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Running title: Topographic anatomy of the infracardiac bursa

Revisiting the infracardiac bursa using multimodal methods: topographic anatomy for surgery of the esophagogastric junction

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Abstract

In embryology, the infracardiac bursa (ICB) is a well-known derivative separated from the omental bursa. During surgeries around the esophagogastric junction (EGJ), surgeons often encounter a closed space considered to be equivalent to the ICB, but the macroscopic anatomy in adults is hardly known. This study aimed to revisit the ICB using multimodal methods to show its development from the embryonic to adult stage and clarify its persistence and topographic anatomy. Histological sections of 79 embryos from the Carnegie stage (CS) 16 to 23 and magnetic resonance (MR) images of 39 fetuses were examined to study the embryological development of the ICB. Horizontal sections around the EGJ obtained from three adult cadavers were examined to determine the topographic anatomy and histology of the ICB. Further, 32 laparoscopic surgical videos before (n = 16) and after (n = 16) the start of this study were reviewed to confirm its remaining rate and topographic anatomy in surgery. The ICB was formed in 1 out of 10 CS17 samples and in 8 out of 10 CS18 samples. Further, it was observed in all CS19-23 except one CS23 sample and in 25 (64%) out of 39 fetus samples. Three-dimensional reconstructed MR images of fetuses revealed that the ICB was located at the right alongside the esophagus and the cranial side of the diaphragmatic crus. In one adult cadaver, the caudal end of the ICB arose from the level of the

esophageal hiatus and the cranial end reached up to the level of the pericardium. The inner surface cells of the space consisted of the mesothelium. In laparoscopic surgery, the ICB was identified in only 11 (69%) out of 16 surgeries before. However, subsequently we were able to identify the ICB reproducibly in 15 (94%) of 16 surgeries. Thus, the ICB is the structure commonly remaining in almost all adults as a closed space located at the right alongside the esophagus and the cranial side of the diaphragmatic crus. It may be available as a useful landmark in surgery of the EGJ.

Keywords: infracardiac bursa; esophagogastric junction; embryology; macroscopic anatomy; endoscopic surgery

Introduction

In the recent decades, the incidence of adenocarcinoma arising at the esophagogastric junction (EGJ) has rapidly increased in Japan (Kusano et al. 2008; Yamashita et al. 2017) as well as in Western countries (Blot et al. 1991; Hansson et al. 1993; Pera et al. 1993; Devesa et al. 1998; Vizcaino et al. 2002; Steevens et al. 2010). Surgical resection along with mediastinal lymphadenectomy may be the most effective treatment for the cancer. However, the surgery is technically demanding because the operative field is the most secluded area of either the thoracic cavity or the abdominal cavity cramped by the diaphragm. Therefore, the topographic anatomy around the EGJ still have some unclear parts.

One of the parts is a closed space on the right side of the EGJ. During transhiatal approach from the abdominal cavity, surgeons often confuse this space with the right thoracic cavity. According to embryology, the right pneumato-enteric recess, the superior part of the omental bursa, is cut off as the diaphragm develops, forming a closed space (Hamilton et al. 1972; Moore et al. 2008). More than 100 years ago, a Swedish embryologist, Ivar Broman, named the space the infracardiac bursa (ICB) (Broman, 1904). Some older reports indicate that it lies medial to the base of the right lung if it persists after birth (Zschokke, 1920; Viikari, 1950; Patten, 1953;

Kanagasuntheram, 1957). These knowledges strongly support that the closed space is equivalent to the ICB. However, macroscopic anatomy has missed describing the ICB (Snell, 2004; Putz et al. 2008; Agur and Dalley, 2009; Netter, 2010; Moore et al. 2014; Schulte et al. 2016), and most surgeons have not been interested in this unclear space, even though they encounter it during surgeries. Thus, little is known about its persistence, precise location, and surrounding structures in adults.

In the present study, we aimed to revisit the ICB using current multimodal methods to sequentially show that the ICB develops in the embryo, is located above the right diaphragmatic crus in the fetus and is observed consistently in the adult cadaver. We further clarify its persistence and topographic anatomy from the surgical perspective.

