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The authors have no conflict of interest to declare

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**SHORT TITLE:**

Peripancreatic lymphatic pathways exploration in live pigs

**COVER LETTER (June 2019)**

Reims, June 5th, 2019

Dear Professor Friedrich Paulsen, dear editors

Please find attached our revised manuscript entitled "**Exploration of peripancreatic lymphatic pathways in a live porcine model**", by Y Renard and colleagues that we are resubmitting as an original scientific article to ***Annals of Anatomy***.

We sincerely thank again the Editorial Office and Reviewers for their reviews of our manuscript that contributed to improve its quality. In this new revised version, all changes are highlighted in yellow. Our detailed point-by-point answers are given below.

We hope you will find this revised version suitable for publication in ***Annals of Anatomy*** and look forward to hearing from you.

Sincerely yours,

**COVER LETTER (December 2018)**

Reims, December 17st, 2018

Dear Editors

Please find attached our manuscript, entitled "Exploration of peripancreatic lymphatic pathways in a live porcine model", by Y Renard and colleagues that we are submitting as an original scientific article to *Annals of Anatomy*.

Pancreatic cancer is associated with a poor prognosis, mainly due to lymph node invasion and lymph node recurrence after surgical resection. In this study, we hypothesized that the pancreas could be divided into several segments each with its own lymphatic drainage. Our goal was to evaluate the feasibility of defining these pancreatic segments following lymphatic drainage in a live animal model, by mapping the peripancreatic lymphatic pathways using Patent Blue. We reviewed the current knowledge of peripancreatic lymphatics focusing on the exploratory techniques used in the literature. In the discussion section we show the difficulty of obtaining a routine description of the peripancreatic lymphatic pathways and of visualizing the node during pancreatic surgical resection.

We demonstrate for the first time that a cartography of the peripancreatic lymphatic pathways can be obtained in a live animal. Our results in pigs suggest that independent anatomical-surgical pancreatic segments with specific lymphatic drainage can be defined and could help the surgeon safely perform limited pancreatic resection procedures with accurate lymph node resections. Applied to humans these results could be of great interest since lymph-node status is the most important prognostic factor for pancreatic cancer. These results were a pilot study for a prospective research project in patients operated on for pancreatic resection (ClinicalTrials.gov Identifier: NCT03597230).

We also concentrated on presenting a concise report of our results.

All authors contributed equally to this work and accepted its submission in *Annals of Anatomy*. We hope you will find it of interest and suitable for publication.

Sincerely yours,

Yohann Renard, MD, in the name of all the authors.

Department of Anatomy, Faculty of Medicine, University of Lorraine, Nancy.

**ABSTRACT (270 words)**

Pancreatic cancer is associated with a poor prognosis, mainly due to lymph node invasion and lymph node recurrence after surgical resection, even after extended lymphadenectomy. The peripancreatic lymphatic system is highly complex and the specific lymphatic drainage of each part of the pancreas has not been established.

The aim of this study was to determine the lymphatic drainage pathways specific to each part of the pancreas on live pigs using Patent Blue.

The pancreases of 14 live pigs were injected in different parts of the gland. The technique was efficient and reproducible. The diffusion patterns were similar for each location and were reported. Our results in pigs allowed us to define specific nodal relay stations and lymphatic drainage for each part of the pancreas and confirm that independent anatomical-surgical pancreatic segments can be described. It is interesting to note that lymphatic drainage for the upper part of the proximal part of pancreas (duodenal lobe) occurred on the left side of the portal vein. This suggests that lymph node resection during cephalic duodenopancreatectomy in humans should be extended to the left side of the mesenteric vein, and probably to the right side of the superior mesenteric artery, as recently suggested.

These results could help surgeons perform safe anatomical-segmental pancreatic resections with accurate lymphadenectomies and improve survival in patients with pancreatic cancer. Based on these results we will perform an innovative prospective study. Patent Blue will be injected into different parts of the gland in patients operated for pancreatic resection, and lymphatic diffusion of the dye will be recorded in relation to their origin from the theoretical pancreatic segments (ClinicalTrials.gov Identifier: NCT03597230).

## 1. INTRODUCTION

Pancreatic ductal adenocarcinomas (PDAC) are the most frequent pancreatic cancers with a 5-year survival rate of 7% and the poorest prognosis of all cancers (Neuzillet et al., 2015). When possible, surgical resection is the best chance of cure (Siegel et al., 2016). Prognosis is determined by tumor stage, in particular lymph-node status (Fink et al., 2016; Pu et al., 2018) as well as the quality of lymph node resection (Tol et al., 2014; Zhan et al., 2015). The network of the supra-mesocolic lymphatic vascular system is highly complex (Kanda et al., 2011)(Cesmebasi et al., 2015). The high rate of loco-regional lymph-node recurrence after cancer resection, even following extended lymphadenectomy (Riediger et al., 2009) has promoted interest in the anatomical and radiological knowledge of the peripancreatic lymphatic circulation (Akcali et al., 2006; Borghi et al., 1998; Delpero et al., 2017; Pu et al., 2018; Samra et al., 2008; Tol et al., 2014) and the peri-operative detection of lymph node metastases around the pancreas (Beisani et al., 2016; Tomson et al., 2015).

