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Endoscopic Ultrasound guided perivascular pancreatic Radiofrequency Ablation using a Hydroxyethyl Starch Solution prior to Pancreatectomy


Affiliations below.

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Abstract:
Background and study aim. Pancreatic surgery remains complex, particularly for borderline resectable and locally advanced tumours. Vascular invasion compromises resectability, and vascular resection entails increased morbidity and mortality. Following a feasibility and safety demonstration of an augmented endoscopic ultrasound (EUS) guided radiofrequency ablation (RFA) using hydroxyethyl starch (HES) in porcine pancreatic parenchyma, the present study assesses whether this approach (EUS-sugar-RFA) in the pancreatic perivascular space is safe and creates a controllable margin of necrosis to enable a vessel-sparing resection.

Methods. EUS-sugar-RFA in the pancreatic parenchyma adjacent to the splenic artery and vein was performed in a live animal model. Following different survival periods (0-4 days) in the interventional group (n=3), open pancreatectomy was carried out. The control group (n=4) included open pancreatectomies in two pigs with non-treated pancreases and in two with pancreatic RFA alone on the same day.

Results. All procedures were completed successfully, without intra- or postoperative complications. Survival periods were uncomplicated. Histopathological examination showed local necrosis and inflammatory reaction at the ablation sites. Vascular wall integrity was preserved in all specimens. The untreated pancreatic zones in the interventional group were no different from the normal pancreases in the control group.

Conclusions. Preoperative perivascular augmented RFA using HES was safe, and in the pancreatic animal model the best timeframe was within 24h prior to pancreatic surgery. This technique might improve resectability in selected borderline and locally advanced pancreatic cancers.

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INTRODUCTION

Pancreatic surgery is complex, with a non-negligible risk of complications [1]. Although pancreatic surgery centralization in high-volume centres helped reducing morbidity and mortality rates, up to 35% of these patients develop postoperative complications [1]. Pancreatic resection offers the only curative option, so its indications remain important. Peripancreatic vessel involvement has prompted vascular resection and reconstruction techniques, however, with increased postoperative morbidity/mortality [2].

Patients with borderline resectable pancreatic cancer (BRPC) and locally advanced pancreatic cancer (LAPC) (i.e., unresectable non-metastatic pancreatic cancer), initially undergo chemotherapy +/- radiotherapy for attempted downstaging and potential subsequent radical surgery. Nevertheless, only 12% of them proceed to surgery, and a R0 resection is achieved in 70% of these [3]. The main impediment is increasing the clean resection margin, particularly for arteries. Therefore, there is growing interest in complementary minimally invasive downstaging therapies.

Endoscopic ultrasound (EUS) enables high precision guidance of interventional therapies, and it can enhance radiofrequency ablation (RFA) by allowing real-time visualization for localised, controlled ablation while preserving surrounding structures. RFA causes coagulative necrosis and fibrotic changes. Its current indications include functional neuroendocrine tumours (NET) and percutaneous debulking in LAPC of pancreatic body after failed chemotherapy. As the use of sucrose in isotonic solutions combined with RFA was shown to reduce conductivity and increase heating rates, we have tested the feasibility and safety of adding hydroxyethyl starch (HES) to pancreatic parenchyma RFA (EUS-sugar-RFA) in an animal model (n=4), followed by pancreatic biopsies and resection [4-5].

The present study assesses the effects of EUS-sugar-RFA applied to the perivascular space of the splenic vessels before pancreatectomy, with the objective of causing targeted necrosis while maintaining vascular integrity.

Primary outcomes: a) Safety of EUS-sugar-RFA applied to the perivascular pancreatic space; b) Capacity to create a controllable margin of perivascular necrosis to facilitate R0 resection; c) Best treatment timing before pancreatectomy, d) Histopathological effect on pancreas and perivascular space.

Secondary outcomes: Effect visibility in post-interventional imaging studies.

MATERIAL AND METHODS

Study approved by the institutional Animal Care and Ethics Committee (reference #28599-202012101222760 v2). Seven pigs (sus scrofa domesticus) were included (interventional group n=3; control group n=4, 2x normal pancreas and 2x standard RFA), and managed according to French regulations, European Community Council directives (2010/63/EU), and ARRIVE guidelines [6].

