

# EFSUMB Guidelines on Interventional Ultrasound (INVUS), Part II

## Diagnostic Ultrasound-Guided Interventional Procedures (Long Version)

### EFSUMB Leitlinien interventioneller Ultraschall (INVUS), Teil II

#### Diagnostische Ultraschall-gestützte Interventionen (Langversion)

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#### Key words

- guideline
- biopsy
- ultrasound
- aspiration
- CT
- MRI
- needle
- catheter
- cancer
- transplant
- adrenal
- liver
- kidney
- pancreas
- lymph node
- gastrointestinal tract
- retroperitoneal
- spleen

#### Bibliography

**DOI** <http://dx.doi.org/10.1055/s-0035-1554036>  
 Published online: 2015  
 Ultraschall in Med 2015; 36: E15–E35 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0172-4614

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#### Abstract

This is the second part of the series on interventional ultrasound guidelines of the Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB). It deals with the diagnostic interventional procedure. General points are discussed which are pertinent to all patients, followed by organ-specific imaging that will allow the correct pathway and planning for the interventional procedure. This will allow for the appropriate imaging workup for each individual interventional procedure (Long version).

#### Introduction

This is the second of three guidelines (parts I – III) within the framework of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Guidelines on Interventional Ultrasound (INVUS) describing percutaneous ultrasound (US)-guided diagnostic and therapeutic abdominal interventions. Part II gives evidence-based recommendations for the safe and efficient performance of US-guided diagnostic interventions based on the available evidence at the time of manuscript preparation. It is preceded by guidelines on general principles and necessities of INVUS (part I) [1] and followed by US-guided therapeutic abdominal interventions (part III) [2]. Methods of guideline development are described in the introduction to the EFSUMB Guidelines on Interventional Ultrasound (INVUS) [3]. Levels of Evidence (LoE) and Grades of Recommendations (GoR) have been assigned according to the Oxford Centre for Evidence-based Medicine criteria (March 2009 edition) [<http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009>].

#### Zusammenfassung

Der zweite Teil der Serie von Leitlinien der European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) zur interventionellen Sonografie beschreibt die Vorbereitung, Indikationen, Durchführung und Nachsorge ultraschallgestützter diagnostischer Interventionen am Abdomen. Nach Darstellung allgemeiner, für alle Patienten gültiger Voraussetzungen werden organbezogenen Bildgebung, Planung und Ablauf der verschiedenen diagnostischen Interventionen dargestellt (Langversion).

#### General Principles of Diagnosis for Ultrasound-Guided Interventional Procedures

Diagnostic interventional ultrasound (INVUS) procedures are efficient, minimally invasive techniques with the purpose of acquiring a diagnosis. Ultrasound (US) is the ideal imaging modality to guide interventional procedures with several advantages: the absence of radiation and lack of potentially nephrogenic contrast agents, US is inexpensive and real-time imaging ensures the visualization of needles, thus improving diagnostic accuracy with a reduction of complications [1 – 6]. Details are given in part I [1].

#### Essential Rules

- ▶ There must be a clearly defined indication for the diagnostic procedure and the risk should not outweigh the potential benefits.
- ▶ Accurate planning for INVUS procedures is essential to avoid complications. The appropriate imaging guidance modality has to be chosen after the target has been evaluated. The operator should select the image guidance and interventional access pathway with the lowest risk.

- ▶ INVUS procedures require informed consent. In emergency INVUS procedures, particularly in patients who are uncommunicative with a significant morbidity or mortality susceptibility, informed consent can be waived.
- ▶ Normal coagulation indices and platelet count are necessary to reduce bleeding risk [7]. There is no consensus regarding the threshold values that preclude interventional procedures, but platelet count < 50 000/ $\mu$ L and Quick time < 50 % are commonly used indices [8]. In patients with < 50 000 platelets, prior to a high-risk procedure (e.g. liver or kidney biopsy, nephrostomy, complex radiofrequency ablation (RFA)), a transfusion of platelets is necessary [9]. For patients undergoing a moderate risk procedure (e.g. chemoembolization, venous interventions, chest, lung and intra-abdominal biopsy, drainage, direct RFA, spine procedures) or low bleeding risk procedures (e.g. thoracentesis, paracentesis, superficial abscess drainage, venography), a platelet transfusion is recommended [7]. The International Normalized Ratio (INR) value should be corrected to < 2.0 prior to low-risk procedures and < 1.5 prior to moderate to high-risk procedures. In patients with a Quick time < 50 %, vitamin K or administration of fresh plasma is recommended before the procedure. In most abdominal INVUS procedures, it is recommended to discontinue antiplatelet therapy in the peri-procedural period.
- ▶ INVUS procedures that have an increased risk of septic complications (e.g. prostate biopsy) should include prophylactic antimicrobials to reduce post-INVUS procedure infection.
- ▶ The use of sedation has to be considered in non-cooperative patients or when performing an INVUS procedure where an immobilized patient is crucial. Local anesthetic administration is recommended for biopsies. Fine needle aspiration (FNA) may be performed without local anesthesia but is recommended when multiple passes are necessary.
- ▶ All personnel performing any interventional procedure must observe aseptic conditions, and the puncture site must also be sterile. Sterile gowns, disposable US covers, sterile US gel, meticulous hand cleaning and patient skin preparation with antiseptic are mandatory to avoid infection.
- ▶ Whenever possible, the use of continuous US guidance is recommended to reduce the risk of complications. The use of contrast-enhanced US (CEUS) or fusion techniques may be helpful in large tumors with necrosis, or in tumors that are invisible or poorly visible on grayscale US to improve the accuracy in obtaining adequate tissue samples [11, 12].
- ▶ Diagnostic interventional procedures can often safely transgress the stomach and small or large bowel with fine needles (22 gauge) [13].
- ▶ Correct identification and suitable transportation of the tissue samples in an appropriate medium are essential.
- ▶ The most common complication of the INVUS procedure is puncture site pain requiring simple analgesia. Other complications include a vasovagal reaction, sepsis, inadvertent puncture of surrounding viscera and intra-parenchyma vascular complications, such as arteriovenous fistulas or pseudoaneurysm formation. A major complication is hemorrhage [14] and normal coagulation indices do not preclude bleeding complications.
- ▶ Following a diagnostic INVUS procedure, the patient should remain under medical observation to detect early complications. INVUS procedures can be performed safely on a day-case or out-patient basis, as the majority of complications occur in the first few hours [15]. Some centers prefer to perform INVUS procedures only as an inpatient procedure [16].

### Multidisciplinary decision

The multidisciplinary setting should be the standard to discuss INVUS procedures to confirm the necessity of the procedure, possible alternatives and complications.

### What defines the probability of performing an INVUS procedure?

- ▶ Availability of a safe needle path governs the performance of an INVUS procedure.
- ▶ The target structure should be visible during the procedure.
- ▶ Risk of bleeding should be taken into account.
- ▶ Patient cooperation is needed.

If the patient cannot remain immobile during the procedure or control breathing, the risk of patient complications increases as does the potential of operator harm, e.g. needle stick injury [17].

### Fine needle biopsy or aspiration

Different sample types may be obtained either with a fine needle biopsy (FNB) or FNA depending on indication and local protocol; cytology is often adequate but insufficient when tissue architecture is essential, e.g. lymphoma. A combination of techniques is frequently performed to improve diagnostic accuracy.

### Specimen preparation

The preparation and care of specimens depend on the local laboratory services, proximity to the procedure room, and availability of specialist technicians.

### Cytology specimen preparation

Perform 1–2 passes. For each needle pass performed, prepare  $\geq 2$  good quality slides, with fixation according to the standard of the local cytology laboratory. Rinse the remainder of the material from the needle and syringe into a preservation solution. Label the slide holders and fixative containers with patient identification including the specimen source. Submit to the laboratory with a completed cytology requisition. For optimal results, two air-dried slides, two ethanol-fixed slides and one container are dispatched to the laboratory [11–20].

### Histology specimen preparation

Specimens should be submitted in an adequate amount of 10% neutral-buffered formalin fixative. The volume ratio of fixative to specimen size is very important for proper preservation of the tissue, i.e., a minimum of at least twice the volume of fixative as tissue is required.

Small biopsies should be placed in at least 20 mL of formalin. There are numerous causes for an inconclusive result: insufficient material, necrotic lesion and not sampling the area of malignancy. This will require a repeat biopsy. This should be explained to the patient during the consent process and critical assessment of any failure should be undertaken to improve the success of a second procedure. Consider the presence of cytopathology during the repeat procedure [17].

### Microbiology specimens

Proper specimen collection, identification, transport, and storage are necessary. A strict aseptic collection technique is necessary to avoid contamination. It is essential to obtain sufficient material for cultures [21] and perform the appropriate culture depending on the clinical suspicion.

## Follow-up imaging

Immediate post-procedural imaging is not routinely recommended. Patients should be observed following a standard protocol in a dedicated unit with appropriately trained staff. Standard procedure-specific post-biopsy observation sheets which highlight the management of suspected complications should be available [22].

### Recommendation 1

Informed consent is mandatory in all ultrasound-guided interventional procedures with variation of forms as indicated in general ethical and national legislative documents (LoE 5, GoR D). Strong consensus (100%).

