A "mysterious" intrabdominal mass with infectious origin, in a patient with HIV infection under control. A "delayed diagnosis" allows to enlarge our knowledge, by assessing a rare disease

Clinical Management Issues

Case report

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Abstract

A probable case report of an abdominal botryomycosis has been hypothesized in a patient with a stable HIV infection under an effective antiretroviral therapy. Hyperpyrexia, abdominal pain and tenderness, and a thickening of small intestinal walls associated with multiple mesenteric adenopathies and a peritoneal involvement, prompted an ultrasonography-guided fine needle biopsy, and later a laparoscopy-laparotomy which excluded a neoplastic or lymphoproliferative disorders, showing only abundant fibrotic and necrotic-steatonecrotic tissue, with sparse multinuclear giant cells type Langhans. The prompt response to surgical intervention and a treatment with i.v. meropenem alone might be referred to a concurrent gram-negative infection of abdominal origin, until a late culture of an atypical Mycobacterium came to our attention over one month after the end of hospitalization. An updated literature search is presented and discussed, in relationship with the observed, extremely infrequent case reports of botryomycosis in different clinical settings.

Keywords: *Intrabdominal mass; Peritoneal involvement; Inflammatory signs; Surgical treatment; Meropenem; Botryomycosis; Atypical mycobacteriosis*

Una "misteriosa" massa intraddominale a eziologia infettiva, in un paziente con infezione da HIV controllata. Un "ritardo diagnostico" consente di approfondirne la conoscenza studiando una patologia rara

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INTRODUCTION

An organizative hitch characterized by an unintentional delayed communication, did not affect the positive clinical evolution of a patient, and allowed us to study in depth an atypical clinical case in terms of differential diagnosis. A literature search and the discussion among all clinicians which come from this clinical presentation enabled us to contribute with personal, professional knowledge of every specialist, and may represent a stimulating subject for a debate also for readers. Only after writing down this contribution, we were finally informed of the exact microbiological diagnosis, so that we voluntary introduced this short premise. When postponing the communication of the final microbiological diagnosis to the "Discussion" section, we aim to leave some

Why do we describe this case

The modern medicine makes use of sensitive and specific laboratories technologies, which allow to make important diagnosis in short periods of time. But sometimes this isn't true. The late availability of a microbiological specimen has allowed to establish the clinical features by the definitive diagnosis of atypical mycobacteriosis. The treatment for a long period with only one carbapenem antibiotic did not affect the clinical response of the patient

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time and some "suspense" to the readers too, in order to underline the adjunctive diagnostic difficulties potentially descending from apparently lacking laboratory data in an extremely complicated diagnostic "puzzle", and the need to always maintain an elevated, broad spectrum mind in the clinical management of "difficult to treat" patients.

Botryomycosis has been described since 1950s as an uncommon bacterial infection mimicking actinomycosis and fungal infections, characterized by one or multiple aspecific suppurative-granulomatous foci containing sulphur-like granules, usually with eosinophilic infiltrates, where in many cases either Gram-positive organisms (i.e. *Staphylococcus aureus*, coagulase-negative Staphylococci, *Streptococcus* spp., *Bacillus* or *Corynebacterium* spp.), or Gram-negative organisms (i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* or *Neisseria* spp.), and also anaerobe bacteria (including *Actinobacillus* and *Peptostreptococcus* spp., and *Propionibacterium acnes)*, might be cultured: sometimes a mixed bacterial flora may be found [1-4].

Actually, after the early observations carried out in animals (especially cattle and horses), the term "botryomycosis" has been proposed by Rivolta in 1884 [1,5], after noticing the "grapelike" appearance of its macroscopic lesions, which resembled those caused by fungi (hence the suffix "mycosis"). Later, Magrou proved the most common bacterial origin of botryomycosis, by isolating *S. aureus* from pulmonary lesions [6], and also demonstrated that the unusual histopathological picture of botryomycosis was the result of a sort of "symbiotic" relationship between the inoculum microorganism dose, the virulence of the different pathogens, and the immune response of the affected host [1,6].

Although primarily considered as a veterinary disease, over one hundred of human cases have been described in the past century, in form of single reports or small case series. The majority of described episodes involved mainly skin and skin structures [2,7], and more infrequently the thorax and the abdomen (the so-called visceral botryomycosis, which remains a rare disease, often described in the compromised host, although the specific role of host immune response in the pathogenesis of visceral botryomycosis is not fully understood) [2,3,8,9]. Possible adjunctive host risk factors associated with both cutaneous and visceral botryomycosis include: diabetes mellitus, cystic fibrosis, malnutrition, alcoholism, HIV infection, major or minor trauma, a chronic granulomatous disease, and prior surgery [2,8,10-16].

Also the pathogenesis of botryomycosis is not completely known: the process is thought to involve a combination of supporting factors including an inciting event (i.e. a major or minor trauma, including piercing for example), the amount of inoculated microorganisms, the intrinsic virulence of infecting pathogens, and the intrinsic host susceptibility [1-3,6,16].

