. Usually hepatectomy was performed in patients with well-compensated liver function (Child Pugh A/B) with platelet count $\geq 80,000$. When future liver remnant (FLR) volume would not be sufficient, portal vein embolization (PVE) was performed to ensure FLR $\geq 0.5\%$ of body weight or $\geq 20\%$ of standard liver volume in non-cirrhotic liver.^{22,24}

Liver metastasis: Once the tumor board confirmed the radiological diagnosis of liver metastasis, our policy was to propose potentially curative resections of all both hepatic and extra-hepatic disease (EHD) with combination chemotherapy (CTx) ± biologic agents. Response to CTx was evaluated every 2-3 months according to response evaluation criteria in

solid tumors criteria and with serum tumor markers.²⁵ When downsizing by CTx would not be sufficient to allow curative resection owing to contact with major vessel or bile duct, three specific techniques were practiced to increase resectability. Portal vein embolization (PVE) was performed when estimated FLR volume was <30% especially in patients who have received prolonged CTx.²⁶ Hemi-hepatectomy would be combined with the use of radiofrequency ablation (RFA) for few (≤ 3) contralateral, deeply located lesions ≤ 3 cm in diameter. Two-stage hepatectomy was considered when multinodular bi-lobar metastases were neither be completely resected by a single procedure nor treated with hepatectomy combined with ablation.²⁷ Liver-first strategy was utilized in certain cases of synchronous presentation of colorectal cancer liver metastasis when colorectal primary tumor was not complicated and hepatic tumor burden was significant.²⁸ Disease progression during CTx was considered as a relative contra-indication to curative surgery.

Hepatocellular carcinoma: LRs were performed when MELD ≤ 12 , platelet count $\geq 80,000$ and ICG-R15 $\leq 20\%$.²² In general, anatomic resections were preferred to wedge resections. Transarterial chemoembolization followed by PVE was the treatment strategy when planning major hepatectomy in patients with cirrhosis.²⁶

Cholangiocarcinoma: In patients with peri-hilar cholangiocarcinoma, pre-operative biliary drainage was performed in patients with jaundice and/or before PVE to ensure adequate hypertrophy of FLR. Systematic resections of portal vein bifurcation and extra-hepatic bile ducts was carried out in these patients. *En bloc* lymphadenectomy was performed in patients with intra-hepatic and peri-hilar cholangiocarcinoma.

Operative technique: The technique of hepatectomy has been standardized in our unit since 1982.^{24,30} At surgery, exploration of the abdominal cavity was performed to detect extrahepatic spread. Systematic intra-operative liver ultrasound was carried out in all patients. Enlarged hepatic lymph nodes and any suspicious nodules were excised for frozen section

histology. Hepatic venous pressure gradient (HVPG) was measured at individual surgeon's discretion before the commencement of hepatectomy during laparotomy. Parenchymal dissection was done using the ultrasonic dissector (Cavitron Ultrasonic Surgical Aspirator, Valley Lab Inc., Boulder, USA), and resections are usually performed under intermittent clamping of the portal triad (Pringle maneuver).^{22s} Concomitant extra-hepatic resections for EHD (peritoneal or lymph node metastasis) were performed if it would ensure curative R0 resections.

Post-operative course: After LR, follow-up was at one month and then every four to six months with physical examination, blood tests, serum tumor markers, and imaging. Usually a CECT scan was performed at 1-week and 1-month after hepatectomy and then alternating abdominal ultrasonography and CECT/MRI were performed every four months along with serum tumor markers and liver function tests. The patients with liver metastasis were usually offered a course of chemotherapy after surgery. In case of local or distant recurrence, resection was performed if treatment strategy could be curative.³¹

Definitions

Post-operative complications were graded according to the Clavien-Dindo classification of surgical complications and severe morbidity was defined by the presence >Grade 2 complication in the 90-day post-operative period.^{5,32} Any death in the 90-day post-operative period was considered as post-operative mortality.⁵

Statistical analysis

Discrete variables are reported as counts (percentage) and parametric variables as median (range). Patients were compared by unpaired Student's *t* test for continuous variables with parametric distribution, Mann–Whitney *U* test for those with nonparametric distribution, and *Chi* square or Fischer's exact test for quantitative variables. The potential pre- and intra-operative risk factors of associated with severe morbidity and mortality that were identified by univariate logistic regression were entered into multivariate binary logistic regression by

backward elimination (Wald) method and the variables that remained in the model were considered as independent predictors of the binary outcome. Internal validation was performed by bootstrapped (1000) multivariate logistic regression. Receiver operating characteristic (ROC) analysis with area under ROC curve as main readout was performed to test the model performance. Calibration plots assessed the performance of nomograms in the training and validation cohorts. SPSS 21.0 package (SPSS Inc., Chicago, IL) and R statistical package version 3.2.5 were utilized to perform the analysis. p value <0.05 was considered significant.

Results

Pre-operative and operative details in the study cohort

The most common indications in 418 LRs (58.4% men; 41.6% women) performed during the study period were colorectal metastasis (35.6%), HCC (25.6%), benign liver tumors (14.5%), non-colorectal liver metastases (8.9%), peri-hilar (4.8%) and intra-hepatic cholangiocarcinoma (4.6%). Median (range) age of the resected patients was 62 (16-87) years. Majority of patients (78%) undergoing LR have concomitant co-morbidities like diabetes mellitus, hypertension and half of the patients had received pre-operative anti-tumoral treatment including transarterial chemoembolization (6%) and chemotherapy (44.3%). Median (range) of size and number tumors resected were 27.5(0-190) mm and 1(0-14), respectively. Pre-operative TE was performed successfully in 95 patients with valid LS evaluation possible in 320 patients (76.6%). In 70 patients (16.7%) due to previous right hepatectomy or due to the presence of large right liver tumors, TE was not feasible and in 28 patients (6.7%) measurement were considered invalid (IQR/M $\geq 30\%$). Minor hepatectomy (≤ 2 segments) was performed in majority of 254 patients (60.8%) and major hepatectomy (>2 liver segments) in 164 patients (39.2%). Median (range) of LS was 6.4 (2.7-75) kPa while median (range) of ICG-R15 was 8.4 (0.5-54.1) %. Majority of the LRs (80.4%) was performed by laparotomy and 19.6% of LR was by laparoscopic approach. The types of LRs

performed were partial hepatectomy (33.9%), right (extended) hepatectomy (21.1%), left (extended) hepatectomy (16.5%), uni-segmentectomy (13.6%), bi-segmentectomy (13.2%), and central hepatectomy (1.7%). Two-stage hepatectomy was carried out in 12 patients (2.9%). Concomitant extra-hepatic or vascular procedures were performed in 59 patients (14.1%). Most of the patients (48.1%) undergoing LR had no or minimal underlying liver disease. Advanced liver fibrosis (F3) and cirrhosis were present in 23.7% of the patients. One patient who developed severe PHLF after right hepatectomy extended to segment one and four for peri-hilar cholangiocarcinoma was transplanted. Table 1 summarizes the demographic, laboratory, and operative details of the resected patients along with their post-operative outcomes during this study.

Characteristics of the external cohort

Supplementary table 1 summarizes the clinical, laboratory, operative details and post-operative outcomes of 113 patients (98 men, 15 women) from an external cohort who underwent LR during the period 2012-2016 for various indications and had pre-operative LS measurement. The most common indications for hepatectomy were HCC (81.4%) and intra-hepatic cholangiocarcinoma (8.8%). Median (range) age of patients was 64 (29-80) years. Median (range) of LS was 13.6 (2.5-75) kPa. The most common types of LRs were partial hepatectomy (60.2%) and uni-segmentectomy (8.8%). Major hepatectomy was performed in 12 patients (10.6%) and median (range) number of Couinaud segments resected was one (0-6). Severe morbidity was observed in 19.5% of patients with five patients (4.4%) dying in the 90-day post-operative period. The severe complications observed were hemorrhage (7), ascites (6), renal insufficiency (6), jaundice (5), sepsis (4), biliary fistula (3), intra-abdominal collection (3), encephalopathy (2), pulmonary infection (1), pulmonary embolism (1), portal vein thrombosis (1), and acute pulmonary edema (1). The causes of death were not available.

The comparison of the study (training) cohort with the external validation cohort is summarized in the supplementary table 2.

90-day severe morbidity: Severe morbidity was observed in 87 patients (20.8%) with 19.6% hepatic having hepatic decompensation (ascites, jaundice, and/or encephalopathy) and 4.5% developing PHLF by 50-50 criteria or peak bilirubin >7 mg/dL.^{33,34} The severe complications observed were intra-abdominal collection (47), jaundice (36), sepsis (30), ascites (22), biliary fistula (21), pulmonary infection (16), hemorrhage (14), renal insufficiency (14), encephalopathy (8), portal vein thrombosis (6), cardiac insufficiency (4) and acute pulmonary edema (2). Independent predictors analyzed as continuous variables were age [Odds ratio (OR):1.02, $p=0.06$], LogLS [OR:1.23, $p=0.04$], number of resected segments [OR:1.28, $p=0.004$], and operative time [OR:1.01, $p=0.01$] as shown in Table 2. A predictive nomogram was constructed with age in years, LogLS, number of segments to be resected and estimated operative time (Figure 1). The AUROC of this model was 0.70 (Supplementary Figure). The nomogram had an excellent Goodness of fit in the study cohort and an external validation cohort with p value of 0.64 and 0.70, respectively (Figure 2).

90-day mortality: Nine patients (2.2%) patients died during 90-day post-operative period with PHLF (4) being most cause followed by biliary sepsis (1), pulmonary sepsis (1), hepatic decompensation (1) and suspected cardiac event in two patients after discharge from hospital. Independent predictors analyzed as categorical variables were diabetes mellitus [OR: 6.16, $p=0.04$], tumor size >50 mm [OR: 4.78, $p=0.08$], LS ≥ 22 kPa [OR: 7.02, $p=0.04$], and operative time ≥ 360 min [OR: 6.06, $p=0.05$] as shown in Table 3. A nomogram with AUROC 0.80 was developed using three parameters - diabetes mellitus, LS, and estimated operative time (Figure 3). There was a good calibration of this model in both the study cohort and the external cohort with p value of 0.80 and 0.60, respectively (Figure 4).

Discussion

In the era of personalized medicine, the present study has provided two nomograms capable of predicting accurately the risk of post-operative severe morbidity and mortality in patients undergoing LR for various indications. The factors included in the models were readily available, pre-operative clinical parameters and a factor under surgeon's control – estimated operation time, a surrogate marker for the complexity of planned LR. Therefore, these nomograms would enable a surgeon to estimate the patient's risk pre-operatively and modify the type of LR or offer an alternative treatment in a patient when the risk of severe morbidity or mortality is above the acceptable limits of their center. Thus, the surgeons can use them to plan the operative strategy, obtain informed consent, and adapt surgical technique according to an individual patient's risk and to the expertise available in their centers.

Liver stiffness by TE was an effective non-invasive predictor of post-operative outcomes in patients undergoing LR. Wong *et al* demonstrated that LS was a better predictor of severe morbidity after LR than ICG-R15 in patients operated for HCC and they proposed a LS cut-off 12 kPa.²⁰ Similarly, Cescon *et al* reported that $LS \geq 15.7$ kPa was an independent pre-operative predictor of PHLF in patients undergoing LR for HCC.²¹ Recently, others studies have have established that LS evaluated by FibroScan[®] or MR elastography as an important predictor of liver failure.^{35,36} Donadon *et al* have published a score model called “Humanitas score” that includes type hepatectomy, $LS \leq 9.7$ kPa and BILCHE score ≤ 2 . Humanitas score ≥ 7 was associated with $\leq 54\%$ risk of morbidity after hepatectomy.³⁷ We have previously reported $LS \geq 22$ kPa was an independent predictor of persistent hepatic decompensation after LR for HCC.²² The differences in the study population (all types of tumors or HCC only), endpoint (PHLF only or all post-operative morbidity), methodology and variability in LS could explain the variations in the cut-off values from 10 to 25 kPa.^{20-22,3-}

³⁸ Yokoo *et al* on the other hand developed several models for predicting post-operative

transfusion over five units, unexpected intubation, renal failure, cardiac events, septic shock, and pneumonia using different combinations of multiple pre- and intra-operative parameters.¹⁴ We constructed nomograms to predict adverse post-operative outcomes as they have superior performance when compared to risk-grouping, look-up tables or classification and regression tree analysis as these analyses are limited by variable coding limitations.³⁹ In the nomogram to predict severe morbidity, we have utilized only four easily available variables on a continuous scale and thereby avoiding potential loss of valuable information associated with the use of binary predictors. LS was transformed into Log_{10} as the relationship between the portal venous pressure was defined by the equation $1.746+5.031(\text{Log}_{10}\text{LS})$. Age was included in the nomogram despite its p value being not significant because age remained in the model during multivariate regression and AUROC of the model improved when age was added to the nomogram. A similar study by Breitenstein *et al*, a risk-stratified patients into three risk groups using a score (0-10) that was developed with four pre-operative categorical parameters and they demonstrated patients with a score ≥ 6 were at high risk of severe morbidity. They further highlighted that costs of the entire procedure doubled among the three risk groups. However, cirrhotic patients were excluded from this study and the model has not yet been validated in an external cohort.⁴⁰ With respect to nomogram for mortality, binary predictors were utilized as the events (death) were rare and the model had better performance when compared the model with continuous parameters. Tumor size was not included in the model as the data on tumor size was missing in the external cohort and the performance of model did not change significantly without it. Dhir *et al* have proposed a pre-operative nomogram with 15 parameters to risk of peri-operative mortality following LR for malignancy but it has not yet been validated.¹⁸ Similarly, Hyder *et al* have proposed a composite integer-based risk score to predict 90-day mortality based on post-operative day 3 biochemical and

complications.¹⁹ In comparison, the present nomogram has only three easily available pre-operatively parameters and its performance was validated in an external cohort.

To the best of our knowledge, this is the first study to develop and validate two nomograms for predicting clinically pertinent outcomes - 90-day severe morbidity and mortality in patients (non-cirrhotic and cirrhotic) undergoing LR for various indications. This mono-centric study recruited prospectively patients with various etiology and indications enabling the results to be extrapolated to wide range of clinical situations. Another unique feature of this study all patients in whom TE was programmed were analyzed in intention-to-treat manner. Further, internal validation by bootstrapping the multivariate analysis and external validation using calibration plot boost the robustness of the results. Despite the patients in the external cohort being not similar to our study population, these two nomograms demonstrated good fit of the models. The criteria utilized to select patients for LR such as MELD score and FLR volume were not assessed as predictors of post-hepatectomy outcomes. The limitations of this study was HVPG was not measured in all patients and missing data in the external cohort. These facts should be considered while interpreting the predictive values.

Conclusion

The two easy-to-use LS-based nomograms were accurate to predict 90-day severe morbidity and mortality both in the study and external cohorts. They can utilized pre-operatively to offer personalized surgical strategy in patients planned for LR and improve post-operative outcomes.

Acknowledgements: L'Association Nationale de Recherché et de la Technologie, Paris, France and Echosens™, Paris, France, funded this research.

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Table 1: Patient, primary tumor, and treatment characteristics

Variables [#]	N = 418(100%)
Patient Demographics	
▪ Age in years, median (range)	62(16-87)
▪ Gender, male : female	244(58.4) : 174(41.6)
▪ Body mass index in kg/m ² , median (range)	25(13-41)
▪ Diabetes Mellitus	71(17)
▪ Hypertension	168(40.2)
▪ Dyslipidemia	87(20.8)
▪ Pre-operative transarterial chemoembolization	25(6)
▪ Pre-operative chemotherapy	185(44.3)
Indications for Hepatectomy	
▪ Colorectal liver metastasis	149(35.6)
▪ Hepatocellular carcinoma	107(25.6)
▪ Benign liver tumors	60(14.3)
▪ Non colorectal liver metastasis	37(8.9)
▪ Perihilar cholangiocarcinoma	20(4.8)
▪ Intra-hepatic cholangiocarcinoma	19(4.6)
▪ Gall bladder carcinoma	12(2.9)
▪ Living donors	11(2.6)
▪ Others	3(0.7)
Tumor Characteristics	
▪ Tumor number, median (range)	1(0-14)
▪ Diameter of largest nodule in mm, median (range)	27.5(0-190)
▪ Serum α -fetoprotein in ng/mL, median (range)	3.7(0.8-33930)
▪ Serum carcinoembryonic antigen in IU/L, median (range)	2.5(0.5-1945.4)
▪ Serum carbohydrate antigen 19-9 in IU/L, median (range)	12.7(1-5775700)
Laboratory tests	
▪ Serum total bilirubin in μ mol/L, median (range)	10(2-90)
▪ Serum aspartate aminotransferase in IU/L, median (range)	33(11-780)
▪ Serum alanine aminotransferase in IU/L, median (range)	30(10-776)
▪ Serum γ -glutamyl transpeptidase in IU/L, median (range)	74(9-2597)
▪ Serum alkaline phosphatase in IU/L, median (range)	97(19-993)
▪ Prothrombin activity in %, median (range)	90(53-99)
▪ International normalized ratio, median (range)	1.1(0.9-1.6)
▪ Serum fibrinogen in g/L, median (range)	3.7(1-11.3)
▪ Blood urea in mmol/L, median (range)	5(1-48)
▪ Serum creatinine in μ mol/L, median (range)	70(46-95)
▪ Glycaemia in mmol/L, median (range)	6.1(1-16)
▪ Serum albumin in g/L, median (range)	37.4(18.3-66)
▪ Serum protein in g/L, median (range)	73(38-99)
▪ Hemoglobin in g/L, median (range)	13.3(7.2-18.8)
▪ Platelet count in 10 ⁹ /L, median (range)	231(34-811)
▪ Indocyanine green retention at 15 mins in %, median (range)	8.4(0.5-54.1)
▪ Liver stiffness measurement in kPa, median (range)	6.4(2.7-75)
▪ Controlled attenuation parameter in dB/m, median (range)	252(100-400)
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	336(80.4) : 82(19.6)
▪ Re-hepatectomy	68(16.3)

▪ Portal vein embolization		39(9.3)
▪ Pre-operative biliary drainage		16(3.8)
▪ Hepatic venous pressure gradient in mmHg, median (range)		1(0-14)
▪ Partial hepatectomy		142(33.9)
▪ Right hepatectomy		88(21.1)
▪ Left hepatectomy		69(16.5)
▪ Uni-segmentectomy		57(13.6)
▪ Bi-segmentectomy		55(13.2)
▪ Central hepatectomy		7(1.7)
▪ Duration of Pringle maneuver in min, median (range)		32(0-171)
▪ Operative time in min, median (range)		292(100-755)
▪ Blood loss in mL, median (range)		500(50-7500)
▪ Intra-operative RBC transfusion		52(12.4)
Post-operative outcomes		
▪ 90-day severe morbidity ¹		87(20.8)
▪ Hepatic decompensation ²		82(19.6)
▪ Post-hepatectomy liver failure ³		19(4.5)
▪ 90-day mortality		9(2.2)
▪ Maximum Clavien Dindo grade, median (range)		2(0-5)
▪ Comprehensive complication index, median (range)		13.5(0-100)
▪ Duration of ICU stay in days, median (range)		1(0-48)
▪ Length of hospitalization in days, median (range)		8(1-77)
▪ METAVIR Fibrosis score: F0/F1/F2/F3/F4/Missing data	78(18.7)/123(29.4)/97(23.2)/37(8.9)/62(14.8)/21(5)	
▪ METAVIR Activity score: A0/A1/A2/A3/Missing data	133(31.8)/115(27.5)/83(19.9)/17(4.1)/70(16.7)	
▪ NAS steatosis grade: S0/S1/S2/S3	182(43.5)/153(36.6)/38(9.1)/1(0.3)/44(10.5)	

¹Data are expressed as counts (percentage) unless otherwise stated, 1-Clavien-Dindo grade of surgical complications >2, 2- presence of ascites, jaundice, and/or encephalopathy, 3-50-50 criteria or peak total bilirubin >7 mg/dL., METAVIR-meta-analysis of histological data in viral hepatitis, NAS-non-alcoholic fatty liver disease activity score

Table 2: Independent predictors of 90-day major complications in patients undergoing hepatectomy for various indications (N=418)

Variables	Univariate		Multivariate Logistic			Bootstrapped Multivariate	
	Regression [#]		Regression [#]			Logistic regression [#]	
	<i>p</i>	<i>Beta</i>	<i>p</i>	OR	95% CI	<i>p</i>	95% CI
Age in years	0.02	0.02	0.06	1.02	0.99 – 1.05	0.07	-0.002 – 0.05
Gender, Male/Female	0.59						
Body mass index, kg/m ²	0.44						
Diabetes Mellitus, Yes/No	0.18						
ASA physical status	0.50						
Pre-operative chemotherapy cycles	0.21						
Tumor size in mm	0.82						
Tumor number	0.82						
Tumor type, Benign/Metastasis/Primary	0.03						
Serum total bilirubin, μmol/L	0.03						
Serum aspartate aminotransferase, IU/L	0.07						
Serum alkaline phosphatase, IU/L	0.003						
Hemoglobin, g/L	0.19						
Platelet count, 10 ⁹ /L	0.83						
Indocyanine green retention at 15 min, %	0.01						
Liver stiffness measurement, kPa in Log ₁₀	0.02	1.23	0.04	3.43	1.02 – 11.51	0.03	-0.21 – 2.64
Controlled attenuation parameter, dB/m	0.69						
Number of liver segments to be resected	<0.001	0.25	0.004	1.28	1.08 – 1.51	0.01	0.07 – 0.44
Re-hepatectomy, Yes/No	0.55						
Duration of Pringle maneuver, min	0.06						
Operative time, min	<0.001	0.003	0.01	1.01	1.00 – 1.01	0.01	0.001 – 0.01
Blood loss, mL	0.001						
RBC transfusion	0.02						
Constant			<0.001			0.001	-8.30 – -3.78

[#]All unrelated variables with $P \leq 0.05$ in the univariate regression was entered into multivariate logistic regression by Backward elimination (Wald) method and the variables that remained in the equation were validated by multivariate logistic regression bootstrapped at 1000, ASA-American society of anesthesiologists, OR-Odds ratio, CI-confidence interval.

Table 3: Independent predictors of 90-day mortality in 418 patients undergoing hepatectomy for various indications

Variables	Prevalence	Univariate Regression [#]		Multivariate Logistic Regression [#]				Bootstrapped Multivariate Logistic Regression [#]		
		p	Beta	p	Odds Ratio	95% Confidence Interval		p	95% Confidence Interval	
						Lower	Upper		Lower	Upper
Age in years	< 60 184	0.2								
	≥ 60 234									
Gender	Male 244	0.3								
	Female 174									
Body mass index in kg/m ²	< 30 344	0.05								
	≥ 30 74									
Diabetes Mellitus	No 347	0.04	1.81	0.04	6.16	1.06	35.82	0.004	-16.29	18.74
	Yes 71									
Pre-operative chemotherapy cycles	< 8 cycles 328	0.9								
	≥ 8 cycles 90									
Tumor size in mm	≤ 50 307	0.06	1.67	0.08	4.78	0.81	28.18	0.02	-15.49	18.12
	> 50 111									
Tumor number	≤ 1 253	0.9								
	> 1 165									
Tumor type	Benign Disease 72									
	Liver Metastasis 186	0.07								
	Primary cancer 160									
Serum total bilirubin in μmol/L	< 34 406	0.2								
	≥ 34 12									
Serum aspartate aminotransferase in IU/L	≤ 50 338	0.8								
	> 50 80									
Serum alkaline phosphatase in IU/L	< 100 222	0.6								
	≥ 100 195									
Hemoglobin in g/L	< 10 14	0.01								
	≥ 10 404									
Platelet count in 10 ⁹ /L	< 150 60	0.5								
	≥ 150 358									
Indocyanine green retention at 15 min in %	< 15 266	0.3								
	≥ 15 51									
Liver stiffness measurement in kPa	< 22 304	0.03	1.95	0.04	7.02	1.09	45.32	0.01	-17.23	17.24
	≥ 22 24									
Controlled attenuation parameter in dB/m	< 250 181	0.3								
	≥ 250 138									
Number of liver segments to be resected	< 3 253	0.8								
	≥ 3 165									
Duration of Pringle maneuver in min	< 60 333	0.9								
	≥ 60 85									
Operative time in min	< 360 291	0.03	1.80	0.05	6.06	1.09	35.99	0.01	-0.001	18.78
	≥ 360 127									
Blood loss in ml	< 1000 340	0.06								
	≥ 1000 77									
Constant				<0.001				0.001	-49.19	-5.05

[#]All unrelated variables with $P \leq 0.15$ in the univariate regression was entered into multivariate logistic regression by backward elimination (Wald) method and those that remained in the equation were validated by multivariate logistic regression bootstrapped at 1000

Table 4: Comparison of characteristics of the patients who are alive with those who died within 90 days of hepatectomy

Variables [#]	Alive N=409	Dead N=9	<i>p</i> [*]
Age in years, mean±SD	60.7±13.7	68.1±11.1	0.13
Gender: Male/Female	237(57.9)/172(42.5)	2(22.2)/7(77.8)	0.23
Body mass index in kg/m ² , mean±SD	25.2±4.9	28.2±5.1	0.08
Diabetes Mellitus	67(16.4)	4(44.4)	0.03
Tumor Type			
▪ Benign Disease	72(17.6)	0(0)	0.05
▪ Liver Metastasis	183(44.7)	3(33.3)	
▪ Primary Liver Cancer	154(37.7)	6(66.7)	
Tumor size in mm, mean±SD	40.3±36.6	60.8±49.7	0.13
Tumor number, mean±SD	2.0±1.9	1.9±1.7	0.96
Pre-operative chemotherapy cycles, mean±SD	1.4±0.8	1.8±1.5	0.75
Serum total bilirubin in µmol/L, mean±SD	11.8±9.6	14.4±13.5	0.43
Serum aspartate aminotransferase in IU/L, mean±SD	46.5±64.3	40.6±18.9	0.63
Serum alkaline phosphatase in IU/L, mean±SD	132.5±123.8	174.9±206.2	0.38
International normalized ratio, mean±SD	1.0±0.2	1.0±0.0	0.63
Serum creatinine in µmol/L, mean±SD	78.4±47.9	86.3±39.9	0.33
Blood sugar in mmol/L, mean±SD	6.5±2.1	8.1±2.0	0.03
Serum albumin in g/L, mean±SD	37.4±5.1	33.8±8.3	0.13
Hemoglobin in g/L, mean±SD	13.3±1.8	11.7±2.5	0.04
Platelet count in 10 ⁹ /L, mean±SD	240.5±94.4	205.9±83.5	0.21
ICG-R15 in %, mean±SD	9.8±7.0	12.5±9.2	0.39
Liver stiffness measurement in kPa, mean±SD	9.4±8.6	18.8±15.7	0.02
Controlled attenuation parameter in dB/m, mean±SD	243.2±62.4	289.7±102.2	0.29
Surgical approach: Open/Laparoscopic hepatectomy	327(79.9)/82(20.1)	9(100)/0(0)	0.14
Number of liver segments, mean±SD	2.0±1.9	2.6±2.3	0.46
Pringle maneuver	311(76.0)	6(66.7)	0.52
Duration of Pringle maneuver in min, mean±SD	35.0±30.5	41.6±33.9	0.46
Maximum serum lactate in mmol/L, mean±SD	3.1±1.7	3.7±1.7	0.19
Operative time in min, mean±SD	309.9±118.9	359.1±95.4	<0.001
Blood loss in ml, mean±SD	668.9±766.7	1300.0±1154.3	0.12
Post-hepatectomy liver failure	15(3.7)	4(44.4)	<0.001
90-day severe morbidity	78(19.1)	9(100)	<0.001
90-day comprehensive complication index, mean±SD	17.2±18.9	100±0.0	<0.001
Length of hospital stay in days, mean±SD	10.2±8.2	28.1±20.2	0.01