Methods

Human embryo specimens

The Kyoto Collection of Human Embryos comprises approximately 44,000 human embryos stored at the Congenital Anomaly Research Center of Kyoto University (Nishimura et al. 1968; Shiota, 1991; Yamada et al. 2004). The embryos were staged according to Carnegie stages (CS), which was proposed by O’Rahilly et al. (1987). The

embryos were fixed in Bouin's fluid for a day and transferred to 10% formalin for storage. Then they were dehydrated, embedded in paraffin by standard methods, serially sectioned at 10 μ m, and stained with hematoxylin and eosin (HE). Histological sections of well-preserved CS16-23 embryos that were found to be externally normal were scanned and digitized with an Olympus virtual slide system (VS120-S5-J; Olympus, Tokyo, Japan) to observe the histological findings. Two authors (T.N. and T.T.) evaluated 79 samples (10 samples per CS16-22 and 9 samples in CS23) to investigate the embryological changes of the ICB from the right pneumato-enteric recess.

Human fetus specimens

Thirty-nine well-preserved human fetuses with crown-rump length of 30-87 mm were selected from the Kyoto Collection of Human Embryos for observation of the ICB. Magnetic resonance (MR) images were acquired using a 7-T MR system (BioSpec 70/20 USR; Bruker Biospin MRI GmbH, Ettlingen, Germany). The three-dimensional (3D) images of the ICB and its adjacent anatomical structures were reconstructed using the Amira software, version 6.4.0 (Thermo Fisher Scientific, Bordeaux, France).

Adult cadaver specimens

Three adult cadavers bequeathed to the Ehime University, Ehime, Japan, were subjected to precise investigation of the ICB anatomy. The cadavers were embalmed using a

standard mixture containing 10% formalin within 24 h of death. In each cadaver, a portion surrounding the EGJ was harvested en bloc, embedded in 10% gelatin, refrigerated at 4°C, and frozen at −20°C (Zhang et al. 2004). The samples were then sliced into 4 mm-thick sections using a slicer (NF-385K; Nantsune, Osaka, Japan) to identify the location of the ICB. An approximately 4 cm² area including the ICB was excised. Specimens were dehydrated through stepwise series of ethanol solutions and embedded in paraffin wax. Subsequently, 5 µm-thick specimens were prepared for HE and immunohistochemical staining of the mesothelium with monoclonal antibodies to calretinin (rabbit, lot no. 1779337A; Invitrogen, Carlsbad, CA, USA) and podoplanin (mouse, code: 413451; Nichirei, Tokyo) (Fig. 1).

Surgical videos

Two experienced surgeons (T.N. and H.S.) reviewed 32 surgical videos of the laparoscopic transhiatal lower mediastinal lymphadenectomy to verify the remaining rate and topographic anatomy of the ICB, of which 16 were performed at the Kyoto University Hospital from September 2014 to October 2016 before the start of this study and 16 performed subsequently at the Kyoto University Hospital (8 videos) and Hyogo College of Medicine (8 videos).

Surgical specimen

The inner surface cells of the closed space which is probably equivalent to the ICB were histologically validated using the surgical specimen. They were stained with HE and the calretinin and podoplanin antibodies.

Ethics

This study was approved by the Committee of Medical Ethics of the Kyoto University Graduate School of Medicine, Kyoto, Japan (E986, R0316, and R0823), and the Institutional Review Board of Ehime University, Ehime, Japan (no. 1701001). This study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient included in the study.

Results

Development of the ICB

First, we investigated the formation of the ICB from the CS16 to 23 (Table 1). The right pneumato-enteric recess was connected to the omental bursa in all the CS16 samples. Among the CS17 and CS18 samples, the right pneumato-enteric recess was separated by the developing diaphragm (Fig. 2a). The ICB was formed in 1 out of 10 CS17 samples and in 8 out of 10 CS18 samples. Further, it was observed in all CS19-23

samples, except one CS23 sample (Fig. 2b). The inner surface of the ICB was lined with simple squamous epithelium (Fig. 2c).

3D reconstruction of the ICB in fetus

To understand the location and outline of the developing ICB, we examined the MR images of the human fetuses. The ICB was successfully detected in 25 (64%) of 39 samples. Figure 3a shows a representative coronal section where the ICB was depicted as a space existing at the right alongside the esophagus and the cranial side of the diaphragmatic crus. It was spindle-shaped in a 3D image reconstructed from the MR images (Fig. 3b).

Topographic anatomy of the ICB in adult cadavers

We then investigated the topographic anatomy of the ICB in human adult cadavers using consecutive slices. Figure 4a shows the macroscopic findings in the cadaver identified the ICB. The caudal end arose from the level of the esophageal hiatus and the cranial end reached up to the level of the pericardium. The location was consistent with the 3D reconstruction by the MR images of fetus. Like embryo specimens, the simple squamous epithelia covering the space were observed as shown in Figure 4b, c. Immunohistochemical staining with calretinin and podoplanin revealed the presence of mesothelium (Fig. 4d, e).

