

Safety of endoscopic ultrasound-guided tissue acquisition with uninterrupted direct oral anticoagulation in a swine model



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ABSTRACT

Background and study aims The risk of bleeding with endoscopic ultrasound-guided tissue acquisition (EUS-TA) in patients on uninterrupted antithrombotic agents is unknown. This study aimed to assess the safety of EUS-TA without discontinuing direct oral anticoagulants.

Methods Twenty pigs were randomized (1:1) to oral apixaban or placebo. Treatment was administered for 3 days before and 3 days after EUS-TA of the pancreas. Primary endpoint was occurrence of clinically significant bleeding events (CSBEs), defined as a composite outcome of drop in hemoglobin ≥ 2 g/dL and evidence of intraprocedural or post-procedure bleeding. Secondary outcome was cytopathological adequacy of acquired specimens. Endoscopists and pathologists were blinded to the treatment arm.

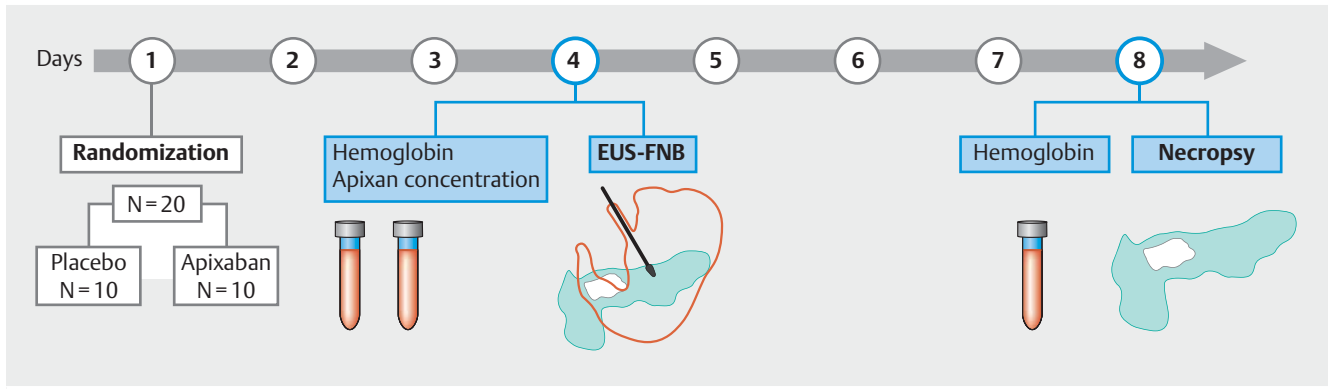
Results Total of 80 tissue samples were obtained. CSBE occurred in one animal with apixaban levels above the upper limit ($P=1$). Intraprocedural bleeding occurred in five animals (25%), four of which from the apixaban group. Hematoma at necropsy was found in 10 animals (50%), six of which were on apixaban. Median drop in hemoglobin was similar in both groups. Adequacy of specimens for cytology and pathology evaluation was confirmed in 20 of 20 and 19 of 20 animals, respectively.

Conclusions In this pilot preclinical trial, EUS-TA under apixaban showed a trend toward higher minor bleeding events but did not increase CSBE or prevent adequate cytopathological evaluation.

Introduction

Bleeding in patients undergoing high-risk gastrointestinal endoscopy while on antithrombotic therapy remains a major concern. Data on the need to interrupt direct oral anticoagulants (DOACs) before an endoscopic procedure are scarce. Cur-

rent guidelines recommend discontinuation tailored to the bleeding risk of the procedure versus the thrombotic risk of the patient based on low-quality evidence. Recent studies highlight the risk of thrombotic events associated with transient interruption of anticoagulants with a 30-day incidence of up to 5.4% [1]. Early evidence suggests it may not be necessary to



► Fig. 1 Study design.

withhold anticoagulants for certain therapeutic procedures such as small colorectal polypectomies [2]. Bleeding from endoscopic ultrasound-guided tissue acquisition (EUS-TA) is infrequent occurring in 0.1% of cases [3], and usually mild and self-limiting.

