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Case report

The kinetoplast in the diagnosis of visceral leishmaniasis

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ABSTRACT

In visceral leishmaniasis (as in all leishmanial infections), microscopic diagnosis is made by observing the intracellular amastigote form, complete with a kinetoplast, in aspirate smears or biopsied tissue. In the 2 clinically-ill patients described here, intracellular inclusions were demonstrated in a bone marrow aspirate or a colon tissue biopsy. Kinetoplasts associated with the inclusions were not identified in the marrow aspirate smear (although the patient was treated for visceral leishmaniasis), but were identified retrospectively in the colonic tissue (although the patient was treated for histoplasmosis). Both cases illustrate the importance to clinical consultants of microscopically observing (or not) an associated kinetoplast when faced with a tissue aspirate or biopsy specimen showing intracellular inclusions.

Introduction

In visceral leishmaniasis, microscopic diagnosis is made by demonstrating the intracellular amastigote form in stained smears or tissue obtained by aspiration or biopsy. Amastigotes are small (2–4 υm), oval-shaped forms surrounded by a plasma membrane containing a single, dense eccentric nucleus and rod-shaped kinetoplast. The kinetoplast may be perpendicular to or parallel with the nucleus. For clinical consultants, the 2 cases presented here underscore the diagnostic value of microscopically observing (or not) a kinetoplast when faced with ill patients with a tissue aspirate or biopsy specimen showing intracellular inclusions.

Case reports

Patient 1

A bone marrow aspirate and smear was performed in a previously healthy, HIV-negative adult admitted to the New York Presbyterian Hospital with an acute-onset, febrile illness. The patient had hepatosplenomegaly, pancytopenia, elevated inflammatory markers and evidence of progressive multi-organ involvement – together suggesting hemophagocytic lymphohistiocytosis (HLH). In addition to containing ingested erythrocytes, bone marrow macrophages (histiocytes) also showed numerous intracellular inclusions (Fig. 1). This finding, along with travel in the previous 6 months in both southern Europe and the Caribbean region, raised the question of visceral leishmaniasis (kala-azar) as well as disseminated histoplasmosis, both of which have been associated with secondary HLH [1–4].

Patient 2

This previously reported adult with fully-established AIDS had undergone a colonic biopsy at another hospital because of fever and diarrhea [5]. Histopathologic examination of the colonic tissue was reported as showing forms consistent with *Histoplasma capsulatum* (which often involves the colon [6]), and itraconazole was begun for AIDS-related disseminated histoplasmosis. After treatment for 3 months, he was re-admitted with a possible drug reaction, but within days, deteriorated with presumed sepsis, prompting antibacterial therapy and transfer to this institution where amphotericin B deoxycholate was also begun. Blood cultures were negative. However, a routine peripheral blood smear unexpectedly showed intracellular forms characteristic of *Leishmania* amastigotes within monocytes, prompting continued amphotericin B treatment and bone marrow aspiration – the aspirate smear also demonstrated amastigotes [5]. The patient was critically ill and died 3 days after admission. Autopsy was not permitted, but a histopathology slide from the original colonic biopsy 3 months before was subsequently provided and reviewed (Fig. 2). The patient had been raised in urban Peru, spent 8 adult years in southern Spain and France (where subclinical *L. infantum* infection was presumably acquired), and then immigrated to and lived for decades in the United States.

Abbreviations: AIDS, acquired immunodeficiency syndrome; HLH, hemophagocytic lymphohistiocytosis.

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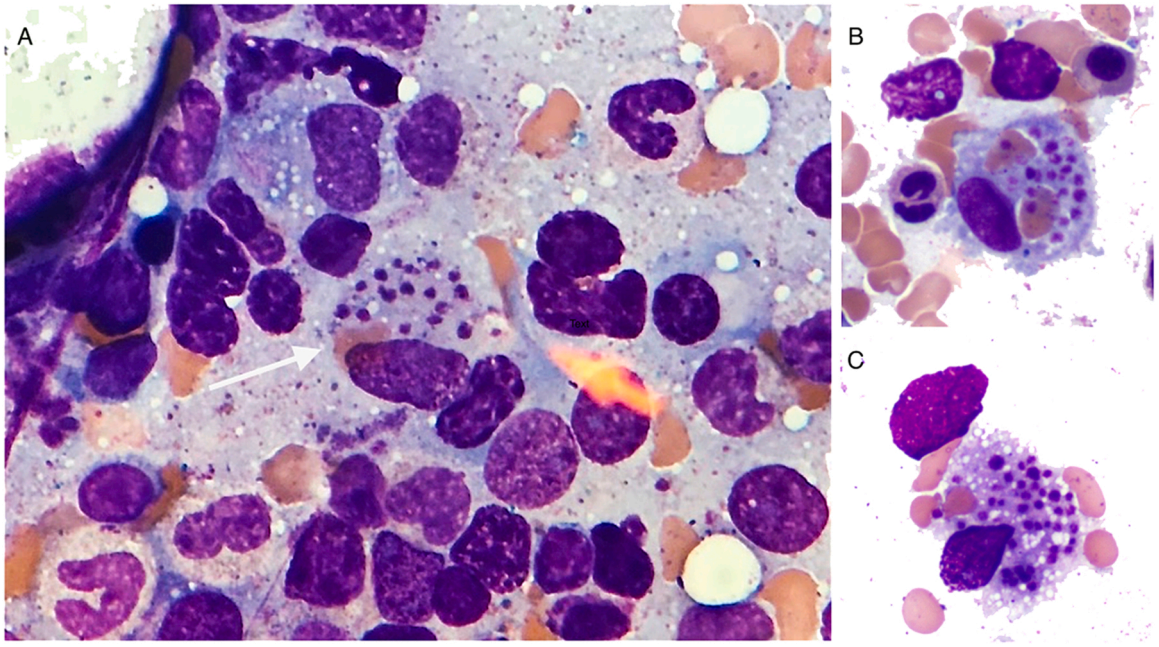

