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Establishment and Assessment of the HVPG using Biofluid Mechanics ($HVPG_{BFM}$)

Study Protocol and Statistical Analysis Plan

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Introduction

Portal hypertension (PH), an increased blood pressure of the portal vein and its branches, is one of the most severe syndromes caused by chronic liver diseases. Hepatic venous pressure gradient (HVPG) is currently the gold standard to detect portal hypertension.(1) HVPG value greater than 5 mmHg is defined as portal hypertension; HVPG value higher than 10 mmHg is considered as clinically significant portal hypertension (CSPH), which is highly associated with hepatocellular carcinoma and severe complications including gastroesophageal variceal haemorrhage, hepatic encephalopathy and ascites.(2)

However, the HVPG measurement is only available in few hospitals due to its invasiveness and technical difficulty.(3) In recent years, there are already several non-invasive portal hypertension assessment methods, including clinical examination, ultrasound, elastography, CT and MRI,(4) few of which, however, was proved to be accurate enough to replace the invasive HVPG measurement. Therefore, a less-invasive and accurate assessment method is needed and would be useful in the diagnosis and evaluation of portal hypertension.

Biofluid mechanics is the study of mechanisms of biological flows (liquid and gas) and their interrelationships with physiological and pathological processes by using fundamental principles of fluid mechanics.(5) Fortunately, recent advances in biofluid mechanics and image-based modelling make it possible for cardiologists to calculate fractional flow reserve, which is the gold standard assessment of the haemodynamic significance of coronary stenoses.(6) Moreover, biofluid mechanics may offer a new method for physicians to make an accurate assessment of HVPG noninvasively. The aim of the present study is to establish and validate the HVPG using biofluid mechanics ($HVPG_{BFM}$) model.

Methods and analysis

Study design overview

This study is a prospective, non-controlled, multicentre trial in patients with cirrhosis or portal hypertension.

Study population

Consecutive patients scheduled for HVPG measurement for clinical indications at Shanghai Ninth People's Hospital, Renji Hospital and Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine are screened daily for study eligibility. Recruitment began on March 20th, 2018 and will continue until 200 participants have been recruited.

Inclusion criteria:

- Be at least 18 years of age.
- Patients with cirrhosis or portal hypertension, scheduled for HVPG measurement.

Exclusion criteria:

- Female patients who are pregnant or nursing.
- Patients who are medically unstable, terminally or seriously ill, or patients whose clinical course is unpredictable.
- Patients with clinically unstable cardiac disease, for example, congenital heart defect,

arrhythmia, uncontrolled heart failure (NYHA Class IV).

- Patients with respiratory distress syndrome or clinically unstable pulmonary disease, for example, pulmonary hypertension, pulmonary emboli, pulmonary vasculitis, emphysema.
- Patients with severe coagulation disorders.
- Patients with unstable occlusive disease or thrombosis within the hepatic, portal, or mesenteric veins.
- Patients who are allergic to iodinated contrast.

Ethics and informed consent

The trial complies with the latest Declaration of Helsinki. Written informed consent form will be obtained from patients or patients' legal guardian or patients' next of kin before the study begins. All participants can quit the study at any time without penalty or impact on the treatment. The study protocol, statistical analysis plan, informed consent form and case report form have already been approved by Scientific Research Projects Approval Determination of Independent Ethics Committee of Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. (Approval Number: 2017-430-T326)

Objectives

The primary objective of this study is to determine the numeric correlation between $HVPG_{BFM}$ and HVPG. The secondary objective is to determine the diagnostic accuracy of $HVPG_{BFM}$ for the diagnostics of PH and CSPH. The gold standard for the diagnosis will be the HVPG measurement.

