Accessory spleen

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Abstract
An accessory spleen (AS), or splenunculus, is a small nodule of splenic tissue found outside of the spleen. Post-mortem examinations, also known as autopsies, are carried out to identify a cause of death, and to assess the state of the organs of the deceased. We present two incidental cases of splenunculi, one localized in greater omentum and a second one localized in the hilum of the spleen, diagnosed during a histopathological examination. An immunohistochemical profile of the normal and accessory spleen was followed where the expression of the CD20, CD3, CD23, α-SMA were analyzed.

Keywords: accessory spleen, spleen, immunohistochemical profile, autopsy, greater omentum.

Introduction
The spleen consists of a large encapsulated mass of vascular and lymphoid tissue situated in the upper left quadrant of the abdominal cavity between the fundus of the stomach and the diaphragm [1]. The spleen can display various developmental anomalies, including complete agenesis, multiple spleens or polysplenia, isolated small additional splenunculi, and persistent lobulation. Accessory spleens or splenunculi (AS) may be formed during embryonic life, they rise from the left side of the dorsal mesogastrium as a result of imperfect fusion of separate splenic masses [2, 3]. The localization varies widely, but the most common locations are hilus and vascular pedicle of the spleen, around the tail of the pancreas, greater omentum, along the greater curvature of the stomach, mesentery of the small and the large intestine, near the left ovary or left testis and in the pouch of Douglas. Accessory spleens are usually few in number, maximum six and they have normal splenic histological features with blood supply uniformly arising from a branch of the splenic artery [4]. Ectopic splenic tissue can be either congenital (accessory spleen or splenunculi) or acquired (splenosis). The congenital type is seen in 10–44% of all autopsies [5].

Materials, Methods and Results
Case No. 1
A 55-year-old male, with no history of personal or family diseases, was subjected to an autopsy on October 24, 2010, by the Legal Medicine Bihor County Service, Oradea, Romania.

The cause of death was mechanical asphyxiation and the manner of death was suicide by hanging.

Autopsy examination: on gross examination at the level of the greater omentum, an oval-shaped formation of 0.5/0.4 cm, purple-violet and with a spleen-like macroscopic aspect, a splenunculus (AS) was identified. The histopathological examination showed a normal spleen structure with a pulp aspect: 10/7 cm, with an incision of 0.7 cm and a splenunculus (AS) with similar structure to the main spleen. Other organs did not reveal any significant pathological change. All other viscera were grossly unremarkable (Figure 1).

Figure 1 – (A and B) Accessory spleen at greater omentum level.
Case No. 2

A 52-year-old male was subjected to an autopsy on September 28, 2011, by the Legal Medicine Bihor County Service, Oradea.

The cause of death as of cardiac origin. According to data obtained from the family, the deceased had exposed himself to prolonged physical effort and showed signs of exhaustion. The subject went to sleep without complaining about any other symptoms.

Autopsy examination: the spleen had the dimension of 14/9/2.5 cm, poly-lobed, and with three incisions of 4.5 cm, 2.5 cm and 1 cm. A splenunculus (AS) in the hilum, measuring 0.5 cm, was detected. All other viscera were grossly unremarkable (Figure 2).

Figure 2 – (A and B) Accessory spleen in the hilum.

An immunohistochemical analysis was performed on 4 μm-thick sections prepared from formalin-fixed paraffin-embedded tissue by using an automated immunostainer (Bechmark XT, Ventana Medical Systems Inc., Tucson, AZ, USA). Immunohistochemical assays were performed on a Ventana Benchmark XT automated staining instrument according to the manufacturer’s instructions. Slides were de-paraffinized using EZprep solution (Ventana Medical Systems, Inc.) at 90°C, and all reagents and incubation times were chosen as directed on antibody package inserts. Slides were developed using the OmniMap DAB (3,3'-diaminobenzidine) detection kit (Ventana Medical Systems, Inc.) [6] and counterstained with Hematoxylin.

The CD3 reactivity sections were incubated with the primary polyclonal rabbit antibody, clone 2GV6 (ready-to-use Ventana), according to the manufacturer’s instructions. The CD20 reactivity sections were incubated with the primary polyclonal mouse antibody, clone L26 (ready-to-use Ventana), according to the manufacturer’s instructions. The CD23 reactivity sections were incubated with the primary polyclonal rabbit antibody, clone SP23 (ready-to-use Ventana), according to the manufacturer’s instructions. The CD68 reactivity sections were incubated with the primary polyclonal mouse antibody, clone 1A4 (ready-to-use Ventana), according to the manufacturer’s instructions.

The white pulp is composed of three sub-compartments: the periarteriolar lymphoid sheath (PALS), the follicles, and the marginal zone. The PALS is a T-cell dependent region and the follicles are more a B-cell dependent region [7].

