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EUS-Guided Portal Venous Sampling of Circulating Tumor Cells

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Abstract

Purpose of Review Although liquid biopsies hold significant promise in the management of patients with cancer, peripheral blood analyses remain dependent on the degree of tumor burden with prohibitively low yields until the cancer is widely metastatic. Multiple lines of evidence support a dynamic, spatiotemporal localization of circulating tumor cells (CTCs) supporting specific targeting of vascular compartments, such as the portal vein. This review discusses the literature evaluating the possibility of portal venous blood as a new, potentially higher yield liquid biopsy and the current devices and techniques for endoscopic ultrasound (EUS)-guided portal venous sampling for CTC detection.

Recent Findings Two recent studies in pancreatic cancer have demonstrated that portal venous blood can be safely sampled via EUS and consistently yields significantly higher CTC counts compared with matched peripheral blood. EUS-acquired samples can be used for molecular testing, clinical prognostication, and drug sensitivity analyses.

Summary Portal venous CTCs are identified in higher quantity relative to peripheral blood and can be safely obtained via EUS. Further studies are required to demonstrate the clinical utility of EUS-guided portal venous tumor material enrichment and analysis; however, obtaining EUS-guided "liquid biopsies" appears to merit significant consideration for procedural adoption.

Keywords Pancreatic cancer · Endoscopic ultrasound · EUS · Circulating tumor cells · CTC · Circulating tumor DNA · Portal Vein · Liquid Biopsy

Abbreviations

CTC	Circulating tumor cell
ctDNA	Circulating tumor DNA
EUS	Endoscopic ultrasound
FNA	Fine-needle aspiration

Introduction

Endoscopic ultrasound (EUS) provides high-resolution, precise access to major abdominal vasculature, such as the portal vein, allowing for minimally invasive access for diagnostic, and therapeutic vascular interventions. Over the past 5 years, an increasing number of studies have reported on the feasibility and safety of EUS-guided vascular interventions, including portal venous sampling for diagnostic and surveillance purposes in gastrointestinal cancer, portal venous angiography, portal pressure gradient measurement, transhepatic intrahepatic portosystemic shunt creation, portal vein embolization, or targeted drug delivery [8, 23]. Although the technical aspects of EUS-guided vascular interventions continue to be refined, the relatively low risk of complications combined with the shortcomings of currently available peripheral blood tumor markers makes EUS-guided "liquid biopsies" of the portal vein an increasingly attractive approach for management of cancer. This review will discuss the literature on portal venous CTCs and current devices and methods for EUS-guided portal venous sampling for CTC detection.

Circulating Tumor Cells: Vascular Localization Determines Yield

Circulating tumor cells are among several cancer-derived materials shed from primary tumors that circulate through the

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vasculature. Intact CTCs disseminate into the blood stream as single cells or clusters/microemboli and have been reported in multiple malignancies including lung [31], breast [20, 25], prostate [10], colorectal [19], pancreatic [3•, 13], and hepatobiliary cancers [12]. When isolated and enriched from the blood, CTCs can serve as a minimally invasive tool providing solid tumor molecular characterization and prognostication.

In the peripheral blood, however, CTCs are extremely rare—with an estimation of one to ten tumor cells interspersed with billions of normal circulating red blood cells and millions of white blood cells [18]. Peripheral blood draws are ideal due the ease of access and minimal risk and multiple studies have demonstrated that peripheral blood CTC enumeration can correlate to overall survival, risk of metastasis, and guide therapy decisions. Despite studies demonstrating importance to cancer management, in many studies patients have metastatic disease burden with very low CTC counts. For example, in the study of metastatic breast and prostate cancers by Weissenstein et al., the "unfavorable" cohort had CTC counts ranging from 3–5 CTCs per 7.5 mL [30]. Similarly, Hiraiwa et al. reported the "high-CTC" cohort containing 2 or more CTCs/7.5 mL of peripheral blood were associated pleural and peritoneal dissemination [9]. Thus, depending on the malignancy and particularly in the non-metastatic disease state, peripheral blood specimens are frequently too low yield for clinical utility.