### Recommendation 2

Specific assessment of bleeding risk and considerations for the use of blood products or other hemostatic agents must be individualized to the patient. The INR value should be corrected to <2.0 prior to low-risk procedures and <1.5 in moderate to high-risk procedures. In patients with <50 000 platelets, a transfusion of platelets is necessary prior to high bleeding risk procedures (LoE 2a, GoR C). Strong consensus (100%).

### Recommendation 3

Repeat biopsy is recommended when there is an inconclusive result or insufficient or non-diagnostic material. Critical evaluation of the first attempt is mandatory before considering an optimized repeated procedure (LoE 5, GoR D). Broad agreement (94%).

### Recommendation 4

Adequate material for a microbiology specimen is essential, and should be collected in sterile tubes, with correct labelling to assure appropriate analysis (LoE 5, GoR D). Strong consensus (100%).

### Recommendation 5

Post-procedural care is essential to detect complications and should be part of appropriate patient management (LoE 2b, GoR B). Strong consensus (100%).

## Liver



### Diffuse liver disease

Liver biopsy (LB) for diffuse liver disease can be performed percutaneously, laparoscopically or by a transjugular approach.

### Percutaneous liver biopsy

#### Indications for percutaneous liver biopsy

1. Evaluation of chronic liver diseases for staging and grading
2. Confirmation of diagnosis and prognosis
3. Evaluation of abnormal liver function tests
4. Diagnosis of cholestatic liver disease
5. Evaluation of infiltrative or granulomatous disease

6. Post-liver transplantation to evaluate and manage rejection
7. Evaluation of unexplained jaundice or suspected drug reactions

### Contraindications for percutaneous liver biopsy

1. Patient refusal or uncooperative patient
2. Ascites
3. Infection of the hepatic bed
4. Severe coagulopathy
5. Platelet count <70 000/ $\mu$ L, transfusion is recommended [23].

### Antithrombotic agents

Antithrombotic agents should be stopped or substituted before IN-VUS procedures, ensuring optimal risk/benefit ratio for the patient. In 15 181 percutaneous liver biopsies, the incidence of bleeding in patients taking aspirin (acetyl salicylic acid) within 10 days prior to the biopsy was 0.6%, not statistically different from the incidence of bleeding in those not taking aspirin (0.4%;  $p=0.34$ ) [24]. When anticoagulant therapy cannot be discontinued, a transjugular LB is the preferred approach.

### Post Liver Biopsy

After LB, a period of four hours of observation, including measurement of pulse and blood pressure, is recommended [23]. Performing LB in an outpatient setting is standard practice.

### Technical aspects of a liver biopsy

Important aspects of percutaneous LB include:

1. LB under US guidance is safer than a blind biopsy [21 – 28];
2. LB specimen size is related to the diameter of the needle; a 11 – 18-gauge needle will provide sufficient portal tracts for histological diagnosis [29];
3. Operator experience has an influence on the quality of the sample [29, 30];
4. An optimal specimen should be  $\geq 25$  mm long and include  $\geq 11$  portal tracts [25].

### Complications

Complications following LB performed by experienced operators are low. Serious complications occur in 1%, and the overall mortality is <0.2% [31 – 33]. Operator experience influences the rate of complications [34]. The main complications following percutaneous LB are: pain, vasovagal reactions, liver hematoma (symptomatic or asymptomatic), hemoperitoneum, pneumothorax, hemobilia, bile leakage, organ perforation (gallbladder, colon) and arterio-venous fistula. Pain is the most frequent complication; 25% of patients experience some pain, usually mild to moderate, in the right upper quadrant or in the right shoulder. Non-opiate painkillers are sufficient to alleviate the pain.

### Laparoscopic liver biopsy

This can be performed during a laparoscopic procedure (e.g. cholecystectomy) or during a diagnostic laparoscopy. Diagnostic laparoscopy has the advantage that it visualizes the superior and inferior surfaces of the liver and enables guidance of the biopsy. Post-biopsy bleeding can be appreciated and controlled. Usually a suction needle is used for this biopsy.

### Transjugular liver biopsy

This is performed in patients at high risk of bleeding and in whom percutaneous LB is hazardous. The technique is complex and an experienced operator is needed. The quality of the specimen is essential for diagnosis. Optimal specimens should be  $\geq 15$  mm long and

should include  $\geq 6$  complete portal tracts [35]. In 81–100% of cases, transjugular liver biopsies are diagnostic [36]. The rate of complications after this procedure is 1–20%, with a mortality of 0.1–0.5% [35].

### Focal liver lesions

Despite the evolution of imaging methods, such as CEUS, contrast-enhanced computed tomography (CE-CT), contrast-enhanced magnetic resonance imaging (CE-MRI), as well as the availability of tissue elastography for focal liver lesion (FLL) assessment, histological evaluation is often required. FLL biopsy is performed under guidance (usually by US). In exceptional cases ( $< 10\%$ ), if the lesion is not seen by US, computed tomography (CT) or magnetic resonance (MR) imaging can be used to guide biopsy. Alternatively, fusion methods can be performed for lesions not seen on grayscale US, combining this with CEUS to target the lesion for biopsy.

### Indications for FLL biopsy

- ▶ Diagnosis not established on any imaging
- ▶ Lesion immune-histochemical analysis needed for therapy
- ▶ Histological assessment is needed for a therapeutic decision (e.g. hepatocellular carcinoma vs. cholangiocarcinoma).

### Contraindications for FLL biopsy

Identical as for percutaneous LB.

### Technique

The lesion is biopsied under US guidance, always passing through healthy liver, to avoid bleeding. Tru-Cut needles with an automatic device (“gun”) are normally used. The needle is advanced to the surface of the tumor, and then the automatic “gun” is armed and triggered. The ideal site to perform the biopsy is close to the tumor margin, where the risk of sampling necrotic tissue is reduced. CEUS guidance can be useful to avoid necrotic areas. The needle size used to biopsy an FLL can vary from thin needles 21–20 gauge for FNA to large needles 11–15 gauge for core biopsy. Generally, FNA provides cytology (or micro-histology), with less diagnostic value than core biopsy [23]. Previously FNA of focal lesions was used to demonstrate malignancy. As oncologic treatment is dependent on assessment of the cell type, large needle biopsies are performed in order to assess specific tumor markers [37].

### Complications of FLL biopsy

Complications include shoulder pain, bleeding, tumor seeding, organ perforation (gallbladder, colon) and sepsis. The incidence of complications varies depending on operator experience, needle type and tumor location. More frequent complications include: pain ( $< 20\%$ ) and liver hematoma (1–20%). The following other complications are seldom encountered: intraperitoneal bleeding ( $< 1\%$ ), pneumothorax ( $< 1\%$ ), death (0.0081–0.03%) [37, 38]. The risk of malignant seeding during biopsy is rare (0.003–0.009%) [39, 40], but can be problematic, especially in patients who are candidates for liver surgery or transplantation. Similar to biopsy for diffuse liver disease, FLL biopsy may be performed in an outpatient setting, but a follow-up of  $\geq 4$  hours post-procedure is recommended [41].

#### Recommendation 6

Liver biopsy is associated with a low rate of complications (LoE 2b, GoR B). Broad agreement (94%).

#### Recommendation 7

The discontinuation of acetyl salicylic acid (aspirin) is not necessary when performing a liver biopsy (LoE 2b, GoR B). Broad agreement (81%).

#### Recommendation 8

Liver parenchymal biopsy should be performed with ultrasound, either guided or assisted (LoE 2b, GoR C). Broad agreement (88%).

## Spleen



### Introduction

Focal lesions of the spleen are rarely encountered but can be difficult to characterize. The risks of splenic biopsy are lower than generally thought and can be undertaken safely in most patients while achieving high levels of diagnostic accuracy. Percutaneous splenic biopsy carries significantly less risk than diagnostic splenectomy [42, 43].

### Background

Focal lesions of the spleen are uncommon, encountered in only 0.1–1.0% of abdominal US examinations [44]. Benign lesions are slightly more common than malignant ones [45].

### Sonographic features

Focal lesions may be solid, cystic or mixed in nature. Although certain focal lesions have distinctive US features, definitive characterization is often impossible based on the clinical history, laboratory tests and imaging characteristics. Cystic lesions are frequently benign but in the setting of infection or immunosuppression abscess formation must be excluded. Small ( $< 2$  cm) well-defined echogenic lesions are usually benign vascular tumors and are managed with US surveillance [46]. Focal echo-poor, solid lesions in the spleen are difficult to characterize; lymphoma is the most common malignancy [47] and is almost always echo-poor [48].

### Contrast-enhanced ultrasound

The use of CEUS can be very helpful in identifying and characterizing focal splenic lesions, as summarized in previous guidelines [49]. Typically benign splenic lesions show either no enhancement or enhancement which persists in the late (parenchymal) phase. Malignant splenic lesions usually show early-phase enhancement followed by washout in the parenchymal phase [50].

