CASE REPORTS

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Intrapancreatic accessory spleen. Report of four cases diagnosed by ultrasound-guided fine-needle aspiration biopsy

JOSÉ-FERNANDO VAL-BERNAL[1)], MARÍA MARTINO[2)], ELENA YLLERA-CONTRERAS[3)], BEATRIZ CASTRO-SENOSIAIN[4)], PABLO BUENO-ORTIZ[2)]

[1)] *Pathology Unit, Department of Medical and Surgical Sciences, University of Cantabria and IDIVAL Research Institute, Santander, Spain*

[2)] *Service of Anatomical Pathology, Marqués de Valdecilla University Hospital, Medical Faculty, University of Cantabria and IDIVAL Research Institute, Santander, Spain*

[3)] *Service of Radiodiagnosis, Marqués de Valdecilla University Hospital, Santander, Spain*

[4)] *Service of Digestive System, Marqués de Valdecilla University Hospital, Santander, Spain*

Abstract

Intrapancreatic accessory spleen (IPAS) is a congenital anomaly usually misdiagnosed as a pancreatic neoplasm. For five years and four months, we collected seven IPASs located in the tail of the pancreas in four patients diagnosed by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). All cases had associated cell block preparations. Each patient underwent endoscopic ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) studies. The patients ranged in age from 57 to 73 years (mean age 65.7 years old). All lesions were well-defined, 1–1.9 cm in size (mean 1.5 cm). To our knowledge, a case with four IPASs in the tail of the gland has not been previously reported. Cytological features of IPAS included a polymorphous population of hematopoietic cells admixed with occasional blood vessels. Cell blocks comprised spleen red pulp. CD8 immunostaining of cell blocks highlighted splenic endothelial cells and confirmed the diagnosis. IPAS presented as an asymptomatic lesion detected on imaging studies. It may mimic a pancreatic neoplasm, mainly a neuroendocrine tumor. The use of EUS-FNA is an essential tool in the diagnosis of the lesion. The endothelial cells of the splenic sinuses characterized by their positivity for CD8 are evident in the sections of the cell blocks. This staining is considered specific and can be used as a confirmatory marker. EUS-FNA biopsy provides a reliable diagnosis that prevents unnecessary surgery.

Keywords: intrapancreatic accessory spleen, ultrasound-guided fine-needle aspiration, immunohistochemistry.

Introduction

Accessory spleen is a developmental anomaly in which splenic tissue is found outside the spleen. Accessory spleens are present in about 10.4% of the population. The majority of cases (80%) are found in the perihilar area of the spleen, while the second most common location is the tail of the pancreas (16.7%) [1]. Intrapancreatic accessory spleen (IPAS) is a congenital anomaly that usually comprises a well-delimited nodule within the tail of the pancreas or close to it. This nodule constitutes a portion of normal splenic tissue separated from the principal body of the spleen. Although IPASs are usually asymptomatic, their presence may be noted incidentally on radiological imaging [2]. Despite the fact that they are clinically innocuous, they can pose a challenge in the imaging studies by mimicking a pancreatic neuroendocrine tumor or diverse types of malignant pancreatic neoplasms [3, 4]. Thus, it is essential to differentiate IPAS from other pancreatic tumors to avoid unrequired surgery [2–11]. The only safe diagnostic method is direct sampling. The fine-needle aspiration (FNA) biopsy findings of IPAS have been uncommonly reported. However, aspiration cytology may be misleading due to poor sampling or difficulty in interpretation of the sample [12, 13]. On the other hand, the use of endoscopic ultrasound to guide FNA biopsy has become a very important tool in the diagnosis of pancreatic lesions. With this method, it is possible to obtain a cell block that allows studying the architecture of the tissue and its immunohistochemical reactivity. However, experience with this procedure is still scarce [14, 15].

We report herein four cases of IPAS diagnosed by endoscopic ultrasound-guided (EUS)-FNA biopsy.

