Infection (2019) 47:507–518
https://doi.org/10.1007/s15010-019-01279-5

REVIEW

Focal spleen lesions in visceral leishmaniasis, a neglected manifestation of a neglected disease: report of three cases and systematic review of literature

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Received: 4 September 2018 / Accepted: 5 February 2019 / Published online: 15 February 2019

Abstract

Background The Mediterranean area is endemic for the zoonotic form of visceral leishmaniasis (VL) caused by *Leishmania infantum*, a species which has been recently associated with unexpected epidemiological and clinical features.

Method We report the result of a systematic review of the literature on VL unusually presenting with spleen focal lesions, including three previously unpublished cases.

Results A total of 28 cases of VL with multiple spleen focal lesions were retrieved. Twenty-six (93%) were acquired in the Mediterranean area, where *L. infantum* is endemic. Thirteen cases were diagnosed in children, and the remaining 15 cases in middle-aged adults, of whom 9 were immunosuppressed. Four patients (14%) underwent diagnostic splenectomy, while the diagnosis was confirmed by less invasive techniques in the remaining patients. The most common radiological patterns were: multiple subcentimetric or centimetric spleen lesions (size ≤ 1 cm in 14 of 19 cases, 74%), hypoechoic at ultrasonography (25 of 26 cases, 99%) and hypodense at CT scan (9 of 10 cases, 90%). PET-CT (available for four patients) showed an intense FDG spleen uptake. MRI and contrast-enhanced ultrasonography, available for two and one cases each, showed complex patterns.

Conclusions VL must be added to the list of possible differential diagnosis of spleen focal lesions and health care provider awareness about this condition will avoid unnecessary invasive diagnostic procedures.

Keywords Visceral leishmaniasis · Focal spleen lesions · Spleen · Haematologic disorder · Atypical

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s15010-019-01279-5) contains supplementary material, which is available to authorized users.

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Introduction

The Mediterranean area is endemic for the zoonotic form of visceral leishmaniasis (VL), caused by *Leishmania infantum* [1]. In Italy, southern regions are classically considered the most endemic areas for VL, and in this setting the human disease usually affects mostly pediatric population and adult immunosuppressed individuals, such as people living with HIV [2]. *L. infantum* is increasingly showing a potential to present unexpected epidemiological features and unusual clinical findings. From the epidemiological point of view, several outbreaks have recently been reported in the Mediterranean area, with unusual reservoir host, as hares [3], or a northern diffusion instead of the typical epidemiology [4]. From the clinical point of view, the parasite has shown to be able to affect nearly any organ, including skin, gastrointestinal and oral mucosa, but also profound organs such as lung, mediastinum, pericardium, pleura, and lymph nodes [3–6].

Here, we report three cases of VL, presenting with focal spleen lesions, and the results of a systematic literature review on this infrequently reported imaging feature.

Methods

Besides describing three previously unpublished cases of VL presenting with focal spleen lesions, a systematic review of literature was conducted to identify all reported cases of VL presenting with focal spleen lesions. The detailed protocol followed for the systematic review of literature, including search strategy, selection criteria, and extraction protocol is reported in Supplementary File 1. Figure 1 shows the flow chart of search and selection process.

Data obtained were finally summarized and presented in an aggregate way in the text of the article, and in detail in Table 1.