### Indications

The most common indications for biopsy are:

- ▶ Focal lesion in a patient with known or suspected lymphoma
- ▶ Focal lesion in a patient with a known extrasplenic malignancy
- ▶ Focal lesions in immunocompromised patients
- ▶ Pyrexia of unknown origin with splenic abnormality
- ▶ Cystic lesion where there is concern of malignancy or abscess

### Contraindications

Contraindications to biopsy include:

- ▶ Uncorrectable coagulopathy
- ▶ Lack of a safe biopsy pathway
- ▶ Uncooperative patient

- ▶ Hemodynamic instability
- ▶ Severe cardiopulmonary compromise

## Materials and Technical Issues

### Pre-biopsy planning

Prior to biopsy all imaging studies should be reviewed to identify the safest route of access. In patients with imaging abnormalities at multiple sites, a non-splenic biopsy site is usually preferred. Risk factors for bleeding must be assessed. Splenic biopsy is rarely an emergency procedure. Anticoagulation medication should be withheld for an appropriate period prior to biopsy and coagulation abnormalities should be corrected wherever possible. Different authorities recommend different acceptable levels for platelet count and coagulation prior to splenic biopsy. Some recommend that these should be within the normal range, while others recommend that some degree of derangement is acceptable but that a minimum platelet count of 50 000 – 70 000/ $\mu$ L, INR < 1.2 – 1.6 and APTT 20 – 33 sec are required [51, 52]. In all patients with deranged coagulation, a risk to benefit assessment must be considered prior to biopsy.

### Biopsy technique

Biopsy is usually possible with local anesthesia. Patient positioning is frequently in the lateral decubitus position but will depend on the site of the biopsy target. Subcostal puncture minimizes the risk of pleural transgression but higher punctures may be necessary to target specific lesions. Biopsy is performed with suspended respiration to minimize the risk of shearing injury to the spleen, facilitated by US guidance rather than CT. Hemorrhage is minimized by targeting a peripheral lesion [51 – 54] but it is desirable to cross normal splenic parenchyma to achieve a tamponade effect [55]. Lesions close to the splenic hilum are a relative contra-indication to biopsy. Injection of hemostatic gelatin sponge along the biopsy tract has been described, but there are no trials in humans to confirm that this is beneficial.

### Fine needle aspiration cytology versus core needle biopsy

Both fine needle aspiration cytology (FNAC) and core needle biopsy (CNB) can be used [51 – 53, 53 – 61]. A meta-analysis involving 741 splenic biopsies in 639 patients [51] found that 95% provided sufficient material for analysis, with an overall sensitivity of 87.0% and specificity of 96.4%. CNB performed slightly better than FNAC. In 389 biopsies, an overall diagnostic accuracy rate of 90.9% with the best results obtained by “double biopsy” (cytological and histological sampling) was reported. The results of FNB and CNB were similar except for lymphoma where CNB gave statistically superior results [62]. Other studies have also found CNB to be superior to FNAC in lymphoma [61 – 65]. Biopsy procedures are accurate in the specific settings of pediatric patients [66], HIV [67] and non-lymphomatous metastases [68, 69]. FNB has also been shown to be safe and effective in the diagnosis of diffuse involvement of the spleen in sarcoidosis [70, 71] and kala-azar [72]. The CNB needle size should be 18 gauge or smaller to minimize the risk of hemorrhagic complications [51, 64, 73]. The complication rate of 18-gauge biopsies does not appear to be greater than with smaller needle sizes and provides greater diagnostic accuracy [65]. The FNB needle size is usually 21 – 22 gauge. The use of co-axial needle systems allows multiple passes through a single cannula but this technique does require a larger caliber needle [55].

### Sample preparation

CNB samples are usually sent to the laboratory in formalin solution. Several FNAC aspirates are optimal for cytology prepared as 1 – 4 smeared air-dried slides and an aspirate in cytology collection fluid to allow preparation of a micro-pellet. A sample in saline permits immunohistochemistry to be performed for lymphoma characterization. Where infection is suspected, abscess fluid can be sent to the laboratory without delay in a sterile container for processing. In difficult cases, particularly in immunocompromised patients, prior discussion with a microbiologist is desirable to ensure that a small specimen is optimally presented for processing.

### Post-procedure care

Post-procedure the patient should be carefully observed for a minimum of 4 hours. Discharge is possible at this stage [52, 54] provided that the patient is asymptomatic and discharged to a responsible caregiver.

### Complications

The most common major complications are hemorrhage and splenic rupture. While hemorrhage is usually self-limiting, splenectomy (or endovascular embolization) is occasionally required. Rarely splenic biopsy may result in a pneumothorax. Other major complications are very unusual. A meta-analysis of 859 biopsies in 741 patients calculated an overall complication rate of 4.2% and a major complication rate of 2.2% [51]. When studies excluding needles larger than 18 gauge were omitted, the CNB major complication rate (1.9%) was only slightly greater than that of all biopsies (1.3%). For biopsies performed with 14-gauge needles, the pooled total complication rate was 60.6% (major complications 12.5%). In an analysis of 389 biopsy procedures, an overall complication rate of 5.2% with a major complication rate of < 1% was reported [62]. The results of 1000 FNB procedures on the spleen without imaging guidance reported no major complications [74]. No reports of needle tract tumor seeding from splenic tumors were identified.

#### Recommendation 9

Focal lesions of the spleen are uncommon; definitive diagnosis based on imaging appearances may not always be possible and biopsy may be considered if a definitive diagnosis is required (LoE 3b, GoR C). Strong consensus (100%).

#### Recommendation 10

Ultrasound is the imaging modality of choice for most splenic biopsy procedures (LoE 5, GoR D). Strong consensus (100%).

#### Recommendation 11

Biopsy of focal splenic lesions has high levels of diagnostic accuracy. Overall, core needle biopsy is slightly superior to fine needle aspiration for cytology particularly if lymphoma is suspected (LoE 2a, GoR B). Strong consensus (97%).

**Recommendation 12**

The complications of splenic biopsy are predominantly due to bleeding, with the complication rate of core needle biopsy being slightly greater than fine needle aspiration for cytology but lower than splenectomy (LoE 2a, GoR B). Strong consensus (100%).

**Recommendation 13**

For core needle biopsy a needle size of 18G or smaller should be used to minimize the risk of splenic bleeding (LoE 2a, GoR B). Strong consensus (100%).

**Pancreas****Biopsy of focal pancreatic lesions****Solid pancreatic lesion**

Patients with a ductal adenocarcinoma characterized as resectable on imaging should have no preoperative sampling performed (avoiding false-negative results) with surgical referral instituted [71–79]. Histopathological confirmation is necessary for inoperable pancreatic cancer and for patients who are unsuitable for surgery prior to non-surgical neoadjuvant treatments [80]. Diagnostic intervention may be considered in suspected uncommon lesions (e.g. lymphoma or metastases) which are managed non-surgically particularly if the differentiation between a solid neoplasm and focal pancreatitis is uncertain on imaging. FNA or CNB can be performed to determine the Ki-67 value of neuroendocrine neoplasms for prognosis. The Ki-67 index must be evaluated in the most cellular areas of the neoplasm. Multiple samples may be needed; multiple “safe” passes with an FNA needle are more productive than a single pass with a biopsy needle [81, 82].

**Cystic pancreatic lesion**

Percutaneous sampling of cystic pancreatic lesions has limited supporting evidence and endoscopic ultrasound (EUS)-guided sampling is performed in these cases [83]. EUS-FNA cytology is more accurate than fluid analysis in the differentiation of benign and malignant cystic pancreatic lesions. The combination of cytology and fluid analysis is the best method for malignant lesions [83, 84]. EUS-FNA is also indicated when a previous diagnostic modality has shown suspicious features (other than enhancing solid component), when other diagnostic modalities fail to obtain a definite diagnosis (e.g. between mucinous and non-mucinous lesions), or in cases of advanced malignant lesions when chemotherapy is considered [83]. Cystic neoplasms requiring surgery with typical imaging appearances do not require EUS-FNA before resection; most pancreatic cystic tumors should be resected without the need for cystic fluid analysis [85].

**Imaging and sampling accuracy**

Focal pancreatic lesions (FPL) are initially identified on transabdominal US examinations. The addition of elastography may evaluate the stiffness of the lesion. A distinction between solid and cystic masses is crucial [86, 87]. Further evaluation of solid pancreatic lesions relies on CECT [88]. Better results for the diagnosis of ductal adenocarcinoma can be obtained when CT is combined with CEUS [89]. MR imaging and EUS are second-line examinations [88]. With cystic lesions, MRCP represents the gold

standard for noninvasive assessment; EUS can be vital for further characterization [84]. While confirmation of malignancy for a solid lesion is mandatory in the presence of borderline resectable lesions prior to treatment with neoadjuvant therapy, biopsy proof is not required in a resectable pancreatic lesion [90, 91]. A percutaneous US-guided approach is preferred for minimal invasiveness, low cost, and duration of the procedure, and allows appropriate cytology assessment of solid lesions [91–94]. FNA is superior to core-needle or open biopsy in terms of cost, procedure-associated morbidity, and timeliness of diagnosis [95]. Percutaneous US-FNA is performed without anesthetic in < 30 minutes, allowing for rapid diagnosis of unresectable pancreatic masses [93, 96, 97]. Biopsy may be performed in FNA cytology failure. FNA should also target any focal liver lesion, suspicious of metastases, allowing diagnosis of histotype and stage.

Cystic lesions that require pathological diagnosis are sampled via EUS [91–102]. The accuracy of percutaneous US-FNA of pancreatic masses reaches 99.4% [92, 93, 97, 101–106]. A sensitivity of 89%, a specificity of 98%, a positive predictive value of 99%, and a negative predictive value of 74%, for an overall diagnostic accuracy of 91%, have been reported [94]. In 545 focal pancreatic lesions, US-guided FNA cytology had 99.4% sensitivity, 100% specificity, and 99.4% accuracy but sampling was non-diagnostic in 6.6% (36/545) of procedures [93]. The accuracy of percutaneous sampling varies depending on the lesion position: 91–94% for body-tail lesions, 81–84% for head lesions [97, 107]. Microbiological evaluation of a cystic pancreatic lesion (i.e., pseudocyst) when infection is suspected may be assessed with aspiration of the cyst content either percutaneously or endoscopically.

