Fulminant atypical Cryptococcus neoformans pneumonia confirmed by PLEX-ID.

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Abstract
Use of the PLEX-ID system can lead to a rapid molecular diagnosis in microbiology. To illustrate the clinical implications of this new diagnostic tool, we present the case of a 46-year-old patient admitted with severe respiratory failure and septic shock. Cryptococcal pneumonia was diagnosed by Fungi-Fluor™ staining of the bronchoalveolar lavage (BAL) and the patient tested positive for HIV. Unfortunately, he died 12h after admission despite intensive care support and treatment with broad-spectrum antibiotics, amphotericin B, and flucytosine. Retrospective use of the PLEX-ID on the BAL, bronchial aspirate, and blood yielded Cryptococcus neoformans in all fluids tested. Rapid molecular diagnosis with PLEX-ID, especially when performed on the blood of septic patients, may reduce the time to adequate treatment and limit the number of diagnostic procedures needed.

Reference

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Case Report

Fulminant atypical Cryptococcus neoformans pneumonia confirmed by PLEX-ID

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1. Introduction

The PLEX-ID system is a novel technology using multiplexed and broad-range PCR amplifications, coupled with electrospray ionization-mass spectrometry (ESI-MS) and computer triangulation to analyze the amplification products. It allows the identification of various pathogens within 4-6 h. This diagnostic tool should contribute to the earlier identification of pathogens, without the need to wait for culture. This may have a major impact on patient management and outcome, especially in immunocompromised patients, for whom empirical treatment cannot cover all possibilities.

2. Case report

A 46-year-old Mediterranean Caucasian man was seen in the emergency unit of our hospital with headache and dizziness for 15 days, without fever, impaired vision, or diploria. His history was notable for a 10-kg weight loss in the preceding 2 months, without night sweats. The patient reported no prior medical visits and denied illicit drug use or high-risk sexual behavior. His blood pressure (BP) was 134/97 mmHg, heart rate (HR) 116 beats/min, temperature 37.6 °C, respiratory rate 14/min, and oxygen saturation 98% on ambient air; weight was 78 kg. Examination revealed no oral thrush, no lymphadenopathy, normal cardio-pulmonary auscultation, a supple neck, and normal neurological findings. The white blood cell count was 7.2 × 10³/μL, hemoglobin 128 g/l, and platelet count 120 × 10⁹/μL. Creatinine was 69 μmol/l and C-reactive protein (CRP) was 49 mg/dl. A cerebral computed tomography (CT) scan showed thickening of the mucosa of the left sphenoid sinus consistent with sinusitis. The patient was discharged with a prescription for oral amoxicillin–clavulanic acid, 625 mg three times daily, and consultation with his primary care physician to investigate the weight loss.

Five days later the patient developed severe dyspnea requiring ambulance transport to our emergency unit. Upon arrival his oxygen saturation was 72% on ambient air. BP was 125/61 mmHg, HR was 125 beats/min, and temperature was 37.8 °C. His respiratory rate was 35/min. Laboratory results showed a white blood cell count of 17.1 × 10³/μL, hemoglobin level of 156 g/l, and a
platelet count of $207 \times 10^9$/L. Creatinine was 160 µmol/l and CRP was 112 mg/dl. Liver tests were normal and the lactate dehydrogenase was 663 U/L.

Immediate interventions included endotracheal intubation, empiric broad-spectrum antibacterial therapy with imipenem–cilastatin and clarithromycin, and pressure support with norepinephrine and saline. CT of the brain, chest, and abdomen revealed ground glass opacities obscuring the inferior lobes of both lungs. There was also hepatosplenomegaly and retroperitoneal, mesenteric, mediastinal, and hilar lymphadenopathy (Figure 1). The patient was admitted to the intensive care unit where bronchoalveolar lavage (BAL) showed $125 \times 10^6$/l cellular elements comprising 88% macrophages and 10% polymorphonuclear cells. Gram stain revealed no organisms. Fungi-Fluor™ stain for *Pneumocystis jiroveci* was negative, but revealed yeasts compatible with *Cryptococcus neoformans*. PLEX-ID was also performed 5 days after patient admission. This was performed with the BAL, bronchial aspirate, and blood, which were all positive for *C. neoformans*. Respiratory virus PCR was positive for parainfluenza 2. PCR for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were negative, as were serum and urinary antigens for *Streptococcus pneumoniae* and *Legionella* spp.