Procedure of the study

Consecutive patients are randomly assigned 1:1 to either the original cohort or the validation cohort. Randomization is based on the computer-generated random digits table. This study consists of two independent and consecutive stages:

1. Establishment and improvement of the $HVPG_{BFM}$ model: For 100 patients in the original cohort, biofluid mechanics specialists will use each patient's computed tomography, blood tests, Doppler ultrasound and HVPG results to adjust the parameters of the $HVPG_{BFM}$ model in order to make each patient's $HVPG_{BFM}$ and HVPG values match well.
2. Assessment of the $HVPG_{BFM}$ model: For 100 patients in the validation cohort, biofluid mechanics specialists will use each patient's computed tomography, blood tests and Doppler ultrasound results to calculate each patient's $HVPG_{BFM}$ according to the $HVPG_{BFM}$ model established previously. Biofluid mechanics specialists will make no changes to the $HVPG_{BFM}$ model and will have no access to patients' HVPG results in this cohort. Finally, the researchers will compare each patient's $HVPG_{BFM}$ and HVPG value and make an assessment of the $HVPG_{BFM}$ model.

Each patient's HVPG measurement will be performed after finishing computed tomography, blood tests and Doppler ultrasound. These results and other clinical data will be inaccessible to professionals for HVPG measurements in order to prevent certain biases. Each patient's computed tomography, blood tests, Doppler ultrasound and HVPG measurement will be performed within 30 days and treatments that may affect HVPG value will be avoided during this period.

The study design and procedure are shown in Figure 1.