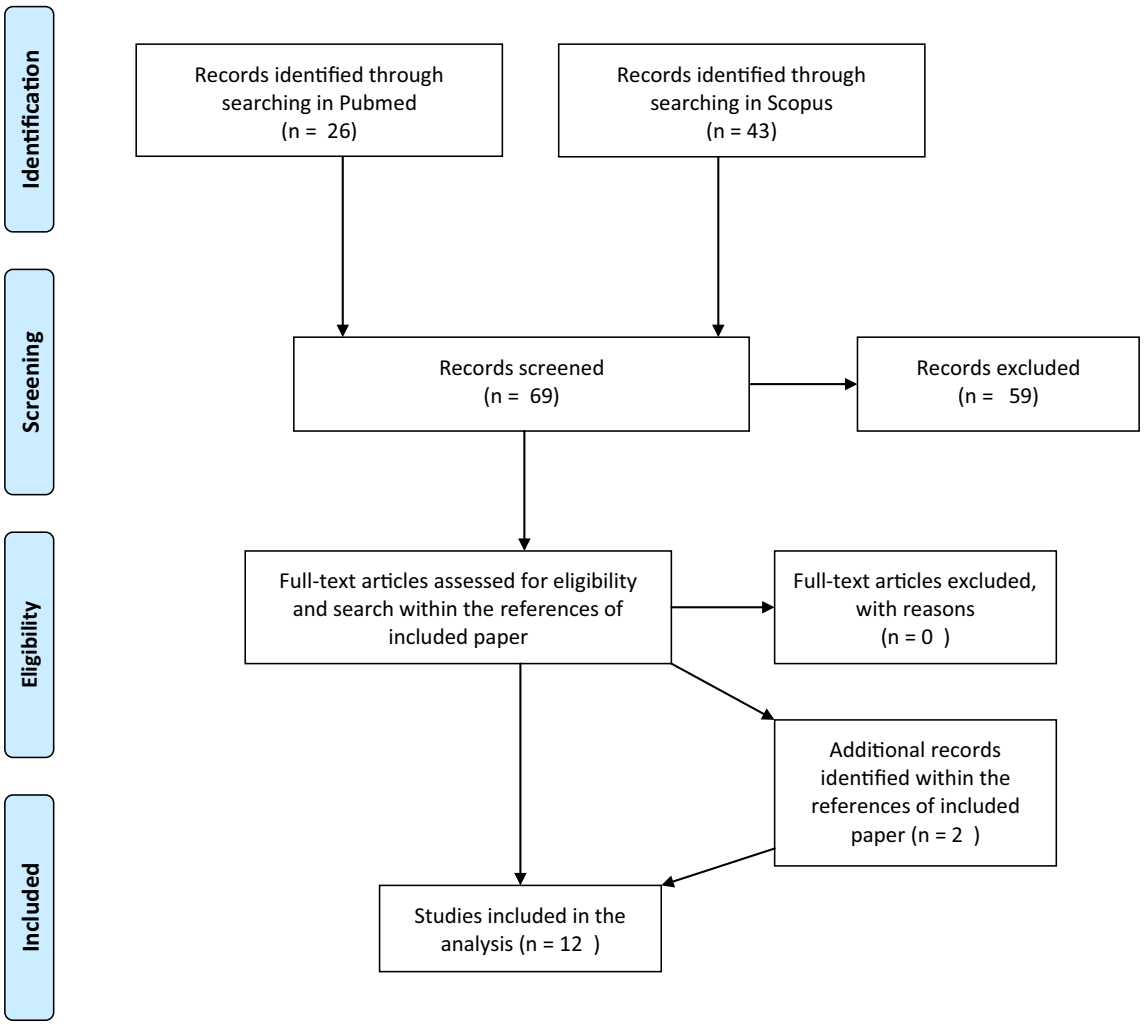

Fig. 1 Flow chart showing the steps of the search and selection process of the systematic review

Table 1 Main features of human visceral leishmaniasis cases with focal spleen lesions reported in the literature

Authors (year)	Patient age/sex	Country	Clinical features	Immuno-depressive factor	Pancytopenia	Splenomegaly (diameter)	Splenic focal lesions	Hepatic focal lesions	US pattern
Bernabeu-Wittel et al. (1999) [7]	Adults (median age 33 yo) 7 cases	Spain	Fever	HIV	ND	ND	Yes	No	Multiple hypoechoic nodules (< 5 mm)
Fretzayas et al. (2001) [8] case 1	10 yo/M	Greece	Fever, fatigue, weight loss	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules (~ 20 mm)
Fretzayas et al. (2001) [8]: case 2	17 mo/M	Greece	Fever, fatigue, weight loss	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules (5, 9 and 5 × 12 mm)
Fretzayas et al. (2001) [8]: case 3	3 yo/M	Greece	Fever	None	Only anemia + thrombocytopenia	Yes (ND)	Yes	No	Multiple hypoechoic nodules (5, 5, 9 and 12 mm)
Bukte et al. (2004) [9]	28 yo/M	Turkey	Fever, abdominal pain	None	Only anemia + thrombocytopenia	Yes (ND)	Yes	Yes	Multiple hyperechoic splenic nodules with hypoechoic haloes. Multiple isoechoic hepatic necrotic nodules with hypoechoic haloes (the largest 61 × 50 mm)
Yazici et al. (2008) [10]	43 yo/F	Turkey	Fever, abdominal pain	None	Yes	Yes (ND)	Yes	No	Multiple splenic nodules and intracapsular lacerations within
Saxena et al. (2011) [11]	7 yo/F	India	Fever, abdominal pain	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules (2–5 mm)
Raeymaeckers et al. (2012) [12]	15 mo/F	Morocco	Fever, swollen abdomen	None	ND	Yes (12 cm; nv for 1–2 yo: 5.1–8.2 cm)	Yes	No	Multiple hypoechoic nodules
Mao et al. (2014) [13]	45 yo/M	Zhejiang province (China)	Fever, abdominal pain, weight loss	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules
Melchiondaet al. (2014) [14]: case 1	5 mo/F	Italy	Fever, weight loss	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules

Table 1 (continued)

Authors (year)	Patient age/sex	Country	Clinical features	Immuno-depressive factor	Pancytopenia	Splenomegaly (diameter)	Splenic focal lesions	Hepatic focal lesions	US pattern
Melchionda et al. (2014) [14]: case 2	1 yo/F	Morocco	Fever	None	No	Yes (ND)	Yes	No	Multiple iso-hypoechoic nodules
Melchionda et al. (2014) [14]: case 3	18 mo/F	Italy	Fever	None	Only anemia	Yes (ND)	Yes	No	Multiple iso-hypoechoic nodules
Melchiondaet al. (2014) [14]: case 4	18 mo/M	Italy	Fever	None	No	Yes (ND)	Yes	No	Multiple iso-hypoechoic nodules
Fuertes et al. (2014) [15]	44 yo/F	Spain	Fever	None	ND	Yes (ND)	Yes	No	ND
Gibson et al. (2014) [16]	48 yo/M	Australia (Spain 2–3 years early)	Fever, weight loss	None	Yes	Yes (ND)	Yes	No	ND
Alkan et al. (2016) [17]	22 mo/F	Turkey	Fever	None	Yes	Yes (ND)	Yes	No	Multiple hypoechoic nodules (the largest 10 mm)
Krepis et al. (2018) [18]	Children (median age 3.7 yo) 3 cases	Greece	ND	ND	ND	Yes (range 11–18.5 cm)	Yes	ND	Multiples hypoechoic nodules (4–5.4 mm)
Our case 1	63 yo/M	Italy	Fever, nocturnal sweating	COPD	Only thrombocytopenia	Yes (15 cm)	Yes	No	Multiple hypoechoic nodules (~ 10–15 mm)
Our case 2	32 yo/M	Italy	Fever	T1DM, Hashimoto thyroiditis	Yes	Yes (20 cm)	Yes	No	Multiple hypoechoic nodules ~ 1 cm
Our case 3	53 yo/M	Italy	Fever, nocturnal sweating	CLL	Yes	Yes (16 cm)	Yes	No	Multiple hypoechoic nodules ~ 1 cm

