The usefulness of blood culture in diagnosing HIV-related systemic mycoses: evaluation of a manual lysis centrifugation method

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The results of 5034 blood cultures, implementing a lysis-centrifugation method with saponin, are summarized in this paper. Three hundred and twenty two blood samples (6.3%) obtained from a pool of human immunodeficiency virus (HIV)-positive patients yielded fungi. Cryptococcus neoformans was isolated in 199 samples (3.95%), Histoplasma capsulatum in 95 (1.89%), Candida parapsilosis in 12 (0.23%), C. albicans in 7 (0.13%), C. tropicalis in 2, C. krusei in 1, C. guilliermondii in 1, and Prototheca wickerhamii in 4 (0.07%). Blood cultures were positive for C. neoformans in 76.23% of patients having a diagnosis of cryptococcosis and in 89.65% of those who had histoplasmosis. The blood culture was the first means of confirming the diagnosis in 23.8% of the patients with cryptococcosis and in 54% with histoplasmosis. In the four patients in whom P. wickerhamii was isolated, a diagnosis of disseminated protothecosis was not achieved by other findings. Catheter infections were responsible for the majority of recovered Candida spp.

Keywords blood cultures, cryptococcosis, histoplasmosis, HIV infection, lysis-centrifugation, systemic mycoses

Introduction

Prior to the mid-1970s blood cultures were used infrequently in the diagnosis of systemic mycoses. In general, they were less sensitive than blood cultures for bacteria, and yeast-like fungi were recovered much more frequently than mycelial and dimorphic fungi [1]. The endemic, systemic mycoses, such as histoplasmosis, usually manifested clinically as chronic pulmonary or chronic disseminated forms, in which blood cultures rarely contributed to the isolation of the etiological agents [2]. Renewed interest in blood cultures for the isolation of fungi has occurred because of the change in the clinical spectrum of systemic mycoses in the last 20 years and also as a result of the rise in infections associated with neutropenia, oncohematological diseases, and the acquired immune deficiency syndrome (AIDS) pandemic. The evolution of the systemic mycoses in the compromised patients is more acute with greater possibilities for obtaining positive blood cultures [3,4].

Changes in the clinical presentation of mycoses were concomitant to innovative technological changes in blood culture procedures which notably increased their efficacy. Initially, broth blood cultures were vented and subcultured on to a solid medium after 7 and 14 days of incubation at 37 °C. Diverse techniques of lysis-centrifugation continued to be developed and, finally, automated blood culture procedures were introduced which could rapidly detect microbial growth, either by the radiometric (Bactec®) or photochromatic (Bact-Alert®) detection of CO2 [5–9]. These innovative techniques improved the yield of blood cultures and increased their use in the diagnosis of opportunistic mycoses associated with various predisposing factors [10].
The diagnosis of histoplasmosis and cryptococcosis is usually achieved by identifying the causal agent in focal cutaneous lesions or in cerebro-spinal fluid (CSF), bronchial secretions, etc. [11,12]. Blood cultures are an important diagnostic tool in the absence of focal mycotic manifestations or when foci are not easily reached [13].

Earlier studies evaluated the efficacy of four blood culture techniques for diagnosing AIDS-related mycoses: the classic technique in liquid medium, the automated Bact-Alert® and two lysis-centrifugation techniques, Isolator® and a saponin system standardized in our laboratory [14–16].

The two lysis-centrifugation systems were equivalent in efficacy and superior to the other procedures. The cost of the saponin system was 16 times less than for Isolator® [15].

Materials and methods

Patients

Only HIV-positive patients were enrolled in this study using the following two inclusion criteria:

1. Systemic mycosis mycologically confirmed and without previous anti-fungal treatment: 293 cases.

2. Non-confirmed diagnosis, fever of unknown origin and/or headache of ≥7 days duration, meningitis, hepatosplenomegaly, interstitial pulmonary infiltrates and micronodules and/or necrotic cutaneous papules: 3033 patients.

Test tube preparation

Plastic, 10 ml capacity, screwtop centrifuge tubes were used. One milliliter of isotonic saline solution was placed in each tube with 5% saponin and 0·4% sodium salt polyethyle sulfonic acid. The tubes were autoclaved at 1 atmosphere for 20 min. A total of 5034 blood samples were studied during the last 5 years.

Drawing and processing of blood samples

Blood samples were drawn by venous puncture, approximately 9 ml of blood being removed with a dry syringe and placed immediately in the tubes for culturing. The tubes were inverted several times to ensure mixing of their contents, which were then sent to the laboratory.

Having remained at 28 °C for 1 h, the tubes containing the blood samples were then centrifuged at 3000 rpm for 30 min. Supernatants were discarded and the sediments were seeded in four test tubes containing Sabouraud honey agar [17] and brain–heart infusion agar (Difco). The tubes were then incubated at 28 and 37 °C, respectively, for 4 weeks, with controls twice a week.

The Sabouraud honey agar medium consisted of: honey 20 g, peptone 10 g, yeast extract 5 g, agar 18 g, water 1 l.

Cultures of Histoplasma capsulatum were identified by micromorphological characteristics and, in the case of yeast-like fungi, the following tests were performed: urease reaction, phenol-oxidase, observation of capsule in India ink preparations, pseudohyphae and chlamydospore production on milk-tween 80 agar [18] and API 20C Aux® (bio Mérieux s.a., Marcy l’Etoile, France).