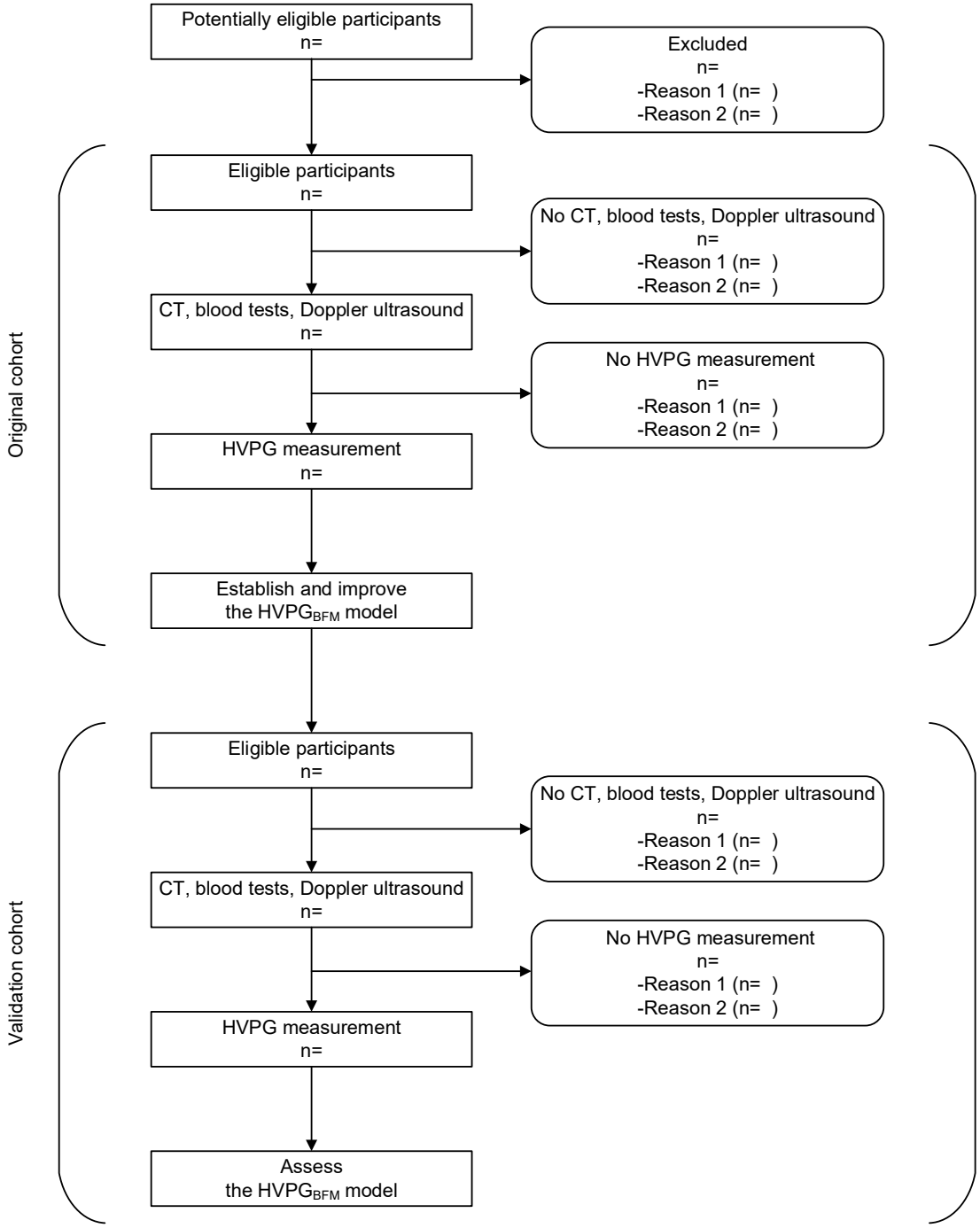

Figure 1 Study design and procedure.

Examination

Computed Tomography

Each patient will undergo an abdominal contrast-enhanced computed tomography (CT) by multi-detector row CT scanners according to the protocol.(7, 8) The following parameters will be used: voltage, 120 kVp; current, automatic; collimation, 1.24-1.25 mm; Slice thickness, 0.625-2.5 mm; Slice interval, 1.0-2.0 mm; Matrix size, 512 × 512; rotation time, 0.5 s. A nonionic iodinated contrast agent (600 mg of iodine per kilogram of body weight, 300-370 mg

of iodine per ml, 3-5 ml per second) will be injected. Portal venous phase imaging will be performed 60-70 seconds after the beginning of intravenous contrast injection.

Blood tests

Each patient's blood samples will be collected from the cubital vein for blood viscosity test and density measurement. The blood viscosity test will be done by a ZL9100C blood rheology analyser (Zhongchi, China). The blood density will be done by weighing one millilitre of blood: one millilitre of blood will be pipetted by a Thermo Scientific™ Finnpipette™ F1 Pipettor onto a YP-B2002 electronic balance (Guangzheng, China) for weight measurement. The blood density measurements will be repeated at least three times and then be averaged.

Doppler Ultrasound

Each patient will undergo an abdominal Doppler ultrasound scan to measure the inner diameters and the maximum blood flow velocity of the inferior vena cava, the hepatic veins, the portal vein and its main branches after an overnight fast. The 12 measurement positions are: right branch of the portal vein, left branch of the portal vein, the portal vein, proximal part of the splenic vein, distal part of the splenic vein, the superior mesenteric vein, the inferior mesenteric vein, the right hepatic vein, the middle hepatic vein, the left hepatic vein, the suprahepatic inferior vena cava and the infrahepatic inferior vena cava. Each measurement will be done during deep inspiration breath-hold. All measurements will be repeated at least twice and then be averaged.

HVPG Measurement

HVPG will be measured by professionals according to established standards.(3) After finishing the computed tomography, blood tests and Doppler ultrasound, each patient will undergo an HVPG measurement after an overnight fast and local anaesthesia. The right internal jugular vein will be cannulated using a 6-French introducer (TERUMO Radifocus RS*A60K10SQ, Japan) under ultrasonographic guidance, then a 5.5-French compliant balloon-tipped catheter (Edwards Lifesciences Fogarty 12TLW805F35, USA) connected with a pressure monitoring set (Edwards Lifesciences TruWave PX260, USA) will be guided into the right or middle hepatic vein for the measurement of wedged hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP). WHVP will be measured when inflating the balloon to totally occlude the hepatic vein; FHVP will be measured when placing the catheter tip freely in the hepatic vein, at approximately 3 cm from its opening into the inferior vena cava. All measurements will be taken at least in duplicate and HVPG will be calculated from the difference between average WHVP and average FHVP. HVPG = WHVP - FHVP.