Case presentations

We collected a series of four cases of IPAS diagnosed by EUS-FNA biopsy over a five-year and four-month period (January 2013 to April 2018). In this study period, 435 EUS-FNA biopsies had been made. There were seven lesions in four patients. All the cases were seen in-house. Each patient underwent endoscopic ultrasound, computed tomography (CT) scan, and magnetic resonance imaging (MRI) for evaluation of abdominal pain, tumor extension, urinary incontinence or epigastralgia. Rapid on-site evaluation was performed in the four cases and the specimen was considered adequate. In each case, there were smears of the aspirate that were stained with Diff–Quik and Papanicolaou method. In all the cases, we had cytoblock preparations. Immunopathological study was performed on formalin-fixed, 4-µm-thick, paraffin-

embedded tissue sections, using the EnVision FLEX Visualization System (Dako, Agilent Technologies, SL, Las Rozas, Madrid, Spain). Antibodies used in the immunohistochemical study are indicated in Table 1.

Table 1 – ***Antibodies used in this study***

Antibody	Source	Clone	Dilution	Retrieval solution pH (Dako)
CD8	Dako*	C8/144B	FLEX RTU	High
Cytokeratin	Dako	AE1/AE3	FLEX RTU	High
CD56	Dako	123C3	FLEX RTU	High
Chromogranin	Dako	Polyclonal	FLEX RTU	High
Synaptophysin	Dako	SY38	FLEX RTU	High
Ki-67	Dako	MIB-1	FLEX RTU	Low
CD31	Dako	JC70A	FLEX RTU	Low
Factor VIII	Dako	Polyclonal	FLEX RTU	Low

*: Dako, Agilent Technologies, SL, Las Rozas, Madrid, Spain; RTU: Ready-to-use.

Immunohistochemical reactions were carried out using pertinent tissue controls. Concerning the antibody for CD8, tonsillar tissue was used as an external positive control and the T-cells of the splenic tissue under study as an internal positive control. Normal skin was used as an external negative control. Automatic staining was performed in a Dako Omnis autostainer (Agilent Technologies, SL).

Clinical imagistic and follow-up profiles

Case No. 1

A 64-year-old woman presented with abdominal pain and flatulence of one-month duration. The pain was not severe and was not related to food. There was no associated fever, chills, night sweats, nausea or vomiting. She had been diagnosed with Sjögren's syndrome. The patient underwent echoendoscopy evaluation, which revealed a rounded, solid, isoechogenic nodular lesion with well-defined limits of 1.5 cm in maximum diameter within the tail of the pancreas. The nodule was hypervascular in contrast-enhanced abdominal CT scan. MRI showed a hypointense caudal nodule in T1 and isointense in T2. The pancreatic body and duct appeared normal. The first diagnostic consideration was a nonfunctioning neuroendocrine tumor. Three passes were made using a 22-gauge needle for EUS-FNA. The microscopic study revealed an IPAS. Follow-up demonstrated 51 months of stability in the imaging actualization including the size of the tail lesion.

Case No. 2

A 69-year-old man was diagnosed with rectal adenocarcinoma. In the tumor extension study, four solid, rounded, nodular lesions were detected in the tail of the pancreas. They were well-delimited, solid, hypoechogenic, measuring 1 to 1.9 cm in maximum diameter (Figure 1). A CT scan of the abdomen revealed that the lesions were hyperdense with respect to fatty tissue and muscle, and isodense with respect to the pancreas and splenic parenchyma (Figure 2). MRI showed that they were hypointense in T1 after administration of paramagnetic contrast (gadolinium), and hyperintense in T2 with identical hyperintense signal with respect to the pancreas and spleen, and early enhancement after the introduction of the contrast (Figure 3). The lesions were suggestive of multiple IPASs. Five passes were made using a 22-gauge needle for EUS-FNA. The microscopic study revealed an IPAS of the selected lesion. Follow-up showed 22 months of stability in the imaging actualization including the size of the tail lesions.

