Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(13)60088-X

Giemsa and Grocott in the recognition of *Histoplasma capsulatum* in blood smears

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PEER REVIEW

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Comments

Histoplasmosis is the most common endemic mycosis in the Argentina and has recently emerged as an important opportunistic infection among human immunodeficiency virus-infected persons living in areas where it is endemic. In this article, the authors describe the epidemiologic and ecologic features of histoplasmosis, highlight the implications for prevention. Surveillance and education of the public and health care providers are needed to determine the disease burden of histoplasmosis. Development of better diagnostic tests for detection of disease in humans and of the organism in the environment will help in designing better prevention strategies.
(Details on Page 420)

ABSTRACT

Objective: To facilitate the recognition of intracellular yeasts of *Histoplasma capsulatum* and differentiate it from *Leishmania* amastigotes and other parasites, using the combination of Giemsa and a rapid modification of Grocott stains to peripheral blood smears in a hematological study. **Methods:** The combination of both stains was applied consecutively (first Grocott and then Giemsa) to previously fixed peripheral blood smears. Microscopy was performed with 400× and 1000×, the latter using immersion oil. **Results:** The yeasts of *Histoplasma capsulatum* were observed into the cytoplasm of leukocytes as brownish oval elements, with 3-4 μm in diameter. **Conclusions:** The combination of both techniques is a simple and fast method to facilitate recognition of intracellular yeasts and it is different from intracellular parasitic elements. Moreover, it allows distinguishing the cell elements that are in the microscopic preparations. It may be very helpful in those cases in which the presumptive diagnosis of histoplasmosis has not been established yet and where other more sophisticated methods are not available.

KEYWORDS
Histoplasmosis, Peripheral blood smear, Visceral leishmaniasis, HIV

1. Introduction

The Giemsa stain is usually applied to peripheral blood smears in order to carry out the cytological study of the blood formula. Occasionally, the operator can identify fungal elements, which may be important for the diagnosis, especially in the yeast-like phase of *Histoplasma capsulatum* (*H. capsulatum*), as well as parasitic structures, such as different stages of the agents that cause the visceral leishmaniasis, the African and American trypanosomiasis, and paludism[1].

The diagnosis of visceral leishmaniasis is based on the view of *Leishmania* sp. amastigotes in blood smears (or preferably in those carried out with material from a splenic or bone marrow puncture). In these cases, the causal agent can be seen in the Giemsa stain as oval or round elements, of about 2-3 μm in diameter, located in the monocytes and macrophages, and inside they have a bacilliform structure called kinetoplast, which does not appear in yeasts[2].

Likewise, the *Leishmania* sp. amastigotes can be clearly

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Foundation Project: Supported by grant from the Louis Albert Foundation, Tolosa, Argentina (Grant No. 165381).

Article history:
Received 11 Apr 2013
Received in revised form 17 Apr, 2nd revised form 23 Apr, 3rd revised form 27 Apr 2013
Accepted 12 May 2013
Available online 28 May 2013

seen with this stain in microscopic preparations that are carried out with material from the scrapping of the skin lesions that appear in the cutaneous leishmaniasis[3].

In the case of histoplasmosis, the fungal parasitic phase can be seen with the Giemsa stain as oval elements of 3-4 μm in diameter, with a typical coloration as a cap (the nuclear chromatin moved to one of the poles) and a small light halo around it (a false capsule), which are located in the phagocytic cells[4].

Furthermore, the fact of applying the Giemsa stain reveals these fungal structures in the phagocytic cells in microscopic preparations that are carried out based on material that is obtained from the histoplasmosis skin lesions, in patients with AIDS and in patients with classical histoplasmosis[5].

Even though the microscopy does not establish a histoplasmosis diagnosis with certainty, the findings make a high diagnostic presumption possible, which will be later on corroborated by the *H. capsulatum* isolation based on cultures[5].

In the daily practice, structures, yeasts and amastigotes can be mistaken in coloured smears with the Gimsa technique if the observer is not properly trained in the microbiological diagnosis.

The Grocott technique or its rapid modifications are usually applied to histopathological sections in order to recognize the fungal structures (yeasts, pseudohyphae, hyphae, *etc*)[6,7].

Also, they can be applied to smears of different clinical materials, which should be previously fixed, although in opposition to the Giemsa stain, they do not allow to view cellular elements which are in the samples (leukocytes, macrophages, epithelial cells, *etc*.) neither to view trophozoite parasitic structures.

In order to facilitate the recognition of these intracellular yeasts, the different cell types that appear in the microscopic preparations and their differentiation from amastigotes, we propose a combination of the Giemsa technique and a rapid modification of Grocott, applied to either blood smears or other types of microscopic preparations, fixed to the microscope slide[1,7].

2. Materials and methods

The involved techniques, in the first place, is a rapid modification of the Grocott stain, which briefly consists covering the microscopic preparation, which was previously fixed, with an aqueous solution of 10% chromic acid for 10 min, and then washing with tap water.

After that, the preparation is covered with an aqueous solution of methenamine-silver (composed by equal parts of an aqueous 3% methenamine solution and 5% silver nitrate, with the addition of a few drops of 5% aqueous sodium tetraborate), and it is heated by applying heat below the microscope slide, with a cotton swab on, as it is done in a Ziehl-Neelsen stain, preventing the overheating of the preparation and the release of bubbles until the material that is fixed to the microscope slide acquires a brownish coloration[7].