[#]Data are expressed as counts (percentage) unless otherwise stated; ICG-R15-indocyanine green retention at 15 minutes, ^{*}Mann Whitney U test with asymptomatic bilateral *p* value

Supplementary table 1: Patient, primary tumor, and treatment characteristics in the external validation cohort

Variables [#]	N=113 (100%)
Patient Demographics	
▪ Age in years, median (range)	64(29-80)
▪ Gender, male : female	98(86.7) : 15(13.3)
▪ Body mass index in kg/m ² , median (range)	27(17-40)
▪ Diabetes Mellitus	46(40.7)
▪ Hypertension	NA
▪ Dyslipidemia	NA
▪ Pre-operative transarterial chemoembolization	NA
▪ Pre-operative chemotherapy	NA
Indications for Hepatectomy	
▪ Colorectal liver metastasis	6(5.3)
▪ Hepatocellular carcinoma	92(81.4)
▪ Benign liver tumors	2(1.8)
▪ Non colorectal liver metastasis	2(1.8)
▪ Perihilar cholangiocarcinoma	1(0.9)
▪ Intra-hepatic cholangiocarcinoma	10(8.8)
Tumor Characteristics	
▪ Tumor number, median (range)	NA
▪ Diameter of largest nodule in mm, median (range)	NA
▪ Serum α -fetoprotein in ng/mL, median (range)	5.3(1.5-2000)
▪ Serum carcinoembryonic antigen in IU/L, median (range)	NA
▪ Serum carbohydrate antigen 19-9 in IU/L, median (range)	NA
Laboratory tests	
▪ Serum total bilirubin in μ mol/L, median (range)	11(2-155)
▪ Serum aspartate aminotransferase in IU/L, median (range)	33.5(13-119)
▪ Serum alanine aminotransferase in IU/L, median (range)	29(11-138)
▪ Serum γ -glutamyl transpeptidase in IU/L, median (range)	98(13-866)
▪ Serum alkaline phosphatase in IU/L, median (range)	NA
▪ Prothrombin activity in %, median (range)	90(27-100)
▪ International normalized ratio, median (range)	1.1(0.9-2.9)
▪ Serum fibrinogen in g/L, median (range)	NA
▪ Blood urea in mmol/L, median (range)	5(2.3-16.2)
▪ Serum creatinine in μ mol/L, median (range)	79(44-229)
▪ Glycaemia in mmol/L, median (range)	NA
▪ Serum albumin in g/L, median (range)	NA
▪ Serum protein in g/L, median (range)	NA
▪ Hemoglobin in g/L, median (range)	14.2(9.5-16.4)
▪ Platelet count in 10 ⁹ /L, median (range)	162(63-538)
▪ Indocyanine green retention at 15 mins in %, median (range)	NA
▪ Liver stiffness measurement in kPa, median (range)	13.6(2.5-75)
▪ Controlled attenuation parameter in dB/m, median (range)	NA
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	101(89.4) : 12(10.6)
▪ Re-hepatectomy	7(6.2)
▪ Portal vein embolization	3(2.7)
▪ Pre-operative biliary drainage	NA

▪ Hepatic venous pressure gradient in mmHg, median (range)	NA
▪ Partial hepatectomy	68(60.2)
▪ Right hepatectomy	8(7.1)
▪ Left hepatectomy	3(2.7)
▪ Uni-segmentectomy	16(14.2)
▪ Bi-segmentectomy	17(15)
▪ Central hepatectomy	1(0.9)
▪ Number of Couinaud segments resected, median (range)	1(0-6)
▪ Duration of Pringle maneuver in min, median (range)	0(0-276)
▪ Operative time in min, median (range)	231(71-480)
▪ Blood loss in mL, median (range)	500(25-2800)
▪ Intra-operative RBC transfusion	5(4.4)
Post-operative outcomes	
▪ 90-day severe morbidity ¹	22(19.5)
▪ Hepatic decompensation ²	11(9.7)
▪ Liver failure ³	NA
▪ 90-day mortality	5(4.4)
▪ Maximum Clavien-Dindo grade, median (range)	2(0-7)
▪ Comprehensive complication index, median (range)	25.7(0-100)
▪ Duration of ICU stay in days, median (range)	NA
▪ Length of hospitalization in days, median (range)	8(2-147)
▪ METAVIR fibrosis score: F0/F1/F2/F3/F4/Missing data	31(27.4)/17(15)/6(5.3)/9(7.9)/47(41.6)/3(2.7)
▪ METAVIR activity score: A0/A1/A2/A3	NA
▪ NAS steatosis grades: S0/S1/S2/S3	78(69)/25(22.1)/8(7.1)/2(1.8)

¹Data are expressed as counts (percentage) unless otherwise stated, 1-Clavien-Dindo grade of surgical complications >2, 2-presence of ascites, jaundice, and/or encephalopathy, 3-50-50 criteria or peak total bilirubin >7 mg/dL, METAVIR-meta-analysis of histological data in viral hepatitis, NAS-non-alcoholic fatty liver disease activity score, NA-data not available

Supplementary table 2: Comparison of characteristics of the patients in the study (training) and in the external validation cohorts

Variables [#]	Training Set N=418	Validation Set N=113	<i>p</i> *
Age in years, mean±SD	60.8±13.7	62.5±9.2	0.49
Gender: Male/Female	244(58.4)/174(41.6)	98(86.7)/15(13.3)	<0.01
Body mass index in kg/m ² , mean±SD	25.3±4.9	27.5±4.5	<0.01
Diabetes Mellitus	71(17)	46(40.7)	<0.01
Tumor type			
▪ Benign disease	72(17.2)	2(1.8)	<0.01
▪ Liver metastasis	186(44.5)	8(7.1)	
▪ Primary liver cancer	160(38.3)	103(91.2)	
Tumor size in mm, mean±SD	NA		
Tumor number, mean±SD	NA		
Serum total bilirubin in μmol/L, mean±SD	11.9±9.7	14.5±17.4	0.15
Serum aspartate aminotransferase in IU/L, mean±SD	46.4±63.7	36.8±17.3	0.58
Serum γ-glutamyl transpeptidase in IU/L, mean±SD	173.9±274.2	158.8±165.6	0.06
International normalized ratio, mean±SD	1±0.2	1.1±0.3	0.28
Serum creatinine in μmol/L, mean±SD	78.5±47.8	80.1±24.8	0.82
Hemoglobin in g/L, mean±SD	13.8±1.8	14±1.4	<0.01
Platelet count in 10 ⁹ /L, mean±SD	239.7±94.3	171.6±811.8	<0.01
ICG-R15 in %, mean±SD	NA		
Liver stiffness measurement in kPa, mean±SD	9.5±8.8	18.6±15.0	<0.01
Controlled attenuation parameter in dB/m, mean±SD	NA		
Surgical approach: Open/Laparoscopic hepatectomy	336(80.4)/82(19.6)	101(89.4)/12(10.6)	0.03
Re-hepatectomy	68(16.3)	7(6.2)	0.01
Number of Couinaud segments, mean±SD	2±1.9	1.5±1.4	0.10
Pringle maneuver	317(75.8)	33(29.2)	<0.01
Duration of Pringle maneuver in min, mean±SD	35.1±30.5	19.1±34.7	<0.01
Operative time in min, mean±SD	311.4±117.8	243.5±83.4	<0.01
Blood loss in ml, mean±SD	682.6±780.6	605.4±504.7	0.88
Intra-operative RBC transfusion	52(12.4)	5(4.4)	0.36
90-day severe morbidity	87(20.8)	22(19.5)	0.75
90-day mortality	9(2.2)	5(4.4)	0.18
90-day comprehensive complication index, mean±SD	18.8±21.9	28.6±25.7	<0.01
Length of hospital stay in days, mean±SD	10.6±9.0	10.8±15.5	0.37

[#]Data are expressed as counts (percentage) unless otherwise stated; ICG-R15-indocyanine green retention at 15 minutes, NA-not applicable, *Mann Whitney U test with asymptomatic bilateral *p* value

Figure 1: Nomogram to predict 90-day post-operative severe morbidity

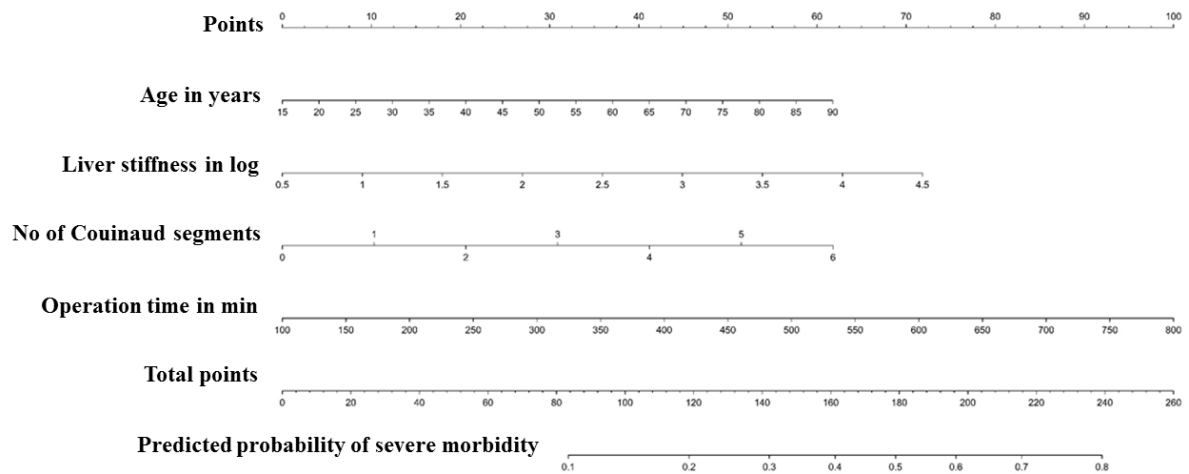


Figure 2: Calibration plots of the study (training) and external (validation) cohorts

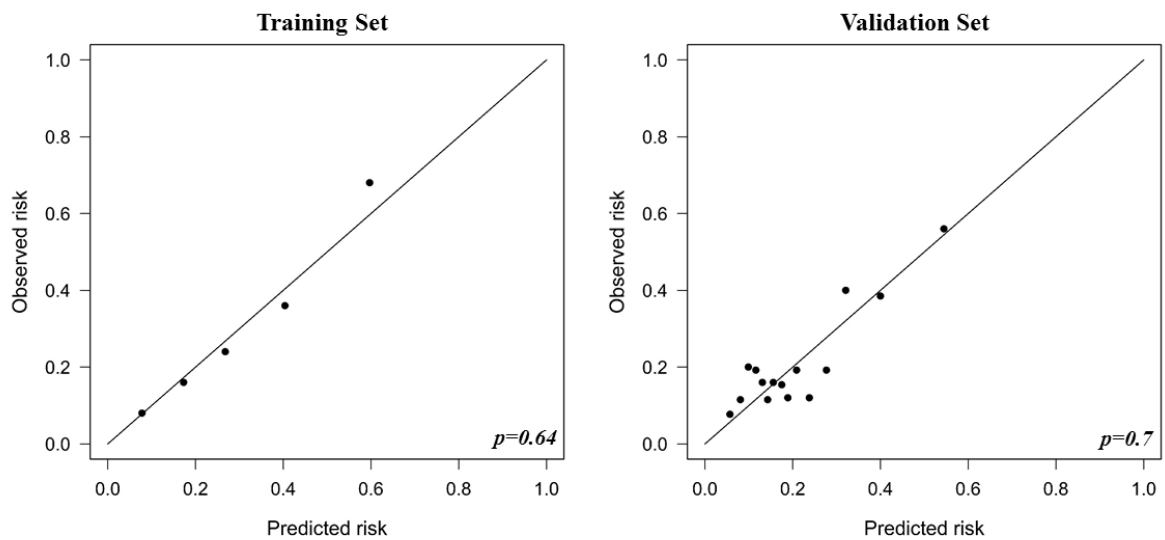


Figure 3: Nomogram to predict 90-day post-operative mortality

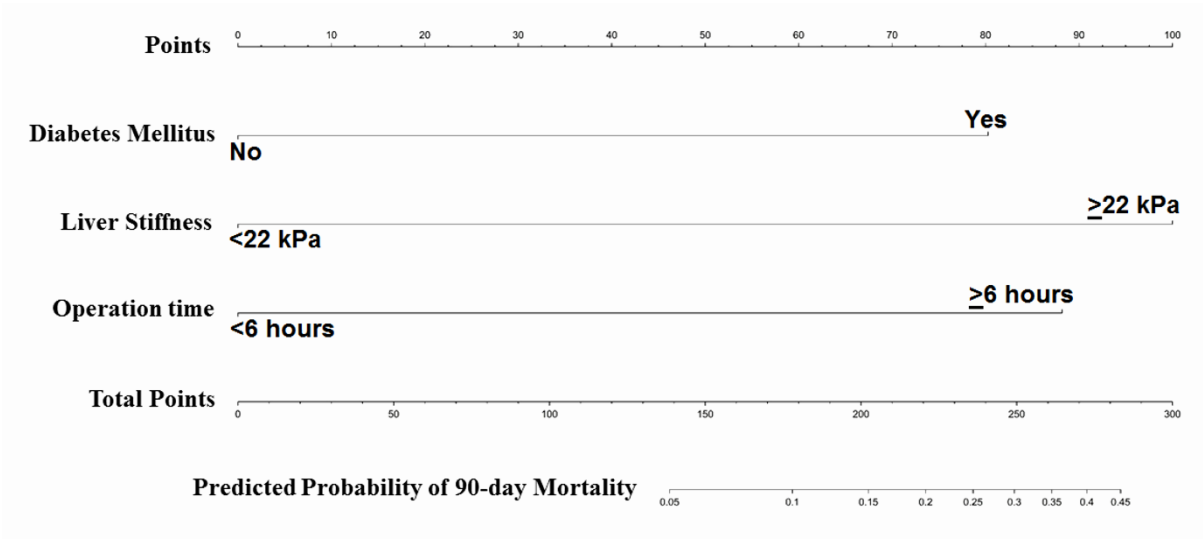
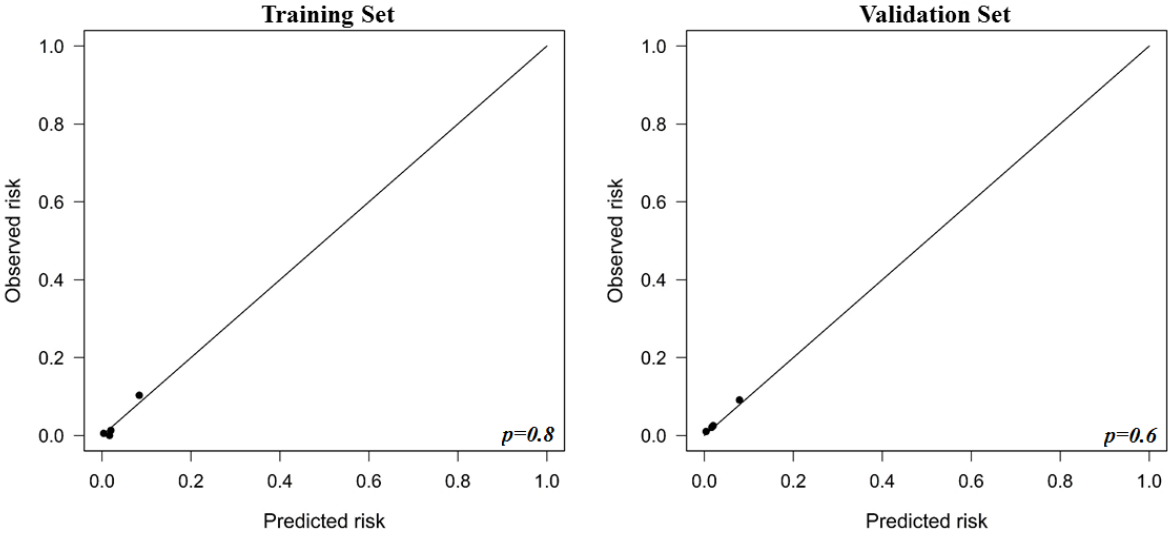


Figure 4: Calibration plots of the study (training) and external (validation) cohorts



Article 5:

Title: Preoperative nomogram to predict post-hepatectomy liver failure in all patients undergoing liver resection

Authors:

Muthukumarassamy Rajakannu,^{1,2,3} MBBS, MS, DNB, MRCS, Daniel Cherqui,^{1,3} MD, Oriana Ciacio,¹ MD, Nicolas Golse,¹ MD, Gabriella Pittau,¹ MD, Marc Antoine Allard,¹ MD, Teresa Maria Antonini,¹ MD, Audrey Coilly,^{1,2} MD, Antonio Sa Cunha,^{1,3} MD, Denis Castaing,^{1,2,3} MD, Didier Samuel,^{1,2,3} MD, PhD, Catherine Guettier,^{1,2,3} MD, PhD, René Adam,^{1,3,4} MD, PhD, Eric Vibert,^{1,2,3} MD, PhD

Affiliations:

¹Centre Hépato-Biliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

Running title: Nomograms to predict post-hepatectomy severe morbidity and mortality.

Keywords: Liver resection, liver stiffness, post-operative complication, transient elastography.

Financial support: L'Association Nationale de la Recherche et de la Technologie, Paris, France and EchosensTM, Paris, France funded this study.

Correspondance:

Prof. Eric Vibert

Centre Hépato-Biliaire

AH-HP Hôpital Paul Brousse

12 Avenue Paul Vaillant Couturier

94800 Villejuif

France

Abstract

Background: Post-hepatectomy liver failure (PHLF) is a rare but serious complication after liver resection (LR) and a leading cause of mortality. Our aim was to define preoperative predictors of PHLF and propose a predictive nomogram.

Methods: Consecutive patients planned for LR from October 2014 to August 2016 were prospectively recruited. Clinical and laboratory data including liver stiffness and indocyanine green retention at 15 min (ICG-R15) were collected at inclusion and until three months after LR.

Results: Four hundred and eighteen hepatectomies were performed in 244 men and 174 women whose median age was 62 years. PHLF was observed in 19 patients (4.6%) after major LR in 17 and minor LR in two. Mortality rate in patients developing PHLF was 21.1% while mortality rate in the entire cohort of 418 patients was 2.2%. Independent predictors of PHLF were diabetes mellitus (odds ratio (OR): 6.6; 95% confidence interval (CI):1.1-39.3), pre-operative chemotherapy cycles ≥ 8 (OR: 4.1; 95%CI:0.8-20.9), tumor size ≥ 51 mm (OR: 4.8; 95%CI:0.9-26.1), platelet count $< 150,000$ /mL (OR: 8.7; 95%CI:1.3-56.8), ICG-R15 (OR: 10.4; 95%CI:1.9-58.1) and number of resected liver segments ≥ 3 (OR: 12.9; 95%CI:1.3-125.4). Nomogram built with these six factor had an excellent discriminatory performance with area under receiver operating characteristic curve of 0.92 and satisfactory goodness-of-fit ($p=0.44$).

Conclusion: Predictive nomogram incorporating ICG-R15 would improve the safety of LR by enabling surgeons to identify high-risk patients and adapt the surgical strategy.

Introduction

With considerable reduction in post-operative morbidity and mortality over the past two decades due to improvements in surgical expertise and peri-operative management, increasing number of major and extended liver resections (LR) are being performed in diseased or injured liver.¹⁻⁶ Post-hepatectomy liver failure (PHLF) is the most dreaded complication in these patients as it depends on the volume and function of future liver remnant (FLR).⁷ Despite the introduction of pre-operative assessment of the volume and function of FLR and use of portal vein embolization (PVE) when the volume is insufficient, PHLF occurs in 1.2-32% of hepatectomies depending upon the definition utilized and the mortality associated with it can be as high as 10-20%.⁷⁻¹⁶ One of the commonly utilized tests in Eastern countries to assess liver function preoperatively is indocyanine green retention at 15 min (ICG-R15).¹⁷⁻¹⁹ Imamura *et al* reported that strict application of a decision tree incorporating ICG-R15 had resulted in near zero mortality in patients undergoing LR in Japan.¹⁷ Recently, Cescon *et al* and Chong *et al* have demonstrated that liver stiffness (LS) measured by transient elastography (TE) was an independent predictor of PHLF.^{20,21} The aim of the present study was to assess the role of ICG-R15 and LS in predicting PHLF and propose a predictive nomogram using pre-operative parameters.

Patients and methods

Consecutive adult patients undergoing LR for various indications in our institute from October 2014 to August 2016 were prospectively recruited after obtaining an informed consent. Clinical and laboratory data were obtained at inclusion and at post-operative follow-up visits. Final follow-up was on November 30, 2017. The hospital Ethics committee approved the study protocol according to the principles of Helsinki declaration.

Liver resection

Pre-operative evaluation consisted of laboratory tests including liver function tests, imaging studies like ultrasonography, contrast-enhanced abdominal computer tomography (CECT) ± volumetry, magnetic resonance imaging (MRI), LS, and ICG-R15. ICG clearance test was usually performed the day before the planned hepatectomy. An intravenous bolus of 0.5 mg/kg of patient's body weight of ICG was injected into a large vein in an arm and the blood samples were collected from the other arm every four minutes from time 0 until 16 minutes. ICG-R15 was calculated as the ratio between ICG concentration at 15 min after injection and its initial concentration (normal range, 0%-10%).^{17,19} On the other hand, TE measured LS on the right liver under fasting condition using FibroScan[®] Touch 502 with M/XL probes (Echosens[™], Paris, France). Median of ≥ 10 valid measurements was considered as LS and expressed within the range 0.5-75 kPa.^{20,22} The operator was blinded to the patient's clinical data. All patients were operated within two weeks of TE.

The objective of LR was to resect all visible lesions with tumor-free margins while leaving behind adequate liver parenchyma to ensure normal post-operative liver function except in benign liver tumors where enucleation were preferred. Usually hepatectomy was performed in patients with well-compensated liver function with platelet count $\geq 80,000$ and ICG-R15 $\leq 20\%$. When FLR volume was insufficient, PVE was performed to ensure FLR $\geq 0.5\%$ of body weight or $\geq 20\%$ of standard liver volume in non-cirrhotic liver.²² Transarterial chemoembolization followed by PVE was the treatment strategy when planning major LR for hepatocellular carcinoma (HCC) in cirrhotic patients.²³ Pre-operative biliary drainage was carried out in patients with jaundice and/or to facilitate adequate regeneration of FLR after PVE.²⁴ Patients with liver metastasis were usually operated after a course of neoadjuvant combination chemotherapy.²⁵

The technique of hepatectomy has been standardized in our unit since 1982.^{22,24,25,26} At surgery, systematic exploration of the abdominal cavity and intra-operative liver ultrasound

were performed to assess the liver tumor and to detect extrahepatic spread. Any enlarged hepatic lymph nodes and suspicious nodules were excised for frozen section histology. Intra-operative measurement of hepatic venous pressure gradient was performed at individual surgeon's discretion before the commencement of hepatectomy during open LR. Parenchymal dissection was done using the ultrasonic dissector (Cavitron Ultrasonic Surgical Aspirator, Valley Lab Inc., Boulder, USA), and was usually performed under intermittent clamping of the portal triad (Pringle maneuver). In general, anatomic resections were preferred to wedge resections in patients with primary liver malignancy. In patients with intra-hepatic or peri-hilar cholangiocarcinoma, lymphadenectomy of the hepatic pedicle was performed *en bloc* with hepatectomy. Further, systematic resection of portal bifurcation along with resection of the extra-hepatic bile duct and hepaticojejunostomy was carried out in patients with peri-hilar cholangiocarcinoma.

After LR, regular follow-up of patients was ensured to monitor the patient's recovery, development of post-operative complications and disease recurrence. A CECT scan was performed at 1-week and 1-month following LR and then alternating abdominal ultrasonography and CECT/MRI were performed every 3-4 months along with serum tumor markers and liver function tests. The patients with liver metastasis were usually offered 6-8 cycles of post-operative chemotherapy.

Definitions

Clavien-Dindo classification of surgical complications was utilized to grade 90-day post-operative complications.²⁷ The primary end-point of this study was PHLF and it was defined by 50-50 criteria of Balzan *et al* and/or peak total bilirubin >7 mg/dL criterion of Mullen *et al*.^{7,8} PHLF manifested as nonobstructive jaundice, coagulopathy, fluid retention (ascites), encephalopathy, and an increased susceptibility to sepsis and multiple organ failure.^{8,9}

Statistical analysis

Discrete variables are reported as counts (percentage) whereas parametric variables are expressed as median (range). The potential pre-operative risk factors of associated with PHLF were identified by univariate logistic regression and those with $p \leq 0.10$ were entered into multivariate binary logistic regression by backward elimination (Wald) method and the variables that remained in the model were considered as independent risk factors for PHLF. Internal validation and overfitting of the model was tested by bootstrapped (1000) multivariate logistic regression. Receiver operating characteristic (ROC) analysis with area under ROC curve (AUROC) as main readout was performed to test the model performance. Calibration plots assessed the performance of nomograms in the training and validation cohorts. Spearman's rank correlation and were performed respectively to evaluate the correlation between LS and ICG-R15 and ROC analysis to test their diagnostic performance. Patients without and with PHLF were compared by Mann–Whitney U test. SPSS 21.0 package (SPSS Inc., Chicago, IL) and R statistical package version 3.2.5 (www.rproject.org) were utilized to perform the analysis. p value < 0.05 was considered significant.