There is a lack of data on bleeding outcomes when DOAC therapy is uninterrupted with EUS-TA. We hypothesized that EUS-TA performed without discontinuing DOACs would not be associated with an increased risk of significant bleeding. We conducted a randomized study in a swine model aimed to assess the safety of EUS-TA without interruption of DOACs.

Methods

Study design

This randomized controlled pilot study was performed according to ARRIVE 2.0 recommendations for animal studies. All experiments followed the American Association for Laboratory Animal Science guidelines and the study protocol was approved by the Institutional Animal Care and Use Committee (IACUC protocol #A00005862–21).

Twenty domestic pigs (weight 35–40 Kg) were randomized (1:1) to receive anticoagulant treatment with oral apixaban (0.5 mg/Kg/12 h) or placebo. Treatment was administered 3 days before EUS-TA of the pancreas, the day of the procedure, and three days after the procedure. The study design and timeline are detailed in ► Fig. 1.

Procedure

All animals underwent EUS fine-needle biopsy (EUS-FNB) of the pancreas tail using a 22G needle (Sharkore, Medtronic, Minnesota, United States) using the same approach of 4 passes with 10 to-and-fro movements and 3 to 5 cc of negative pressure. Two each of the EUS-FNB samples were submitted to cytologic and histologic evaluation. Smear slides for cytology were prepared for rapid on-site evaluation (ROSE) while specimens for histology were placed in formalin. Endoscopists performing EUS-TA and necropsy, and pathologists were blinded to the treatment arm. All procedures were performed with a linear echoendoscope (GF-UCT260, Olympus, Tokyo, Japan). Detailed information is displayed in **Supplementary material**.

Definitions and outcomes

The primary endpoint was occurrence of clinically significant bleeding events (CSBEs), defined as a composite outcome of drop in hemoglobin level ≥ 2 g/dL [4, 5] and evidence of intraprocedural or post-procedure bleeding. Intraprocedural extraluminal bleeding on EUS was defined as a new hypoechoic area at the site of needle puncture. Post-procedure bleeding was defined as clinical signs of blood loss during the survival period and/or hematoma documented at necropsy. Bleeding at the puncture site without a drop in hemoglobin of ≥ 2 g/dL was considered a minor adverse event.

The secondary endpoint was the adequacy of acquired specimens defined as confirmation of pancreas tissue by cytopathological evaluation (**Supplementary Material**).

