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MEASUREMENT OF THE HEPATIC VENOUS PRESSURE GRADIENT AND TRANSJUGULAR LIVER BIOPSY

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TITLE:

Measurement of the Hepatic Venous Pressure Gradient and Transjugular Liver Biopsy

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SUMMARY:

Here, we present a protocol for measurement of hepatic venous pressure gradient (HVPG),the gold standard to diagnose clinically significant portal hypertension. Moreover, we describe how to perform a transjugular liver biopsy within the same session.

ABSTRACT:

Here we provide a detailed protocol describing the clinical procedure of hepatic venous pressure gradient (HVPG) measurement in patients with advanced chronic liver disease followed by an instruction for transjugular biopsy. Under local anesthesia and ultrasound guidance, a catheter introducer sheath is placed in the right internal jugular vein. Under fluoroscopic guidance, a balloon catheter is advanced in the inferior vena cava (IVC) and inserted into a large hepatic vein. Correct and sufficient wedge position of the catheter is ensured by injecting contrast media while the balloon is blocking the outflow of the cannulated hepatic vein. After calibrating the external pressure transducer, continuous pressure recordings are obtained with triplicate recordings of the wedged hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP). The difference between FHVP and WHVP is referred to as HVPG, with values ≥10 mm Hg indicating

clinically significant portal hypertension (CSPH). Before removing the catheter, pressure readings obtained in the IVC at the same level, as well as the right atrial pressure are recorded.
Finally, a transjugular liver biopsy can be obtained via the same vascular route. Different systems are available; however, core biopsy needles are preferred over aspiration needles, especially for cirrhotic livers. Again, under fluoroscopic guidance a biopsy needle introducer sheath is advanced into an hepatic vein. Next, the transjugular biopsy needle is gently advanced through the introducer sheath: (i) in case of aspiration biopsy, the needle is advanced into the liver parenchyma under aspiration and then removed quickly, or (ii) in case of a core biopsy, the cutting-mechanism is triggered inside the parenchyma. Several separate passages can be safely performed to obtain a diagnostic liver specimens via transjugular biopsy. The combination of these procedures takes about 30–45 min.

INTRODUCTION:

Patients with cirrhosis are at risk for developing complications mostly related to portal hypertension (PHT), such as ascites or bleeding from gastric or esophageal varices[1-3]. The risk of hepatic decompensation is related to the degree of PHT[2]. Measurement of the hepatic venous pressure gradient (HVPG) is the gold standard to estimate portal venous pressure in patient with cirrhosis, i.e. assessing the severity of intrahepatic portal hypertension[4]. An HVPG of ≥6 mm Hg to 9 mm Hg indicates elevated portal pressure ('preclinical portal hypertension'), while an HVPG ≥10 mm Hg defines CSPH. This protocol provides a detailed description of the equipment and the procedure and also highlights potential pitfalls and offers advice for troubleshooting.

Clinically, measurement of HVPG is indicated (i) to establish the diagnosis of intrahepatic portal hypertension, (ii) to identify patients at risk for hepatic decompensation by diagnosing CSPH (HVPG ≥10 mm Hg), (iii) to guide pharmacological therapy in primary or secondary prophylaxis of variceal bleeding, and (iv) assess the risk of hepatic failure after partial hepatectomy[2,4]. HVPG is used as an established surrogate marker for improvement and/or worsening of liver fibrosis/function, since a decrease in HVPG translates into a clinically meaningful benefit[5], whereas higher HVPG values are associated with an increased variceal bleeding risk[6]. Based on observations on changes in HVPG in patients under non-selective beta-blocker (NSBB) therapy, a decrease in HVPG of 10% is considered to be clinically relevant[7,8].

To date, there are no alternative, non-invasive parameters reflecting the degree of portal pressure with similar accuracy as HVPG. Even if HVPG is actually an 'indirect' way to measure portal pressure, it strongly correlates and thus accurately reflects 'directly' measured portal pressure in patients with cirrhosis[9]. Importantly, HVPG measurements should be performed using a balloon catheter to maximize the assessed amount of liver parenchyma[10-12]. Although HVPG measurements are invasive, resource-intensive, and require interventional skills and expertise in interpreting the reliability and plausibility of pressure readings, this method is the current gold standard for diagnosing and monitoring portal hypertension in patients with cirrhosis[13-15].

Simple laboratory values, such as platelet count, may help to estimate the likelihood for CSPH. However, platelet count, or non-invasive scores that include platelet count, have limited

predictive value[16]. Imaging modalities showing splenomegaly[17] or portosystemic collaterals[18] in patients with cirrhosis suggest the presence of CSPH, but are not helpful for quantifying the actual degree of portal hypertension. Novel non-invasive imaging tools, such as elastography of the liver[19] and/or of the spleen[20] are useful for ruling-in or ruling-out the presence of CSPH. Still, none of the available methods is able to directly measure dynamic changes in portal pressure[21].

The prognostic value of HVPG has been underlined by several landmark studies, showing that a HVPG ≥10 mm Hg (i.e. CSPH) is predictive for the formation of varices[8] (and for the development of complications related to portal hypertension[22], while a (pharmacologically-induced) decrease of HVPG modulates the respective risk of variceal growth[23] and decompensation[7]. HVPG-response is the only established surrogate for the effectiveness of NSBBs in preventing (recurrent) variceal bleeding. If HVPG decreases to a value of ≤12 mm Hg or is reduced by ≥10% during NSBB treatment, patients are protected from variceal bleeding and survival is increased[24,25]. Similarly, HVPG-response also decreases the incidence of ascites and related complications in patients with compensated cirrhosis[26]. Several studies have provided evidence supporting the use of HVPG-guided therapy[27-32]. Thus, in centers with sufficient experience, HVPG-response should guide treatment decisions, facilitating personalized medicine for patients with portal hypertension.

Moreover, measuring of HVPG might serve as a surrogate endpoint for proof-of-concept studies assessing the effectiveness of novel treatments for cirrhosis and/or portal hypertension being translated from bench to bedside, such as sorafenib[33,34], simvastatin[35,36], taurine[37], or emricasan[38]. Ultimately, measurements of HVPG can also provide important prognostic information about the risk for development of HCC[39] and for liver failure post hepatic resection [40].