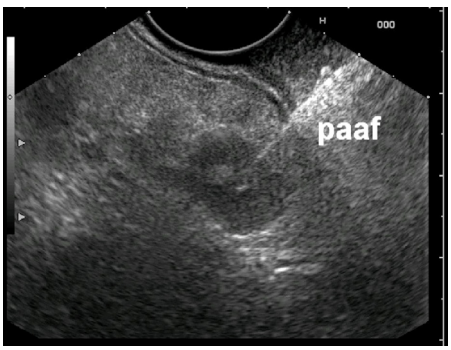

Figure 1 – ***Nodular, hypoechogenic lesion of the pancreatic tail. The biopsy needle appears within the lesion (paaf). In this figure, only a nodule is shown.***

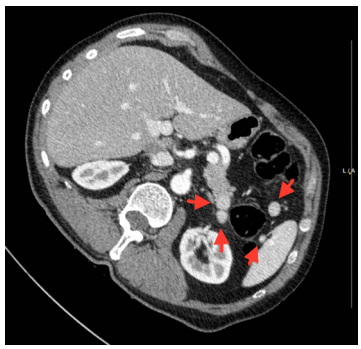

Figure 2 – ***Abdominal CT scan in portal phase: axial oblique multiplanar reconstruction. The arrows indicate four pancreatic, solid nodules.***

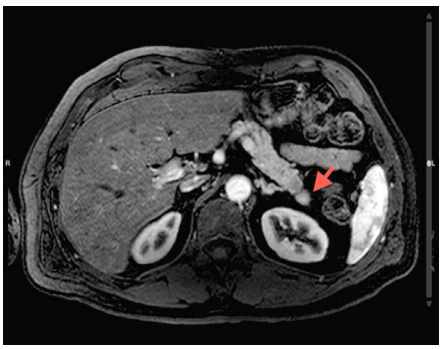

Figure 3 – ***Abdominopelvic MRI. Potentiated sequence in T1 after administration of paramagnetic contrast (gadolinium). The arrow indicates a solid, nodule in the pancreatic tail. In this figure, only a nodular lesion is shown.***

Case No. 3

A 57-year-old woman, gravida 2 para 2, was referred for evaluation of urinary incontinence. The patient had suffered urinary incontinence since the last pregnancy that occurred 20 years earlier. She was not under medical treatment at the time of consultation. Previous clinical history was significant for delivery by Cesarean section in the last pregnancy. Endoscopic ultrasound revealed a 1.7 cm, round, well-defined, hypoechoic, homogeneous mass in the tail of the pancreas. CT evaluation showed that it was hypervascular, and MRI demonstrated a T1 hypointense and T2 hyperintense-enhancing lesion. The primary diagnostic consideration was a nonfunctioning neuroendocrine tumor. Four passes were made using a

22-gauge needle for EUS-FNA. The microscopic study revealed an IPAS. Follow-up demonstrated 16 months of stability in the imaging actualization including the size of the tail lesion.

Case No. 4

A 73-year-old woman presented with epigastric pain irradiated to the right upper quadrant for several months. Pain was not always related to dietary intake. The patient reported a lack of appetite with a loss of 13 kg in the few last months. Past medical history was significant for arterial hypertension, psoriasis, osteoporosis, and cholecystectomy due to biliary lithiasis. Endoscopic ultrasound revealed a 1.2 cm, round, well-defined, hypoechoic, homogeneous mass in the tail of the pancreas. In addition, ectasia of the pancreatic duct in the periampullary area was observed. CT evaluation showed that the mass was hypervascular, and MRI demonstrated a T1 hypointense and T2 hyperintense-enhancing lesion. The primary diagnostic consideration was a nonfunctioning neuroendocrine tumor. Four passes were made using a 22-gauge needle for EUS-FNA. The microscopic study revealed an IPAS. Follow-up demonstrated one month of stability in the imaging appearance including the size of the tail lesion.