Since its first report in humans published in 1913 [17], botryomycosis remained difficult to distinguish from actinomycosis and fungal diseases, in both cutaneous and visceral localizations. When the respiratory tract is involved, actimomycosis usually has an aspiration origin, while the factors prompting botryomycosis have not been identified yet, with host factors and foreign bodies probably playing some role in its pathogenesis [1-3,7,18,19].

A retrospective, historical re-appraisal of botryomycosis, may be found in the narration of the Philoctetes's diseases by Sophocles masterpiece [20,21], with reference to the long-term granulomatous, non-healing cutaneous wounds of the Greek hero Philoctete, which occurred after a painful but not lethal snake (viper) bite at his foot. The superinfection of this lesion caused the legendary, very prolonged stay at the isle of Lesmos of the Greek hero, where Philoctete was reclaimed by his companions in order to prompt a positive course to the long-lasting Troy war [20-22]. The limb lesion of Philoctete was described as a painful and extremely chronic ulcer, not lethal in its course but still present after around one decade, and complicated by bleeding and a discharge of malodorous and purulent material, so that it caused severe functional impotence. Some Homer's commenters interpreted the lesion of Philoctete as caused by maduromycosis, mycetoma, chromoblastomycosis, and also botryomycosis. A comparison between the description of the clinical features of Philoctetes's disease and that of very similar afflictions (also called actinophytosis, or bacterial pseudomycosis, pyogenic granuloma, or granular bacteriosis, in some narrations) [1,23] shows a clinical resemblance of botryomycosis, since each of the considered diseases has a chronic course, may frequently affect the extremities, may be caused by an initial trauma, may present with ulcers, and may discharge purulent-haematic material.

As examined by Urso and Farella in their 1996 contribution on Philoctetes's disease [22], actually botryomycosis is primarily localized at limbs with cutaneous ulcers, has a long-term course in absence of an effective treatment, is complicated by purulent and sero-hematic discharge, has an anamnestic trauma, and a foul odour, but usually it is not painful.

Anecdotal cases of primary botryomycosis (especially cutaneous localizations) have been reported also in patients without any known underlying illness. However, immunocompromised patients as a whole [2,7], and especially subjects with an underlying cystic fibrosis [24], those with diabetes mellitus [2], and patients with HIV and AIDS [8,10,25-32], seem to be more prone to develop botryomycosis (in particular its visceral form), compared with the general population. With regard to life age, episodes of botryomycosis have been described from infancy to old age.

Aim of our report is to describe a patient with a stable HIV infection under an effective antiretroviral therapy, who developed a gross abdominal mass with peritoneal involvement, potentially caused by a visceral botryomycosis, as suggested by multiple, repeated diagnostic procedures (including imaging and histopathology studies), and whose aetiology might be attributed to a gram-negative pathogen, due to the prompt response to a treatment with i.v. meropenem alone, after laparotomy and biopsy. A comprehensive literature search has been performed and discussed, in relationship with the observed, extremely infrequent case report of possible botryomycosis during HIV disease, whose diagnosis has been finally modified by the delayed knowledge of a microbiological isolation.

CASE REPORT

A 37-year-old homosexual male patient was initially diagnosed with HIV infection four years ago, and was treated with a powerful association antiretroviral therapy shortly after his referral to our HIV outpatient clinic (7 months later). At that time, the HIV replication rate proved elevated (as showed by plasma HIV-RNA levels of 620,000 copies/ml), and the patient's immune defence was somewhat compromised (as demonstrated by a $CD4^+$ T-lymphocyte count of 254 cells/μl), so that a treatment with the fixed association tenofovir-emtricitabine (200-300 mg/day), plus the protease inhibitor atazanavir (300 mg/day), boostered with ritonavir (100 mg/day), was recommended, and taken by our patient with optimal adherence and no relevant clinical and laboratory adverse events.

The past clinical history of our patient included a previous, cured syphilis five years before, and a phlemmonous appendicitis which required surgery, one year before the hospitalization in our Division. An allergy to amoxicillin-clavulanate was also reported.

Starting from one month before admission, our patient complained of an irregular, elevated hyperpyrexia (up to 40°C of body temperature), not responsive to broad spectrum empiric antibiotics (mostly beta-lactames and macrolides), and poorly responsive to antipyretics too, associated with mild abdominal pain and tenderness, but in absence of diarrhoea, stipsis, nausea and vomiting.

Upon admission, a normal leukocyte count was shown (6,560 cells/μl), with a tendency towards neutrophilia (81.6%), together with an elevated erythrocyte sedimentation rate (ESR) (75 mm/hour), significantly elevated C-reactive protein levels (20.2 mg/dl), and overt increased serum fibrinogen levels (648 mg/dl), in absence of other relevant laboratory abnormalities, when excluding a moderate anaemia (haemoglobin level 10.7 g/dl), and elevated ferritin levels (up to 780 mg/ml). The absolute $CD4^+$ T-lymphocyte count raised to 399 cells/μl, while HIV-RNA tested extremely low (370 copies/ml), after a the 7-month successful antiretroviral treatment performed with tenofovir-emtricitabine plus atazanavir-ritonavir.

An abdominal ultrasonography, and a contrast-enhanced abdominal computerized tomography (CT) study showed a mild liver and spleen enlargement, an evident ascitic effusion, and focused on a thickening of several small intestinal loops and the related mesenteric tissue, with involvement of the adjacent peritoneum, located in the left paraumbilical region.