The infrastructure to measure HVPG should be readily available at secondary and tertiary care centers. Since the technique of HVPG measurement requires specialized training and equipment, it seems rational for academic and transplant centers to establish a hepatic hemodynamic laboratory, facilitating state-of-the-art diagnosis and management of portal hypertension. Large volume centers perform several hundred HVPG measurements per year. Based on our experience, sufficient expertise to perform accurate HVPG measurements is usually obtained after 50–100 supervised HVPG measurements.

PROTOCOL

The protocol described here complies with the guidelines of the human research ethics committee of the Medical University of Vienna.

1. Preparations

1.1. Use a specialized room dedicated/approved for procedures using x-ray equipped with a digital x-ray fluoroscope sytem (**Figure 1A**), a monitor system (**Figure 1B**), a pressure recorder and transducer (**Figure 1C**) that are conncected to a printer or recording software, and an

ultrasound device (**Figure 1D**). Also, ensure there is sufficient space for a sterile working area (**Figure 1E**) next to the patient bench.

1.2. Screen patients with suspected advanced chronic liver disease (ACLD) for CSPH by measurement of HVPG. In particular, patients with one of the following features might undergo HVPG measurement: thrombocytopenia <150 G/L, portosystemic collaterals on cross-sectional imaging, gastroesophageal varices prior to initiation of non-selective betablocker therapy, ascites, and hepatic tumors that are scheduled for resection.

1.3. Use the following contraindications for the procedure: (i), absence of vascular access due to jugular or caval vein thrombosis, (ii) clinically evident bleeding disorders (e.g. disseminated intravascular coagulation), (iii) contralateral pneumothorax, and (iv) significant cardiac arrhythmias.

1.4. Ensure that the patient is fastened and gave written informed consent after being informed about the purpose and potential risks of the procedure.

1.5. Explain that the procedure must be performed without general anesthesia. However, low doses of midazolam (up to 0.02 mg/kg bodyweight) might be offered to anxious patients.

1.6. Monitor the patient's vital signs by non-invasive arterial blood pressure measurement, pulse oximetry, and electrocardiography using a standard monitor system.

1.7. Flush the pressure transducer set using sterile saline solution in a pressure bag.

1.8. Calibrate the pressure transducer (if not precalibrated, calibrate against an external pressure reference before starting measurements, e.g. by using a water column where 13.6 cm H_2O equals 10 mm Hg).

1.9. Place the transducer at the level of the right atrium by aiming at the patient's midaxillary line.

1.10. Prepare the pressure recorder/recording software to be ready for recording pressure tracings.

1.11. Ensure that all necessary sterile equipment is ready (see **Table of Materials**).

1.12. Ask the patient to take a supine position on the patient bench.

1.13. Have the operator wash and disinfect her/his hands and forearms.

1.14. Put on surgical cap and face mask, sterile gloves and a sterile coat.

1.15. Use a sterile table cover and prepare a sterile working area for the equipment needed for

the central venous access (**Figure 2A**).

2. Central venous access under sterile conditions

2.1. Have the patient shall turn his/her head slightly to the left side, then disinfect the area of the right anterior and lateral neck with a disinfectant solution.

2.2. Place sterile towels that cover the skin around the carotid triangle.

2.3. Use a ultrasound probe (covered by a sterile US probe cover sheath) to identify the right internal jugular vein and the appropriate puncture site.

2.4. Infiltrate the area of subsequent vascular access with a 21 G needle and apply a local anaesthetic (lidocaine 2%, 5 mL). Then, wait 1–2 min for its full effect.

2.5. Prepare all materials needed for central venous access (see **Table of Materials**).

2.6. Using the equipment of the 7.5 F catheter introducer sheath set, place the needle in the right internal jugular vein using ultrasound guidance and advance the guidewire through the needle using the Seldinger technique (**Figure 3A**).

NOTE: If a transjugular biopsy should be performed after HVPG measurement, a larger diameter 1 F liver biopsy introducer sheath set (with extra 18 G needle and guide wire) has to be used.

2.7. Make a 2–3 mm incision along the guidewire using a blade 11 scalpel to ease introduction of the vascular access sheath loaded with the dilatator.

2.8. Insert the percutaneous sheath introducer loaded with the dilatator into the internal jugular vein over the guidewire.

2.9. Remove the guidewire and the dilatator of the vascular access sheath.

2.10. Make sure that the vascular access sheath remains at a stable intravascular position and orient the side injection port of the vascular access sheath towards the lateral side (**Figure 3B**).

NOTE: Suturing or taping is not needed.

3. Placement of the balloon catheter in a hepatic vein

3.1. Prepare all materials needed for placement of the balloon catheter in an hepatic vein (**Figure 2B**).

3.2. Flush the balloon catheter with contrast media (dye) and check the integrity of the balloon by repetitive insufflation/deflation with the catheter tip immersed in saline solution. No air

bubbles should occur.

NOTE: Depending on the visibility of the catheter itself on x-ray, you may also use just sterile saline or diluted dye to flush the vascular lumen of the catheter.

3.3. Moisten the balloon catheter on the outside with sterile saline solution before inserting it into the vascular access sheath.

3.4. Advance the balloon catheter under fluoroscopic guidance into the inferior cava vein (IVC). Aligning the tip of the balloon catheter towards the back of the patient and slight rotations of the catheter usually allows to advance from the right atrium to the IVC. Instructing the patient to inhale deeply might help in some cases (**Figure 3B**).

3.5. Advance the balloon catheter from the IVC into the hepatic vein. Try to get access to the hepatic veins by repeatedly moving with the tip of the balloon catheter facing to the right towards the suspected area of the junction of the hepatic veins and the IVC (**Figure 3C**).

3.6. Ensure that the catheter is advanced into a stable position that allows the repeated measurement of the free hepatic venous pressure (FHVP) at a 2-4 cm distance from its opening into the IVC and adequate space for the inflated balloon in the lumen of the hepatic vein in order to record the wedged hepatic venous pressure (WHVP).

3.7. Check for an adequate occlusion of the vein (wedge position) by inflating the balloon (about 2 mL of air into the balloon lumen) and dye injection (about 5 mL into the vascular lumen) until the hepatic vein distal to the inflated balloon is visualized (**Figure 3D**).

3.8. Observe the stasis of the contrast media and exclude washout of the contrast media due to insufficient occlusion of the venous lumen by the balloon or due to the presence of shunts. If significant contrast media washout is observed, try to reposition the balloon catheter.