**Indications**

- ▶ Characterization of a solid unresectable pancreatic mass.
- ▶ Differential diagnosis between neoplasm and focal inflammatory conditions.
- ▶ Suspicion of an uncommon entity (i.e., metastases, lymphoma), even if resectable, which could be treated non-operatively.
- ▶ Ki-67 “quantification” for the prognosis of neuroendocrine neoplasms [108].
- ▶ Cystic lesions that are undefined or suspicious for malignancy after MR imaging evaluation, even if an endoscopic approach is preferable to address this issue.

**Contraindications**

- ▶ Coagulation disorders are absolute contraindications to pancreatic diagnostic interventional procedures.
- ▶ Patient refusal of any therapy is a contraindication for biopsy.

**Ultrasound biopsy procedure**

US evaluation of a lesion includes B-mode and Doppler imaging to evaluate content and identify the safe and most productive biopsy route, with CEUS aiding positioning in viable vascularized areas. The ideal entry point for percutaneous intervention is the epigastric region to the left of the midline, angled depending on tumor location [109]. The FNA needles used vary from 20 to 25 gauge [91–94]. A cytologist during the procedure allows immediate sample evaluation. Biopsy needles may be of the Menghini or Tru-cut type between 16 and 22 gauge.

**Complications**

Percutaneous US-guided FNA complications are rare [93]. No major complications were reported in a multicenter study [94]. In 96.7% (85/88) of cases, the procedure was uneventful with no

major complications [92]. US guidance has lower complication rates as compared to CT guidance: 1.1 – 5.0% versus 2.1 – 19.0% [97, 106, 107, 110, 111]. The size of the needle is less important than the mechanism of sampling. A cutting needle is more traumatic [106, 107, 110, 111]. The risk of tumor seeding is reported in both percutaneous and endoscopic procedures [112, 113]. In percutaneous abdominal FNA of abdominal lesions, the frequencies of needle tract seeding in the four questionnaires were 0.005%, 0.006%, 0.003%, and 0.009%, respectively [112] and no significant difference was found in the frequency of peritoneal seeding in the EUS FNA group and the no sampling group in the management of IPMN [114].

### Follow-up imaging

At the end of a percutaneous intervention, a complete US evaluation of the abdomen should be performed, especially when the procedure was considered difficult (e.g. poor breath holding), in order to detect immediate complications and a CECT should be performed if warranted by the clinical condition of the patient.

### Pancreas parenchyma biopsy Indications and contraindications

Diagnostic intervention is not required for the diagnosis of diffuse pancreatic diseases (i.e., acute and chronic pancreatitis) except for the diffuse form of autoimmune pancreatitis (AIP). The International Consensus Diagnostic Criteria (ICDC) for autoimmune pancreatitis [115] emphasize the importance of histological samples as well as imaging criteria for diagnosis (CT, MRI, MRCP, ERCP). FNA is not considered useful for the diagnosis of AIP [116], while pancreatic biopsy performed with percutaneous or EUS guidance is fundamental. Biopsy can be performed after FNA if required.

### Diagnostic puncture for pancreatitis-associated fluid

Fine needle aspiration culture of pancreatic fluid collections is useful if the diagnosis is uncertain allowing optimized antibacterial therapy, but is not routinely indicated, as sampling has a 25% false-negative result and rarely leads to an alteration in clinical management [117, 118] and can be performed percutaneously under US or CT guidance or via EUS. Diagnostic intervention in pseudocysts is indicated when noninvasive imaging cannot reliably differentiate from cystic neoplasms, especially mucinous cystic neoplasms or unilocular serous cystadenomas. Pseudocysts almost never develop without a history of acute pancreatitis or signs of chronic pancreatitis.

#### Recommendation 14

In patients with a resectable pancreatic mass with typical imaging aspect of ductal adenocarcinoma, a preoperative sample should not be performed and patients should be directly referred for surgical evaluation (LoE 2b, GoR B). Strong consensus (100%).

#### Recommendation 15

Resectable pancreatic masses with atypical features at imaging should be referred for EUS and EUS-guided sampling (LoE 3b, GoR A). Strong consensus (97%).

#### Recommendation 16

Borderline resectable pancreatic masses in candidates for neoadjuvant treatment should be referred for EUS and EUS-guided sampling (LoE 2b; GoR C). Strong consensus (100%).

#### Recommendation 17

Unresectable locally advanced pancreatic solid masses should be referred for diagnostic biopsy in candidates for oncological treatment (LoE 2b, GoR B). Strong consensus (100%).

#### Recommendation 18

Unresectable locally advanced pancreatic masses should be evaluated for percutaneous ultrasound-guided biopsy. If a percutaneous route is not feasible, EUS should be considered (LoE 5, GoR D). Strong consensus (100%).

#### Recommendation 19

Percutaneous US guidance of the pancreas should be preferred to CT owing to the lower complication rates (LoE 2b, GoR B). Broad agreement (83%).

#### Recommendation 20

Biopsy should be targeted to the suspected liver metastases for diagnosis and staging (LoE 5, GoR D). Strong consensus (100%).

#### Recommendation 21

Sampling of cystic pancreatic masses should be performed under EUS guidance (LoE 5, GoR D). Strong consensus (100%).

#### Recommendation 22

Cystic pancreatic masses typical at imaging and requiring surgery should not be sampled before resection (LoE 5, GoR D). Strong consensus (96%).

## Kidney



### Introduction

The clinical aspects of renal intervention are described elsewhere in the guideline series, and this section deals with aspects of US guidance for renal biopsies [111 – 121]. Renal biopsy will be performed in both the native and transplant kidney [122].

### Imaging modalities

Alternative imaging options should be considered as appropriate if US does not provide the required information. For drainage of an abscess or the collecting system and biopsy of the renal parenchyma in the assessment of renal impairment, US is adequate. A combination of CECT and MR imaging is useful for focal lesion assessment and for planning RFA.

### Multidisciplinary decision

The decision for INVUS related to tumor treatment should be made in an interdisciplinary tumor meeting. When INVUS is performed to relieve obstructive disease, the discussion should involve and be led by urology and nephrology should lead the process for INVUS to obtain histological information for suspected renal parenchymal disease.

### What defines the possibility of performing an INVUS procedure?

The INVUS procedures for diagnostic workup are limited by absolute and relative contraindications. INVUS is available at a reasonable cost and in low resource settings, yet requires investigators experienced in the procedure [4].

### Diffuse renal disease

Percutaneous renal biopsy has become the gold standard for the diagnosis and classification of diffuse renal diseases, in the absence of a major contraindication, particularly when specific treatment can be initiated [123].

### Indications and contraindications

#### Indications

There is no generally accepted standard protocol for selecting patients for renal biopsy. The decision for renal biopsy is largely made by weighing therapeutic benefit against potential complications.

#### Contraindications

The most common contraindications for percutaneous renal biopsy are mentioned elsewhere [123] and include an uncooperative patient, uncontrolled arterial hypertension (>150 mmHg systolic and >95 diastolic), an abnormal coagulation status, renal or systemic bacterial infection (except when used to diagnose infectious or pseudo-lesions). Relative contraindications include solitary native kidney [124, 125], hydronephrosis, and anatomic abnormalities of the kidney which may increase the risk of bleeding [121, 126, 127].

#### Pathology

The biopsy report for non-neoplastic kidney diseases represents a complex integration of clinical data with light microscopy, immunofluorescence, and other (electron) microscopic findings. We refer to the practice guidelines for the handling and processing of the renal biopsy (Renal Pathology Society) [128]. A renal biopsy specimen should always be interpreted within the context of the clinical presentation and laboratory findings.

### Ultrasound guidance

Real-time US is superior to the "blind" approach (using US for localization only) with a higher diagnostic yield (100% vs. 84%) and a lower complication rate [5]. US can localize the lower pole and tangential position to minimize vascular structures and to avoid cysts that might necessitate altering to biopsy of the contralateral kidney [129].

### Biopsy technique

The choice of biopsy needle is largely one of individual preference. Most studies have been performed with semi-automated biopsy needles with a size of 11–18 gauge in order to ensure a sufficient number of glomeruli [131–134].

### How many passes?

It is recommended to obtain two core renal biopsies from the lower pole of the left kidney in the absence of local contraindications, such as polar atrophy, arteriovenous fistula or cyst.

### Needle size

Renal biopsy produces the highest diagnostic yield with more glomeruli per core biopsy using 14-gauge Tru-cut needles compared to 16- and 18-gauge needles without a difference in complication rates [131–134]. A meta-analysis of 34 retrospective (n=21) and prospective (n=13) studies, including 9474 biopsies, revealed an increased need for blood transfusion following 14G compared with either 16- or 18-gauge biopsy [135]. A trend to less complications in the case of smaller needles has been recognized in other studies [132]. "Biopince" full-core biopsy instruments with a diameter of 18 gauge might be sufficient but there is insufficient evidence in the literature.

### Fine needle aspiration cytology versus core needle biopsy

There is no role for FNAC in the evaluation of diffuse renal disease.