The large majority of all performed microbiological investigations tested negative or not significant in relationship with the underlying clinical situation. They included: blood, sputum, urine, and stool culture, stool search for parasitic diseases (including *Cryptosporiudium* spp. and *Clostridium difficile*), Widal-Wright serology, *Histoplasma*, *Entamoeba*, Enterovirus

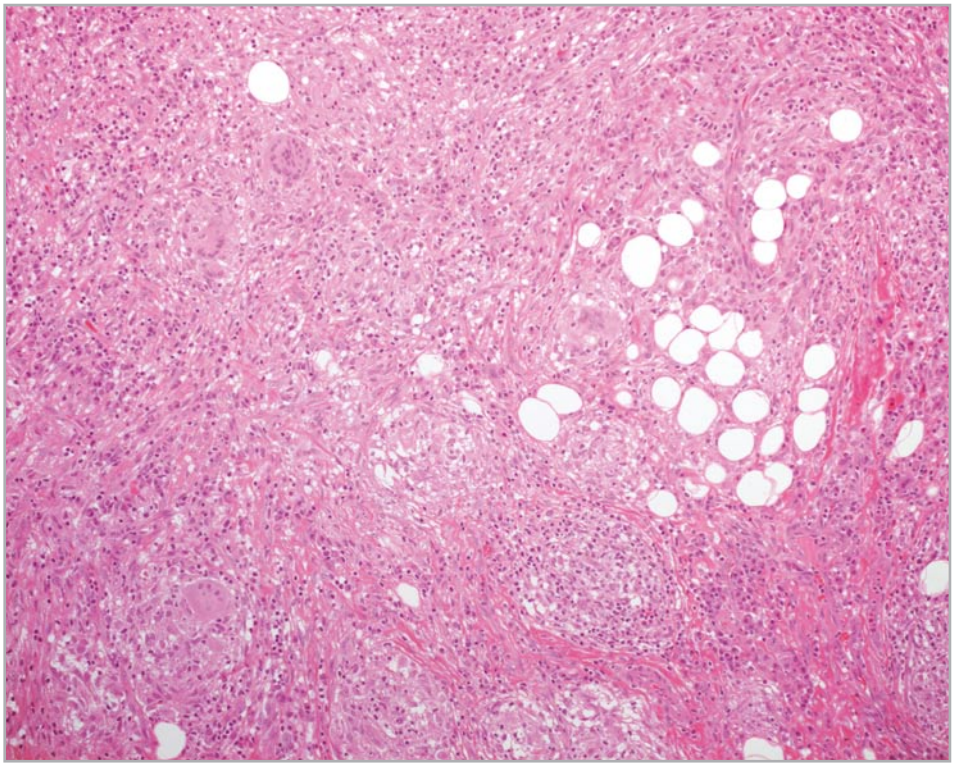

Figura 1
The microscopic examination of the abdominal mass biopsy shows wide areas of steatonecrosis, granulation tissue and a diffuse, chronic inflammatory, granulomatous reaction, with areas of colliquative, non-caseation necrosis. Numerous granulomas are apparent. Haematoxylin-eosin stain. Original magnification 10×

and Adenovirus serology, and *Cryptococcus neoformans* serum antigen search. Signs of the previous known syphilis infection were retrieved (as demonstrated by a low 1:320 TPHA titre, with negative treponemal tests), serum Quantiferon test proved negative, as well as the intradermal Mantoux reaction. Only the Epstein-Barr virus molecular biology tested positive, by disclosing 3,250 genome equivalents/ml, while the molecular assay for Cytomegalovirus infection proved negative (< 500 genome equivalents/ml). All laboratory oncology markers proved negative.

An esophagogastroduodenoscopy showed an erosive gastritis-duodenitis (in absence of *Helicobacter pylori* infection), and a pancolonoscopy with multiple biopsies disclosed an aspecific colitis. An ultrasonographic heart study showed a mild pericardial effusion, a high-resolution thorax CT scan tested not significant, while a further contrast-enhanced abdominal TC scan, carried out 10 days after the first examination, showed

