Co-infection by Cryptococcus neoformans and Mycobacterium avium intracellulare in AIDS
Clinical and epidemiological aspects

Keikawus Arasteh¹, Christiane Cordes¹, Ursula Futh², Gernot Grosse³, Ekkehart Dietz⁴ & Friedrich Staib⁵
Auguste Viktoria Hospital (AVK), D-12157 Berlin, Germany; ¹Department of Gastroenterology and Infectiology, ²Department of Laboratory Diagnostics and ³Department of Pathology; ⁴Institute of Social Medicine, Epidemiology Unit, Free University Berlin, Germany; ⁵Former Chief, Mycology Unit, Robert Koch Institute, Berlin, Germany

Received 7 July 1997; accepted 27 January 1998

Abstract
In the observation of various opportunistic pathogens in HIV-positive persons, co-infection by Cryptococcus neoformans together with Mycobacterium avium intracellulare was found if there was a CD4 lymphocyte count as low as 3–20/µl. In 1540 HIV-positive patients under treatment at a Berlin hospital (Auguste–Viktoria–Krankenhaus) during 1985–1994, all AIDS-relevant diseases were examined in a multivariate analysis as variables of influence on the manifestation of a systemic Mycobacterium avium complex (MAC) infection. The analysis involved data on 36 cases of cryptococcosis and 202 cases with a typical clinical course in whom MAC had been detected at sterile body sites. As significant and independent factors of influence, the following were identified: C. neoformans infection, wasting syndrome, lower age, low CD4 lymphocyte count and preceding Pneumocystis carinii pneumonia (PcP) prophylaxis. Cryptococcosis ranged first with an odds ratio of 2.75. The concomitant manifestation of cryptococcosis and systemic MAC infection in six patients is shown. Because both opportunists, C. neoformans and avian mycobacteria, may have their common habitat in droppings of defined species of pet birds, a common source of infection deserves further clinical and epidemiological attention.

Key words: Avian mycobacteriosis, cryptococcosis, co-infection, droppings of pet birds.

Introduction
In recent studies on opportunistic infections in AIDS patients involving 36 subjects who had become affected by cryptococcosis, it became obvious that co-infection by Cryptococcus neoformans and the Mycobacterium avium complex (MAC) was found only if there were uniformly low CD4 lymphocyte counts (between 3 and 30/µl) [1]. It is known that with a severe decrease of the CD4 lymphocyte count, not only the incidence but also cases with a life-threatening, disseminated course of the MAC infection (DMAC) will increase [2, 3]. C. neoformans var. neoformans, the causative agent of cryptococcosis in AIDS patients, may find its habitat in droppings of feral pigeons not exposed to weather conditions, and in those from pet birds [4, 5]. Pet birds, like AIDS patients, may fall sick from mycobacteriosis caused by MAC, e.g. M. avium intracellulare (frequently with a massive involvement of the digestive tract) [6]. Therefore, droppings from pet birds have to be considered in epidemiological studies of these two different opportunistic pathogens. Recent results of veterinary-pathological studies on the occurrence of mycobacteriosis among pet birds in Berlin [6] have raised the question of the significance of a common source of co-infection by the two opportunistic pathogens, C. neoformans and MAC, in AIDS patients.
Patients and methods

From 1985 until the end of 1994, 1540 HIV-positive persons were treated once or repeatedly at the Dept. of Gastroenterology and Infectiology of the Auguste Viktoria Hospital, Berlin. The patient population consisted of 1453 males (94.4%) and 87 females (5.6%). At the time of the first diagnosis of AIDS (Fig. 1), their average age was 37.95 years and the median CD4 lymphocyte count was about 42 cells/µl (SD = 116.42). The average Karnofsky index of the patients was 57.21% (SD = 24.24). 202 cases with typical symptoms of MAC infection and detection of MAC by blood culture were analyzed by Cox regression models regarding the relationship to 36 cases of cryptococcosis in the patient population [7, 8]. The assessment of disseminated MAC infection is based on the detection of MAC by blood culture according to Nightingale [9].

Clinical-chemical, pathological and microbiological examinations

The various laboratory examinations for the diagnosis of opportunistic infections were performed by standard methods [10, 11].

Mycological examinations

The procedures of clinical and environmental examinations for C. neoformans have been described elsewhere [10, 12].

Procedures used for the detection and identification of mycobacteria from clinical specimens

As recommended by the Centers for Disease Control and Prevention (CDC), Atlanta, USA [13], the specimens from non-sterile body sites were decontaminated with N-acetyl-L-cysteine (NALC)–NAOH and concentrated. From the sediment, the following inocula with different culture media (agar-based (Stonebrink), egg-based (Löwenstein–Jensen), and liquid (Bactec 12 B)) were used [14, 15], and smears prepared for examination with the Kinyoun stain. Blood specimens (NH₄ Heparin vials) were directly transferred into the Bactec 13 A medium (Becton Dickinson Diagnostic Instrument Systems, USA) [16]. When acid-fast rod-like bacteria were present in the Bactec 460 TB vials with a GI > 999, identification with a commercially available nucleic-acid probe assay (GEN-Probe, USA) was performed.

CD4 lymphocyte count

CD4 lymphocyte counting (normal count 650–1250/µl) was performed with the aid of an FACScan flow cytometer and reagents from Becton Dickinson Immunocytometry Systems, San Jose, California 955113 USA (Supplier: Becton Dickinson GmbH, Tullastr. 8-12, D-69126 Heidelberg, Germany [1,10].

Results

In the stage of the first AIDS diagnosis, the frequency of the various AIDS-defining diseases are shown in Fig. 1. On the basis of these 17 diagnoses, in addition to the MAC infection, all AIDS-relevant diseases were examined in a multivariate analysis as variables of influence on the manifestation of a systemic MAC infection. The analysis involved data on 202 cases with a typical clinical course, in whom MAC had been detected at sterile body sites. As significant and independent factors of influence, the following were identified: C. neoformans infection, wasting-syndrome, lower age, low CD4 cell count and preceding PcP prophylaxis. Cryptococcosis ranged first, with an odds ratio of 2.75 (Table 1).

In Table 2, the concomitant manifestation of cryptococcosis and DMAC in six patients is shown. An exposure to pet birds could be proven in only two out of the six patients (LM and LHP); in the other four patients, this question could not be clarified. Unfortunately, in the two cases of pet bird exposure, subsequent examinations of birds and cages for C. neoformans and avian mycobacteria could not be performed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcosis</td>
<td>2.75</td>
<td>1.195–6.327</td>
<td>0.0173</td>
</tr>
<tr>
<td>Wasting syndrome</td>
<td>2.49</td>
<td>1.710–3.634</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.97/year</td>
<td>0.952–0.996</td>
<td>0.02</td>
</tr>
<tr>
<td>CD4 cell count</td>
<td>2.21</td>
<td>1.516–3.217</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PcP prophylaxis</td>
<td>1.61</td>
<td>1.089–2.399</td>
<td>0.0172</td>
</tr>
</tbody>
</table>

DMAC = disseminated Mycobacterium avium complex infection.
PcP = Pneumocystis carinii pneumonia prophylaxis (pentacarinate, cotrimoxazole).