Computation

Geometry

Biofluid mechanics specialists will use IQQA-Liver software (version 2.0, EDDA Technology, Inc., USA) to convert the CT images from Digital Imaging and Communications in Medicine (DICOM) format files into stereolithography (STL) format files and create the simulation model of blood flow area in blood vessels accordingly. The model surface will then be meshed into triangle surface grids, each measuring from 0.2-1.0 mm, and the body meshes will be created accordingly. One case is shown in Figure 2.

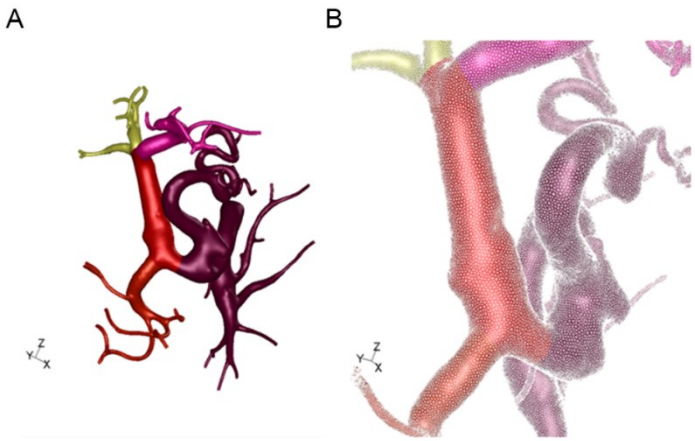

Figure 2 An example of the simulation model of a portal venous system and its body meshes. (A) The simulation model of a portal venous system. (B) The body meshes of the simulation model.

Physical property

Biofluid mechanics specialists will use the blood viscosity of different shear forces (high, medium and low) to calculate the overall viscosity (Formula 1). Define a, b and c (a + b + c = 1) as weighting coefficients.

Formula 1: $overall\ viscosity = a \times viscosity_{high} + b \times viscosity_{medium} + c \times viscosity_{low}$

Boundary conditions

Biofluid mechanics specialists calculate the blood flow velocity at the boundaries of the main blood vessels according to the maximum blood flow velocity (measured by Doppler ultrasound), the inner diameter (obtained from the STL format files) and the principle of mass conservation (Formula 2). Define d ($0.7<d<1$) as the maximum blood flow velocity attenuation coefficient. One case is shown in Figure 3.

Formula 2: $average\ blood\ flow\ velocity = d \times blood\ flow\ velocity_{maximum}$

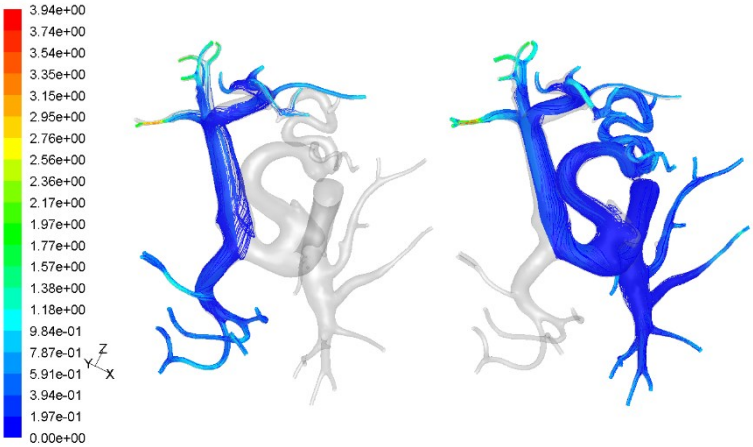

Figure 3 An example of the blood flow velocity of the portal vein and its branches.