3.9. Deflate the balloon and flush the lumen of the catheter with saline.

4. Hemodynamic readings for assessing the HVPG

4.1. Connect the vascular lumen of the balloon catheter to the pressure transducer using an infusion line.

4.2. Start recording the FHVP with the tip of the balloon 2-4 cm from the opening of the hepatic vein to the IVC. The waveform of the curve must be stable without variations over time.

NOTE: Stable values are usually obtained after 15 s.

4.3. Inflate the balloon and continue recording the WHVP until the measurement becomes a stable horizontal line with no variations over time.

NOTE: Stable tracings of the wedged pressure are usually obtained after >40 s.

4.4. Repeat the measurement of FHVG (>15 s) and WHVP (>40 s) at least three times to obtain triplicate high-quality readings (**Figure 3E**).

NOTE: If significant discrepancies of ≥2 mm Hg are observed between the single FHVP-WHVP readings, additional measurements should be obtained. Note the reasons for potential artefacts, such as coughing, moving, or talking at the respective timepoints of hemodynamic recording.

4.5. Record pressure in the IVC at the level of the ostium of the hepatic vein as well as the right atrial pressure (RAP).

4.6. Stop recordings.

4.7. Remove the balloon catheter.

4.8. Calculate HVPG (FHVP subtracted from WHVP) as the mean of 3 measurements.

NOTE: If significant differences ≥2 mm Hg are noted between the sequentially obtained HVPG readings, additional measurements should be obtained.

4.9. Proceed to transjugular biopsy (step 5.1) or remove the catheter introducer sheath from the internal jugular vein.

NOTE: Inserting a stiff guidewire may help to reach the same intrahepatic position for subsequent transjugular liver biopsy.

4.10. Apply pressure on top of the vascular insertion site of the internal jugular vein for at least 5 min using a sterile gauze.

5. Preparation for transjugular liver biopsy

NOTE: Two different biopsy methodologies may be used to obtain a transjugular liver biopsy: aspiration (step 6) or core biopsy (step 7). First decide which system to use and then select the appropriate biopsy needle introducer sheath before proceeding with steps 5.1 to 5.3).

5.1. Prepare the respective transjugular liver biopsy (TJLBX) set (aspiration TJLBX: **Figure 4A**-B,; core TJLBX: **Figure 4C**; see **Table of Materials**).

5.2. Flush the biopsy needle introducer sheath with sterile saline or, in case of transjugular aspiration liver biopsy, with dye for better visualization.

5.3. Advance the biopsy needle introducer sheath into a hepatic vein using the same technique

as described in 3.4.

5.4. Depending on the biopsy set used, proceed either to step 6.1 for transjugular aspiration liver biopsy or to step 7.1 for transjugular core liver biopsy.

6. Transjugular aspiration liver biopsy

6.1. Use a 10 mL Luer-lock syringe to flush the aspiration TJLBX needle with sterile saline.

6.2. Advance the biopsy needle gently through the biopsy needle introducer sheath until the tip of the needle reaches the end of the introducer sheath.

NOTE: Avoid any force or fast movements while advancing the needle. Asking the patient to take a deep breath will decrease the angle between the IVC and the hepatic veins for easier advancement of the biopsy needle.

6.3. Ask the patient to hold his breath.

6.4. Apply suction using the 10 mL Luer-lock syringe and advance the needle into the liver parenchyma.

6.5. Retract the needle while still applying suction (negative pressure).

6.6. Advice the patient to continue breathing normally.

6.7. Remove the needle (but not the needle introducer sheath) and harvest the liver sample.

NOTE: The liver sample is usually retained in the syringe, not in the needle (**Figure 4D**).

6.8. Repeat steps 6.1 to 6.6 for additional needle passages until sufficient liver specimens are obtained.

6.9. Inject 5–10 mL of contrast media over the catheter introducer sheath to rule out perforation of the liver capsule.

6.10. Remove the biopsy needle introducer sheath.

6.11. Remove the 10 F liver biopsy set introducer sheath and apply pressure on top of the vascular insertion site of the internal jugular vein for at least 5 min using sterile gauzes.

7. TRANSJUGULAR CORE LIVER BIOPSY

7.1. Load the core TJLBX needle by pulling the grip until the shooting mechanism is locked. Advance the core biopsy needle gently through the biopsy needle introducer sheath until the tip

of the needle approaches the end of the introducer sheath.

NOTE: Avoid any force or fast movements while advancing the needle. Asking the patient to take a deep breath will decrease the angle between the IVC and the hepatic veins for easier advancement of the biopsy needle.

7.2. Ask the patient to hold his/her breath.

7.3. Advance the needle into the liver parenchyma.

7.4. Perform the core biopsy by pulling the trigger of the shooting mechanism.

7.5. Advice the patient to continue breathing normally.

7.6. Remove the needle (but not the needle introducer sheath) and harvest the liver sample. A small needle might help to remove the liver sample (**Figure 4D**).

7.7. Repeat steps 7.1 to 7.6 for additional needle passages until sufficient liver specimens are obtained.

7.8. Inject 5–10 mL of contrast media over the catheter introducer sheath to rule out perforation of the liver capsule.

7.9. Remove the biopsy needle introducer sheath.

7.10. Remove the 10 F liver biopsy set introducer sheath and apply pressure on top of the vascular insertion site of the internal jugular vein for about 5 min using sterile gauzes.

REPRESENTATIVE RESULTS:

In compensated patients with well-preserved liver function (i.e. without any history of hepatic decompensation, such as ascites or variceal bleeding) the measured HVPG values might be normal or in the range of subclinical portal hypertension (HVPG 6–9 mm Hg). However, compensated patients might develop CSPH (HVPG ≥10 mm Hg) which indicates an increased risk for developing varices or hepatic decompensation. In turn, patients with esophageal or gastric varices, HVPG is usually in the range of CSPH, if not patients should be assessed for the presence of portal vein thrombosis or other reasons for prehepatic portal hypertension. In patients with a history of bleeding from esophageal varices, HVPG is usually at least ≥12 mm Hg. Similarly, in patients with ascites due to cirrhosis, i.e., portal-hypertensive ascites, the HVPG values are usually expected to be at least ≥10 mm Hg (**Figure 3E**). HVPG values of ≥20 mm Hg indicate high risk of variceal and/or recurrent variceal bleeding and patients might be candidates for transjugular intrahepatic portosystemic shunt (TIPS) placement. While HVPG values might rise to values >30 mm Hg in patients with advanced cirrhosis and severe portal hypertension, HVPG values of >40 mm Hg are very unusual and the accuracy of the measurement and the pressure tracings should be critically assessed.