Authors (year)	CT pattern	MRI pattern	PET pattern	Leishmania serology	Leishmania amastigotes on BMA/ other tissues	PCR for *Leishmania* spp.on BMA	Anti-leishmanial treatment	Outcome
Bernabeu-Wittel et al. (1999) [7]	ND	ND	ND	ND	Positive	ND	ND	6/7 patients recovered and lesions resolved 1/7 acute pancreatitis and death
Fretzayas et al.(2001) [8] case 1	ND	ND	ND	Positive	Positive	ND	Antimonial agents	Patient recovered and lesions resolved

Table 1 (continued)

Authors (year)	CT pattern	MRI pattern	PET pattern	Leishmania serology	Leishmania amastigotes on BMA/ other tissues	PCR for *Leishmania* spp.on BMA	Anti-leishmanial treatment	Outcome
Fretzayas et al. (2001) [8]: case 2	ND	ND	ND	Positive	Positive	ND	Antimonial agents	Patient recovered and lesions resolved
Fretzayas et al. (2001) [8]: case 3	ND	ND	ND	Positive	Positive	ND	LAMB	Patient recovered and lesions resolved
Bukte et al. (2004) [9]	Multiple hypodense nodules	ND	ND	Positive	Positive on BMA and hepatic sample	ND	LAMB + antimonial agents	MOF and death
Yazici et al. (2008) [10]	Multiple splenic nodules and intracapsular lacerations within	ND	ND	Positive	Negative on BMA and splenic sample	ND	Splenectomy, LAMB	Patient recovered
Saxena et al. (2011) [11]	ND	ND	ND	Positive	Positive	ND	LAMB	Patient recovered and lesions resolved
Raeymaeckers et al. (2012) [12]	Multiple hypodense nodules in the delayed phase of contrast-enhanced CT scan	Multiple heterogeneous hypointense nodules on T2WI	ND	Seroconversion 1 month later	Negative	Positive	LAMB	Patient recovered and lesions resolved
Mao et al. (2014) [13]	Multiple hypodense nodules with slight ring-like enhancement	Concentric rings lesions on T2WI, no diffusion restriction on DWI and gradually increasing ring-like enhancement	Diffuse splenic FDG uptake	Positive	Positive	ND	Antimonial agents	Patient recovered and lesions resolved
Melchiondaet al. (2014) [14]: case 1	ND	ND	ND	Positive	Negative	Positive	LAMB	Patient recovered and lesions resolved
Melchionda et al. (2014) [14]: case 2	ND	ND	ND	Positive	Negative	Positive	LAMB	Patient recovered and lesions resolved
Melchionda et al. (2014) [14]: case 3	ND	ND	ND	Positive	Negative	Positive	LAMB	Patient recovered and lesions resolved
Melchiondaet al. (2014) [14]: case 4	ND	ND	ND	Positive	Negative	Positive	LAMB	Patient recovered and lesions resolved

Table 1 (continued)

Authors (year)	CT pattern	MRI pattern	PET pattern	Leishmania serology	Leishmania amastigotes on BMA/ other tissues	PCR for *Leishmania* spp.on BMA	Anti-leishmanial treatment	Outcome
Fuertes et al. (2014) [15]	Multiple hypodense nodules	ND	Multifocal splenic FDG uptake	ND	Positive on splenic and hepatic samples	ND	Splenectomy	ND
Gibson et al. (2014) [16]	Multiple hypodense nodules	ND	Diffuse and superimposed multifocal splenic FDG uptake	ND	Positive on splenic sample	Positive	Splenectomy, LAMB	Patient recovered
Alkan et al. (2016) [17]	Multiple hypodense nodules	ND	ND	Positive	Negative	ND	LAMB	Patient recovered and lesions resolved
Krepis et al. (2018) [18]	ND	ND	ND	ND	ND	ND	LAMB	Patient recovered and lesions resolved
Our case 1	Multiple hypodense nodules	ND	ND	Positive	Negative	Negative on BMA, positive on splenic tissue	Splenectomy, LAMB	Patient recovered
Our case 2	Multiple hypodense nodules with inhomogeneous pattern in arterial phase and loss of definition in venous phase	ND	Diffuse and superimposed multifocal splenic FDG uptake	Negative	Negative	Positive	LAMB	Patient recovered and lesions resolved
Our case 3	Multiple hypodense nodules	ND	ND	Negative	Negative	Positive	LAMB	Patient recovered and lesions resolved