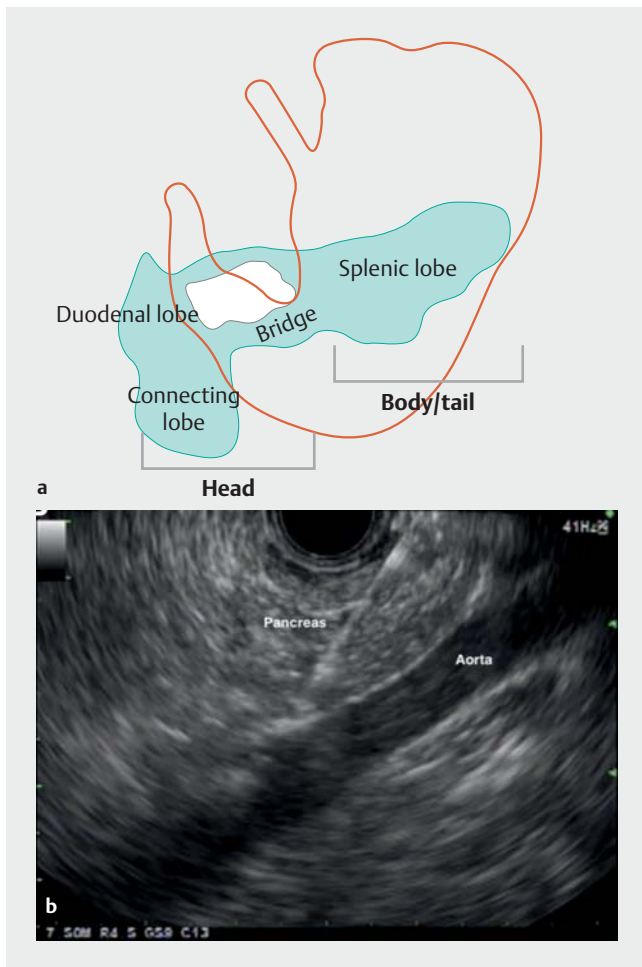
Statistical analyses

Randomization sequence using blocks was applied. Categorical variables are displayed as absolute and relative frequencies. Intergroup comparisons were made using the Fisher's exact test. Quantitative variables are reported as mean and standard deviation or as median and interquartile range (IQR), when appropriate. Comparisons for independent continuous variables were performed using the Mann-Whitney U test. All analyses were two-tailed and significance was set at $P < 0.05$. A sample size of 10 pigs per arm provided an E value (E value = total number of animals-total number of groups) of 18 and allowed non-parametric tests (further information on sample size calculation is detailed in **Supplementary Material**).

Results

Study population

Twenty pigs (55% females; mean weight 38.1 Kg, standard deviation 1.7) were randomized to oral apixaban (n = 10) or placebo (n = 10). Median hemoglobin before the procedure was 10.9 g/dL (IQR, 10.2–11.2). Median apixaban concentration on day of EUS-FNB in treatment pigs was 365.15 ng/mL (IQR, 252.5–484.8) with a median time between the last apixaban dose and blood draw of 2.2 hours (IQR, 1.08–3.25). Two pigs had an apixaban concentration > 500 ng/mL (on therapy range in humans: 20 to 500 ng/mL [6]).



► **Fig. 2** a Cartoon of pig pancreas anatomy. b Endoscopic ultrasound-guided fine-needle biopsy of the pancreas body/tail.

Procedure and follow-up

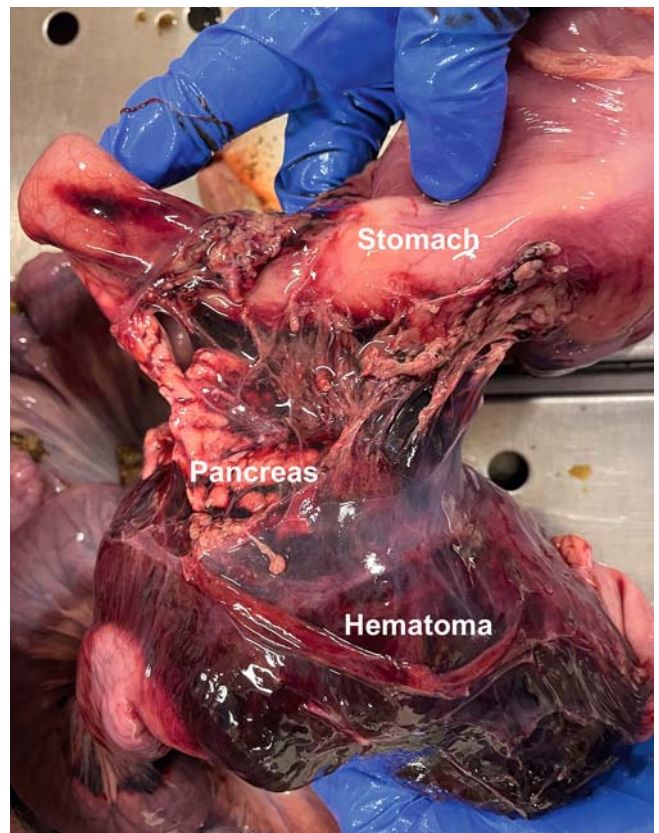
EUS-FNB sampling of the pancreas body/tail was successfully performed in all 20 pigs (four samples/pig) (► **Fig. 2**). A total of 80 tissue samples were obtained.

