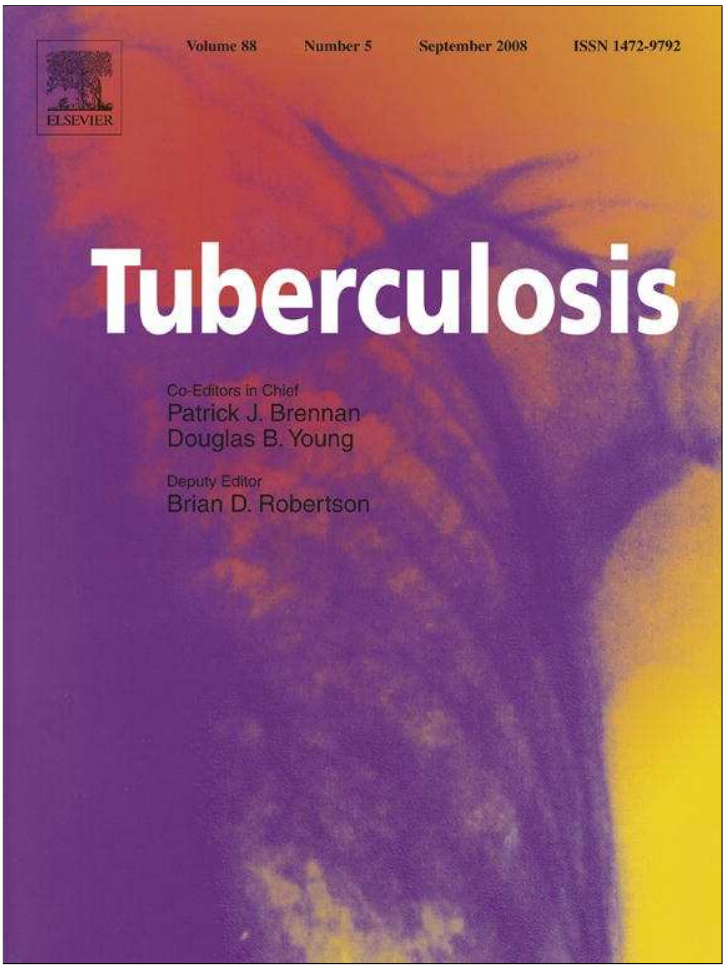

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Pulmonary tuberculosis: Virulence of *Mycobacterium africanum* and relevance in HIV co-infection

Christian G. Meyer [a,*,h], Genevieve Scarisbrick [b,h], Stefan Niemann [c,h], Edmund N.L. Browne [d], Margaret Amanua Chinbuah [e], John Gyapong [e], Ivy Osei [e], Ellis Owusu-Dabo [d,f], Tanja Kubica [c], Sabine Rüsch-Gerdes [c], Thorsten Thye [a,g], Rolf D. Horstmann [a]

[a] *Department of Molecular Medicine, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
[b] *Department of Radiology, Komfo Anokye Teaching Hospital, Kumasi, Ghana*
[c] *National Reference Center for Mycobacteria, Research Center Borstel, Borstel, Germany*
[d] *Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[e] *Health Research Unit, Ghana Health Service, Accra, Ghana*
[f] *Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana*
[g] *Institute of Medical Biometry and Statistics, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany*

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Summary
Although *Mycobacterium africanum* is being isolated in a significant proportion of cases of pulmonary tuberculosis in West Africa, its pathogenic potential remains a matter of discussion. Recent reports leave the question of whether *M. africanum* causes more severe pathology than *M. tuberculosis* or resembles opportunistic pathogens and might gain importance in the course of the HIV pandemic.

Patients with pulmonary tuberculosis associated with *M. africanum* ($n = 556$) and *M. tuberculosis* ($n = 1350$) were studied in Ghana, West Africa, and compared regarding self-reported signs and symptoms, chest radiography, HIV status, mycobacterial drug resistance and mycobacterial clustering as determined by spoligotyping and IS*6110* fingerprints.

The rate of *M. africanum* infections was similar in HIV-positive (27%) and HIV-negative (30%) patients. *M. africanum* clustered less than *M. tuberculosis* (21% vs 79%; OR, 0.38; 95% CI, 0.3–0.5; $p < 0.001$) corresponding to its lower prevalence (29% vs 70%). Clinically and radiographically, no

* Corresponding author. Department of Molecular Medicine, Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Str. 74, 20359 Hamburg, Germany. Tel.: +49 40 4281 8501; fax: +49 40 4281 8512.
E-mail address: c.g.meyer@bni-hamburg.de (C.G. Meyer).
[h] Dr. Meyer, Dr. Scarisbrick and Dr. Niemann equally contributed to this work.