Appearance of the ICB during surgeries

Lastly, we examined the remaining rate and the surrounding structures of the ICB in living humans by reviewing videos of laparoscopic surgery. In 16 surgeries performed before the start of the present study, we recognized the ICB in only 11 (69%) surgeries. However, in 16 surgeries performed subsequently, the recognition rate increased to 94% (15 cases) and we were able to identify the ICB reproducibly. At first, the caudal edge of the ICB appeared as a thick whitish membrane after dissecting the phrenico-esophageal ligament (PEL), which connects the esophagus to the right crus (Fig. 5a). Then, by cutting into the membrane, surgeons could enter the closed space enveloped with the serosa (Fig. 5b). The right lung was occasionally seen on the lateral side through the space (Fig. 5c). The size of the space and the accumulation of adipose tissue outside the space varied across cases.

Histological validation of the closed space in a surgical specimen

Figure 6a shows a surgical specimen with the closed space completely resected by lower mediastinal lymphadenectomy. Immunohistological staining with calretinin and podoplanin definitely showed that the inner surface cells of the space consisted of mesothelium, like the adult cadaver (Fig. 6b–d).

Discussion

In this study, we revisited the ICB using multimodal methods in the fields of embryology, anatomy, and surgery. We sequentially showed that the ICB was formed by separation from the cranial part of the omental bursa in the embryo, located above the right diaphragmatic crus in the fetus and observed consistently in the adult cadaver. In the laparoscopic surgery based on these results, we reproducibly identified the ICB in almost all surgeries.

In embryology, the ICB is a well-known space derived from the omental bursa during the development of the diaphragm (Zschokke, 1920; Viikari, 1950; Patten, 1953; Kanagasuntheram, 1957; Hamilton et al. 1972; Moore et al. 2008). The background knowledge has been provided by Ivar Broman more than one hundred years ago (Broman, 1904). However, for unknown reason, most authoritative anatomical atlases have neglected this small bursa (Snell, 2004; Putz et al. 2008; Agur and Dalley, 2009; Netter, 2010; Schulte et al. 2016). Therefore, few surgical literatures have focused on the structure. Only two old textbooks showed the ICB on the anatomical chart (Palmer and Anderson, 1952; Edwards et al. 1972), but they failed to reflect knowledge from embryology, since the bursa was drawn on the caudal side of the right crus inside the phreno-esophageal membrane. Considering that the ICB is cut off by the diaphragm in

embryos, the ICB should be located on the cranial side of the PEL and the diaphragmatic crus. In the present study, we demonstrated that the closed space encountered by surgeons was equivalent to a remnant of the ICB in the embryo, based on the location and histology. We herein propose a new chart of surgical anatomy around the EGJ as Figures 7a and b, in which the ICB is depicted at the right alongside the esophagus and the cranial side of the diaphragmatic crus. This will help surgeons to recognize the ICB during surgeries around the EGJ.

Does the ICB persist throughout development in all human individuals? Favaro (1909) reported that it was observed in 15 (60%) of 25 cadavers from the embryonic to adult stage. Viikari (1950) reported it in 64 (82%) of 78 prenatal cadavers and in 35 (47%) of 74 postnatal cadavers. In adult cadavers, Zschokke (1920) reported that it persisted in 5 (70%) of 7 cases. In the present study, we could not conclude whether the ICB disappeared at a certain rate during development. The limitation of this study is that we could not observe it in most fetuses and adult cadavers, because MR images and tissue slices could not detect small spaces, compared with histological sections and laparoscopic surgeries. However, the ICB may be one of the common anatomical structures rather than an anomaly, because the development of the ICB was confirmed in all embryos, except one, and it was identified in almost all surgeries once it was

recognized. In the future, more surgeries should be prospectively evaluated for the true remaining rate of the ICB in adults.

Recognizing the ICB may increase accuracy of surgeries around the EGJ. If surgeons overlook the existence of the ICB, they are often confused by the unintentional entry into the closed space lying in front of the thoracic space. By contrast, intentional entry into the ICB provides surgeons with a landmark to identify the location of the pleura, right lung, and inferior vena cava. Moreover, surgeons can choose one of three routes based on the malignancy of the EGJ disease; the medial route of the ICB can be used as route in benign diseases, whereas the inside or lateral routes of the ICB can be used in malignant diseases (Fig.7b). In addition, it is reported that the ICB is associated with a hiatus hernia or hydrothorax, which is a complication of peritoneal dialysis (Kunath, 1977; Gagnon and Daniels, 2004). The precise topography of the ICB may promote future studies to understand those pathogeneses.