The summary of demographic, clinical, and radiological data of these cases are included in Table 2.

Table 2 – ***Clinicopathological features of IPAS cases diagnosed on fine-needle aspiration biopsy***

Case No.	Age [years]/ Gender	Presentation	Main ailment	No. of lesions	Lesion size [cm]	Imaging diagnosis	Follow-up [months]/ Outcome
1.	64/F	Incidental (investigation of abdominal pain and flatulence)	Sjögren's syndrome	1	1.5	Neuroendocrine tumor	51/NEP
2.	69/M	Incidental (investigation for cancer staging)	Rectal carcinoma	4	1.9 1.5 1.4 1	IPASs	22/NEP
3.	57/F	Incidental (urological investigation)	Urinary incontinence	1	1.7	Neuroendocrine tumor	16/NEP
4.	73/F	Incidental (abdominal investigation for epigastralgia)	Epigastric pain irradiated to right hypochondrium	1	1.2	Neuroendocrine tumor	1/NEP

IPAS: Intrapancreatic accessory spleen; F: Female; M: Male; NEP: No evidence of progression of the lesion.

In conclusion, the patients ranged in age from 57 to 73 years (mean age 65.7 years old) and three of the four patients were women. All the lesions were detected incidentally. The lesions ranged in size from 1 to 1.9 cm (mean size 1.5 cm). All the cases were in the tail of the pancreas. One case showed four lesions. In three cases, the clinical-imagistic differential diagnosis included a nonfunctioning neuroendocrine tumor. The follow-up of the four patients varied from one to 51 months (mean 22.5 months). All the patients showed no evidence of lesion progression.

Microscopic study

Conventional smears demonstrated in all cases cellular aspirates with single cells and clusters of cells. A light-to-moderate cellularity with a prominent background of red blood cells was observed. There was a polymorphous population of small-to-medium lymphocytes admixed with other inflammatory cells, including, macrophages, plasmacytoid cells, and occasional eosinophils and neutrophils (Figure 4). Dense aggregates of predominantly lymphohistiocytic tissue with occasional traversing structures suggestive of sinuses were seen in Case No. 2. Cell blocks showed small fragments of splenic tissue with well-formed red pulp. The red pulp was composed of cords between sinusoidal vascular spaces. Frequently, the reticular sinusoidal pattern was not evident on Hematoxylin and Eosin (HE) staining (Figure 5). CD8 highlighted the sinus (littoral) endothelial cells (Figure 6). These cells were also reactive for CD31 and factor VIII (Figure 7). The reactivity for CD31 was not discriminative because the staining showed other positive cells besides the endothelial cells. Ki-67 proliferation index was about 1%. Reactivity for cytokeratin AE1/AE3, CD56, chromogranin, and synaptophysin was not observed. Small fragments of acinar tissue without abnormalities were occasionally present.

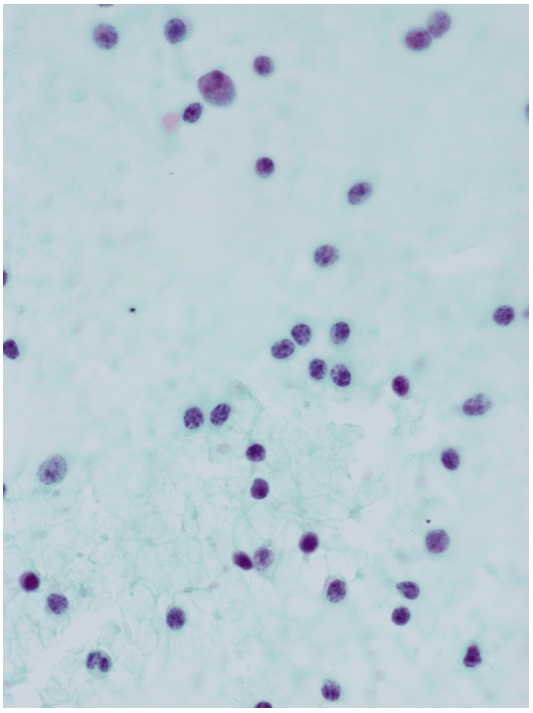

Figure 4 – ***Papanicolaou stained conventional smear demonstrating a polymorphous population of lymphocytes admixed with other inflammatory cells, including plasmacytoid cells, and macrophages. This single-cell aspirate shows low-cellular density on a background of red blood cells (×1000).***