Very high values of FHVP and IVC may hint to incorrect calibration of the pressure transducer but might also indicate right heart failure or tricuspide insufficiency. A difference of more than ≥4mm Hg between the FHVP and the IVC pressure indicates an outflow obstruction/stenosis of the hepatic vein or Budd-Chiari Syndrome. However, in case of severe Budd-Chiari Syndrome with complete thrombotic obstruction of the hepatic veins, the insertion of the balloon catheter is usually not possible. Thus, in case Budd-Chiari Syndrome or other causes of hepatic outflow obstruction are suspected, a Doppler ultrasound examination of the hepatic veins is recommended.

If shunts are observed during dye injection while the balloon is inflated, the HVPG is typically underestimated. However, while in this case the absolute value of HVPG can not be used to estimate prognostis or guide pharmacological therapy, the diagnosis of CSPH can still be made if the HVPG is recorded at ≥10 mm Hg.

In primary prophylaxis of variceal bleeding, hemodynamic response to non-selective betablocker therapy (e.g. with carvedilol 12.5 mg once daily) is defined as an HVPG decrease of at least ≥10% compared to baseline, or as a decrease to absolute values <12 mm Hg: e.g., patient A (who had an episode of variceal bleeding) has a baseline HVPG of 20mm Hg. Following administration of carvedilol 12.5 mg/day for 4 weeks, a second HVPG (on carvedilol) of 16 mm Hg is recorded. Thus, HVPG decreased by 4 mm Hg (i.e. 20% of baseline HVPG), indicating a hemodynamic response.

In secondary prophylaxis of variceal bleeding, hemodynamic response requires an HVPG decrease of ≥20% (or to absolute values <12 mm Hg): e.g., patient B has a baseline HVPG of 26 mm Hg and under propranolol 40 mg b.i.d. (80 mg cumulative daily dose) HVPG drops to 24 mm Hg, which is a decrease of just 8%, indicating hemodynamic non-response. In absence of a hemodynamic response, patient B has a higher risk of rebleeding if just treated with propranolol 40 mg b.i.d.

It has been shown that HVPG-guided pharmagological therapy plus endoscopic band ligation is more effective than ‘uncontrolled‘ combined therapy of NSBB plus endoscopic band ligation in secondary prophylaxis and even improves survival[32].

Transjugular liver biopsy can be safely performed in the same session using the same vascular access sheath after recording HVPG. Liver biopsy specimens obtained with transjugular aspiration biopsy needles might be fragmented, especially in patients with advanced cirrhosis, and thus, less representative than biopsies obtained with core biopsy needles. However, in patients in whom less pronounced liver fibrosis is expeted, aspiration needle biopsy yields good sample quality with usually larger diameter specimens being obtained.

FIGURE AND TABLE LEGENDS:

Figure 1: Equipment needed for a hepatic hemodynamic laboratory. (A) Room dedicated/approved for procedures using x-ray with a digital x-ray fluoroscope system. **(B)** Monitor system for vital signs, i.e., ECG, non-invasive blood pressure, and oxygen saturation. **(C)**

Pressure transducer with pressurized sterile saline flush. **(D)** Ultrasound device for guiding vascular access. **(E)** Sterile working area.

Figure 2: Sterile equipment needed for HVPG measurement. This figure shows the sterile working area prepared with the materials needed for **(A)** central venous access and **(B)** placement of the balloon catheter in a hepatic vein.

Figure 3: Placement of the catheter introducer sheath and correct positioning of the balloon catheter. (A) Catheter introducer sheath correctly placed in the right internal jugular vein. **(B)** Balloon cather advanced into the IVC. **(C)** Balloon catheter advanced into the right hepatic vein. **(D)** Balloon catheter with inflated balloon and hepatic vein visualized by contrast media injection. **(E)** Representative pressure tracing of the free and wedged hepatic pressure readings.

Figure 4. Equipment needed for transjugular liver biopsy. (A) 10 F transjugular liver biopsy set introducer sheath. **(B)** Transjugular aspiration biopsy system including the biopsy needle introducer sheath and the transjugular aspiration biopsy needle connected to a 10 mL Luer-Lock syringe. **(C)** Transjugular core biopsy system including the needle introducer sheath with a side port and the core biopsy needle. **(D)** Liver specimen obtained by transjugular biopsy.

DISCUSSION:

While HVPG measurements require considerable resources and trained personal with interventional skills and expertise in the reading of pressure tracings, it improves prognostication and might guide treatment decisions, and thus, facilitates personalized medicine. In addition, the opportunity to safely obtain liver biopsy specimens via the transjugular route in the same session is another argument in favour of implementing hepatic hemodynamic laboratories at tertiary care centers. Indeed, guidelines support the use of HVPG measurements in centers with adequate expertise and resources[2,4]. The safety of the procedures is largely related to the vascular access to the internal jugular vein. Once correctly placed, the risk of the remaining procedure is negligible and patient's comfort is mostly limited by the duration of the procedure, if placement of the balloon catheter in the hepatic vein takes longer as expected. While a recently published study on patient-reported outcomes demonstrated that the HVPG procedure is well-tolerated[41], low dose midazolam sedation (up to 0.02 mg/kg body weight) may be used to relief anxiety and to promote patient comfort[42]. However, general anesthesia and/or deep-sedation with propofol and remifentanil cannot be used for HVPG measurements, since this would impact on hemodynamic readouts[43].

In case the right internal jugular vein cannot be used for venous access (e.g. in case of thrombosis), the left internal jugular vein or even the antecubital/brachial veins may be used instead. Importantly, HVPG can also be measured via the femoral veins given specialized catheters are used.