Surgery is a branch of medicine aimed at treating the living human body by modifying its structure. It references the field of macroscopic anatomy, which is a science based on studies of cadavers. Macroscopic anatomy is substantiated by the field of embryology, which is a branch of biology that studies the formation and development of embryos and fetuses. There is occasional discontinuity between macroscopic

anatomy and embryology, and this can affect what surgeons know and how they perform surgeries. Despite recent advantages in endoscopic surgery that allow surgeons to recognize the enhanced anatomy that far surpasses their comprehension obtained from classical anatomy (Sakai et al. 2009, 2016; Shinohara et al. 2013, 2015; Cuesta et al. 2015; Haruta et al. 2015; Weijs et al. 2017), surgeons tend to overlook structures that are not described in the anatomical charts. The ICB may be a representative example. Further developments in clinical anatomy should not only occur from technological advancements in optical instruments, but also from feedback and exchange of information among the fields of embryology, anatomy, and surgery.

Conclusions

We revisited the ICB using the current multimodal methods. The ICB is a closed space located at the right alongside the esophagus and the cranial side of the diaphragmatic crus and commonly remains in almost all adults. It may be a surgical landmark around the EGJ, and further studies are required to determine its usefulness in lower mediastinal lymphadenectomy for cancer.

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Author contributions

Study conception and design: Nakamura, Shinohara, Kurahashi, Sakai

Acquisition of data: Nakamura; Makishima (embryo); Okada, Shimokawa, Takai (adult cadaver); Hisamori, Tsunoda, Obama, Kurahashi, Shinohara (surgery)

Analysis and interpretation of data: Nakamura, Shinohara, Takakuwa, Yamada, Matsuda

Drafting of manuscript: Nakamura, Shinohara

Critical revision: Sakai

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Table 1. Number of samples from Carnegie stage (CS) 16 to 23 showing the infracardiac bursa (ICB) formation

	CS16	CS17	CS18	CS19	CS20	CS21	CS22	C23
Pneumato-enteric recess	10	9	2	0	0	0	0	0
ICB	0	1	8	10	10	10	10	8
Total number	10	10	10	10	10	10	10	9

Values are presented as n.