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significant differences were found except that *M. africanum* caused lower-lobe disease less frequently than *M. tuberculosis* (OR, 0.39; 95% CI, 0.2—0.7; $p_c = 0.01$), whereby this association applied to HIV-negative patients only. No difference in virulence, as assessed by the severity of radiological presentation, was found when the two *M. africanum* subtypes West African 1 and West African 2 were compared.

In the population studied, *M. africanum* closely resembled *M. tuberculosis* in pathology and cannot be considered an opportunistic pathogen.

Introduction

Mycobacterium africanum causes up to 50% of cases of human pulmonary tuberculosis (TB) in West Africa.[1—3] It can be distinguished from *M. tuberculosis* by a number of biochemical characteristics, which show some similarities to those of *M. bovis*, a species primarily affecting cattle. More recently, the use of genetic analyses has indicated that: (i) East African isolates of *M. africanum* are closely related to *M. tuberculosis* and should not be termed *M. africanum* therefore; and (ii) West African isolates can be separated into two clades with clade West African 2 resembling *M. bovis* more closely than West African 1.[4]

There is considerable uncertainty about the virulence of *M. africanum*. In a recent report from The Gambia, it was described that *M. africanum* was at least as virulent as *M. tuberculosis* and tended to cause more extensive radiographic changes.[5] In contrast, an increased rate of *M. africanum* infections among HIV-positive patients has also been reported, suggesting that *M. africanum* may be less virulent than *M. tuberculosis* and may resemble opportunistic pathogens and, therefore, may gain importance in populations with high HIV endemicities.[6]

Here we present findings from a study in Ghana, West Africa, where we compared 556 patients with pulmonary TB associated with *M. africanum* to 1350 cases associated with *M. tuberculosis* regarding clinical and radiographic findings, HIV infection status and infection dynamics as estimated by a cluster analysis of mycobacterial isolates.

Patients and methods

Study population

With the primary objective to study human and mycobacterial genetic factors relevant to disease development, consecutive patients with pulmonary TB were from September 2001 to July 2004 recruited at Korle Bu Teaching Hospital, Accra, Komfo Anokye Teaching Hospital, Kumasi, 15 additional hospitals or policlinics in Accra and Kumasi and at the hospitals of Obuasi, Agona, Mampong, Agogo, Konongo and Nkawie (Ashanti Region), Nkawkaw and Atibie (Eastern Region) and Assin Fosu and Dunkwa (Central Region).

Characterization of patients included: (i) documentation on standardized structured questionnaires of the medical history including self-reported duration of cough, occurrence of dyspnea, chest pain, night sweats, fever, hemoptysis, and weight loss; (ii) two examinations of non-induced sputum specimens for acid-fast bacilli; (iii) determination of HIV serology and confirmation of positive results by an alternative test; (iv) culturing and subsequent genotyping of mycobacterial species; (v) an assessment of mycobacterial drug resistance; and (vi) a standard posterior—anterior chest radiography. Extrapulmonary manifestations of TB were not recorded.

Inclusion criteria were two sputum smears positive for acid-fast bacilli, no history of previous TB or antimycobacterial treatment and an age between 6 and 60 years. As the primary objective of the study included host genetics, exclusion criteria were, in addition to incomplete information provided on the questionnaire, evidence of alcoholism or drug addiction, concomitant diabetes or other apparent generalized disease.

Of 2335 patients primarily enrolled, mycobacterial cultures, clinical information and radiological data were available from 1921, 1906 and 1600 individuals, respectively. All clinical, radiological and bacteriological data were available from a total of 1597 patients, whereby, because of their low frequency ($n = 10$), *M. bovis* cases were not analysed further. Biases due to missing parameters (mycobacterial culture, clinical information, X-ray film) in the various groups (HIV status, mycobacterial species) were, as far as determinable, not present in comparisons between patients included and patients excluded. This applied to age, gender, ethnicity and the various recruitment sites in Accra and Kumasi. Based on these comparisons, the study group may be considered largely representative for the area surveyed.