Fig. 1. (A-C). Bone marrow aspirate smear from Patient 1 showing macrophages (arrow) in adjacent fields with numerous intracellular inclusions but no identifiable or clearly-associated kinetoplast-like structures. Hematoxylin-eosin stain; original magnification, x1000.

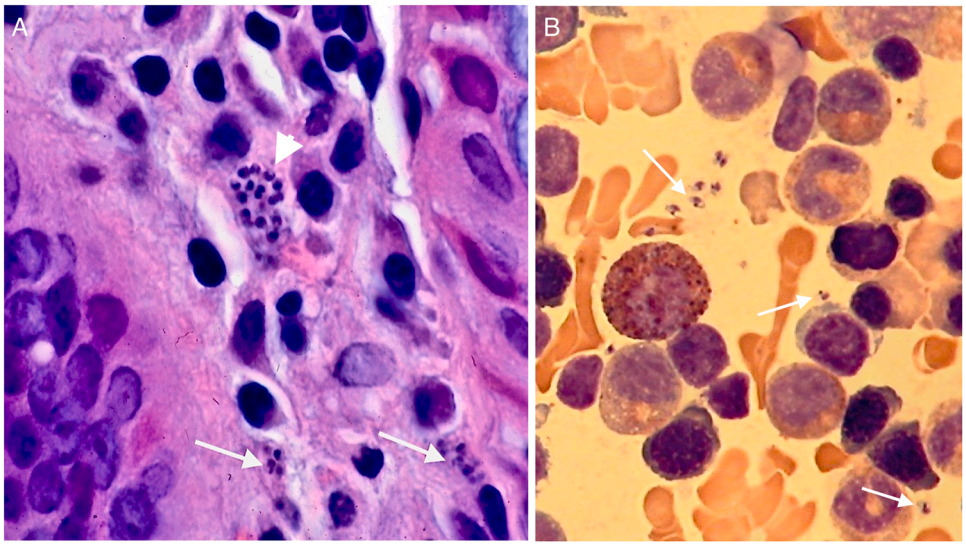

Fig. 2. Colonic biopsy tissue (A) and bone marrow aspirate smear (B) from Patient 2. In (A), arrows indicate two amastigotes with identifiable kinetoplasts. Note adjacent "nest" of forms (arrowhead) possibly resembling a *T. cruzi* "pseudocyst." In (B), arrows indicate characteristic *Leishmania* amastigote morphology in extracellular forms released during smear preparation. Hematoxylin-eosin (A) and Wright-Giemsa (B) stains; original magnification, x1000.

Discussion

Absence of a kinetoplast

In Patient 1′s bone marrow aspirate smear, microscopic fields such as those illustrated in Fig. 1A were thought by some initial observers (from the hematology and infectious diseases clinical services) to suggest the presence of intracellular *Leishmania* amastigotes within macrophages. However, careful examination of this and other fields containing numerous intracellular inclusions (Fig. 1B, C) using high magnification (100x objective, oil immersion) and focusing up and down failed to show associated kinetoplasts. In addition, none of the inclusions were within a discernible plasma membrane (see Fig. 2B), their sizes were highly variable, and while some inclusions were irregular, few were oval and most were homogenously-stained and perfectly round. None of the inclusions resembled *H. capsulatum* yeast forms, and were thought to represent phagocytized cellular and/or some form of lipid debris. Despite the microscopic conclusions, however, liposomal amphotericin B (10 mg/kg on 2 consecutive days [7]) was nevertheless given for the remote possibility of Mediterranean visceral leishmaniasis associated with HLH [1–3]. Urgent treatment by the medical staff was prompted out of an abundance of clinical caution – at the time of bone marrow examination, the patient was febrile, rapidly deteriorating and requiring intensive care unit support. Two days later, however, the patient was begun on treatment for HLH with dexamethasone, etoposide and emapalumab and over time showed a good clinical response. Ultimately, no microbial trigger for secondary HLH was demonstrated; all cultures and extensive serologic and antigen testing, including for *Leishmania* and