Results

Study population and operative details

Four hundred and eighteen hepatectomies were performed during the study period. The study cohort consisted of 58.4% men and 41.6% women with a median (range) age of 62 (16-87) years. Concomitant co-morbidities like diabetes mellitus (17%), hypertension (40.2%) and dyslipidemia (20.8%) were observed in majority of the resected patients. Colorectal metastasis (35.6%) was the most common indication for LR followed by HCC in 25.6%, benign liver tumor in 14.5%, non-colorectal liver metastasis in 8.9%, and peri-hilar or intra-hepatic cholangiocarcinoma in 9.4% of the patients. Pre-operative combination chemotherapy was administered in 185 patients (44.3%) with half of them receiving ≥ 8 cycles. Median (range) of size and number tumors resected were 27.5(0-190) mm and 1(0-14), respectively.

Pre-operative TE was performed successfully in 348 patients (83.2%) with valid LS evaluation possible in 320 patients (76.6%) and in 28 patients (6.7%) measurement were considered invalid as interquartile range/median $\geq 30\%$. In 70 patients (16.7%) due to previous right hepatectomy or due to the presence of large right liver tumors, TE was not feasible. Successful evaluation ICG-R15 was possible in 317 patients (75.8%). Median (range) of LS was 6.4 (2.7-75) kPa while median (range) of ICG-R15 was 8.4 (0.5-54.1) %. There was a significant but moderate correlation between LS and ICG-R15 (Spearman $\rho=0.4$, $p<0.001$). Majority of the hepatectomies (80.4%) was performed by laparotomy while 19.6% was by laparoscopic approach. The types of LRs performed were partial hepatectomy (33.9%), right hepatectomy (21.1%), left hepatectomy (16.5%), uni-segmentectomy (13.6%), bi-segmentectomy (13.2%), and central hepatectomy (1.7%). Most of the patients (48.1%) undergoing LR had no or minimal underlying liver disease. Thus, minor LRs (≤ 2 segments) were performed in majority of 254 patients (60.8%) and major LRs (>2 liver segments) in 164 patients (39.2%). Overall 90-day severe morbidity and mortality in the study cohort were 20.8% and 2.2%, respectively. Table 1 summarizes the demographic, laboratory, and operative details of the resected patients in this study along with their 90-day post-operative outcomes.

Post-hepatectomy liver failure

Post-operative liver failure developed in 19 out of 418 LRs (4.6%) which included two (0.5%) minor and 17 (4.1%) major hepatectomies. While 14 patients (73.7%) recovered eventually, four patients (21.1%) died due to PHLF and multiple organ failure in the 90-day post-operative period. The indications for hepatectomy in these patients were HCC (6), colorectal metastases (5), intra-hepatic cholangiocarcinoma (4), non-colorectal metastasis (2), and peri-hilar cholangiocarcinoma (2). The degree of fibrosis in non-tumoral liver parenchyma was F1 in six, F2 in nine, F3 in one and F4 in three patients according to

METAVIR scoring system. One patient each with colorectal and non-colorectal liver metastases died after right extended hepatectomy. Two patients with HCC in cirrhosis (F4) expired after posterior sectionectomy and right hepatectomy, respectively. One patient who developed PHLF after extended right hepatectomy for peri-hilar cholangiocarcinoma underwent liver transplantation. Table 3 compares the characteristics of patients who developed PHLF with those who did not. Independent predictors of PHLF as shown in table 2 were diabetes mellitus (odds ratio (OR): 6.6; 95% confidence interval (CI):1.1-39.3), ≥ 8 pre-operative chemotherapy cycles (OR: 4.1; 95%CI:0.8-20.9), tumor size ≥ 51 mm (OR: 4.8; 95%CI:0.9-26.1), platelet count $< 150,000$ /mL (OR: 8.7; 95%CI:1.3-56.8), ICG-R15 (OR: 10.4; 95%CI:1.9-58.1) and ≥ 3 number of resected liver segments (OR: 12.9; 95%CI:1.3-125.4). A predictive nomogram was constructed with these six factors (Figure 1). Area under ROC curve of the predictive model was 0.92 (Figure 2A). The calibration plot also demonstrated satisfactory Goodness-of-fit of this model with p value of 0.44 (Figure 2B). Discriminatory performance of ICG-R15 (AUROC=0.73, $p=0.02$) was better than LS (AUROC=0.63, $p=0.19$).

Discussion

Statement of principal findings: Six independent readily available pre-operative factors of PHLF identified were from this prospective study and a nomogram was developed to be utilized during pre-operative assessment of patients programmed for hepatectomy. Indocyanine green clearance, a test routinely utilized in Eastern countries was evaluated prospectively in Western patients and had better performance than LS. We propose that ICG-R15 must be added to the routine pre-operative workup of all patients planned for LR because the present nomogram incorporating ICG-R15 would help to identify patients at risk of this serious complication pre-operatively and to adapt surgical strategy in high-risk patients: 1. Obtain informed consent, 2. Prepare patients with PVE to increase FLR, 3. Perform more

conservative resections, and 4. Avoid LR in patients with very high risk and choose an alternative treatment like local ablative therapy or chemotherapy.

Comparison with published literature: A decision-tree incorporating ICG-R15 was published by Imamura *et al* in 2005 and it established what type of LR to be performed depending on ICG-R15.¹⁷ Pre-operative assessment of hepatic functional reserve with ICG-R15 has been widely implemented in the East and has resulted in considerable reduction of PHLF. Strict compliance with this protocol has helped to achieve near-zero mortality in patients undergoing LR for various benign and malignant hepatobiliary diseases.^{5,17,28} However, ICG-R15 is not yet part of routine pre-operative workup in Occidental countries where LS has emerged recently as a non-invasive predictor of PHLF.^{19,20,22} Assessment of LS by either ultrasound or MR elastography has recently been utilized to predict post-hepatectomy outcomes especially PHLF. Chong and his colleagues have proposed a cutoff 12 kPa for LS measured by TE to predict high-grade (Grade B and C) liver failure defined by international study group for liver surgery (ISGLS) group criteria in patients undergoing LR for HCC.²¹ Another study by Cescon *et al* have proposed a LS cutoff 15.7 kPa to predict post-operative liver failure. Another new technique of assessing LS is two-dimensional shear wave elastography (SWE) which has demonstrated good performance to predict liver failure.^{29,30} Hu *et al* from China have proposed a nomogram with platelet count, bilirubin, γ -glutamyl transpeptidase, clinical signs of portal hypertension and SWE to predict Grade A and B liver failure according to ISGLS criteria. None of these studies has compared LS with ICG-R15.³⁰ Different endpoints were utilized in various publications and study population was predominantly HCC patients. Further, the definition utilized in their studies is not utilized in day-to-day clinical practice in most hepatobiliary centers and therefore, these results cannot be extrapolated to all patients in routine clinical practice.^{7,8,11,14,16,20,21,29-31} For example, incidence of PHLF in our study was about 5% whereas in the publications by Cescon *et al* and Hu *et al* it was about

30%.^{20,30} Kim and his colleagues evaluated the role of LS and ICG-R15 in patients undergoing hepatectomy for HCC and demonstrated LS ≥ 25.6 kPa was the sole independent predictor of PHLF.³¹ In the present study, LS did not emerge as an independent predictor as patients undergoing LR for all hepatobiliary diseases were included and the primary endpoint was not ISGLS criteria unlike the above-mentioned studies. Liver stiffness probably plays a more important role in patients having underlying liver disease (fibrosis) as demonstrated by various authors evaluating PHLF in patients with HCC.^{20,21,30,31}

In our study, diabetes mellitus was an important risk factor for PHLF probably because it adversely affects liver regeneration. Strazl *et al* have established by an animal experiment that insulin was the most important hepatotrophic factor in the portal blood and its absence decreased liver regeneration in hepatectomized dogs.³² Similarly the duration of chemotherapy has a negative impact on liver recovery after LR and Shindoh *et al* have proposed to ensure >30% FLR in patients who have received >12 weeks of combination chemotherapy.^{33,34} Interestingly in our study FLR was not a significant factor probably it was a selection criteria for LR and patients with insufficient FLR were not resected. The main objective of LR should be to bring the incidence of PHLF down to zero and widespread utilization of ICG-R15 as in Eastern countries would ensure safe hepatectomy in all patients. The proposed nomogram would help in pre-operative preparation and selection of the patients and help in achieving this goal.

Study highlights and limitations: To the best of our knowledge, the present study evaluated both LS and ICG-R15 prospectively for the first time in Western patients. Nomogram was built with readily available binary factors and it predicts clinical relevant endpoint with excellent discriminatory performance (AUROC=0.92) and good calibration. A nomogram is considered as an evidence-based, accurate, and individualized risk prediction model that is better than risk grouping, look-up tables or classification and regression tree

analysis.^{30,35} The other unique features of this study are: 1. This study included all types of hepatectomies for various etiologies enabling the results to be extrapolated to wide range of clinical situations, 2. Internal validation by bootstrap resampling was performed to boost the robustness of our results, 3. Only pre-operative parameters were included in the analysis, and 4. All patients were analyzed in intention-to-treat manner. The main limitation of this study is that the nomogram could not be validated in an external cohort, as ICG-R15 was not practiced routinely in other centers.

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Table 2: Independent pre-operative predictors of post-hepatectomy liver failure in 418 patients

Pre-operative variables [#]	Prevalence		Univariate	Multivariate Logistic					Bootstrapped Multivariate		
			Regression	Regression		Regression			Regression		
			<i>p</i>	<i>Beta</i>	<i>p</i>	Odds Ratio	95% Confidence Interval		<i>p</i>	95% Confidence Interval	
						Lower	Upper		Lower	Upper	
Age in years	< 60	184	0.1								
	≥ 60	234									
Gender	Male	244	0.7								
	Female	174									
Body mass index, kg/m ²	< 30	344	0.4								
	≥ 30	74									
Diabetes Mellitus	No	347	0.02	1.9	0.04	6.6	1.1	39.3	0.04	-16.5	17.3
	Yes	71									
Hypertension	No	250	0.8								
	Yes	168									
Dyslipidemia	No	331	0.6								
	Yes	87									
Pre-operative chemotherapy cycles	< 8 cycles	328	0.03	1.4	0.09	4.1	0.8	20.9	0.05	-1.3	27.9
	≥ 8 cycles	90									
Tumor size in mm	≤ 50	307	0.001	1.6	0.07	4.8	0.9	26.1	0.05	-0.9	30.7
	> 50	111									
Tumor number	≤ 1	253	0.8								
	> 1	165									
Tumor type	Benign Disease	72	0.05								
	Liver Metastasis	186									
	Primary cancer	160									
Serum total bilirubin, μmol/L	< 34	406	0.003								
	≥ 34	12									
Serum aspartate aminotransferase, IU/L	≤ 50	338	0.01								
	> 50	80									
Serum alkaline phosphatase, IU/L	< 100	222	0.1								
	≥ 100	195									
Hemoglobin, g/L	< 10	14	0.1								
	≥ 10	404									
Platelet count, 10 ⁹ /L	< 150	60	0.04	2.2	0.02	8.7	1.3	56.8	0.001	-0.5	18.6
	≥ 150	358									
Indocyanine green retention at 15 min, %	< 15	266	0.01	2.3	0.01	10.4	1.9	58.1	0.004	-0.2	18.5
	≥ 15	51									
Liver stiffness measurement, kPa	< 22	304	0.01								
	≥ 22	24									
Controlled attenuation parameter, dB/m	< 250	181	0.8								
	≥ 250	138									
Number of liver segments to be resected	< 3	253	<0.001	2.6	0.03	12.9	1.3	125.4	0.004	1.3	21.2
	≥ 3	165									
Constant											
					<0.001				0.001	-40.1	-5.6

[#]All unrelated variables with $P \leq 0.15$ in the univariate regression was entered into multivariate logistic regression by Backward elimination (Wald) and the variables that remained in the model were validated by multivariate logistic regression bootstrapped at 1000

Table 3: Comparison of characteristics of patients with and without persistent post-hepatectomy liver failure (PHLF)

Variables [#]	NO PHLF N=399	PHLF N=19	<i>p</i> *
Age in years, mean±SD	60.5±13.9	66.8±7.7	0.05
Gender: Male/Female	232(58.1)/167(41.9)	12(63.2)/7(36.8)	0.6
Body mass index in kg/m ² , mean±SD	25.3±5	25.7±4.6	0.7
Diabetes Mellitus	64(16)	7(36.8)	0.02
Tumor size in mm, mean±SD	39.3±36.6	57.6±42.6	0.06
Tumor number, mean±SD	2±2	2.1±1.9	0.9
Pre-operative chemotherapy cycles, mean±SD	4.5±6.7	4.8±6.2	0.8
Serum total bilirubin in µmol/L, mean±SD	11.5±8.6	19.8±21.3	0.05
Serum aspartate aminotransferase in IU/L, mean±SD	43.5±52	107.3±173.3	<0.001
Serum alkaline phosphatase in IU/L, mean±SD	130.8±123.7	188.4±160.1	0.01
International normalized ratio, mean±SD	1±0.2	1±0.0	0.5
Serum creatinine in µmol/L, mean±SD	78.5±48.6	79.2±25.5	0.4
Blood sugar in mmol/L, mean±SD	6.5±2.1	7.3±2.8	0.2
Serum albumin in g/L, mean±SD	37.4±5.1	36.3±6.2	0.6
Hemoglobin in g/L, mean±SD	13.3±1.8	12.5±2.2	0.1
Platelet count in 10 ⁹ /L, mean±SD	241.5±94.5	202.4±81.9	0.1
ICG-R15 in %, mean±SD	9.7±7	13.9±6.9	0.01
Liver stiffness measurement in kPa, mean±SD	9.3±8.5	16±13.8	0.08
Controlled attenuation parameter in dB/m, mean±SD	243.7±63.6	254.3±63	0.7
Surgical approach: Open/Laparoscopic hepatectomy	317(79.4)/82(20.6)	19(100)/0(0)	0.03
Number of liver segments, mean±SD	1.9±1.8	4.5±1.3	<0.001
Pringle maneuver	306(76.7)	11(57.9)	0.06
Duration of Pringle maneuver in min, mean±SD	34.3±28.4	53.7±58.3	0.4
Operative time in min, mean±SD	305.7±115.2	419.9±136.8	<0.001
Blood loss in ml, mean±SD	622.1±615.1	1950±1990	<0.001
90-day severe morbidity	71(17.8)	16(84.2)	<0.001
90-day mortality	3(0.8)	4(21.1)	<0.001
90-day comprehensive complication index, mean±SD	17±19.9	56.1±27.6	<0.001
Length of hospital stay in days, mean±SD	9.8±7.7	28.1±14.7	<0.001

[#]Data are expressed as counts (percentage) unless otherwise stated; ICG-R15-indocyanine green retention at 15 minutes; * Mann-Whitney U test with asymptomatic bilateral significance

Figure 1: Nomogram to predict post-hepatectomy liver failure

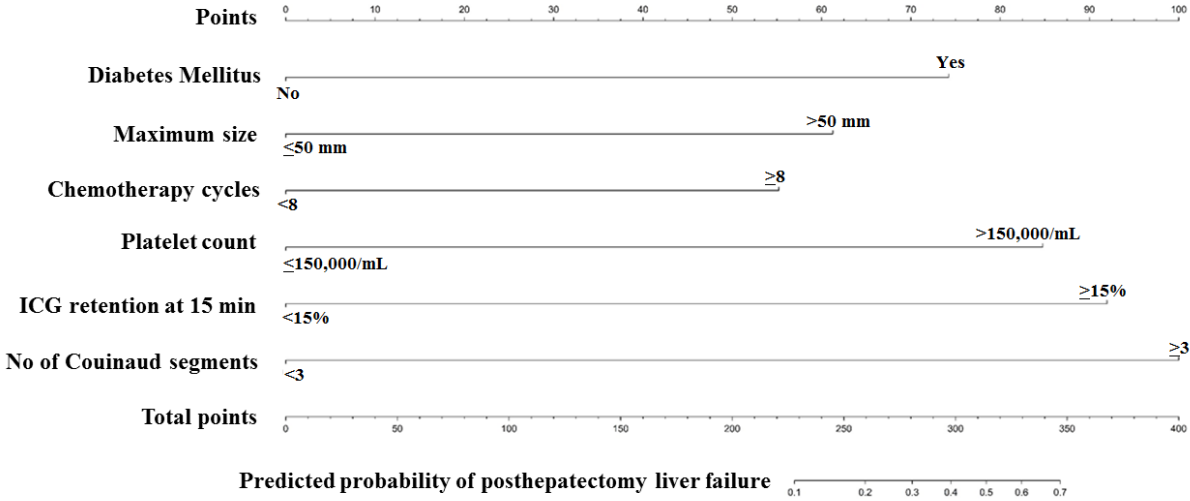
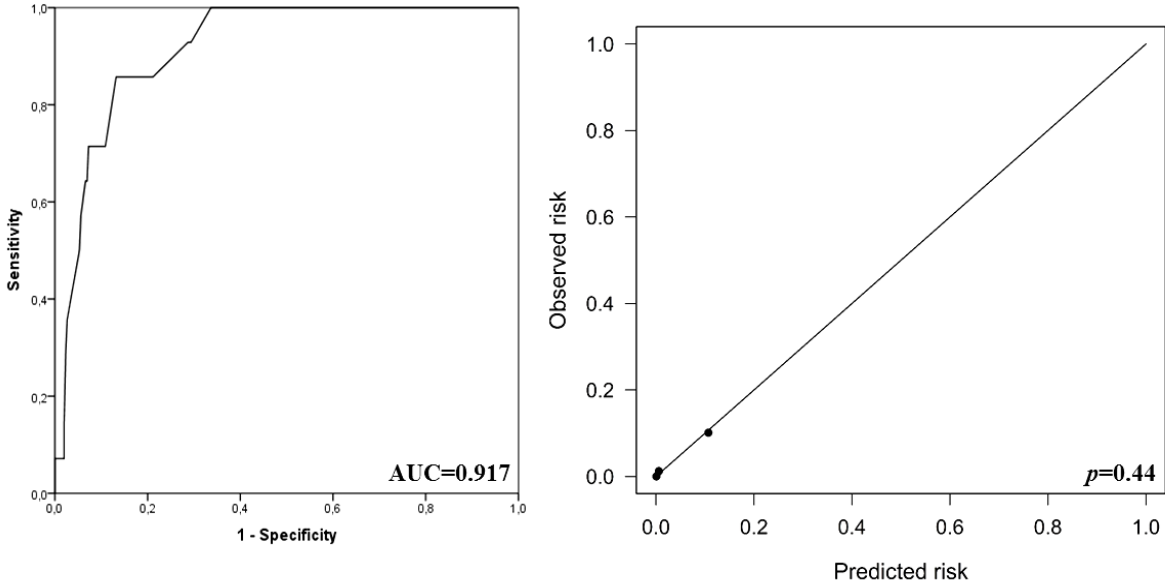


Figure 2: Receiver operating characteristic analysis and calibration plot of the predictive nomogram



Supplementary results:

Table 1: Independent predictors of post-operative hepatic decompensation in 418 patients undergoing hepatectomy for various indications

Variables	Prevalence	Univariate Regression [#]		Multivariate Logistic Regression [#]				Bootstrapped Multivariate Logistic Regression [#]			
		<i>p</i>	<i>Beta</i>	<i>p</i>	Odds Ratio	95% Confidence Interval		<i>p</i>	95% Confidence Interval		
						Lower	Upper		Lower	Upper	
Age in years	< 60	184	0.001	1.11	0.01	3.05	1.407	6.591	0.004	0.401	1.959
	≥ 60	234									
Gender	Male	244	0.13								
	Female	174									
Body mass index in kg/m ²	< 30	344	0.08								
	≥ 30	74									
Diabetes Mellitus	No	347	0.05								
	Yes	71									
Hypertension	No	250	0.02								
	Yes	168									
Dyslipidemia	No	331	0.14								
	Yes	87									
Pre-operative chemotherapy cycles	< 8 cycles	328	0.48								
	≥ 8 cycles	90									
Tumor size in mm	≤ 50	307	0.08								
	> 50	111									
Tumor number	≤ 1	253	0.24								
	> 1	165									
Tumor type	Benign Disease	72									
	Liver Metastasis	186	<0.001								
	Primary cancer	160									
Serum total bilirubin in μmol/L	< 34	406	0.01								
	≥ 34	12									
Serum aspartate aminotransferase in IU/L	≤ 50	338	<0.001								
	> 50	80									
Serum alkaline phosphatase in IU/L	< 100	222	<0.001								
	≥ 100	195									
Hemoglobin in g/L	< 10	14	0.01								
	≥ 10	404									
Platelet count in 10 ⁹ /L	< 150	60	0.43								
	≥ 150	358									
Indocyanine green retention at 15 min in %	< 10		0.04								
	≥ 10										
Liver stiffness measurement in kPa	< 10	304	<0.001	1.17	0.003	3.23	1.496	6.965	0.003	0.356	1.890
	≥ 10	24									
Controlled attenuation parameter in dB/m	< 250	181	0.86								
	≥ 250	138									
Re-hepatectomy	No	350	0.89								
	Yes	68									
Surgical approach	Laparoscopy	82	0.003								
	Laparotomy	336									
Number of liver segments to be resected	< 3	253	<0.001	1.84	<0.001	6.32	2.925	13.670	0.001	1.017	2.512
	≥ 3	165									
Duration of Pringle maneuver in min	< 60	333	0.31								
	≥ 60	85									
Operative time in min	< 360	291	<0.001	0.79	0.04	2.203	1.041	4.664	0.01	0.181	1.526
	≥ 360	127									
Blood loss in ml	< 1000	340	<0.001								
	≥ 1000	77									
RBC transfusion	No	366	0.001								
	Yes	52									
Constant					<0.001				0.001	-4.732	-2.849

[#]All unrelated variables with $p \leq 0.05$ in the univariate regression was entered into multivariate logistic regression by backward entry of Wald and those that remained in the equation were validated by multivariate logistic regression bootstrapped at 1000

Figure 1: Nomogram to predict post-operative hepatic decompensation

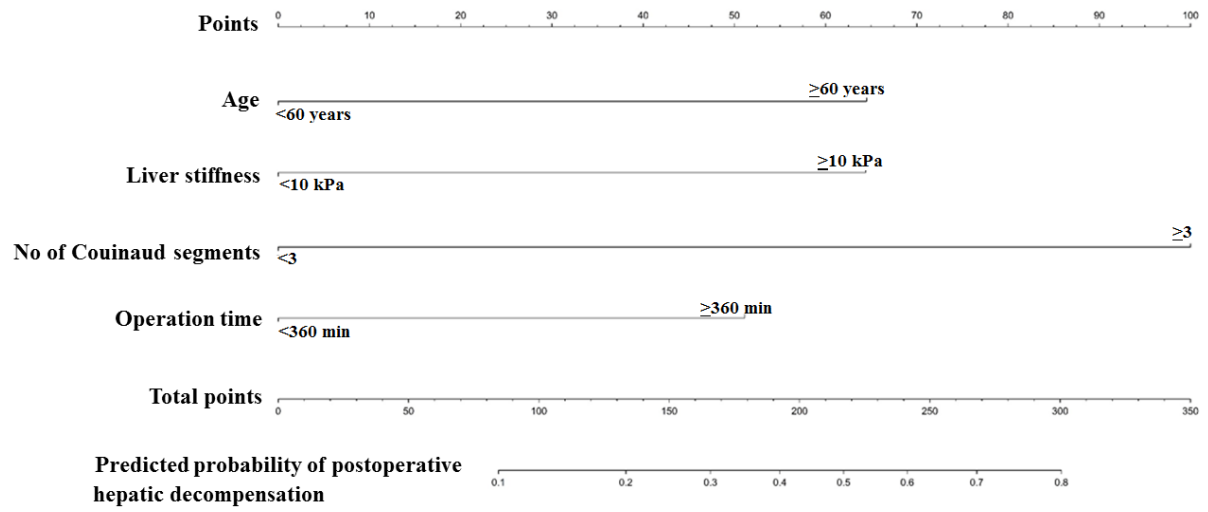


Figure 2: Receiver operating characteristic analysis and calibration plot of the predictive nomogram

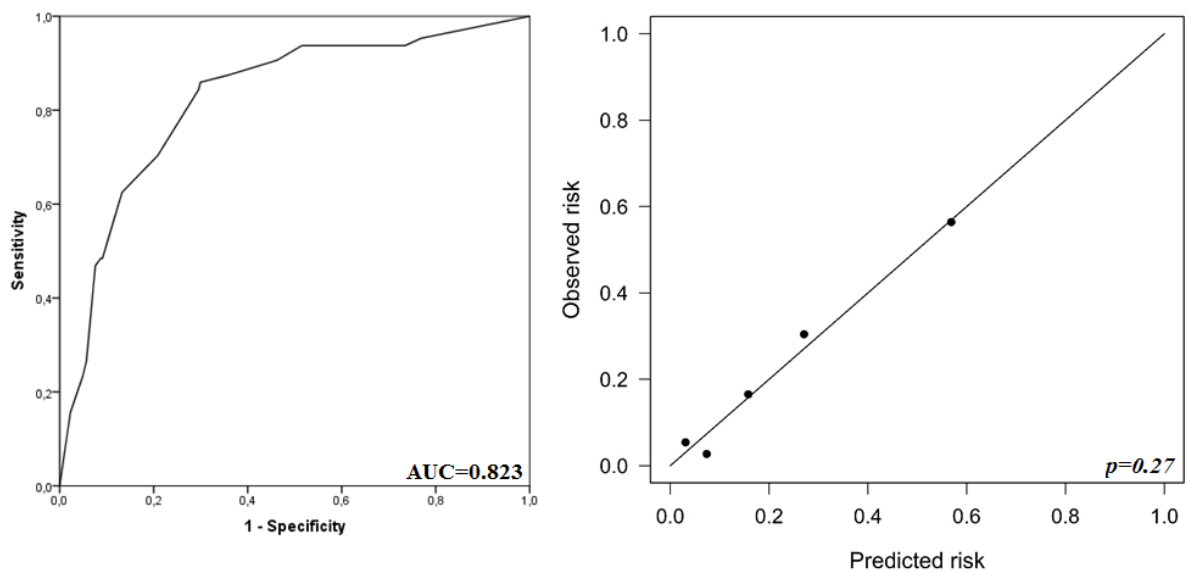


Table 2: Demographic, primary tumor, and treatment characteristics of patients undergoing major hepatectomy