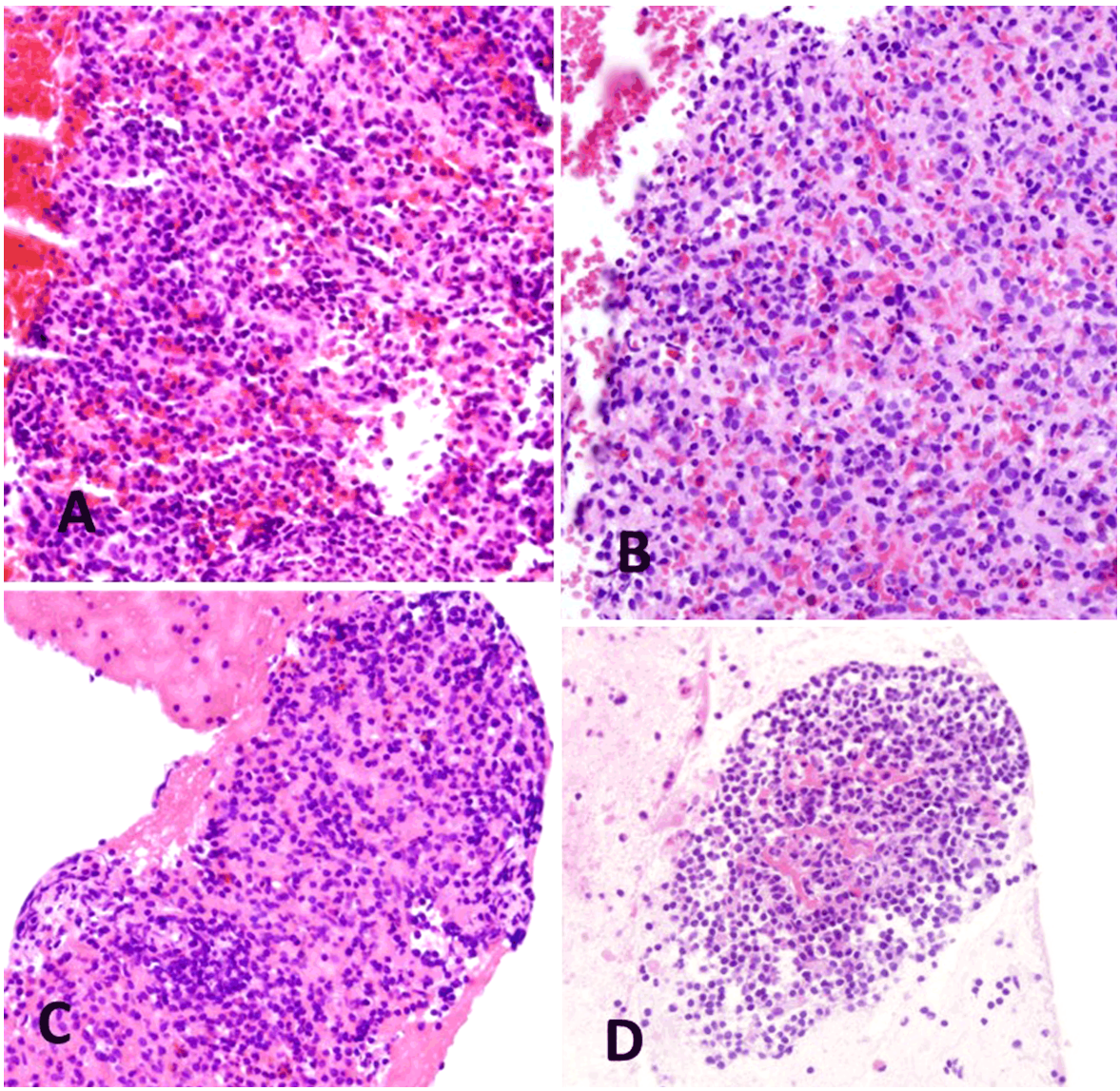

Figure 5 – ***Routine HE staining of the cell blocks. Presence of aggregates of splenic constituents in a background of red cells: (A) From Case No. 1 (×400); (B) From Case No. 2 (×400); (C) From Case No. 3 (×400); (D) From Case No. 4 (×400).***