The Navier-Stokes equations

The whole blood can be assumed to be an incompressible Newtonian fluid and blood flow can be modelled by the Navier-Stokes equations as follows:

Formula 3: $\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{v}) = S_m$

Formula 4: $\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho v_x) + \frac{\partial}{\partial r}(\rho v_r) + \frac{\rho v_r}{r} = S_m$

Formula 5: $\frac{\partial}{\partial t}(\rho E) + \nabla \cdot (\vec{v}(\rho E + p)) = \nabla \cdot \left(k_{eff}\nabla T - \sum_j h_j \vec{J}_j + (\bar{\bar{\tau}}_{eff} \cdot \vec{v})\right) + S_h$

Formula 6: $E = h - \frac{p}{\rho} + \frac{v^2}{2}$

Formula 7: $\frac{\partial}{\partial t}(\rho\vec{v}) + \nabla \cdot (\rho\vec{v}\vec{v}) = -\nabla p + \nabla \cdot (\bar{\bar{\tau}}) + \rho\vec{g} + \vec{F}$

Formula 8: $\bar{\bar{\tau}} = \mu\left[(\nabla\vec{v} + \nabla\vec{v}^T) - \frac{2}{3}\nabla \cdot \vec{v}I\right]$

ρ: density; v: velocity; S_m: mass added to the continuous phase; x: axial coordinate; r: radial coordinate; v_x: axial velocity; v_r: radial velocity; h: enthalpy value; p: static pressure; $\bar{\bar{\tau}}$: stress tensor; $p\vec{g}$: gravitational body force; $\vec{F}$: external body force; μ: molecular viscosity; I: unit tensor.

Establishment and improvement of the HVPGBFM model in the original cohort

Empirical coefficients in initial: a = 0.05; b = 0.05; c = 0.9; d = 1. Calculate the overall viscosity and average blood flow velocity (formula 1 & 2) of one patient in the original cohort. Use the FLUENT software (version 6.3, ANSYS, Inc., USA) to solve the Navier-Stokes equations (formula 3-8) and get the simulated blood pressure on each volume grid. One case is shown in Figure 4. The simulated HVPG will be calculated from the difference between simulated portal pressure (PP_{BFM}) and simulated hepatic venous pressure (HVP_{BFM}), namely, $HVPG_{BFM}$ = PP_{BFM} - HVP_{BFM}. Compare the simulated and measured HVPG. If they don't match well (their difference greater than 5%), then modify the value of a, b, c and d until they do. Record the value of a, b, c and d and the whole process for one patient is completed. Apply this process to each patient in the original cohort and record the value of a, b, c and d of each patient.

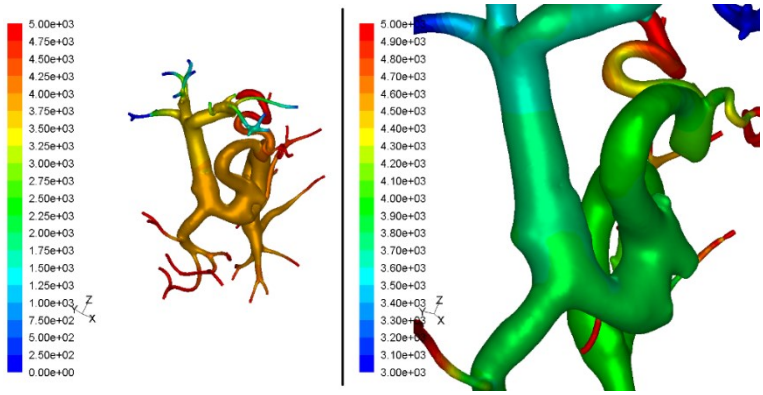

Figure 4 An example of the blood pressure of the portal vein and its branches.

