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AIDS-Associated Cryptococcal Meningitis in Rwanda (1983–1992): Epidemiologic and Diagnostic Features

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Objectives: to document the trend of AIDS-associated *Cryptococcus neoformans* meningitis (CM) in Kigali, Rwanda, during 1983–1992, and to highlight some diagnostic and epidemiological features of the disease.
Methods: during the study period, 3476 cerebrospinal fluid (CSF) specimens from 2824 adults (1578 men, 1246 women) were analysed in the Laboratory of Microbiology at the Centre Hospitalier de Kigali, Rwanda, Central Africa, using direct examination, culture and detection of the cryptococcal antigen (CrAg) in the CSF.
Results: CM was diagnosed among 549 (19%) patients (347 men, 202 women) and was by far the leading cause of meningitis before *Neisseria meningitidis* (n=115), *Streptococcus pneumoniae* (n=68), *Mycobacterium tuberculosis* (n=26), *E. coli*, *Klebsiella pneumoniae*, non-typhoid *Salmonella* (n=15) and streptococci (n=4). The number of CM increased from one case in 1983 to 130 new cases in 1992. All 293 tested CM patients had HIV-1 antibodies. The male/female ratio declined from 3.31 during 1983–1987 to 1.58 during 1988–1992. CM showed a seasonal fluctuation, the highest number of infections being observed during the long rainy season. The sensitivity and specificity of the latex test for diagnosing CM was 98% and 99%, respectively. *Cryptococcus neoformans* var. *gattii* was cultured from eight (1.6%) of the 499 culture positive patients.
Conclusion: CM is an important opportunistic infection among AIDS patients in Central Africa. It remains a problematic diagnosis in areas with limited diagnostic facilities.

Introduction

Cryptococcosis is a worldwide fungal infection caused by the yeast *Cryptococcus neoformans*. There are two varieties, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* which differ in their geographical distribution, morphology, ecology biochemical characteristics and serotype distribution. *Cryptococcus neoformans* var. *neoformans* occurs worldwide whereas the variety *gattii* is limited to subtropical and tropical areas (South California, Australia, Asia, South America, Africa). Before the advent of the acquired immunodeficiency syndrome (AIDS) cryptococcosis was a rare disease, mainly diagnosed among persons with impaired cell-mediated immunity. At the present time, it is one of the most frequently diagnosed opportunistic infections among AIDS patients in Central Africa. Extensive reviews on AIDS associated cryptococcosis have been published recently.[1–3]

In the Democratic Republic of Congo, the former Zaire, only 47 clinical isolates of *C. neoformans* were reported between 1951–1985.[4] In Rwanda and Burundi, despite sufficient laboratory infrastructure, CM was not observed before 1983 when infection with the human immunodeficiency virus (HIV) was diagnosed for the first time.[5,6] *Cryptococcus neoformans* var. *neoformans* is widespread in the environment of Central Africa.[7–9] *Eucalyptus camaldulensis* trees are the natural habitat of the variety *gattii* in Australia.[10] Although these trees are ubiquitous in Rwanda the yeast could not be isolated from this source.[9]

A national HIV serosurveillance carried out in 1986, showed that 30% of the Rwandan adult urban population aged between 26 and 40 years, and 10% of the children aged between 0 and 5 years were infected with the human immunodeficiency virus (HIV-1). Overall, women were more frequently infected with HIV-1 than men (20% vs. 16%).[11] The prevalence of HIV-1 among antenatal care women in Kigali, reflecting the prevalence in the general urban population, varied between 32% in 1986–1987 and 34% in 1992–1993.[12,13] During 1983–1991 a nearly 10-fold increase of active tuberculosis was observed for which HIV-1 infection was mainly accountable.[14]

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Patients and Methods

Patients

During 1983–1992, adults who attended the Outpatient Department of Internal Medicine of the Centre Hospitalier de Kigali (CHK), Kigali, with symptoms or signs of meningitis, severe headache, fever or altered consciousness underwent systematically a lumbar puncture. The CHK is a general reference hospital with about 450 beds and until 1992 was the only hospital in Kigali. Most patients were living in the city or in overcrowded suburbs. Kigali (population ± 350 000 in 1992) stands around 1800 m above sea level and has a moderate tropical climate with two rainy (February–May/September–November) and two dry (December–January/June–August) seasons. The mean temperature is ± 21°C and is fairly stable throughout the whole year.