The study protocol had been approved by the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University, Kumasi, and the Ethics Committee of the Ghana Health Services, Accra. Patients were treated free of charge according to the ''Directly Observed Treatment Short-Course'' (DOTS) strategy organized by the National Tuberculosis Programme. Samples were taken only after a detailed explanation of the study aims and written or thumb-printed consent obtained for participation including HIV testing. Disclosure of HIV test results was dependent on the documented willingness of participants to be informed and included referral to counseling and treatment provided by the Ghanaian AIDS Control Programme.

Laboratory tests

Sputum smears were stained by the modified Ziehl—Neelsen stain at recruitment sites and subsequently confirmed in an independent sample at the Kumasi Centre for Collaborative

Research in Tropical Medicine (KCCR) in Kumasi. Smear positivity was not quantified. *Mycobacterium* isolates were cultured on solid Löwenstein–Jensen (LJ) media and read weekly for growth at KCCR. Cultures were then shipped to the German National Reference Centre for Mycobacteria (Borstel, Germany) for analyses of biochemial, molecular and growth patterns, including drug sensitivity testing of first-line drugs (proportion method on LJ media).

For HIV-1/-2 testing, a capillary test system (Capillus, Trinity Biotech, Bray, Co Wicklow, Ireland) was applied. According to local standards, HIV positivity was confirmed by the Organon Teknika Vironostika HIV-1/-2 EIA (Organon Teknika, Turnhout, Belgium). The confirmation rate was 100%.

Strain differentiation and cluster analysis

Mycobacterial strains were analysed by spoligotyping and IS*6110* fingerprinting as described previously.[7,8] Differentiation of *M. tuberculosis* and *M. africanum* was based on growth characteristics and biochemical test results as well as on specific molecular typing patterns.[9] Molecular typing data were analysed (cluster analysis) with the Bionumerics software (version 4.5; Applied Maths, Kortrijk, Belgium). Ten *M. africanum* isolates could not unambiguously be assigned to the clades of West African 1 and 2.

Clusters were defined as groups of patients infected with mycobacterial strains showing identical IS*6110* fingerprinting patterns. If less than five bands were identified, analyses were supported by spoligotyping. Identical strains recovered from two or more patients were regarded as a cluster and strains found in one patient only were considered ''unique'' as previously defined.[10]

Radiography

All patients underwent posterior–anterior chest radiography. Pseudonymized films were read by an experienced radiologist (G.S.) who was not informed about the mycobacterial strains and the HIV status. Opacities, cavities, nodular lesions, pulmonary shrinkage (loss of lung volume as assessed by hilus dislocation or displacement of fissures), calcifications, and pleural thickening were individually assigned to the upper right, lower right, upper left, and lower left thoracic quadrants and, according to the severity of lesions rated ''0'' (no lesion detectable), ''1'' (mild lesion), ''2'' (moderate lesion), and ''3'' (severe lesion). Enlargements of mediastinal lymph nodes, effusions, and miliary lesions were assessed qualitatively.

Databases, statistical analyses

Demographic data, self-reported signs and symptoms as documented on a structured questionnaire as well as laboratory results were double-entered into a Fourth Dimension database (San Jose, CA, USA). Bacteriological data and radiological evaluations were provided as datasheets. Data were locked before using them in a pseudonymized form for statistical analyses.

Multivariate logistic and ordinal logistic regression analyses were performed to calculate odds ratios (OR; STATA 8.2 software; Stata Corporation, College Station, TX, USA). As age, sex, ethnicity, self-reported duration of coughs prior to recruitment (for example, $\leq$5 weeks vs $>$5 weeks, opacities, OR, 1.7; CI, 1.3–2.2; $p < 0.001$; cavities OR, 2.4; CI, 1.9–3.1; $p < 0.001$), and the recruitment sites of Accra and Kumasi (Accra vs Kumasi, opacities, OR, 0.68; CI, 0.6–0.8; $p < 0.001$; cavities OR, 0.49; CI, 0.4–0.6; $p < 0.001$) significantly influenced one or more of the parameters studied, all calculations included adjustments for these variables.