Variables [#]	N = 164 (100%)
Patient Demographics	
▪ Age in years, median (range)	62 (17-86)
▪ Gender, male : female	93 (56.7) : 71 (43.3)
▪ Body mass index in kg/m ² , median (range)	24 (13-41)
▪ Diabetes Mellitus	25 (15.2)
▪ Hypertension	59 (36)
▪ Dyslipidemia	32 (19.5)
▪ Pre-operative transarterial chemoembolization	13 (7.9)
▪ Pre-operative chemotherapy	70 (42.7)
Indications for Hepatectomy	
▪ Colorectal liver metastasis	51 (31.1)
▪ Hepatocellular carcinoma	35 (21.4)
▪ Perihilar cholangiocarcinoma	20 (12.2)
▪ Intra-hepatic cholangiocarcinoma	14 (8.5)
▪ Non colorectal liver metastasis	11 (6.7)
▪ Living donors	11 (6.7)
▪ Benign liver tumors	5 (3.1)
▪ Gall bladder carcinoma	3 (1.8)
▪ Others	14 (8.5)
Tumor Characteristics	
▪ Tumor number, median (range)	1 (0-14)
▪ Diameter of largest nodule in mm, median (range)	36.5 (0-190)
▪ Serum α -fetoprotein in ng/mL, median (range)	4 (1-33930)
▪ Serum carcinoembryonic antigen in IU/L, median (range)	2 (0.5-1945.4)
▪ Serum carbohydrate antigen 19-9 in IU/L, median (range)	23.5 (1-5775700)
Laboratory tests	
▪ Serum total bilirubin in μ mol/L, median (range)	10 (4-90)
▪ Serum aspartate aminotransferase in IU/L, median (range)	38 (14-780)
▪ Serum alanine aminotransferase in IU/L, median (range)	39 (10-776)
▪ Serum γ -glutamyl transpeptidase in IU/L, median (range)	153(10-2597)
▪ Serum alkaline phosphatase in IU/L, median (range)	121 (32-955)
▪ Prothrombin activity in %, median (range)	91 (53-100)
▪ International normalized ratio, median (range)	1 (1-2)
▪ Serum fibrinogen in g/L , median (range)	4 (1-11)
▪ Blood urea in mmol/L, median (range)	5 (1-10)
▪ Serum creatinine in μ mol/L, median (range)	67.5 (46-126)
▪ Blood glucose in mmol/L, median (range)	6 (1-15)
▪ Serum albumin in g/L, median (range)	37.4 (18.3-66)
▪ Serum protein in g/L, median (range)	73.5 (57-96)
▪ Hemoglobin in g/L, median (range)	13 (8-19)
▪ Platelet count in 10 ⁹ /L, median (range)	249 (55-648)
▪ Indocyanine green retention at 15 mins in %, median (range)	8 (1-38)
▪ Liver stiffness measurement in kPa, median (range)	6 (3-75)
▪ Controlled attenuation parameter in dB/m, median (range)	236.5 (100-400)
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	151 (92.1) : 13 (7.9)
▪ Re-hepatectomy	19 (11.6)

▪ Portal vein embolization	37 (22.6)
▪ Pre-operative biliary drainage	14 (8.5)
▪ Hepatic venous pressure gradient in mmHg, median (range)	4 (1-14)
▪ Right hepatectomy	55 (33.5)
▪ Extended right hepatectomy	33 (20.1)
▪ Left hepatectomy	31 (18.9)
▪ Extended left hepatectomy	38 (23.2)
▪ Central hepatectomy	7 (4.3)
▪ Duration of Pringle maneuver in min, median (range)	34 (0-171)
▪ Operative time in min, median (range)	360 (126-755)
▪ Blood loss in mL, median (range)	700 (50-7500)
▪ Intra-operative RBC transfusion	26 (15.9)
Post-operative outcomes	
▪ 90-day severe morbidity ¹	49 (29.9)
▪ Hepatic decompensation ²	63 (38.4)
▪ Liver failure ³	17 (10.4)
▪ 90-day mortality	4 (2.4)
▪ Comprehensive complication index, median (range)	21 (0-100)
▪ Duration of ICU stay in days, median (range)	1 (0-36)
▪ Length of hospitalization in days, median (range)	9 (3-77)

¹Data are expressed as counts (percentage) unless otherwise stated, 1-Dindo-Clavien grade of surgical complications >2, 2-presence of ascites, jaundice, and/or encephalopathy, 3-50-50 criteria or peak total bilirubin >7 mg/dL.

Table 3: Independent predictors of 90-day major complications in patients undergoing major hepatectomy for various indications (N=164)

Variables#	Univariate		Multivariate Logistic			Bootstrapped Multivariate	
	Regression		Regression			Logistic regression	
	<i>p</i>	<i>Beta</i>	<i>p</i>	OR	95% CI	<i>p</i>	95% CI
Age in years	<0.001	0.06	0.01	1.06	1.02 – 1.11	0.02	0.01 – 0.13
Gender	0.27						
Body mass index, kg/m ²	0.25						
Diabetes Mellitus	0.09						
ASA physical status	0.09						
Pre-operative chemotherapy cycles	0.63						
Tumor size in mm	0.61						
Tumor number	0.60						
Tumor type	0.03						
Serum total bilirubin, μmol/L	0.19						
Serum aspartate aminotransferase, IU/L	0.29						
Serum alkaline phosphatase, IU/L	0.05						
Hemoglobin, g/L	0.39						
Platelet count, 10 ⁹ /L	0.92						
Indocyanine green retention at 15 min, %	0.01	0.06	0.07	1.06	0.99 – 1.13	0.04	0.00 – 0.13
Liver stiffness measurement, kPa	0.27						
Controlled attenuation parameter, dB/m	0.54						
Number of liver segments to be resected	0.002	0.43	0.08	1.54	0.95 – 2.50	0.07	-0.11 – 1.02
Re-hepatectomy	0.22						
Surgical approach, Laparotomy	0.99						
Duration of Pringle maneuver, min	0.31						
Operative time, min	0.002	0.004	0.02	1.01	1.00 – 1.01	0.04	0.00 – 0.01
Blood loss, mL	0.04						
RBC transfusion	0.02						
Constant			<0.001			0.001	-14.95 – -5.55

All unrelated variables with $P \leq 0.05$ in the univariate regression was entered into multivariate logistic regression by Backward elimination (Wald) method and the variables that remained in the equation were validated by multivariate logistic regression bootstrapped at 1000, ASA-American society of anesthesiologists, OR-Odds ratio, CI-confidence interval.

Figure 3: Nomogram to predict 90-day severe morbidity after major hepatectomy

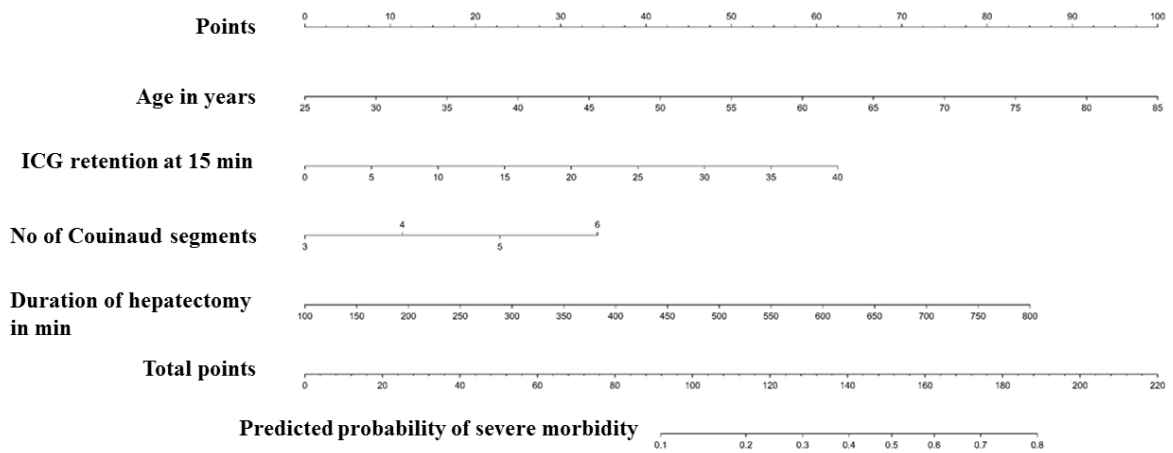
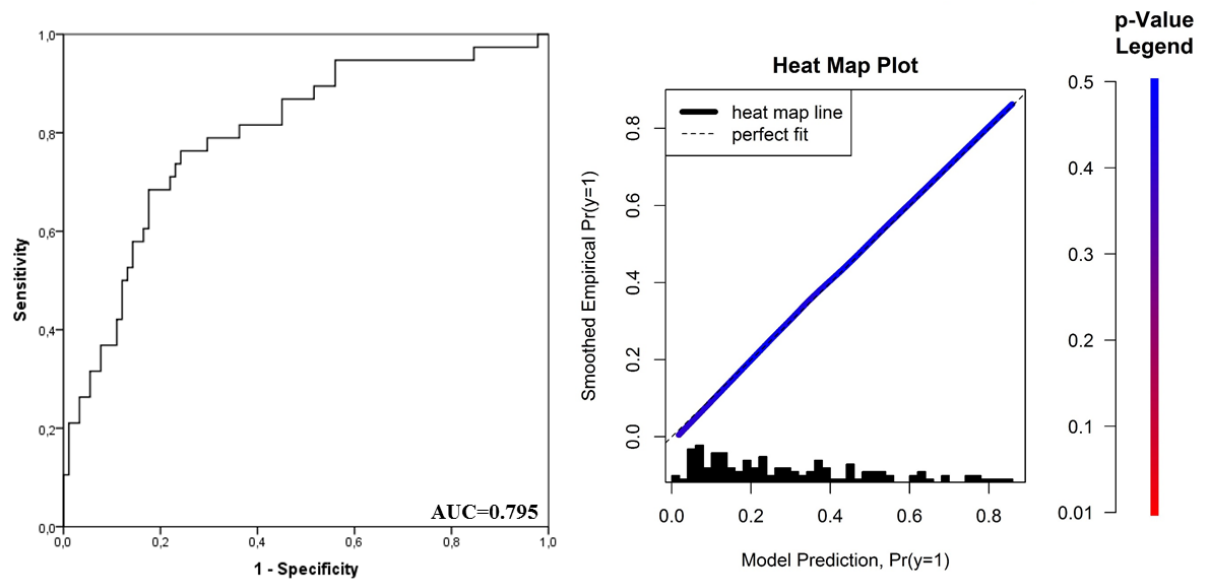


Figure 4: Receiver operating characteristic analysis and heat map plot of the predictive nomogram



Article 6: Liver stiffness measurement by transient elastography predicts late post-hepatectomy outcomes in patients undergoing resection for hepatocellular carcinoma

Authors:

Muthukumarassamy Rajakannu,^{1,2,3} MBBS, MS, DNB, MRCS, Daniel Cherqui,^{1,3} MD, Oriana Ciacio,¹ MD, Nicolas Golse,¹ MD, Gabriella Pittau,¹ MD, Marc Antoine Allard,¹ MD, Teresa Maria Antonini,¹ MD, Audrey Coilly,^{1,2} MD, Antonio Sa Cunha,^{1,3} MD, Denis Castaing,^{1,2,3} MD, Didier Samuel,^{1,2,3} MD, PhD, Catherine Guettier,^{1,2,3} MD, PhD, René Adam,^{1,3,4} MD, PhD, Eric Vibert,^{1,2,3} MD, PhD

Affiliations :

¹Centre Hépatobiliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

Running title: Role of transient elastography in the management of hepatocellular carcinoma

Keywords: Liver resection, post-operative complication, liver decompensation, cirrhosis, FibroScan

Correspondance :

Prof. Eric Vibert

Centre Hépatobiliaire,

AH-HP Hôpital Paul Brousse,

12 Avenue Paul Vaillant Couturier,

94800 Villejuif,

France

Abstract

Background: Post-operative hepatic decompensation is a serious complication of liver resection in patients undergoing hepatectomy for hepatocellular carcinoma(HCC). Liver fibrosis and clinical significant portal hypertension(CSPH) are well-known risk factors for hepatic decompensation. Liver stiffness measurement(LSM) is a non-invasive method of evaluating hepatic venous pressure gradient(HVPG) and functional hepatic reserve by estimating hepatic fibrosis. Effectiveness of LSM in predicting persistent post-operative hepatic decompensation(PHD) has not yet been investigated.

Methods: Consecutive patients with resectable HCC were recruited prospectively and LSM of non-tumoral liver was measured using FibroScan[®]. HVPG was measured intra-operatively by direct puncture of portal vein and inferior vena cava. HVPG \geq 10mmHg was defined as CSPH. Primary outcome was PHD defined as the presence of at least one of the following unresolved ascites, jaundice, and/or encephalopathy beyond three months following hepatectomy.

Results: One hundred and six hepatectomies, including 22 right-hepatectomy(20.8%), 3 central-hepatectomy(2.8%), 12 left-hepatectomy(11.3%), 11 bi-segmentectomy(10.4%), 30 uni-segmentectomy(28.3%) and 28 partial hepatectomy(26.4%), were performed in patients for HCC(84 men and 22 women with median age of 67.5 years; median model for end-stage liver disease score of eight). Ninety-day mortality was 4.7%. Nine patients(8.5%) developed PHD. Multivariate logistic regression bootstrapped at 1000 identified LSM($P=0.001$) as the only pre-operative predictor of PHD. Area under ROC curve for LSM and HVPG was 0.81[95%CI=0.506-0.907] and 0.71[95%CI=0.646-0.917] respectively. LSM \geq 22kPa had 42.9% sensitivity and 92.6% specificity while HVPG \geq 10mmHg had 28.6% sensitivity and 96.3% specificity.

Conclusion: In selected patients undergoing liver resection for HCC, transient elastography is an easy and effective test to predict PHD pre-operatively.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world, with an incidence of more than 500,000 new cases.¹ Surgical resection and liver transplantation (LT) are standard curative therapies for HCC.^{2,3} Although LT is considered the best treatment for HCC in patients with underlying liver disease, it is hampered by severe organ shortage.² Curative liver resection (LR) for HCC is the treatment of choice in non-cirrhotic patients and in cirrhotic patients having very early stage (Barcelona Clinic Liver Cancer [BCLC] stage 0) and early stage (BCLC stage A) tumors.^{2,3} The outcome of LR has improved significantly over the years because of better patient selection, improved surgical techniques and peri-operative management.²⁻⁵ Post-hepatectomy liver failure (PHLF), defined by 50-50 criteria/peak post-operative total bilirubin ≥ 120 $\mu\text{mol/L}$, and hepatic decompensation as defined by Bruix *et al* are the two important post-operative complications that impact the patients' survival and quality of life after LR.^{3,7,8,9} Further, the prognosis of patients depends not only tumor biology but also quality of the liver remnant and presence of portal hypertension.^{3,6} Five-year survival after LR in patients with and without clinically significant portal hypertension (CSPH) were $>70\%$ and 50-60% respectively.^{3,5,7,8} Bruix *et al* and Boleslawski *et al* have demonstrated that HVPG was an independent risk factor for postoperative hepatic decompensation and 90-day mortality in cirrhotic patients undergoing LR for BCLC stage 0/A HCC. Therefore, evaluation of HVPG is considered as a key step in the pre-operative assessment of patients for hepatectomy in compensated cirrhosis.^{7,8} However, it is not routinely used in most centers, as it is an invasive procedure and the role of LSM was not explored in these two studies. Recently liver stiffness measurement (LSM) by transient elastography (TE) has emerged as a non-invasive test to assess liver fibrosis, HVPG and predict post-hepatectomy outcomes.¹⁰⁻¹⁵ Kim *et al* and Cescon *et al* have demonstrated

that elevated LSM to be an independent predictor of PHLF with or without hepatic decompensation.^{14,15} In this study we investigated the effectiveness of TE to non-invasively predict post-operative hepatic decompensation which persist beyond three months in patients undergoing hepatectomy for HCC.

Patients and Methods

Consecutive adult patients with potentially resectable HCC referred to our institute from November 2014 to August 2016 were included prospectively in the study. The Ethics Committee of the hospital approved the study protocol and from each patient gave an informed consent according to the principles of the Helsinki Declaration. Clinical and laboratory data were obtained at inclusion and at post-operative follow-up visits. Final follow-up was on February 28, 2017.

Transient Elastography

In all patients, TE was performed pre-operatively in fasting condition using FibroScan[®] Touch502 with M/XL probes. Liver stiffness was measured on the right lobe of the liver through intercostal spaces by placing the probe on the skin between the ribs while the patient was lying supine with the right arm in maximal abduction. A liver portion of at least 6 cm thick, free of large vascular structures and tumor, was located by an ultrasonic time-motion image and ten successful measurements were performed on each patient. LSM was expressed in kilopascal (kPa) and median value was kept as representative of liver stiffness. Only measurements with a success rate of at least 60% and an interquartile range (IQR)/median <30% were considered valid.^{10,15} The operator was blinded to the patient's clinical data. All patients were operated within two weeks of TE.

Liver resection

Pre-operative evaluation consisted of laboratory tests including liver function tests, imaging studies like ultrasonography, dynamic computed tomography±volumetry, magnetic resonance imaging, indocyanine green retention at 15 minutes (ICG-R15), and LSM but it did not

include HVPG measurement. Usually hepatectomy was performed when MELD ≤ 12 , platelet count $\geq 80,000$ and ICG-R15 $\leq 20\%$. When future liver remnant (FLR) would not be sufficient, portal vein embolization (PVE) was performed to ensure FLR was 0.5% of body weight or $\geq 20\%$ of standard liver volume in non-cirrhotic liver. Transarterial chemoembolization followed by PVE was the treatment strategy when planning major LR in patients with cirrhosis.¹⁶ The technique of liver resection for HCC in our unit has been standardized since 1982.^{17,18} At surgery, exploration of the abdominal cavity is performed to detect extrahepatic spread. Systematic intra-operative liver ultrasound is carried out. Enlarged hepatic lymph nodes and any suspicious nodules are excised for frozen section histology. The objective of hepatectomy was to resect all detectable lesions with tumor-free margins of ≥ 1 cm while leaving behind adequate liver parenchyma to ensure normal liver function. In general, anatomic resections were preferred to wedge resections. In our center, there was no protocol to measure HVPG in all patients. It was feasible only during laparotomy and was performed before the commencement of hepatectomy at individual surgeon's discretion. HVPG was measured intra-operatively by direct puncture of portal vein in the hepatic pedicle and retro-hepatic inferior vena cava using a 25-gauge needle connected to a manometer with the patient in supine neutral position and after zeroing to the atmospheric pressure at the level of heart. HVPG was obtained by calculating the difference between the measured portal and hepatic venous pressures. Parenchymal dissection is done using the ultrasonic dissector (CUSA, Cavitron Ultrasonic Aspirator, Valley Lab Inc., Boulder, CO), and resections are usually performed under intermittent clamping of the portal triad. After LR, serial follow-up of patients were provided to monitor the post-operative complications and HCC recurrence. A contrast-enhanced abdominal computer tomography (CECT) was performed at 1-week and 1-month after LR and then alternating abdominal ultrasonography and CECT were performed every four months along with serum α -fetoprotein and liver function tests.

Definitions

Persistent post-operative hepatic decompensation (PHD) was defined as the presence of at least one of the following unresolved ascites, jaundice, and/or encephalopathy beyond three months following hepatectomy.⁷ HVPG ≥ 10 mmHg was defined as CSPH.⁷

Statistical analysis

Discrete variables are reported as counts (percentage) and parametric variables as median (range). To identify the variables associated with development of PHD, patients compared by unpaired Student's *t* test for continuous variables with parametric distribution, Mann–Whitney *U* test for those with nonparametric distribution, and *Chi* square or Fischer's exact test for quantitative variables. The pre-operative parameters entered into analysis were age, gender, body mass index, tumor number, tumor size, total bilirubin, prothrombin activity, γ -glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, fibrinogen, blood urea nitrogen, creatinine, plasma albumin, hemoglobin, platelet count, ICG-R15 and LSM. The parameters that were significant at 20% in this univariate analysis were entered into multivariate binary logistic regression (Forward entry) bootstrapped at 1000 to determine independent predictors of PHD. Pearson test was performed to estimate the correlation between LSM, ICG-R15 and HVPG. Receiver operating characteristic (ROC) analysis was performed to compare LSM with HVPG and to determine LSM cut-off value and the ROC curves were compared using DeLong test. Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratios for PHD prediction were calculated for previously described cut-off of LSM and HVPG. Mann Whitney *U* compared the two groups with and without PHD. SPSS 21.0 package (SPSS Inc., Chicago, IL) was utilized to perform the analysis. *P* value < 0.05 was considered significant.

Results

Pre-operative and operative details

One hundred and six patients, consisting of 84 men and 22 women with a median age of 67.5 years, underwent curative-intent LR for HCC during the study period. All patients, including 52 of them (49.1%) with cirrhosis (METAVIR F4), were in Child Pugh class A or B with compensated pre-operative liver function. Median MELD score was eight (range, 6-20) and platelet count was $215.5 \times 10^3/\text{mL}$ (range, $34\text{-}618 \times 10^3/\text{mL}$). Majority of patients under LR had underlying chronic liver disease with viral hepatitis being the most common etiology. Twelve patients (11.3%) had no known liver disease. Pre-operative TE was performed successfully in 95 patients with valid LSM evaluation possible in 89 patients (83.9%) including 69 patients with right-sided HCC. In 11 patients (10.4%) due to previous right hepatectomy, TE was not feasible and in 6 patients (5.7%) measurement were not considered valid as IQR/median was $>30\%$ due to presence of large right liver tumors. Minor hepatectomy (≤ 2 segments) was performed in majority of patients (65.1%) and major hepatectomy (> 2 liver segments) in 37 patients (34.9%). Majority of the LR (72.6%) was performed by laparotomy and 27.4% of LR was by laparoscopic approach. Uni-segmentectomy (28.3%) was the most common type of LR. Table 1 summarizes the clinical, laboratory, and operative details of the operated patients. The discriminatory performances of LSM for various METAVIR grades for fibrosis were as follows: $F \geq 1$ (area under ROC curve (AUROC): 0.84, 95%CI: 0.71-0.97), $F \geq 2$ (AUROC: 0.87, 95%CI: 0.77-0.96), $F \geq 3$ (AUROC: 0.86, 95%CI: 0.78-0.94), and $F = 4$ (AUROC: 0.88, 95%CI: 0.81-0.95) [Fig 1].

Post-hepatectomy decompensation

Twenty-nine patients (27.4%) developed post-operative liver decompensation in form of ascites in 18, jaundice in 18 and/or encephalopathy in four. Five patients (4.7%) died during 90-day post-operative period with two of them having sepsis associated with PHLF, one patient with ascites and sepsis due to ischemic colonic perforation, one patient from biliary sepsis due to endobiliary recurrence and the last patient died at his home after discharge from

hospital due to suspected cardiac event. At last follow-up, three more patients had died beyond 90-day post-operative period due to multiple intra-hepatic tumor recurrence in two and sepsis due hepatic necrosis in the third patient.

Nine patients (8.5%) who had initial decompensation had manifestations of persistent hepatic decompensation beyond 90-day post-operative period with eight patients having ascites requiring diuretics (including three patients who died within 90-day post-operative period) and one having persistent jaundice. Three patients, who had hepatic decompensation in the form of PHLF in two and ascites in one, at the time of their death were included in this cohort. The other two patients without signs of decompensation and with normal liver function at the time of their demise were excluded from the analysis. None of the patients, who did not develop early decompensation within three months of hepatectomy, developed it later. None of the patients had developed post-operative portal vein thrombosis. The types of LR performed in these nine patients were right hepatectomy (n=3), uni-segmentectomy (n=3), bi-segmentectomy (n=2) and left hepatectomy (n=1). Further, all patients who developed PHD had advanced compensated liver disease (METAVIR F3/F4). At last follow-up, three patients have died, three patients are alive with PHD, two patients have recovered from PHD and one patient had developed intra-hepatic recurrence.

Univariate analysis of the pre-operative parameters identified body mass index, alanine transaminase, hemoglobin, platelet count, and LSM as the probable risk factors. LSM was the only independent pre-operative parameter predictive of PHD beyond three months by bootstrapped multivariate analysis. There was a significant correlation between LSM and ICG-R15 ($P<0.0001$, $r=0.7$). When LSM and HVPG were compared in 34 patients who had both measurements, there was a significant correlation between LSM and HVPG ($P<0.0001$, $r=0.6$). However, there was no significant difference in their diagnostic ability when the AUROC for LSM (0.8) and HVPG (0.7) were compared ($P=0.5$). The cut-off value for LSM

was determined as 22 kPa and it was compared to previously established HVPG cut-off of 10 mmHg. LSM \geq 22 kPa had 42.9% sensitivity and 92.6% specificity whereas HVPG \geq 10 mmHg had 28.6% sensitivity and 96.3% specificity. Positive predictive value for LSM and HVPG was 60% and 66.7%. Negative predictive value (NPV) of LSM (86.2%) was better than that of HVPG (83.8%). Positive and negative likelihood ratios (LR) of LSM were 5.8 and 0.6 while those of HVPG were 0.4 and 0.7 respectively. Majority of the patients with LSM \geq 22 kPa (66.7%) developed complications after hepatectomy with two patients dying due to PHLF in the 90-day post-operative period.

Table 3 summarizes the characteristics of patients with and without PHD.