Multiple lines of evidence support a dynamic, spatiotemporal localization of CTCs suggesting that specific targeting of vascular compartments may provide higher yields of tumor signature material [26••]. The spatial localization (central/mesenteric vs. peripheral) differences are suggested by the clinical observation that in many gastrointestinal malignancies (pancreaticobiliary, colon), the most frequent site of distant metastatic spread is to the liver, the first-pass organ for gastrointestinal venous blood drainage via the portal system. Contrarily, in patients with distal rectal cancer, in which the blood drainage bypasses the liver via the internal iliac vein directly to the inferior vena cava, there is a higher propensity for lung metastases [21]. Sized-based first-pass organ sequestration is supported by animal models in which radiolabeled cancer cell injection into the portal vein and tail vein resulted in entrapment in the first-pass organ and massive cancer cell death [29]. Most recently, Sun et al. mapped the preoperative CTC distribution in patients with hepatocellular carcinoma by performing simultaneous blood draws from the peripheral vein, peripheral artery, hepatic veins, infrahepatic inferior vena cava, and portal vein [26••]. The different location of blood draws demonstrated varied distribution of CTCs along the dissemination pathway, with 80.8% of patients having CTCs in the hepatic vein (pre-first-pass organ, i.e., the lung) compared with 39.7% and 58.9% of patients in the infrahepatic inferior vena cava and portal vein, respectively (post-first-pass organ). Contrarily, in malignancies in which the first-pass organ is the liver (colorectal and pancreatic cancer), surgically acquired portal venous sampling has consistently revealed CTC detection at a significantly higher rate in portal than in peripheral venous blood [1, 19, 27].

Advances in enrichment techniques and analysis tools have yielded nearly 50 methods for CTC detection [7]; however, the Change to CellSearch system (Menarini Silicon Biosystems, Huntingdon Valley, PA, USA) remains the only FDA-approved method for CTC enumeration in whole blood. As enrichment and molecular genomic profiling technology are progressing toward single-cell analyses, the desire to move beyond enumeration and towards molecular characterization currently remains in large part dependent on the quantity of tumor material available. In summary, to overcome the limitations of peripheral blood assessment for CTCs, a targeted approach to site-directed blood acquisition will result in higher yields of tumor material for analysis.

Obtaining Liquid Biopsies of the Portal Vein for Tumor Analysis

During surgery, the extrahepatic portal vein can be directly punctured for simple, safe extraction of blood from the portal vein. However, minimally invasive access to the portal vein could provide personalized risk stratification and therapeutic decision-making prior to the use of neoadjuvant chemotherapy and/or surgery. As EUS provides both real-time imaging of the abdominal vasculature and the ability to take directed biopsies, it appears to be an optimal method for minimally invasive diagnostic sampling of the portal vein. Beginning in 2015, there have been two published reports detailing the acquisition and assessment of circulating tumor materials by accessing the portal vein via EUS guidance.

Catenacci et al. published the first assessment of EUS for the sampling of portal venous blood and enumeration of CTCs in patients with pancreaticobiliary cancers. Portal venous blood specimens were obtained using a 19G EUS-FNA needle, and in conjunction with the CellSearch EpCAM-based enrichment platform, CTCs were identified in portal vein samples from all 100% of patients (18 of 18) as opposed to only 22.2% of (4 of 18) matched peripheral blood samples. Among patients with confirmed malignancy, there was an average of 118.4 ± 36.8 CTCs per 7.5 mL of portal venous blood compared with an average of 0.8 ± 0.4 CTCs per 7.5 mL of matched peripheral blood ($P < .01$). Among patients with resectable, or borderline resectable pancreaticobiliary cancers, there were on average less portal venous CTCs per 7.5 mL (83.2, median, 62.0; range, 1–265) as opposed to patients with unresectable pancreaticobiliary cancers (157.9, median, 73.5; range, 9–156), ($P = .23$). This study has

provided results that not only prove portal venous blood can be acquired safely via EUS but also proves that portal venous blood yields significantly higher CTC counts compared with peripheral blood.