Under anaesthesia, a blood collection (hemogram, creatinine, amylase, lipase) and a contrast-enhanced triphasic thoraco-abdominal CT scan were done.
The three porcine pancreatic segments (duodenal lobe -DL-, connecting lobe -CL-, and splenic lobe -SL-) and vascular landmarks were identified using a EUS therapeutic linear scope (EG38-J10UT, Pentax, Japan; processor Arietta V70, Hitachi, Japan) [7] (Figure 1-A). (Two to three target zones (TZ) according to the individual anatomy were defined adjacent to the splenic artery (SA) and to the portal vein (PV) and splenic vein (SV), respectively. The first site was 15mm distal to the spleno-porto-mesenteric confluence (SPMC). The 2nd and 3rd TZ were determined in caudal direction, leaving a 10-15mm distance between them (Figure 1-B and C).

A 22G needle (Expect Slimline, Boston Scientific Corporation, USA; SonoTip ProFlex, MediGlobe GmbH, Germany) was used to inject 1-1.5cc of HES 130/0.4. Then, 50 Watts were applied through a 19G EUSRA needle (Taewoong Medical, USA) placed in the TZ for 6 seconds (VIVA COMBO RF Generator System). Colour Doppler was routinely used to determine the TZ and to check for bleeding. Finally, an adapted GAPS-EUS assessment tool was completed [8].

**Pancreatectomy**

Under anaesthesia, the blood sample was repeated. A thoraco-abdominal CT scan and a diagnostic EUS were done to document changes.

Open pancreatectomy was performed en bloc with vascular axes, using a vessel sealing device (Ligasure, Covidien, Ireland). Then, an Objective Structured Assessment of Technical Skills (OSATS) and a questionnaire created by us evaluating the subjective perception regarding the difficulty of pancreatectomy between interventional and control groups were completed (Annex 1) [9].

The control pancreatectomy group included 2 normal specimens and 2 after RFA alone (50 Watts applied in the splenic vessels’ perivascular space, 10-15 seconds), obtained from educational courses.

**Statistics**

Due to the pilot character of the study with purposedly low sample size and variable survival period durations, no statistical analysis was performed. Descriptive results are provided as mean±standard deviation.

**RESULTS**

All procedures were successfully completed. Pigs 1 and 2 had a survival of 4 and 1 days respectively between both procedures. They had appetite and tolerated liquid diet. No vital sign alterations occurred. On the second follow-up day, pig 1 showed mild abdominal tenderness during palpation, with soft abdomen, which resolved within one day. No abnormalities were found in the blood samples. Pig 3 underwent a non-survival protocol (both procedures on the same day).
The first TZ was 15mm distally to the SMPC, and the following at 10-15mm in direction of the SL. With the SA as the landmark, 3 TZ were defined for pigs 1 & 3, and 2 TZ for pig 2, varying according to the pancreas' length; and 3 TZ adjacent to the SV. Consequently, 5-6 injections/RFAs were performed in each pig. The mean procedure duration was 48.3±10.89 minutes. Classic hyperechogenic bubbles were observed during RFA. No bleeding was observed under Colour Doppler control. Two EUS experts performed the procedures, one with extensive (pig 1) and one with less experience on animal models (pigs 2 and 3). Completion of EUS-sugar-RFA was represented by a GAPS-EUS overall score of 71/75 for the interventional group (Figure 2).

Pancreatectomy

Normal pancreas control:

30' and 45' procedures. In the latest, metal stents gastrojejunostomies (EUS-GJ) had been placed during an EUS course, which reduced manoeuvrability.

Surgical difficulty (Annex 1): normal complexity for one (score 0), harder exposure for the EUS-GJ sample (score 3).

RFA alone control:

20' and 55' procedures. In the longer one, splenic vessels dissection was more difficult due to previous educational coil + glue treatment, without entailing complications.

Surgical difficulty: normal complexity (score 0), slightly increased difficulty with the coiled vessel (score 1).