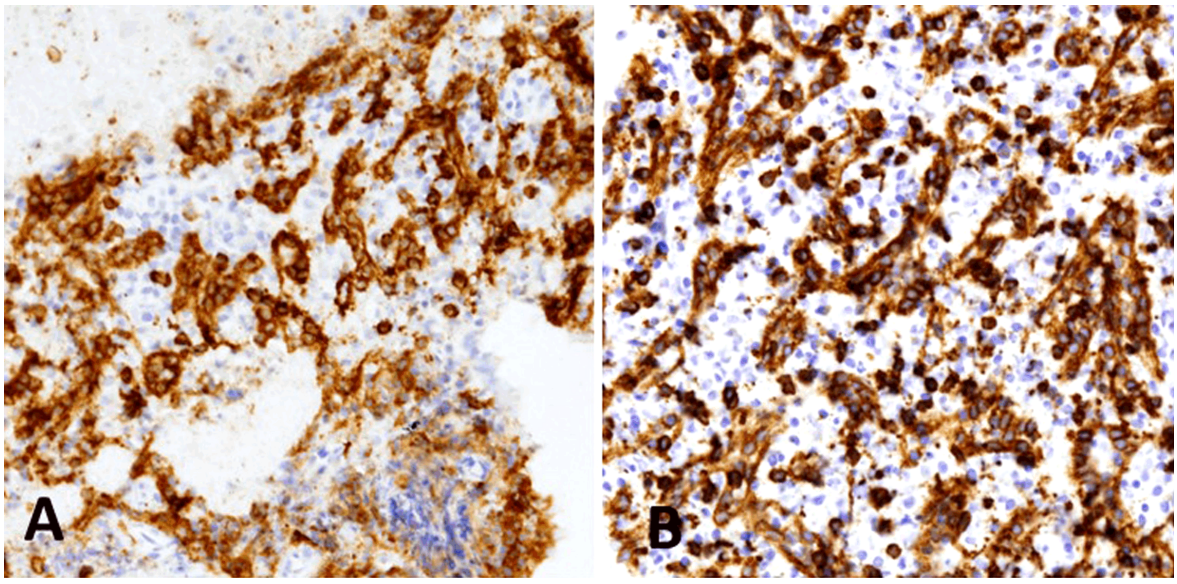

Figure 6 – ***Immunohistochemical staining of the cell block for CD8 in Cases Nos. 1 & 2. This marker highlights splenic sinus endothelial cells: (A) Case No. 1 (×400); (B) Case No. 2 (×400).***