In 2018, Liu et al. published their findings on EUS-guided portal venous blood acquisition for CTC analysis in patients with pancreatic cancer [16••]. In this study, peripheral blood samples were acquired simultaneously with EUS-acquired portal venous blood. Blood samples were acquired with a 20G FNA needle and a sized-based, label-free enrichment method was used. Showing significant similarities to what was reported in the study by Catenacci et al., CTCs were identified in 100% of portal venous samples as opposed to only 54% of corresponding peripheral blood samples. Portal venous blood also contained significantly more CTCs per 7.5 mL relative to peripheral blood with 282.0 CTCs compared with 21.0, respectively. Further, patients with metastatic disease had significantly more CTCs (449.0 per 7.5 mL) compared locally advanced pancreatic carcinoma (161.0 per 7.5 mL). Advancing the field beyond enumeration, Liu et al. provides novel data detailing the potential use of EUS-guided CTC analysis for prognostication and therapeutic guidance. Patients with more than 150 CTCs per 7.5 mL of portal venous blood had a significantly shorter overall survival (19.8 weeks vs. 9.2 weeks). Furthermore, using the acquired portal venous CTC samples, the authors managed to test ex vivo CTC cultures to a panel of therapeutic agents to determine drug sensitivity. This testing was only successful in 24% of patients, however, in one patient the ex vivo testing demonstrated resistance to standard chemotherapy but sensitivity to a small molecule inhibitor of the KRAS-PDE-δ interaction.

Finally, although not technically designed for the assessment of CTCs, in 2018 Levy et al. utilized EUS-guided portal venous blood sampling to assess the effects of EUS-FNA of primary, solid pancreatic masses on primary tumor shedding into the portal vein [15]. To assess this issue of potential iatrogenic tumor dissemination, the authors performed EUS-guided portal venous blood acquisition before and after (within 15 min) EUS-FNA of suspected primary pancreatic tumors. Multiple relevant procedural issues are discussed including safety, as well as technical decisions (e.g., FNA needle selection and access approach), and challenges including premature blood clotting. For example, the authors report the technical issues with blood clotting using a 22-G needle: one of the most common issues with EUS-guided portal vein blood sampling. In the study, 10 patients underwent portal vein EUS-guided FNA; however, only 5 patients were able to complete molecular analysis due to addition of heparin (10,000 USP) to the Streck tubes which is known to inhibit downstream molecular analyses [28]. The authors noted "slow" aspiration from the portal vein requiring transition in technique to add 2 mL of heparin to prevent blood clotting.

EUS-Guided Portal Venous Sampling Technique

Pre-Procedural Considerations

The following methods discussed for EUS-guided portal vein access for blood sampling are based upon our personal experience [3•], additional published reports including other EUS-guided portal vein procedural data [8, 11, 15, 16••], and extrapolated safety data from percutaneous, transhepatic access by interventional radiology [5]. There is limited data on the safety and the technical methods are continuously evolving; therefore, we recommend this novel technique be performed under an IRB-approved protocol with adequate explanation of risk and benefits. Prior to starting the procedure, we suggest only considering EUS-guided vascular procedures under monitored anesthesia care or general anesthesia, utilizing only CO_2 insufflation, and only after any bleeding risks have been addressed (i.e., coagulopathy, use of anticoagulants). Additionally, appropriate supplies for collection, stabilization, and transport of nucleated blood cells or cell-free nucleotides need to be obtained and ready for immediate use. Although not routinely done in our endoscopy unit, some endoscopists have advocated administering a dose of prophylactic intravenous antibiotics.