Interventional Group:

Mean duration of the pancreatectomies = 54±27 minutes. No signs of bleeding or peritonitis during exploration. Regular pancreas' consistency. No visible signs of inflammation, neovascularization or tissue scarring for pig 3 (acute study), that allowed the selective use of vessel-sealing device. Pigs 2 and 1 (1 and 4-day survival respectively) had neovascularisation around the TZ.

OSATS score: 35/35 (all cases).

Surgical difficulty: Normal for 2 pigs (score 0); harder dissection of TZ for pig 1(score 1).

Pre-EUS CT scan: Normal; pre-surgical: Hypodense areas adjacent to the splenic vessels, corresponding to the TZ (Figure 3).
Post-interventional EUS: Doppler-negative hypoechogenic zone adjacent to the splenic vessels, which were slightly larger when compared to the initial EUS control after EUS-sugar-RFA (Figure 3).

**Pathology**

*Normal pancreas specimens:*

Signs of peripancreatic adiponecrosis and slight coagulation necrosis at the pancreatic margins, consistent with the use of monopolar cautery and a vessel-sealing device during dissection. Minimal foci of acute lymphadenitis.

Overall, the pancreatic parenchyma was homogeneous and served as a reference for comparison with the study group and RFA controls.

*RFA alone specimens:*

The vascular coil + glue treatment sample showed a SL haematoma and local peritonitis consistent with the splenic vessel injury.

**Interventional group**

Specimen #1: 3x4 cm yellowish, necrotic zone in the posterior part of the pancreas.

Specimen #2: 9x3 mm congestive zone in perivascular pancreatic tissue.

Specimen #3: 1 cm pancreatic haematoma adjacent to treated vessels, and a 1-2 mm necrotic area (see Figure 4).

Vessel walls: mild mesenterico-portal phlebitis in specimen #1; others normal. Wall integrity maintained in all specimens (see Figure 5).

Microscopic specimens of pigs 1 and 2 showed acute pancreatitis and peripancreatic fat necrosis. The mesenterico-portal phlebitis (pig 1) revealed polymorphonuclear infiltration up to the tunica intima and foci of neoangiogenesis. Pig 2 had signs of acute peritonitis. Pig 3 presented a perivascular hematoma as well as early perivascular pancreatic and fat cell necrosis (Figures 4 and 5).

Detailed histopathological results are shown in Table 1.

**DISCUSSION**

This study assesses the impact of adding a starch solution to RFA (EUS-sugar-RFA) applied to the perivascular space of the splenic vessels before pancreatectomy. After proving feasibility and safety along a 4-day survival period (pig 1), the following survival periods were shortened to minimise local inflammatory response. The best timeframe for EUS-sugar-RFA was within 24h prior to pancreatectomy, where the lower inflammatory response and neovascularisation limited the use of a vessel-sealing device during dissection. Vascular wall integrity was maintained for all specimens.
Our previous study suggested that the interaction between starch and RFA generated a demarcated necrosis that allowed a clear separation of necrotic from normal tissue, but that study was performed within the parenchyma [5]. The present study focused on assessing the effect when applied to the perivascular space, specifically on the vascular axes adjacent to the pancreas, for potential application in pancreatic cancer with vascular compromise.

As the present study targeted the perivascular space, in contrast to pancreatic parenchyma or neoplasia, the energy was applied for a shorter time to avoid potential vascular complications. The result was a perivascular 5-mm charred layer composed by fibrin and granulation tissue.

The interaction between sugar solutions and RFA has not been extensively explored in in vivo, but an ex vivo study using a porcine vascular model concluded that the addition of carbohydrates to a solution enabled a selective higher cell death rate and lower conductivity when exposing a tissue to RF energy [4]. Therefore, we have subsequently assessed sugar-boosted RFA, targeting a specific zone where a circumscribed, augmented effect is desired.

Perivascular space injection may benefit from the local fluid spread adjacent to the initial injection site, dissecting the space, and thereby supporting energy transmission to the perivascular space. Moreover, the sugar/RF interaction allows an augmented ablation while remaining limited to the TZ.