US Ultrasonography, *CT* computed tomography scan, *MRI* magnetic resonance imaging, *PET* positron emission tomography, *PCR* polymerase chain reaction, *yo* years old, *mo* months old, *ND* no data, *BMA* bone marrow aspirate, *LAMB* liposomal amphotericin B, *MOF* multiple organ failure, *nv* normal value, *T2WI* T2 weighted image, *DWI* diffusion weighted imaging, *FDG* 18F-fluorodeoxyglucose, *COPD* chronic obstructive pulmonary disease, *T1DM* type 1 diabetes mellitus, *CLL* chronic lymphocytic leukemia

Case series

Case 1 A 63-year-old Italian man living in the countryside near Florence, Italy with chronic obstructive pulmonary disease, was referred to Infectious and Tropical Diseases Unit (ITDU) outpatient clinic of Careggi University Hospital in Florence, for 2-month history of fever, chills and nocturnal sweating, unresponsive to a domiciliary antibiotic course. Blood tests showed normal white blood cell (WBC) count and hemoglobin (Hb), thrombocytopenia (67,000 platelets (PLT)/μl; normal value 140,000–440,000), high liver enzymes (AST 137 UI/L, ALT 243 UI/L, n.v. 12–65), increased LDH (1068 UI/mL, n.v. 84–246), increased beta2-microglobulin (4.13 mg/L, n.v. < 2.53) and a slight hypergammaglobulinemia (22%, n.v. 11.1–18.8). Chest X-ray was negative for pneumonia. Abdominal ultrasonography (US) and computed tomography (CT) scan showed splenomegaly (diameter 15 cm) with multiple ~ 1 to 1.5 cm focal lesions, hypoechoic at US and hypodense at CT scan. A contrast-enhanced ultrasound (CEUS) was performed, confirming nodular hypoechoic non-enhancing spleen lesions (see Fig. 2). Indirect immunofluorescence assay for leishmania was positive (titer 1:160), while bone marrow (BM) histology resulted negative both for malignancies and leishmania amastigotes. Polymerase chain reaction (PCR) for *Leishmania* spp. was negative on peripheral and BM blood. We prescribed intravenous (iv) liposomal amphotericin B (L-AmB) 3 mg/kg per day which was immediately discontinued due to an abrupt onset of lumbar pain and skin rash, suggesting a hypersensitivity reaction. After multidisciplinary discussion involving hematology and surgery specialists, a diagnostic splenectomy was performed in the suspicion of a spleen lymphoma, after adequate vaccinations for asplenic patients. Histology was negative for lymphoma, and PCR for *Leishmania* spp. on splenic tissue resulted positive. Therefore, the patient received a complete course of L-AmB 3 mg/kg iv for 5 days with steroid pre-medication, followed by additional doses at day 14 and 21. His clinical conditions improved, and 3 months later serology for *Leishmania* spp. decreased in titer (from 1:160 to 1:80). The patient developed para-physiologic post splenectomy thrombocytosis, and he is currently on good health condition.

Case 2 A 32-year-old Italian man with Type 1 diabetes mellitus and Hashimoto thyroiditis, living in the countryside near Florence, was admitted to the ITDU with a 10-day history of high fever, shivers and headache, unresponsive to oral antibiotics. At admission, he had a palpable splenomegaly, and blood test showed slight pancytopenia (WBC 3,280/μl, Hb 12.3 g/dL, PLT 138,000/μl), high liver enzymes (AST 97 UI/L, ALT 150 UI/L) and high C-reactive protein (37 mg/L, n.v. < 9). Complete laboratory work-up was negative for HIV, brucellosis and typhoid fever, malaria, and recent infections with EBV, CMV, Parvovirus B 19 and HHV6. Immunofluorescence leishmania serology was negative too. Abdominal US confirmed splenomegaly (diameter 20 cm, area 90 cmq) with multiple hypoechoic lesions (see Fig. 3), confirmed by CT scan showing multiple hypointense splenic lesions. Positron emission tomography (PET)-CT imaging showed a diffuse and superimposed multifocal splenic FDG uptake (see Fig. 4). Based on laboratory and imaging results, a haematologic disorder was suspected and a BM biopsy was performed. Histology was negative for malignancies, while PCR for *Leishmania* spp. on medullary and peripheral blood resulted positive. The patient received iv L-AmB 3 mg/kg per day for 5 days, followed by two further doses (day 14 and 21), achieving complete disappearance of fever after 72 h from the first infusion. During

