Some undesirable traps which can mislead the pathologist

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Abstract

In clinical laboratories, the diagnosis of parasite diseases can sometimes be challenging for non-expert microbiologists. Indeed in spite of the advent of the molecular biology, macro- and microscopic examination still remains essential. Nonetheless, it is usually not automated and requires great skills to complete the correct diagnosis. It is not infrequent that inert elements mislead to erroneous diagnoses. Through three different concrete examples, this article aims at underscoring the actual risk of parasite misidentification and at highlighting the systematic approach to be conducted in order to enable reliable diagnosis.

Keywords: rice-bodies, ciliocytophthoria, starch grains

Introduction

In clinical laboratories, the diagnosis establishment of parasite diseases can be challenging for pathologists or non-expert microbiologists. Thorough macro- and microscopic observation and histological examination of miscellaneous biological specimens, such as bronchial-alveolar lavage fluids (BALF), feces or biopsies of various natures, are the key-steps for reaching an accurate diagnosis. So far, these methods have been usually non-automated yet and both require extensive experience. Misidentification pitfalls are numerous, and pathologists or microbiologists should always be aware of them to avoid failure in the diagnosis process. Indeed, several inert or non-pathogenic elements can frequently mislead to erroneous diagnoses, and thus subsequently to inadequate curative therapies. Diagnostic difficulties are primarily emphasized when managing a patient who is used to live or has lived in tropical areas; the imported diseases are often neglected, and thus the pathologists and microbiologists are less seasoned with them and their diagnosis.

Therefore to reinforce the accuracy of their diagnostic conclusions, all laboratory staff should be informed of few very-practical tricks to enable better recognition of pathogenic elements and for distinguishing them easily from non-parasite structures. In this article, we will expose three concrete examples that focus on thorough observations of fake parasites in biological samples, and we will discuss more the differential diagnosis for each one.

Maggots or cestode vesicles?

A 58-year-old woman underwent a total left hip replacement due to coxarthrosis. In the following months, she began to feel chronical pain next to the joint, in spite of the painkiller medications. No clear etiology was found through the magnetic resonance imaging (MRI). Likewise, a punction was not contributive. Twenty-two months after the initial surgery, the hip prothesis was removed in order to avoid a septical issue. During this second intervention, numerous little white inert bodies, measuring seven to eight millimeters in length, were found around the soft tissues (Figure 1 A) and above the prothesis (Figure 1 B). The samples – which look like maggots or cestode vesicles at first glance – were sent to the hospital laboratory for parasite identification (Figure 1 C), but were finally

characterized as rice-bodies. The *in vitro* bacterial culture of the tissue surrounding the rice-bodies eventually isolated *Cutibacterium acnes* colonies, *i.e.* natural saprophyte bacterium of the skin, whereas the mycological culture remained sterile. Based on periodic acid Schiff (PAS) and hematoxylin and eosin safron staining, the histopathological examination showed fibrin deposition with inflammatory cells, but no parasite. To definitively exclude cysticercosis, the research of *Taenia solium* antibodies was conducted, but was found negative. A second total hip replacement was programmed, four months later. No complication occurred, and the patient left the hospital with favourable outcome.

Ciliated protozoans?

A 65-year-old woman underwent surgical resection of womb fribroma that was evolving over one year. There was no known underlying infection at time of the surgery. Concomitantly to the intervention, aspirate of the pertioneal liquid was systematically addressed to the hospital laboratory for biological investigations. At the fresh mounting examination, several mobile structures of 10-25 µm were observed. At higher magnification, they appeared ciliated on their whole circonference (Figure 2 – video 1), a little bit like a protozoan. However, the motions were quite unatural, depending mostly on the Brownian flow. Finally, these structures were identified as ciliocytophthoria artifacts coming from uterine tubes or endometrium. No *in vitro* cultures were positive. There was no complications for the patient and no occurrence of infection. The outcome was favourable.

Segmented helminth?

A 58-year-old male was found dead at home and subsequently autopsied by forensic scientists for suspicion of COVID-19 (coronavirus infectious disease – 2019) infection. During necropsy, some lung lesions were clearly observed, and biopsies were then performed for further investigations. Histopathology examination confirmed the presence of foci of acute pneumonitis. Moreover, several

unusual round- or oval-shaped structures, with size ranging from 2-3 µm to 10-15 µm, were noticed within the lumen of alveoli and bronchioles (Figure 3). Due to the size and the regular section, helminth larvae or nematode remnants were first suspected. However at higher magnification, the appearance was rather suggestive of starch grains. The grains had been probably unintentionally aspirated into the lungs, and thus were not assumed to be responsible for the death. The latter was finally inferred to a stroke.

Discussion

Correct identification of parasites in clinical samples can be critically challenging, including for quite-skilled microbiologists. The latter have to conduct a systematic thorough approach during the macro- and microscopic examination of all the specimens that are addressed to them. Indeed, the risk of confusion can be really detrimental in some circumstances.