As observed in our previous study, the addition of starch allowed the delivery of a lower amount of energy to achieve the desired effect.

The present study is limited by the absence of pancreatic neoplasia. The proof of concept was therefore achieved without assessing its capacity to downstage pancreatic tumours. Survival after pancreatectomy was omitted due to the expected complex management of insufficiencies in accordance with ethical considerations.

A detailed assessment of the vascular area is essential prior to treatment and avoiding areas close to the pancreatic ducts. The maintenance of a stable position during HES injection, needle retrieval and RFA catheter insertion is also fundamental. Such technical precision requires a high level of EUS expertise and a second operator assistance, which is a disadvantage. However, the high overall GAPPS-EUS score obtained by the second operator reflects that the procedure can be quickly learned by expert endoscopists.

The small number of animals is also a limitation, as it does not allow taking significant conclusions as to the ideal timeframe of application. Also, the control animals were taken from educational courses for ethic reasons, but still hinder comparison. However, feasibility, safety and histopathological findings are consistent along the previous and present study, with an overall of 7 animals treated with EUS-sugar-RFA. A multicentric study with several experts and higher number of procedures is required to assess generalisability of the procedure and further biological aspects before clinical translation. In conclusion, perivascular EUS-sugar-RFA of the pancreas is an emerging neoadjuvant supportive technique. Although not conclusive, the best observed time to perform it was within the day of surgery, as it efficiently induced perivascular necrosis, without complicating inflammation/haemorrhage. Potential applications are preoperative treatment before distal/partial pancreatectomies for NETs, selected patients with BRPC and LAPC, and metastases treatment of body/tail. Larger studies, with follow-up periods after EUS-sugar-RFA and surgery are needed to evaluate the impact on the residual parenchyma. Only then, further
clinical protocols can be planned. If also safe and feasible in clinical contexts, this approach may become part of the multidisciplinary treatment of pancreatic disease in the future.
References


LEGENDS

Caption Figure 1:

A) Porcine pancreas specimen with the duodenal (DL), connecting (CL) and splenic lobe (SL) as well as the portal vein (PV) section. B) Latero-anterior vision of 3D CT reconstruction showing the planned EUS-sugar-RFA ablation strategy. Orange transparent: Porcine pancreas; red: arterial system, blue: venous system; green points: Sites of injection that were both planned and performed; red point: Site injection planned, not performed after considering the individual anatomy of the pig. C) Schematic design of the hypothesized EUS-sugar-RFA’s effect. Superior image: Pancreatic tumour compromising the adjacent vessel. Purple arrows: EUS-sugar-RFA application in the perivascular space. Inferior image: Necrotic effect with vascular wall preservation, allowing vessel-preserving dissection and tumour resection.

Caption Figure 2:

GAPS-EUS assessment tool adapted from [21]. Ratings are shown as orange bars representing the absolute numbers for the 3 interventional group procedures.

Caption Figure 3:

Treatment sites, visualised in EUS and contrast-enhanced CT before (A,D), during (B), and after (C,E, F) the EUS-sugar-RFA procedure in pig 1. A) EUS assessment prior to the EUS-sugar-RFA with identification of the course of the splenic vessels’ course, and choice of the target zones. B) First part of the EUS-sugar-RFA treatment: injection in the target zone adjacent to the splenic vein. C) Second step of the EUS-sugar-RFA treatment: after needle retrieval, the RFA probe is inserted in the target zone and 50 watts are applied for 6 seconds. D) Contrast-enhanced CT scan prior to EUS-sugar-RFA: the pancreas is normal. E & F) Contrast-enhanced CT scan prior to pancreatectomy (4 days after the EUS-sugar-RFA): the hypodense area adjacent to the splenic vessels is indicated by the yellow arrows. P: Pancreas; SV: Splenic vein; SA: Splenic artery; A: Aorta; N: Needle; SS: Sugar solution; RFA-N: Radiofrequency ablation needle; RFA: Radiofrequency Ablation effect; HES: hydroxyethyl starch.