### Post-procedural care

After biopsy, an observation time of 6 hours is thought sufficient but up to 24 hours may be considered in patients with a higher risk of bleeding. An observation period of <8 hours may miss up to 33% of minor and major complications when performed with a 14-gauge Tru-Cut needle [16].

### Out- or inpatient

There is a trend to perform biopsies in outpatient clinics [136]. Post-procedural care is recommended for at least 1–12 hours, since 81–85% complications occur within 8 hours [16, 131–139]. An observation time of 24 h is advisable in patients with an abnormal coagulation status and end-stage renal insufficiency.

### Complications

High blood pressure, female gender, younger age, abnormal coagulation (prolonged bleeding time) and both acute and chronic renal failure are associated with a higher complication rate [140, 141].

The main complication of renal biopsy is bleeding [135, 136] which may be subcapsular, peri-renal hematoma or gross hematuria, manifested clinically by hypotension. Other complications include flank pain, arterio-venous fistula and aneurysm, urinary tract obstruction, acute renal failure or even death [142, 143].

### Focal renal lesions

The differentiation between benign and malignant renal lesions is of utmost importance. Solid renal masses are malignant with a probability of >90%, whereas the rate for benign lesions is reportedly low [144], possibly accounted for by the increasing rate of small renal lesions detected incidentally [144, 145]. Diagnostic biopsy success is reported between 71–100% and has improved with a significant reduction of indeterminate biopsies (around 10%) [141–148]. A review of the current rationale, indications, and outcomes of percutaneous biopsies and histologic characterization of renal tumors (112 papers) found performing >2 biopsy cores with an 18-gauge needle was no different under CT or US guidance [149]. 152 renal lesion biopsies were performed using a coaxial 18-gauge core needle technique in 125 patients. ≤4 cores were obtained from each tumor, with 3 or 4 cores obtained in most patients with success and a low complication rate [150].

It is preferable to use a needle introducer to limit the risk of track seeding.

### Indications

Renal lesion biopsy is indicated when management will change under the following circumstances:

- ▶ Small renal masses that are indeterminate on imaging
- ▶ Known extrarenal malignancy
- ▶ Candidates for active surveillance or local ablative techniques
- ▶ Metastatic disease to select the optimal systemic therapy when the renal tumor is the most suitable site
- ▶ Unresectable retroperitoneal tumors involving the kidney
- ▶ In infection without response to antibiotic treatment
- ▶ When partial vs. radical nephrectomy is discussed (solitary kidney)

### Needle size

Usually 14- to 18-gauge core biopsy needles are used but data regarding complications following multiple biopsies are not available [151, 152]. Three biopsy cores in one patient are better than a single biopsy core [153]. In patients with cystic lesions, a percutaneous biopsy with fluid aspiration is of limited value due to a high rate ( $\leq 50\%$ ) of false-negative findings [116]. Some studies found biopsy helpful in lesions of Bosniak category III [154]. The risk of track seeding has not been evaluated. If renal lesions are biopsied, a combination of fluid aspiration for cytology and biopsy of the wall or nodules in the cyst should be used.

### Contrast-enhanced ultrasound

The role of CEUS has been described in the EFSUMB guidelines and is useful to delineate necrotic areas [49].

#### Recommendation 23

Percutaneous renal biopsy should be performed under ultrasound guidance (LoE 3a, GoR B). Strong consensus (100%).

#### Recommendation 24

Spring-loaded needles for native parenchymal kidney biopsies are superior to manual needles (LoE 2b, GoR B). Strong consensus (100%).

#### Recommendation 25

Two adequate samples should be obtained with parenchymal kidney biopsies (LoE 3b, GoR B). Strong consensus (100%).

#### Recommendation 26

18G needles should be used as they combine a high diagnostic yield and a relatively low complication rate in native kidneys (LoE 2a, GoR B). Broad agreement (90%).

#### Recommendation 27

Post-procedural care is recommended for at least 1 – 12 hours after renal biopsies (LoE 3a, GoR B). Strong consensus (96%).

#### Recommendation 28

Percutaneous biopsy should be considered in cases of solid focal renal masses when there is a significant probability for a change in patient management (LoE 2a, GoR C). Strong consensus (100%).

#### Recommendation 29

18G needles are recommended for solid focal renal lesions (LoE 4, GoR C). Strong consensus (100%).

## Adrenal Gland

### Imaging modalities

Adrenal masses can be detected by transabdominal grayscale US with high accuracy [151 – 159]: 99% and 69% for the right and left adrenal glands, respectively [155]. Ultrasound, although sensitive, is not capable of accurately differentiating adrenal lesions [160]. Follow-up of a CT or MRI diagnosis of an adrenal adenoma with US is feasible but CT or MRI needs repeating if the lesion increases in size [161]. Contrast-enhanced ultrasound for the characterization of adrenal masses has been evaluated [162, 163], demonstrating no specific patterns distinguishing benign from malignant lesions [162], although analysis of time-intensity curves showed early arterial contrast enhancement and rapid wash-out in all malignant lesions [163, 164]. The detection and the characterization of adrenal lesions are traditionally achieved on CECT and MR imaging, with nuclear medicine being useful for pheochromocytoma and positron emission tomography (PET) being valuable.

### Multidisciplinary decision

Most adrenal masses not typical for adenoma and not characteristic for a pheochromocytoma on CECT and MRI may require biopsy, especially with a background of known or suspected malignancy [165, 166]. A biopsy of a possible pheochromocytoma is contentious because of the risk of severe hypertension [167] and clinical and laboratory evaluation is advised prior to biopsy [161 – 170]. With thorough pre-procedural planning, careful intra-procedural monitoring and availability of adrenergic blockade or anesthesia assistance, biopsy can be safe. The proximity of the adrenal gland to the diaphragm presents a challenge for the patient to cooperate with breathing instructions [171, 172].

### Indications for adrenal biopsy

- ▶ Staging a known malignancy.
- ▶ Identifying an unknown primary malignancy.
- ▶ Differentiating benign from malignant lesions in equivocal cases [170].

### Relative contraindications to adrenal biopsy

- ▶ Uncorrectable coagulopathy.
- ▶ Inability to reach the tumor using a safe path.
- ▶ An unsafe target [170, 171].

### INVUS procedure

The benefits of US guidance include real-time multi-planar imaging, absence of radiation, low cost, portability, and the ability to rapidly confirm complications such as bleeding. The drawbacks of US guidance include inadequate visualization of the target or

needle due to operator experience, lesion depth, or intervening bowel gas or bony structures. Both CT and US are used with high success to biopsy adrenal lesions and operators combine CT and US (including fusion imaging) to attain the spatial resolution of CT and the temporal resolution of US [170]. Use of US identifies the pleural reflection and lung edge to avoid diaphragmatic penetration [170]. Adrenal biopsy using EUS through a transgastric approach may also guide biopsy of left adrenal masses, but the right adrenal is poorly visualized [173, 174]. In clinical practice, the choice of imaging modality is based on equipment availability, cost, lesion conspicuity, and physician preference. US and MRI readily allow for complex oblique angles of approach but US may be limited in large patients, while MRI is expensive and requires MRI-compatible equipment and needles [172, 175, 176].

### Materials and technical issues

Routine pre-procedural blood investigations including full blood count (FBC), metabolic panel and coagulation studies (PT, PTT, INR) are performed. Prior to elective biopsy, anticoagulant use is altered at the appropriate time [177, 178].

### Description of the intervention

Right-sided adrenal biopsies can be performed through a transhepatic, direct posterior or right-decubitus (target side down) approach. Placing the patient in a slight right-decubitus position restricts diaphragmatic motion. Left-sided adrenal biopsies can be approached with the patient in the left-decubitus position, posteriorly or anteriorly/transgastric [179, 180]. With US guidance, a free-hand approach, a needle guide, or fusion guidance technique may be chosen. The use of spatial compounding markedly improves both lesion and needle conspicuity [170]. Smaller FNA needles (21–23G) may be preferred when sampling hypervascular lesions, especially when surrounded by bowel or blood vessels, or in the setting of malignancy [172, 175]. If FNA is chosen, a capillary pass technique is used. Syringe aspiration may traumatize the lesion so that a bloody sample is obtained. A core biopsy may be preferable depending on local cytology expertise [170–181].

### Role of cytology

The overall sensitivity of FNA in detecting the presence of malignancy is 85% [181–184]. Fine needle aspiration cytology is useful in patients with bilateral adrenal lesions, especially in the presence of adrenal insufficiency. The most common causes of adrenal insufficiency are infections, e.g. cytomegalovirus, HIV/AIDS, Mycobacterium tuberculosis and Mycobacterium avium-intracellulare, Cryptococcus neoformans, Histoplasma capsulatum, Pneumocystis jirovecii, and Toxoplasma gondii, or neoplastic diseases (Kaposi's sarcoma and lymphoma) and bilateral adrenal hemorrhage [185, 186]. Around 10% of cases of Addison's disease have an infectious etiology.

### Complications

The most frequent complications following adrenal biopsy are hemorrhage and pneumothorax. Less common complications include pancreatitis, and rarely, needle tract seeding. The overall complication rate is 5.3%. Most are minor, self-limiting complications. The rate of major complications requiring further treatment is 0.1–2% [179, 180, 187]. The risk of hematoma and the rate of major complications increases with a transhepatic approach and pneumothorax is associated with prone positioning. Pancreatitis has been reported when the needle transgresses the

pancreas during an anterior approach [188]. This technique reduces the risk of needle tract seeding in adrenocortical carcinoma [170, 187].