Twenty-four hours post-procedure, one animal from the apixaban group presented with behavioral changes of reduced activity and poor oral intake which subsequently improved within 24 hours with intravenous analgesia and liquid diet.

Endoscopic ultrasound-related bleeding

Clinically significant bleeding

Only one animal in the apixaban group presented with CSBE (10%; 95% confidence interval [CI] 1.8–40.4% versus 0%; 95% CI 0–27.7% in the placebo group). This animal experienced a drop in hemoglobin level ≥ 2 g/dL (3.4 g/dL) and a large hematoma (120×11 mm) at the puncture site noted at necropsy (► **Fig. 3**). This pig had an apixaban concentration above the upper range (> 500 ng/mL) and was the same animal that presented with post-procedure lethargy and poor oral intake. No intra-



► **Fig. 3** Large hematoma in pig that experienced a clinically significant bleeding event.

procedural extraluminal bleeding was observed during EUS in this animal.

Median drop in hemoglobin was similar between both groups, **Supplementary Fig. 1**.

Non-clinically significant bleeding

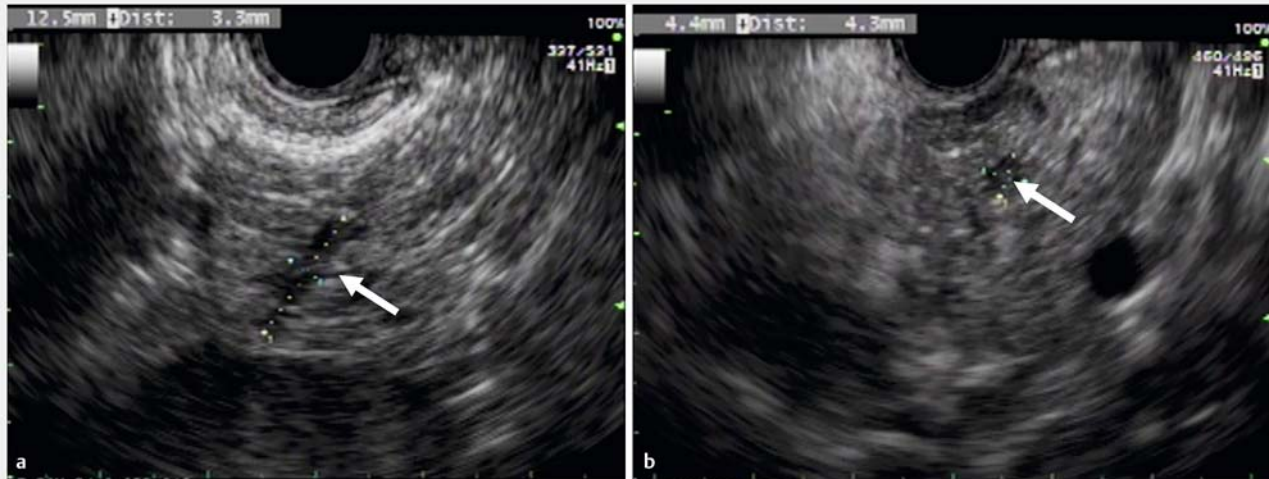
Minor extraluminal bleeding observed at EUS occurred in five pigs (25%), of which four were from the apixaban group ($P=0.3$). Bleeding manifested in the form of a new hypoechoic area at the puncture site (median size 5.5 mm [IQR, 4.8–6.3]), all of which were self-limited (► **Fig. 4**; **Supplementary Table S1**).

Hematoma at necropsy was observed in 10 pigs (50%) (median size 17.5 mm [IQR, 11–20]), six of which occurred in the apixaban group ($P=0.65$) (► **Fig. 5**; **Supplementary Table S1**). Four of the 10 animals that had a hematoma at necropsy had documented intraprocedural extraluminal bleeding.

