



## Horse spleen segmentation technique as large animal model of preclinical trials

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### ABSTRACT

The parenchymal distribution of the splenic artery was studied in order to obtain anatomical basis for partial splenectomy. Thirty two spleens were studied, 26 spleens of healthy horses weighing 320 to 450kg, aged 3 to 12 years and 6 spleens of fetus removed from slaughterhouse. The spleens were submitted to arteriography and scintigraphy in order to have their vascular pattern examined and compared to the external aspect of the organ aiming establish anatomo-surgical segments. All radiographs were photographed with a digital camera and the digital images were submitted to a measuring system for comparative analysis of areas of dorsal and ventral anatomo-surgical segments. Anatomical investigations into the angioarchitecture of the equine spleen showed a paucivascular area, which coincides with a thinner external area, allowing the organ to be divided in two anatomo-surgical segments of approximately 50% of the organ each.

**Key words:** arteriography, equine, scintigraphy, spleen, splenic artery.

### INTRODUCTION

The segmental nature of the mammalian spleen was confirmed a long time ago (Lewis 1957, Dreyer and Van 1961, Cubillos 1969). This segmental organization shows a simple arterial arborizing pattern down to the finest arterial capillaries (Lewis 1957). Anatomical investigations into the angioarchitecture of parenchymatous organs will support partial resections which will be able to be performed in less vascularized areas in an easier manner (Christo and Di Dio 1997). Partial splenectomy in horses has not

been reported (Wilson and Constantinescu 1999), although the effects of splenectomy in horses are well documented (Persson and Bergsten 1975, Davis and Manohar 1988, Hanzawa et al. 1995, Wagner et al. 1995), including the immune response to a disease caused by babesia, a blood protozoa (Dennig and Brocklesby 1965, Simpson et al. 1980, De Waal et al. 1988, Guimarães et al. 1997). Saving spleen procedures are justifiable, depending on the type and extent of splenic injury (Schwartz et al. 1974).

The spleen is a falciform shaped organ. The parietal or lateral surface of the spleen is convex, going along the costal arch, in intimate contact

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with the diaphragm. The visceral or medial surface is generally concave and contains the hilus; the vessels and nerves run in the hilus from the dorsal extremity to the ventral extremity. The hilus protects the vessels and nerves. The splenic artery, a branch of the celiac artery, runs within the groove of the hilus and sends branches supplying the spleen (*rami lienalis*) and the short gastric arteries supplying the greater curvature of the stomach (*arterie gastrici breves*). It is important to highlight that in the equine, the amount of fat surrounding the vessels as well the omentum, gastrolial and lienorenal insertions in the spleen does not allow the sight of the hilus area (Witzel and Mullenax 1964, Rigg et al. 1987). The length of the spleen varies from 40 to 70cm, its width from 20 to 25cm, the thickness 2-6cm and its weight from about 0.5 to 3.5kg (Ghoshal 1975, Saar and Getty 1975, Nickel et al. 1979). Regarding its both functions, defense and storing, its size will vary depending on the conditions given to the organism of the animal. It can store up to 60% of circulating red blood cells and it is a rich source of erythrocytes to the horse during exercises (Irvine 1958, Cunningham 1992). In this research the investigations aiming to establish the area more favorable will support partial resections according of the vascular pattern.

#### MATERIALS AND METHODS

This study used 26 spleens of healthy horses weighing 320 to 450kg, aged 3 to 12 years and 6 spleens of fetus removed from slaughterhouse Pomar located in Araguari-MG. The material was donated for slaughterhouse because the spleen is a organ that doesn't have commercial value.

Splenic arteriography was performed in 14 fresh spleens of adult animals, by injecting barium sulfate (Celobar<sup>®</sup>) through the splenic artery at a rate of 1mL/s. We stopped injecting the solution when the short gastric arteries were filled (about 40 to 60mL). Radiographs were obtained using a Raicenter 100Ma machine (Medical Minas<sup>®</sup>,

Brazil, 1998) at 90cm focal-film distance, and a 50kV and 3MaS.

Scintigraphic images were obtained injecting approximately 6ml of a solution of 5 mCi (185MBq) Technetium 99m and 10mL of Dextran 70 (Fresenius Laboratory S/A) through the splenic artery of the 6 fetus spleens. Images were obtained using a gamma camera and computer-assisted image analysis immediately after injection. All radiographs were photographed with a digital camera and the digital images were submitted to the measuring system Image Pro- Plus 4.0 (Media-Cyberneticsb, USA, 1993).