#### Recommendation 30

Adrenal masses incidentally detected at US or indeterminate at CT should be characterized with MR imaging and/or PET imaging (LoE 2b, GoR B). Strong consensus (97%)

#### Recommendation 31

An ultrasound-guided adrenal biopsy should be considered in lesions that are indeterminate at imaging (LoE 2b, GoR B). Strong consensus (100%).

#### Recommendation 32

Prior to adrenal biopsy, pheochromocytoma should be excluded by biochemical assessment in patients with a clinical suspicion (LoE 5, GoR D). Strong consensus (100%).

## Gastrointestinal tract



### Indications and contraindications

Most neoplastic lesions of the gastrointestinal (GI) tract develop as mucosal masses and endoscopic biopsy is the traditional procedure to characterize and obtain a tissue sample. Ultrasound or CT guidance is reserved for specific situations where an appropriate approach by endoscopy or EUS is not feasible [189].

The indications for US-guided biopsy of GI tract lesions are:

- ▶ Beyond easy reach of the endoscope (small bowel lesions)
- ▶ Submucosal, subserosal and exophytic lesions, especially gastric tumors, e.g. gastrointestinal stromal tumors (GIST) or lymphoma
- ▶ Failed biopsy attempts by endoscopic means [181–191]

Absolute contraindications are abnormal coagulation parameters. Relative contraindications are operable GI tract lesions with high suspicion of malignancy (to avoid seeding of neoplastic cells into the peritoneal cavity) and core biopsy of lesions that requires needle passage through the colon. It is usually safe to pass through stomach and small bowel segments with 18-gauge needles [192].

### Imaging modalities

EUS-guided biopsy is the procedure of choice for submucosal, subserosal, or exophytic lesions [193]. Enteroscopy (double balloon or spiral) allows sampling of small intestinal tumors. This technique is invasive and is associated with an increase in complications (e.g. perforation) [193]. CT guidance may be preferred for some lesions, especially those located deep in the pelvis or behind a gas-filled bowel. If the patient has excess abdominal fat, CT may be the better choice for guidance [189]. For perirectal or pelvic lesions, transrectal US may be an alternative [194].

### Multidisciplinary decision

The indication for US-guided biopsy of a GI tract lesion should be determined by a multidisciplinary team (gastroenterologist, surgeon, radiologist and oncologist) taking into account several factors:

- ▶ Availability of advanced endoscopic techniques (i. e., EUS and enteroscopy) [195, 196]
- ▶ Suspicion of malignancy and assessment of operability
- ▶ Probability that the result of the biopsy will alter management (i. e., starting systemic antibiotic therapy in a tuberculous lesion instead of surgery)

### Materials and technical issues

Sampling may be performed by means of FNA or core biopsy [181 – 191]. A graded compression technique should be used especially for mobile and deep lesions or in the presence of excessive bowel gas [191].

### Results

Sensitivity and accuracy between 81 – 99% have been reported for GI tract biopsies with large needles in retrospective series [181 – 191, 197]. Fine needles perform less well with sensitivities of 41 – 50% [191]. To increase the sensitivity, CEUS guidance may be used in larger lesions (especially gastric GIST tumors) to target non-necrotic, viable tissue [198].

### Complications

Complications are rare (<1%) for GI tract diagnostic interventions and include hemorrhage and infection related to perforation [197]. To avoid complications, biopsies should be performed at the thickest area of the abnormality and along the longitudinal axis of the GI tract, so that the wall is not traversed into bowel lumen. Patients should be monitored for 11 – 24 hours following the procedure [191].

#### Recommendation 33

GI tumors not characterized by endoscopic biopsy can alternatively be biopsied by percutaneous or endoscopic US guidance (LoE 4, GoR C). Strong consensus (100%).

## Peritoneal cavity and mesentery



### Indications and contraindications

The peritoneum, including the omentum and mesentery, is a common site for secondary disease extension from adjacent visceral organs and distant metastatic deposits, and is an unusual site of primary neoplastic disease. Non-neoplastic processes (e.g. granulomatous diseases, hematomas, infectious or inflammatory conditions) may also involve the peritoneum, mimicking neoplastic disorders. Detection of peritoneal dissemination is essential in the staging and management of primary tumors. Tumors known to cause solid masses or thickening of the peritoneum include the ovary, uterus, cervix, stomach, colon, pancreas and lymphoproliferative malignancies (primary or secondary processes). Peritoneal disease is discovered in many patients with ascites and/or abdominal distention of unknown cause [199].

### Imaging modalities

Imaging plays an important role in the evaluation of patients with suspected or proven peritoneal disease. Contrast-enhanced computed tomography is the modality of choice for diagnosis, supplemented by MRI and PET/CT techniques [200]. Imaging does not provide phenotype information essential for targeted therapy and a tissue diagnosis is desirable before treatment. Laparoscopy can

identify lesions and allow multiple biopsies. Percutaneous imaging-guided biopsy is safe with a sensitivity of 93%, specificity of 86%, and negative predictive value (NPV) of 50%. In patients with a known primary malignancy, the sensitivity of the biopsy procedure is 93%, the specificity is 100% and the NPV is 38%. In patients without a known primary neoplasm, the sensitivity is 96%, the specificity is 75% and the NPV is 75% [201 – 203].

### Multidisciplinary decision

Peritoneal mass biopsy should be considered at an early stage in the investigation of any patient with no diagnosis. Biopsy is not required if the mass is part of progressive disease and histological diagnosis has previously been obtained. Biopsy is performed if there is uncertainty of recurrence or possible new disease.

In ascites of unknown origin, routine biochemical tests of blood, urine, and ascites and imaging assessment including X-rays, US, CT, MRI must be performed to obtain a general impression of the disease. If a definitive diagnosis is not possible, biopsy of the peritoneum usually confirms the source of the ascites [204]. Peritoneal masses in patients with a history of cancer are nearly always malignant (86%) [203]. Biopsy is still indicated; 10% of patients with a known primary malignant neoplasm will have a second malignant tumor. Biopsy is also indicated in patients without a known primary cancer; benign-appearing peritoneal tissue is predictive of a benign lesion in 75% of cases [203].

### What defines the possibility of performing an INVUS procedure?

The criteria for performing biopsy are a thick peritoneum or presence of a mesenteric mass on diagnostic imaging. Although US plays a minor diagnostic role in the imaging of peritoneal malignancy, it is the modality of choice for imaging-guided biopsy for histological diagnosis. US is cost- and time-effective (no repeated needle position check like CT) and is radiation-free [201, 205]. The multiplanar capability of US allows the operator to avoid vessels, the bowel and solid viscera. Real-time visualization of the needle tip ensures that the targeted mass is not displaced during biopsy [206]. CT should be reserved for small lesions or disease that is inaccessible to US.

### Materials and Technical Issues

Peritoneal masses are localized with US using graded compression to displace overlying tissue and bowel, employing either a low-frequency or high-frequency transducer. The needle path is assessed with color Doppler US to ensure blood vessels are avoided. Local anesthetic (1 – 2% lidocaine hydrochloride) can be administered subcutaneously into the abdominal wall. Conscious sedation is seldom necessary. Peritoneum lesions are best sampled if thickened, hard and fixed during biopsy. If there are any mobile lesions, the peritoneum should be kept stable through compression. Fine needle aspiration is typically performed using 21 – 25-gauge needles and provides samples for cytologic examination, whereas CNB is performed using 11 – 20-gauge needles and provides tissue for histologic assessment [207]. Although both techniques are safe, FNA is preferred for sampling deeply placed lesions, those adjacent to major vessels, and when it is necessary to traverse the bowel wall [208]. In the case of a known malignancy with prior histological material, an FNA procedure is usually sufficient, but in the case of undiagnosed metastatic malignancy or when a definition of the specific cancer subtype is required, a histological sample is necessary. The number of needle passes depends on the quality of the specimen and the volume of tissue obtained at first pass [208].

## Complications

In those patients with large-volume ascites, biopsy should not be performed until the ascites is reduced. The anatomical features of the peritoneum will result in a superficial location of the lesions, adhering to the abdominal wall, thus avoiding underlying organs during biopsy. Ultrasound-guided peritoneal percutaneous biopsies have a lower complication rate in comparison to other biopsy methods. Minor complications related to percutaneous biopsy procedures are seen in 2.7% patients, unrelated to needle size. Severe abdominal discomfort, an episode of hypotension, a small hematoma anterior to the mass that resolved on follow-up have been described [199, 203, 209]. If bleeding occurs, the US transducer can be used to compress the biopsy site and control bleeding.

## Follow-up

In patients with a known malignancy, obtaining benign-appearing peritoneal tissue has a low NPV, which means that with a negative biopsy result a repeat biopsy or surgery should be considered to exclude a malignant process [203].

False-negative results after percutaneous imaging-guided biopsy of masses  $\geq 4$  cm may result from sampling typically centrally located necrotic portions. CEUS can be used to guide the needle away from the necrotic areas [198].

### Recommendation 34

Imaging-guided percutaneous biopsy of the peritoneum is a safe and effective means of providing a tissue diagnosis (LoE 2b, GoR B). Strong consensus (100%).

### Recommendation 35

Ultrasound can be used for peritoneal mass biopsy (LoE 3b, GoR B). Broad agreement (87%).