Certain studies, based on child and adult cadaveric dissections as well as lymph-node spreading in PDAC resected specimens, have hypothesized that the pancreas may be divided into several segments (Renard et al., 2017) each with their own lymphatic drainage (Borghi et al., 1998; Cesmebasi et al., 2015; Cubilla et al., 1978; Donatini and Hidden, 1992; Nagakawa et al., 1994; Pissas, 1984). Theoretically, the ability to distinguish independent segments of the pancreatic parenchyma could help surgeons perform safe segmental pancreatic resections and adequate lymphadenectomy to reduce the loco-regional lymph-node recurrence rate. Nevertheless, no independent peripancreatic lymphatic pathways have been demonstrated thus far (Renard et al., 2017).

The goal of this study was to evaluate the feasibility of defining pancreatic segments following lymphatic drainage in a live animal model by mapping the peripancreatic lymphatic pathways using the lymphophil dye, Patent Blue (Beisani et al., 2016).

## 2. MATERIAL AND METHODS

The pig pancreas is composed of three lobes (Fig. 1). The splenic lobe is located posteriorly and to the left, close to the spleen and stomach, and corresponds to the tail and body in the human pancreas. The duodenal lobe is located closest to the duodenum, and corresponds to the head of the pancreas. The connecting lobe is an extension located on the anterior border of the portal vein and should correspond to the uncinate process (Ferrer et al., 2008; Tsuchitani et al., 2016).

Fourteen live pigs were included in this study. They came from the School of Surgery, Lorraine University, Nancy, France. All experiments on pigs were performed in accordance with National Institute of Health recommendations and approved by the corresponding ethics committee. All pigs were initially enrolled for laparoscopic surgical training sessions and were injected 10 min before animal sacrifice (Pissas et al., 1982). The pig was not included in case of surgical dissection of the upper part of the abdomen or in the event of the death of the animal before injection. A median xypho-pubic laparotomy was performed under general anesthesia and precautions were taken to avoid any dissection around the peripancreatic area.

Reproducibility of our protocol was excellent and included an injection of 1 mL of pure Patent Blue (*Guerbet®, Villepinte, France*) in the pancreatic parenchyma at a depth of 0.5 to 1cm from the surface, using a 2mL syringe (*BD Medical®, Le Pont-de-Claix, France*) and a 23G needle (*Fine-Ject, Henke Sass Wolf®, Tuttlingen, Germany*). We performed the injections to make sure that the results may be applied to human pancreatic surgery. The injections were performed in the upper part of the duodenal lobe and the lower part of this lobe in 4 pigs each, in the distal parts of the splenic lobe in 3 pigs and in the proximal parts of this latter lobe in 3 pigs. The pigs were sacrificed and dissected. Three hours were necessary to make sure that all locations of the blue diffusion were explored. The patterns of blue diffusion were recorded for each pig and pictures were taken.

## 3. RESULTS

The technique was highly efficient and reproducible. For each location of injection, the diffusion patterns of the blue injection were similar:

- Injection in the superior part of the duodenal lobe (Fig. 2A) resulted in lymphatic diffusion around the hepatic pedicle (Fig. 2B) and towards but not into the splenic lobe until beyond the portal vein (Fig. 2C).
- Injection in the lower part of the duodenal lobe (Fig. 3A) resulted in lymphatic diffusion towards the connecting lobe (Fig. 3C).
- Injection in the distal part of the splenic lobe (Fig. 4A) resulted in lymphatic diffusion around the splenic pedicle and towards the connecting lobe (Fig. 4B).
- Injections in the proximal part of the splenic lobe (Fig. 5A) resulted in lymphatic diffusion around the splenic pedicle and towards the portal vein without crossing the left border (Fig. 5B).

## 4. DISCUSSION

Most pancreatic tumors (95%) are ductal adenocarcinomas (PDAC) (Amundadottir, 2016; Neuzillet et al., 2015) which is the eleventh cause of cancer and the fourth leading cause of death from cancer worldwide (Ferlay et al., 2016; Siegel et al., 2016). PDAC has the poorest prognosis of all cancers, with a 5-year survival rate of 7% (Neuzillet et al., 2015; Siegel et al., 2016). Lymph node status, including malignant spread and the total number of retrieved lymph nodes as well as the lymph node ratio, is the most important prognostic factor (Fink et al., 2016; Pu et al., 2018; Santi et al., 2011). A prospective multicenter study performed by Delpero et al. (2017) including 147 patients operated for pancreatic cancer showed that lymph node status was the greatest independent prognostic factor (Delpero et al., 2017). Surgical resection with lymphadenectomy seems to be the best curative treatment option (Kayahara et al., 1992; Riediger et al., 2009; Samra et al., 2008; Sauvanet, 2008; Tol et al., 2014; Zhan et al., 2015) in patients with localized cancer (i.e. without spreading to regional and distant lymph node locations, representing up to 10% of cases (Fink et al., 2016; Siegel et al., 2016)). The importance of lymph node status and the high rate of local lymph node

recurrence following surgical resection (Delpero et al., 2017; Nimura et al., 2012; Pu et al., 2018; Riediger et al., 2009) have renewed interest in the peripancreatic lymphatic circulation (Akcali et al., 2006; Borghi et al., 1998; Samra et al., 2008). However, because of the absence of strong evidence on the optimal lymphadenectomy to be performed during pancreatic resection, better anatomical understanding of lymphatic spreading around the pancreas, depending on the location of the tumor is still needed (Renard et al., 2017; Tol et al., 2014) .