However, the most critical part of HVPG measurements is the correct recording of pressure tracings while the balloon is insuflated and deflated, ensuring that propper placement and sufficient time are assured to obtain the correct WHVP and FHVP. The waveforms obtained in

485 the “free” position of the catheter might be slightly impacted by the heart beat or a physiologic
486 tricuscipde valve regurgitation during systole, however, should still follow a straight line. In
487 contrast the “wedged” pressure curve obtained when the balloon is inflated should follow a fast
488 increase during the first 3–5 s followed by a more slowly increase over 10–30 s. Finally, a straight
489 and stable line should be obtained which reflects the correct sinusoidal pressure. Any
490 inconsistancies occurring during triplicate measurements should prompt the operator to obtain
491 additional pressure readings in order to identify the correct FHVP and WHVP.
492
493 The complication rate of HVPG measurement is low and the risks are almost exclusively related
494 to the venous access usually performed at the right internal jugular vein[11,13]. Common side effects
495 of the HVPG procedure that should be discussed with the patients prior to the procedure include
496 pain at the incision site or slight thoracic or abdominal discomfort when advancing the catheter
497 through the vena cava system into the hepatic veins and while inflating the balloon. Specific but
498 rare complications related to the venous access include hemotomas at the access site,
499 pneumothorax requiring a chest tube and cardiac arrhythmias.
500
501 The four main indications for HVPG measurements are (i) to establish the diagnosis of
502 intrahepatic portal hypertension, (ii) to identify patients at risk for hepatic decompensation by
503 diagnosing CSPH (HVPG ≥10 mm Hg), (iii) to guide pharmacological therapy in primary or
504 secondary prophylaxis of variceal bleeding, and (iv) assess the risk of hepatic failure after hemi-
505 partial hepatectomy. While CSPH diagnosis and risk stratification might also be performed by
506 imaging/Laboratory studies or by endoscopy[2,44], currently, there are no adequate alternative
507 means to monitor the response to NSBB therapy. While some patients might be readily excluded
508 from major hepatic resection by considering signs of hepatic impairment (i.e. ascites or
509 jaundice)[45], measurement of HVPG represents an important predictor of postsurgical morbidity
510 and mortality in patients with otherwise well-preserved liver function (i.e. compensated
511 patients)[46].
512
513 HVPG correlates well with directly measured portal pressure, as it has been shown for patients
514 with alcoholic etiology and viral etiology of liver disease[9]. However, certain liver diseases (e.g.
515 nodular regenerative hyperplasia) might also affect presinusoidal resistance which impacts on
516 the severity of portal hypertension but is not adequately reflected by HVPG[47]. Furthermore,
517 HVPG is also not able to detect the presence of (additional) prehepatic portal hypertension, as
518 caused by portal vein thrombosis or mechanical compression of the portal vein. Thus, abdominal
519 imaging with a special focus on the splenoportal vascular axis, on the mesenteric veins, as well
520 as on spleen size and the presence of ascites should be performed in unclear cases and whenever
521 a prehepatic component of portal hypertension is suspected[48].
522
523 Finally, the use of transjugular liver biopsy has been demonstrated to be safe in patients with
524 contraindications to percutaneous liver biopsy, e.g., due to inherited or acquired bleeding
525 disorders or in case of severe ascites[49,50]. In addition, the development of core biopsy needles
526 has increased the diagnostic yield of transjugular biopies[51,52]. In case of acute liver failure of
527 unknown etiology, or in case of suspected cirrhosis, transjugular biopsy should be favoured over
528 percutaneous liver biopsy for safety reasons in most cases. In decompensated patients,

percutaneous liver biopsy is associated with a high risks of severe bleeding or procedure-related complications, while in compensated patients, the respective risks are lower. Still, we prefer to perform transjugular liver biopsies in all patients with cirrhosis (including compensated patients) as important prognostic information through HVPG measurements can be simultaneously obtained.