Results

Of the 5034 blood samples examined, 322 (6·39%) contained fungi. They were Cryptococcus neoformans in 199 samples (3·95%), H. capsulatum in 95 (1·89%), Candida spp. in 34 (0·67%), C. tropicalis in two, C. glabrata in one, C. krusei in one, C. guilliermondii in one, and Prototheca wickerhamii in four (0·07%). (Table 1). The average period and the range of time needed to identify the different etiological agents isolated from blood cultures were as follows: C. neoformans 6·3 (3–13) days, H. capsulatum 16·4 (11–24), Candida spp. 1·5 (1–3), P. wickerhamii 6·1 (4–7) days.

The percentage of positive blood cultures was higher in patients with histoplasmosis. The blood culture was the sole diagnostic tool in 17 patients from this group; in the
remaining patients, *H. capsulatum* was identified in other materials, especially biopsy samples and cutaneous scrapings. Most of the patients with cryptococcosis had positive findings on direct microscopic examination and in CSF cultures; however, in 20 of them, the diagnosis was only made by the isolation of *C. neoformans* in blood cultures.

Isolation of *Candida* spp. (predominantly *C. parapsilosis*) corresponded almost entirely to candidemias caused by catheter infections. Disseminated candidiasis was evident in patients with *C. tropicalis* infections. This fungal species was recovered from a 3-year-old, HIV-positive, female patient, with a history of recurrent respiratory infections, neutropenia of more than 15 days duration, refractory fever which was unresponsive to a variety of antibacterial drugs, and persistent fever once the neutrophil count rose. The child presented hepatosplenomegaly and urinary casts. Abdominal ultrasonography showed multiple hypoechoic images compatible with chronic disseminated candidiasis. The other case was a 38-year-old, HIV-positive, male patient, hospitalized in the intensive care unit with treatment-refractory sepsis, in whom *C. tropicalis* was recovered repeatedly from urine and blood cultures. Recovery of the remaining *Candida* spp., especially *C. albicans*, occurred in HIV-positive patients with various predisposing factors, such as venous catheterizations, broad-spectrum antibiotic therapy, colonization of digestive tract mucosa, prolonged hospitalization, etc.

*P. wickerhamii* was identified in four patients with advanced HIV disease. Numerous opportunistic infections had seriously deteriorated the general health of these patients and three of them died shortly after enrollment. The isolation of this achlorophyllous alga was obtained only once in each patient and no data from autopsy of these cases are available. The four isolations occurred during a 45-day time period in 1997 and have not repeated since then, which makes the interpretation of these findings difficult.

**Discussion**

This research was conducted in the medical mycology unit of an infectious disease hospital in Buenos Aires, a city of 12 million inhabitants and the largest urban center in Argentina. The hospital receives approximately half of the country’s total cases of advanced AIDS with infectious complications [19]. The majority of the patients come from low socio-economic conditions. The prevalence of disseminated cryptococcosis in advanced AIDS patients in our hospital varies between 10 and 13%, while the prevalence of histoplasmosis is between 3 and 5% [13]. The city of Buenos Aires and its surroundings are an endemic zone for histoplasmosis. More than 30% of apparently healthy adults have positive histoplasmin skin tests [2].

The use of blood cultures to diagnose HIV-related systemic mycoses, as well as those caused by serious immune deficiency, has increased notably in the last two decades [10].

Previous studies have demonstrated the sensitivity of this diagnostic tool, particularly lysis-centrifugation [15,16]. This blood culture technique has proven to be much more sensitive than other procedures, including Bact-Alert®, for the isolation of dimorphic fungi [7,15]. An earlier study demonstrated both the sensitivity and economy of the lysis-centrifugation method used for the present study [15].

It is well known that disseminated candidiasis is rarely seen in HIV-positive patients unless other predisposing factors exist [20]. The results presented in this study could be explained by the clinical and epidemiological characteristics of the patient population studied. The major portion of isolations corresponded to *C. neoformans* and *H. capsulatum* while the recovery of *Candida* spp. corresponded to transitory candidemias from infected catheters.

The present study demonstrates a higher frequency of positive blood cultures in patients with disseminated histoplasmosis than that observed in previous studies (89–7 vs. 64–7%). Furthermore, in 54% of the cases, the positive blood culture was the first diagnostic evidence of the disease and, in 17 patients (20%), blood culture was the only procedure which confirmed the diagnosis. In the latter cases, existing focal lesions were not easily accessible (hepatosplenomegaly and retroperitoneal adenopathies). This problem has been discussed in a previous work [21].

The percentage of positive blood cultures in patients with disseminated cryptococcosis was also higher than that reported in previous studies (76–3 vs. 67–8%), but was less pronounced than in histoplasmosis. The blood cultures which provided the first diagnostic evidence of the disease constituted less than one-fourth of the cases studied (48 patients). Many were patients with suspected cerebral masses, which precluded lumbar puncture; in 20 patients from this group, blood culture yielded the only evidence of systemic mycosis. This figure corresponds to 10% of the patients with AIDS-related cryptococcosis with no meningoecephalic compromise.

Protothecosis is an infection produced by an achlorophyllous alga which is rarely seen in humans. Eighty cases have been reported in the world literature. The clinical manifestations described are either dermoepidermal lesions or bursitis of the olecranon. Systemic protothecosis is observed sporadically and involves seriously immuno-compromised patients, very few of whom are HIV-positive [22–25]. As we pointed out their isolation in this study did not confirm disseminated protothecosis.
Once again, we have been able to prove the usefulness of this simple and inexpensive blood culture technique for diagnosing HIV-related, systemic mycoses. This substantiation is of particular interest to an institution such as ours, Hospital Muñiz, which works with limited economic resources and lacks advanced technologies, such as the polymerase chain reaction or other molecular biological procedures, which are routinely used in medical centers with bigger budgets.

References