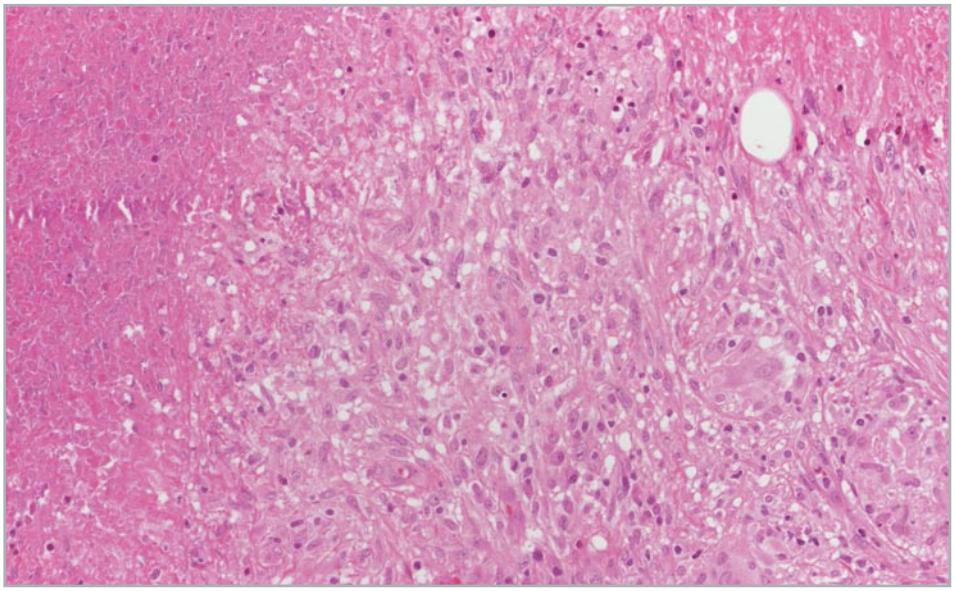

Figura 2
In the specimen also extensive necrotic and steatonecrotic processes, together with granulation tissue and a diffuse, chronic inflammatory, granulomatous reaction are recognizable, with areas of colliquative, non-caseation necrosis. Focus on the upper area of necrosis. Haematoxylin-eosin stain. Original magnification 20×

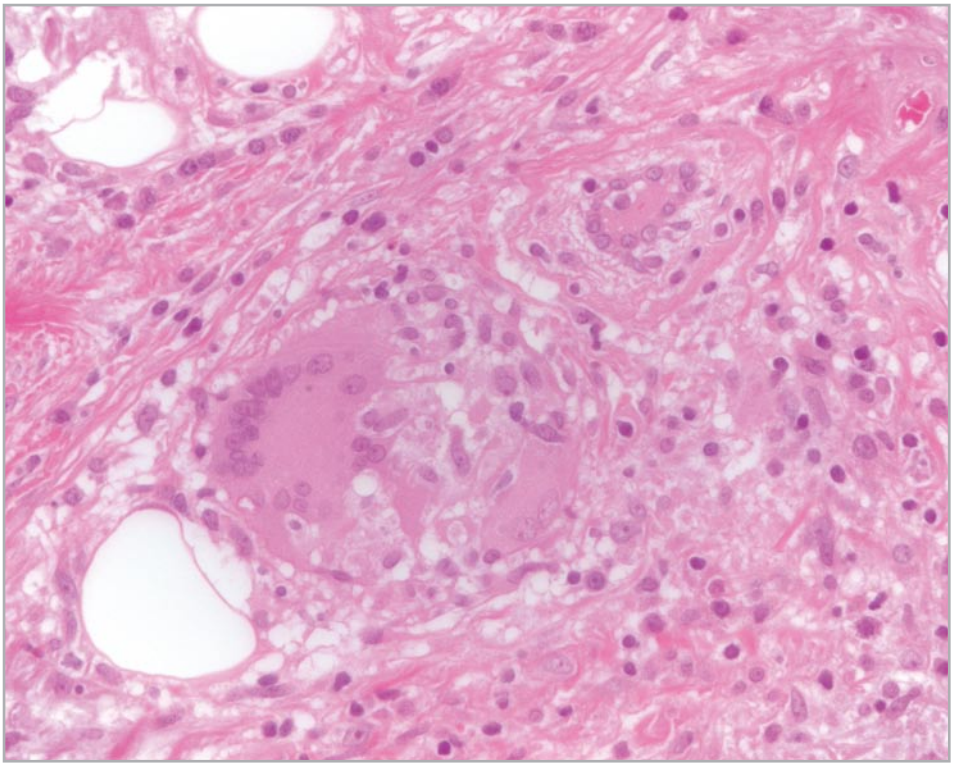

Figura 3
When observing the slide with a greater magnification, the granulomas are composed of monocytes, epithelioid macrophages and numerous Langhans' giant cells. Langhans' giant cells have multiple nuclei, with a "horse-shoe-like" configuration. Haematoxylin-eosin stain. Original magnification 40×

a progressively increased amount of ascitic fluid, a number of enlarged mesenteric and para-aortic lymph nodes (up to 16-18 mm of maximum diameter), and a hypodense, round intrabdominal mass at the root of mesenterial branch, primarily compatible with a lymphoproliferative origin. A subsequent total-body tomoscintigraphy (positron emission tomography, or PET), disclosed a diffuse and intense hypercaptation of the ^{18}F-FDG radiocompound at all abdominal sites (with a maximum SUV – Standardized uptake values – index of 17), especially at lower left abdomen, where an intestinal and peritoneal involvement were confirmed.

The peritoneal fluid was repeatedly tapered and examined: an elevated protein content (5,280 mg/dl) was associated with an increased leukocyte count (960 cells/µl), composed by 75% lymphocytes, 10% neutrophils, and 15% monocyte-macrophages. At the microscopic examination, a prevalence of phlogistic and necrotic material was found (poorly represented granulocytes, lymphocytes, and plasmacells, with a predominant $CD3^+$ T-lymphocyte number, and a regular $CD4^+/CD8^+$ T-lymphocyte ratio), in absence of neoplastic cells. Neither bacteria, nor mycobacteria, nor fungi, or other microorganisms were observed at Gram stain, Ziehl-Nielsen stain, and Grocott stain, and all cultures tested repeatedly negative for all searchable microorganisms (as well as molecular biology probes for *Mycobacterium tuberculosis* and atypical mycobateria).

