Case Report

Subcutaneous dirofilariosis in Italy: a diagnostic hypothesis to remind when the anamnesis is misleading

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Summary
Subcutaneous parasitic infections are possible matches in routine pathology and can be detected not only in patients coming from or travelling in tropical countries, but also when the anamnesis does not suggest at first worm infestations.
Here we report the case of a young man from the north-west of Italy, with a negative anamnesis for travels but the presence of dogs at home, who presented a subcutaneous nodule showing a nematode surrounded by a fibrous capsule; the morphology was suggestive for Dirofilaria repens. Dirofilariosis is a zoonosis caused by D. repens or D. immitis, which can be acquired by dogs and cats through mosquitoes bites; the disease is widespread in developing countries, but it is also emerging in Western countries, becoming an important public health issue.

Key words: Dirofilariasis, Dirofilaria repens, subcutaneous nodules, zoonosis, one health

Introduction

Subcutaneous parasitic infection are possible matches in routine pathology. Even though most of them are related to journeys in tropical countries, the clinical suspect should be considered even when the anamnesis is negative for travel episodes. Dirofilariosis is a zoonosis caused by Dirofilaria repens or Dirofilaria immitis, which can be acquired by dogs and cats through mosquitoes bites; the disease is widespread in developing countries, but it is emerging in Western countries too, becoming an important public health issue. We report the clinical case of a young man with a temporal subcutaneous nodule.

Clinical presentation and methods

In March 2019 the surgical specimen of an 18-year-old man coming from the north-west of Italy was accepted in our Pathology Unit. It was a subcutaneous nodule excised from the right temporal region, close and firm to the cranial theca. The lesion had been present for about a year but had become painful in the last two months. He lived in a farmhouse with dogs and had not travelled abroad. No other clinical information was available. The sample was formalin fixed in 10% formalin and at gross examination the nodule was a cystic lesion, brownish and measuring 0.8 cm in maximum diameter.
The whole nodule was assessed for paraffin embedding and histological
examination. 3 µm sections were cut and stained with hematoxylin and eosin.

Results

Microscopically, at low magnification (Fig. 1) a peripheral rim of fibrosis with inflammatory infiltrate surrounded multiple sections of a worm-like organism. At higher magnification, the inflammatory population was reminiscent of the granulomatous chronic type and was represented by lymphocytes, plasma cells and histiocytes, with occasional polymorphonuclear neutrophils (PMNs), and hemorrhagic extravasation occurred (Fig. 2). At deeper examination, the worm was recognizable as a nematode and was composed by a thick multi-layered cuticle with indented surface, a muscle layer and the pseudocoelom containing intestine and paired uteri with dots resembling oocytes (Figs. 3, 4).

At microscopic digital analysis, the maximum diameter of the nematode was 460 µm (Fig. 5). No other staining was necessary to establish the diagnosis and the morphology was clear for a *Dirofilaria*.

**Figure 1.** Cystic lesion (H/E, 1X). A thick layer of fibrous tissue surrounds the nematode.

**Figure 2.** Detail of the inflammatory infiltrate (H/E, 20X). The fibrous capsule show blood extravasation and chronic infiltrate represented mainly by plasma cells and lymphocytes. PMN cells can occur.

**Figures 3, 4.** Anatomy of the nematode (H/E, 20X). The worm of the case is a mature female of *D. repens*, in which are clearly visible cuticle with peculiar external ridges (ER), resembling a gear-wheel, lateral chords (LT), muscular layer (ML) and intestinal tubule (I); the female genital tubules (FT) are well represented and contain oocytes (arrows).
Discussion

Since clinical history had few significant details, the differential diagnosis rested principally on microscopic morphology. It excluded a foreign body reaction because of the dimension of the worm and the intact material let the diagnosis be made without ancillary techniques.

*Dirofilaria* spp is a group of nematodes normally affecting animals but can cause a zoonosis due to transmission by mosquitoes. While *D. immitis* develops in deep organs, such as lungs and heart, *D. repens* and *D. tenuis* cause mostly subcutaneous nodules, in particular in periorcular area, or conjunctival lesions; a single case of pulmonary involvement by *D. repens* was reported in literature 1. *D. repens* is a parasite of dogs in South Europa, Africa and Asia. Microscopically, recent lesions show eosinophils and neutrophils in the fibrous capsule, while old abscesses display chronic infiltrate, with lymphocytes, histiocytes, eosinophils, epithelioid cells and giant cells, with necrotizing granuloma when degenerated. Peculiar is the presence of longitudinal stripes on the surface, giving a “gear wheel” – like aspect in transversal section. Surgical removal is a sufficient treatment and does not require anthelmintic drugs because the infestations do not cause severe sequelae.