### Recommendation 36

In the case of ascites of unknown origin, a biopsy of thickened peritoneum may be considered an alternative to laparoscopic biopsy (LoE 3b, GoR B). Broad agreement (93%).

## Lymph Nodes

### Indications and contraindications

Cross-sectional imaging examinations reveal abdominal (mesenteric/retroperitoneal) lymph nodes with increasing frequency entailing further diagnostic workup as many neoplastic, inflammatory and infectious diseases produce abdominal lymphadenopathy [210]. Almost any malignancy may produce abdominal lymphadenopathy, with lymphoma being the most frequent. Percutaneous imaging-guided biopsy is used in the diagnosis as an alternative to surgical biopsy, particularly of deep-seated lymph nodes or in critically ill patients.

### Imaging modalities

Imaging evaluation is an important part of the workup of patients with abdominal lymphadenopathy. Chest X-ray and CECT imaging of the neck, chest and abdomen are mandatory to evaluate the stage of the disease. A baseline PET examination should be carried

out according to the recommendations for staging and response assessment [211]. Pathological analysis of the disease process is of paramount importance and is the reference standard for diagnosis [212]. In patients who have known lymphoma, other important management considerations, such as staging, response to therapy, malignant transformation, and identification of recurrent disease, are also important and biopsy plays a crucial role [211].

### Multidisciplinary decision

With any primary carcinoma it is important to identify abdominal lymphadenopathy as this affects staging and management. Lymph node biopsy is adequate for the diagnosis of metastatic carcinoma. In the assessment for lymphoma, an entire lymph node is desirable, but studies have shown that biopsy is useful and accurate in the initial diagnosis of lymphoma, and also in the progression or recurrence of previously diagnosed lymphomas [211–217], with advantages in terms of morbidity and costs. Biopsy is a viable alternative when the number and size of specimen cores for morphological and molecular studies are not compromised.

### What defines the possibility of performing an INVUS?

Ultrasound-guided biopsy of abdominal lymph nodes is considered feasible if the lymph nodes are visible and a safe route is available [218] but CT-guided biopsy is the preferred technique [211–222]. CT has the advantage of imaging posterior to bowel gas, bone and impenetrable soft tissue and better delineating the lesion, the adjacent structures and the needle. CT-guided CNB is adequate to establish a diagnosis in 82.5% of patients with lymphoproliferative disorders and should be deployed first in the diagnosis of any lymphoma [220]. Use of CT as a guidance modality precludes real-time visualization during needle placement and biopsy. Ultrasound allows continuous real-time visualization of the needle tip throughout the procedure, minimizing injury to adjacent critical structures and contamination with blood or extraneous tissue [223].

### Materials and technical Issues

Fine needle aspiration with adjuvant flow cytometry for diagnosing and sub-typing malignant lymphomas has been reported [212] but CNB provides additional diagnostic and prognostic information that may not be easily derived from FNA [224].

Core needle biopsy yields large cores for histological analysis, samples various parts of the node and therefore allows for a WHO classification of lymphoma [225] with additional tests, e. g. immunohistochemistry and receptor analysis.

With CNB, a diagnostic rate of 81–96% is reported for lymphoma and should be the procedure of choice for histological sampling in the absence of peripheral lymphadenopathy [216, 226, 227].

A free-hand or a needle guide US technique can be used. The free-hand technique offers the advantage of fine adjustments while maneuvering the needle to compensate for an imperfect trajectory and patient movements. The choice between devices is mainly a matter of the experience of the operator and the target location.

Core needle biopsy is performed most often with large core needles ( $\leq 14$  gauge), while smaller needles ( $\leq 25$  gauge) are used more readily for FNA.

### Description of technique

Grayscale imaging and color Doppler are used to localize the lymph node and to select the shortest route free of vascular structures. Applying pressure with the transducer displaces and mini-

mizes intervening bowel loops and fatty tissue. An approach through solid organs is avoided except for the porta hepatis nodes, where a transhepatic route can be used. The biopsy needle is advanced manually under real-time US guidance visualizing the needle tip at all times. Shallow breathing by the patient during insertion of the needle into the lymph node and a short breath-hold only for specimen acquisition help to achieve better patient compliance and reduce procedure time. Sedation is not used routinely. 1–2% lidocaine hydrochloride can be instilled into the skin and subcutaneous tissues as local anesthetic. Usually two needle passes are performed, avoiding any necrotic area of the target lymph node. CEUS can be used [198]. The operator should evaluate the specimen visually both before and after placing the sample into a 10% formalin solution. The specimen should have a white (generally pathologic) or brown tan (lymph node tissue) component, possibly with a yellow component representing adjacent fatty tissue. If the samples appear not to contain lymph node tissue (cortex) or tumor at visual assessment, additional sampling is advised. The material aspirated with FNA is smeared over several glass slides, which are either air dried or fixed in alcohol or other agents according to the cytopathology preference and sent for cytopathology evaluation.

### Complications

An abdominal lymph node biopsy is usually well tolerated with a low rate of complications [220]. Local hematoma and post-procedural pain are described in 1.8% of cases, while bleeding requiring surgery is seen in 1% [228]. Complications may develop if major vessels, bowel loops, or bile ducts are transgressed.

### Follow-up

Patients must be monitored for 4 hours after biopsy procedures to check vital signs and assess for complications.

#### Recommendation 37

Percutaneous ultrasound provides accurate and safe guidance for abdominal lymph node biopsy (LoE 3b, GoR B). Strong consensus (100%).

#### Recommendation 38

Percutaneous core needle lymph node biopsy should be used as the method of choice if lymphoma is suspected (LoE 3b, GoR B). Strong consensus (100%).

#### Recommendation 39

In suspicious lymph nodes either core needle biopsy or fine needle biopsy/aspiration may be considered in the presence of known malignancy (LoE 3b, GoR B). Strong consensus (100%).

## Retroperitoneum

### Indications and contraindications

Retroperitoneal tumors cause symptoms or become palpable only when they have reached a significant size. The most common malignant lesions are sarcomas and lymphomas, while neurogenic tumors, paragangliomas and fibromatosis are the most

frequently encountered benign lesions [229]. Retroperitoneal tumors are best evaluated using cross-sectional imaging with preoperative histology by CNB being required when imaging is non-diagnostic. The main indications for a biopsy are: a) masses with an uncertain radiological appearance; b) tumors with a radiological appearance suggesting pathology where neo-adjuvant treatment may be indicated as induction therapy (e.g. gastrointestinal stromal tumor, Ewing's sarcoma, teratoma); c) unresectable tumors or tumors with distant metastases; d) diagnosis and subtyping of lymphomas [229].

### Other guiding modalities

CT-guided biopsy of retroperitoneal masses is well-established with good outcome. CT guidance uses typically the safer posterior approach. Contraindications to CT-guided biopsy are the lack of patient cooperation, coagulation abnormalities and the lack of a safe biopsy route (interruption by major vessels, bowel and vertebral bodies adjacent to the target lesions). Due to lower accuracy and increased risk, the puncture of small lesions (< 1 cm) is not suitable [230]. MRI-guided biopsy is an emerging technique with both advantages and disadvantages over CT [231]. Fine needle aspiration guided by EUS has a high diagnostic accuracy with lower complications particularly for small lesions [232, 233].

### Multidisciplinary decision

The decision to perform a biopsy of a retroperitoneal mass should be made by a multidisciplinary team consisting of a surgeon, oncologist and radiologist. Essentials to support this decision are: imaging features, potential resectability, the probability that the lesion is chemotherapy-sensitive (lymphoma, GIST) or a benign tumor and tumor size [229]. For retroperitoneal sarcomas, although evidence supports biopsy in the setting of preoperative and intraoperative radiotherapy, percutaneous biopsy is still controversial [234].

### Materials and technical considerations

With US guidance an anterior approach must be used [234]. Due to the risk of injury to large vessels (with subsequent intraperitoneal bleeding) or the bowel, fine needles are usually chosen. Core biopsy with 11–18-gauge needles may be performed in large tumors, provided there is a safe needle track [235].

### Complications

In retroperitoneal tumors percutaneous US-guided FNA has a sensitivity of 61–95.8% depending on the frequency of different diseases in the study population [223, 236, 237]. The accuracy of FNA in diagnosing lymphoma, sarcoma and benign tumors is low. FNA is not indicated when these tumors are suspected [234]. The overall diagnostic rate of US-guided core biopsy was 88.5% for retroperitoneal tumors and 86% for lymphomas, similar to surgical biopsy [238]. Using CT guidance core biopsy yields a correct diagnosis in 91–96% of cases [230, 234]. For sarcomas and lymphomas the sensitivities are lower: 82% and 87%, respectively [230, 235]. Complications include bleeding (intraperitoneal, retroperitoneal or in abdominal wall), injury of the bowel wall and pain. Using an appropriate technique the complications are mostly of minor importance, and major events are rare [198, 230, 231, 234, 238]. The performance of percutaneous biopsy in retroperitoneal tumors may be improved using larger needles (especially for lymphoma subtyping) [234], more passes [198, 234, 238] and CEUS to avoid necrotic areas [198]. The use of a coaxial technique increases both the diagnostic rate (allowing a higher number of passes) and improves safety [234, 238].

## Conclusion

In the management of retroperitoneal tumors, percutaneous biopsy should be performed in certain circumstances. Ultrasound is a valid guidance alternative to CT when biopsy is indicated. To increase diagnostic accuracy, larger needles should be used whenever possible. In the absence of a safe pathway, FNA performed either via the percutaneous route or through EUS may be an alternative.