Divide all patients into 9 groups according to their overall blood viscosity (high, medium and low) and their average blood flow velocity (high, medium and low): HighVis&HighVel, HighVis&MidVel, HighVis&lowVel, MidVis&HighVel, MidVis&MidVel, MidVis&lowVel, LowVis&HighVel, LowVis&MidVel, LowVis&lowVel. Calculate the statistical mean value of a, b, c and d of each group.

Calculation of the HVPGBFM in the validation cohort

First, determine each patient's group according to the blood viscosity and blood flow velocity. Secondly, use the value of a, b, c and d of the specific group to calculate the geometry, physical property and the boundary conditions of each patient. Thirdly, use the FLUENT software to solve the Navier-Stokes equations and get the simulated blood pressure on each volume grid.

Finally, calculate the HVPG$_{BFM}$.

The summarized computation process is shown in Figure 5.

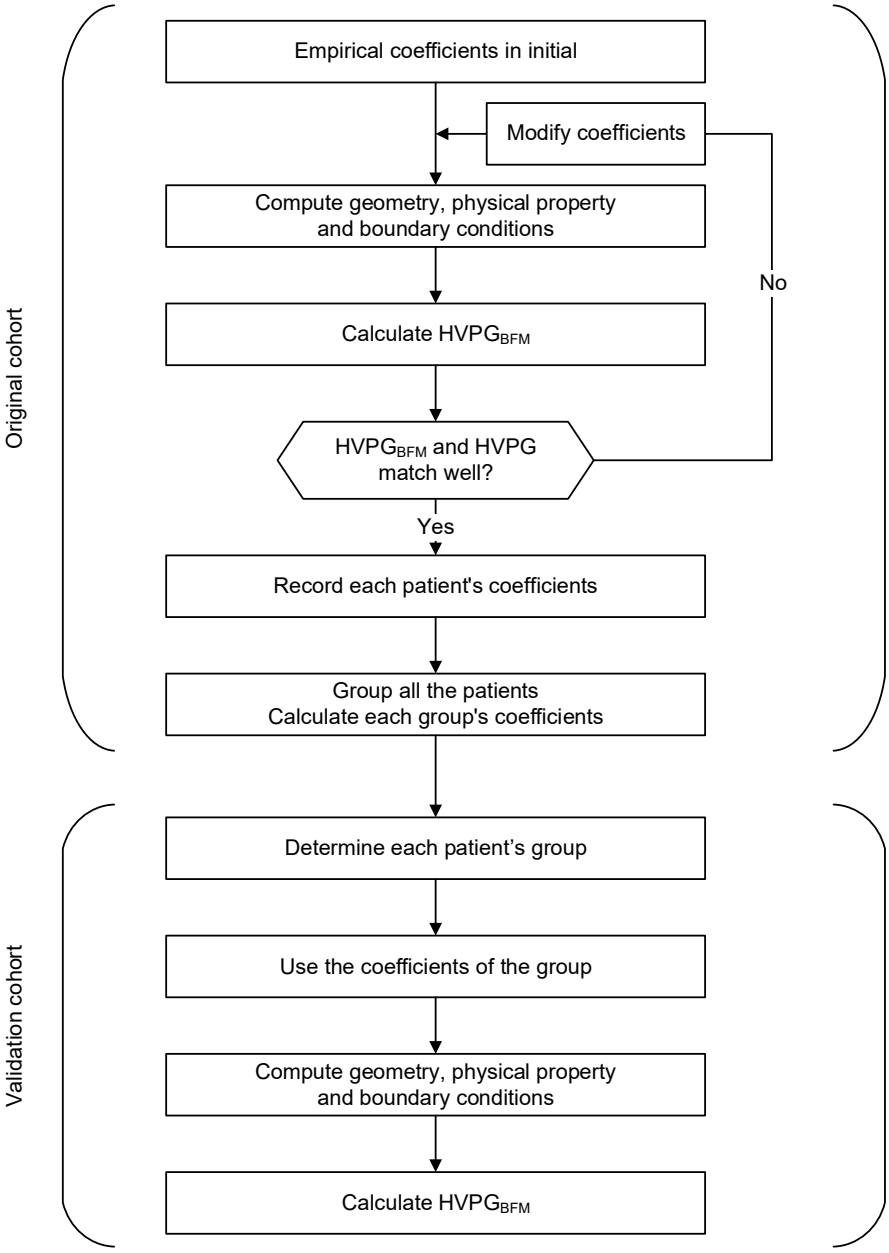

Figure 5 The process of the HVPGBFM computation

Sample size calculation

There will be 9 groups in the model. In order to obtain a precise model, no less than 10 samples for each group is needed in the original cohort, so the sample size estimated for the original cohort is 90 patients. It is anticipated that 10% of the patients recruited are likely to be excluded due to various reasons and therefore the target recruitment for the original cohort is 100 patients. The same number of patients will be recruited for the validation cohort. The total sample size is 200 patients.

Statistical Analysis Plan

Discrete variables will be summarized by frequencies and percentages and analysed by the $\chi 2$

test. Continuous variables will be checked for normal distribution and summarized by either mean and standard deviations or median and interquartile range as appropriate. Comparison of continuous variables will be performed by using Student's t-test or ANOVA for normally distributed variables and the Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed variables as appropriate.