The working hypotheses on which the aim of the study, namely a comparison of infections due to different mycobacterial species, was based were that: (i) male sex is positively associated with an increased prevalence and an increased severity of clinical and radiographic signs of TB;[11–13] (ii) HIV positivity is negatively associated with the sizes of cavities and pleural thickening;[14,15] and (iii) HIV positivity is positively associated with exclusive lower-lobe.[16] Corrections for multiple testing were made if an OR did not fulfill the working hypotheses. Then, p values were corrected by multiplication with 12 and 10, respectively, i.e. the product of six clinical signs and five radiographic signs evaluated, respectively, times 2 for the stratifications made (mycobacterial species, HIV status). If a significant correlation was subsequently analysed by stratification, no further correction for multiple testing was made because the result of the initial correlation was considered the working hypothesis.

Results

Frequency, clustering and drug resistance of *M. africanum*

Studying 1921 newly diagnosed cases of pulmonary TB in Ghana, West Africa, *M. tuberculosis*, *M. africanum*, and *M. bovis* were recovered from sputum cultures in 70.3%, 28.9% (West African 1, 20.5%; West African 2, 8.4%), and 0.8% of the patients, respectively. The mycobacterial species were similarly distributed among the ethnic groups studied. Because of their low frequency ($n = 10$), *M. bovis* cases were not analysed further.

Using mycobacterial spoligotyping and IS*6110* fingerprinting, 63% of infections were found to be included in clusters of cases with identical isolates. There were 219 separate clusters with a median size of 2. *M. africanum* was found less frequently in clusters than *M. tuberculosis* (21% vs 79%; OR, 0.38; 95% CI, 0.3–0.5; $p < 0.001$).

An evaluation of mycobacterial drug resistance in the study group has previously been published;[17] 14.7% of the isolates were mono-drug resistant, 2.2% were multi-drug resistant. The frequencies did not significantly differ between *M. africanum* and *M. tuberculosis* or between isolates from 279 HIV-positive and 1627 HIV-negative patients.

Clinical and radiographic findings in *M. africanum* infections

Overall, demographic data, self-reported signs and symptoms (Table 1) as well as radiographic findings (Table 2) were

Table 1 Demographic data and clinical findings in pulmonary TB associated with *M. africanum* and *M. tuberculosis*, and stratification for concomitant HIV infection.

	M. africanum	*M. tuberculosis*	HIV-negative		HIV-positive	
			M. africanum	*M. tuberculosis*	*M. africanum*	*M. tuberculosis*
n (%)	556 (29)	1350 (71)	480 (30)	1147 (70)	76 (27)	203 (73)
Age (mean ± SD)	35.8 ± 11.8	33.8 ± 11.0	35.7 ± 12.1	33.4 ± 11.3	35.9 ± 9.8	35.9 ± 9.1
Gender (male)	66	68	68	69	54	61
Ethnicity (*n* [%])						
Akan	350 (63)	901 (67)	299 (62)	761 (66)	51 (67)	140 (70)
Ga	88 (16)	170 (13)	80 (17)	154 (14)	8 (11)	16 (8)
Northerners[#]	64 (12)	163 (12)	58 (12)	141 (12)	6 (8)	22 (11)
Ewe	41 (7)	83 (6)	34 (7)	73 (6)	7 (9)	10 (5)
Unknown[†]	13 (2)	31 (2)	9 (2)	18 (2)	4 (5)	13 (6)
Duration of cough[§]	12 (8–20)	12 (8–20)	12 (8–20)	12 (8–20)	12 (5.5–24)	12 (8–20)
Fever	477 (86)	1193 (89)	414 (87)	1009 (88)	63 (84)	184 (93)
Hemoptysis	178 (32)	446 (33)	157 (33)	390 (34)	21 (29)	56 (28)
Chest pain	495 (90)	1219 (91)	427 (89)	1030 (90)	68 (91)	189 (95)
Weight loss	536 (97)	1319 (98)	462 (97)	1123 (98)	74 (99)	196 (98)
Shortness of breath	456 (83)	1084 (81)	391 (82)	915 (80)	65 (87)	169 (85)
Night sweats	435 (79)	1060 (79)	373 (79)	893 (78)	62 (83)	167 (84)

p values were determined by logistic regression, adjusted for age, sex, ethnicity, recruitment center and for the duration of cough, because the latter was found to correlate to various of the signs/symptoms. When *p* values were corrected for 12 comparisons, including six possibly independent signs/symptoms and two stratifications, no significant differences were found.

[#] Immigrants from the Sahel zone of Northern Ghana, comprising Dagomba, Sissala, Gonja, and Kusasi.

[†] Participants of whom no unambiguous information on ethnicity was obtained.