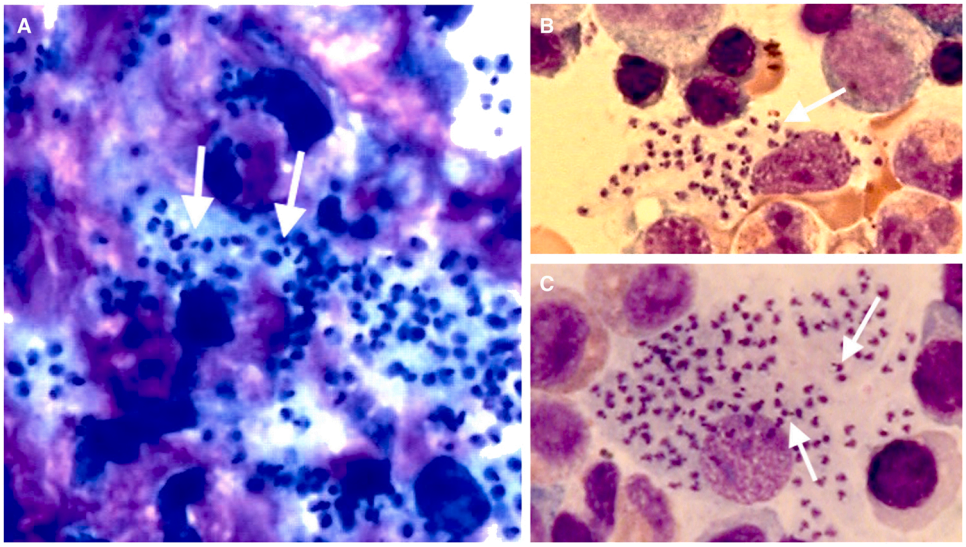

Fig. 3. Characteristic amastigotes with identifiable kinetoplasts (arrows) in liver biopsy tissue (A) and within macrophages in bone marrow smears (B, C). Giemsa (A), and Wright-Giemsa (B, C) stains; original magnification x1000.

Histoplasma infection, proved negative.

Presence of a kinetoplast

Kinetoplasts may be difficult to visualize in fixed sections of *Leishmania*-parasitized tissue irrespective of the staining method employed but in particular in sections stained with hematoxylin and eosin. Nonetheless, in the postmortem review of Patient 2's colonic biopsy (which had been performed 3 months before his final admission), kinetoplasts associated with characteristic amastigote nuclei were identifiable (Fig. 2A). The same intracellular forms were also present in peripheral blood and bone marrow aspirate smears in the days before he died [5]. Fig. 2B, showing amastigotes released from his bone marrow macrophages during smear preparation, well illustrates amastigote morphology including nucleus, kinetoplast and parasite plasma membrane.

In immunocompetent patients with visceral leishmaniasis, amastigotes are primarily demonstrated in tissues accessible to aspiration or biopsy – bone marrow, spleen, liver and lymph nodes. In immunodeficient patients, however, including those with fully-established AIDS, intracellular amastigotes can be observed in a variety of other tissues, ranging from the gastrointestinal tract to the skin as well as in peripheral blood smears [8–10]. Although not considered at the time [5], it is worth noting that the intracellular amastigote form of *Trypanosoma cruzi*, which also contains a nucleus and rod-shaped kinetoplast, is microscopically indistinguishable from *Leishmania* amastigotes in tissue [1]. Given: (a) Patient 2′s upbringing in Peru, (b) gastrointestinal (colon) involvement, and (c) a nest-like collection of organisms (a possible "pseudocyst" [1]) in the biopsied tissue (Fig. 2A), AIDS-related reactivation of chronic Chagas disease should also have been considered [11, 12]. However, bone marrow involvement is thought uncommon in *T. cruzi* infection [13] and, if parasitemic (as Patient 2 was [5]) the extracellular, flagellated trypomastigote form of *T. cruzi* and not amastigotes would be expected in a peripheral blood smear [12,14,15].

Finally, 3 other adult patients with visceral leishmaniasis have been seen at this institution since 2000 – a long-term New York City resident originally from South Asia who, in the setting of advanced HIV infection, developed fever, skin lesions and hepatosplenomegaly (Fig. 3A); a New York City resident who presented with splenomegaly and marked anemia months after an extended stay in Southern Europe (Fig. 3B); and a recent immigrant also from the Mediterranean region who presented with fever, hepatosplenomegaly and pancytopenia (Fig. 3C). In each, along with consistent clinical findings and laboratory results, the presence of intracellular inclusions with kinetoplasts on microscopic examination of a tissue (liver) biopsy or bone marrow aspirate permitted the initial diagnosis of and prompt treatment for visceral leishmaniasis.

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Ethical approval

None.

Consent

None.

CRediT authorship contribution statement

Henry Murray – Conceptualization, Writing – original draft.

Conflicts of interest

None.

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