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Figure 1

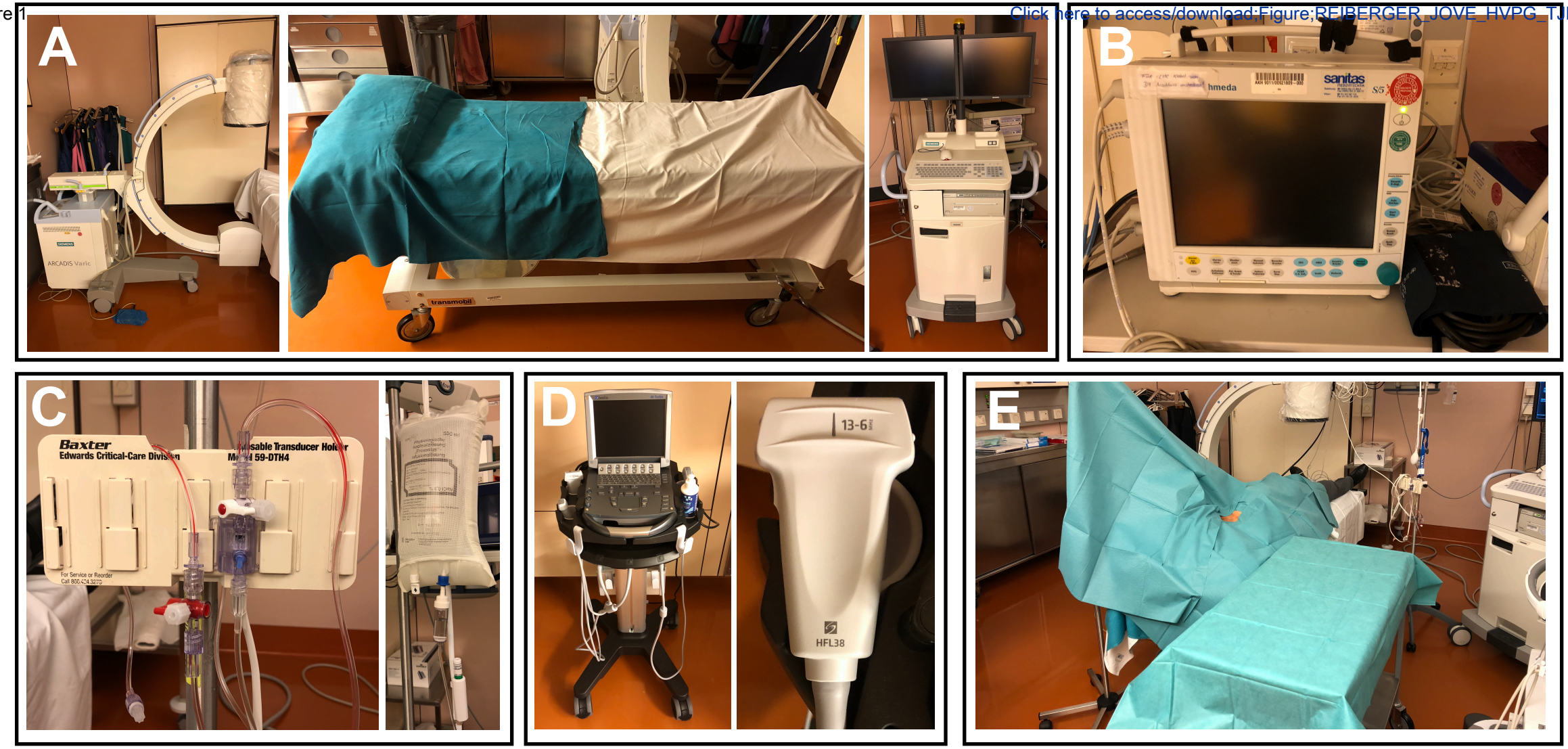

Figure 2

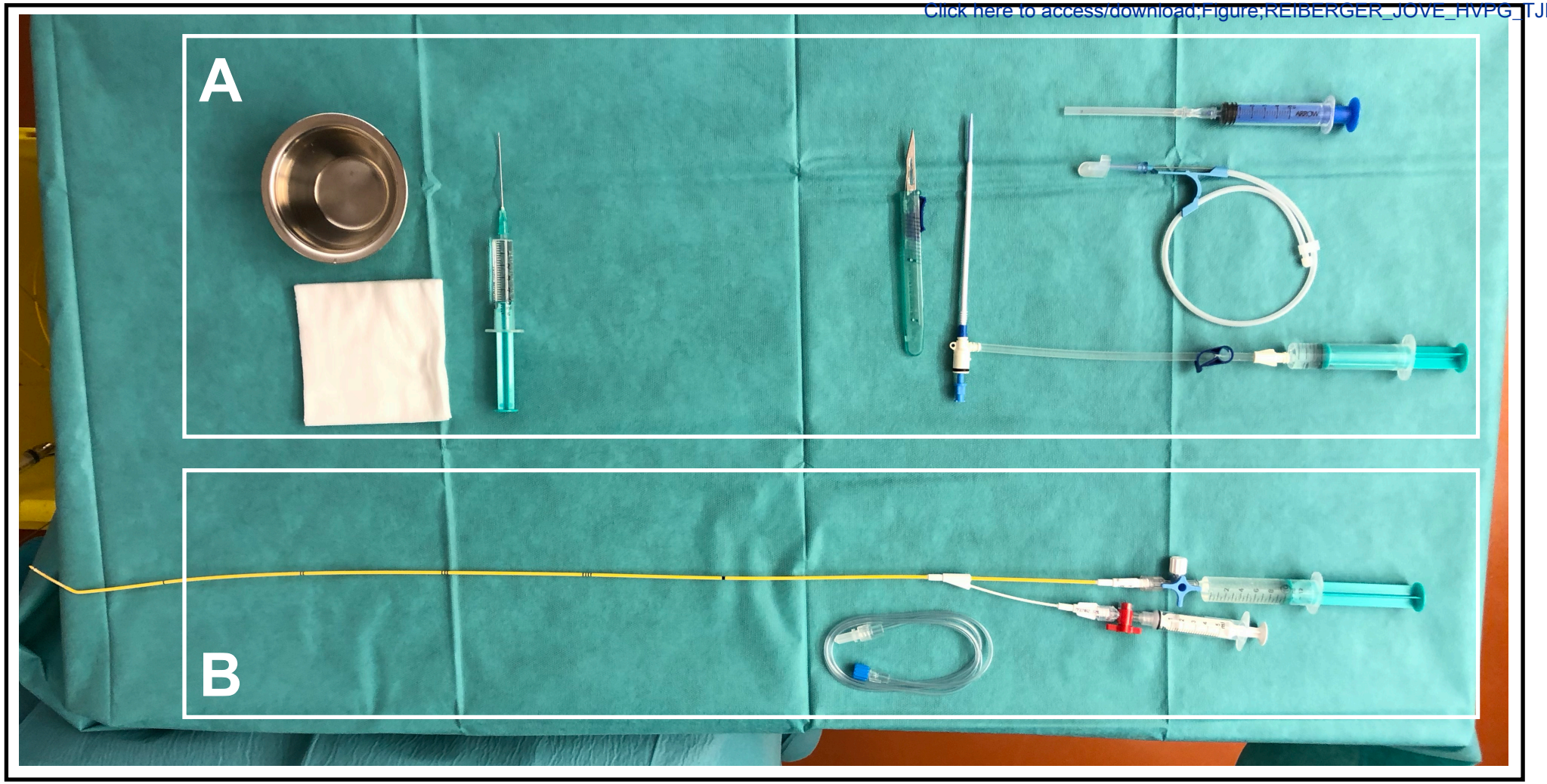

Figure 3

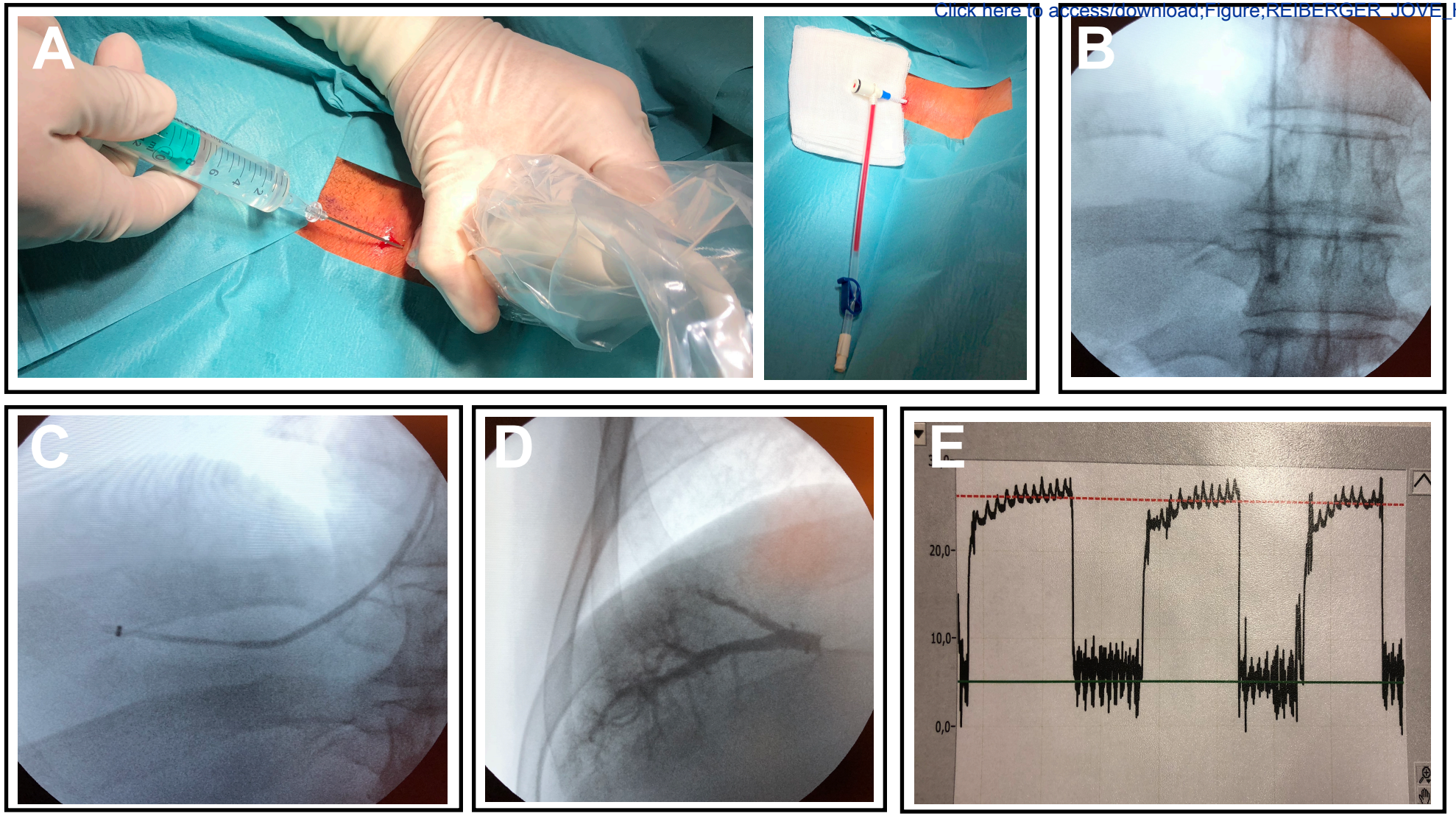

Figure 4

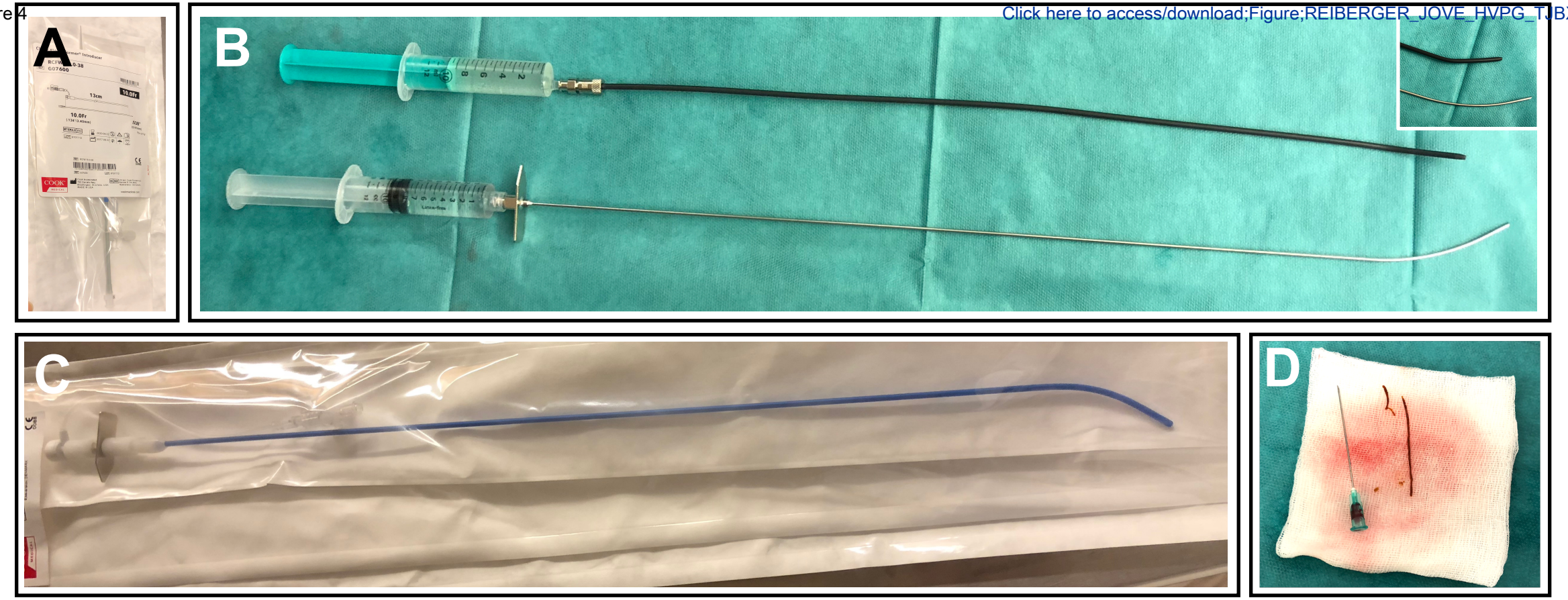

Table of Materials

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
10 mL Luer-Lock syringe	Braun	REF 4617100V, LOT 17G03C8	Luer-Lock Syringe for connection with the aspiration biopsy set
10 mL Syringe 2x	Braun	REF 4606108V	Snonpyrogenic, nontoxic 10 mL syringe
10 F liver biopsy introducer sheath set	Cook Medical	REF RCFW-10.0-38, REF G07600	Percutaneous Sheath Introducer Set (TJBX), 10F Port, 13cm, Check-Flo Performer Introducer
18 G needle for biopsy introducer sheat	Arrow International	REF AN-04318	Introducer Needle for TJBX Set, 18 G, 6.35 cm
21 G needle	Henke Sass Wolf	REF 0086, Fine-Ject 21Gx2"	Sterile injeciton needle, 21 Gx2", 0.8 x 50 mm, for local anesthesia
3-way channels	Becton Dickinson	BD Connecta Luer-Lok, REF 39402	Three way channel with Luer-Lok connection system
7.5 F catheter introducer sheath set	Arrow International	REF SI_09875-E	Percutaneous Sheath Introducer Set, 7.5 F Port
Aspiration TJLBX set	Cook Medical	REF RMT-16-51.0-TJL, REF G20521	TJ Liver Access and Biopsy Needle Set (Aspiration Set), 9 F-45 cm, 16 G-50.5 cm
Balloon catheter	Gerhard Pejcl Medizintechnik Austria	REF 500765B	Ferlitsch HVPG Catheter, 7F-65cm, Balloon:2.5mL, Pressure 50-90 kPa, GW: 0.032"
Blade 11 scalpel	Medi-Safe Surgicals	MS Safety Scalpel REF /Batch 18012424	11 blade safety scalpels, retractable, single use, 10 scalpels per package
Blunt tip fill needle	Becton Dickinson	REF 303129	Sterile blunt tip fill needle, single use
Contrast media (dye)	Dr. Franz Köhler Chemie GmbH, Bensheim, GER	ZNR 1-24112	Peritrast 300 mg Iod/mL, 50 mL, contrastmedia
Core TJLBX set	Cook Medical	REF RMT-14XT-50.5-LABS-100, REF G08285	TJ Needle Introducer and Bx-Needle 7 F-53.5 cm, 14 G-53.5 cm/20 mm, 18 G-60 cm
Digital x-ray fluoroscope system	Siemens	Model No 07721710	ARCADIS Varic, mobile x-ray fluoroscope system
Disinfectant solution	Gebro Pharma	1-20413	Isozid-H
Face mask	MSP Medizintechnik GmbH	REF HSO36984	Surgical face mask from double fleece, with tie, 50 pieces