A lymphoprolipherative disease was therefore suspected, due to the underlying HIV disease, the positivity of Epstein-Barr virus viraemia, and especially the aspect of the abdominal-peritoneal lesion at all instrumental examinations (ultrasonography, contrast-enhanced CT scan, and especially the PET scan).

As a consequence, an ultrasonography-guided biopsy of abdominal wall close to the thickened mesenteric tissue was performed, but all microbiological and histopathological studies performed on biopsy material did not disclose any infectious or neoplastic disorder, showing only abundant fibrotic and necrotic-steatonecrotic tissue only, with sparse multinucleated giant cells type Langhans.

Thereafter, an explorative laparoscopy and laparotomy was finally deemed necessary twenty days after admission, in order to have a definite diagnosis and approach a specific treatment. Thick, hard, white-grayish membranes involving the parietal peritoneum and some intestinal loops which appeared conglomerated were seen in the left paraumbilical region, close to the peritoneal wall of the left hemiabdomen. Once again, all intraoperative material and many tissue biopsies

involving also the colonic wall, proved negative at all microbiological examinations and culture and molecular biology testings for all searchable microbial pathogens, while histopatological studies demonstrated a diffuse granulomatous inflammatory process with a non-specific aspect, and a diffuse oedema of small intestinal walls. On macroscopic examination, the fibrotic-adipose tissue showed multiple areas of steatonecrosis. Microscopic examination disclosed wide areas of steatonecrosis, granulation tissue and a diffuse, chronic inflammatory, granulomatous reaction, with areas of colliquative, non-caseation necrosis (Figures 1 and 2).

The granulomas were composed of monocytes, epithelioid macrophages and numerous Langhans' giant cells. Langhans' giant cells have multiple nuclei, with a "horseshoe-like" configuration (Figure 3).

When considering the clinical course of hospitalization, a first empirical attempt performed with i.v. levofloxacin (500 mg twice daily for one week) apparently did not act significantly. Later, i.v. meropenem (at 3 g/day) plus i.v. fluconazole (at 400 mg/day) were introduced under the suspicion of a bacterial and/or fungal aetiology, but fluconazole was discontinued 10 days later after obtaining repeated, negative microscropy and culture assays for fungal organisms, while i.v. meropenem was carried out at the same dosage for two more weeks, and acted favourably on both the febrile reaction, and all the phlogistic parameters (especially C-reactive protein, ESR, and serum fibrinogen levels, which remained remarkably altered since patient's admission). Notably, both hyperpyrexia, and abdominal signs rapidly disappeared after laparoscopy/laparotomy itself, and especially during the prolonged, single-agent antibiotic therapy.

After the explorative laparoscopy/laparotomy with multiple biopsies, i.v. therapy with meropenem was continued for two further weeks, and finally allowed to reach a stable, complete disappearance of fever and all abdominal complaints, together with a complete normalization of all inflammatory indexes, so that our patient was discharged without any antimicrobial therapy (when excluding the unmodified antiretroviral combination treatment). A repeated a contrast-enhanced abdominal CT scan four weeks after the end of his hospitalization confirmed the complete resolution of the acute episodes, with isolated fibrotic remnants involving the site of the pathological process.

DISCUSSION

Classically, botryomycosis may present with cutaneous or visceral (mainly pulmonary) involvement.

When considering cutaneous botryomycosis, feet, hands, inguinal and gluteal areas are the most frequently affected. Infrequent complications may occur under the appearance of subcutaneous invasion, or by a local lymph node or bone involvement (osteomyelitis), including also skull, mandible, or orbit, as well as tendons and muscle [2,33-35]. Cutaneous botryomycosis sometimes occurs after skin inoculation of microorganisms following trauma, surgery, or in presence of foreign bodies (including piercing practices), or positioning of medical devices like a pacemaker or orthopaedic biomaterials [7,19,23,33,36]. The majority of patients present with skin or subcutaneous nodules, but in other cases verrucous lesions or non-healing ulcers associated with draining fistulae may develop, with purulent discharge and the frequent presence of yellowish "grains", resembling the "sulphur grains" typical of actinomycosis [37]. Cutaneous lesions have a slow clinical progression, and may evolve for several months to years (in rare cases). Five episodes complicated by fistulisation and deep, bone infection have been described in 2006 in men aged over 70 years, who had their long-term infection resolved after extensive surgery and prolonged antimicrobial administration [23]. A paediatric case presenting with hyperpyrexia, elevated inflammatory indexes, and an inguinal inflammatory mass associated with pruritic papules, evolved in a prominent lymphadenitis, which was successfully treated with oxacillin and surgery, which material yielded the growth of a *S. aureus* strain, although showed a granulomatous process at histopatology examination [38]. A single case of muscular botryomycosis of the abdominal wall followed visceral surgery, and involved primary the rectus abdominis muscle [35]. Mucosal involvement (i.e. that of nasal septum and tongue, or a more extensive oral-facial involvement) has also been infrequently reported [16,39,40], as well as conjunctival lesions [41]. Patients with HIV infection and AIDS may present with multiple pruritic papules on neck, trunk, and limbs, difficult to be diagnosed until a biopsy is performed [28], or a pyoderma-like appearance in the genital region (successfully treated with dapsone in one case) [29], as well as complicated forms including

both skin and pulmonary involvement with concurrent isolation of *S. aureus* and *Pneumocystis carinii* in a patient with full-blown AIDS [30]. Only one case with lethal course followed an isolated cutaneuos localization of botryomycosis, in the setting of a severe AIDS-related immunodeficiency [26].