Discussion

In this prospective study LSM was the only independent predictor of PHD among the pre-operative demographic, tumor and laboratory parameters in patients undergoing LR for HCC. A LSM cut-off of \geq 22 kPa was demonstrated to have better sensitivity, NPV and positive LR in discriminating patients with different risks of hepatic decompensation when compared to HVPG \geq 10 mmHg. TE was feasible in majority of our patients without any change in the pre-operative workup schedule and in planned operative strategy. With widespread availability of FibroScan[®] in hepatobiliary centers, the simplicity of its use and reliability of the results, LSM must be added to the routine pre-operative workup of patients with HCC, as it would help in adapting the surgical strategy to reduce post-operative morbi-mortality in high-risk patients. PVE could be performed to better prepare high-risk patients, liver-conserving non-anatomic resection preferred over wide-margin and anatomic resections, choose an alternative surgical treatment like local ablative therapy instead of LR, or plan an early salvage LT in patients who are likely to decompensate after hepatectomy. In surgical patients, major limitation to TE would be in the patients having large right liver mass and in patients having recurrence after right hepatectomy as observed in 16.1% of patients in the present study.

Therefore, LSM would be a good noninvasive surrogate for invasive HVPG measurement in patients planned for LR.

Persistent post-operative hepatic decompensation, defined by the presence of ascites, jaundice and/ or hepatic encephalopathy beyond 90-day post-operative period, is a serious complication of hepatectomy especially in patients having CSPH with an incidence of about 38% in cirrhotic patients.⁷ Five-year survival and the quality of life were significantly reduced in these patients.^{7,8} Therefore, several authors have recommended the invasive measurement of HVPG pre-operatively to predict PHD in LR candidates.^{7,8,19,20}

Recently LSM measured by TE has been demonstrated to be the non-invasive marker of liver fibrosis, HVPG, hepatic functional reserve and a pre-operative predictor of early post-hepatectomy outcomes.¹²⁻¹⁵ LSM has been proven to have an excellent performance in diagnosing cirrhosis (AUROC=1), and significant fibrosis (AUROC=0.9), equivalent to the best blood markers.^{10,11,21} In the present study, LSM demonstrated similar performance in differentiating various stages of hepatic fibrosis according to METAVIR scoring system. A prospective study by Robic *et al* demonstrated that LSM proved as effective as HVPG in predicting clinical decompensation and CSPH related complications in patients with chronic liver disease.¹² Vizzutti *et al* proposed a LSM cut-off of 13.6 kPa having 97% sensitivity and 92% specificity to predict CSPH in patients with hepatitis C virus-related cirrhosis.²² However, a recent study by Llop *et al* questioned the usefulness of LSM in the evaluation of CSPH in patients with compensated cirrhosis and resectable liver tumors despite a significant correlation between LSM and HVPG.²⁰ There are various cut-off values for LSM reported in the literature and they vary from 12 to 25 kPa depending on the study population and endpoint. The differences in the study population (HCC only or all types of tumors), endpoint (PHLF only or all post-operative morbidity), methodology and variability in LSM could explain these observations.^{13-15,20} However, LSM has not been specifically evaluated in the

context of PHD.^{7,8,20} In a prospective study of patients with HCC undergoing curative LR, Kim *et al* concluded that LSM as an independent risk factor PHLF and proposed a cut-off of 25.6 kPa.¹⁴ In another study evaluating the impact of LSM in the prediction of PHLF, proposed a cut-off >15.7 kPa to risk stratify the patients.¹⁵ In recent publication by Wong *et al*, LSM >12 kPa predicted worse early post-operative outcomes in 105 patients undergoing hepatectomy for various indications.¹³ When we tested various cut-offs reported for predicting post-hepatectomy complications and PHLF such as 12 kPa and 15.7 kPa in our cohort, we found that these cut-offs had a better sensitivity but a lower specificity of 66.7% and 85.2% respectively when compared to HVPG in prediction of PHD.^{13,15} Further, there is an overlap between the two groups in the grey zone of 12-22 kPa as demonstrated by Llop *et al* in their study comparing LSM with HVPG.²⁰ Therefore in the present study, we propose a cut-off of 22 kPa to help the surgeons in clinical decision-making for the patients with potentially resectable HCC because it had similar performance to the gold standard, HVPG ≥ 10 mmHg.^{7,20} Moreover we have demonstrated that all patients who developed PHD had advanced liver disease and it would be ideal to risk stratify these patients who would have higher LSM values.

Another test commonly adopted in Eastern countries to measure hepatic reserve is ICG clearance and it is being routinely used to predict post-operative outcomes.^{23,24} Lau *et al* demonstrated that ICG-R15 was the single best predictor of post-hepatectomy mortality in 127 non-cirrhotic and cirrhotic patients with HCC.²³ In another recent study of 28 LRs for HCC, LSM was demonstrated to have a moderate correlation with ICG-R15 but both of these tests failed to predict early post-operative complications.²⁵ By contrast, in the present study LSM was the only independent predictor of PHD and ICG clearance was not a risk factor despite having a moderate correlation with LSM. While HVPG evaluates the degree of portal

hypertension and ICG-R15 estimates functional hepatic reserve, LSM evaluates both HVPG and functional hepatic reserve by quantifying the degree of liver fibrosis accurately.^{10-12,22}

To the best of our knowledge, this is the first study to evaluate specifically the effectiveness of LSM in predicting delayed post-operative outcomes in patients undergoing LR for HCC in prospective manner and demonstrate better performance of non-invasive LSM over invasive HVPG measurement and ICG-R15. This mono-centric study recruited HCC patients with various etiology enabling the results to be extrapolated to wide range of indications. The other unique features of this study are that only pre-operative parameters were included in the analysis and all patients in whom TE was programmed were analyzed in intention-to-treat manner. Traditional risk factors for post-hepatectomy complications like MELD score and FLR volume were not tested, as they were criteria used to select patients for LR. Further, internal validation by bootstrapping the multivariate analysis was performed to boost the robustness of its results. The limitations of this study were the low prevalence of the primary endpoint and HVPG was not measured in all patients. These facts should be considered while interpreting the predictive values.

In conclusion, TE should be part of pre-operative workup to risk-stratify the patients with HCC as LSM predicts accurately persistent post-hepatectomy decompensation before LR.

Acknowledgements: Echosens™, Paris, France, funded this research.

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Table 1: Patient, primary tumor, and treatment characteristics

Variables [#]	N = 106 (100)
Patient Demographics	
▪ Age in years, median (range)	67.5 (26-87)
▪ Gender, male : female	84 (79.2) : 22 (20.8)
▪ Body mass index in kg/m ² , median (range)	15.5 (16-41)
▪ MELD score, median (range)	8 (6-20)
▪ Child Pugh score, median (range)	5 (5-8)
Etiology	
▪ Viral hepatitis (HBV, HCV, HEV)	41 (38.7)
▪ Non-alcoholic fatty liver disease	26 (24.5)
▪ Alcohol	22 (20.8)
▪ Others (Hemochromatosis, Wilson's disease)	5 (4.7)
▪ No known liver disease	12 (11.3)
Hepatocellular Carcinoma	
▪ Tumor number, median (range)	1 (1-6)
▪ Diameter of largest nodule in cm, median (range)	4 (1.1-19)
▪ Localization, right liver : left liver : bilateral	75 (70.8) : 28 (26.4) : 3 (2.8)
▪ Serum α -fetoprotein in ng/mL, median (range)	10 (1.5- 333930)
Laboratory tests	
▪ Serum total bilirubin in μ mol/L, median (range)	12 (4-36)
▪ Serum aspartate aminotransferase in IU/L, median (range)	35 (21-132)
▪ Serum alanine aminotransferase in IU/L, median (range)	37.5 (10-110)
▪ Serum γ -glutamyl transpeptidase in IU/L, median (range)	102.5 (18-2400)
▪ Serum alkaline phosphatase in IU/L, median (range)	96 (37-580)
▪ Prothrombin activity in %, median (range)	82 (59-100)
▪ International normalized ratio, median (range)	1.1 (0.9-1.6)
▪ Serum fibrinogen in g/L, median (range)	3.5 (1.1-8.4)
▪ Blood urea in mmol/L, median (range)	5 (2-24.7)
▪ Serum creatinine in μ mol/L, median (range)	72 (50-163)
▪ Glycaemia in mmol/L, median (range)	6.2 (1-16)
▪ Serum albumin in g/L, median (range)	36.6 (18.3-49.7)
▪ Hemoglobin in g/L, median (range)	13.5 (7.2-18.8)
▪ Platelet count in 10 ⁹ /L, median (range)	215.5 (34-618)
▪ Indocyanine green retention at 15 minutes in %, median (range)	12.1 (0.7-54.1)
▪ Liver stiffness measurement in kPa, median (range)	11.6 (3.3-48.8)
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	77 (72.6) : 29 (27.4)
▪ Portal vein embolization	14 (13.2)
▪ Pre-operative transarterial chemoembolization	22 (20.8)
▪ Future liver remnant as % of standard liver volume, median (range)	51.2 (26.3-76.2)
▪ Hepatic venous pressure gradient in mmHg, median (range)	4 (1-18)
▪ Uni-segmentectomy	30 (28.3)
▪ Partial hepatectomy	28 (26.4)
▪ Right hepatectomy	22 (20.8)
▪ Left hepatectomy	12 (11.3)
▪ Bi-segmentectomy	11 (10.4)
▪ Central hepatectomy	3 (2.8)
▪ Resection margin in mm, median (range)	4 (0-70)
METAVIR Fibrosis score of non-tumoral liver	
▪ F0	9 (8.5)
▪ F1	9 (8.5)
▪ F2	21 (19.8)
▪ F3	15 (14.2)
▪ F4	52 (49.1)

[#] Data are expressed as counts (percentage) unless otherwise stated; MELD-model for end-stage liver disease; HBV-hepatitis B virus; HCV-hepatitis C virus; HEV-hepatitis E virus

Table 2: Pre-operative variables as independent predictors of persistent post-operative hepatic decompensation by bootstrapped logistic regression in 106 patients

Pre-operative variables [#]	Univariate	Multivariate	95% Confidence interval	
	<i>P</i> value	<i>P</i> value	Lower	Upper
Age in years	0.727			
Female gender	0.486			
Body mass index in kg/m ²	0.189	0.337	-0.41	5.93
Viral hepatitis	0.731			
Tumor size in mm	0.867			
Tumor number	0.313			
Serum α -fetoprotein in ng/mL	0.828			
Serum total bilirubin in μ mol/L	0.408			
Serum alanine aminotransferase in IU/L	0.138	0.15	-0.15	0.67
Serum alkaline phosphatase in IU/L	0.972			
Serum fibrinogen in g/L	0.826			
International normalized ratio	0.365			
Serum creatinine in μ mol/L	0.206			
Blood sugar in mmol/L	0.873			
Serum albumin in g/L	0.628			
Hemoglobin in g/dL	0.116	0.785	-0.61	24.71
Platelet count in 10 ⁹ /L	0.179	0.223	-0.34	0.02
Indocyanine green retention at 15 minutes in %	0.321			
Liver stiffness measurement in kPa	0.005	0.001	-0.01	3.75
Major hepatectomy	0.373			
Constant		0.012	-603.99	-1.23

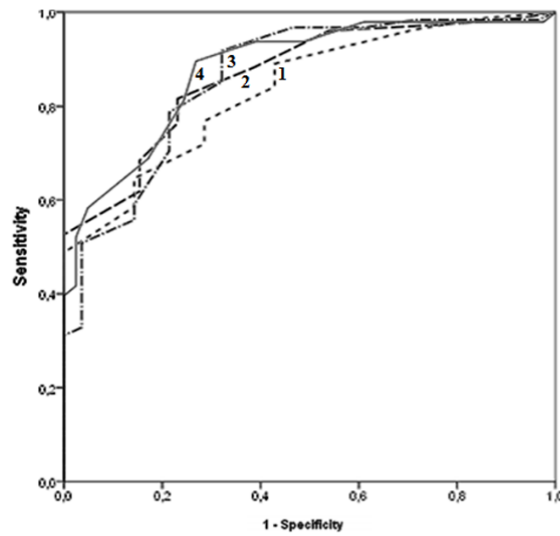
[#] all pre-operative variables in Table 1 were entered in to the univariate analysis and the variables with $p \leq 0.2$ were entered in to multivariate analysis

Table 3: Comparison of characteristics of patients with and without persistent post-operative hepatic decompensation (PHD)

Variables [#]	NO PHD	PHD	P value*
	N=97	N=9	
Age in years, mean±SD	65.1±12.5	66.7±8.9	0.82
Gender: Male/Female	76(78.4)/21(21.6)	8(99.9)/1(1.1)	0.45
Body mass index in kg/m ² , mean±SD	25.2±5.0	27.6±4.9	0.15
Viral hepatitis	39(40.2)	3(33.3)	0.73
MELD score, mean±SD	8.3±2.6	8.0±1.6	0.86
Child Pugh score, mean±SD	5.5±0.7	5.4±0.7	0.81
Tumor size in mm, mean±SD	56.7±43.3	54.2±43.5	0.89
Tumor number, mean±SD	1.4±0.7	1.7±1.7	0.99
Serum α-fetoprotein in ng/mL, mean±SD	3787.5±33882.7	622.3±816.5	0.30
Serum total bilirubin in μmol/L, mean±SD	11.2±5.6	12.8±4.8	0.21
Serum alanine aminotransferase in IU/L, mean±SD	42.4±31.8	61.9±63.1	0.57
Serum alkaline phosphatase in IU/L, mean±SD	115.3±80.1	114.3±59.7	0.68
International normalized ratio, mean±SD	1.1±0.2	1.1±0.3	0.34
Serum creatinine in μmol/L, mean±SD	90.8±72.5	70.1±8.1	0.19
Blood sugar in mmol/L, mean±SD	7.0±2.5	6.8±1.7	0.96
Hemoglobin in g/L, mean±SD	13.4±1.8	14.4±3.5	0.25
Platelet count in 10 ⁹ /L, mean±SD	226.3±98.7	180.8±81.1	0.05
ICG-R15 in %, mean±SD	12.9±8.1	16.6±6.6	0.16
Liver stiffness measurement in kPa, mean±SD	14.325±9.2	26.6±18.8	0.04
Controlled attenuation parameter in dB/m, mean±SD	237.7±65.9	275.4±84.6	0.27
Surgical approach: Open/Laparoscopic hepatectomy	67(69.1)/30(30.9)	8(99.9)/1(1.1)	0.21
Hepatic venous pressure gradient in mmHg, mean±SD	4.7±2.9	8.1±5.6	0.10
Type of hepatectomy: Minor/Major hepatectomy	68(70.1)/29(29.9)	5(55.6)/4(44.4)	0.37
Operative time in min, mean±SD	270.7±101.1	350±72.2	<0.01
Pringle maneuver	86(88.7)	9(100)	0.28
Duration of Pringle maneuver in min, mean±SD	44.3±37.1	48.2±21.2	0.38
Maximum serum lactate in mmol/L, mean±SD	3.1±1.7	2.9±1.6	0.77
Blood loss in ml, mean±SD	698.5±1079.9	1100±847.1	0.01
Resection margin in mm, mean±SD	8.8±12.2	2.4±4.8	0.02
90-day severe morbidity	16(16.5)	8(99.9)	<0.01
90-day mortality	2(2.1)	3(33.3)	<0.01
90-day comprehensive complication index, mean±SD	19.2±21.9	62.4±29.5	<0.01
Length of hospital stay in days, mean±SD	9.8±6.1	19.9±17.0	0.13

[#] Data are expressed as counts (percentage) unless otherwise stated; * Mann-Whitney U test with asymptomatic bilateral significance; MELD-model for end-stage liver disease; ICG-R15-indocyanine green retention at 15 minutes

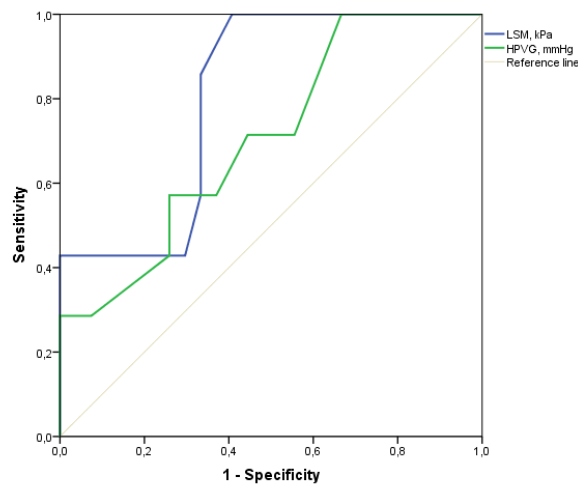
Figure 1: Receiver operating characteristic analysis of the performance of liver stiffness measurement by transient elastography



Curve	Metavir Grade	Prevalence %	AUROC	95% CI	Cut-off *	Sensitivity %	Specificity %
1	F \geq 1	97	0.835	0.706-0.965	7.5	84.1	57.1
2	F \geq 2	88	0.866	0.774-0.959	8.5	81.6	76.9
3	F \geq 3	67	0.861	0.779-0.944	9.5	85.2	67.9
4	F = 4	52	0.879	0.808-0.950	11.5	81.3	75.6

AUROC-area under receiver operating characteristic curve, CI-confidence interval, *Cut-off determined at 80% sensitivity

Figure 2: Receiver operating characteristic analysis comparing the performance of liver stiffness measurement by transient elastography and hepatic venous pressure gradient



Parameter	AUROC	95% CI	Cut-off	Sensitivity,%	Specificity,%
LSM, kPa	0.81	0.64 - 0.97	12	85.7	66.7
			15	42.9	81.3
			22	42.9	92.6
HVPG, mm Hg	0.71	0.49 - 0.91	10	28.6	96.3

LSM-liver stiffness measurement, HVPG-hepatic venous pressure gradient, AUROC-area under receiver operating characteristic curve, CI-confidence interval

Article 7: Prognostic impact of transient elastography by FibroScan® in the surgical management of patients with resectable hepatocellular carcinoma

Authors:

Muthukumarassamy Rajakannu,^{1,2,3} MBBS, MS, DNB, MRCS, Daniel Cherqui,^{1,3} MD, Nicolas Gölse,¹ MD, Oriana Ciaccio,¹ MD, Gabriella Pittau,¹ MD, Antonio Sa Cunha,^{1,3} MD, Denis Castaing,^{1,2,3} MD, Didier Samuel,^{1,2,3} MD, PhD, Catherine Guettier,^{1,2,3} MD, PhD, René Adam,^{1,3,4} MD, PhD, Eric Vibert,^{1,2,3} MD, PhD

Affiliations:

¹Centre Hépatobiliaire, AH-HP Hôpital Paul Brousse, Villejuif, France

²Inserm, Unité UMR-S 1193, Villejuif, France

³Université Paris-Sud, Faculté de Médecine, Le Kremlin-Bicêtre, France

⁴Inserm, Unité UMR-S 776, Villejuif, France

Running title: Survival after hepatectomy for HCC

Keywords: Controlled attenuation parameter, cirrhosis, liver stiffness, liver transplantation, Fibroscan

Correspondance:

Prof. Eric Vibert

Centre Hépatobiliaire,

AH-HP Hôpital Paul Brousse,

12 Avenue Paul Vaillant Couturier,

94800 Villejuif,

France

Abstract

Background: Liver fibrosis and portal hypertension are the important prognostic factors of survival and recurrence in patients with hepatocellular carcinoma(HCC). The aim of the present study was to evaluate transient elastography(TE) as prognostic factors for survival and recurrence after hepatectomy.

Methods: Liver stiffness(LS) and controlled attenuation parameter(CAP) were prospectively assessed in patients planned for liver resection(LR) or transplantation(LT). Predictive factors for overall survival(OS), disease-free survival(DFS), early recurrence(ER) after LR and dropout in patients waiting for LT were analyzed.

Results: 105 and 103 patients with HCC planned LR and LT, respectively were included in the study. In patients undergoing LR, 1- and 2-year OS in patients undergoing LR were 88% and 80.9%, respectively whereas 1- and 2-year DFS were 59.5% and 39.5%, respectively. Median duration of follow-up after LR was 16.3 months. Poor prognostic factor for OS were AFP ≥ 100 ng/mL (HR:6.03, 95%confidence interval[CI]:2.13–17.09), LS ≥ 30 kPa (HR:3.46, 95%CI:1.15–10.42), open LR (HR:5.31, 95%CI:1.13–25.04), and post-operative severe complications (HR:3.48, 95%CI:1.24–9.79). Independent predictors of poor DFS were size >50 mm (HR:2.22, 95%CI:1.12–4.44), AFP ≥ 100 ng/mL (HR:2.42, 95%CI:1.17–4.99), CAP <240 dB/m (HR:2.49, 95%CI:1.21–5.16), Pringle maneuver ≥ 75 min (HR:5.29, 95%CI:1.95–14.37), and intra-operative RBC transfusion (HR:4.67, 95%CI:1.39–71). In the subgroup patients with F3/F4 fibrosis and eligible for LT, LS ≥ 30 kPa (HR:5.33, 95%CI:1.27–184.84), and CAP <240 dB/m (HR:9.46, 95%CI:0.98-91.52) were predictors of ER. In similar patients enlisted for LT, elevated platelet count and LS ≥ 30 kPa were predictors of dropout due to tumor progression.

Conclusion: Pre-operative TE should become a part of pre-operative work-up in patients awaiting surgery and transplantation for HCC as LS and CAP are potential prognostic biomarkers of survival and recurrence.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver with a worldwide incidence of about 850,000 new cases per year^{1,2}. Curative liver resection (LR) is the treatment of choice in non-cirrhotic patients with HCC and in cirrhotic patients having very early stage [Barcelona Clinic Liver Cancer (BCLC) stage 0] and early stage (BCLC stage A) tumors.³⁻⁵ Although liver transplantation (LT) is considered the best treatment for HCC in patients with underlying liver disease and portal hypertension (PH), it is hampered by severe organ shortage.^{3,4} Prognosis of the patients undergoing LR depends on the tumor biology, completeness of resection, degree of hepatic fibrosis and PH.⁶⁻¹⁰ Kluger *et al* recently demonstrated that tumor biology [elevated α -fetoprotein (AFP), microvascular invasion, poor differentiation, and satellite nodules] and the presence of cirrhosis were the most important determinants of overall survival.⁷ Similarly, clinically significant portal hypertension (CSPH) is a risk factor not only for post-hepatectomy morbidity and mortality but also for decreased 5-year survival after LR in patients with underlying liver cirrhosis.^{9,10} Further, Faitot *et al* have proposed that patients with CSPH have increased rate of tumor progression and dropout from the waiting list of LT due to poor response to transarterial chemoembolization.¹¹ A proper selection of patients for LR would provide results similar results to LT whose results are significantly hampered by the increasing rates of dropout while waiting for a liver graft.⁴ Liver stiffness (LS) assessed by transient elastography (TE) has recently emerged as an accurate predictor of hepatic fibrosis and CSPH.¹²⁻¹⁵ Pre-operative LS has been proven to predict of post-operative complications including post-hepatectomy liver failure.¹⁵⁻¹⁹ Some studies have even demonstrated that LS could be a non-invasive method to assess the risk of

HCC development in patients with underlying liver disease and risk recurrence after hepatectomy.^{20,21} The aim of this monocentric clinico-pathological study was to determine whether pre-operative TE would help in the choice of surgical strategy (LR or LT) for HCC patients by predicting the post-operative oncological outcomes after LR for HCC.

Patients and Methods

Prospective recruitment of consecutive adult patients with potentially resectable or transplantable HCC was performed after obtaining informed consent according to the principles of the Helsinki Declaration during the study period October 2014 to June 2017. Clinical and laboratory data of LR patients were obtained at inclusion and at post-operative follow-up visits. Clinical and laboratory data of LT eligible patients were collected at the time of inscription into the LT waiting list and they were followed-up until transplantation, dropout from the list and/or death. Final follow-up was on June 30, 2017. The Ethics Committee of the hospital approved the study protocol. The primary objective of this study was to determine the prognostic factors for overall survival (OS) after hepatectomy. The secondary objectives were to find out the predictive factors for disease-free survival (DFS) R1 resection, early recurrence (ER) after hepatectomy, microvascular invasion in the resected surgical specimen, and dropout in waiting list of LT for HCC.

Transient Elastography:

In all patients, TE was performed pre-operatively in fasting condition using FibroScan[®] Touch 502 with M or XL probes (Echosens[™], Paris, France). LS was measured on the right lobe of the liver through intercostal spaces by placing the probe on the skin between the ribs while the patient was lying supine with the right arm in maximal abduction. A liver portion of at least 6 cm thick, free of large vascular structures and tumor, was located by an ultrasonic time-motion image and ten successful measurements were performed on each patient. LS was expressed in kilopascal (kPa) and median value was kept as representative of

LS. Only measurements with a success rate $\geq 60\%$ and an interquartile range/median (IQR/M) $< 30\%$ were considered valid.^{12,18} Controlled attenuation parameter (CAP) was assessed simultaneously and was expressed in decibels per meter (dB/m). The operator was blinded to the patient's clinical data. All patients underwent LR within two weeks of TE.

Liver resection:

Pre-operative evaluation consisted of laboratory tests including liver function tests, imaging studies like ultrasonography, contrast-enhanced abdominal computer tomography (CECT) \pm volumetry, magnetic resonance imaging, TE, and indocyanine green retention at 15 minutes (ICG-R15). Usually hepatectomy was performed when MELD ≤ 12 , platelet count $\geq 80,000$ and ICG-R15 $\leq 20\%$. When future liver remnant (FLR) would not be sufficient, portal vein embolization (PVE) was performed to ensure FLR was $\geq 0.5\%$ of body weight or $\geq 20\%$ of standard liver volume in non-cirrhotic liver. Transarterial chemoembolization (TACE) followed by PVE was the treatment strategy when planning major LR in patients with cirrhosis.²² The technique of hepatectomy for HCC has been standardized in our unit since 1982.^{22,23} At surgery, exploration of the abdominal cavity was performed to detect extrahepatic spread. Systematic intra-operative liver ultrasound was carried out. Enlarged hepatic lymph nodes and any suspicious nodules were excised for frozen section histology. The objective of hepatectomy was to resect all detectable lesions with tumor-free margins of ≥ 1 cm while leaving behind adequate liver parenchyma to ensure normal liver function. In general, anatomic resections were preferred to wedge resections. In our center, there was no protocol to measure pre-or intra-operative hepatic venous pressure gradient (HVPG) in all patients. It was feasible only during laparotomy and was measured before the commencement of hepatectomy at individual surgeon's discretion. Parenchymal dissection was done using the ultrasonic dissector (CUSA, Cavitron Ultrasonic Aspirator, Valley Lab Inc., Boulder, CO), and resections were usually performed under intermittent clamping of the portal triad. Serial

follow-up of patients was ensured to monitor the post-operative complications and HCC recurrence. A CECT scan was performed at 1-week and 1-month after LR and then alternating abdominal ultrasonography and CECT were performed every four months along with serum AFP and liver function tests.