Many reports have described the peripancreatic lymphatic system with various results (Deki and Sato, 1988). Recently, certain studies have focused on the main lymphatic pathways around the pancreas, with discrepant results on the location of the regional lymph nodes (Cubilla et al., 1978; Deki and Sato, 1988; Donatini and Hidden, 1992; Kanda et al., 2011; Kayahara et al., 1992; Nagakawa et al., 1994; O'Morchoe, 1997; Pissas, 1990, 1984):

- Certain authors have named lymph groups according to their location around the pancreas. Based on 33 pancreatectomy specimens, Cubilla et al. (1978) described 5 main groups: superior, inferior, anterior, posterior and splenic (Cubilla et al., 1978). Borghi et al. (1998) and Pissas et al. (1984) reported a similar regional classification (Borghi et al., 1998; Pissas, 1984).
- Other authors have developed numerical classifications (O'Morchoe, 1997). Based on lymph-node spreading in 44 PDAC resected specimens, Kayahara et al. (1992) and Nagakawa et al. (1994) described 11 nodal areas, forming 4 lymphatic pathways around the head of the pancreas (Kayahara et al., 1992; Nagakawa et al., 1994, 1993). After meticulous macroscopic dissection of 4 cadavers, Deki and Sato (1988) provided a detailed description of 8 lymphatic pathways running towards the abdomino-aortic (para-aortic) lymph-nodes (Deki and Sato, 1988; Pavlidis et al., 2011). They reported 3 lymphatic pathways in the anterior surface of the head, 3 in the posterior surface and two major lymphatic routes in the left half of the pancreas (Deki and Sato, 1988).
- Finally, the Japan Pancreatic Society developed an anatomical classification dividing regional and juxtaregional lymph nodes into 35 groups including 23 subgroups based on data from 18,629 cases of carcinoma of the pancreas (Cubilla et al., 1978; Isaji et al., 2004; Nagakawa et al., 1994; Tol et al., 2014).

These authors only reported the location of the lymph nodes around the pancreas and did not try to determine the origin of these pathways from the precise location of the tumors in the pancreatic parenchyma. Further, their works weren't based on a live model.

Very few other studies have evaluated the correlation between these lymphatic pathways and their origin in the different areas of the pancreas, suggesting that each theoretical pancreatic segment could have its own lymphatic drainage (Borghi et al., 1998; Cesmebasi et al., 2015; Cubilla et al., 1978; Donatini and Hidden, 1992; Nagakawa et al., 1994; Pissas, 1984). This segmentation would help surgeons perform accurate targeted lymph node resection during segmental pancreatectomy (Fink et al., 2016; Renard et al., 2017). These studies were mainly based on adult, child or embryo cadaveric dissections, or surgical specimens from patients with pancreatic carcinomas. In pilot studies, lymphatic channels were shown to come from two distinct portions of the pancreas, one on the right side and one of the left (head/neck, body/tail, respectively)(Cesmebasi et al., 2015; Fink et al., 2016). Other authors have proposed more precise theories: Cubilla et al. (1978) and Pissas (1984) proposed a division into 5 segments with specific and independent lymphatic drainage (Cubilla et al., 1978; Pissas, 1984), but with significant differences between the 2 theories. Donatini and Hidden (1992) proposed 4 segments with independent pathways of lymphatic drainage (Donatini and Hidden, 1992), which differed from the two earlier theories.

These complex and non-standardized (Fink et al., 2016) classifications show the difficulty of obtaining a routine description of the peripancreatic lymphatic pathways, and suggest that more accurate and detailed studies are needed to improve staging (Pavlidis et al., 2011).

The goal of the present study was to show the feasibility of defining the origins of the peripancreatic lymphatic pathways in live animals. The pig pancreas includes duodenal, splenic and connecting lobes (Ferrer et al., 2008; Tsuchitani et al., 2016) and is considered a highly suitable model for experimental pancreatic research (Bensley, 2005; Shokouh-Amiri et al., 1989; Troisi et al., 2000), in particular for the lymphatic pathways (Ji and Kato, 1997; Regoli et al., 2001). To the best of our knowledge, this is the first time that several parts of the pancreas of a live animal have been injected with Patent Blue to describe a cartography of lymphatic drainage for each theoretical segment (Renard et al., 2017). Pissas (1982) reported a similar study in dogs, with a different goal, whose pancreatic anatomy has been

already shown to be segmented and highly different from humans (Galindo-Pacheco et al., 1992; Pissas et al., 1982).

The identification of lymphatic vessels is difficult in cadavers and surgical samples (Cesmebasi et al., 2015; Fink et al., 2016; Hirai et al., 2001; O'Morchoe, 1997; Pissas, 1984). Thus several vital staining dyes have been used to distinguish lymphatic vessels from other structures (Evans and Ochsner, 1954; Pissas, 1984; Pissas et al., 1982; Tomson et al., 2015). Although they have been found to be efficient to achieve this goal when injected in the lung and stomach, they are ineffective in recognizing the lymphatic vessels when injected in the pancreas (Pissas et al., 1982). Pissas (1984) reported a cadaveric study of lymphatic drainage of the pancreas using the previous manufacturing process of Patent Blue dye (Pissas, 1984). More recently, Nagakawa's group (1994) used carbon particles or radioactive insulin in surgical specimens from patients with pancreatic tumors (Kayahara et al., 1999; Nagakawa et al., 1994, 1993). At present lymphoscintigraphy is the technical reference for the detection of the sentinel node. However, it is difficult to use routinely, in particular during gross dissection and anatomical studies (Niebling et al., 2016; Peek et al., 2017; Tomson et al., 2015).