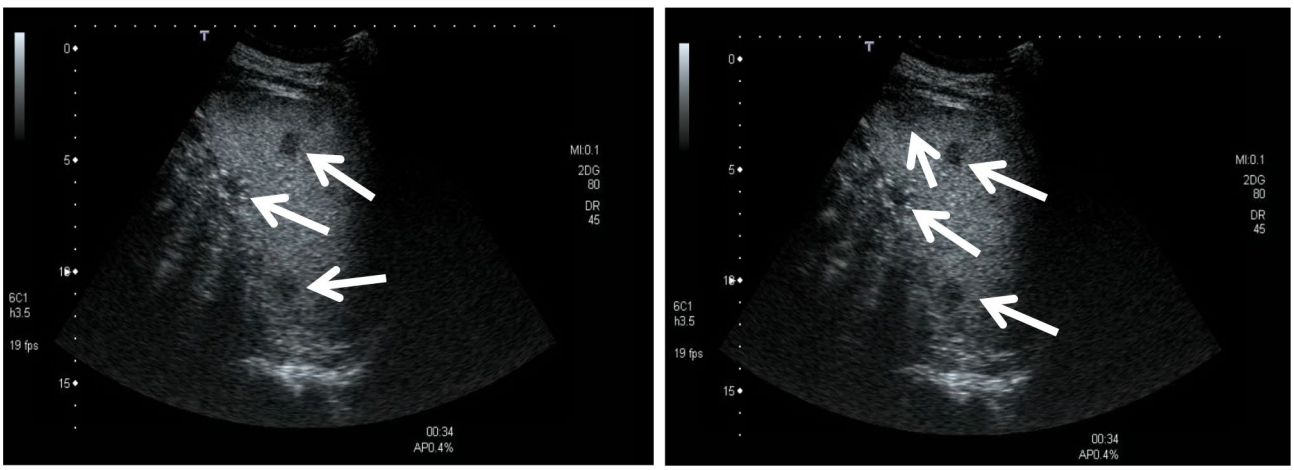

Fig. 2 Case 1: Contrast-enhanced ultrasound (CEUS) of spleen: nodular hypoechoic non-enhancing spleen lesions (arrows)

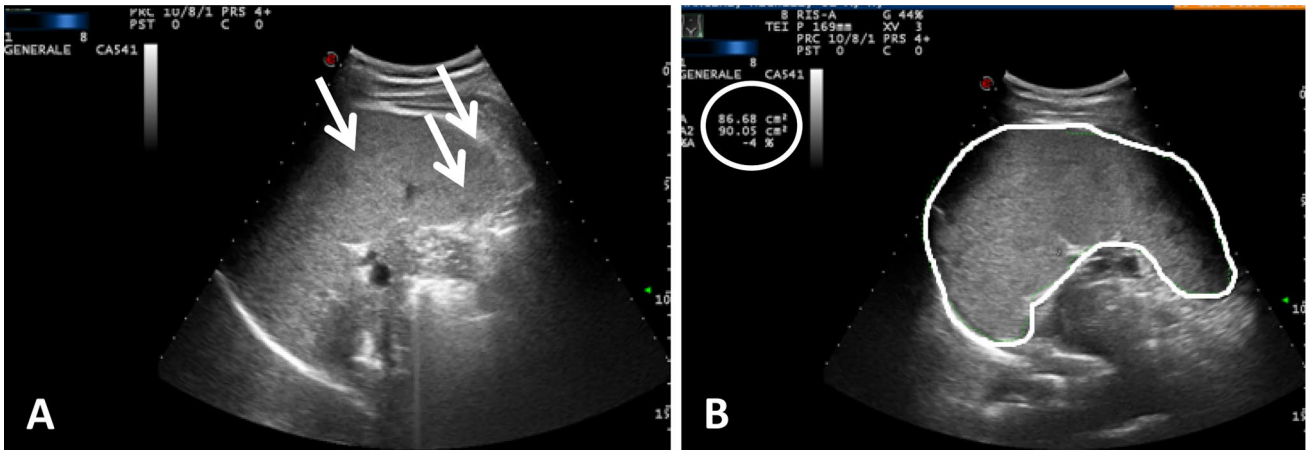

Fig. 3 Case 2: Ultrasonography. **a** Multiple hypoechoic lesions (arrows); **b** splenomegaly: splenic area 90 cmq

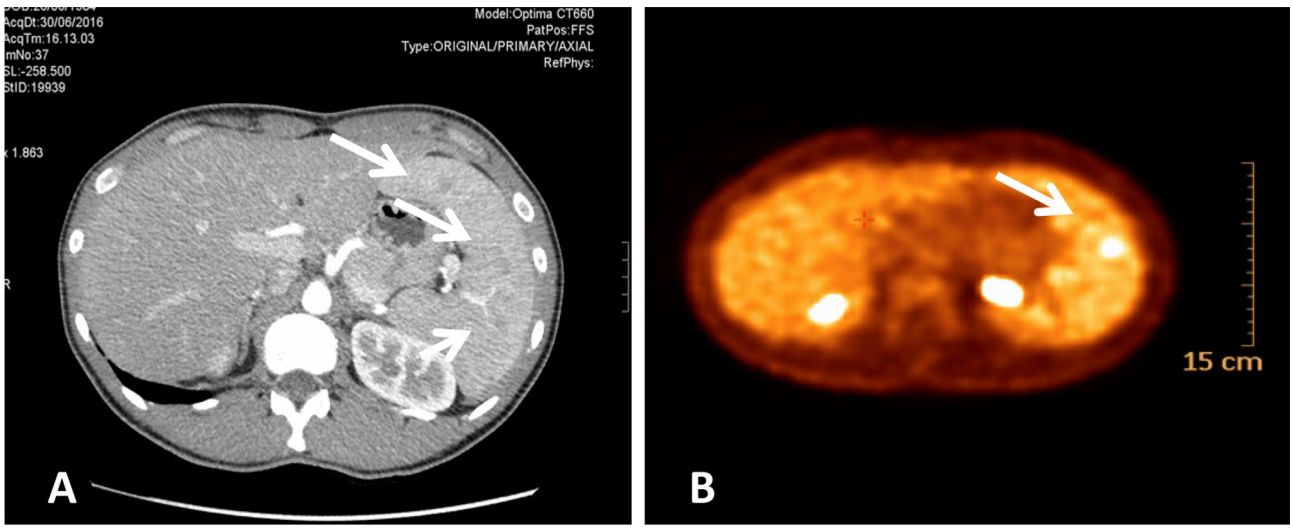

Fig. 4 Case 2: Positron emission tomography-computed tomography (PET-CT) imaging. **a** Multiple hypointense splenic lesions (arrows); **b** diffuse and superimposed multifocal splenic FDG uptake (arrow)