Figure Legends

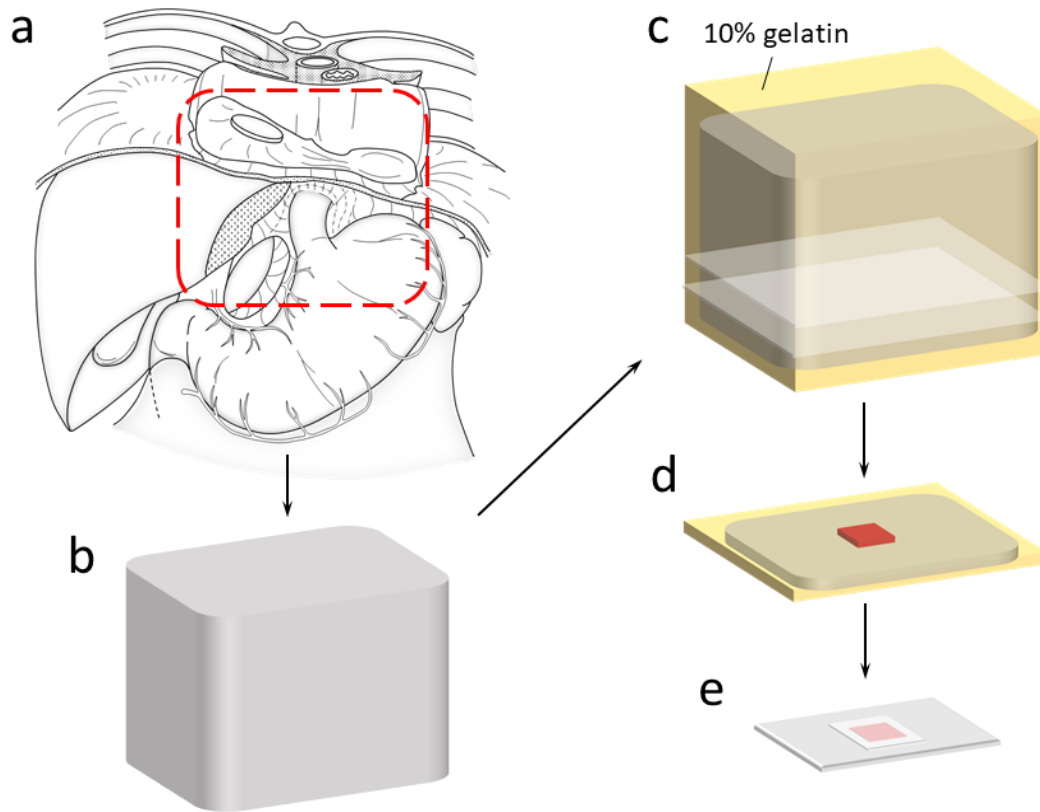


Figure 1

Production of cadaver slices (a) Depiction of the esophagogastric junction with surrounding structures removed heart, lung, and lateral segment of the liver. (b) The rectangular portion, depicted as the area in the red square in (a), is harvested en bloc. (c) The portion is embedded in 10% gelatin, refrigerated at 4°C, and frozen at −20°C. (d) The slice is cut transversely into 4-mm-thick sections. The red square represents the 4 cm² area macroscopically, including the infracardiac bursa. (e) The red area is retrieved, cut into 5 μm-thick sections, and mounted.

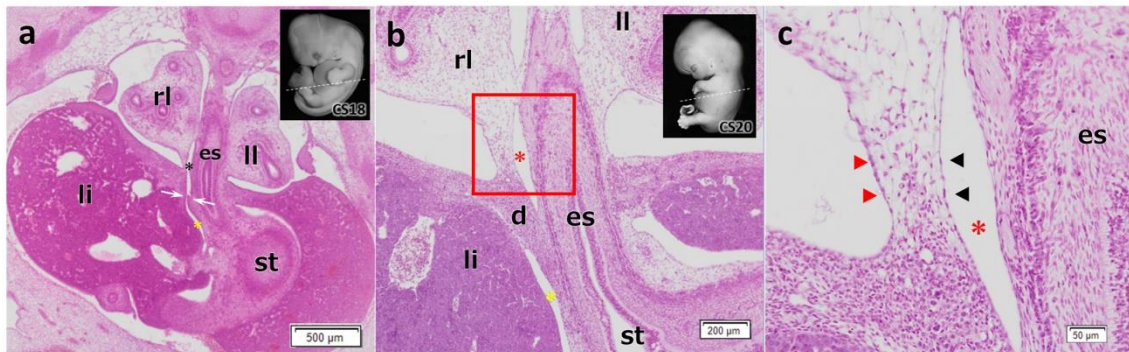


Figure 2

Development of the infracardiac bursa (ICB) (a) The CS18 (#24992) section shows the right pneumato-enteric recess separated by development of the diaphragm (white arrow), stained with hematoxylin and eosin (HE). (b) The CS20 (#4330) section showing the ICB separated from the omental bursa, stained with HE. (c) A higher magnification of (b) showing the inner cells of the ICB. They are simple squamous epithelia like the pleura.

d, diaphragm; es, esophagus; li, liver; ll, left lung; rl, right lung; st, stomach; black asterisk, right pneumato-enteric recess; yellow asterisk, omental bursa; red asterisk, ICB; red arrow head, pleural surface cell; black arrow head, inner surface cell of the ICB.