In the present study we used Patent Blue, first described by Weinberg and Greaney for gastric cancer (Weinberg and Greaney, 1950), which is now a frequently used lymphophil dye, alone or combined with a radiocolloid tracer or lympho-fluoroscopy for sentinel lymph node identification in breast cancer and melanoma (Niebling et al., 2016; Peek et al., 2017; Tomson et al., 2015).

In our study, we were able to describe the peripancreatic lymphatic pathways and show the different pancreatic origins of each. These results confirm the presence of pancreatic segmentation and suggest that independent anatomical-surgical pancreatic parts could be defined with their own specific lymphatic drainage. If these results are confirmed in humans, they could help the surgeon perform limited pancreatic resection procedures safely as well as increase the accuracy of lymph node resections.

For example, there is an ongoing debate on the extent of lymphadenectomy to be performed during pancreatoduodenectomy, in particular around the superior mesenteric artery (Tol et al., 2014). Our results in live pigs show that the upper part of the duodenal

lobe drains towards the splenic lobe, beyond the portal vein. If these results are applied to humans this suggests that during surgical resection of a tumor located in the head of pancreas, lymph node resection should be extended to the left, beyond the portal vein up to the right side of the superior mesenteric artery, confirming several recent studies (Borghi et al., 1998; Cesmebasi et al., 2015; Hirai et al., 2001; Kanda et al., 2011; Kayahara et al., 1999; Nimura et al., 2012; Pu et al., 2018; Samra et al., 2008; Tol et al., 2014).

This study presents limitations inherent to the use of live animal. Although pigs are considered as interesting model for experimental pancreatic research, is it not known, to the best of our knowledge, whether the 3 anatomical lobes of the pig's pancreas can be good correlated to human's one. In particular, we did not injected the connecting lobe, probably corresponding the uncinate process, since numerous authors demonstrated that the uncinate process should be an extension of the posterior part of the head (Ferrer et al., 2008; Tsuchitani et al., 2016; Renard et al., 2017). Uchida et al. (1999) and Suda et al. (2006) demonstrated that during embryogenesis, the posterior part of the head and the uncinate process, come from the ventral bud (Uchida et al., 1999; Suda et al., 2006). Moreover, Holt and Varadarajulu (2014) and Rana and Vilmann (2015) reported that the embryological dorsal pancreas is generally more echogenic than the embryological ventral pancreas: a transition area can be distinguished between the darker anterior head to the brighter posterior pancreas (body and tail, including the uncinate process) (Holt and Varadarajulu, 2014; Rana and Vilmann, 2015). In addition, based on cadaveric injections, Donatini and Hidden (1992) showed that the uncinate process was an integral part of the inferior portion of the head (Donatini and Hidden, 1992).

## 5. Conclusions and perspectives

Mapping the peripancreatic lymphatic pathways with the lymphophil dye, Patent Blue,(Beisani et al., 2016) can offer a reproducible description of pancreatic segments following lymphatic drainage in a live animal model. If these results are confirmed in the human anatomy, they could help to define patient-specific lymph node dissection during pancreatic adenocarcinoma surgical resections. Based on these results we will perform a

prospective study in patients undergoing pancreatic resection. During surgery, different parts of the pancreas will be injected with Patent Blue and the lymphatic pathways will be identified in relation to their origin from the theoretical pancreatic segments (ClinicalTrials.gov Identifier: NCT03597230).


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## Disclosure of interests

The authors have no conflict of interest to declare.

## Author contributions

Y.R., C.P., M.P. and M.B. designed research, collected data and performed data analysis. M.L., C.A. and M.P. performed analysis/interpretation of data and provided critical revisions of the manuscript. Y.R., R.R. and M.P. performed drafting of the manuscript. All authors approved the manuscript.


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**FIGURES**

Figure 1

Anatomy of the pig's pancreas.

1: portal vein. 2: duodenum. 3: aorta. 4: splenic vein. 5 : superior mesenteric artery. 6: superior mesenteric vein. 7: inferior mesenteric vein.

A: duodenal lobe. B: connecting lobe. C: splenic lobe.

Figure 2

Lymphatic diffusion of the Patent Blue after injection in the superior part of the duodenal lobe.

A1: Injection in the superior part of the duodenal lobe. A2: Schematic illustration of A1.

B1: Diffusion around the hepatic pedicle (white arrow 1). B2: Schematic illustration of B1.

C1: Diffusion beyond the portal vein (white arrow 2). C2: Schematic illustration of C1.

The blue line corresponds to the portal vein.

a, pancreas; b, duodenum; c, portal vein; d, stomach.

Figure 3

Lymphatic diffusion of the Patent Blue after injection in the lower part of the duodenal lobe.

A1: Injection in the lower part of the duodenal lobe. A2: Schematic illustration of A1.

B1: Diffusion towards the connecting lobe (white arrow). B2: Schematic illustration of B1.