Histopathological evaluation of surgical specimen:

Two expert pathologist, blinded to patients' TE results, evaluated hematoxylin and eosin (H&E) stained sections of the tumor and non-tumoral explant liver from hepatectomy. Macroscopic features like number, size, percentage of necrosis, presence of capsule, and tumor-free parenchymal margin were assessed. Microscopic tumor characteristics studied included differentiation, presence of satellite nodules, macro- or microscopic vascular invasion. Non-tumoral liver distant from the resected tumor was evaluated for fibrosis and steatosis. Hepatic fibrosis and steatosis were graded according to meta-analysis of histological data in viral hepatitis (METAVIR) scoring system and non-alcoholic fatty liver disease (NAFLD) activity score (NAS), respectively.

Definitions:

Overall survival was the time interval from hepatectomy to the last follow-up or death. Disease-free interval from the date of hepatectomy to the date of first hepatic or extra-hepatic recurrence was defined as disease-free survival. When there was no tumor-free margin between the resected liver parenchymal surface and tumor in the surgical specimen it was called R1 resection. Any intra- or extra-hepatic recurrence occurring within 12 months of hepatectomy was defined as early recurrence. CSPH was defined by an HVP ≥ 10 mmHg.⁶

Statistical analysis:

Continuous variables are expressed as median with range and discrete variables are presented as counts with percentage. Survival analysis was performed using Kaplan–Meier

method and survival curves were compared using the log-rank test. Cox proportional hazard model used to define predictors of survival. Potential predictors of R1 resection, ER, microvascular invasion, and dropout in waiting list of LT for HCC were identified by univariate logistic regression and entered into multivariate logistic regression by backward elimination (Wald) method was performed to determine the independent predictors of the binary outcome. The variables that remained in the model during multivariate regression were considered to the independent predictors. Internal validation of the model was performed by bootstrapped (1000) multivariate logistic regression. *p* value <0.05 was considered significant. SPSS 21.0 package (SPSS Inc., Chicago, IL) was utilized to perform the analysis.

Results

Characteristics of study population:

One hundred and five patients with HCC underwent hepatectomy during the period October 2014 to August 2016 and were followed-up up to June 2017. The patients' and tumor characteristics are summarized in the table 1. Eighty-three men (79.1%) and 22 women (20.9%) with a median age of 68 (range, 26-87) years underwent LR during the study period. All patients, including 52 of them (49.5%) with cirrhosis (METAVIR F4), were in Child Pugh class A or B with compensated pre-operative liver function. Median model for end-stage liver disease (MELD) score was eight (range, 6-20) and platelet count was $214 \times 10^3/\text{mL}$ (range, $34\text{-}618 \times 10^3/\text{mL}$). Majority of patients undergoing LR had underlying chronic liver disease ($F \geq 2$ in 83.8%) with viral hepatitis (39%) and NAFLD (24.8%) being the most common etiologies. Median (range) size and number of tumors resected were 38 (11-190) mm and 1 (1-6), respectively. Pre-operative TE was performed successfully in 94 patients (89.5) with valid LS evaluation possible in 88 patients (83.8%) including 69 patients with right-sided HCC. TE was not feasible in 10 patients (9.5%) due to previous right hepatectomy, and in seven

patients (6.7%) measurement were not considered valid as IQR/M was $\geq 30\%$ due to presence of large right liver tumors.

During the same period, 103 patients with HCC were enlisted for LT, among whom 64 patients (51 men and 13 women, median age of 62.2 years) had MELD ≤ 15 (Supln. table 1). Median (range) size and number of tumors resected were 22 (10-70) mm and two (1-10), respectively. Viral hepatitis (45.3%) and alcohol (40.6%) were the most common etiologies of the underlying liver disease. Transient elastography was performed with success in 60 patients (93.8%) with 52 patients (81.3%) having a valid LS and CAP and TE was not feasible in four patients (6.3%) due to treatment.

Minor hepatectomy (< 3 segments) was performed in majority of patients (68.6%) and major hepatectomy (≥ 3 liver segments) in 33 patients (31.4%). Most common approach utilized for LR was laparotomy and uni-segmentectomy (28.6%) was the most common type of hepatectomy. Nine patients (8.6%) who had undergone LR received LT afterwards. Similarly, majority of patients (79.7%) waiting for LT received a treatment with an intention of decreasing HCC progression and reducing the dropout rate. Transarterial chemoembolization (56.3%) was the most common treatment offered to these patients while on the waiting list.

Overall and disease-free survival:

One- and 2-year OS in patients undergoing hepatectomy were 88% and 80.9%, respectively. One- and 2-year DFS in patients undergoing hepatectomy were 59.5% and 39.5%, respectively. Median duration of follow-up after LR was 16.3 months. Early recurrence was observed in 34 out of 105 patients (34.4%) with a year of LR. Figure 1 illustrates OS and DFS in the entire study population and in patients with solitary HCC after hepatectomy. Poor prognostic factor for OS were serum AFP ≥ 100 ng/mL (HR:6.03, 95%

confidence interval [CI]: 2.13–17.09), LS \geq 30 kPa (HR: 3.46, 95% CI: 1.15–10.42), open LR (HR: 5.31, 95% CI: 1.13–25.04), and post-operative severe complications (HR: 3.48, 95% CI: 1.24–9.79) (Table 1). Independent predictors of poor DFS were size $>$ 50 mm (HR: 2.22, 95% CI: 1.12–4.44), serum AFP \geq 100 ng/mL (HR: 2.42, 95%CI: 1.17-4.99), CAP $<$ 240 dB/m (HR: 2.49, 95% CI: 1.21–5.16), Pringle maneuver \geq 75 min (HR: 5.29, 95% CI: 1.95–14.37), and intra-operative RBC transfusion (HR: 4.67, 95% CI: 1.39–71). Although LS \geq 30 kPa ($p=0.09$) and macrovascular invasion ($p=0.15$) were remained in the multivariate model, their p -value did not attain the level of significance after bootstrap (Table 2).

Risk factors for R1 resection:

Pre-operative predictive factors for margin-positive resections were determined in patients undergoing LR for solitary HCC whose characteristics are summarized in Suppln. table 2. Liver stiffness \geq 12 kPa (HR: 5.5, 95% CI: 1.01–27.91) was the only independent predictor of R1 resection. Similarly, LS \geq 12 kPa (HR: 6.37, 95% CI: 1.22–33.29) was the only independent risk factor for R1 resections in the overall cohort (Suppln. table 3).

Risk factors for Microscopic vascular invasion:

Predictive factors of the presence of microvascular tumoral invasion was assessed in patients undergoing LR solitary HCC and the patients who received neoadjuvant anti-tumoral treatment (15-TACE, 2-Sorafenib) were excluded from this analysis. Serum AFP 100 ng/mL (HR: 5.0, 95% CI: 1.31–19.07) and CAP $<$ 200 dB/m (HR: 4.0, 95% CI: 1.08–14.78) were the independent predictors of microvascular invasion in the resected HCCs (Table 4).

Liver resection versus Liver transplantation:

In the subgroup LR patients with METAVIR F3 and F4 liver fibrosis and within Duvoux criteria for LT in HCC, LS \geq 30 kPa (HR: 15.33, 95% CI: 1.27–184.84), and CAP $<$ 240 dB/m (HR: 9.46, 95% CI: 0.98-91.52) were the independent predictors of ER (Table 6).

Supplementary table 4 summarizes the independent predictors of ER after hepatectomy in the entire study cohort. In the LT patients with MELD ≤ 15 (similar to LR group), elevated platelet count and LS ≥ 30 kPa were the independent predictors of dropout due to tumor progression from the waiting list of LT for HCC, even though LS did not achieve the level of significance after bootstrapped multivariate regression (Table 7).

Discussion

Transient elastography (LS and CAP) was demonstrated to be an important prognostic factor for disease recurrence and survival in patients undergoing LR for HCC. Elevated LS was an independent predictor of the quality of LR, and OS. Tumor-free surgical margin was not ensured when the LS ≥ 12 kPa resulting in inadequate R1+ surgical resection. Along with serum AFP ≥ 100 ng/mL, open LR, and severe post-operative morbidity, LS ≥ 30 kPa was an important predictor (HR: 5.3) of poor OS. Elevated serum AFP level and lower CAP were surrogate markers of the aggressive tumor biology in these patients. Serum AFP ≥ 100 ng/mL and CAP < 240 dB/m were the independent predictors of microvascular invasion, early recurrence and thus poor DFS. Moreover, LS ≥ 30 kPa was an independent predictor of dropout from the list of LT for HCC. Therefore, *Ab initio* LT should be planned for the patients with LS ≥ 30 kPa and CAP < 240 dB/m with LR being offered as bridge to transplantation. In patients with LS < 30 kPa and CAP ≥ 240 dB/m, surgical resection would be treatment of choice as they seem to have less aggressive HCC and lower rate of ER, and lower risk of dropout while waiting for LT. TE was feasible in majority of our patients without any change in the pre-operative workup of the patients programmed for LR and LT. With widespread availability of FibroScan[®], the simplicity of its use, its non-invasiveness, and reliability of its results, TE must be added to the routine pre-operative workup of patients with HCC, as it would help in choosing the surgical strategy according to LS and CAP values. Further, TE could also be utilized to screen patients for post-operative HCC recurrence and

evaluate resolution of underlying liver disease with newer antiviral therapies. Major limitation to TE would be the presence of large tumor in the right liver and the absence of right liver after right hepatectomy.

Hepatocellular carcinoma is one of the leading causes of cancer-related death and is one of the most important causes of death in patients with cirrhosis.^{2,25} Progressive hepatic fibrosis due to viral hepatitis, alcohol or NAFLD is the common pathway for all form chronic liver disease.^{26,27} HCC developing in patients with NAFLD had a larger volume, showed more often an infiltrative pattern, and was detected outside specific surveillance at a late stage.²⁸ However, they were better differentiated and could arise in the absence of cirrhosis having have similar or better prognosis after LR when compared to the tumors associated with hepatitis C infection.^{28,29} Recently, LS measured by TE has been proven to be an accurate non-invasive predictor of hepatic fibrosis, hepatic functional reserve and thus post-hepatectomy outcomes.¹²⁻²¹ LS has an excellent performance in diagnosing significant fibrosis and cirrhosis with area under the receiver operating characteristic curve of 0.9 and 1, respectively.^{12,13,19} Baveno VI consensus recommendations for diagnosing advanced chronic liver disease (ACLD) and cirrhosis (F4) were LS ≥ 10 kPa and ≥ 15 kPa, respectively.³⁰ Several studies have demonstrated that LS to be an effective predictor of CSPH, clinical decompensation and PH-related complications and survival in patients with chronic liver disease.^{9,10,14,15} In the present study, we demonstrate for the first time that LS is an effective predictor of short and mid-term survival in patients undergoing resection. These results suggest that LS being a surrogate marker of degree of fibrosis and HVPG it was able to predict the patients' survival. On the other hand, CAP is an emerging technique being utilized to evaluate hepatic steatosis. The attenuation of ultrasonic signals is measured in the same region of interest where LS is estimated using a novel proprietary algorithm and expressed in dB/m as CAP.³¹ A recent meta-analysis by Karlas *et al* determined a cut-off of ≥ 248 dB/m to

detect hepatic steatosis in patients with various liver diseases.³² In an interesting study by Min *et al*, the presence of intra-tumoral fat was associated with less microvascular invasion.³³ In the present study, CAP <240 dB/m and AFP \geq 100 ng/mL were consistently associated with poor prognostic factors such as microvascular invasion and ER and thereby suggesting that the tumors arising in non-steatotic livers were more aggressive and had poor DFS. Another important finding from this study was that uncomplicated cirrhosis (F4) *per se* was not a risk for short- and mid-term survival as suggested by Vigano *et al* and Kluger *et al*.^{7,29} Advanced cirrhosis (LS \geq 30 kPa) and probably CSPH were the determinants of the nature of HCC and the post-operative oncological outcomes in these patients. However, in patients with advanced liver fibrosis and cirrhosis (LS \geq 12 kPa) the surgical strategy must be adapted to avoid margin positive resections. Although the width of the resection margin did not influence post-operative recurrence rate, positive histologic margin had been proven to increase post-operative recurrence due to the presence of microsatellites.³⁴

Recurrence of HCC can arise from the primary tumor (early recurrence) or de novo from liver remnant after LR (late recurrence). The most common site of first recurrence after LR is the liver. Clinico-pathological factors associated with tumor invasiveness such as large tumor, multifocal lesions, tumor rupture, elevated AFP, peri-operative transfusion, venous invasion, and presence of satellite nodules are known predictors of HCC recurrence.³⁴⁻³⁸ Recently molecular and genetic factors such as high proliferating cell nuclear antigen activity, telomerase activity, and DNA ploidy have been reported to predict recurrence.^{25,34} The factors associated with ER (<1 year) were non-anatomical resection, R1 resection, elevated AFP and vascular invasion. In our study, we found that the tumors developing in non-steatotic livers (CAP<240 dB/m) and pre-operative serum AFP 100 ng/mL were the predictors of ER. These findings are consistent with the fact that they are surrogate markers of microscopic vascular invasion.

The strategy for patients with high probability of recurrence after LR due to the presence of pejorative histological factors such as microvascular invasion and/or satellite nodules is *ab initio* LT.³⁹ Ferrer-Fabrega *et al* prospectively validated this strategy of salvage transplantation and reported an excellent 5-year survival of 77.1% after LT. The main advantage of this approach was that the patients with high risk of recurrence were prioritized while reducing the number of patients enlisted and thus reducing number of dropouts and unnecessary LT.⁴⁰ In a recent review, Mazzaferro highlighted that the main obstacle to a smooth organ allocation was the lack of credible instruments able to determine exactly how sick a patient was and how likely the tumor was progress despite various treatments while waiting for a liver graft.⁴¹ Faitot *et al* suggested that CSPH was an important factor determining tumor progression and subsequent dropout from LT waiting list.¹¹ Therefore, the patients should be prioritized as according the degree of PH. The current allocation of liver grafts for HCC in France is based on Duvoux criteria, which takes into consideration serum AFP level in addition to tumor size and number. LT transplantation is indicated in patients with a Duvoux score of ≤ 2 , which means that any patient with serum AFP ≥ 1000 ng/mL will not eligible for LT.⁴² In the present study, LS ≥ 30 kPa was an independent risk factor for dropout from the waiting list due to tumor progression. Interestingly serum AFP was not risk factor for dropout as in previous literature probably because the patients with AFP ≥ 1000 ng/mL were excluded by the new criteria for LT in HCC. Moreover, elevated LS was also risk factor for inadequate surgical resection and poor survival. Therefore, pre-operative LS would be an effective method in selecting and adapting surgical strategy in patients with ACLD and HCC.

To the best of our knowledge, this is the first study to evaluate specifically the effectiveness of TE in predicting oncological outcomes in patients undergoing LR for HCC in prospective manner and demonstrate better performance of non-invasive LS and CAP as

prognostic factors of OS and DFS after hepatectomy. These results could be extrapolated to wide range of indications as HCC patients with various etiology were included in this study. The other unique features of this study are that pre-operative LS and CAP were surrogate markers of quality of LR and the nature of HCC invasiveness. All patients in whom TE was programmed were analyzed in intention-to-treat manner. Further, internal validation by bootstrapping the multivariate analysis was performed to boost the robustness of its results. The limitations of this study were the small number of patients resulting in non-significant *p* value in certain subgroups analysis and HVPG was not measured in all patients.

In conclusion, LS and CAP were accurate pre-operative predictors of post-operative OS and DFS, respectively. TE should be part of pre-operative workup to risk-stratify the patients with HCC along with other traditional biomarkers and to choose appropriate surgical strategy (LR or LT) to enable personalized treatment of each patient with HCC.

Acknowledgements

L'Association Nationale de la Recherche et la Technologie, Paris, France and Echosens[™], Paris, France, funded this research.

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Table 1: Patient, primary tumor, treatment characteristics and post-operative outcomes

Variables [#]	N=105 (100)
Patient Demographics	
▪ Age in years, median (range)	68 (26-87)
▪ Gender, male : female	83 (79.1) : 22 (20.9)
▪ Body mass index in kg/m ² , median (range)	25 (15-41)
▪ Diabetes Mellitus	28 (26.7)
▪ Arterial Hypertension	56 (53.3)
▪ Model for end-stage liver disease score, median (range)	8 (6-20)
Etiology of underlying liver disease	
▪ Viral hepatitis (HBV, HCV, HEV)	41 (39)
▪ Non-alcoholic fatty liver disease	26 (24.8)
▪ Alcohol	21 (20)
▪ Others (Hemochromatosis, Wilson's disease)	5 (4.8)
▪ No known liver disease	12 (11.4)
Hepatocellular Carcinoma	
▪ Tumor number, median (range)	1 (1-6)
▪ Diameter of largest nodule in cm, median (range)	38 (11-190)
▪ Localization, right liver : left liver : bilateral	74 (70.5) : 28 (26.7) : 3 (2.9)
▪ Serum α -fetoprotein in ng/mL, median (range)	10 (2-33930)
Laboratory tests	
▪ Hemoglobin in g/L, median (range)	14 (7-19)
▪ Platelet count in 10 ⁹ /L, median (range)	214 (34-618)
▪ Serum total bilirubin in μ mol/L, median (range)	10 (4-36)
▪ Serum aspartate aminotransferase in IU/L, median (range)	39 (16-706)
▪ Serum alanine aminotransferase in IU/L, median (range)	35 (10-229)
▪ Serum alkaline phosphatase in IU/L, median (range)	96 (37-580)
▪ International normalized ratio, median (range)	1 (1-2)
▪ Serum creatinine in μ mol/L, median (range)	72 (48-719)
▪ Serum albumin in g/L, median (range)	36 (18-50)
▪ Indocyanine green retention at 15 minutes in %, median (range)	12 (1-54)
▪ Liver stiffness measurement in kPa, median (range)	12 (3-54)
▪ Controlled attenuation parameter in dB/m, median (range)	228 (100-400)
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	77 (73.3) : 28 (26.7)
▪ Re-hepatectomy	10 (9.5)
▪ Portal vein embolization	14 (13.3)
▪ Pre-operative transarterial chemoembolization	22 (20.9)
▪ Uni-segmentectomy	30 (28.6)
▪ Partial hepatectomy	28 (26.6)
▪ Right hepatectomy	21 (20)
▪ Left hepatectomy	9 (8.6)
▪ Bi-segmentectomy	14 (13.3)

▪ Central hepatectomy	3 (2.9)
▪ Duration of Pringle maneuver in min, median (range)	42 (0-240)
▪ Operative time in min, median (range)	250 (103-590)
▪ Blood loss in mL, median (range)	400 (50-7500)
▪ RBC transfusion	11 (10.5)
▪ Number of Couinaud segments resected, median (range)	1 (0-6)
▪ Resection margin in mm, median (range)	3 (0-55)
METAVIR Fibrosis score of non-tumoral liver	
▪ F0 : F1 : F2 : F3 : F4	9 (8.6) : 8 (7.6) : 21 (20) : 15 (14.3) : 52 (49.5)
METAVIR Activity Score of non-tumoral liver	
• A0 : A1 : A2 : A3 : Missing data	32 (30.5) : 35 (33.3) : 22 (20.9) : 11 (10.5) : 5 (4.8)
NAS Steatosis grade of non-tumoral liver	
• S0 : S1 : S2 : S3 : Missing data	41 (39) : 40 (38.1) : 17 (16.2) : 0 (0) : 7 (6.7)
Post-operative Outcomes	
• 90-day severe morbidity*	24 (22.9)
• 90-day mortality	5 (4.8)
• Comprehensive complication index, median (range)	21 (0 -100)
• ICU stay in days, median (range)	1 (0 -48)
• Duration of hospitalization in days, median (range)	8 (3 -48)
• Two-year overall survival	80.9%
• Two-year disease-free survival	39.5%

*Data are expressed as counts (percentage) unless otherwise stated; HBV-hepatitis B virus; HCV-hepatitis C virus; HEV-hepatitis E virus, METAVIR-meta-analysis of histological data in viral hepatitis, NAS- nonalcoholic fatty liver disease activity score,*Dindo-Clavien grade of surgical complications, ICU-intensive care unit

Figure 1: Short and mid-term overall and disease-free survival in patients undergoing liver resection for hepatocellular carcinoma

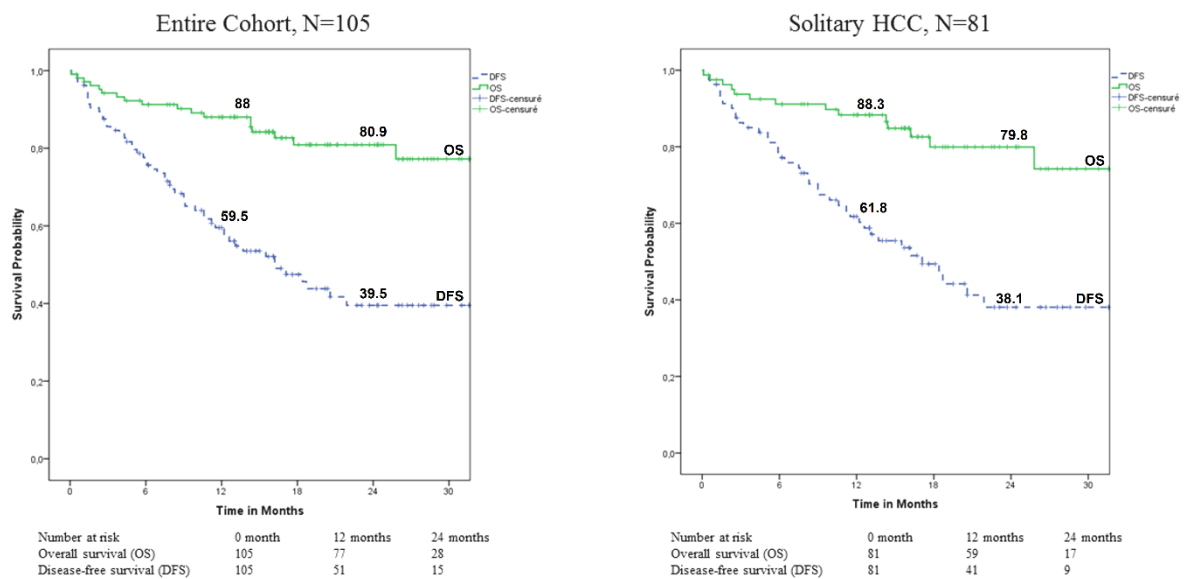


Table 2: Independent predictors of poor short- and mid-term overall survival in the entire study cohort (N=105)

Variables	Prevalence (%)		Overall Survival					
			Uni- variate <i>p</i>	Multivariate Cox Regression*			Bootstrapped Multivariate Cox Regression*	
				<i>Beta</i>	<i>p</i>	HR (95% CI)	<i>p</i>	95% CI
Age, yr	<60	31 (29.5)	0.8					
	≥60	74 (70.5)						
Gender	M	83 (79.1)	0.3					
	F	22 (20.9)						
Body mass index, kg/m ²	<30	88 (83.8)	0.1					
	≥30	17 (16.2)						
diabetes mellitus	No	77 (73.3)	0.3					
	Yes	28 (26.7)						
Viral hepatitis	No	64 (61)	0.1					
	Yes	41 (39)						
Non-alcoholic fatty liver disease	Yes	26 (24.8)	0.8					
	No	79 (75.2)						
Model for end-stage liver disease score	<12	94 (89.5)	0.4					
	≥12	11 (10.5)						
Maximum size, mm	<50	67 (63.8)	0.14					
	≥50	38 (36.2)						
Tumor number	≤1	64 (61)	0.8					
	>1	41 (39)						
Pre-operative TACE	No	83 (79.1)	0.5					
	Yes	22 (20.9)						
α-fetoprotein, ng/mL	<100	75 (71.4)	0.005	1.79	0.001	6.03 (2.13 – 17.09)	0.001	0.68 – 3.72
	≥100	29 (27.6)						
Hemoglobin, g/dL	≥10	101(96.2)	0.6					
	<10	4 (3.8)						
Platelet count, 10 ⁹ /L	≥150	82 (78.1)	0.7					
	<150	23 (21.9)						
Total bilirubin, μmol/L	≤18	93 (88.6)	0.4					
	>18	12 (11.4)						
Aspartate aminotransferase, IU/L	≤50	69 (65.7)	0.3					
	>50	36 (34.3)						
Alkaline phosphatase, IU/L	<75	29 (27.6)	0.3					
	≥75	76 (72.4)						
ICG-R15, %	<15	56 (56.3)	0.6					
	≥15	25 (23.8)						
Liver stiffness, kPa	<30	78 (74.3)	0.02	1.24	0.03	3.46 (1.15 – 10.42)	0.02	-0.16 – 2.83
	≥30	10 (9.5)						
CAP, dB/m	≥240	50 (47.6)	0.3					
	<240	36 (34.3)						
Re-hepatectomy	No	95 (90.5)	1.0					
	Yes	10 (9.5)						
Laparotomy	No	30 (28.6)	0.13	1.67	0.04	5.31 (1.13 – 25.04)	0.03	0.19 – 13.59
	Yes	75 (71.4)						
Major hepatectomy	No	72 (68.6)	0.9					
	Yes	33 (31.4)						
Pringle maneuver, min	≤75	94 (89.5)	0.9					
	>75	11 (10.5)						
Operative time, min	<240	43 (41)	0.8					
	≥240	62 (59)						
Blood loss, mL	≤500	66 (62.9)	0.3					
	>500	39 (37.1)						
RBC transfusion	No	94 (89.5)	0.3					
	Yes	11 (10.5)						
90-day severe morbidity	No	81 (77.1)	0.01	1.25	0.03	3.48 (1.24 – 9.79)	0.02	-0.01 – 3.13
	Yes	24 (22.9)						
METAVIR F4	No	53 (50.5)	0.9					
	Yes	52 (49.5)						
METAVIR A2/3	No	67 (63.8)	0.8					
	Yes	33 (31.4)						
Steatosis, %	≤5	41 (39)	0.5					
	>5	57 (54.3)						
Steatohepatitis	No	42 (40)	0.2					
	Yes	25 (23.8)						
Moderate/Poor-differentiation	No	52 (49.5)	0.7					
	Yes	53 (50.5)						
Satellite nodule	No	85 (81)	0.2					
	Yes	20 (19)						
Microvascular invasion	No	57 (54.3)	0.14					
	Yes	48 (45.7)						
Macrovascular invasion	No	92 (87.6)	0.9					
	Yes	13 (12.4)						
Presence of intact capsule	Yes	40 (38.1)	0.6					
	No	65 (61.9)						
Tumor-free margin	R0	85 (81)	0.9					
	R1	20 (19)						

