Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 3, 317–323
ISSN 1311-1477; DOI: 10.15547/bjvm.2305

Original article

VARIATIONS AND SOME CLINICALLY RELEVANT RELATIONS OF *A. CYSTICA* IN PIGS – A CORROSION CAST STUDY

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Summary

Stefanov, I. S., N. S. Tsandev & A. P. Vodenicharov, 2021. Variations and some clinically relevant relations of *A. cystica* in pigs – a corrosion cast study. *Bulg. J. Vet. Med.,* **24**, No 3, 317–323.

The aim of this study was to investigate the variations as well as the length of *A. cystica* and its branches in pigs using corrosion casting method with the self-polymerising resin Duracryl® Plus. The method included several steps: hepatectomy, precasting treatment, injection of Duracryl, polymerisation of casting medium, corrosive treatment, cleaning of the corrosion casts, air-drying and preservation of casts. The livers were collected from 12 male 6-month-old pigs (crossbred Landrace×Danube White). With regards to the beginning of *A. cystica*, 4 variations were observed and grouped as follows: variation A – *A. cystica* detached from *R. dexter medialis*, together with *R. quadratus* (variation A1), or alone (variation A2); variation B1 – *A. cystica* originated from *A. gastroduodenalis*, or was a branch of the common trunk (*R. dexter*) (variation B2). The metric data were processed by GraphPad Prism 6 for Windows. Clinically relevant relations between *A. cystica*, *Ductus cysticus, A. celiaca* and *R. sinister* also were described. The new information received about the blood supply of the gallbladder would contribute to the understanding of the etiology of postoperative complications as a result of surgical interventions in this location and for their prevention.

Key words: cystic artery, gallbladder, morphometry, swine, vascularisation

INTRODUCTION

The morphological studies of microcirculation of extrahepatic bile ducts using various resins involved predominantly animal species such as the rabbit (Jackowiak & Lametschwandtner, 2005), the guinea pig (Akarinejad & Lametschwandtner, 1992) and the rat (Gaudio *et al.*, 1993). Some authors identified anatomical variations of *A. hepatica* and its branches in domestic pigs using the advantages of methods for making corrosion casts (Osman *et al.*, 2008) and defined *A. cystica* as the main vessel supplying the gallbladder and its excretory duct, with no information about its length and branching. According to Tejaswi *et al.* (2013) however,

this information is extremely valuable in surgery where the knowledge of variations of *A. cystica* and its branches would help surgeons to prevent uncontrolled bleeding from the vessels, which can be fatal during cholecystectomy. Moreover, according to Park *et al.* (2005) the pig is a suitable species for experiments related to development of new surgical techniques that could be very useful for cholecystectomy in humans. Making corrosion casts of blood vessels and hollow organs in different species of animals and in humans, facilitated not only the establishment of their microanatomic and gross anatomical features but also their topographic peculiarities (Hagras & Swielim, 1990; Gaudio *et al.*, 1993; Osman *et al.*, 2008).

This study was performed to supplement the information about the variations and length of *A. cystica* and its branches, as well as to study the clinically relevant relations between *A. cystica*, *Ductus cysticus, A. celiaca* and *R. sinister*.

MATERIALS AND METHODS

Animals

A set of fresh liver with gallbladder, stomach, spleen and cranial part of the duodenum was collected from 12 male 6-month-old crossbred pigs (Landrace × Danube White) at a slaughterhouse. The study was supported by Scientific Project № 19-15/Faculty of Veterinary Medicine, Trakia University, Bulgaria.

Corrosion casting method

The corrosion casting method of Tsandev *et al.* (2015) using the resin Duracryl® Plus (Spofa Dental Product, Czech Republic) was adapted for preparing corrosion casts of blood vessels supplying the aforementioned organs together with casts of bile ducts and gallbladder. Six sets of organs were used for preparing corrosion casts of arteries, veins, bile ducts and gallbladder (Fig. 1) and 6 sets for corrosion casts of arteries and bile ducts without the veins (Fig. 2).

Fig. 1. Corrosion cast of arteries (in red) and veins (in blue) of porcine liver, stomach and spleen together with bile ducts (in green) and gallbladder (in green).

- Hepatectomy

Immediately after slaughtering a set of fresh liver with gallbladder, stomach, spleen and cranial part of the duodenum were removed.

- Precasting treatment

The livers were perfused with saline solution through a cannula placed into the portal vein, celiac artery and common bile duct using a syringe. After washing with saline, the liver vascular system was again clamped, a self-polymerising resin (casting medium) was tested.