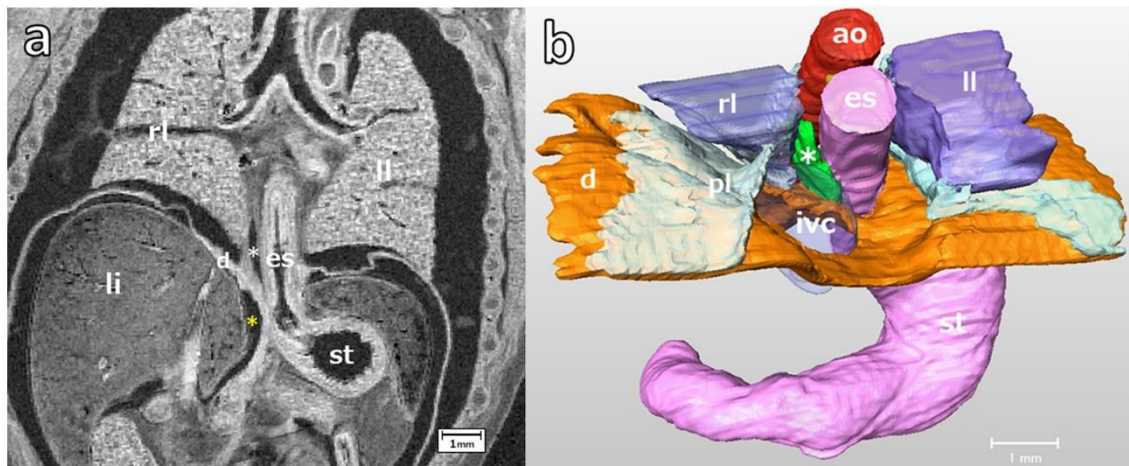


Figure 3

Magnetic resonance (MR) image of the infracardiac bursa (ICB) and its three-dimensional reconstruction (a) Coronal MR image of the fetus with 43.5 mm crown-rump length (#33563). The ICB is located at the right alongside the esophagus and the cranial side of the diaphragmatic crus. (b) It is spindle-shaped in the three-dimensional reconstruction of (a).

ao, aorta; d, diaphragm; es, esophagus; ivc, inferior vena cava (semitransparent); li, liver; ll, left lung; pl, pleura; rl, right lung; st, stomach; white asterisk, ICB; yellow asterisk, omental bursa.