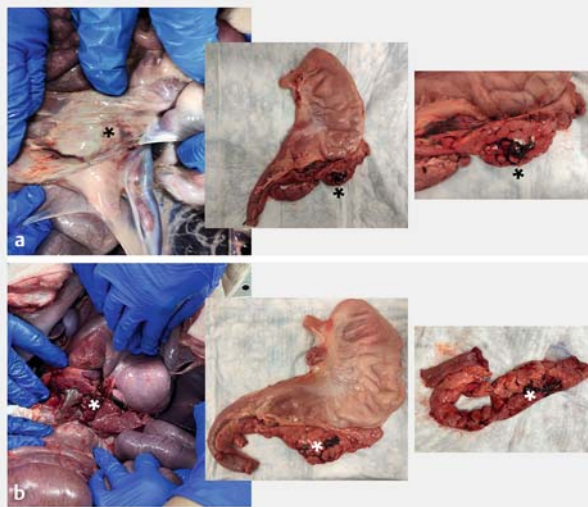
Cytopathological assessment

An adequate specimen for cytological evaluation and confirmation of pancreatic tissue was acquired in all 20 animals (100%) (median number of smear slides 5 [IQR, 4–7]).

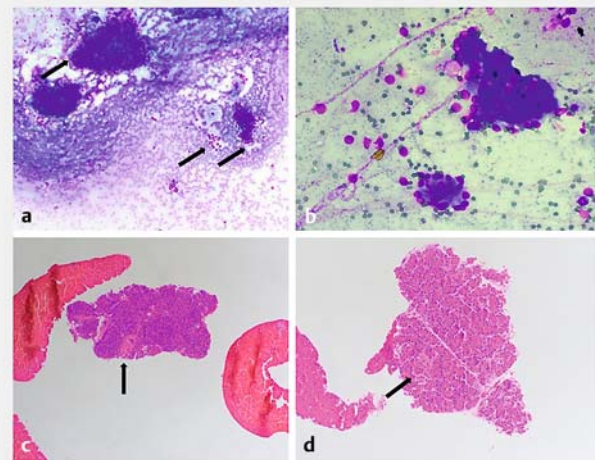
An adequate specimen for histological assessment and confirmation of pancreatic tissue was acquired in 19/20 (95%) animals. Core area median size was 4.6 mm² (IQR, 1.2–4.6), and



► Fig. 4 Intraprocedural minor bleeding at the puncture site.



► Fig. 5 Non-clinically significant hematoma at the puncture site in animal on **a** apixaban and **b** placebo.



► Fig. 6 **a, b** Cytology and **c, d** pathology images confirming pancreatic tissue.

pancreatic tissue represented 6% (IQR, 1.6–2.5) of the core area (► Fig. 6, Supplementary Table S1).

Discussion

Bleeding related to EUS-TA is rare and the risk in patients treated with antithrombotic drugs is unknown. Adverse events associated with transient interruption of anticoagulants may outweigh the potential hemorrhagic complications. In our pilot preclinical trial with a limited sample size EUS-TA on apixaban did not significantly increase the occurrence of CSBE or prevent adequate cytopathological evaluation.

Only one animal with levels of apixaban above the upper limit of detection experienced CSBE. Of note, the majority of ani-

mals with a hematoma at necropsy did not have documented intraprocedural bleeding highlighting that bleeding may be missed, may occur beyond the endosonographic field of view, or result from delayed hemorrhage. Although more animals in the apixaban group had minor bleeding events, there was no significant difference between the groups. Furthermore, the difference in the median drop of hemoglobin post-procedure between the groups was similar.