[§] Duration in weeks (median, interquartile range).

very similar among patients infected with *M. africanum* and *M. tuberculosis*. This applied to all major radiographic signs of disease severity including opacities, cavities, pulmonary shrinkage and pleural thickening. Findings were similar also for signs of lymph node enlargements, nodular pulmonary lesions, calcifications, pleural effusions, and miliary dissemination, which were identified in 0.8%, 0.8%, 0.4%, 5%, and 0.3% of patients, respectively (data not shown). An exception was the occurrence of exclusive lower-lobe disease, which was found substantially less frequently in patients with *M. africanum* than in those with *M. tuberculosis* infections (OR, 0.39; CI, 0.2–0.7; $p = 0.011$). A multivariate analysis showed that this was not correlated to the lower clustering of *M. africanum* (OR, 1.09; CI, 0.7–1.7; $p = 0.72$).

M. africanum and HIV coinfections

Fifteen percent of the patients were HIV-positive. The relative frequencies of *M. africanum* and *M. tuberculosis* did not differ in HIV-negative and HIV-positive ones (Table 1). Similarly, clinical and radiographic signs of *M. africanum* and *M. tuberculosis* infections resembled each other when separately analysed in the subgroups of HIV-negative and HIV-positive patients (Tables 1 and 2). This stratification revealed, however, that an association of exclusive lower-lobe disease with *M. tuberculosis* infection showed in HIV-negatives but not in HIV-positives (Table 2).

M. africanum subtypes

None of the radiological parameters studied showed a significant difference when 315 cases associated with *M. africanum* clade West African 1 were compared to 129 cases associated with *M. africanum* West African 2, whereby the numbers of individuals infected with either genotype did not allow valid statistical evaluation in HIV-positive patients (Table 3).

Additional findings

Both *M. tuberculosis* and *M. africanum* were isolated from men approximately twice as often as from women (69% and 67%, respectively, infections of men). The sex difference also applied to the severity of disease as indicated by the frequency of hemoptyses (males, 37%; females, 25%; $p < 0.001$) and the severity of radiographic signs of disease. The differences in the severity of opacities, cavities, pulmonary shrinkage and pleural thickening were statistically significant in the entire patient group and in the *M. tuberculosis* subgroup (for example, opacities, *M. tuberculosis* subgroup, male-to-female, OR, 1.42; CI, 1.1–1.8; $p = 0.04$). In the *M. africanum* subgroup, sex differences were less pronounced and did not reach statistical significance (for example, opacities, *M. africanum* subgroup, male-to-female, OR, 1.20; CI, 0.8–1.8).

HIV-positive patients were marginally older and showed a smaller age range, and male preponderance was less pronounced. Radiographically, HIV-positive patients had significantly less severe cavities (OR, 0.49; CI, 0.3–0.7; $p < 0.001$) and less severe pleural thickening (OR, 0.55; CI, 0.4–0.8; $p = 0.002$) than HIV-negative ones. Conversely, HIV-positives had more frequently exclusive lower-lobe disease (OR, 2.06; CI, 1.0–4.2; $p = 0.05$). Exclusive

Table 2 Major radiographic findings in pulmonary TB associated with *M. africanum* and *M. tuberculosis*, and stratification for concomitant HIV infection.