### Recommendation 40

In the case of indeterminate retroperitoneal masses (e. g. sarcoma), the indication for biopsy versus primary resection should be individually assessed (LoE 4, GoR C). Strong consensus (100%).

### Recommendation 41

Ultrasound is a valid retroperitoneal biopsy guidance alternative to CT (LoE 4, GoR C). Broad agreement (87%).

### Recommendation 42

An ultrasound retroperitoneal core biopsy is more accurate than fine needle aspiration and should be performed whenever possible (LoE 3b, GoR C). Broad agreement (84%).

### Recommendation 43

Fine needle aspiration retroperitoneally either percutaneous or by EUS may be an alternative in difficult cases (LoE 4, GoR C). Strong consensus (100%).

## Liver, renal, pancreas and bowel transplant

### Imaging modalities

Ultrasound is the first-line imaging modality in evaluating all abdominal organ transplants to detect postoperative complications and most interventional procedures will be performed guided by US [231 – 242]. A multimodality approach is often required to evaluate the transplant and diagnose complications when US studies are inconclusive. CT is crucial for the detection of fluid collections [243, 244], abscesses and fistulae. Contrast-enhanced MR and CT angiography can diagnose vascular complications. Non-enhanced MR angiography and CEUS can help identify vascular complications when renal dysfunction is present. PET-CT can be used when there is suspicion of neoplastic disease. CEUS or combined techniques such as fusion imaging when there is low-lesion conspicuity may facilitate intervention.

### Multidisciplinary decision

Multidisciplinary teams are involved from the preoperative evaluation and discussion of potential candidates in donor transplant programs to the management of complications throughout hospitalization and follow-up. The multidisciplinary team should include transplant physicians, surgeons, hemato-oncologists, histopathologists, and radiologists with experience in treating transplant patients. Patients should be discussed with the transplant team to decide whether the potential benefits of the INVUS procedure outweigh the risks [245, 246].

## Indications and contraindications

### Liver transplant

#### Indications

- ▶ Percutaneous LB is indicated to diagnose diffuse parenchymal abnormality to differentiate between allograft rejection, reperfusion injury, drug-induced toxicity, viral infection or recurrent disease.
- ▶ FNA is indicated in the presence of perihepatic collections with suspicion of infection or bile leakage.
- ▶ FNB or FNA is indicated with suspicion of neoplastic complications (e.g. hepatocellular carcinoma or post-transplant lymphoproliferative disease (PTLD)).
- ▶ Protocol LB with normal liver function is accepted to reveal unexpected abnormalities such as progressive fibrosis [247].

### Kidney transplant

#### Indications

- ▶ Renal transplant biopsy is indicated when renal dysfunction is attributable to parenchymal disease, to differentiate between acute rejection and acute tubular necrosis as well as between chronic rejection and immunosuppression toxicity.
- ▶ Worsening of renal function or absence of improvement after treatment [241 – 250].
- ▶ Prior to altering immunosuppression treatment.
- ▶ Protocol transplant biopsies at 1 – 12 months despite normal renal function to diagnose subclinical allograft dysfunction [248, 251, 252].
- ▶ FNA is indicated in the presence of peri-renal collections with suspicion of infection.
- ▶ FNB or FNA are indicated with suspicion of neoplastic complications (e.g. PTLD).

### Pancreas transplant

#### Indications

- ▶ Suspected rejection: persistently or significantly elevated blood glucose level and/or significant reduction in insulin level.
- ▶ Follow-up of rejection.
- ▶ Clinical protocol in some institutions.
- ▶ Suspicion of PTLD.
- ▶ FNA to differentiate between the different types of fluid collections (e.g. abscess).

### Combined kidney/pancreas transplant

The majority of pancreas transplants are simultaneous pancreas-kidney transplants.

#### Indications

- ▶ Suspected rejection.
- ▶ Follow-up of rejection.

### Bowel transplant

Surveillance endoscopies for the first few months after intestinal transplantation are performed and endoscopically guided biopsy is required for rejection [251 – 255]. Percutaneous biopsies are uncommon due to the risk of bleeding, perforation, and possible abscess formation, but may be considered for small bowel lesions in which endoscopy is not feasible, or in patients with non-diagnostic endoscopic biopsies [189, 256].

#### Indications

- ▶ To differentiate between acute rejection, chronic rejection, infections, and a variety of other inflammatory conditions.

### Contraindications to all transplant interventions

- ▶ Uncorrectable coagulopathy.
- ▶ Lesions not detected by US (contraindicated to perform the procedure by US). Fusion imaging with CEUS may allow this to be performed.

### Guided biopsy in focal and diffuse lesions

Biopsies are indicated to diagnose diffuse parenchymal disease and post-transplant focal or diffuse neoplasia including organ malignancy or PTLD.

### Description of the intervention

A variety of needles with different lengths and caliber can be used for INVUS procedures in transplant patients. End-cutting or side-cutting needles can be used with or without a coaxial technique. Biopsy guns are frequently used with diameters ranging from 20–14 gauge, 18 gauge being most commonly used. Color Doppler US imaging is helpful to select a biopsy area with relatively few vessels. In patients with an increased risk of bleeding, a plugged biopsy in which the biopsy track is plugged with collagen or thrombin may be safer than a standard percutaneous procedure [257].

### Liver transplant biopsy

A biopsy of a liver transplant is performed in the same way as a biopsy of a native liver [258, 259]. Local anesthesia is administered down to the liver capsule. An intercostal or subcostal approach is selected. If a focal lesion is targeted, a rim of normal liver tissue should be punctured before entering the lesion. A single biopsy with the use of a biopsy gun (18G needle) is preferred. Following a liver transplant biopsy, the patient should remain in bed and be monitored for  $\geq 4$  hours. The most common serious complication is post-biopsy bleeding, occurring in  $< 0.3\%$  of patients. CEUS may be helpful in diagnosing ongoing bleeding which may be managed with embolization.

### Kidney transplant biopsy

The lower renal pole area is preferred, but the upper pole or other regions of the kidney transplant may be selected [260]. Color Doppler will display vessels to be avoided, both inside the kidney and along the entire biopsy path. Local anesthesia is administered down to the kidney. An automated biopsy gun with a 16–18-gauge needle should be used. A cortical tangential needle approach to the kidney is preferred, and the needle should remain within the cortex when the biopsy is sampled. The direction of the deviation of the needle caused by the bevel should be towards the periphery of the kidney to reduce the risk of bleeding [260]. The specimen should ideally be examined immediately, under a stereo microscope, to verify the presence of a sufficient number of glomeruli for different pathological analyses (light microscopy, C4D, electron microscopy etc.). Following a renal transplant biopsy, the patient should remain in bed and be monitored for  $\geq 4$  hours. Hemorrhage and arteriovenous fistula are the two most common complications. Immediately after biopsy, color Doppler US or CEUS can identify any significant bleeding along the puncture tract which may be treated by US-guided compression [261]. CEUS may be helpful in diagnosing persistent ongoing bleeding, which may be treated by embolization.

### Biopsy of pancreatic transplant

The pancreatic transplant may be located behind the bowel and firm transducer pressure often allows bowel displacement to visualize the transplant.

Transcolic biopsy should be avoided. To reduce the risk of bleeding, the biopsy should be taken from an area with few vessels. To avoid the needle penetrating the dorsal aspect of the transplant, an oblique needle trajectory and an appropriate biopsy sample length should be selected. The deviation of the needle due to the bevel should be considered as the needle should remain entirely within the transplant during the biopsy. The most common needle diameter is 18 gauge, but 20 gauge needles have also been successfully used. The biopsy specimen is placed in formalin, and one biopsy is usually sufficient. The complications are hemorrhage and fistula formation. Color Doppler could be useful to identify ongoing bleeding after biopsy [261–267].

#### Recommendation 44

Ultrasound should be the first-line imaging modality to detect postoperative complications in organ transplants (LoE 5, GoR D). Strong consensus (100%).

#### Recommendation 45

A biopsy of a liver transplant should be performed using ultrasound (LoE 3b, GoR B). Strong consensus (100%).

#### Recommendation 46

Percutaneous ultrasound-guided biopsy of a renal transplant is a low-risk procedure (LoE 3b, GoR B). Broad agreement (100%).

#### Recommendation 47

Color Doppler should be used prior to transplant biopsy to reduce the risk of vascular complications (LoE 5, GoR D). Broad agreement (86%).

#### Recommendation 48

Percutaneous ultrasound-guided pancreatic transplant biopsies are to be performed in expert transplant centers (LoE 5, GoR D). Strong consensus (96%).

### Intervention in the elderly

When considering an invasive US-guided procedure in an elderly person (defined as  $> 75$  years), the benefit of making a precise diagnosis should generally have impact on the treatment plan. Based on the current limited literature focusing on the outcome of INVUS in elderly patients, ultrasound-guided tissue sampling and treatment is as safe and accurate as in younger patients [268–271].

#### Recommendation 49

The accuracy and complication rate of interventional ultrasound are similar in elderly ( $> 75y$ ) and younger patients. US-guided therapeutic procedures may replace more invasive and radical treatment methods, with an adequate outcome and better patient tolerance (LoE 4, GoR C). Strong consensus (100%).

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## Acknowledgement

We would like to acknowledge the advice from Lynne Rudd, EFSUMB general secretary.

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