Methods

CSF was collected in a sterile disposable plastic tube and centrifuged at 1400 × *g* for 10 min. After transfering the supernatant into a sterile disposable vial, which was stored at −20°C, following diagnostic procedures were carried out on the sediment: direct examination with and without India ink, Gram staining when the specimens were cloudy, culture on blood agar, chocolate agar, thioglycollate medium, trypticase soy broth (TSB), Sabouraud agar at 25°C and 37°C and Löwenstein medium. The Sabouraud and Löwenstein media were incubated for 6 weeks, the thioglycollate and TSB media for 7 days. The blood and chocolate agars were incubated in a candle jar at 37°C for 3 days. Diagnostic methods remained unchanged over the years except that a latex agglutination test for the detection of the CrAg was performed on the CSF supernatant (Latex Crypto Antigen System, Immuno-Mycologics, Norman, OK, U.S.A.) and was introduced in 1987. Specimens were pretreated with pronase to avoid false-positive results due to interfering factors.[15,16] Isolates and an aliquot of the supernatants were submitted to the Laboratory of Mycology, Institute of Tropical Medicine, Antwerp, Belgium, and, in 1992, to the Laboratory of Microbiology of the University of Leuven, Belgium, for confirmation and titration of the CrAg. In Kigali, the morphology of the yeasts was assessed and the isolates were tested for the presence of an urease. D-proline assimilation was used for the identification of the two varieties. All pathogens were identified according to recommended methods.[17] At initial diagnosis, specimens were collected before starting antifungal treatment. The diagnosis of CM was considered when CSF culture was positive or, in the absence of a positive culture, when fresh examination and CrAg test were positive. A positive CrAg test or fresh examination alone was considered as indicating CM if, besides clinical evidence of meningitis or suggestive neurological signs and symptoms, *C. neoformans* was demonstrated in other body tissues or fluids (blood, urine, pleural exudate, lung or skin biopsy) by culture or histological examination. Results of non-specific laboratory tests such as determination of CSF glucose, protein and cell count as well as the results of clinical examination are not reported in the present paper.

Statistical analysis was performed using the statistical package Epi-Info (Version 6, Centers for Disease Control and Prevention, Atlanta, Georgia, U.S.A). For comparison of proportions the Chi-square test was used. The odds ratio (OR) was calculated for measuring associations.

Results

Patients characteristics

CM was more frequently diagnosed among men than women (22% or 347/1578 vs. 16% or 202/1246; $P<0.001$; OR: 1.46), the overall male/female ratio being 1.7. However, the male/female ratio declined from 3.31 (53/16) during 1983–1987 to 1.58 (294/186) during 1988–1992. The mean ages of men and women was 36.3 ± 7.9 vs. 33.2 ± 7.7 year respectively ($P<0.001$) and remained unchanged over time. All 293 tested CM patients, including those with *C. neoformans* var. *gattii* and mixed *Mycobacterium tuberculosis* infection, had HIV-1 antibodies vs. 60% of the patients with pneumococcal or *M. tuberculosis* infection. Interestingly, the male/female ratio for tuberculous (TB) meningitis was 1.17 (14/12), the two periods combined; the ratio for pneumococcal meningitis was 1.5 (6/4) and 1.07 (30/28) during the successive periods.

New C. neoformans infections

During 1983–1992, 3476 CSF specimens from 2824 new patients (1578 men, 1246 women) were submitted to the laboratory. The annual number of new specimens, obtained from both genders, increased from 23 in 1983 to 761 in 1992. The first CM patient was identified in December 1983, the second one in February 1984. After a break of 12 months, a third patient was diagnosed in March 1985, which revealed the start of the CM outbreak. The overall annual prevalence of CM increased from 4% to 22% during 1983–1987 (X^2_{trend}, $P=0.01$) and varied between 17% and 24% during 1988–1992, no trend being observed during the latter period. Overall, 549 (19%) patients (347 men, 202 women) were diagnosed

Table I. CSF sampling rates and relative frequency of *C. neoformans* meningitis in Kigali (1983–1992).

	Men		Women	
	CSF*	CM†	CSF*	CM†
Year	No.	No. (%)	No.	No. (%)
1983	15	1 (7)	8	0 (0)
1984	15	1 (7)	16	0 (0)
1985	44	10 (23)	28	4 (14)
1986	82	16 (20)	36	3 (8)
1987	103	25 (24)	52	9 (17)
1988	141	30 (22)	117	14 (11)
1989	268	57 (21)	188	27 (14)
1990	253	67 (25)	216	47 (22)
1991	231	61 (26)	250	47 (19)
1992	426	79 (18)	335	51 (13)
Total	1578	347 (22)	1246	202 (16)

*CSF, Cerebrospinal fluid. †CM, Cryptococcal meningitis.