follow-up, US showed a reduction of the splenomegaly (diameter from 20 to 14–12 cm). Complete regression of splenic lesions and splenomegaly was seen 3 months after the end of treatment. Blood test showed negativization of PCR for *Leishmania* spp. in peripheral blood.

Case 3 A 53-year-old Italian man with chronic lymphocytic leukemia (CLL) was admitted to the Emergency department of our hospital with 1-month history of high fever with shivers, headache and nocturnal sweating not responsive to oral antibiotic and corticosteroid treatment. He was followed up since 5 years by the Hematology department with a watch and wait approach, without receiving any treatment for CLL. He reported alcohol abuse (1 L wine/day). He lived in the countryside near Florence, and had never traveled outside Italy.

At admission to ITDU, blood analysis showed pancytopenia (WBC 3150/µl, Hb 10.1 g/dl, PLT 91,000/µl) and high liver enzymes (ALT 303 UI/L). Chest X-ray was negative for pneumonia and trans thoracic echocardiogram was negative for endocarditis, while an abdominal CT scan showed splenomegaly (diameter 16 cm) with multiple, partially merging hypodense splenic nodules and subcentimetric celiac lymphnodes. In the suspicion of CLL progression, a BM biopsy was performed. Histological examination was suggestive for CLL/lymphocytic lymphoma, compatible with patient's history. The analysis of BM with Giemsa coloration was negative for leishmania amastigotes, but PCR for *Leishmania* spp. on BM aspiration and peripheral blood resulted positive. Immunofluorescence leishmania serology was negative. The patient was transferred to ITDU, where an abdominal US confirmed the presence splenomegaly (area 76 cmq) and

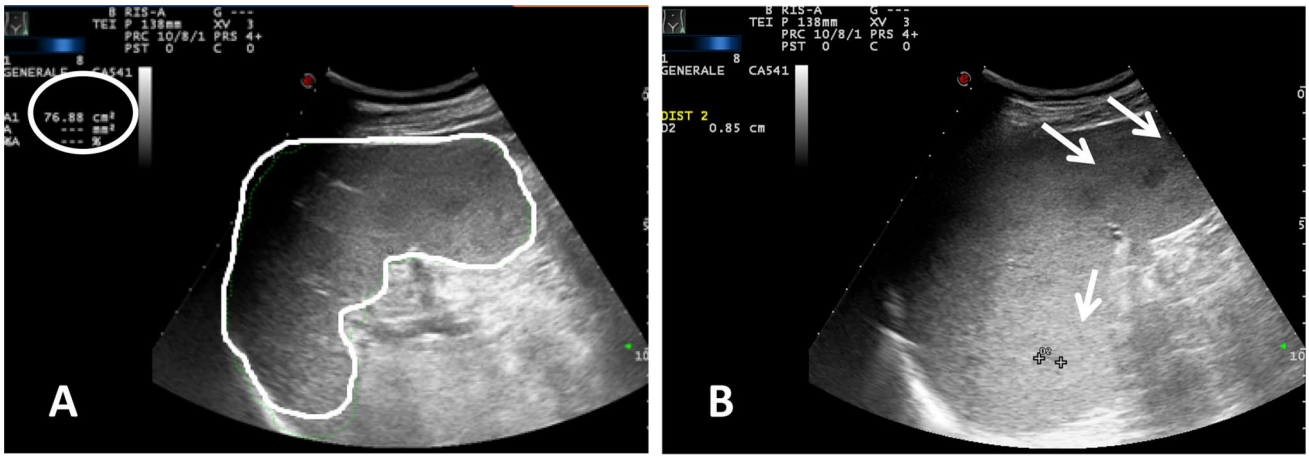

Fig. 5 Case 3: Ultrasound. **a** Splenomegaly: splenic area 76 cmq; **b** subcentimetric hypoechoic splenic nodules (arrows)

the presence of multiple nodular hypoechoic splenic lesions (see Fig. 5). The patient was treated with L-AmB 4 mg/kg per day on days 1–5, 10, 17, 24, 31 and 38 according to the treatment schedule for immunocompromised patient, with immediate clinical improvement. PCR for *Leishmania* spp. on peripheral blood turned negative after treatment, but splenomegaly with nodular hypoechoic lesions (diameter 1 cm) persisted 1 month after treatment. Fifteen months later he was readmitted to ITDU for moderate anemia, thrombocytopenia and splenomegaly with hypoechoic lesions detected by both US and CT scan. PCR for *Leishmania* spp. resulted positive on BM and peripheral blood. He was re-treated with L-Amb per day on days 1–5, 10, 17, 24, 31, 38, 45 and 52 with negativization of PCR for *Leishmania* spp. on peripheral blood and resolution of anemia and thrombocytopoenia. Follow-up is ongoing.