Visceral cases of botryomycosis account for a non-negligible portion of referred, but usually anecdotal cases, burdened by a proportionally greater severity and mortality rate when compared with cutaneous episodes. Clinical presentations involving liver, spleen, kidney, brain, and prostate have been described together with the more frequent pulmonary localizations [2,3,7]. Systemic symptoms such as fever, fatigue, or weight loss, may accompany all forms of visceral disease.

In particular, signs and symptoms associated with pulmonary botryomycosis include chronic cough, dyspnoea, haemoptysis, and chest pain. Clinical examination may be negligible, or demonstrate reduced breath sounds or rhonchi, should a consolidated parenchyma is of concern. Given the prolonged disease duration, lung botryomycosis may be mistaken for a mycosis, tuberculosis, actinomycosis, or a malignancy (especially pulmonary cancer) [4,10,15,42,43]. A literature search performed by Bersoff-Matcha in 1998, allowed to record 7 cases of apparently primary pulmonary botryomycosis, 5 of them treated with surgery, and responsive to a concomitant antibiotic treatment, after staining and/or culture positive for either Gram-positive organisms (*S. aureus*, non-haemolytic Streptococci, *Bacillus* spp.), or Gram-negative bacteria (*P. aeruginos*a, *Serratia* spp., other unidentified Gram-negative rods) [3], as initially supposed in our case report. A positive outcome was registered after a combined medical-surgical management in the large majority of cases [3]. A thoracic case of botryomycosis was described with a pleural lung mass presentation complicated by bone invasion into the thoracic spine and two posterior ribs [3]. The cultures tested negative for all bacterial, mycobacterial, and fungi, as well as for *Actinomyces* and *Nocardia* spp. Malignancies were excluded through a mediastinoscopy and lymph node biopsy and examination. No immunological abnormalities were detected, save an absolute $CD4^+$ T-lymphocyte count of 290 cells/μl (but the patient tested HIV-negative). An extensive pulmonary-pleural-spine intervention finally yielded *P. aeruginosa*, so that a ceftazidime treatment was administered postoperatively, and continued for a prolonged time. A diagnosis of botryomycosis was posed on the ground of the appearance of the multiple biopsy and surgical specimens, enforced by the presence of bright eosinophilic clubs at the periphery of granules [3]. Another primary pulmonary case of botryomycosis complicated by parietal pleural involvement was attributed to viridans Streptococci, and was cured with surgery plus antibiotic treatment [43]. A further case of primary lung botryomycosis with multiple continuous organ involvement (parietal pleura, chest wall, diaphragm, liver, and costovertebral junction) was successfully treated with a three-month long antibiotic therapy, after obtaining the diagnosis through a CT-guided biopsy of the pulmonary mass [44]. A particular case of lung botryomycosis secondary to a foreign body aspiration, and cured by the sole extraction of the foreign body, without any surgical-medical intervention, has been also reported [19].

When considering concomitant or underlying disorders in the field of pulmonary botryomycosis, Paz et al. reported one patient whose first manifestations of chronic granulomatous disease were represented by a lung botryomycosis, thus recommending a concurrent evaluation for this underlying disease [11]. On the other hand, patients with cystic fibrosis are well known to be at risk for respiratory botryomycosis, since different anatomic and immune defence defects, and iatrogenic causes are expected to support a pulmonary botryomycosis [24]. Katzenelsen et al. reported 7 pulmonary cases of botryomycosis, with even 5 of 7 complicated by a lethal course, despite a frequent resort to surgery and antimicrobial chemotherapy. A gram-positive (*Micrococcus pyogenes* var. *aureus*) or a gram-negative (*P. aeruginosa*) aetiology was found in all cases. As expected, all episodes of suspected lung botryomycosis have to be assessed in a differential diagnosis process with actinomycosis and fungal infections, as well as malignancies [14,15,24,37,43-47].