Apixaban was selected being the most frequently prescribed DOAC in clinical practice [7]. Oral apixaban has been used in swine in two prior studies that demonstrated lower bioavailability, shorter half-life, and a larger volume of distribution compared to humans, and thus requirement of higher doses [8]. Specifically, a dosage of 0.5 mg/Kg/12 h was found to maintain an adequate anti-factor Xa activity [8]. Apixaban has been associated with a lower overall bleeding risk [9], with a lower in-

cidence of upper gastrointestinal bleeding and post polypectomy bleeds compared to other DOACs. We determined apixaban blood concentration before EUS-TA for the purposes of the study, even though apixaban is generally given at a fixed dose without monitoring in clinical practice, to ensure animals had increased anti-factor Xa activity relative to a sham.

The porcine pancreas was the target puncture site as solid pancreatic lesions are the most common indication for EUS-TA. In this pilot study we sampled normal pancreatic parenchyma which may result in higher incidence of adverse events compared to focal tissue acquisition of a solid pancreatic neoplasm. Fine-needle biopsy was chosen over fine-needle aspiration (FNA) as this approach would provide both cytological and histological specimens. There are no reported significant differences in diagnostic yield and adverse events between FNA or FNB techniques. The 22G needle was studied as it represents the predominant needle of choice in clinical practice.

Current guidelines consider EUS-TA a high-risk procedure and recommend against sampling in patients on DOACs and, if sampling is indicated, discontinuation is recommended (last dose \geq 48 hours before the procedure and resumption up to 48 hours after the procedure). The available evidence on bleeding secondary to EUS-TA in patients on antithrombotics discontinued according to guidelines is very limited, as most studies include a small number of patients and specifically DOACs are underrepresented. The reported bleeding rate ranges between 2.4% to 3.5% and, in most cases, the bleeding was not severe and could be managed conservatively with no need for blood transfusion [5, 10]. A prospective multicenter study on 85 patients on antithrombotics (14 on DOACs) reported two bleeding events in patients on antithrombotics (one on dual antiplatelet therapy and one on warfarin, 2.4%; 95% CI 0.6–8.3%). Both patients experienced bleeding despite discontinuation of thienopyridine and warfarin [10]. Another single-center retrospective Japanese study evaluated 908 patients undergoing EUS-FNA of which 114 were on antithrombotics (eight on DOACs). The authors observed higher bleeding events in the antithrombotic group (3.5%) compared to the non-antithrombotic group (0.8%), $P=0.02$. All bleeding was post-procedure and was significant in six patients, four from the antithrombotic group and two from the non-antithrombotic group (odds ratio: 9.59; 95% CI 2.12–43%). However, none of the patients required blood transfusion and were treated conservatively [5].

Our pilot study has several limitations including a small sample size, that was intentionally designed to minimize animal use while providing both meaningful and relevant data consistent with the objectives of the study. Future clinical trials with a larger population size are needed to support or refute these findings. We opted for a DOAC with the theoretically lowest bleeding risk and as such bleeding may differ with other DOACs. Also, societal recommendations to assess endoscopy-related adverse events is 14 days post-procedure [4]; however, bleeding related to EUS-TA generally occurs during the procedure or within 72 hours [3] which was the basis for our survival interval of 4 days.

Conclusions

There is a clinical need to minimize the risk of periprocedural thromboembolic events in anticoagulated patients undergoing high-risk endoscopy. Continuous anticoagulation seems safe for some endoscopic procedures, but no data is available for patients on uninterrupted DOACs undergoing EUS-TA. Although our study showed a trend toward higher minor bleeding events in animals on apixaban, EUS-TA with apixaban did not increase the incidence of clinically significant bleeding or prevent adequate cytopathological evaluation. This pilot study may set the framework for future large population human trials.

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Competing interests

Dr. Storm has received research grant support from Apollo Endosurgery, Boston Scientific, Endogenex, Endo-TAGSS and Enterasense and is a consultant for Apollo Endosurgery, Boston Scientific, ERBE Elektromedizin, GI Dynamics, Intuitive Surgical and Olympus. Dr. Rajan has intellectual property with Medtronic and Ruhoff Corporation and is a consultant for Olympus and Ethicon.

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