Table II. Results of CSF examination for *Cryptococcus neoformans* in Kigali (1983–1992).

Diagnostic features at initial diagnosis		
Culture positive	*n=490*	*CrAg**
Microscopy		
positive	384	258/264
negative	99	64/64
not done	7	not done
Culture negative	*n= 59*	
Microscopy		
positive	22†	13/13‡
negative	37§	37/37

*Number of positive/tested CSF.
Follow-up:
†9 Patients were not tested for CrAg: 6 were lost and 3 were culture positive.
‡3 Patients were culture positive.
§3 Patients were culture positive, 2 patients showed a positive direct examination.

as having CM. The rate of CSF sampling and relative frequency of CM among both genders over time is represented in Table I.

Cryptococcus neoformans was isolated from 91% (499/549) of the patients, from whom eight (1.6%) (five men, three women) harboured *C. neoformans* var. *gattii*. In the absence of a positive culture, the diagnosis of CM was based on the direct demonstration of encapsulated yeasts with a positive CrAg test (n=12 or 2%), only a positive CrAg test (n=32 or 6%) or only a positive direct examination (n = 6 or 1%). The results of culture, microscopic examination and detection of the CSF CrAg at initial diagnosis and follow-up are summarized in Table II. Two patients had a mixed non-typhoid *Salmonella*, and two others a mixed *M. tuberculosis* meningitis. Other pathogens isolated from CSF were: *N. meningitidis* (n=115), *S. pneumoniae* (n=68), *M. tuberculosis* (n=26, including two mixed infections with *C. neoformans*), enterobacteriaceae (n=15) and streptococci (n = 4). The frequency and aetiology of adult meningitis over time is represented in Figure 1.

Eighty-six per cent (99/115) of the meningococcal infections were diagnosed during 1991–1992 when a new outbreak of serotype A meningitis among children and adults hit the country. Pneumococcal and TB meningitis increased over time, 85% (58/68) and 81% (21/26) of the respective cases being identified during 1988–1992. The annual number of these cases was too small for trend analysis. During 1983–1987 and 1988–1992 the number of culture proven TB meningitis was five and 21 respectively; the number of pneumococcal meningitis, 10 and 58. The relative frequency of TB meningitis was 1.3% and 0.9% and of pneumococcal meningitis 2.5% and 2.4% during the successive periods. Since a higher number of CSF specimens was received during 1988–1992, the increasing number of infections was not translated into a higher relative frequency.

CM showed a clear seasonal picture among men but not among women. In the former group, the relative frequency of new cases, expressed as the proportion of CM among the number of new CSF specimens received during the specific period of the year, was significantly higher during January–June than July–December (27% or 191/718 vs. 18% or 156/860; OR: 1.64; P<0.001). Similarly, 55% (191/347) of the male CM cases were diagnosed in the first half of the year whereas 46% (718/860) of the CSF specimens were received. In contrast, 40% (80/202) of the female CM cases were diagnosed in the first part of the year, with 43% (530/1246) of the annual number of CSF samples. The higher number of male CM during the first half of the year was observed during each individual year. This period coincides with the end of the short dry season (January), the main rainy season (February–May) and the beginning of the dry season (June). The relative frequency of CM according to gender and the period of the year is represented in Figure 2.

Laboratory findings

Results of culture, direct examination and CrAg detection on the initial CSF specimen. Cryptococcus neoformans was isolated from 91% (499/549) of the CM patients, 89% (490/549) had a positive culture at the first visit. Culture, direct microscopic examination and detection of the CrAg was performed on 71% (2008/2824) of the initial samples.