C1 showed no diffusion around the hepatic pedicle. C2: Schematic illustration of C1.

a, pancreas; b, duodenum; c, portal vein; d, stomach; e, colon.

Figure 4

Lymphatic diffusion of the Patent Blue after injection in the distal part of the splenic lobe.

A1: Injection in the distal part of the splenic lobe. A2: Schematic illustration of A1.

B1: Diffusion around the splenic pedicle (white arrow 1) and towards the connecting lobe (white arrow 2). B2: Schematic illustration of B1.

a, pancreas; d, stomach; f, small intestine; g, spleen; h, superior mesenteric vein.

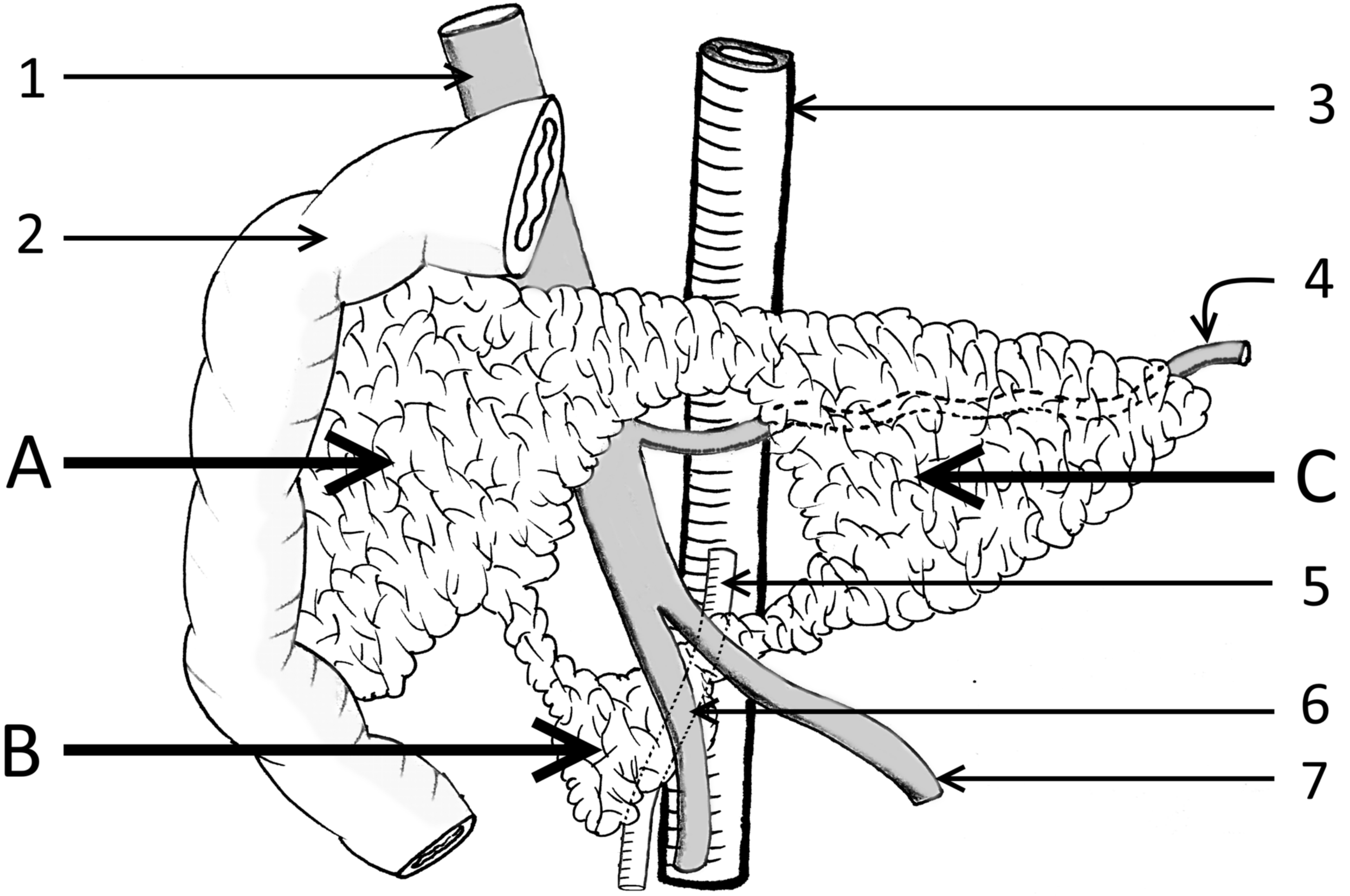
Figure 5

Lymphatic diffusion of the Patent Blue after injection in the proximal part of the splenic lobe.

A1: Injection in the proximal part of the splenic lobe. A2: Schematic illustration of A1. B1: Diffusion around the splenic pedicle (white arrow 1) and towards the portal vein but without crossing it's left border (white arrow 2). B2: Schematic illustration of B1.