Only a few cases of visceral botryomycosis have been reported as intrabdominal abscesses, but detailed aetiological, clinical, and outcome notices were often lacking in their short descriptions [2]. When the liver, spleen, or kidney are involved, a chronic abdominal pain and local tenderness to palpation are usually present [33,48], as in the patient observed by us. One case of cecal botryomycosis [49], and one episode of rectal botryomycosis [47] have been also

described: the last one responded to erythromycin despite the absence of positive cultures [48]. In these cases, ultrasonography and CT scans of the abdomen reveal a mass lesion suspicious for an abscess or a malignant process, as in the patient reported by us. A fatal case of disseminated visceral botryomycosis probably caused by *P. aeruginosa* has been described in detail by Winslow and Chamblin [50], in an 80-year-old man who underwent prostatectomy, and with post-mortem examination showing multiple, scattered granules involving the lower respiratory tract, the heart, and the urinary tract (as the supposed origin of the systemic infection), which tested negative for fungi and *Actinomyces* spp., but proved repeatedly positive for *P. aeruginosa* cultures. Botryomycosis complicated by central nervous system involvement has been also described, in association with dental caries or after oral surgery [33,51,52]; focal neurological deficits, seizures, or also a meningeal involvement have been reported, as well as a rare, fulminant episode [33]. A unique case of autoptic diagnosis of heart botryomycosis has been reported recently by Gupta et al. [53]: in this anecdotal case, further botryomycotic abscesses involved the lungs and the bone marrow, leading to a picture of disseminated disease, occurring in absence of an apparent immunodeficiency.

From a pathogenetic point of view, a concomitant immunodeficiency is known to prompt the onset and the progression of botryomycosis. Brunken et al. [2] reviewed some of the immunologic abnormalities possibly retrieved in botryomycosis, and also postulated a nonspecific host reaction, possibly on a hypersensitivity basis, or the establishement of a sort of symbiosis status between the infecting organisms and the host defences. In one cutaneous case report of the year 1983, a reduced absolute B e T lymphocyte count was found, together with a blunted response to concanavalin A stimulation [2]. In particular, a concurrent HIV disease or AIDS is thought to be a severe risk factor for a predominantly cutaneous [25,26,31,32], but also visceral (pulmonary only) botryomycosis [10], with the multiple immunologic abnormalities of HIV infectious probably implicated in its pathogenesis. Ahdoot et al. reported the successful treatment of a case of mucocutaneous botryomycosis with an atypical presentation, occurred in a 21-year-old HIV-infected Somalian woman followed in the pre-HAART era, and attributed to a *S. aureus* infection [25]. Patients infected with HIV may present atypical skin lesions mimicking those of prurigo nodularis, lichen simplex chronicus, varicella-zoster virus, or sporotrichosis [8,26], although no clear relationship has been demonstrated between the severity of immunodeficiency (as expressed by the peripheral, absolute $CD4^+$ T-lymphocyte count), and the susceptibility to botryomycosis. Medical and combined medical-surgical treatment has been successful in the large majority of the described cases.

The diagnosis of botryomycosis is usually based on one or more of the following procedures [7,39]: identification of non-filamentous bacteria in purulent granules from draining sinuses or in biopsy specimens, culturing bacteria from ulcers or exudates in patients with clinical findings of botryomycosis, or on histopathological basis, after tissue biopsy, in patients with a likely clinical picture. Gram staining or silver nitrate staining (by the Gomori-Grocott technique) of the crushed granules is used for morphologic assessment. Botryomycosis may be distinguished from actinomycosis and mycetoma since botryomycosis granules are of variable size and shape, and may reach up to 500 microns in diameter. On the other hand, actinomycetes are branching, filamentous bacteria ≤ 1 micron in diameter, while fungi responsible for mycetoma have hyphae that are at least 2 microns wide. These differences may easily drive a correct recognition. Microscopic evaluation may be combined with routine bacterial, fungal, and mycobacterial cultures for definitive diagnosis. In addition, tissue specimens submitted to histopathological studies may add significantly. The histopathologic appearance of botryomycosis is usually depicted by a central focus of necrosis, surrounded by a chronic inflammatory reaction containing histiocytes, epithelioid cells, multi-nucleated giant cells, and a marked fibrosis [33]. The granules seen in botryomycosis usually contain bacteria within an eosinophilic matrix containing club like projections. This histologic appearance is commonly referred to as the Splendore-Hoeppli phenomenon [3,5-7,38], although this last feature may not be always present [26,41,48], as happened in our atypical case report.

Radiological and imaging procedures usually play a very significant role to evaluate the size and the extent of organ involvement,

also in the view of eventual surgical interventions. Pulmonary lesions may appear as a consolidation or a mass lesion, while other forms of visceral botryomycosis usually present as a mass lesion, with no particular, distiguishing features, as in the abdominal case reported by us.

As a consequence, the clinical differential diagnosis of either cutaneous or visceral botryomycosis, includes a very broad spectrum of disorders, i.e.: actinomycosis, mycetoma, atypical mycobacterial infection, sporotrichosis, cutaneous leishmaniasis, verrucous herpes, cutaneous abscess, nocardiosis (on the side of infectious diseases), and also Kaposi's sarcoma and other malignancies (especially when an underlying HIV disease is of concern, as in our case) [7,14,16,24,27,37,43-47].