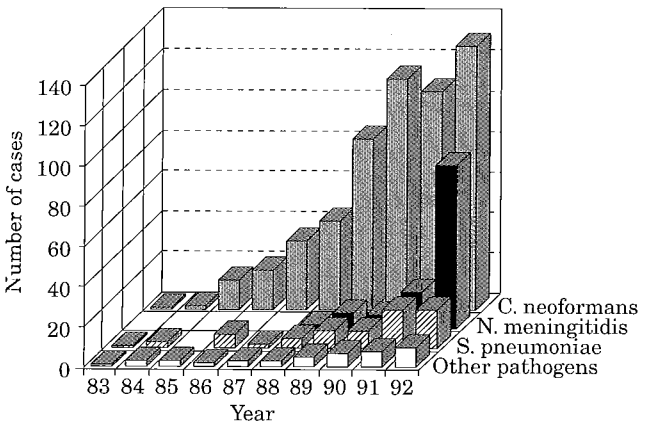

Figure 1. Frequency and aetiology of adult meningitis (1983–1992).

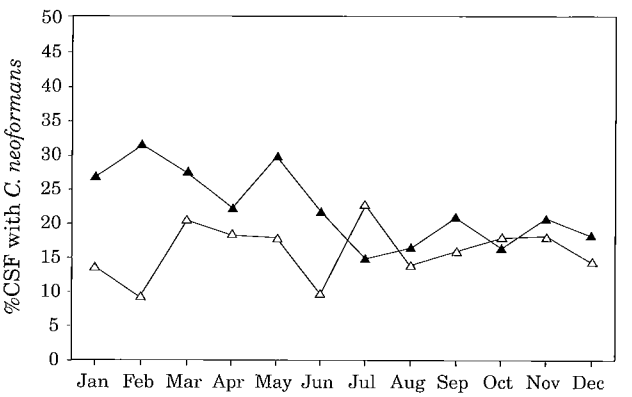

Figure 2. Relative frequency of *C. Neoformas* meningitis according to the period of the year. Key: ▲, men; △, women.

Considering a positive culture or CrAg test as the standard of diagnosis, the sensitivity of direct examination at the initial visit was 73% (277/378) with a 100% (1630/1630) specificity. On the other hand, considering a positive culture or direct examination as the standard, the sensitivity of the CrAg test was 98% (335/341) and the specificity 97% (1621/1667). However, 37 of the 46 'false-positive' CrAg tests were considered as true cryptococcal infections, based on the demonstration of *C. neoformans* in other body sites such as blood, urine, pleural exudate, lung or skin tissue by culture or histologic examination, whereas nine CSF specimens, showing CrAg titres of 1:1, were considered as false-positive since patients had no clinical or microbiologic evidence of cryptococcal infection during follow-up.

Follow-up specimens were available from four of the six patients who were CrAg negative but culture and direct examination positive. One patient had a second CSF sample after 1 month, which was CrAg positive. Another patient had four successive CSF specimens, each with 1 month interval. A negative CrAg test with a positive microscopic examination persisted all over the time. The third patient showed a positive culture and microscopic examination during a single follow-up visit but the CrAg remained negative. The fourth patient, from whom *C. neoformans* var. *gattii* was isolated, had two follow-up CSF specimens. Cultures and microscopic examinations remained positive but the CrAg test negative. Interestingly, the four other tested CSF specimens positive for *C. neoformans* var. *gattii* showed CrAg titres of 1: 2, 1:64, 1:128 and 1:512 respectively, the remaining three *gattii* patients not being tested.

Performance of culture media for isolation of C. neoformans. The performance of the different media used for primary growth of *C. neoformans* was assessed using data obtained from 2012 specimens plated onto all media. A total of 352 isolates were obtained. The isolation rates were: Sabouraud agar 92%, thioglycollate broth 82% and blood agar 80%. Seven (2%) isolates grew exclusively on blood agar, seven (2%) on thioglycollate broth and 23 (7%) on Sabouraud agar. Growth on Sabouraud agar at 25°C and 37°C was strictly similar: 17 and 20 isolates were exclusively cultured at the respective temperatures (X^2 paired samples: 0.25; $P>0.5$). During follow-up the isolation rates on the different media remained similar.

Predictive value of the CrAg titre for culture outcome. At initial diagnosis, 53% (9/17), 80% (82/103) and 95% (218/229) of the CSF specimens showing CrAg titres of <1:4, 1:4–128 and ≥1:256, respectively, had a positive culture of *C. neoformans*, 29 CSF samples were not titrated. Follow-up CSF specimens were taken with 1 month interval from the surviving patients, all receiving antifungal treatment. The CrAg latex test was performed on a total of 335 follow-up specimens: none of eight, 34% (55/164) and 74% (120/163) of the specimens with titres in the above mentioned categories were culture positive.