Guide wire for biopsy introducer sheat	Arrow International	REF AW-14732	Marked Spring-Wire Guide, TJBX Set, 0.032", 0.81 mm, 68 cm
Infusion line	Rosstec Medical Products b Cardea GmbH & Co	REF 220010, 100m	Infusion line, Luer-Lok for connection of balloon catheter and pressure transducer
Lidocaine 2%	Gebro Pharma	Xylanaest, ZNR 17.792, 20mg/1mL (2%)	Sterile vials Xylanest including 2% Lidocain hydrochloride for local anesthesia
Midazolame	Roche	Dormicum, ZNr 1-18809, Midazolam 5mg/5mL	Sterile vials Dormicum including Midazolam for sedation
Monitor system	Datex-Ohmeda by GE	Type F-CMREC1	Patient monitoring system
Patient bench	Silerlen-MAQET	Model No 7474.00A	Mobile patient bench for x-ray fluoroscopy
Pressure bag	Ethox Corp	REF 4005	Pressure Infuser Bag 500 mL
Pressure recorder	Edwards Lifesciences	Ref T001631A, Lot 61202039	TruWave 3 cc/150 cm
Pressure transducer	Edwards Lifesciences TM	REF T001631A	Pressure Monitoring Set (1x), 3 cc/150 cm, TruWave TM
Recording software	Datex Ohmeda by GE	Software S/5 is property of Instrumentarium Corp of Datex-Ohmeda TM	Datex-Ohmeda S/5TM Collect - Software to record pressure tracings of the patient monitor system
Sterile coat	Lohmann & Rauscher International GmbH & Co	REF 19351	Surgical Gown, Different sizes, e.g. L-130 cm
Sterile gauze	Hartmann	REF 401798, 10x10cm gauze	10 x 10 cm sterile gauze, 10 pieces per package
Sterile gloves	Meditrade	REF 9021	Gentle Skin sterile gloves, different sizes, e.g. 7.5
Sterile saline solution	Fresenius Kabi	NaCl 0.9%, B009827 REV 03	Physiologic Saline Solution 0.9% NaCl, 309 mosmol/L, pH-Wert: 4.5-7.0
Sterile saline vessel	KLS Martin	REF K8A, 18/10 Jonas	Sterilizable Metal Vessel for sterile saline
Sterile table cover	Hartmann	REF 2502208	Table Cover, Foliodrape 150 x 100 cm
Sterile towel	BARRIER by Mölnlycke Health Care	REF 706900	Adhesive OP-Towel, 100 x 100 cm
Sterile US probe cover sheath	Websinger	REF 07014	Sterile ultrasound probe cover, 20 x 60 cm, inluding two sterie adhesive tapes
Stiff guidewire	Cook Medical	REF TSMG-35-180-4-LES, G46729	Lunderquist Extra Stiff Wire Guide, 0.035"-.89 mm, 180 cm, 4 cm flexible tip
Surgical cap	BARRIER by Mölnlycke Health Care	REF 620500, PCS 100, Colour Green	Surgical Cap