A positive rapid HIV test was confirmed by immunoblot; quantitative testing showed HIV-1 viremia at $7.3 \times 10^4$ copies/ml. In view of the HIV-positive status with *C. neoformans* pneumonia, our patient was categorized as HIV stage IV.

Immediately after the positive BAL for *C. neoformans*, about 6 h after patient admission, amphotericin B and flucytosine therapy was added. Despite aggressive management, the patient developed acute respiratory distress syndrome (ARDS) requiring increasing inotropic support. This was followed by multiple organ dysfunction with septic shock. He went into cardiorespiratory arrest approximately 12 h after admission and was successfully resuscitated, but went into arrest again and died approximately 13 h after admission. The BAL culture was positive for *C. neoformans* on day 2, bronchial aspirate on day 4, and blood cultures on day 7.

On day 5, for the purpose of evaluation, the PLEX-ID was performed on BAL, bronchial aspirate, and blood of this patient. All were positive for *C. neoformans*.

3. Discussion

The PLEX-ID system was originally developed with the support of the biodefense agencies in the USA. The idea was to combine several broad-range PCR amplifications coupled with ESI-MS detection of the amplicons and computed triangulation, to identify any pathogen (bacterial, viral, fungal) rapidly from specimens.1

Later, Abbott Molecular acquired this tool and developed it towards clinical microbiology, where its use has been focused on clinical samples.2 Because the test uses pattern recognition rather than a categorical readout, it is likely that ESI-MS will eventually allow the detection of multiple agents in the same assay of the same sample. While the method has not yet been reduced to a clinical assay, sample presentation and processing for other MS applications has proven highly cost-effective compared to conventional methods, saving both materials and labor costs as well as improving turnaround times.3 We anticipate that this modality will eventually take its place as a central element in laboratory diagnostics for patients with unknown infections. In this case, had such a test been applied to blood at the patient’s initial presentation, it may have prevented his death.

PLEX-ID detected *C. neoformans* in the BAL, the bronchial aspirate, and the blood culture bottles from this patient. Interestingly, the PLEX-ID also revealed the presence of *C. neoformans* directly from 1 ml of native blood in ethylenediaminetetraacetic acid (EDTA). We are unable to assess whether the fungus would have been detected 5 days earlier when the patient first presented. At that time there was no indication for exhaustive testing and we had no residual sample to test. We note that some public health agencies now recommend universal rapid HIV testing for patients in this age group, regardless of the stated risk factors.4

Had this been standard procedure, the outcome for this patient might have been different.

Cryptococcosis is an opportunistic fungal infection with a ubiquitous distribution mainly affecting AIDS patients, although other immunocompromised and some immunocompetent patients have also been infected. In a large series of 171 patients in Brazil, Rozenbaum and Goncalves found disseminated disease in the vast majority (92%) of patients, whereas localized pulmonary involvement only occurred in 14 patients (8%).5

In our case, the clinical picture of septic shock with ARDS in an immunocompromised patient was atypical enough to be solely attributed to a disseminated cryptococcal infection, based on the direct examination of BAL and later confirmed by blood cultures. This unusual case highlights the potential of using a broad-scale molecular diagnostic method to obtain a rapid microbiological diagnosis in complicated clinical situations, and reinforces the need to have a higher index of suspicion for HIV and its complications in patients with an unknown HIV status.

Conflict of interest: All authors report no conflicts of interest.

References