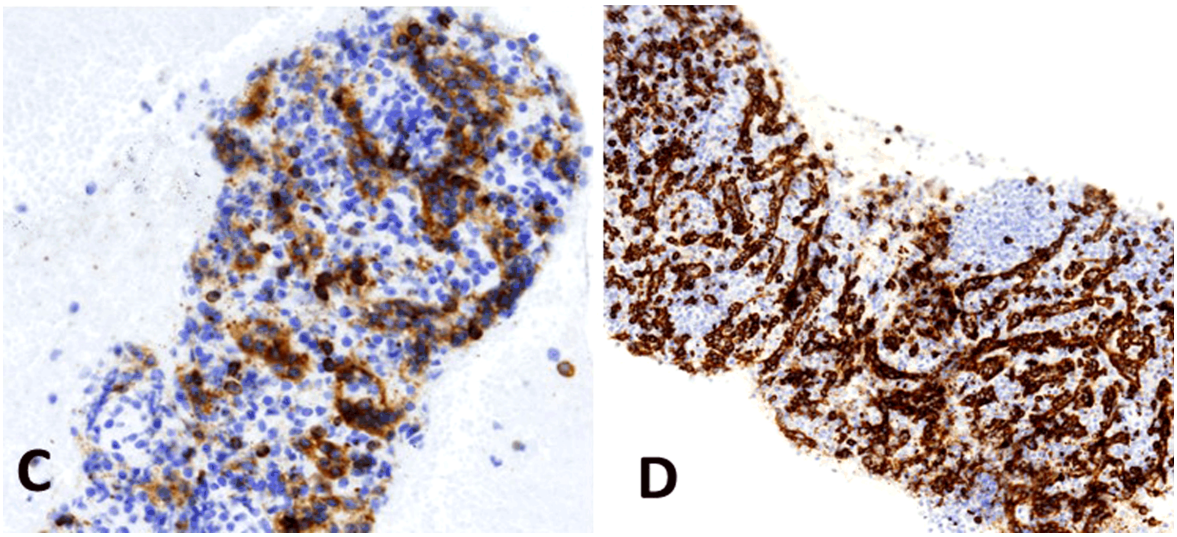

Figure 6 (continued) – ***Immunohistochemical staining of the cell block for CD8 in Cases Nos. 3 & 4. This marker highlights splenic sinus endothelial cells: (C) Case No. 3 (×400); (D) Case No. 4 (×200).***

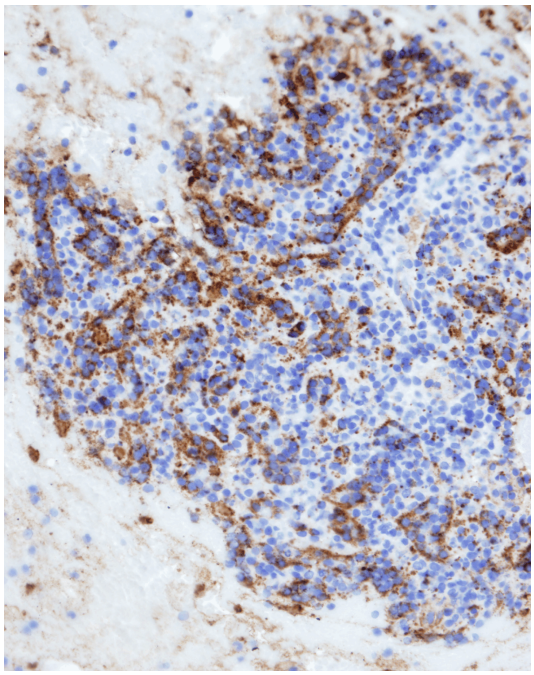

Figure 7 – ***Positivity for factor VIII in endothelial cells (from Case No. 3, ×400).***

Discussions

Accessory spleen is a congenital abnormality in which there is a failure of fusion between a portion of the developing splenic tissue and the principal body of the spleen [16]. The prevalence of an accessory spleen in the pancreatic tail has been reported in 2.9% of cases in postmortem studies [17]. Nevertheless, an IPAS may be occasionally located in the head of the pancreas [18].

IPASs are commonly asymptomatic and clinically harmless. Their presence is usually noted incidentally on radiological imaging during an unrelated workup. Thus, the IPAS is becoming more commonly observed due to the frequent use of CT and/or MRI with better contrast and spatial resolution. Currently, there are no reliable clinical or radiological criteria for the diagnosis or differentiation of an IPAS from a malignant tumor [12, 19–21].