Timing of EUS-Guided Portal Vein Sampling: Pre- vs. Post-EUS-FNA Diagnosis Confirmation Accessing of the Portal Vein

Although EUS-guided portal venous sampling for cancer may have future applications to non-pancreaticobiliary cancers, such as colon cancer, a primary indication for EUS is for the tissue acquisition of pancreatic and biliary mass lesions. EUS-FNA is believed to be safe for tumor diagnosis with a recent study reporting that preoperative EUS-guided FNA does not impair survival of patients with resected pancreatic cancer [17]. However, derived from concerns that surgical resection techniques can increase the risk of CTCs in the portal vein [6], there remains concern regarding EUS-FNA-induced tumor material dissemination increasing the potential risk of metastasis or in the diagnostic setting, artificially increasing the yield of portal venous tumor material. The aforementioned report by Levy et al. is the first to directly assess if EUS-FNA results in iatrogenic tumoremia [15]. The authors identified no statistically significant change in median peripheral or portal blood plasma circulating free DNA concentration in samples obtained within 15 min of EUS-FNA (portal vein - pre: 1100 (430–3210) ng/mL vs. post: 1300 (320–3010) ng/mL; $P = .853$).

In our initial pilot and feasibility study [3•] as well as the recent study by Liu et al. [16••], EUS-FNA of the primary lesion with diagnosis confirmation via rapid on-site evaluation

was completed prior to accessing the portal vein. Given the data published by Levy et al. demonstrating no significant change in portal venous cell-free DNA after EUS-FNA of a primary lesion, we hypothesize that timing of EUS-guided portal vein sampling relative to EUS-FNA of solid malignant lesions does not significantly alter tumor material yield. However, Levy et al. did identify several patients with a post-FNA ≥ 2-fold increase in cell-free DNA and new KRAS mutations in the peripheral blood, suggesting that further research is necessary for confirmation.

EUS Needle Selection

With the advent of EUS-guided tissue acquisition techniques including FNA and more recently, fine-needle biopsy (FNB), EUS can provide real-time cytologic and histologic sampling. There are multiple EUS-FNA and FNB needles currently available for use including needles with variable sizes (19 G, 22 G, and 25 G sizes) and with proprietary bevels/tips, materials, or sheaths [4]. The selection of EUS-FNA needle is typically dependent on the target lesion characteristics and a balance of providing the largest sample size, while minimizing adverse events.

In the available reports on EUS-guided portal vein sampling, three different needle sizes were used. In our practice, we use a 19 G Echotip Ultra (Cook Endoscopy, Winston-Salem, NC), while both Liu et al. and Levy et al. reported using smaller sized needles, 20–21-G FNA needles and 22-G EUS-FNA needle, respectively. In our experience, the 19-G EUS-FNA needle allows adequate blood flow to minimize time within the vessel and appears to reduce clotting compared with smaller gauge needle sizes. Although Liu et al. did not report any issues with clotting, Levy et al. demonstrated the smaller size needles were more prone to clotting—with the authors noting, "slower" aspiration with the 22-G needle. This resulted in an adjustment to their acquisition technique by adding 2 mL of heparin (10,000 USP) to Streck tubes, which unfortunately then interfered with downstream molecular analyses. Given the safety profile and adequate biospecimen acquisition in our feasibility trial, we encourage use of a larger bore, 19-G FNA needle.

Portal Vein Access Location: Transhepatic vs. Extrahepatic Portal Vein

Given the anatomic proximity to the bowel, the extrahepatic portal vein can often be visualized from the proximal duodenum or distal stomach and can be traced into the liver as it becomes the intrahepatic portal vein and subsidiary branches. Both transgastric or transduodenal transhepatic access to the intrahepatic portal vein and transduodenal extrahepatic access to the extrahepatic portal vein have been published [3•, 14, 15, 16••, 22].