After washing it with water, the preparation is covered with an aqueous solution of sodium bisulfite at 1% for 1 min, and then it is washed again with tap water. Once the preparation is dry, it is filled with an aqueous solution of Giemsa 1:10 for 20 min, and after this time it is washed with water and dried, before observing it with the microscope with immersion objective (1 000×).

3. Results

In the microscopy of peripheral blood smear, with the histoplasmosis suspicion, the dark yellow or brownish yeasts shall be looked for in the cytoplasm of leukocytes, as it can be seen in Figures 1 and 2.

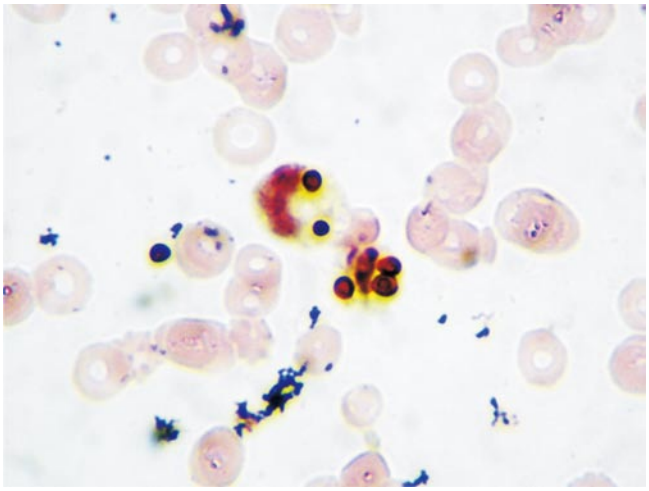

Figure 1. *H. capsulatum* yeasts present within the cytoplasm of leukocytes in a blood smear, carried out for blood test, stained with a combination of Grocott and Giemsa stains (1 000×).

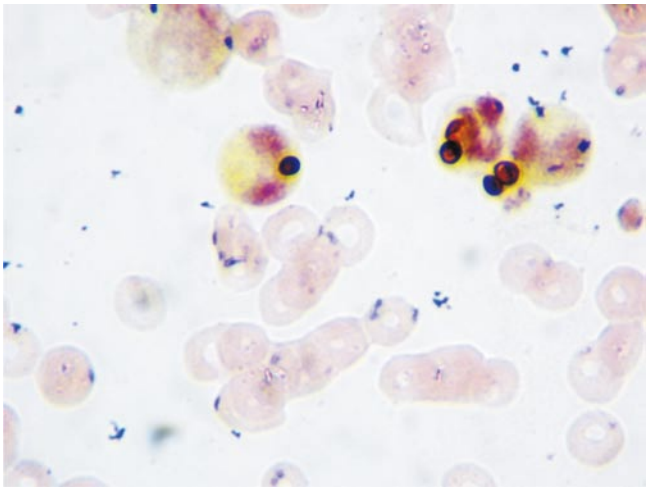

Figure 2. *H. capsulatum* yeasts in stained smear (1 000×).

4. Discussion

The view of the parasitic phase of *H. capsulatum* in blood smears constitutes an exceptional event in patients with histoplasmosis, which is usually observable in patients with severe clinical forms of the disease and associated with states of severe immunosuppression[4]. In our experience, these are patients with histoplasmosis associated with AIDS, who are hospitalized in the intensive care unit, with low counts of CD4+ T lymphocytes, which

means a delicate health, and whose prognosis, after the discovery of yeasts in the blood smear, is bleak in spite of an appropriate antifungal treatment[8].

The exceptional of the finding and the fact that the people who perform the microscopy are professionals who are not trained in microbiological diagnosis (but usually they are used to carrying out blood tests of blood smears) mean that they can ignore the presence of yeasts (even more when they are scant) or confuse them with amastigotes[9].

The combination of both techniques is a simple, fast (it takes about 45 min) and economic method, which is within the reach of laboratories with low complexity and it facilitates intracellular yeast recognition, and its differentiation from intracellular parasitic elements, moreover, it allows distinguishing the cell elements that are in the microscopic preparations[10,11]. Even though this methodology does not provide a definitive diagnosis of histoplasmosis, it may be very helpful in those cases in which the presumptive diagnosis has not been established yet and where other more sophisticated methods are not available[12,13].

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Histoplasmosis is a common infection endemic in many regions of America, Asia, India and Africa, with sporadic cases also occurring throughout the world. Although excellent laboratory methods for diagnosis are available, there are deficiencies that must be met by continued research. Clinicians and laboratory directors must be familiar with the uses and limitations of a battery of serologic and mycological tests to accurately diagnose histoplasmosis. Research is needed to reduce false-negative and false-positive results and to improve the identification of the organism in tissues. Approaches to the diagnosis of histoplasmosis and areas that require further research will be necessary.

Research frontiers

As it is true of all fungal infections in immunosuppressed patients, heightened awareness of the epidemiology and clinical manifestations of histoplasmosis is essential in making an early diagnosis.

Related reports

Culture remains definitive and should always be performed to confirm the results of the rapid diagnostic studies.

Innovations and breakthroughs

The identification of the yeast phase of *Histoplasma capsulatum* in tissue biopsy samples and, uncommonly, in circulating blood phagocytes is also helpful in establishing a diagnosis quickly.

Applications

If strategies for the prevention of disseminated histoplasmosis in HIV-infected patients are to be improved, studies must better define the risk factors for this opportunistic infection, describe its natural history and develop more reliable tests to predict its development.

Peer review

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