Regarding the first case, one could have first suggested vesicles due to any cestod infection, *e.g.* cysticercosis, when facing the oval white structures. However in the medical history of the patient, there were no clear clues that could have reinforced this diagnostic hypothesis. In contrast, the observation was finally more in adequation with rice-bodies description.[1, 2] Rice-bodies appear rubbery when cut into pieces, and they are theoreteically not hollow. However, there contain neither liquid nor parasite elements, wether living or dead, inside. Rice-bodies are rarely observed; they sometimes form during inflammatory processes of the synovial liquid, like infectious arthritis, rheumatoid arthritis, non-specific arthritis or osteoarthritis.[3] The pathogenesis may be explained by microinfarcted synovium and release of tissue into the joint, and subsequent encasement by fibrin deposition.[4] Another hypothesis suggests a *de novo* formation in synovial fluid independently of any synovial elements, and progressive enlargement with aggregation of inflammatory nodules consisting of fibrin, collagen, inflammatory cells and blood vessels.[5] In the present case, the rice-bodies probably developed after the first hip surgery. Usually, rice-bodies are isointense on T1-weighted MRI

and slightly hyperintense on T2-weighted MRI relative to muscles;[6] but in the present case, rice-bodies were unperceivable during medical imaging.

At first glance, the second observation reported herein could have suggested a vegetative stage of ciliated protozoans,[7] like trichomonids or paramecia.[8] Actually, the microscopic structures were rather consistent with ciliocytophthoria artefacts.[9] Ciliocytophthoria are usually the result of the degenerative process of ciliated cells consequently to various common infections, like miscellaneous virus diseases.[10] They are produced from the triangular top part of the bronchial or uterine cells, at the luminal border, which is separated from the cytoplasm bearing the decaying nucleus. Ciliocytophthoria size is quite variable. The cilia are straight, sometimes only represented on a limited part of the outer perimeter, and have a uniformed short length. They express a coordinate and synchronous rhythmic beating which does not lead to any active movement.[11] All these features should theoretically allow a clear distinction with (non-)pathogenic flagellated or ciliated protozoans. For example, the vegetative stage of *Giardia duodenalis* displays a flat pear-shape with two transverse, claw-shaped median bodies, and with four pairs of flagella. *Lophomonas blattarum* bears irregular long flagella arising over the anterior part, and which are longer at center and shorter at sides.[11] Furthermore, the motility of flagella is much more active.[12] Among the ciliate phylum, *Balantioides coli* trophozoites are large, up to 150 μm in length, and their entire cell surface is covered by short cilia.[13]

The third case about the observation of starch grains highlights how histopathology examination can be tricky for pathologists and parasitologists. Starch grains are quite common structures. Their morphologic presentation is the same for all vegetables; they are stained in dark pink color by the PAS method, which indicates high concentration in carbohydrates. Starch grains contain a lobulated and empty internal part with no clear well-defined structures inside, and there is a lack of inflammatory response surrounding these exogenous particles.[14] The presence of such starch particles has already been described in inhalation pneumonia,[15] but with no clear demonstration of any pathogenic role. Likewise, starch grains may be sometimes confused with pinworm remnants. However, *Enterobius vermicularis* pinworm is quite different, regarding its morphology: the transversal section of its body

displays a pair of cuticular crests. Usually, it also shows typical eggs in the uterus and the characteristic narrow meromyarian layers (two to three muscle layers *per* quarter section divided by four cords).[16]

In conclusion, in case of any doubt about the parasite etiology, the clinical specimens have to be systematically addressed to the parasitology laboratory in parallel before the tissue fixation which is mandatory for histological examination, and in order to get a specialized advice on a fresh sample. Although the multiplex real-time PCRs have been increasingly used as first-line diagnostics,[17] the macro- and microscopic examination still play a major role in the diagnostic process. The three aforementioned cases show how numerous can be the pitfalls leading to erroneous identification. In such a context, one should remember that the microbiologists own a critical expertise added to an habit of systematic approach of biological analysis.

Ethics

The patients were informed and did not oppose to the publication process. As the study is retrospective with no impact on the routine healthcare, no specific approval was required by the authorities.

Conflict of interest

None

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Figure legends

Figure 1: Rice-body elements. A) Dissection of the left hip compartment showing multiple surrounding white bodies. B) Same hip after removing the superficial white bodies. C) Numerous rice body-like structures removed from the hip prosthesis.

Figure 2: Observation of ciliocytophthoria particles at the fresh mounting examination x400. Ciliocytophthoria artifacts are degenerated from bronchial cells and each one is constituted by the nucleus (nc) with small cytoplasm that bears the cilia (ci). The latter arise from the terminal bar (tb).

Figure 3: Histological observation of starch grains (sg) within the lumen of bronchioles at time of necropsy A) Hematoxylin and eosin (H&E) stain x125; B) Grocott-Gomori's methenamine silver (GMS) stain x125; C) H&E stain x250, showing more clearly a lobulated and optically empty internal part (‡); D) Periodic acid Schiff (PAS) stain x250, indicating the high concentration in carbohydrates of the particle through the dark pink colour.

Video 1: Observation of ciliocytophthoria particles at the fresh mounting examination x400. Note the unilateral disposition of the cilia and the unnatural movements of their beating.