Ultrasound device	FUJIFILM SonoSite	Model M-Turbo, REF P17000-17	Mobile ultrasound system

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Author(s): Thomas REIBERGER, Markus PECK-RADOSAVLJEVIC, Michael TRAUNER, Mattias MANDORFER

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Signature: [signature] Date: 03-APR-2018

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Vienna, February 1st 2019

Dear Editorial Board of *JoVE*,

Thank you for providing us with feedback on our submitted manuscript on "MEASUREMENT OF THE HEPATIC VENOUS PRESSURE GRADIENT AND TRANSJUGULAR LIVER BIOPSY"

We provide you with a point-by-point response to the comments of the Editors and Reviewers. The revised manuscript has been submitted with all changes tracked (green background, in the "changes marked" version) and in addition we have also provided a clean version. We are looking forward to receiving your comments on our revised protocol.

Sincerely,

Thomas Reiberger
on behalf of all authors

1. Formatted according to JoVE guidelines (all text aligned to the left margin, spaces between all steps and substeps; see attached document), the highlighted protocol is ~3.5 pages long, which is over our limit for filming. Please reduce the highlighted portion to 2.75 pages.

The manuscript has been revised accordingly.

2. Protocol: Please rewrite and incorporate the paragraphs starting 'A specialized room…' and 'Patients with…' into the numbered protocol steps.

There passages are now included as steps of the protocol.

3. 5.1: Can you clarify which biopsy set/sheath is used with which procedure, either here or in the Table of Materials?

The respective biopsy sets used for aspiration or core liver biopsy are now clearly stated.

4. Please reference all Figures in the manuscript text; currently, only Figure 3E is.

All figures are now cited in the text.

5. Figure 1: The panel labels in the Figure and Figure Legend don't match (A-F in the legend and A-E in the Figure). Please correct this.

Thank you. The figure legend been revised accordingly.

6. Figure 2: What is labeled by A-E in the Figure? What are the white boxes indicating? Please explain in the legend.

The figure labels and legend have been simplified.