- Injection of corrosion casting media (Duracryl® Plus)

Bile ducts were washed with saline, and then filled with resin during the opening of the *Papilla duodeni major* or retrogradely through the bottom of the gallbladder, depending on the purpose of the

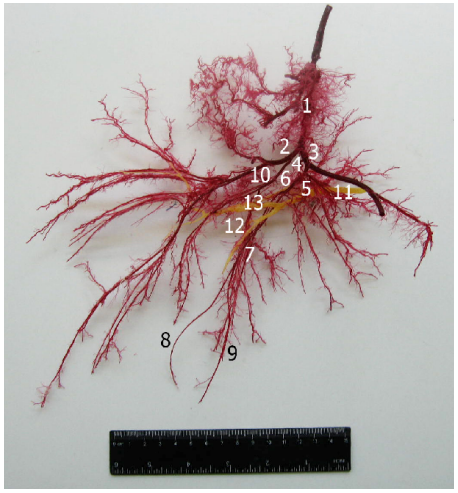

Fig. 2. Corrosion cast of *A. hepatica* (1) branches. 2. *R. sinister,* 3. *A. gastroduodenalis,* 4. *R. dexter*, 5. *R. dexter lateralis* 6. *R. dexter medialis,* 7. *A. cystica*, 8. left branch of *A. cystica*, 9. right branch of *A. cystica*, 10. *R. quadratus,* 11. *D. choledochus*, 12. *D. cysticus*, 13. *D. hepaticus communis.*

study. The injection of the plastic material was carried out after mixing of base and catalyst at a ratio of 4:1, manually using a syringe. In order to facilitate the differentiation of arteries, veins and bile ducts, they were filled with red, blue and yellow or green colourant, respectively. The rate of injection was 100 mL per 90 min.

- Polymerisation of casting medium

The injected organs were placed in a water bath, left at room temperature for 48 hours until complete polymerisation.

- Maceration

Organs were macerated in 20% NaOH solution at a temperature of 40 °C until complete destruction of the soft tissues.

- Cleaning of the corrosion casts

For the complete removal of macerated tissue, corrosion casts were washed with running tap water.

- Air-drying and preservation of casts in 90% alcohol

Macromorphometric methods

The studied macrometric parameters were the length (in millimeters) of the blood vessels supplying the gallbladder as well as the distance (in millimeters) between *A. cystica* and *Ductus cysticus,* between the beginning of *A. cystica* and the origin of *R. sinister (A. hepatica),* between the origin of *A. cystica* and gallbladder. Macromorphometric studies were carried out on corrosion casts of the studied structures using a caliper (accuracy 0.02 mm).

Statistical analysis

The means and standard deviations (SD) were calculated using GraphPad Prism 6 for Windows (GraphPad Software, Inc., USA) statistical software.

RESULTS

The length of *A. cystica* and its branches as well as the distance between *A. cystica* and *Ductus cysticus,* gallbladder, *A. celiaca, R. sinister* were evaluated. The length of *A. cystica* and its branches was as followed: *A. cystica* – 55.61±7.89 mm, *Ramus dexter* of *A. cystica* – 94.49±5.34 mm, *Ramus visceralis* of *A. cystica* – 32.49±2.26 mm, *Ramus sinister* of *A. cystica:* 99.92±7.17 mm.

Combined corrosion casts from veins, arteries and bile ducts were also made but veins were not of interest in this study. Clinically relevant relations between *A. cystica*, *Ductus cysticus, A. celiaca* and *R. sinister* were presented. It was measured that the distance between *A. cystica* and *Ductus cysticus* (dorsal part) was 3.94± 1.63 mm, the distance between the origin of *A. cystica* and the origin of *A. celiaca* was 101.08 ± 9.99 mm, between the be-

ginning of *A. cystica* and the origin of *R. sinister* (*A. hepatica*) – 25.56±14.30 mm, between the origin of *A. cystica* and the gallbladder – 27.14±7.10 mm.

Concerning the branches of *A. cystica,* when it passed along the *D. cysticus,* 3 to 4 thin branches (*Rami ductus cysticus*) were identified. Near to the gallbladder neck, *A. cystica* ramified into *R. dexter* and *R. sinister.* Also, it was observed that in 50% of cases a well-developed visceral branch (*Ramus visceralis* – ramifying within the visceral surface of the gallbladder) originated from the right *A. cystica* branch. The left *A. cystica* branch gave off 2 to 3 oblique branches on the parietal surface of the gallbladder that anastomosed with those of the right branch. On the visceral surface of gallbladder, the same number of tiny visceral branches originating from *Ramus dexter* and *Ramus sinister* of *A. cystica* also anastomosed.