The blue line corresponds to the portal vein.

a, pancreas; b, duodenum; d, stomach; f, small intestine; g, spleen.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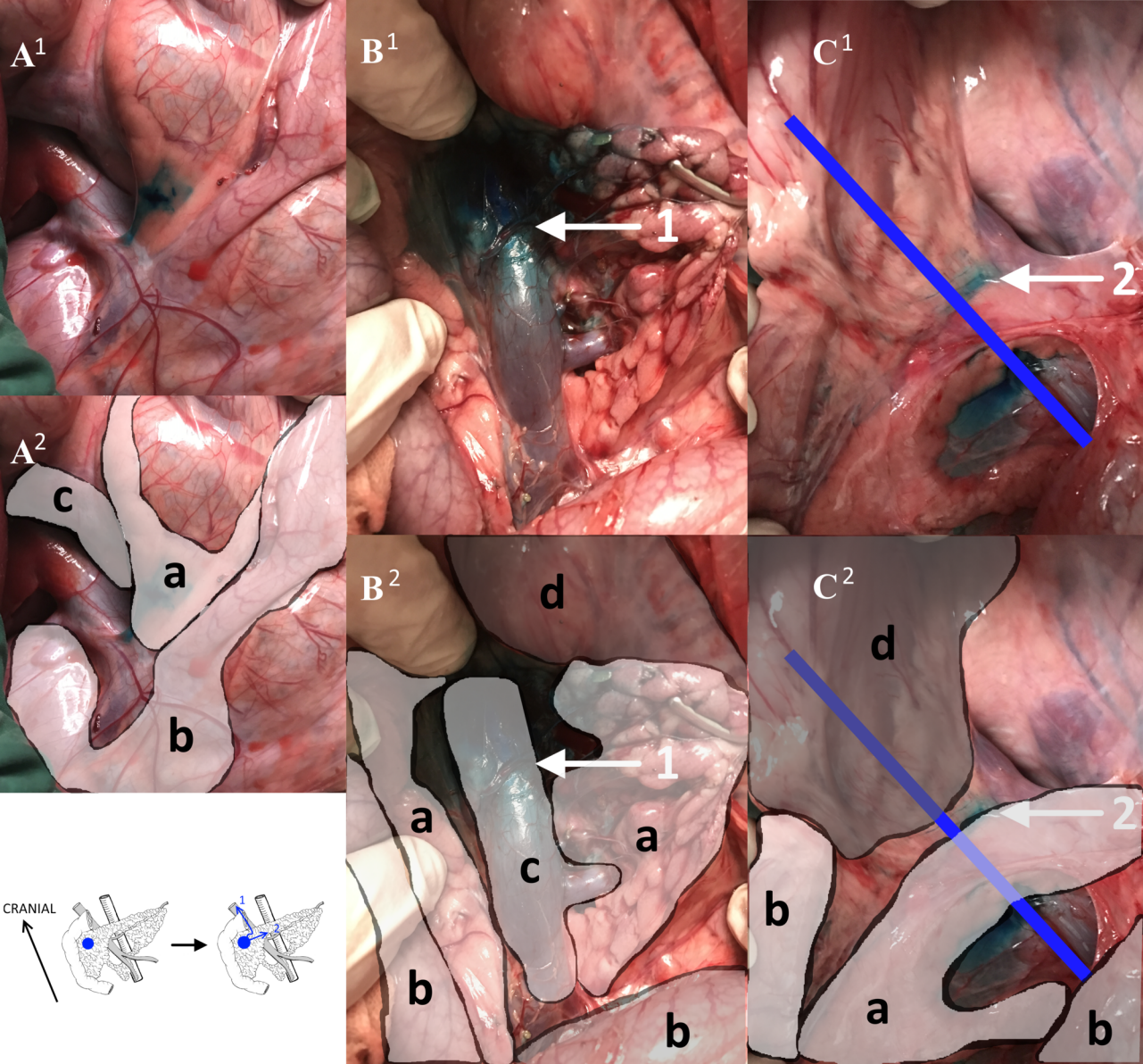
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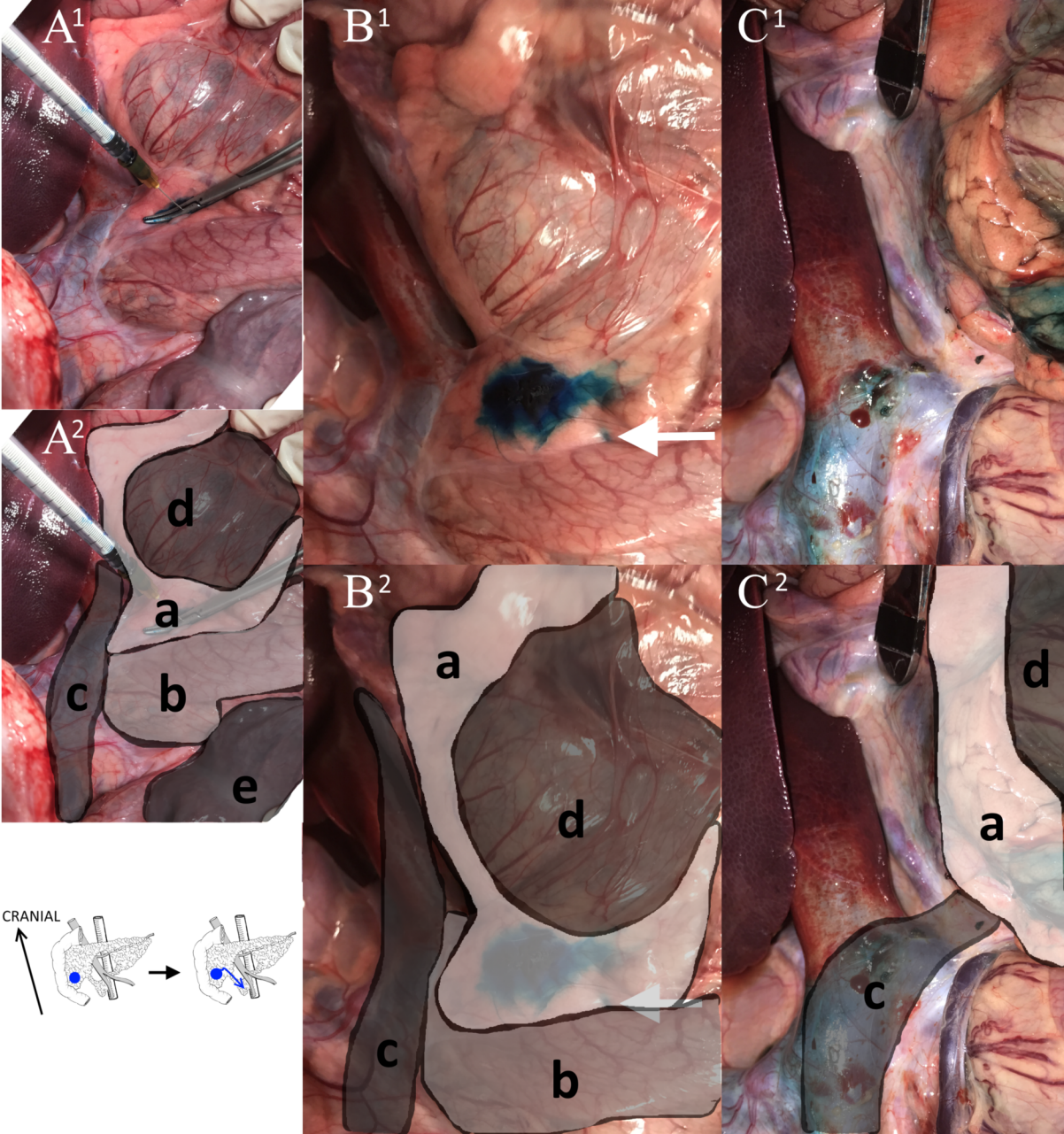
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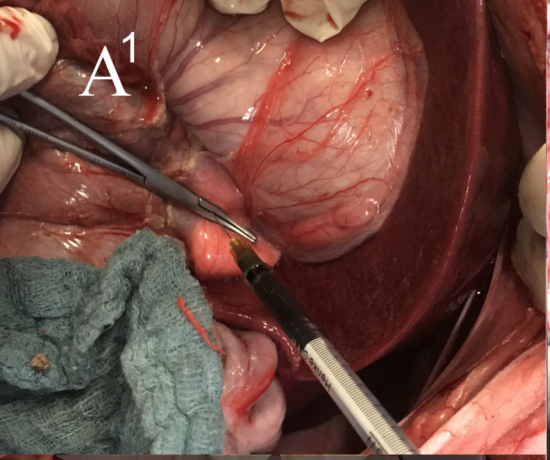