In 2004, Lai et al. published the first report of EUS-guided portal vein access in a porcine model [14]. In this animal study, the authors used a 21-G EUS-FNA needle to access the extrahepatic portal vein via a transduodenal approach. In this study, they obtained EUS-guided portal pressure measurements and upon procedure completion, performed necropsy. At the post-intervention necropsy, there were small subserosal hematomas at the EUS puncture site in every pig. In one anticoagulated pig, there was a small (approximately 25 mL) collection of blood between the PV and duodenum. Additionally, during an intra-operative direct puncture access of the extrahepatic portal vein for CT enrichment using a 21-G needle (PrecisionGlide Needle 21 G 1 1/2 TW; BD Becton, Dickinson) [27], the authors noted bleeding that stopped after digital compression in 65 of 66 patients; however, 1 patient did require placement of a 6–0 Prolene suture.

Thus, while identifying the extrahepatic portal vein offers less technical difficulty, we recommend transhepatic routes due to the benefit of liver parenchyma tamponade of the FNA needle track. Beginning with our 2015 study for EUS-guided portal vein CTC acquisition, as well as the subsequent studies by Liu et al. and Levy at al., transhepatic routes were utilized in all cases for portal venous sampling.

EUS-Guided Portal Vein Sampling Technique

Once the extrahepatic portal vein is identified from the proximal duodenum or distal stomach and traced into the liver, we recommend the following steps to ensure optimal safety and accurate sampling. Prior to EUS-guided sampling of the intrahepatic portal vein, color Doppler evaluation of the liver should be performed to confirm patency of the hepatic artery, portal vein, and hepatic veins. Once a baseline of the major hepatic vasculature is obtained, care must be taken to (i) not go through any visible metastatic lesions, including hepatic parenchymal lesions or lymph nodes, (ii) ensure an absence of interposed vasculature using color Doppler, (iii) identify the left and right portal vein branches with an angle and scope position to allow maximum stability (minimizing scope torque) for blood aspiration without movement and shearing of the vessel, (iv) ensure the target vessel has flow and a venous waveform with Doppler, and (v) minimize the number of passes into the target vessel. In addition, careful attention to the location of the hepatic artery branches and bile ducts relative to the intrahepatic portal vein branches is required as these vessels and ducts course together and potentially could lead to complications or inaccurate sampling if the unintended structures are catheterized (e.g., hemobilia, hepatic artery blood sampling) (Video/Fig. 1).

Negative Suction

Although there is considerable debate and conflicting results on the optimal techniques for EUS-FNA of solid masses, when aspirating blood from the portal vein, negative suction is definitively required. Negative pressure suction has been reported to be standard suction (10–20 mL negative suction pressure) to high pressure suction (up to 50 mL negative suction pressure) [24]. In our experience, we utilized a 10-mL negative suction syringe for portal vein sampling. After the EUS-FNA needle is advanced into the intrahepatic portal vein or subsidiary branch, the stylet is removed and a 10-mL negative suction syringe applied to the FNA needle. If the stylet is withdrawn to sharpen the tip of the FNA needle, it should be advanced prior to collecting blood to remove unintended epithelial "pick-ups" such as hepatocytes or intestinal wall tissue. With the negative suction, blood is aspirated up the shaft of the EUS-FNA needle into the negative suction syringe. Immediately, after aspirating up to 10 mL of blood, an assistant should (i) apply a second pre-prepared negative suction syringe to aspirate a second 10 mL volume and (ii) place the first aspirated volume into a vacutainer tube for downstream application and repeatedly invert to mix the blood.

It is worth noting that Levy et al. implemented a protocol in which the first 5 mL of blood was discarded to ensure a pure portal venous sample [15]. In our study, we did not standardize discarding the initially acquired blood—to minimize time in the vein and lower the risk of clotting within the needle. To test intra-patient variance, in 4 patients, we processed two sequential portal venous samples and found good correlation in CTC yield from the first and second samples [3•].

Post-Procedure Monitoring

After the acquisition of the portal venous blood, the EUS-FNA needle is withdrawn into the sheath under direct EUS visualization. The intrahepatic needle track should be observed with color Doppler to assess for persistent flow. In our practice, the puncture site is monitored under direct EUS color Doppler visualization for complications for a minimum of 5 min in the endoscopy suite. Patients are observed in the GI post-procedure recovery area for a minimum 45 min after the procedure. We routinely made telephone calls 24 h and 7 days after the procedure to further assess recovery. Similarly, in the EUS-FNA-induced tumoremia study, Levy et al. monitored patients during the procedure and in the postoperative recovery area. Patients were also seen in clinic within 7 days and contacted by telephone 15–30 days and if necessary, 2–4 months post-procedure [15].