With regard to the beginning of *A. cystica*, 4 variations were observed which could be grouped as followed; variation A – in 83% of cases, it detached from *R. dexter medialis* passing behind the portal vein and above the common hepatic duct, of which 40% together with *R. quadratus* (variation A1, Fig. 3), and 60% – alone (variation A2, Fig. 4); variation B – in 17% of the cases *A. cystica* arose from *A. gastroduodenalis*, of which 12% *A. cystica* detached 3.07–3.53 mm from the beginning of *A. gastroduodenalis* (variation B1, Fig. 5) and in 5% of the cases *A. cystica* was observed as a branch of the common trunk (*R. dexter* ramifying as *R. dexter lateralis* et *R. dexter medialis)* which originated from *A. gastroduodenalis* (variation B2, Fig. 6). In other cases, when *R. quadratus* originated separately from *R. sinister (A. hepatica),* it was not accompanied by *A. cystica.*

Our results showed that *R. dexter medialis* was the main vessel detaching from *A. cystica*. In most cases (83%), *R. dexter medialis* was directed cranially to *D. hepaticus communis*, and in 17% of cases – caudally.

In terms of syntopic relations of *A. cystica* to *D. cysticus* two variations were identified. In the first variation type (92% of cases), *A. cystica* originated from *R. dexter medialis* and from *A. gastroduodenalis* on the left side of the sagittal plane, passed through the terminal part of *D. cysticus* where was the confluence of *D. cysticus* and *D. hepaticus communis*. It then passed on the left of *D. cysticus* and caudally to *V. portae* and *D. hepaticus communis*. The second variation type (8% of cases) represented the origin of *A. cystica* from the common trunk of *R. dexter lateralis* and *R. dexter medialis.* In this case, the beginning of *A. cystica* was located on the right of the sagittal plane which passed near the end of *D. cysticus*. Then *A. cystica* was directed ventrally, passing cranially to *D. choledochus*, parallel to and on the right of the excretory duct of the gallbladder to the place of its division into two main branches.

DISCUSSION

In terms of *A. cystica* origin, unlike Osman *et al.* (2008) who claimed that in 40% of cases it originated from *R. dexter* and in the rest of cases – from *R. quadratus*, we confirmed the statement of Barone (1996) that in most of cases (83% in the current study) *R. dexter medialis* detached from *A. cystica.* According to Nomina Anatomica Veterinaria (2017) *A. cystica* originates from *R. dexter medialis* in pigs, in carnivores – from *Rami sinistri mediales*, in ruminants – from *R. dexter,* in the ox – from *A. gastroduodenalis*.

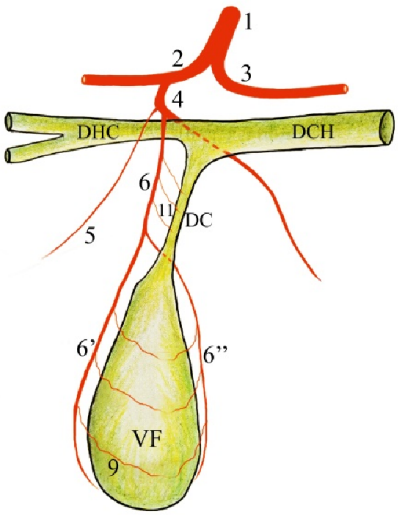

Fig. 3. Variation of *A. cystica* beginning (A1)

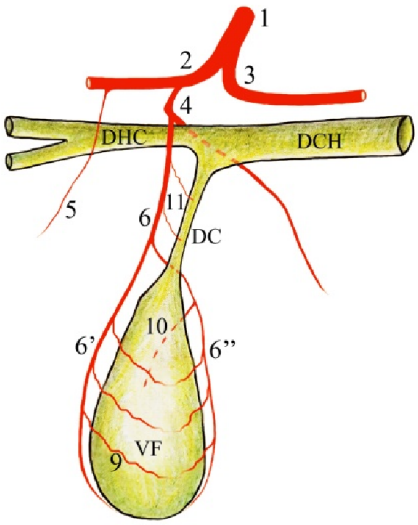

Fig. 4. Variation of *A. cystica* beginning (A2)