*All unrelated variables significant at ≤15% in univariate regression were entered in to multivariate regression by backward elimination (Wald) method and the variables that remained in the model were again analyzed after bootstrapping at 1000, HR-hazard ratio, CI-confidence interval, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Table 3: Independent poor prognostic factors for short- and mid-term disease-free survival in the entire study cohort (N=105)

Variables	Prevalence (%)		Disease-Free Survival					
			Uni- variate <i>p</i>	Multivariate Cox Regression*			Bootstrapped Multivariate Cox Regression*	
				<i>Beta</i>	<i>p</i>	HR (95% CI)	<i>p</i>	95% CI
Age, yr	<60	31 (29.5)	0.4					
	≥60	74 (70.5)						
Gender	M	83 (79.1)	0.09					
	F	22 (20.9)						
Body mass index, kg/m ²	<30	88 (83.8)	0.4					
	≥30	17 (16.2)						
Diabetes mellitus	No	77 (73.3)	0.16					
	Yes	28 (26.7)						
Viral hepatitis	No	64 (61)	0.07					
	Yes	41 (39)						
Non-alcoholic fatty liver disease	Yes	26 (24.8)	0.11					
	No	79 (75.2)						
Model for end-stage liver disease score	<12	94 (89.5)	0.5					
	≥12	11 (10.5)						
Maximum size, mm	<50	67 (63.8)	0.02	0.79	0.02	2.22 (1.12-4.44)	0.04	0.03 – 1.59
	≥50	38 (36.2)						
Tumor number	≤1	64 (61)	0.7					
	>1	41 (39)						
Pre-operative TACE	No	83 (79.1)	0.5					
	Yes	22 (20.9)						
α-fetoprotein, ng/mL	<100	75 (71.4)	<0.001	0.88	0.02	2.42 (1.17-4.99)	0.01	0.33 – 2.04
	≥100	29 (27.6)						
Hemoglobin, g/dL	≥10	101(96.2)	0.13					
	<10	4 (3.8)						
Platelet count, 10 ⁹ /L	≥150	82 (78.1)	0.4					
	<150	23 (21.9)						
Total bilirubin, μmol/L	≤18	93 (88.6)	0.2					
	>18	12 (11.4)						
Aspartate aminotransferase, IU/L	≤50	69 (65.7)	0.05					
	>50	36 (34.3)						
Alkaline phosphatase, IU/L	<75	29 (27.6)	0.14					
	≥75	76 (72.4)						
ICG-R15, %	<15	56 (56.3)	0.9					
	≥15	25 (23.8)						
Liver stiffness, kPa	<30	78 (74.3)	0.03	0.99	0.05	2.71 (1.01 – 7.24)	0.09	-0.28 – 1.88
	≥30	10 (9.5)						
CAP, dB/m	≥240	50 (47.6)	0.12	0.92	0.01	2.49 (1.21 – 5.16)	0.04	-0.08 – 1.82
	<240	36 (34.3)						
Re-hepatectomy	No	95 (90.5)	0.6					
	Yes	10 (9.5)						
Laparotomy	No	30 (28.6)	0.4					
	Yes	75 (71.4)						
Major hepatectomy	No	72 (68.6)	0.09					
	Yes	33 (31.4)						
Pringle maneuver, min	≤75	94 (89.5)	0.08	1.67	0.001	5.29 (1.95 – 14.37)	0.004	0.59 – 3.06
	>75	11 (10.5)						
Operative time, min	<240	43 (41)	0.7					
	≥240	62 (59)						
Blood loss, mL	≤500	66 (62.9)	0.4					
	>500	39 (37.1)						
RBC transfusion	No	94 (89.5)	0.002	1.54	0.01	4.67 (1.39 – 71)	0.001	0.00 – 2.86
	Yes	11 (10.5)						
90-day severe morbidity	No	81 (77.1)	0.2					
	Yes	24 (22.9)						
Metavir F4	No	53 (50.5)	0.4					
	Yes	52 (49.5)						
Metavir A2/3	No	67 (63.8)	0.3					
	Yes	33 (31.4)						
Steatosis, %	≤5	41 (39)	0.02					
	>5	57 (54.3)						
Steatohepatitis	No	42 (40)	0.01					
	Yes	25 (23.8)						
Moderate/Poor-differentiation	No	52 (49.5)	0.13					
	Yes	53 (50.5)						
Satellite nodule	No	85 (81)	0.003					
	Yes	20 (19)						
Microvascular invasion	No	57 (54.3)	0.009					
	Yes	48 (45.7)						
Macrovascular invasion	No	92 (87.6)	0.001	1.12	0.04	3.08 (1.06 – 8.90)	0.15	-0.68 – 2.64
	Yes	13 (12.4)						
Presence of intact capsule	Yes	40 (38.1)	0.5					
	No	65 (61.9)						
Tumor-free margin	R0	85 (81)	0.9					
	R1	20 (19)						

*All unrelated variables significant at ≤15% in univariate regression were entered in to multivariate regression by backward elimination (Wald) method and the variables that remained in the model were again analyzed after bootstrapping at 1000, HR-hazard ratio, CI-confidence interval, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Table 4: Independent pre-operative predictors for R1 resection in patients undergoing hepatectomy for solitary HCC (N=81)

Variables	Prevalence (%)	R1 resection					
		Uni variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
			<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI
Age, yr	<60 25 (30.9)	0.07					
	≥60 56 (69.1)						
Gender	M 64 (79.0)	0.3					
	F 17 (21.0)						
Body mass index, kg/m ²	<30 65 (80.2)	0.16					
	≥30 16 (19.8)						
Diabetes mellitus	No 58 (71.6)	0.3					
	Yes 23 (28.4)						
Viral hepatitis	No 49 (60.5)	0.7					
	Yes 32 (39.5)						
Non-alcoholic fatty liver disease	Yes 22 (27.2)	0.16					
	No 59 (72.8)						
Model for end-stage liver disease score	<12 73 (90.1)	0.9					
	≥12 8 (9.9)						
Maximum size, mm	<50 53 (65.4)	0.4					
	≥50 28 (34.6)						
Pre-operative TACE	No 66 (81.5)	0.001					
	Yes 15 (18.5)						
α-fetoprotein, ng/mL	<100 57 (70.4)	0.8					
	≥100 23 (28.4)						
Hemoglobin, g/dL	≥10 77 (95.1)	0.8					
	<10 4 (4.9)						
Platelet count, 10 ⁹ /L	≥150 65 (80.2)	0.05					
	<150 16 (19.8)						
Total bilirubin, μmol/L	≤18 75 (92.6)	0.4					
	>18 6 (7.4)						
Aspartate aminotransferase, IU/L	≤50 52 (64.2)	0.2					
	>50 29 (35.8)						
Alkaline phosphatase, IU/L	<75 23 (28.4)	0.8					
	≥75 58 (71.6)						
ICG-R15, %	<15 45 (55.6)	0.03					
	≥15 18 (22.2)						
Liver stiffness, kPa	<12 34 (41.9)	0.04	1.71	0.04	5.5 (1.01 - 27.91)	0.02	0.29 - 20.46
	≥12 35 (43.2)						
CAP, dB/m	≥240 28 (34.6)	0.3					
	<240 40 (49.4)						
Re-hepatectomy	No 73 (90.1)	0.6					
	Yes 8 (9.9)						
Laparotomy	Yes 57 (70.4)	0.17					
	No 24 (29.6)						
Major hepatectomy	No 56 (69.1)	0.2					
	Yes 25 (30.9)						
Pringle maneuver, min	≤75 70 (86.4)	0.15					
	>75 11 (13.6)						
Operative time, min	<240 37 (45.7)	0.9					
	≥240 44 (54.3)						
Blood loss, mL	≤500 49 (60.5)	0.7					
	>500 32 (39.5)						
RBC transfusion	No 72 (88.9)	0.06					
	Yes 9 (11.1)						
Metavir F4	No 53 (50.5)	0.3					
	Yes 39 (48.1)						
Metavir A2/3	No 52 (64.2)	0.3					
	Yes 26 (32.1)						
Steatosis, %	>5 32 (39.5)	0.06					
	≤5 46 (56.8)						
Steatohepatitis	Yes 42 (40)	0.4					
	No 25 (23.8)						
Moderate/Poor-differentiation	No 39 (48.1)	0.14					
	Yes 42 (51.9)						
Satellite nodule	No 54 (66.7)	0.3					
	Yes 27 (33.3)						
Microvascular invasion	No 45 (55.6)	0.3					
	Yes 36 (44.4)						
Macrovascular invasion	No 72 (88.9)	0.3					
	Yes 9 (11.1)						
Presence of intact capsule	Yes 28 (34.6)	0.8					
	No 53 (65.4)						
Constant				<0.001		0.001	-21.20 - 1.65

*All unrelated pre-operative variables significant at ≤15% in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Table 5: Independent pre-operative predictors for microscopic vascular invasion in patients with solitary HCC undergoing hepatectomy (excluding those who had neoadjuvant treatment) (N=64)

Variables	Prevalence (%)		Microscopic vascular Invasion					
			Uni- variate <i>P</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
				<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI
Age, yr	<60	25 (30.1)	0.11					
	≥60	58 (69.9)						
Gender	M	62 (74.7)	1.0					
	F	21 (25.3)						
Body mass index, kg/m ²	<30	70 (84.3)	0.6					
	≥30	13 (15.7)						
Diabetes mellitus	No	59 (71.1)	0.2					
	Yes	24 (28.9)						
Viral hepatitis	No	49 (59.0)	0.5					
	Yes	34 (41.0)						
Non-alcoholic fatty liver disease	Yes	63 (75.9)	0.1					
	No	30 (24.1)						
Model for end-stage liver disease score	<12	73 (88.0)	0.4					
	≥12	10 (12.0)						
Maximum size, mm	<50	54 (65.1)	0.3					
	≥50	29 (34.9)						
α-fetoprotein, ng/mL	<100	57 (68.7)	0.003	1.61	0.02	5.0 (1.31 - 19.07)	0.01	0.23 - 3.52
	≥100	25 (30.1)						
Hemoglobin, g/dL	≥10	80 (96.4)	0.9					
	<10	3 (3.6)						
Platelet count, 10 ⁹ /L	≥150	66 (79.5)	0.8					
	<150	17 (20.5)						
Total bilirubin, μmol/L	≤18	93 (88.6)	0.9					
	>18	12 (11.4)						
Aspartate aminotransferase, IU/L	≤50	57 (68.7)	0.5					
	>50	26 (31.3)						
Alkaline phosphatase, IU/L	<75	25 (30.1)	0.07					
	≥75	58 (69.9)						
ICG-R15, %	<15	47 (56.6)	0.8					
	≥15	19 (22.9)						
Liver stiffness, kPa	<30	67 (80.7)	0.3					
	≥30	6 (7.2)						
CAP, dB/m	≥200	51 (61.4)	0.02	1.3	0.04	4.0 (1.08 - 14.78)	0.03	0.05 - 3.14
	<200	21 (25.3)						
METAVIR F4	No	42 (50.6)	0.9					
	Yes	41 (49.4)						
METAVIR A2/3	No	52 (62.7)	0.2					
	Yes	26 (31.3)						
Steatosis, %	≤5	32 (38.6)	0.08					
	>5	46 (55.4)						
Steatohepatitis	Yes	40 (48.2)	0.2					
	No	12 (14.5)						
Constant					0.001		0.01	-2.59 - -0.61

*All unrelated variables significant at ≤10% in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, OR-odds ratio, CI-confidence interval, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Table 6: Independent predictors for early recurrence within 12 months in HCC patients undergoing hepatectomy with advanced liver disease and within Duvoux criteria of ≤ 2 (N=42)

Variables	Prevalence (%)	Early Recurrence					
		Uni-variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
			<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI
Recurrent HCC	No 39 (92.9) Yes 3 (7.1)	0.9					
Viral hepatitis	No 22 (52.4) Yes 20 (47.6)	0.2					
Non-alcoholic fatty liver disease	Yes 5 (11.9) No 37 (88.1)	0.5					
Model for end-stage liver disease score	<12 38 (90.5) ≥ 12 4 (9.5)	0.3					
Platelet count, $10^9/L$	<250 38 (90.5) ≥ 250 4 (9.5)	0.9					
α -fetoprotein, ng/mL	<100 39 (92.9) ≥ 100 3 (7.1)	0.14					
Liver stiffness, kPa	<30 31 (73.6) ≥ 30 6 (14.3)	0.05	2.73	0.03	15.33 (1.27-184.84)	0.01	0.81-40.39
CAP, dB/m	≥ 240 16 (38.1) <240 19 (45.2)	0.1	2.25	0.05	9.46 (0.98-91.52)	0.04	0.33-38.79
Constant				0.01		0.02	-39.67- -1.30

*All unrelated variables significant at $\leq 15\%$ in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Table 7: Independent predictors of drop-out from the waiting list of liver transplantation in patients with MELD ≤ 15 (N=64)

Variables	Prevalence (%)	Drop-out					
		Uni-variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
			<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI
Recurrent HCC	No 44 (68.8) Yes 20 (31.3)	0.7					
Waiting time, months	<12 49 (76.6) ≥ 12 15 (23.4)	0.18					
Platelet count, $10^9/L$	<250 57 (89.1) ≥ 250 7 (10.9)	0.07	1.89	0.04	6.66 (1.09-40.59)	0.01	-0.08-22.76
α -fetoprotein, ng/mL	<100 59 (92.2) ≥ 100 5 (7.8)	0.9					
Liver stiffness, kPa	<30 32 (20) ≥ 30 20 (31.3)	0.16	1.27	0.07	3.56 (0.89-14.12)	0.05	-0.23-3.28
CAP, dB/m	≥ 240 22 (34.4) <240 20 (31.3)	0.9					
TACE/Ablation/Resection	Yes 83 (79.1) No 22 (20.9)	0.5					
Constant				0.001		0.002	-3.43--0.83

*All unrelated variables significant at $\leq 20\%$ in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, CAP-controlled attenuation parameter, TACE-transarterial chemoembolization

Supplementary results:

Supplementary table 1: Demographic and primary tumor characteristics of patients with hepatocellular carcinoma with MELD \leq 15 waiting for liver transplantation

Variables [#]	N=64 (100)
Patient Demographics	
▪ Age in years, median (range)	62.2 (27-72)
▪ Gender, male : female	51 (79.7) : 13 (20.3)
▪ Model for end-stage liver disease score, median (range)	10 (7-15)
▪ Duration of waiting in the list in months, median (range)	8 (0-28)
Etiology of underlying liver disease	
▪ Viral hepatitis (HBV, HCV)	29 (45.3)
▪ Alcohol	26 (40.6)
▪ Non-alcoholic fatty liver disease	4 (6.3)
▪ Others (Hemochromatosis, Fructose intolerance)	3 (4.7)
▪ No known liver disease	2 (3.1)
Hepatocellular carcinoma	
▪ Presentation, Primary : Recurrence	44 (68.8) : 20 (31.3)
▪ Maximum size in mm, median (range)	22 (10-70)
▪ Number, median (range)	2 (1-10)
▪ α -fetoprotein in ng/mL, median (range)	8.5 (1-196.3)
▪ Milan criteria, Within : Outside	59 (92.2) : 5 (7.8)
Laboratory tests	
▪ Serum total bilirubin in μ mol/L, median (range)	18 (6-71)
▪ Serum alkaline phosphatase in IU/L, median (range)	111.5 (31-961)
▪ Serum sodium in mmol/L, median (range)	138 (132-143)
▪ International normalized ratio, median (range)	1.3 (1-1.7)
▪ Serum creatinine in μ mol/L, median (range)	69.5 (53-127)
▪ Platelet count in $10^9/L$, median (range)	117 (38-304)
▪ Liver stiffness measurement in kPa, median (range)	23.5 (3-75)
▪ Controlled attenuation parameter in dB/m, median (range)	245 (100-400)
Outcomes	
▪ Treatment on list, TACE : Ablation : Resection : Combined: Others: None	36 (56.3) : 3 (4.7) : 2 (3.1): 7 (10.9) : 2 (3.1) : 13 (20.3)
▪ Liver transplantation	37 (57.8)
▪ Overall dropout from list	21 (32.8)
▪ Dropout due to HCC progression	17 (26.6)
▪ Death on the list	9 (14.1)

[#]Data are expressed as counts (percentage) unless otherwise stated; HBV-hepatitis B virus; HCV-hepatitis C virus.

Supplementary table 2: Demographic, primary tumor, treatment characteristics and post-operative outcomes in patients with solitary hepatocellular carcinoma

Variables [#]	N = 81 (100)
Patient Demographics	
▪ Age in years, median (range)	67 (31-87)
▪ Gender, male : female	64 (79) : 17 (21)
▪ Body mass index in kg/m ² , median (range)	25 (15-41)
▪ Diabetes Mellitus	23 (28.4)
▪ Arterial Hypertension	42 (51.9)
▪ Model for end-stage liver disease score, median (range)	8 (6-20)
Etiology	
▪ Viral hepatitis (HBV, HCV, HEV)	32 (39.5)
▪ Non-alcoholic fatty liver disease	22 (27.2)
▪ Alcohol	14 (17.3)
▪ Others (Hemochromatosis, Wilson's disease)	4 (4.9)
▪ No known liver disease	9 (11.1)
Hepatocellular Carcinoma	
▪ Diameter of largest nodule in mm, median (range)	36 (11-160)
▪ Localization, right liver : left liver : bilateral	(70.5) : (26.7) : (2.9)
▪ Serum α -fetoprotein in ng/mL, median (range)	10 (2-33930)
Laboratory tests	
▪ Hemoglobin in g/L, median (range)	14 (7-19)
▪ Platelet count in 10 ⁹ /L, median (range)	217 (34-618)
▪ Serum total bilirubin in μ mol/L, median (range)	10 (4-31)
▪ Serum aspartate aminotransferase in IU/L, median (range)	39 (21-706)
▪ Serum alanine aminotransferase in IU/L, median (range)	35 (10-229)
▪ Serum alkaline phosphatase in IU/L, median (range)	96 (37-580)
▪ International normalized ratio, median (range)	1 (1-2)
▪ Serum creatinine in μ mol/L, median (range)	72 (48-719)
▪ Serum albumin in g/L, median (range)	37 (22-50)
▪ Indocyanine green retention at 15 minutes in %, median (range)	12 (3-46) 228 (100-400)
▪ Liver stiffness measurement in kPa, median (range)	
Operative Parameters	
▪ Approach, laparotomy : laparoscopy	57 (70.4) : 24 (29.6)
▪ Re-hepatectomy	8 (9.9)
▪ Portal vein embolization	19 (23.5)
▪ Pre-operative transarterial chemoembolization	15 (18.5)
▪ Uni-segmentectomy	24 (29.6)
▪ Partial hepatectomy	24 (29.6)
▪ Right hepatectomy	19 (23.5)
▪ Left hepatectomy	5 (6.2)
▪ Bi-segmentectomy	8 (9.9)
▪ Central hepatectomy	1 (1.2)
▪ Duration of Pringle maneuver in min, median (range)	44 (0-240)
▪ Operative time in min, median (range)	245 (103-590)
▪ Blood loss in mL, median (range)	410 (50-7500)

▪ RBC transfusion	9 (11.1)
▪ Number Couinaud segments resected, median (range)	1 (0-6)
▪ Resection margin in mm, median (range)	4 (0-55)
METAVIR Fibrosis score of non-tumoral liver	
▪ F0 : F1 : F2 : F3 : F4	6 (7.4) : 7 (8.6) : 18 (22.2) : 8 (9.9) : 42 (51.9)
METAVIR Activity Score of non-tumoral liver	
• A0 : A1 : A2 : A3 : Missing data	29 (35.8) : 23 (28.4) : 17 (21) : 9 (11.1) : 3 (3.7)
NAS Steatosis grade of non-tumoral liver	
• S0 : S1 : S2 : S3 : Missing data	32 (39.5) : 31 (38.3) : 15 (18.5) : 0 (0) : 3 (3.7)
Post-operative Outcomes	
• Duration of hospitalization in days, median (range)	7 (3-48)
• ICU stay in days, median (range)	1 (0-48)
• 90-day severe morbidity*	19 (23.5)
• 90-day mortality	2 (2.5)
• Comprehensive complication index, median (range)	21 (0-100)
• Two-year overall survival	79.9 %
• Two-year disease-free survival	38.1 %

[‡]Data are expressed as counts (percentage) unless otherwise stated; HBV-hepatitis B virus; HCV-hepatitis C virus; HEV-hepatitis E virus, METAVIR-meta-analysis of histological data in viral hepatitis, NAS- nonalcoholic fatty liver disease activity score,*Dindo-Clavien grade of surgical complications, ICU-intensive care unit

Supplementary table 3: Independent pre-operative predictors for R1 resection in patients undergoing hepatectomy in entire cohort (N=105)

Variables	Prevalence (%)	Early Recurrence						
		Uni-variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*		
			<i>Beta</i>	<i>p</i>	OR (95% CI)	<i>p</i>	95% CI	
Age, yr	<60 31 (29.5) ≥60 74 (70.5)	0.1	-1.19	0.1	0.31 (0.08 – 1.24)	0.08	-2.62 – 0.31	
Gender	M 83 (79.1) F 22 (20.9)	0.6						
Body mass index, kg/m ²	<30 88 (83.8) ≥30 17 (16.2)	0.15						
Diabetes mellitus	No 77 (73.3) Yes 28 (26.7)	0.6						
Viral hepatitis	No 64 (61) Yes 41 (39)	0.9						
Non-alcoholic fatty liver disease	Yes 26 (24.8) No 79 (75.2)	0.3						
Model for end-stage liver disease score	<12 94 (89.5) ≥12 11 (10.5)	0.4						
Maximum size, mm	<50 67 (63.8) ≥50 38 (36.2)	0.15						
Tumor number	≤1 64 (61) >1 41 (39)	0.7						
Pre-operative TACE	No 83 (79.1) Yes 22 (20.9)	<0.001						
α-fetoprotein, ng/mL	<100 75 (71.4) ≥100 29 (27.6)	0.8						
Hemoglobin, g/dL	≥10 101(96.2) <10 4 (3.8)	0.7						
Platelet count, 10 ⁹ /L	≥150 82 (78.1) <150 23 (21.9)	0.12						
Total bilirubin, μmol/L	≤18 93 (88.6) >18 12 (11.4)	0.19						
Aspartate aminotransferase, IU/L	≤50 69 (65.7) >50 36 (34.3)	0.3						
Alkaline phosphatase, IU/L	<75 29 (27.6) ≥75 76 (72.4)	0.8						
ICG-R15, %	<15 56 (56.3) ≥15 25 (23.8)	0.07						
Liver stiffness, kPa	<12 43 (41.0) ≥12 45 (42.9)	0.01	1.85	0.03	6.37 (1.22 – 33.29)	0.01	0.63 – 20.59	
CAP, dB/m	≥240 50 (47.6) <240 36 (34.3)	0.3						
Re-hepatectomy	No 95 (90.5) Yes 10 (9.5)	0.9						
Laparotomy	Yes 75 (71.4) No 30 (28.6)	0.5						
Major hepatectomy	No 72 (68.6) Yes 33 (31.4)	0.4						
Metavir F4	No 53 (50.5) Yes 52 (49.5)	0.3						
Metavir A2/3	No 67 (63.8) Yes 33 (31.4)	0.3						
Steatosis, %	≤5 41 (39) >5 57 (54.3)	0.18						
Steatohepatitis	No 42 (40) Yes 25 (23.8)	0.7						
Moderate/Poor-differentiation	No 52 (49.5) Yes 53 (50.5)	0.4						
Satellite nodule	No 85 (81) Yes 20 (19)	0.3						
Microvascular invasion	No 57 (54.3) Yes 48 (45.7)	0.16						
Macrovascular invasion	No 92 (87.6) Yes 13 (12.4)	0.7						
Presence of intact capsule	Yes 40 (38.1) No 65 (61.9)	0.8						
Constant				0.01		0.002	-20.86 – -1.14	

*All unrelated pre-operative variables significant at ≤15% in univariate regression were entered in to multivariate regression by backward elimination (Wald) method and the variables that remained in the model were again analyzed after bootstrapping at 1000, OR-odds ratio, CI-confidence interval, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Supplementary table 4: Independent predictors for early recurrence within 12 months after hepatectomy in patients with solitary HCC (N=81)