Our study, based on a reduce number of cases, try to find out if is any differences between the normal spleen immunoprofile and the accessory spleen profile. In our cases, the white pulp showed a target arrangement, onion shape, with three zones: a germinal center (light), a thin mantle zone (dark) and an outer layer (marginal zone). The red pulp shows the normal aspects with vascular structures (sinuses) [8].

The CD23 staining in normal and accessory spleen is seeing in follicular dendritic cell (FDC) meshwork. Comparing the FDC lymph node networks with the spleen FDC meshworks seems to be more developed in the lymph nodes. The dendritic cell network in spleen and accessory spleen is less dense and looks to be “eat by moth”. The number of cases is minimal and can lead to bias [12].

It is well know that the dendritic cells, which are antigen-presenting cells, play an important role in the human immune response. The changes in the CD23 cells expression according with many studies are involved in pathology. After physiologic germinal cell development, the follicular dendritic cell meshwork expands and follicular dendritic cells in the light zone of the germinal center become CD23 positive. CD23 acts as a B-cell growth and activation factor, promoting differentiation into plasma cells [13]. Our finding, that the number of dendritic cells is reduced compared with the similar structure from lymph node, may lead to the conclusion that the CD23 cells from lymph node play a higher role in this part of immune response comparing with the role played by the CD23 cells from spleen or accessory spleen. This remark needs more studies. Our study reveals also that the accessory spleen is integrated in the immune response and is not an unimportant accessory organ (Figure 5).
We noticed in our cases that are some borderlines between red pulp and white pulp. These borders (septa) are coming directly from spleen capsule. The marginal lines and capsule are highlight by \(\alpha\)-SMA. Only few studies reflect the presence of the myofibroblastic cell without any meaning yet or with the explication of the contractile role. In the study are mentioned the irregularly spaced trabeculae of smooth muscle and fibroelastic tissue emanate from the capsule into the splenic parenchyma. These trabeculae also contain blood and lymph vessels and nerves [7].

The presence of the smooth muscle fibers is similar in normal spleen and in the accessory spleen. In our opinion, the exact role played by the \(\alpha\)-SMA positive cells needs more study to understand the exact role played by these cells (Figures 6 and 7).

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**Discussion**

In both our cases, the accessory spleens were not diagnosed antemortem and the families of the deceased could not offer us details regarding this anomaly. At the same time, the splenunculi were asymptomatic and were discovered accidentally. The accessory spleen structure was histopathologically confirmed.

Literature describes the accessory spleen as common phenomenon for both sexes and at all ages and has been encountered in different causes of death. Therefore, a medical autopsy can be a means to determine the incidence and the characteristics of accessory spleens (location, dimension, etc.) in the population. Splenunculi or accessory spleens are congenital in 10–44% of autopsies [5]. An accessory spleen rises from the side of the dorsal mesogastrium during the embryological period of development.
because of imperfect fusion of separate splenic masses. Most often there is one accessory spleen (85%), sometimes two (14%) and rarely three or more (1%). Their size is not large than 2 cm in diameter. The most common location is the hilum of the spleen in the gastroepiploic ligament (50%), but may be found behind the tail of pancreas (30%) or rarely within the greater omentum [14] of the stomach. It can also be found at the mesentery of the small intestine, mesocolon, pancreas [15], kidney [16] and pelvis as an adnexal mass [17].

Microscopic analyses showed normal splenic red and white pulp components, including lymphoid follicles with germinal center formation. The specimen had a thick capsule with smooth muscle elements and was positive for alpha smooth muscle actin (SMA) in the immunohistochemical analysis. It would occasionally appear difficult to distinguish the accessory spleen from splenosis. Histologically, splenosis is not encapsulated nor does it usually present with smooth muscle elements and supply vascular branches rising from the splenic artery. In contrast, accessory spleens are encapsulated with the smooth muscle elements, which is similar structure of normal spleens.

In the accessory spleen, as in the normal spleen, an equally proportion between red pulp and white pulp can be seen. In general, the ratio is 3:1 red pulp/white pulp as in our cases [11, 18].

Splenunculi are important in hematological disorders where splenectomy is the treatment of choice. If the surgeon is not able to locate or remove them at the time of splenectomy, they will undergo hyperplasia and cause recurrence of the disease. Accessory spleens resemble normal spleen in structure and in immunologic functions.

C Conclusions

We presented these cases because of the rarity of accessory spleen at the level of greater omentum level. It was diagnosed postmortem during autopsy and confirmed by a histopathological examination and the finding of the dendritic cell meshwork pattern in spleen and accessory spleen. The autopsies are useful for determining the incidences and other features of AS in different populations.

References


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