The numeric correlation between $HVPG_{BFM}$ and HVPG will be analysed by using Bland and Altman's limits of agreement analysis.(9) Bias is defined as the mean of the difference between $HVPG_{BFM}$ and HVPG. Upper and lower limits of agreement are defined as average difference $\pm$ 1.96 standard deviation of the difference. The numeric relationship between $HVPG_{BFM}$ and HVPG will also be analysed with linear regression analysis, and to visualize the results, $HVPG_{BFM}$ will be plotted against HVPG.

The diagnostic accuracy of $HVPG_{BFM}$ for the diagnostics of PH (HVPG $\geq$ 5 mmHg) and CSPH (HVPG $\geq$ 10 mmHg) will be assessed by its sensitivity (Se), specificity (Sp), false-negative rate (FNR), false positive rate (FPR), positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, Youden index (YI), likelihood ratio (LR+ and LR-) and Cohen's Kappa.

All tests of significance will be at the 5% significance level. Analyses will be conducted using SPSS Statistics (version 24.0, IBM, USA) and MedCalc Statistical Software (version 18.11, MedCalc Software bvba, Belgium).

References

1. de Franchis R, Baveno VIF. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol. 2015;63(3):743-52.

2. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383(9930):1749-61.

3. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol. 2009;6(10):573-82.

4. Qi X, Berzigotti A, Cardenas A, Sarin SK. Emerging non-invasive approaches for diagnosis and monitoring of portal hypertension. Lancet Gastroenterol Hepatol. 2018;3(10):708-19.

5. Elad D, Bluestein D. Biofluid mechanics: innovations and challenges. J Biomech. 2013;46(2):207.

6. Taylor CA, Fonte TA, Min JK. Computational fluid dynamics applied to cardiac computed tomography for noninvasive quantification of fractional flow reserve: scientific basis. J Am Coll Cardiol. 2013;61(22):2233-41.

7. Smith AD, Branch CR, Zand K, Subramony C, Zhang H, Thaggard K, et al. Liver Surface Nodularity Quantification from Routine CT Images as a Biomarker for Detection and Evaluation of Cirrhosis. Radiology. 2016;280(3):771-81.

8. Sartoris R, Rautou PE, Elkrief L, Pollorsi G, Durand F, Valla D, et al. Quantification of Liver Surface Nodularity at CT: Utility for Detection of Portal Hypertension. Radiology. 2018;289(3):698-707.

9. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10.