For comparative analysis of dorsal and ventral area from anatomo-surgical segments. The thickness of the organ was measured using 10 adults spleens fixed in paraformaldehyde. One fragment was removed from the dorsal, one from the concave (transition) and one from ventral areas. Their thickness was measured using a pachymeter. Comparisons of values of thickness from different regions of spleen were made with ANOVA (One-way ANOVA) followed by Tukey-Kramer test. Comparisons of adult and fetal spleen areas were made with Student t-test. Bartlett test was also used to evaluate whether data were parametric. The level of significance was set at  $p < 0.05$  in all analyses.

Two adults' animals had artery injected with vinyl resin (Zyhh<sup>®</sup> - Union Carbide) and subsequent corrosion in sulfuric acid 20% for a period of 96 hours.

#### RESULTS

##### MORPHOLOGY AND TOPOGRAPHY

The equine spleen is located in the left antimer with its base in the dorsal region of the abdomen and fixed by lienorenal and frenicolial ligaments, presenting close connection with the left kidney and the left pillar of the diaphragm. By having the form of comma or sickle, provisions ventrally and turned to the thoracic cavity, and may move toward the median plane by traction of the gastrolial ligament when the stomach is distended.

The parietal surface of the spleen is smooth and slightly convex, accompanying the costal arch. The visceral surface by the proximity to the abdominal wall is slightly concave showing regions of great anatomical importance and surgical interest.

The suspensory ligament, composed by lienorenal and frenicorrenal ligaments fall in visceral surface on average to 6cm of dorsal margin. These ligaments of fibro elastic tissue and smooth muscle fibers join the capsule of the organ. The gastrolial ligament runs through the cranial edge of the spleen and establishes a connection with the stomach. In this region the short gastric arteries go to the same.

The dorsal segment of the spleen showed average thickness of 3.04cm (minimum 2.4 and

maximum 3.4cm with standard deviation of 0.29). The transition region between the segments dorsal and ventral presents a lower thickness, on average 1.49cm (minimum 1.0 and maximum 2.5cm with standard deviation of 0.41). The ventral segment showed average thickness of 2.96cm (minimum 2.4 and maximum 3.5cm with standard deviation of 0.28) (Figure 1).

#### SEGMENTARY BRANCHES NUMBER

The lienal artery does not have extraparenquimal division in horses, penetrating directly in the hilum and scrolling through the major axis of the organ, which emits segmentary branches both for intestinal face (wider) pointing to the left ascending



**Figure 1** - Macroscopic view of spleen visceral face showing the location of withdrawal of fragments and the lower thickness in concave area.

colon and descending colon, as for the gastric face (closer). The counting of segmentary branches was performed based in arteriogram.

In intestinal face, i.e. the area to vasculo-nervous bundle of organ that comprises the largest part of the parenchyma and shelt a larger contingent of vessels were found average on 23.6 branches (minimum 20 and maximum 28). In gastric face, which comprises a narrow strip cranial to the vasculo-nervous bundle were found average on 25.5 branches (minimum 20 and maximum 31) (Figures 2A, C e D).

#### ANATOMICAL SURGICAL SEGMENTS

The equine spleen presents no division extraparenquimal of lienal artery, all divisions are intraparenquimal. As there were no arterial anastomoses, these branches have the behavior of terminal artery. The presence of a paucivascular area in all observations, adults and fetuses, precisely in the area of lesser thickness, enables the division of the

organ in two surgical segments, once a region with the lowest number of vessels facilitates the resection of the segment. It is worth noting that only the ventral segment is likely to be removed, since the topography of component added to the behavior of lienal artery determines a closer relationship between the base of the spleen with the celiac artery and the lienal artery, the largest branch in this species (Figure 2B).

#### PROPORTIONAL ANALYSIS OF THE AREA AND THE NUMBER OF BRANCHES PER SEGMENT ANATOMO-SURGICAL

The format of organ in comma, determining a wide base and a tapered apex, added to the smaller thickness of recessed area (area of transition between the dorsal/ventral segments). It was best visualized in visceral face. Even from the observation of the arterial distribution that showed in all animals an paucivascular area, it allowed the division of the body in two segments anatomico-surgical, which presented the distribution of segmentary branches described in Table I.