Variables	Prevalence (%)	Early Recurrence					
		Uni variate <i>p</i>	Multivariate Logistic Regression*			Bootstrapped Multivariate Logistic Regression*	
			<i>Beta</i>	<i>p</i>	HR (95% CI)	<i>p</i>	95% CI
Age, yr	<60 25 (30.9)	0.5					
	≥60 56 (69.1)						
Gender	M 64 (79.0)	0.03					
	F 17 (21.0)						
Body mass index, kg/m ²	<30 65 (80.2)	0.1					
	≥30 16 (19.8)						
Diabetes mellitus	No 58 (71.6)	0.01					
	Yes 23 (28.4)						
Viral hepatitis	No 49 (60.5)	0.3					
	Yes 32 (39.5)						
Non-alcoholic fatty liver disease	Yes 22 (27.2)	0.14					
	No 59 (72.8)						
Model for end-stage liver disease score	<12 73 (90.1)	0.7					
	≥12 8 (9.9)						
Maximum size, mm	<50 53 (65.4)	0.2					
	≥50 28 (34.6)						
Pre-operative TACE	No 66 (81.5)	0.4					
	Yes 15 (18.5)						
α-fetoprotein, ng/mL	<100 57 (70.4)	0.01	1.1	0.08	2.98 (0.86-10.33)	0.09	-0.303-2.67
	≥100 23 (28.4)						
Hemoglobin, g/dL	≥10 77 (95.1)	0.1					
	<10 4 (4.9)						
Platelet count, 10 ⁹ /L	≥150 65 (80.2)	0.9					
	<150 16 (19.8)						
Total bilirubin, μmol/L	≤18 75 (92.6)	0.3					
	>18 6 (7.4)						
Aspartate aminotransferase, IU/L	≤50 52 (64.2)	0.3					
	>50 29 (35.8)						
Alkaline phosphatase, IU/L	<75 23 (28.4)	0.9					
	≥75 58 (71.6)						
ICG-R15, %	<15 45 (55.6)	0.9					
	≥15 18 (22.2)						
Liver stiffness, kPa	<30 32 (76.5)	0.1					
	≥30 7 (8.6)						
CAP, dB/m	≥240 28 (34.6)	0.01	1.8	0.01	6.08 (1.51-24.44)	0.01	0.52-20.93
	<240 40 (49.4)						
Re-hepatectomy	No 73 (90.1)	0.2					
	Yes 8 (9.9)						
Laparotomy	No 24 (29.6)	0.8					
	Yes 57 (70.4)						
Major hepatectomy	No 56 (69.1)	0.5					
	Yes 25 (30.9)						
Pringle maneuver, min	≤75 70 (86.4)	0.3					
	>75 11 (13.6)						
Operative time, min	<240 37 (45.7)	0.5					
	≥240 44 (54.3)						
Blood loss, mL	≤500 49 (60.5)	0.7					
	>500 32 (39.5)						
RBC transfusion	No 72 (88.9)	0.1					
	Yes 9 (11.1)						
90-day severe morbidity	No 62 (76.5)	0.5					
	Yes 19 (23.5)						
Metavir F4	No 53 (50.5)	0.3					
	Yes 39 (48.1)						
Metavir A2/3	No 52 (64.2)	0.7					
	Yes 26 (32.1)						
Steatosis, %	>5 32 (39.5)	0.003					
	≤5 46 (56.8)						
Steatohepatitis	Yes 42 (40)	0.9					
	No 25 (23.8)						
Moderate/Poor-differentiation	No 39 (48.1)	0.9					
	Yes 42 (51.9)						
Satellite nodule	No 54 (66.7)	0.4					
	Yes 27 (33.3)						
Microvascular invasion	No 45 (55.6)	0.16					
	Yes 36 (44.4)						
Macrovascular invasion	No 72 (88.9)	0.1					
	Yes 9 (11.1)						
Presence of intact capsule	Yes 28 (34.6)	0.1					
	No 53 (65.4)						
Tumor-free margin	R0 65 (80.2)	0.2					
	R1 16 (19.8)						
Constant				<0.001		0.001	-21.67—1.37

*All unrelated variables significant at ≤15% in univariate regression were entered in to multivariate regression by backward entry of Wald method and the variables that remained in the model were again analyzed after bootstrapping at 1000, TACE-transarterial chemoembolization, ICG-R15- indocyanine green retention at 15 min, CAP-controlled attenuation parameter

Figure 1: Kaplan Meir analysis of overall survival

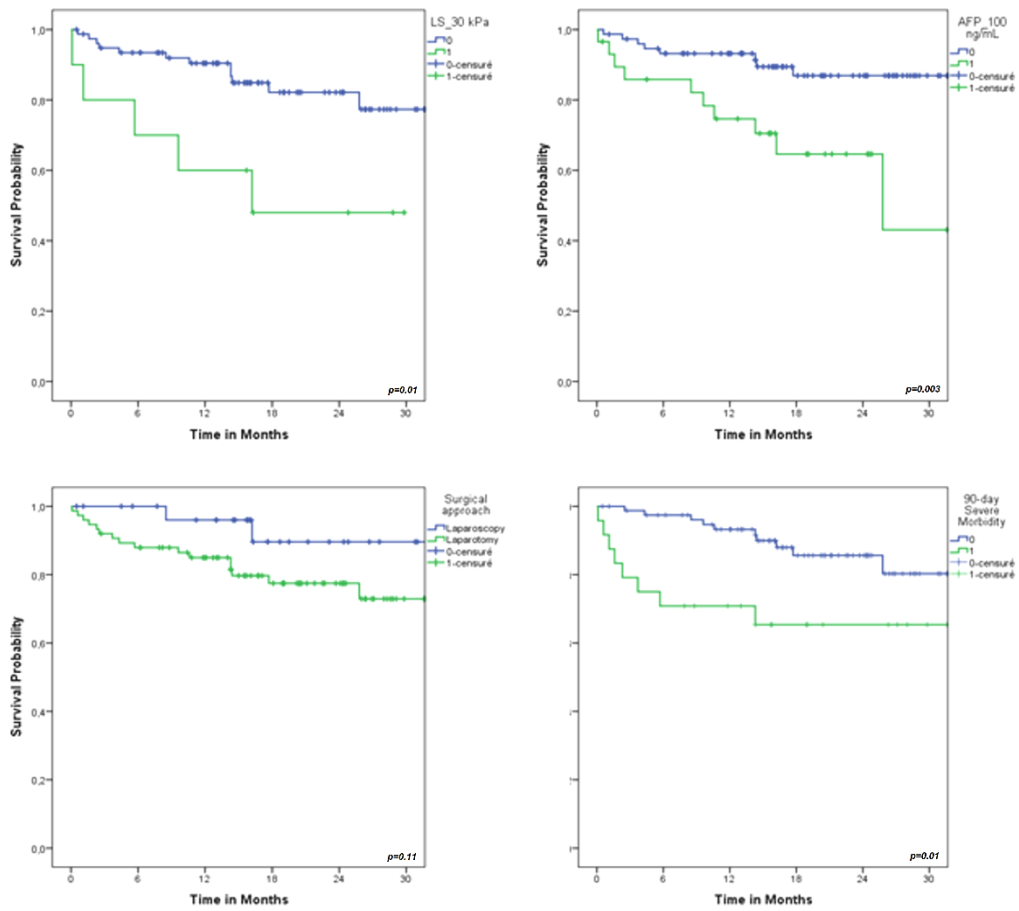
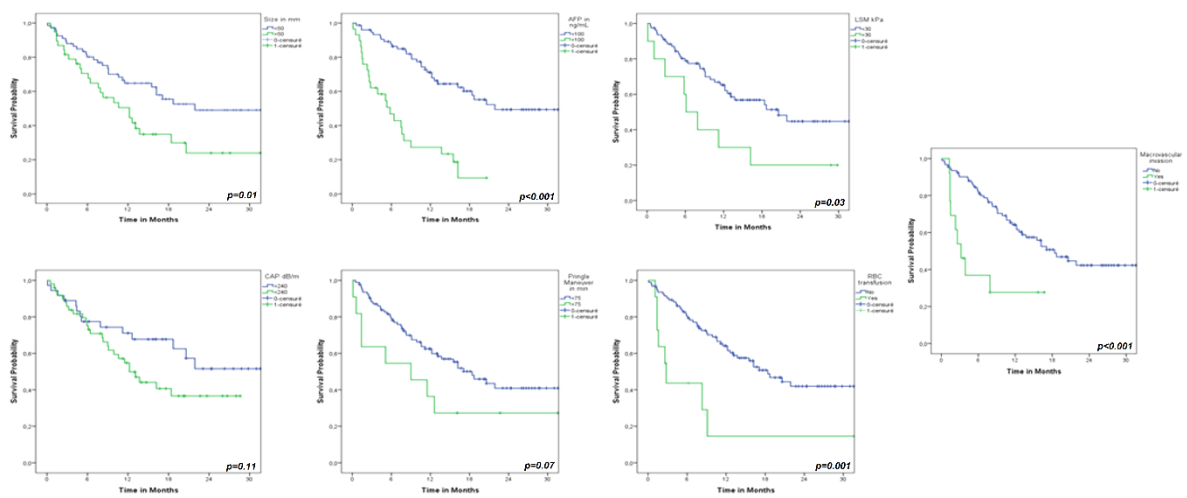


Figure 2: Kaplan Meir analysis of disease-free survival



Discussion

The primary objective of the present study was to develop a statistically model to predict post-operative complications with LS evaluated by FibroScan[®]. A nomogram was built with age [Odds ratio (OR):1.02, $p=0.06$], LS in log [OR:1.23, $p=0.04$], number of resected segments [OR:1.28, $p=0.004$], and operative time [OR:1.01, $p=0.01$] as continuous variables to predict 90-day severe morbidity according Clavien-Dindo classification of surgical complications. This model had a satisfactory discriminatory performance (AUROC=0.7) and Goodness-of-fit ($p=0.7$). External validation of the nomogram had satisfactory calibration. Another nomogram was developed to predict 90-day post-operative mortality with the following factors: diabetes mellitus [OR: 6.16, $p=0.04$], tumor size >50 mm [OR: 4.78, $p=0.08$], LS ≥ 22 kPa [OR: 7.02, $p=0.04$], and operative time ≥ 360 min [OR: 6.06, $p=0.05$]. Similarly, the performance of this model was evaluated in the study and external cohorts with ROC analysis and Goodness-of-fit.

The factors included in the models were readily available, pre-operative clinical parameters and a factor under surgeon's control – estimated operation time, a surrogate marker for the complexity of planned LR. Therefore, these nomograms would enable a surgeon to estimate the patient's risk pre-operatively and modify the type of LR or offer an alternative treatment in a patient when the risk of severe morbidity or mortality is above the acceptable limits of their center. Thus, the surgeons can use them to plan the operative strategy, obtain informed consent, and adapt surgical technique according to an individual patient's risk and to the expertise available in their centers.

When the predictors of liver-specific complication, post-operative hepatic decompensation were assessed, the model with LS as a binary predictor had a better discriminatory performance with AUROC of 0.82. With respect to post-hepatectomy liver failure, ICG-R15 was the important predictor of its development and the predictive nomogram with ICG-R15 had an excellent performance (AUROC=0.92). Similarly, in patients

undergoing major hepatectomy (≥ 3 segments) ICG-R15 was the one of the predictors of major complications. Nomogram developed with age in years, ICG-R15 in percentage, number of segments resected and operation time had a good performance (AUROC=0.8) and an excellent fit.

With respect to 90-day post-operative severe morbidity, LS and ICG-15 play an equally important role in predicting the complications. When LS was replaced in the nomogram for 90-day severe morbidity with ICG-R15, the model had similar performance with AUROC of 0.71. In the analysis for mortality, ICG-R15 did not emerge as a predictor even in univariate analysis. However, in patients undergoing major hepatectomy (usually with minimal underlying liver disease), ICG-R15 is an important predictor. It can be inferred from this study, LS has an important role in patients with underlying liver disease as demonstrated by Cescon *et al*, Kim *et al* and Wong *et al* in patients undergoing LR for HCC.^{173,175,176}

In patients undergoing LR for HCC, we evaluated the role LS as a predictor of persistent hepatic decompensation beyond 90-day post-operative period. Nine out of 29 patients (27.4%) who developed post-operative liver decompensation in form of ascites in 18, jaundice in 18 and/or encephalopathy in four had persistent decompensation. The sole independent predictor of this complication was LS and we identified a cut-off 22 kPa to have similar performance to invasive HVPG measurement. Moreover, majority of the patients with LSM ≥ 22 kPa (66.7%) developed complications after hepatectomy with two patients dying due to PHLF in the 90-day post-operative period. Further, TE was found to be an important prognostic factor for disease recurrence and survival in patients undergoing LR for HCC. Elevated LS (≥ 30 kPa) was an independent predictor of the quality of LR, and overall survival. Tumor-free surgical margin was not ensured when the LS ≥ 12 kPa resulting in inadequate R1+ surgical resection. Along with serum AFP ≥ 100 ng/mL, open LR, and severe post-operative morbidity, LS ≥ 30 kPa was an important predictor (HR: 5.3) of poor OS.

Elevated serum AFP level and lower CAP were surrogate markers of the aggressive tumor biology in these patients. Serum AFP ≥ 100 ng/mL and CAP < 240 dB/m were the independent predictors of microvascular invasion, early recurrence and thus poor DFS. Moreover, LS ≥ 30 kPa was an independent predictor of dropout from the list of LT for HCC. Therefore, *Ab initio* LT should be planned for the patients with LS ≥ 30 kPa and CAP < 240 dB/m with LR being offered as bridge to transplantation. In patients with LS < 30 kPa and CAP ≥ 240 dB/m, surgical resection would be treatment of choice as they seem to have less aggressive HCC and lower rate of ER, and lower risk of dropout while waiting for LT.

FibroScan[®] is an effective non-invasive method of estimating hepatic fibrosis and steatosis by evaluating liver stiffness and controlled attenuation parameter, respectively. In the present study, LS accurately estimated the degree of fibrosis in the non-tumoral liver parenchyma according to METAVIR scoring system in patients undergoing hepatectomy and transplantation. Area under ROC curve for various grades for fibrosis were 0.85 (F ≥ 1), 0.89 (F ≥ 2), 0.95 (F ≥ 3) and 0.97 (F=4). The performance of LS in our cohort was similar to the published literature showing progressive improvement in the performance as the grade increased. This study prospectively validated, in explant liver after surgery, the Baveno VI criteria for non-invasive diagnosis of cACLD (10 kPa) and cirrhosis (15 kPa) using LS not only in virus-related liver disease but also in other aetiologies. The performance of TE in obese patients (BMI ≥ 30) and in steatotic liver ($\geq 34\%$) was also confirmed in this study. The factors affecting LS measurement were BMI, etiology of liver disease, total bilirubin level, AST level, and alkaline phosphatase level. Therefore, LS interpretation must take into account these factors especially in patients with cholestasis and inflammation. However, in our cohort age ≥ 60 years was the only factor that emerged as independent risk factor for false positive results probably reflecting the type of study population. In a pilot study of 22 patients where quantify of fibrosis by digital morphometry was correlated with METAVIR fibrosis score and

LS, there was a significant correlation of fibrosis area in percentage with METAVIR score (Spearman $\rho=0.7$) and LS (Spearman $\rho=0.5$).

The discriminatory performances of CAP to diagnose according to NAS steatosis grades were 0.69 ($S\geq 1$), 0.70 ($S\geq 2$), and 0.67 ($S=3$). The cut-off values for $S\geq 1$, $S\geq 2$, and $S=3$ were 258 dB/m, 278 dB/m, and 287 dB/m, respectively. A recent meta-analysis demonstrated that CAP had good performance to diagnose hepatic steatosis with AUROCs of 0.82 ($S\geq 1$), 0.87 ($S\geq 2$), and 0.88 ($S=3$). The cut-offs in dB/m determined were 248 in $S\geq 1$, 268 in $S\geq 2$ and 280 in $S=3$. The performance of CAP in our study was lower than that of meta-analysis probably due to lower prevalence of grade 3 steatosis (<5%), different etiology, and site of CAP measurement was different from site of biopsy. Despite lower performance of CAP in the present study than the meta-analysis, there was a similar trend in the performance as illustrated in the bar diagram (Figure). There was considerable overlap in the CAP values especially in $S\geq 2$ and $S=3$. The factors influencing the performance of CAP were BMI, etiology of liver disease, and LS measurement. In 83 patients where CAP was compared with histological (NAS) and infrared spectroscopic (FTIR) grades of steatosis, performance of CAP was better in FTIR $G\geq 1$ grade of steatosis and in the higher grades, AUROCs were similar. We conclude that CAP is an evolving technique of estimating hepatic steatosis and it would be able to serve as a screening tool to identify the patients with steatosis ($S\geq 1$) and significant steatosis ($S\geq 2$). Further, confirmatory tests like LS, NAFLD fibrosis score, PAN score etc. would be required in those with elevated CAP before doing a liver biopsy.

A score model was developed to diagnose clinically significant portal hypertension (HVPG ≥ 10 mmHg) with LS and serological tests. The performance of the HVPG₁₀ score was better than LS alone with an excellent discriminatory performance (AUROC=0.91). Using Fagan nomogram, we were able to demonstrate in patients with lower prevalence of CSPH similar to LR patients (pre-cirrhotic and compensated cirrhotic patients), HVPG₁₀ score would

rule out CSPH accurately in 97% of patients. On the other hand, in a cohort with higher prevalence of CSPH (multicentric external cohort), HVPG₁₀ score would rule in CSPH in 97% of patients. When compared with ICG score published by Kim *et al*, HVPG₁₀ score had better discriminatory performance with AUROC of 0.91 compared with 0.71 for ICG score.¹⁷⁹ The present score could be utilized to screen patients suspected of esophageal varices and in patients with a score ≤ 15 , upper GI endoscopy could be avoided, as the risk of missing treatable varices would be $\leq 3\%$. In patients planned for hepatectomy, LS and HVPG₁₀ score would add value to the pre-operative workup as CSPH is an important prognostic factor in patients with HCC undergoing resection. In patients with HCC waiting for LT, LS and HVPG₁₀ score would be able to identify patients likely to progress and dropout of the waiting list. In future, patients with HCC waiting for LT could be prioritized according the degree of portal hypertension. We propose a cut-off of HVPG score of 50 to predict those likely to dropout.

Transient elastography by FibroScan[®] is an important part of pre-operative workup in patients planned for hepatectomy and transplantation. By estimating degree of hepatic fibrosis, portal hypertension and thus hepatic functional reserve, LS was able to predict post-operative outcomes. Along with ICG-R15, it plays an important role in the prediction of overall post-operative morbidity, mortality and specific complications like hepatic decompensation, PHLF and dropout of LT list in HCC patients. TE was feasible in majority of our patients without any change in the pre-operative workup of the patients programmed for LR and LT even in patients with right sided tumors. The major impediments for TE would be the presence of ascites, previous right-sided LR and large right liver tumors. Therefore, TE by FibroScan[®] should be a part of pre-operative workup for LR in hepatobiliary diseases along with ICG-R15.

Conclusions

This PhD research brings new insights into the clinical application of transient elastography by FibroScan[®] in surgical patients:

1. Liver stiffness measured by TE would enable surgeons to identify patients at risk of post-operative complications and death. The predictive nomograms proposed in this study would serve as an effective tool in pre-operative workup of patients.
2. In HCC patients, LS plays a more important role as it not only predicts 90-day post-operative outcomes but also persistent hepatic decompensation beyond the post-operative period.
3. Combination of LS and CAP could be utilized to predict oncological outcomes in patients undergoing LR for HCC. Elevated LS (≥ 30 kPa) and lower CAP (< 240 dB/m) were associated with poor outcomes after LR and thus, could be a surrogate marker for the aggressive nature of HCC.
4. The performance of LS was confirmed by this prospective study in patients undergoing LR and LT. The cut-offs proposed by Baveno VI consensus conference to diagnose cACLD and cirrhosis were validated to have excellent AUROCs of 0.95 and 0.97, respectively.
5. CAP could serve as a screening test to identify patients at risk of developing NASH by diagnosing hepatic steatosis. We propose a CAP cut-off of 278 dB/m to diagnose patients with significant steatosis.
6. Infrared spectroscopic evaluation of hepatic steatosis on tissue sections could be an objective replacement for histological evaluation of hepatic steatosis by pathologists.
7. A new score called HVPG₁₀ score was developed and validated to diagnose clinically significant portal hypertension non-invasively in different cohorts of patients. A cut-off of 15 was demonstrated to rule in or rule out CSPH. Further, HVPG₁₀ score ≥ 50 could predict dropout of patients from waiting list of LT in HCC patients.

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Annexure 1: List of publications and conference abstracts

- Rajakannu M, *et al.* Liver stiffness measurement by transient elastography predicts late posthepatectomy outcomes in patients undergoing resection for hepatocellular carcinoma. *Surgery* 2017 162:766-774. doi: 10.1016/j.surg.2017.06.006.
- Rajakannu M, *et al.* Prospective validation of transient elastography for staging liver fibrosis in patients undergoing hepatectomy and liver transplantation. *J Hepatol* 2017 Aug 23. doi: 10.1016/j.jhep.2017.07.032.
- Rajakannu M, *et al.* Liver stiffness measurement by transient elastography is an effective method of predicting persistent hepatic decompensation in patients undergoing hepatectomy for hepatocellular carcinoma. In: Proceedings from the Asia Pacific Association Hepato-Pancreato-Biliary Association, June 7-10, 2017, Yokohama, Japan. Abstract O-4-3.
- Rajakannu M, *et al.* Quantification of hepatic steatosis in patients undergoing liver resection: A comparative study of preoperative controlled attenuation parameter (CAP) versus Fourier transform-infrared spectroscopy (FTIR) and histopathological estimation (HPE) on surgical specimens. In: Proceedings from the American Association for the study of liver diseases, October 20-23, 2017, Washington, USA. Abstract 2186.
- Rajakannu M, *et al.* A simple score model based on liver function tests (LFT) and liver stiffness measurement (LSM) allows noninvasive estimation of hepatic venous pressure gradient (HVPG). In: Proceedings from the American Association for the study of liver diseases, October 20-23, 2017, Washington, USA. Abstract 457.

Titre : Impact de l'utilisation du FibroScan® dans la prise en charge des tumeurs du foie

Mots clés : Élastographie transitoire, élastométrie, paramètre d'atténuation contrôlée, hépatectomie, fibrose hépatique, hypertension portale, transplantation hépatique

Contexte: Les limites du traitement chirurgical des tumeurs du foie sont définies par la réserve hépatique fonctionnelle qui est déterminée par le volume et la qualité du foie non tumoral restant. L'hépatopathie sous-jacente détermine non seulement la place de la chirurgie mais aussi le risque de récurrence pour les tumeurs hépatiques primitives. Dans les cas des métastases hépatiques, la chimiothérapie précède très souvent la chirurgie et l'hépatotoxicité de cette chimiothérapie est un risque important des complications post-opératoires. Dans le contexte particulier de la transplantation hépatique (TH) pour le carcinome hépatocellulaire (CHC), l'hypertension portale est un risque de progression tumorale pendant la période d'attente et la sortie de la liste. Le foie non tumoral est donc un facteur important dans la prise en charge des patients avec des tumeurs du foie. L'appareil FibroScan® qui mesure l'élasticité du foie pourrait être utilisé pour évaluer le foie et prédire les suites post-opératoires et le risque de progression du CHC dans la liste de TH. **Méthodes:** Les patients consécutifs qui ont été programmés pour subir une hépatectomie ou une transplantation ont été inclus dans l'étude après un consentement éclairé. L'élastométrie (LS) et le paramètre d'atténuation contrôlée (CAP) du foie non tumoral ont été estimés en pré-opératoire par l'élastographie transitoire avec le dispositif FibroScan® 502 Touch en utilisant des sondes M ou XL. **Résultats:** Les nomogrammes basés sur LS qui ont été développés et validés dans cette étude ont

hépatectomie pour les maladies hépatobiliaires. Chez les patients atteints de CHC, LS a joué un rôle plus important car il prédit non seulement les résultats à 90 jours, mais aussi la décompensation hépatique persistante au-delà de la période post-opératoire. En plus, les seuils de LS ≥ 30 kPa et CAP < 240 dB/m ont été associés à un mauvais pronostic oncologique après l'hépatectomie et peuvent ainsi être un marqueur de substitution pour la nature agressive du CHC. La performance de LS pour diagnostiquer la fibrose hépatique avancée (AUROC: 0.95) et la cirrhose (AUROC: 0.97) a été validée dans cette recherche et CAP a eu une performance satisfaisante pour détecter la stéatose hépatique significatif (AUROC: 0.70). Un modèle à base de LS appelé score HVP_{G10} a été développé et validé pour diagnostiquer une hypertension portale significative chez les patients atteints d'une maladie chronique du foie. Avec un seuil de 15, le score HVP_{G10} était précis pour exclure une hypertension portale importante dans $>95\%$ des patients et éviter des investigations supplémentaires et inutiles. **Conclusion:** L'exploration des patients prévus pour l'hépatectomie permet de prévoir des complications sévères et la mortalité après l'hépatectomie. LS ≥ 30 kPa est un facteur de risque important de la récurrence après la résection et de la progression du CHC en attente de TH. Par conséquent, TH devrait être le traitement en première intention avec les patients avec LS ≥ 30 kPa.

Title : Utility of FibroScan[®] in the management of the liver tumors

Keywords : Transient elastography, liver stiffness, controlled attenuation parameter, hepatectomy, hepatic fibrosis, portal hypertension, liver transplantation

Background: The major determinant of the results of surgical resection for liver tumors is the volume and quality of the future liver remnant. The hepatopathy of the non-tumoral liver not only limits the type of surgery but also the risk of recurrence in primary liver tumors. With respect to liver metastasis, pre-operative chemotherapy is the usual treatment strategy and the hepatotoxicity of prolonged chemotherapy is an important risk factor for post-operative morbidity. In patients with hepatocellular carcinoma (HCC) waiting for liver transplantation (LT), clinically significant portal hypertension (CSPH) is a risk for tumor progression and dropout of the waiting list for LT. Overall, degree of liver fibrosis and portal hypertension in the non-tumoral liver are important factors in the management of patients with liver tumors as they determine the prognosis of patients after hepatectomy. FibroScan[®], which estimate the degree of liver fibrosis and steatosis, could be utilized to evaluate the non-tumoral liver and predict the post-operative outcomes and the risk of dropout from the list of LT in HCC patients waiting for LT. **Methods:** Consecutive patients programmed to undergo hepatectomy or LT were included in the present study prospectively after an informed consent. Liver stiffness (LS) and controlled attenuation parameter (CAP) were measured pre-operatively by transient elastography using FibroScan[®] 502 Touch Standard device with M or XL probes. **Results:** LS-based nomograms that were developed and validated in this study were

accurate to predict 90-day severe morbidity and 90-day mortality after hepatectomy for various hepatobiliary diseases. In patients with HCC undergoing hepatectomy, elevated LS ≥ 22 kPa was a risk factor for persistent hepatic decompensation beyond the 90-day post-operative period. Moreover, LS ≥ 30 kPa and CAP < 240 dB/m were associated with poor oncological outcomes after resection and thus could be a surrogate biomarker of more aggressive HCC. The discriminatory ability of LS to diagnose advanced liver fibrosis (AUROC: 0.95) and cirrhosis (AUROC: 0.97) was validated in the present study. Further, CAP had a satisfactory performance to screen significant hepatic steatosis ($S \geq 2$) with AUROC of 0.70. A new LS-based model called HVPG₁₀ score was developed and validated to diagnose CSPH. With a cut-off of 15, it was capable of accurately ruling out CSPH in $>95\%$ of the patients with chronic liver disease and would avoid further unnecessary investigations. **Conclusion:** Pre-operative evaluation of patients with transient elastography would enable surgeons to predict major complications and mortality after hepatectomy with LS-based nomograms. In patients with HCC, LS ≥ 30 kPa was an important risk factor of incomplete surgical resection, early recurrence after hepatectomy and for tumor progression and dropout while waiting for LT. Therefore, LT must be the primary treatment in HCC patients with LS ≥ 30 kPa.