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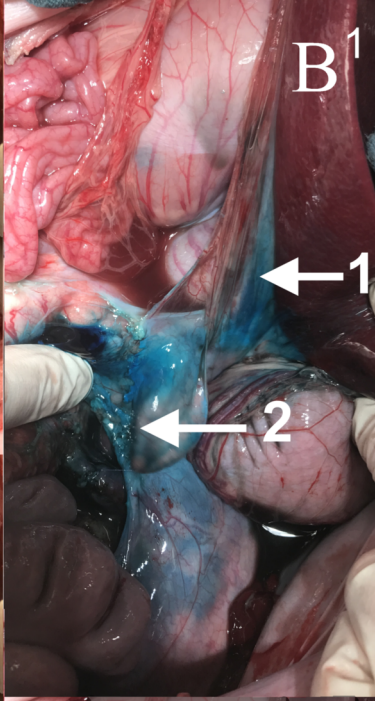
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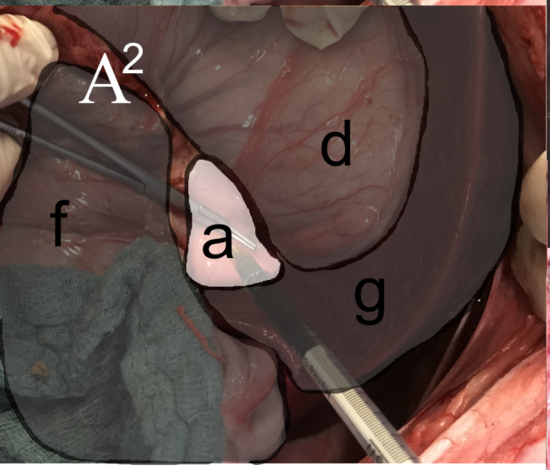
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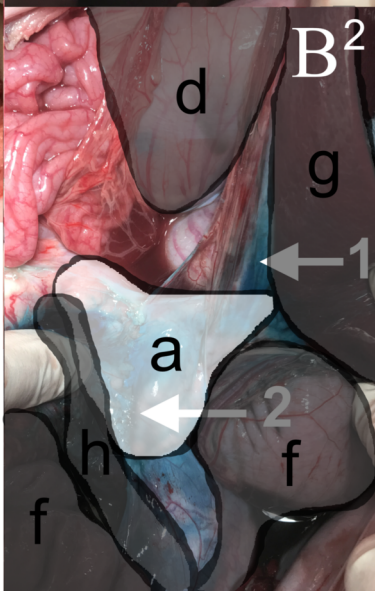
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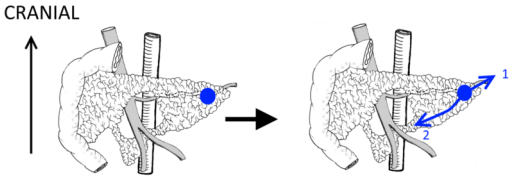
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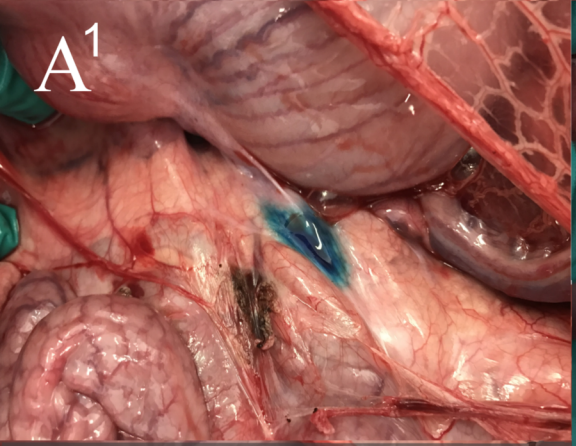