Caption Figure 4:

Histopathological pancreatic findings of the interventional group. Macroscopy: Haematomas in the perivascular treatment zones (A, pig 3), and necrotic area (yellow arrows) developed in the 4-day period between procedures 1 and 2 (B, pig 1). Microscopy (H&E x 100): Acute pancreatitis with necrosis of pancreatic and peripancreatic tissues (C, pig 1); Omentum with acute inflammatory reaction (yellow arrow) and adipose tissue necrosis (blue arrow) adjacent to the pancreas (P) (D, pig 2); early perivascular pancreatic cell necrosis with microscopic foci of perivascular hematomas (yellow circles) (E, pig 3).

Caption Figure 5:
Microscopic vascular findings in the interventional group (pig 1). Transversal cut of the portal vein (PV) (A, H&E x 40); mesenterico-portal phlebitis with thickening of the PV showing polymorphonuclear infiltration and angiogenetic foci (B, H&E x 100); and polymorphonuclear infiltration extending up to the tunica intima of the PV (C, H&E x 200).

Caption Table 1:

**Histopathological findings of the study and control groups.** D0: Day of procedure 1. D1: Day after procedure 1. D4: Four days after procedure 1. EUS-GJ: Endoscopic Ultrasound-guided gastrojejunostomies.

Caption Supplementary Material (Annex 1):

Questionnaire to surgeons for evaluating subjective perception of the surgical procedures. A total score of 0 reflects a surgery of similar complexity to a normal pancreatectomy; the minimum possible score is -14 and it represents a much easier surgery than usual; the maximum possible score is 14, which reflects a much more complex surgery than usual.
Caution: Questionnaire to surgeons for evaluating subjective perception of the surgical procedures. A total score of 0 reflects a surgery of similar complexity to a normal pancreatectomy; the minimum possible score is -14 and it represents a much easier surgery than usual; the maximum possible score is 14, which reflects a much more complex surgery than usual.

1) Procedure-related as opposed to the control group

A- Exposure via laparotomy

2: Very hard access
1: Hard access
0: Normal access
-1: Easy access
-2: Very easy access

B- Manoeuvrability of the organ:

2: very hard to manoeuvre
1: hard to manoeuvre
0: no changes (normal) in manoeuvrability
-1: slightly easier to manoeuvre
-2: much easier to manoeuvre

C- Manoeuvrability of the splenic artery

2: very rigid vessel, very hard to manoeuvre
1: slightly rigid vessel, little harder to manoeuvre
0: normal vessel, no changes in manoeuvrability
-1: softer vessel, slightly easier to manoeuvre
-2: much softer vessel, much easier to manoeuvre

D- Manoeuvrability of the splenic vein

2: very rigid vessel, very hard to manoeuvre
1: slightly rigid vessel, little harder to manoeuvre
0: normal vessel, no changes in manoeuvrability
-1: softer vessel, slightly easier to manoeuvre
-2: much softer vessel, much easier to manoeuvre

2) Study-related vs control group

E- Bleeding:
2: much more bleeding than normal
1: more bleeding than normal
0: same bleeding than normal
-1: slightly less bleeding than normal
-2: much less bleeding than normal

F- Change in tissue consistency:
2: very rigid organ
1: slightly rigid
0: normal organ
-1: softer organ
-2: much softer organ

G- Ease of dissection in pre-treated area:
2: much harder dissection
1: harder dissection
0: normal dissection
-1: easier dissection
-2: much easier dissection
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Procedure done</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated pancreas 1</td>
<td>No particularities</td>
</tr>
<tr>
<td>Non-treated pancreas 2</td>
<td>Modified anatomy (lumen-apossing metal stents with electrocautery enhanced system for EUS-GJ)</td>
</tr>
<tr>
<td>RFA 1</td>
<td>RFA and pancreatectomy (D0); Modified anatomy (artificial attached fluid-filled collections, and a coil + glue treatment in the splenic vessel)</td>
</tr>
<tr>
<td>RFA 2</td>
<td>RFA and pancreatectomy (D0) Modified anatomy (artificial attached fluid-filled collections)</td>
</tr>
<tr>
<td>EUS-sugar-RFA 1</td>
<td>EUS-sugar-RFA (D0); Pancreatectomy (D4)</td>
</tr>
<tr>
<td>EUS-sugar-RFA 2</td>
<td>EUS-sugar-RFA (D0); Pancreatectomy (D1)</td>
</tr>
<tr>
<td>EUS-sugar-RFA 3</td>
<td>EUS-sugar-RFA + pancreatectomy (D0)</td>
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</table>