**TABLE I**  
Number of segmentary branches in spleen of equine adult and fetuses.

	Adults				Fetuses			
	Intestinal face		Gastric face		Intestinal face		Gastric face	
Segment	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
Average	5.3	18.0	7.6	17.5	5.7	18.0	7.8	17.7

#### ANALYSIS OF PROPORTIONAL AREA

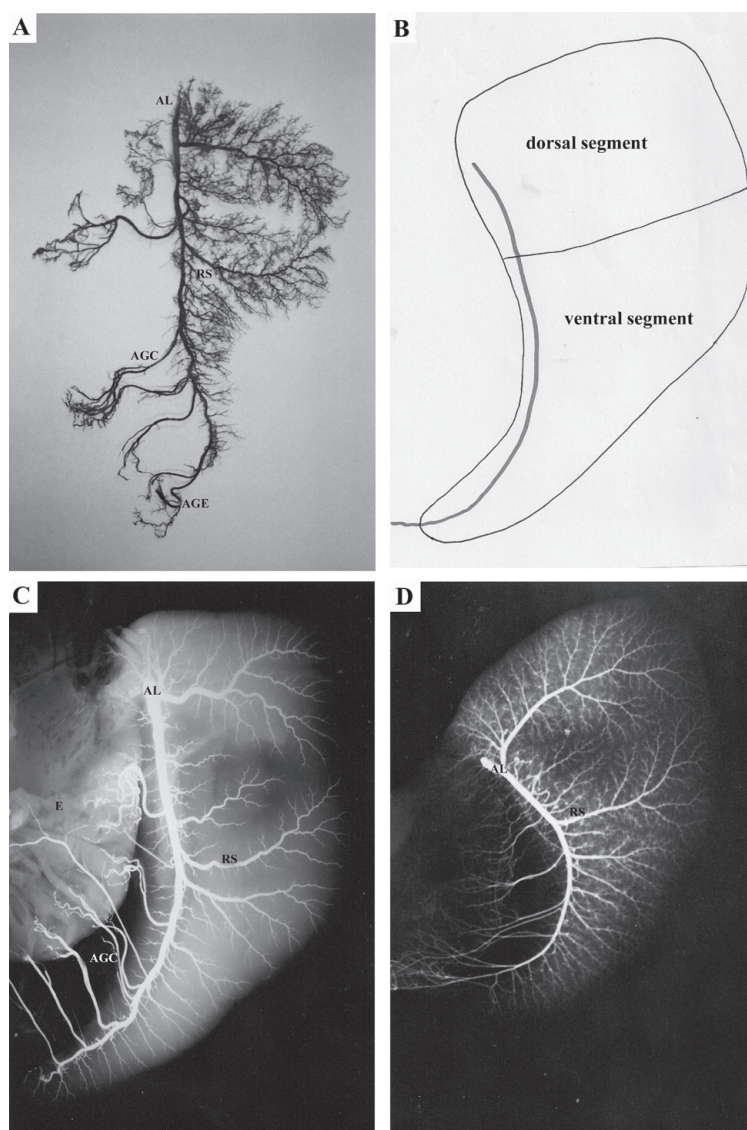
The percentage corresponding to the area of the segments anatomico-surgical dorsal and ventral in relation to the total area of each limbs arteriogram was measured, with the use of Image-Pro Plus. Adult animals presented this correlation on average 52.20% (minimum 47.85% and maximum 56.76%) in dorsal segment and, in ventral segment on average 47.40% (minimum 35.25% and maximum 56.15%).

#### DISCUSSION

Over time the majority of the authors who studied the spleen both in humans and in domestic animals,

regardless of the techniques of research adopted, attempted to establish "parenchymal units" in the spleen. Some classics treaties already brought these evidences when described that the lienal artery is distributed in the spleen while retaining its character terminal (M.C. Christo, unpublished data).

The first work in which the organization and the divisibility of the spleen were described by Assolant (1802). This divisibility reached a series of designations for the "parenchymal units", among them, departments (Assolant 1802), areas (A. Zappalá, unpublished data, M.C. Christo, unpublished data, H.P. Godinho, unpublished data),



**Figure 2** - **A**- Model of vinyl resin arterial vascularization of the spleen, where we can observe the lienial artery (AL), short gastric artery (AGC), left gastroepiploic artery (AGE) and the segmentary branches (RS). **B**- Schematic drawing showing the organ division in two anatomic-surgical segments based on distribution of segmentary branches. **C**- Digital image of arteriogram from equine spleen adult male showing the distribution of segmentary branches, 26 branches in intestinal face and 25 in gastric. We note the absence of conspicuous branches on the parenchyma in gastric face. Lienial artery (A.L.), short gastric artery (A.G.C.), segmentary branches (r.s.) and stomach (e). **D**- Digital image of arteriogram from spleen fetus male, at term, showing the distribution of segmentary branches, with 21 in intestinal and 25 in gastric face and, as in adults we noticed the lack of conspicuous branches on the parenchyma of gastric face. Lienial artery (A.L.) and segmentary branches (r.s.).

regions (Holzchuch 1977), segments (Gupta et al. 1979, A. Ribeiro da Silva Filho, unpublished data) and territories (A.A. Custódio, unpublished data).