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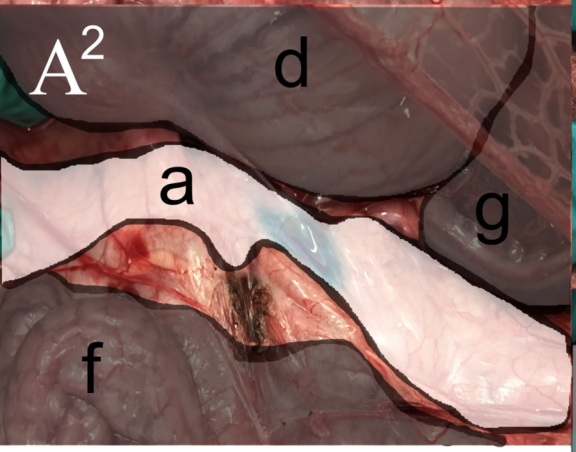
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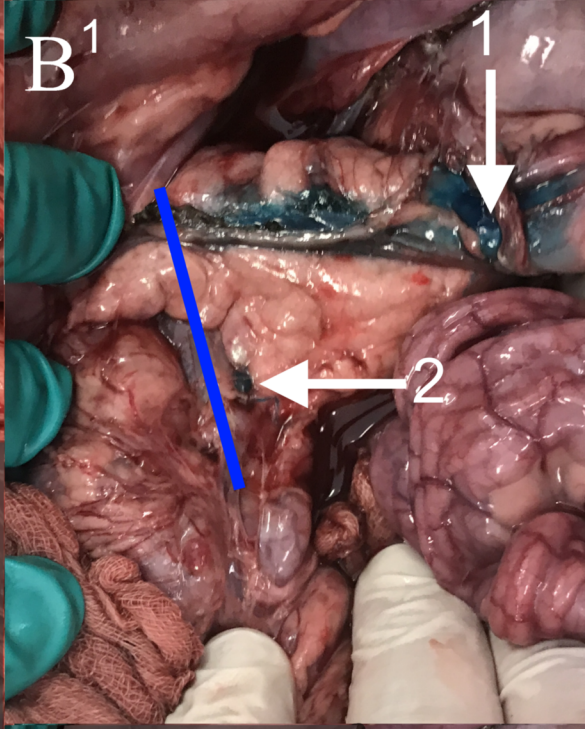
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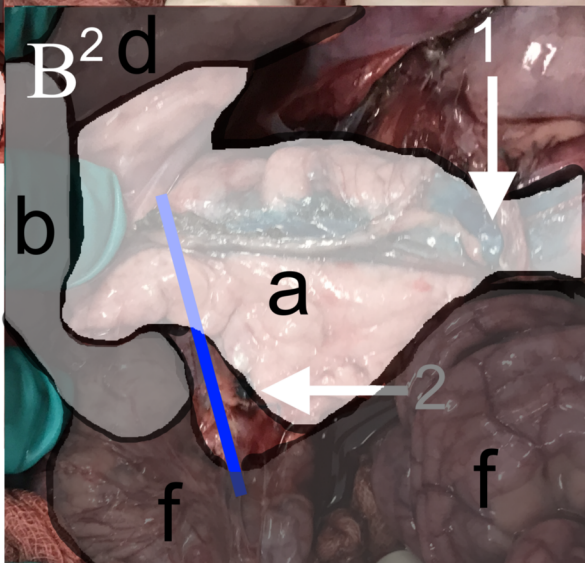
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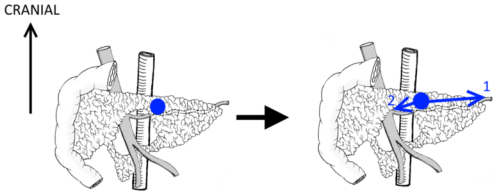
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