Discussion

The present study shows the increasing burden of CM in Kigali, Central Africa, during 1983–1992. Although the number of CSF specimens submitted to the laboratory may result in part from the growing awareness for signs and symptoms suggesting CM, it most probably reflects the increase of central nervous system disorders related to HIV infection. The concomitant increase of both TB and pneumococcal meningitis during the last years of the study may illustrate this hypothesis.

To the best of our knowledge, this is the first report showing a seasonal variation of CM. Since CM was a sporadic disease in Central Africa before the AIDS epidemic, and not diagnosed in Rwanda before 1983, this

phenomenon could not be recognized earlier. In the Kigali area, central Rwanda, high humidity prevails from February until May. This may contribute to a higher proliferation of *C. neoformans* in the environment and, consequently, to a higher risk of exposure.

Cryptococcal infections are more frequently reported from men than women and rarely from children[2]. A protective effect of oestrogens or a reduced environmental exposure have been postulated to explain this difference[3]. In Kigali, *C. neoformans* was not isolated from nearly 2000 new paediatric CSF specimens submitted to the laboratory during the period under review, although at least 10% of the children were HIV infected, whereas the relative and absolute frequency of CM was higher among men than women. Better access to health care, temporary migration, higher exposure to the pathogen and concomitant epidemics of meningitis due to other pathogens, could be responsible for a systematic bias in favour of a specific gender, period of the year or the combination of both. During the observation period, the overall male/female ratio among patients attending the medical department of the CHK was 60/40 or 1.5. The male/female ratio for TB and pneumococcal meningitis was 1.17 and 1.13 respectively whereas the reported male/female ratio of active tuberculosis was 1.51 in 1991.[14] Seasonal labour or temporary migration to other parts of the country or abroad do not exist in Rwanda. In 1988, after several years of complete absence in the country, meningococcal disease re-emerged in Kigali and contributed to the higher number of CSF samples submitted to the laboratory, especially during the last year. This reduced the relative frequency of CM among men and women. Since meningococcal disease was homogeneously spread over the year it had no impact on the seasonal pattern of CM. There is no reason to believe that men were more exposed to the pathogen than women. In the neighbouring Burundi, the yeast was frequently found in the houses of CM patients, which are shared by both genders.[8]

In Rwanda, women are more likely exposed to potential sources of *C. neoformans* outside the house (such as dust, soil, rotting vegetables) than men since they work in the garden and fields. Considering the higher male/female sex ratio in CM than in TB or pneumococcal meningitis, the delayed diagnosis of the first female CM (2 years after the first male case) and the higher frequency of male CM, we may assume that women were less susceptible to cryptococcal infection than men. The change in sex ratio over time in favour of women may tentatively be explained by the fact that women, having a better natural resistance against cryptococcal infection, require a more severe immunodeficiency than men to develop CM. The distribution of such individuals increased progressively over time, bringing the number of susceptible women to about the same level as in the male population. If women are less susceptible to cryptococcal infection than men a seasonal fluctuation is less likely to be observed in the former group.

The diagnosis of CM depends on direct demonstration of encapsulated cells in the CSF, culture of *C. neoformans*, detection of the CrAg in the CSF or histologic demonstration of encapsulated yeasts in the brain or meninges. The reported sensitivities of fresh examination and CrAg detection in the CSF vary around 50–90% and 90–95%, respectively, whereas the sensitivity of CSF culture is only 75–90%.[1,2,18,19] The relative insensitivity of each test means that none can be used solely as the gold standard for CM. A strong variability exists in the sensitivity of different commercial sources of CrAg detection kits.[20,21] Therefore, all CrAg titres obtained in CSF from untreated HIV-infected patients presenting with symptoms of meningitis, severe headache, fever or altered consciousness should be considered as indicating active cryptococcal infection. In the present study, the positive predictive value of a positive CrAg test was 97.6% (372/381) with only 0.5% (9/1630) false-positive results. Infection with *Trichosporon beigelii*, a possible cause of false-positive latex agglutination tests, was not observed.

Molecular identification techniques, combining high sensitivity and specificity, will undoubtedly become a standard practice for the diagnosis of cryptococcal infections. However, these techniques will remain beyond the scope of a clinical laboratory in a developing country, where the burden of cryptococcal infections is unfortunately the highest.

As a consequence of the civil war in 1994, a high proportion of the population succumbed or disappeared and new people from abroad settled in the city of Kigali. Future research cannot, therefore, be linked to the population observed in the present study.

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