Many previously published cases of IPAS were diagnosed by imaging studies of pancreatic neoplasms such as pancreatic endocrine neoplasm, solid-pseudopapillary neoplasm, ductal or acinar carcinoma, pancreatic lymphoma and hypervascular metastasis [20–22]. Nevertheless, pancreatic neuroendocrine neoplasms are in the highest position in the list. Thus, in a study of 11 cases of surgically removed IPAS, only one case was correctly diagnosed preoperatively. The remaining 10 cases were misdiagnosed as pancreatic endocrine neoplasms and accordingly the patients underwent surgical resection [23].

IPASs are usually surrounded by a fibrotic capsule. They are commonly small (1–3 cm; mean 1.5 cm), well-defined, hypervascular lesions that remain stable over time [12]. Multiplicity of lesions in the pancreas is very uncommon. Among 2700 patients, only one case showed four accessory spleens. One was at the hilum of the spleen and the other three were in the transverse mesocolon [17]. As far as we are aware, a case showing four IPASs in the tail of the pancreas has not been previously reported.

Despite the fact that imaging modalities continue to improve these procedures need the assistance of an FNA biopsy for further classification of the lesion.

The cytological features of IPAS were reported by Schreiner *et al.* [24]. These include a moderately cellular polymorphous population of small lymphocytes, dispersed plasma cells, eosinophils, neutrophils, macrophages, erythrocytes, endothelial cells, and traversing vasculatures. Rodriguez *et al.* [25] alerted about the occasional presence of cells with finely granular chromatin and areas mimicking poorly formed rosette, which might suggest the diagnosis of a neuroendocrine tumor. Tatsas *et al.* [15] warned about the monotone unicellular population of inflammatory cells that are observed in the IPAS. These cells can be confused with neuroendocrine elements, but the tumor cells have more abundant cytoplasm than inflammatory cells and show round shaped nuclei with finely dispersed chromatin. Conway *et al.* [26] described the supplementary finding of abundant large CD31+ platelet aggregates as a distinguishing characteristic of splenic tissue on FNA. However, the most advantageous feature that has been described in the diagnosis of IPAS is the study of the scattered tissue fragments of the cell block. The fragments are usually constituted by red pulp because this structure

comprises 75% of the volume of the spleen [27]. These fragments are composed predominantly of splenic sinuses surrounded by cord tissue cells. The lining cells of the splenic sinuses, or specialized littoral cells, stain positive for CD8, a commonly used marker for T-cells. This staining is specific as systemic endothelial cells and all types of hemangioma are negative. Littoral cells with combined phagocytic and endothelial qualities may also express CD4, CD68, CD31, and factor VIII; and they are no reactive for CD34 [28, 29]. Recognition of these features allows for a histopathological diagnosis of IPAS with prevention of unnecessary surgical resection [15, 30].

Conclusions

IPAS is a congenital anomaly that usually presents as an asymptomatic sometimes multiple lesions detected on imaging studies. It may mimic a pancreatic neoplasm. The use of endoscopic ultrasound to guide FNA has become a very important tool in the diagnosis of the lesion. The endothelial cells that line the splenic sinuses are positive for CD8 in sections of the cell block. This staining is considered specific and can be used as a confirmatory marker. Ultrasound-guided FNA biopsy provides a reliable diagnosis that prevents unnecessary surgery.

Conflict of interests

The authors declare that they have no conflict of interests.

Compliance with ethical standards

This study was approved by the Ethics Committee of IDIVAL Research Institute (CI: 2017.231) and confirmed to the provisions of the Declaration of Helsinki.

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Corresponding author

José-Fernando Val-Bernal, Professor, MD, PhD, Unidad de Patología, Departamento de Ciencias Médicas y Quirúrgicas, Universidad de Cantabria, Avda. Cardenal Herrera Oria s/n, 39011 Santander, Spain; Phone +34 942 203492, extension 73232, Fax +34 942 315952, e-mail: fernando.val@unican.es

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