The behavior of lienial artery of equine is not similar to any other kind, with the exception of swine, since all the divisions of the artery are

intraparenchymal as described by Dyce et al. (2004). This feature also does not corroborate with the described by Reginato and Melo (2004) to jawbone, as this species presents vascular pedicles easily identifiable extraparenchymal. With that in mind, some authors consider the spleen as a

single segment (Pelagalli 1966, Gupta et al. 1979, A.P.F. Melo, unpublished data), which comes to corroborate with our predilection for the term anatomico-surgical.

A. Fernandes Filho et al. (unpublished data) studying the northeastern has showed the same results as the absence of large caliber vessels on the gastric face. The major difference in relation to segmentectomy proposals in other species (Holzchuh 1977, A. Zappalá, unpublished data, M.C. Christo, unpublished data, H.P. Godinho, unpublished data, A. Ribeiro da Silva Filho, unpublished data) is that in equine the ventral segment can be removed, because, as described, the extraparenchymal division of the lienal artery does not occur, which penetrates the hilum located at the base of the organ by issuing the segmentary branches in the splenic parenchyma.

Another fact that deserves a proper analysis concerns the presence of anastomosis. As we consider segments anatomico-surgical, we do not care about the anastomosis between vessels of small-sized, like those authors who used the same criterion (Di Dio 1998, M.C. Christo, unpublished data, H.P. Godinho, unpublished data). In any case, anastomosis was not found in any branch of large size that could establish a collateral circulation, nor in the minors branches as described by Holzchuh (1977), A. Fernandes Filho et al. (unpublished data) and M.A.A. Rays (unpublished data). In white-lipped peccary (Reginato and Melo 2004) the anastomoses between the segmental arteries was observed in 9 of 30 cases, the venous in 2 of 30 cases and the intersegmental in 5 of 30 cases. The arterial were of small caliber and the venous with larger caliber than their main root.

In cats, the experimental ligation of lienal artery or of its main branches for the dorsal portions, medium and ventral of the organ do not cause any impairment of the spleen, this is due to the presence of arterial anastomosis between the right and left gastroepiploic arteries, or between the gastric short and collaterals of the left gastric artery (Holzchuh 1977).

The maintenance of the spleen is important from the point of view of the pathophysiology of equine babesiosis, as we saw in previous descriptions (Dennig and Brocklesby 1965, Simpson et al. 1980, De Waal et al. 1988, Franco da Silva et al. 1996, Cunha et al. 1997). The possibility of splitting the organ into two anatomico-surgical segments, each with approximately 50% of the volume of the organ, will allow the permanence of an area of the parenchyma which may be sufficient for the maintenance of immune function of the spleen.

The analysis of studies in horses showing the changes in the cardiovascular system caused by total splenectomy corroborates the maintenance of part of the component whenever possible (Persson and Bergsten 1975, Davis and Manohar 1988, Hanzawa et al. 1995, Wagner et al. 1995).

Despite the advantages of saving viable splenic tissue, the question of the critical minimum mass of normal splenic tissue capable of maintaining efficient immune responses remains undefined (Christo and Di Dio 1997). In the present study, each one of the two anatomico-surgical segments represents approximately 50% of the total area of the organ. However, because of the location of the hilum and vessels, only the ventral anatomico-surgical segment can be resected.

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#### RESUMO

A distribuição parenquimal da artéria esplênica foi estudada com o objetivo de obter base anatômica elementos para a realização de esplenectomias parciais. Trinta e dois baços foram estudados, 26 de equinos saudáveis, com peso entre 320 a 450kg e idade de 3 a 12 anos, e seis baços de fetos obtidos em matadouro. Os baços foram submetidos a arteriografias e cintilografias para examinar o padrão vascular e comparar com o

aspecto externo do órgão, procurando estabelecer segmentos anatomo-cirúrgicos. Todas as radiografias foram fotografadas com câmera digital e as imagens digitais foram submetidas a um programa de mensuração para análise comparativa das áreas dos dos segmentos anatomo-cirúrgicos dorsal e ventral. Investigações anatômicas sobre a angiorquiteta do baço equino mostrou uma área paucivasular que coincide com a área externa mais fina, permitindo que o órgão seja dividido em dois segmentos anatomo-cirúrgicos com aproximadamente 50% do órgão em cada.

**Palavras-chave:** arteriografia, equino, cintilografia, baço, artéria esplênica.

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