With regard to treatment recommendations, cutaneous botryomycosis requires antibiotic administration, and surgical debridement in the majority of cases, since the encapsulated abscesses are thought to protect the eventual microorganisms from the effects of standard courses of antibiotics [7,16,23,34,47]; sulphamidic derivatives like dapsone acted successfully in isolated cases [29]. Antimicrobial therapy alone may be sufficient for superficial, limited episodes, especially when a bacterial pathogen has been identified and a malignancy has been excluded. Should Gram-positive pathogens are implicated, including *S. aureus*, cotrimoxazole, clindamycin, tetracyclines, erythromycin, or beta-lactam derivatives like oxacillin may be used (usually by oral route), after checking the *in vitro* susceptibility testing. In the event of Gram-negative infections including *P. aeruginosa*, an initial therapy with i.v. ceftazidime, ciprofloxacin, aztreonam, or a carbapenem (like imipenem or meropenem) is recommended; if the isolate tests fluoroquinolone-sensitive, a sequential therapy with oral ciprofloxacin is suggested. For infectious due to other Gram-negative organisms (i.e. *Proteus* spp., *Escherichia coli*, *Serratia* spp., or others), an i.v. beta-lactam derivative, a fluoroquinolone, or a carbapenem may be the initial choice, waiting for the *in vitro* sensitivity studies. In our case the potential role of a Gram-negative pathogen was strongly suggested by the relevant activity played by meropenem, whose antibacterial action is primarily directed against these bacterial agents. The antibiotic selection for *Pseudomonas* spp. or other Gram-negative microbial agents is similar to that of cutaneous botryomycosis, but all episodes of visceral infection usually require several months of therapy to have all signs and symptoms of botryomycosis resolved (while in our case report a proportionally rapid response occurred to combined surgery and meropenem administration). There is no conclusive evidence about the duration of medical therapy of botryomycosis, which is usually continued until signs and symptoms of infection have resolved. For superficial infection, 6 to 8 weeks may be sufficient, while subjects suffering from a deep infection and/or a concurrent immunodeficiency may require more prolonged courses. Both antimicrobial chemotherapy and a careful surgical debridement are strongly recommended for the treatment of cutaneous botryomycosis (especially those with deep tissue invasion, including muscle or bone, for those with delayed recovery, and for immunocompromised patients), as well as for almost all visceral episodes [7,23], as in our case. A resection of the mass often occurs prior to diagnosis, given the concern for a malignancy in the majority of cases of visceral localization of botryomycosis.

In the setting of HIV disease and AIDS, as to our knowledge only 10 cases have been reported until now [8,10,25-32], the large majority of them (even nine episodes) with isolated or predominant skin involvement, with only one lethal case associated with a severe form of AIDS [26]. As a consequence, one single case of pulmonary disease has been described in a patient diagnosed with a very advanced form of HIV-related immunodeficiency (as expressed by a $CD4^+$ lymphocyte count of 8 cells/μl), with cough and a blood-streaked sputum, fever, chills, shortness of breath, and weight loss, assessed with a chest CT scan and diagnosed by a fine needle aspiration percutaneous lung biopsy, and attributed to *S. aureus* infection on a microscopical basis only, and successfully treated with amoxicillin-clavulanate and later with erythromycin [10].

Our suspected case of botryomycosis successfully resolved after laparotomy and biopsy, and a prolonged antimicrobial therapy with a carbapenem (meropenem) alone, might have been the first episode of visceral (intraabdominal) botryomycosis ever described in patients living with HIV. The difficult differential diagnosis becomes even more cumbersome when an underlying HIV disease is present, due to the extremely broad spectrum of concomitant and overlapping

conditions, including for instance bacterial, fungal, actinomycotic, tubercular, mycobacterial, and also neoplastic, lymphoproliferative, and dysreactive diseases (as suspected and ruled out in the diagnostic workout of the presented case) [44-46]. The apparent lack of some histopatological hallmarks of botryomycosis, like the macroscopic eosinophilic granuli, and the microscopic Splendore-Hoeppli phenomenon, as well as the impossibility to culture organisms and to search them with molecular diagnostic techniques too, might be also attributed to the concurrent HIV infection and its related immunological abnormalities, possibly modified in their appearance and course due to the prompt and effective activity of the combination antiretroviral therapy already administered to our patient, and the related, remarkable immune system recovery achieved in the meantime by our patient [54]. Moreover, the prompt and durable response to a prolonged treatment with a potent, single antibiotic agent primarily active against a wide spectrum of Gram-negative pathogens could have suggested a potential bacterial aetiology of intestinal-abdominal origin of our case of intrabdominal infection, which remained for a long time with an unknown microbiological diagnosis.

Only 40 days after patient's discharge, from the microbiology laboratory a delayed culture of *Mycobacterium avium-intracellulare* from surgical specimens became finally available, so that a diagnosis of abdominal, atypical mycobacteriosis was unexpectedly confirmed, meropenem was discontinued, and a specific, long-term treatment was established on a day-hospital basis, with associated ethambutol, clarythromycin, and rifabutin. Notably, atypical mycobacteriosis is also included among possible microbiological ethiologies of botryomycosis itself, but in our experience all microscopic, culture, histopathologic, and even molecular biology testings on all available clinical specimens resulted repeatedly negative up to 40 days after patient's discharge, and our patient experienced a prolonged clinical response to a single-agent antimicrobial chemotherapy performed with meropenem alone (which is known to have very limited activity as a monotherapy, against mycobacteria as a whole). After a further two-month follow-up, at this time our patient is still under an effective and well tolerated oral treatment for atypical mycobacteriosis, together with its antiretroviral regimen carried out to ensure a continued control of the underlying HIV disease.

DISCLOSURE

The Authors declare that they have no financial competing interests.

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