Systematic review of literature

Figure 1 shows the steps of the search and selection process followed for the systematic review of literature. Finally, 12 articles reporting 25 cases of VL with focal spleen lesions were included. The first manuscript was published on 1999, and the last on 2018. To those cases, we added the three cases described in this paper, for a total of 28 cases.

While details on identification of the causative leishmania species were reported in only one manuscript [16], the majority of cases ($n=25$; 89%) were probably caused by *L. infantum* based on epidemiological data, such as the history of exposure (residency or travel) in a *L. infantum* endemic country and the lack of history of exposure in areas were *L. donovani* is endemic. In detail, nine cases were most likely acquired in Spain, six in Italy and Greece each, three in Turkey, two in Morocco. Two cases were reported from Asia: one from India, where *L. donovani* is endemic and one from China, from the Zhejiang province, a region where VL is not considered endemic and the causative leishmania species is not easily deductible from the epidemiological criteria, since multiple and peculiar variant of the disease are reported [19]. Thirteen cases (46.4%) were diagnosed in children aged < 10 years, and the remaining 15 cases in middle-aged adults. Median age of pediatric patients was 1.5 years (range 5 months–10 years), median age of adult patients was 44.5 years (range 28–63 years). Nine patients (32.1%), all adults, presented with some immunosuppressive condition (HIV co-infection in seven cases, CLL in one case, type I diabetes mellitus in one case). Of the 18 patients with known gender, 10 (55.5%) were male and the remaining female. Among children with known gender, 6 of 10 patients (60%) were female and the remaining male, while among adult patients 6 of 8 (75%) were male.

As far as clinical presentation is concerned, all the 25 patients with known clinical features presented fever, accompanied by swollen abdomen or abdominal pain in 5 cases (20%), weight loss in 5 cases (20%), nocturnal sweating in 2 cases (8%) and fatigue in 2 cases (8%). Of the 16 cases with available laboratory data, 10 (62.5%) had pancytopenia, and 4 (25.0%) anemia and/or thrombocytopenia.

At the time of patient presentation, leishmania serology was positive in 13 (81.2%) of 16 cases for which serological data are reported. Only one of the three patients with negative serology underwent a second serological test, demonstrating a seroconversion 1 month later. Twenty-two patients underwent BM aspirate. In 59% of them (13 of 22), amastigotes were microscopically detected. PCR for *Leishmania* spp. was performed only in nine BM aspirate samples, and resulted positive in 8 cases (88.9%). Four patients (14%) underwent diagnostic splenectomy, while the diagnosis was confirmed by less invasive techniques in the remaining patients. Direct detection of *Leishmania*

spp was obtained in three of the four spleen tissue samples examined. In detail, two spleen samples were tested with both microscopy and PCR: one was positive with both techniques and one with PCR only. Two samples were tested with microscopy and only one resulted positive. Leishmania amastigotes were detected in two of three liver tissue samples of patients who underwent hepatic biopsy (PCR was not performed in any liver sample). None of the included manuscripts reported data on PCR on peripheral blood, however, we obtained positive results in two patients (Case 2 and Case 3), with negativization after treatment, and a negative result in one patient (Case 1).

Increased size of the spleen was detected by US in all cases with available information ($n = 21$). Multiple focal spleen lesions were described in all the cases ($n = 28$) and were detected in all patients who underwent US ($n = 26$). In all but one patient, spleen lesions were multiple small hypoechoic nodules at US. The size of nodules measured was available for 19 patients. In the majority of cases (14 of 19, 74%), nodule diameter was ≤ 1 cm, in four cases between 1 and 2 cm and in a single case 6.1 cm.

In the latter case, authors described a different ultrasonographic pattern represented by multiple hyperechoic splenic nodules with peripheral hypoechoic haloes and central hypoechoic necrotic area [9]. Moreover, this patient was the only one with hepatic focal lesions too.

CT scan was available for ten patients. In all cases, multiple focal spleen lesions were detected, and in nine cases (90%), the lesions were described as hypodense. In one case, the lesions were accompanied by a slight contrast ring-like enhancement [13].

MRI was available for two patients. In one case, heterogenous hypointense nodules on T2-weighted images (T2WI), clearly deforming the contour of the spleen were reported [12]. In the other case, concentric ring lesions, with dot hyperintensity in central area, and hypointensity in the outer ring in T2WI, without diffusion restriction on diffusion-weighted imaging (DWI), and with a middle ring enhancement after contrast administration, were reported [13].

Data on PET were available for four patients. Two patients presented a diffuse FDG uptake and a multifocal FDG uptake, respectively [13, 15]. A diffuse spleen FDG uptake, with a superimposed intense multifocal splenic FDG uptake, was seen in one patient each [16], as well as in our case 2.

Sixteen of the 20 patients (80%), with available data on pharmacological anti-leishmanial treatment, were treated with intravenous liposomal amphotericin B (L-Amb), 3 patients (15%) were treated with intravenous antimonials and one (5%) with combination of L-Amb and antimonials. Two of 27 patients (7%) with known outcome died, while the remaining recovered. Excluding patients who underwent splenectomy, all but one (our Case 3 with VL reactivation after 15 months) the recovered patients experienced the regression of splenic lesions, regardless of the diagnostic technique.