EUS-Guided Portal Vein Access for Blood Acquisition: Complications and Troubleshooting

Hemorrhage

Immediate or delayed hemorrhage is one of the most significant concerns with EUS-guided vascular procedures. To minimize risk of complications, prior to proceeding with the procedure, patients should be optimized by holding anticoagulation medications and maintaining an international normalized ratio < 1.5, platelet count > 50 × 10^9/L. In the pilot study of portal pressure gradient measurement, Huang et al. suggest selecting patients without evidence post-hepatic/sinusoidal portal hypertension may reduce the risk of needle track bleeding [11].

In all of the available published literature on EUS-guided portal vein sampling via an intrahepatic access point, there has been no report of significant immediate or delayed gastrointestinal bleeding. Thus, although preliminary data appears to not require prophylactic bleeding interventions, sclerosants, cyanoacrylate, thrombin, and coils could theoretically be applied to the site of portal entry under EUS guidance similar to the use of metal coils or gelfoam plugs used to reduce bleeding events in interventional radiology-guided portal vein islet cell transplantation [5].

Blood Sample Clotting

When EUS-FNA is used for blood aspiration, yield may be lower due to clotting of the blood sample. After access to the portal vein is obtained and negative suction is applied, blood has to travel the length of the echoendoscope via a FNA needle not designed to prevent thrombosis. Further, these FNA needles are not designed for blood acquisition and the aspiration in the needle can exert shear forces, creating a predilection to cell lysis [2]. In accordance with this issue, Levy et al. reported "slow" aspiration of central venous blood requiring a protocol adjustment of adding an anticoagulant to the collection tubing. However, the addition of 2 mL of heparin (10,000 USP) resulted in interference with downstream molecular testing in 50% of their portal vein samples [15].

While the methods and devices for portal venous blood sampling continue to be optimized and developed, we recommend at the minimum, rapid transfer from the negative suction syringe into vacutainer tube containing cell preservatives for downstream application. Additional methods that may help reduce clotting include priming the negative suction syringe or EUS-FNA needle by flushing a small amount of (1 mL) of anticoagulant solution (e.g., EDTA or citrate). However, as demonstrated by Levy et al., the choice of anticoagulation solution must be carefully considered and ensured to be safe and compatible for downstream applications [28].

Conclusions

EUS-guided vascular access for diagnostic and therapeutic interventions is an evolving frontier in advanced endoscopy with an increasing number of studies utilizing EUS-guided portal vein access for circulating tumor cell enumeration, cell-free DNA analysis, and portal pressure gradient monitoring. In several gastrointestinal malignancies, portal venous CTCs are identified in higher quantity relative to peripheral blood, including in non-metastatic surgical candidates, and in preliminary studies, have been demonstrated to provide prognostic value. Further studies are required to demonstrate the clinical utility of EUS-guided portal venous tumor material enrichment and analysis; however, obtaining EUS-guided "liquid biopsies" appears to merit significant consideration for procedural adoption given the limitations of peripheral blood sampling, low barrier of entry, technical reproducibility, and low risk of complications when using standardized procedural techniques.

Compliance with Ethical Standards

Conflict of Interest Christopher Chapman reports personal fees from Boston Scientific and Apollo Endosurgery, outside the submitted work. Irving Waxman reports personal fees from Medtronic, Boston Scientific, and Olympus; and grants and personal fees from Cook Endoscopy, outside the submitted work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Disclosures Irving Waxman has served as a consultant to Olympus, Cook Medical, Medtronic, Boston Scientific; Christopher Chapman has served as a consultant to Apollo Endosurgery and Boston Scientific.

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