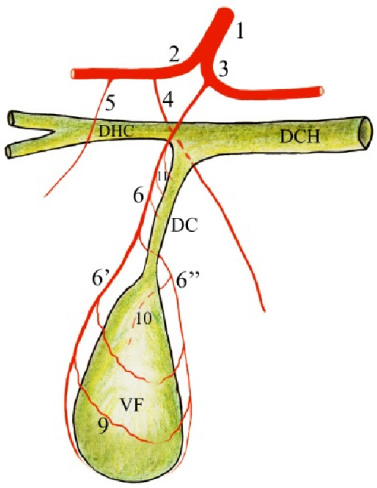

Fig. 5. Variation of *A. cystica* beginning (B1)

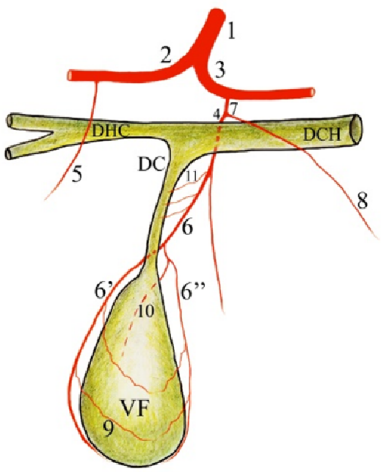

Fig. 6. Variation of *A. cystica* beginning (B2)

1. *A. hepatica*, 2. *R. sinister, 3. A. gastroduodenalis,* 4. *R. dexter medialis,* 5. *R. quadratus,* 6. *A. cystica,* 6`. *R. sinister A. cysticae,* 6``. *R. dexter A. cysticae,* 7. *R. dexter,* 8. *R. dexter lateralis*, 9. *Rami parietalis* of *R. dexter* and *R. sinister A. cysticae,* 10. *R. visceralis* of *R. dexter A. cysticae, 11. Rami ductus cysticus;* DHC: *Ductus hepaticus communis,* DCH: *Ductus choledochus;* DC: *Ductus cysticus;* VF:*Vesica fellea.*

In the current study, we observed that *A. cystica* originated not only from *R. dexter medialis* but from other arteries such as *A. gastroduodenalis* and *R. dexter*. We agree with Osman *et al.* (2008) that *R. dexter* (ramifying mainly into R. *dexter medialis* et *lateralis*) existed in pigs. However, in this study, *R. dexter* was not always present. In humans, mostly *A. cystica* is released from the right branch (*R. dexter*) of *A. hepatica propria* (Vankov & Ovcharov, 2016). Regarding the beginning and the angioarchitectonics of the *A. cystica,* other authors reported 8 different types of arterial supply to the gallbladder in humans (Poston & Blumgart, 2003). Unlike humans, our study showed 4 variations of *A. cystica* origin in swine: from *R. dexter medialis* alone or together with *R. quadratus,* from *A. gastroduodenalis* and from *R. dexter*. In 83% of the examined cases, *A. cystica* was released directly from *R. dexter medialis*, which was in contrast to the study of Osman *et al.* (2008), where *A. cystica* was detached from *R. dexter* in 40% of pigs. The current corrosion cast study in domestic pigs, gave for the first time detailed information about the architectonics of the *A. cystica* branches: *Ramus dexter* and *Ramus sinister* ramifying mainly on the parietal surface of the bladder and less on its visceral surface, which anastomosed with branches of the *Ramus visceralis* – a well-developed branch of the *R. dexter* (*A. cystica*), ramifying within the visceral surface of the gallbladder. The right and left branches of the *A. cystica* released oblique branches on the parietal surface of the gallbladder showing a wavy course that allowed them to adapt to the changes in the volume of the gallbladder in its filling with bile. Data on the length of corrosion casts of *A. cystica* and its branches as well as the distance between *A. cystica* and *Ductus cysticus,* gallbladder*, A. celiaca, R. sinister (A. hepatica)* are also reported for the first time.

The data regarding the anatomical variations of *A. cystica* and its branches supplement the information in Nomina Anatomica Veterinaria (2017). They can be useful for researchers using pigs as experimental models in developing new surgical methods associated with cholecystectomy in humans (Park *et al.* 2005). As Park *et al.* (2005) reported, for cholecystectomy, surgical steps include identification and exposure of the gallbladder; identification and exposure of the cystic duct and artery in order to facilitate dissection; haemostasis, including clipping of the cystic artery and cystic duct as well as gallbladder removal.

CONCLUSION

This study provided new information about the macrovascularisation of the gallbladder, which together with the importance of pigs as experimental animals could be useful in developing new surgical techniques facilitating cholecystectomy. This information would also contribute to the understanding of the etiology of postoperative complications as a result of surgical interventions in this location and their prevention.

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Paper received 07.11.2019; accepted for publication 12.12.2019

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