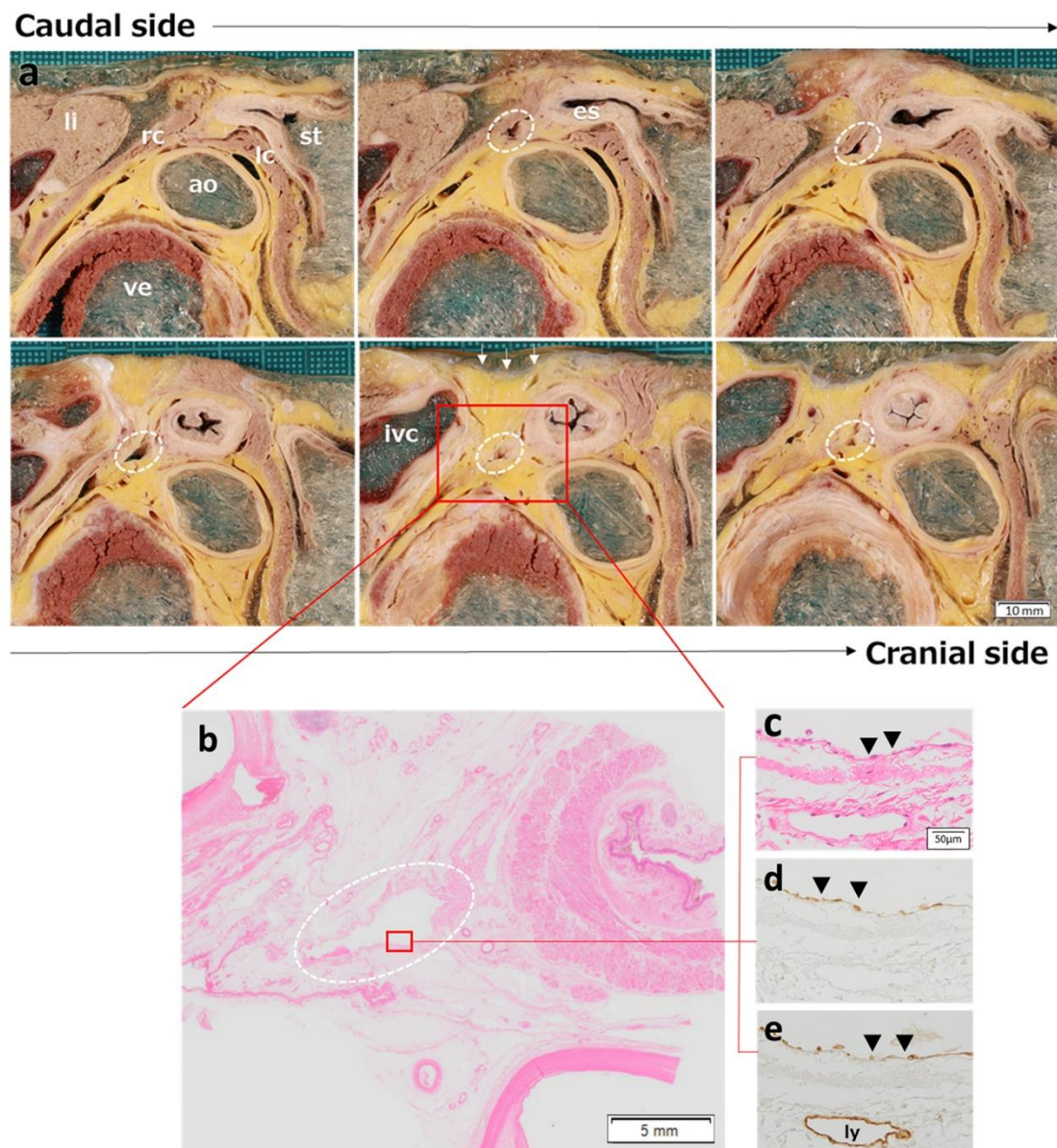


Figure 4

Macroscopic and microscopic findings of the infracardiac bursa (ICB) in the adult cadaver (a) Sequential horizontal slices (4-mm-thick) crossing the esophagogastric junction. Upper left panel, most caudal slice; lower right panel, most cranial slice. The caudal end of the ICB arose from the level of the esophageal hiatus (upper middle slice)

and the cranial end reached up to the level of the pericardium (lower right slice). (b) Tissue section of the red square in (a), stained using hematoxylin and eosin (HE). (c) The inner surface cells of the ICB are covered with the simple squamous epithelium, stained with HE. (d) The inner surface cells of the ICB stained positive for calretinin. (e) The inner surface cells of the ICB and lymphatic vessels stained positive for podoplanin.

ao, aorta; es, esophagus; ivc, inferior vena cava; lc, left diaphragmatic crus; li, liver; ly, lymphatic vessel; rc, right diaphragmatic crus; st, stomach; ve, vertebral body; white circle, ICB; white arrow, pericardium; black arrow head, mesothelium.

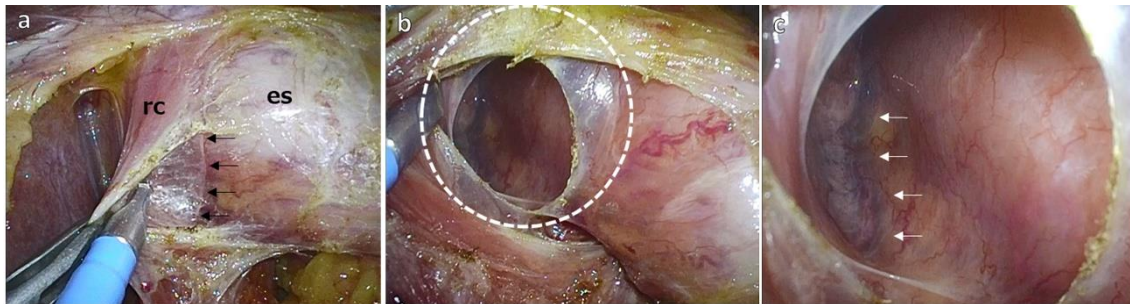


Figure 5

Appearance of the infracardiac bursa (ICB) during a laparoscopic surgery. (a) Caudal edge of the ICB appears as a whitish membrane (black arrows). (b) The opened ICB is enveloped with serosa. (c) The right lung is seen on the lateral side through the space (white arrows).

es, esophagus; rc, right crus of the diaphragm; white circle, ICB.