The unequivocal presence of superficial longitudinal ridges is pathognomonic of *Dirofilaria repens* - just two other filarial worms have them (*D. hongkongensis* and *D. ursi*) 2, but are not epidemiologically compatible with the patient’s history. Furthermore, evaluating the diagnosis report, the clinician required a chest X-ray to exclude systemic involvement, resulting normal, as expected for *D. repens* more than for *D. immitis*. The long-lasting untreated disease in our patient was not exceptional, since the parasite grows slowly and reaches adulthood in 6 months when not damaged by an inflammatory response; however, it is curious that he became symptomatic after almost a year from the appearance of the subcutaneous nodule. It was speculated that humans may have a less efficient immune reaction against a parasite in subcutaneous tissues, so the lack of an immediate reaction could be explained by the localization of the nematode in the human body 3, even because the probability to find microfilarie of *D. repens* in bloodstream is virtually absent. In the literature a case with symptoms due to central nervous system involvement because of a massive eosinophilic reaction has been described. The prolonged permanence in subcutaneous tissue may have evoked in our patient a late antigenic response.

So far, most of the case reports in literature involved Indian, African and Middle Eastern patients, however diffusion of *D. repens* and *D. immitis* autochthonous infections in South Europe 4, in particular Italy, is increasing, thus it should be considered an emerging, self-standing, public health problem.

When microscopic features are not clear, or a poorly conserved sample is available, other tools are available for the pathologist. After accurate clinical data collection from clinicians including age, drugs, travels, contacts with pets and animals and serology, GMS and Giemsa stainings can be useful to distinguish parasites and microorganisms from foreign bodies or atypical tissues, although histochemistry is not currently used for helminths. Additionally, molecular tests are available to define a more accurate diagnosis when the clinical suspect includes few worms, such as ELISA, conventional PCR and real time PCR. To support the worm etiology hypothesis, many other nematodes should be taken into account during the diagnostic process.

*Wuchereria bancrofti* dwells in lymphatic vessels with symptoms ranging from elephantiasis to pneumonia. Microscopically the nematode appears with a complex anatomy and a strong eosinophilic reaction. Very few case reports have described infestation as a singular subcutaneous nodule without lymphatic impairment. *Enterobius vermicularis* infestation is common in children and affects the gut and the anal region. Cutaneous manifestations are rare and indistinct; patients show localized or extended erythematous plaques, unresponsive to antihistamines and steroids. Skin histology shows spongiosis and inflammatory infiltration with no signs of parasites 5.
Gnathostoma is a nematode that can infest travellers of developed countries giving rise to gnathostomiasis, clinically characterized by erythematous or nodular formations with migratory pattern. Dogs and cats are the final hosts; humans can contract the disease by eating raw freshwater fish. Skin biopsy may be useful for diagnosis, with the histological finding of eosinophilic granulocytes infiltrate. The therapy with albendazole or ivermectin increase the possibilities to find larvae leading to the formation of pustules, with larvae inside them.

Onchocerca volvulus is a filariform nematode causing onchocerciasis, a disease endemic in Africa which can lead to blindness. Humans contract the disease through the bite of a Simulium black fly. The microfilariae penetrate through the skin and transform into adult forms. In subdermal tissue the activation of the immune system leads to the formation of focal granulomas and microabscesses resulting in subcutaneous nodules ("onchocercomas"). The most effective method for diagnosing the disease are skin snips to find microfilariae. Histological analysis after the removal of the nodule may show cross sections of microfilariae surrounded by chronic inflammatory infiltration in which lymphocytes, eosinophils, and histiocytes can be found.

Sparganosis is the infestation due to larvae of the Spirometra or Diphyllobothrium genus. The main way in which the westerners can be infected is by drinking water contaminated with larvae. Usually subcutaneous or intramuscular nodules are the clinical manifestation of cutaneous sparganosis. In sections of the body of the larva can be observed: the parenchyma, which is formed by excretory channels and calcareous corpuscles within a loose stroma with smooth muscle cells; microvilli, that surround the tegument; the absence of reproductive organs. Larvae can bring about an inflammatory infiltrate into the surrounding tissues sometimes with granuloma formation.

Conclusions

In conclusion, we present a case of subcutaneous dirofilariasis in a young Italian patient with no history of journeys in endemic areas and considered the differential diagnosis features, explaining the importance of such a diagnosis in terms of public health.

Conflict of interest

The Authors declare no conflict of interest.

Funding

No fundings to declare.

Ethical consideration

None.

Authors’ contribution

NA and LT wrote the paper, GL treated the patient, PF performed the pathological report and supervised the work, CD supervised the work.

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