**Control group**

**Interventional group**
<table>
<thead>
<tr>
<th>Macroscopic description</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 x 3 x 4 cm specimen, no visible lesion. 1.5 cm lymph node</td>
</tr>
<tr>
<td>15 x 4 x 3 cm specimen, with 1.5 cm² pancreatic gray/white lesion + focal congestion. 1.7 cm lymph node</td>
</tr>
<tr>
<td>19 x 4 x 3 cm specimen. 2 x 1 cm gray pancreatic lesion. 6 mm hematoma in the tail. Coil near splenic vessel</td>
</tr>
<tr>
<td>13 x 5.5 x 3 cm specimen, no visible lesion</td>
</tr>
<tr>
<td>15 x 5 x 4 cm specimen. 3 x 4 cm yellowish necrotic zone</td>
</tr>
<tr>
<td>Congestive perivascular pancreatic tissue. Gastric submucosal hematoma</td>
</tr>
<tr>
<td>17 x 5 x 4 cm specimen. 1 cm hematoma around treated vessels, with no other pancreatic lesion</td>
</tr>
</tbody>
</table>
Microscopic evaluation

- Slight pancreatic coagulation necrosis (specimen margins), rest normal
- Peri-pancreatic adiponecrosis
- Minimal acute lymphadenitis
- Slight pancreatic coagulation necrosis (specimen margins) + focus of isolated coagulative necrosis (consequence of EUS-GJ)
- Peri-pancreatic adiponecrosis
- Minimal acute lymphadenitis
  - Pancreatic coagulation necrosis
  - Acute lymphadenitis
  - Acute peritonitis
  - Hematoma in the tail
  - Splenic vessel injury
  - Pancreatic coagulation necrosis
- Acute lymphadenitis
- Acute peritonitis
- Pancreatic & peri-pancreatic coagulation necrosis, nerval and fat tissue necrosis
- Pancreatitis and peripancreatitis
- Focal mesenterico-portal phlebitis
  - Pancreatic coagulation necrosis
- Pancreatic and peri-pancreatic adiponecrosis
- Acute pancreatitis
- Acute peritonitis
- Focal early perivascular pancreatic necrosis, minimal adiponecrosis
- Hematoma
<table>
<thead>
<tr>
<th>Unable to intubate or navigate despite coaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need verbal guidance to intubate, navigate in some areas</td>
</tr>
<tr>
<td>Expertly able to handle scope, intubate, and navigate in all areas and regions of interest</td>
</tr>
<tr>
<td>Unable to visualize and recognize some organs despite coaching</td>
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<tr>
<td>Need verbal guidance to visualize and recognize some organs or structures</td>
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<tr>
<td>Expertly able to visualize and recognize all organs and structures</td>
</tr>
<tr>
<td>Unable to detect, assess and denote the lesion(s) of interest despite coaching</td>
</tr>
<tr>
<td>Need additional information and hints to detect, assess and denote the lesion of interest</td>
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<tr>
<td>Expertly able to detect, assess and denote the lesion(s) of interest</td>
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<tr>
<td>Unable to provide a safe and stable access to a lesion and target it with EUS-FNA/B despite coaching</td>
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<tr>
<td>Need verbal guidance to provide a safe and stable access to a lesion and target it with EUS-FNA/B</td>
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<tr>
<td>Expertly able to provide a safe and stable access to a lesion and target it with EUS-FNA/B</td>
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<tr>
<td>Could not perform a satisfactory examination despite verbal and manual assistance requiring take over</td>
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<tr>
<td>Need verbal guidance to perform some steps</td>
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<tr>
<td>Expertly completes the examination correctly, efficiently and animal friendly</td>
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</table>