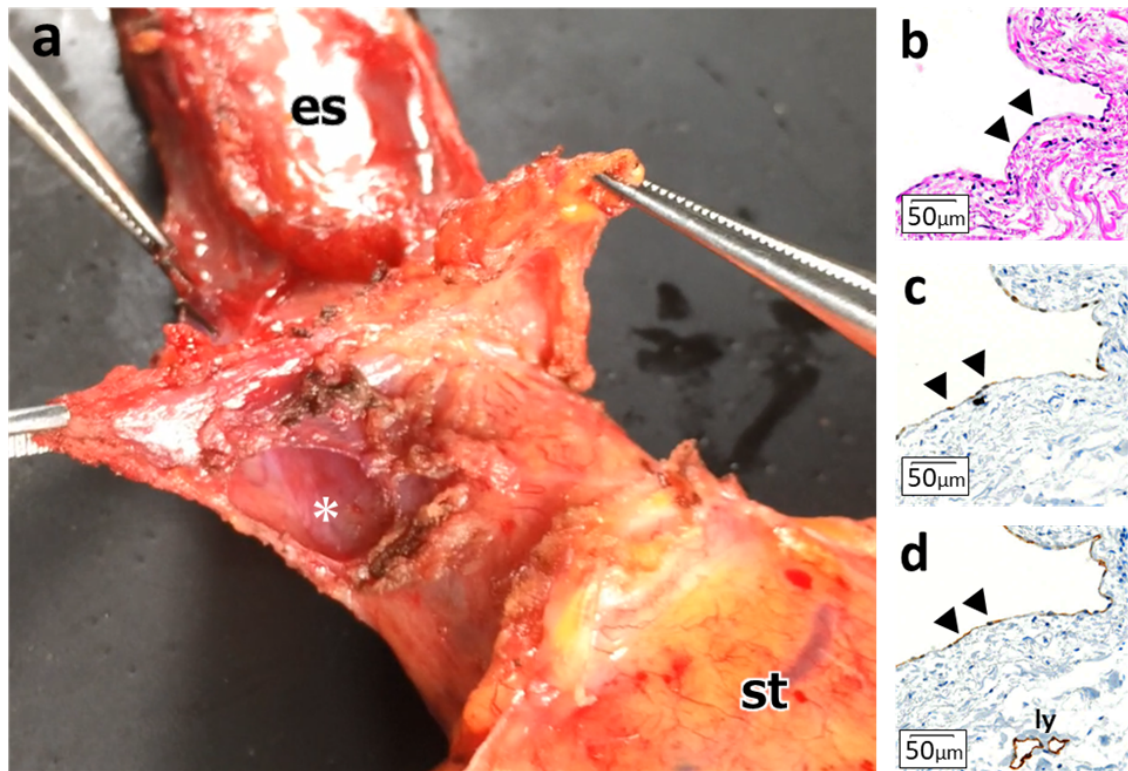


Figure 6

Surgical specimen with the infracardiac bursa (ICB). (a) Excised specimen, including the ICB. (b) Histological section of the ICB showing simple squamous epithelium, stained with hematoxylin and eosin. (c) The inner surface cells of the ICB stained positive for calretinin. (d) The inner surface cells of the ICB and lymphatic vessels stained positive for podoplanin.

es, esophagus; ly, lymphatic vessel; st, stomach; black arrow head, mesothelium; white asterisk, ICB.

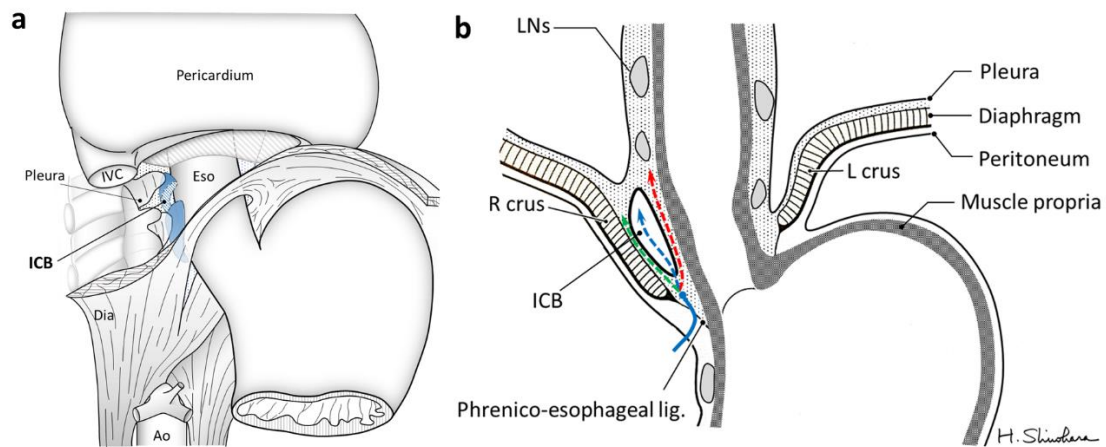


Figure 7

Our proposed anatomical chart of the esophagogastric junction (EGJ) including the infracardiac bursa (ICB) (a) Illustration shows that the ICB is located at the right alongside the esophagus and the cranial side of the diaphragmatic crus. It may be a landmark for the location of the right pleura, right lung, and inferior vena cava (IVC). (b) The coronal section shows that surgeons can choose one of three routes around the ICB.

Ao, aorta; Eso, esophagus; Dia, diaphragm; LNs, lymph nodes; red dotted line, medial route; blue dotted line, inside route; green dotted line, lateral route.