Discussion

In this study, we described three cases of VL case presenting with focal spleen lesions, and we systematically reviewed the literature on this infrequently reported feature of the diseases, retrieving a total 28 cases. The majority of cases was reported from the Mediterranean area, and was most likely due to *L. infantum*. Apart from the presence of focal spleen lesions, the epidemiological and clinical features of the retrieved cases were substantially those of classic VL seen in the Mediterranean area. More in details, the bimodal age distribution with two peaks in pediatric subjects and middle age adults is in line with data on *L. infantum* VL, previously published in the Mediterranean area [2, 20–24]. In the adult group of patients, the higher prevalence of male gender and the high rate of immunosuppressive condition is also consistent with previous reports [2].

Clinical presentation, in the majority of the collected cases, was characterized by classic triad of fever, cytopenia (anemia /leucopenia/thrombocytopenia) and splenomegaly, with similar frequency to those reported in the literature for classic VL without splenic lesions [4, 25]. However, even thus the epidemiological data and the clinical presentation of these cases are those of classic *L. infantum* VL, the presence of spleen lesions posed a challenge for the correct diagnosis in the majority of patients, leading to unnecessary splenectomy in 4 (14%) cases, and was associated with a fatal outcome in 2 (7%) cases.

This uncommon instrumental finding may further complicate the difficult diagnosis of VL, on which clinical manifestations and laboratory alterations largely overlap those of lymphoproliferative and other hematologic disorders. Abdominal US is one of the first investigations that are requested during the work-up of a fever of unknown origin, and the findings of focal spleen lesions may be misleading, suggesting a malignant process, such as a lymphoma, which may even coexist with VL [26]. Several review articles published by radiologists, nuclear medicine specialists and even by infectious diseases specialists expert in US are available, but no paper lists VL among the possible differential diagnosis of focal spleen lesions [27–31]. According to the majority of review articles on this topic, the possible conditions associated to focal spleen lesions, with hypoechoic appearance at US, are cysts, metastasis, hydatid disease, sclerosing angiomatoid nodular transformation, acute hematoma, littoral cell angioma, splenic pleomorphic undifferentiated sarcoma, infarctions, lymphomas, lymphangiomas, and abscess. The latter is most likely caused by hematogenous

metastatic localization of bacterial endocarditis, but also due to *Candida* spp., *Aspergillus* spp., mycobacteria, and *Pneumocystis jirovecii* in immunocompromised subjects, and less frequently *Bartonella* spp [27, 29, 30].

Our study identifies the most common radiologic pattern of VL-related spleen lesions with different instrumental techniques. In brief, VL-related spleen lesions are most frequently multiple subcentimetric or centimetric lesions, hypoechoic at US and hypodense at CT scan. As for MRI, only two cases, with different pattern, are reported, while PET-CT, in the four published cases, showed an intense FDG uptake in the spleen (diffuse in one case, diffuse with superimposed focal uptake in two cases and multifocal in one case). Similar findings on PET-CT have been reported by an Italian study presented as oral communication at the European Congress of Tropical Medicine and International Health in 2015, involving 15 patients with VL. In this study, all but one of the patients with VL undergoing to PET-CT showed an intense FGD uptake on spleen, 8 with diffuse and 6 with focal pattern [32]. We reported for the first time the findings of CEUS on VL spleen lesion, which showed nodular hypoechoic non-enhancing spleen lesions.

Our study suggests that VL must be added to list of possible differential diagnosis of focal spleen lesions. In the presence of this uncommon feature, the combined use of multiple diagnostic tools is recommended to investigate the diagnosis of VL [33], since no single test is sensible enough to confidentially exclude the disease. In the present study, PCR on BM was the test with the higher diagnostic yield, being positive in 88.9% of cases, followed by serology (positive in 81.2% of cases) and microscopic detection on BM (positive only in 59% of cases).

Based on the available literature data, one could speculate that spleen lesions are more typical of *L. infantum* VL than of *L. donovani* VL, since 26 of 28 reported cases (93%) have been reported from the Mediterranean area and only two from Asia (one from China in a region not considered endemic for any *Leishmania* spp. and one form India where *L. donovani* is endemic).

However, it is possible that detection of spleen nodules may be influenced by the wider availability of high resolution US devices in the Mediterranean setting, compared to endemic areas of Asia. It remains unclear why no report on this VL feature is available from Latin America, where the agent of zoonotic VL is genetically indistinguishable from the Mediterranean *L. infantum*.

Conclusions

VL may present with splenomegaly and multiple spleen focal lesions and must be added to the list of possible differential diagnosis of spleen focal lesions. The most common radiological patterns are multiple subcentimetric or centimetric lesions hypoechoic at ultrasonography and hypodense at CT scan. In these cases, PET-CT typically presents with an intense FDG spleen uptake with variable pattern (diffuse, focal or diffuse with superimposed focal uptake). Health care professionals such as internal medicine, infectious diseases, hematology, pediatric and radiology specialists should be aware of this uncommon presentation of VL, and should pursue the diagnosis through the combined use of multiple diagnostic tools (microscopic examination of BM, PCR on peripheral blood and BM, and serology), avoiding unnecessary demolitive diagnostic procedure, such as splenectomy.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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