	M. africanum	*M. tuberculosis*	HIV-negative		HIV-positive	
			M. africanum	*M. tuberculosis*	*M. africanum*	*M. tuberculosis*
n (%)	454 (29)	1133 (71)	428 (29)	1049 (71)	26 (24)	84 (76)
Opacities						
Severity score						
0	2 (0)	16 (1)	1 (0)	15 (1)	1 (4)	1 (1)
1	87 (19)	214 (19)	81 (19)	205 (20)	6 (23)	9 (11)
2	270 (60)	676 (60)	258 (60)	624 (59)	12 (46)	52 (62)
3	95 (21)	227 (20)	88 (21)	205 (20)	7 (27)	22 (26)
	OR, 1.05; CI, 0.8—1.3, ns		OR, 1.08; CI, 0.9—1.3, ns		OR, 0.69; CI 0.3—1.8, ns	
Cavities						
Severity score						
0	22 (5)	70 (6)	20 (5)	65 (6)	2 (8)	5 (6)
1	194 (43)	534 (47)	180 (42)	489 (47)	14 (54)	45 (53)
2	173 (38)	389 (35)	164 (38)	364 (35)	9 (35)	25 (30)
3	65 (14)	140 (12)	64 (15)	131 (12)	1 (4)	9 (11)
	OR, 1.22; CI, 1.0—1.5, ns		OR, 1.23; CI, 1.0—1.5, ns		OR, 0.75; CI, 0.3—1.9, ns	
Exclusive lower-lobe disease	15 (3.3)	87 (7.8)	12 (2.8)	79 (7.7)	3 (12.0)	8 (9.6)
	OR, 0.39; CI, 0.2—0.7; $p_c = 0.011$		OR, 0.34; CI, 0.2—0.6; $p = 0.001$		OR, 2.85; CI, 0.5—14.9; ns	
Shrinkage						
Severity score						
0	96 (21)	231 (20)	86 (20)	208 (20)	10 (38)	23 (27)
1	232 (51)	634 (56)	220 (51)	582 (56)	12 (46)	52 (62)
2	111 (25)	243 (22)	108 (25)	234 (22)	3 (12)	9 (11)
3	15 (3)	23 (2)	14 (3)	23 (2)	1 (4)	0 (0)
	OR, 1.01; CI, 0.8—1.2; ns		OR, 0.99; CI, 0.8—1.2; ns		OR, 0.71; CI, 0.3—1.8; ns	
Pleural thickening						
Severity score						
0	121 (27)	319 (28)	108 (25)	293 (28)	13 (50)	26 (31)
1	201 (44)	545 (48)	192 (45)	499 (48)	9 (34)	46 (55)
2	102 (22)	211 (19)	100 (23)	200 (19)	2 (8)	11 (13)
3	30 (7)	57 (5)	28 (7)	56 (5)	2 (8)	1 (1)
	OR, 1.13; CI, 0.9—1.4; ns		OR, 1.14; CI, 0.9—1.4; ns		OR, 0.46; CI, 0.2—1.2; ns	

Odds ratios (OR) and 95% confidence intervals (CI) were calculated by ordinal logistic regression and adjusted for age, sex, ethnicity, recruitment center and duration of cough, the latter because it was found correlated with the radiographic signs. Cases associated with *M. bovis* were excluded from analyses ($n = 10$). p values were corrected for 10 comparisons, including five possibly independent signs and two stratifications (p_c, corrected p value). If a significant difference was found between *M. africanum* and *M. tuberculosis* infections, the subsequent comparison in the subgroups of HIV-positive and HIV-negative patients was not corrected for multiple comparisons because it was considered hypothesis-driven. ns, not significant.

lower-lobe-disease was not associated with clustering (OR, 1.3; CI, 0.8—2.0; $p = 0.25$).

Discussion

The present study has a number of limitations because it was designed to recruit patients for an analysis of human genetic factors, which required confirmed diagnoses and a clinically unbiased study group (from which HIV-positives were retrospectively excluded). Not including smear negative cases created a bias in the clinical and radiographic signs recorded. In addition, it may have caused an underrepresentation of HIV-positive patients because these are known to be sputum negative more often than HIV-negatives.[18] Furthermore, the exclusion of patients with other concomitant diseases potentially causing immunosuppression may have resulted in an additional bias in the description of TB and may have been a reason to miss associations with immunodeficiencies other than AIDS. Compared to AIDS, such an association would, however, be a relatively small and stable epidemiologic factor and therefore of little public health relevance.

The reliability of our data is supported by correlations: (i) between the duration of symptoms and the severity of radiographic lesions; and (ii) between the reported occurrence of hemoptyses and cavity size as indicated by radiography. It is further underlined by the confirmation of previous observations, including: (i) an increased TB susceptibility of males;[12] (ii) reduced severity of cavitary

Table 3 Radiographic findings in pulmonary TB associated with *M. africanum* genotypes West African 1 and West African 2, and stratification for concomitant HIV infection.

	West African 1	West African 2	HIV-negative		HIV-positive	
			West African 1	West African 2	West African 1	West African 2
n (%)	315 (71)	129 (29)	296 (71)	123 (29)	19 (76)	6 (24)
Opacities						
Severity score						
0	2 (1)	0 (0)	1 (0)	0 (0)	1 (5)	0 (0)
1	63 (20)	21 (16)	57 (19)	21 (17)	6 (32)	0 (0)
2	186 (59)	79 (61)	178 (60)	76 (62)	8 (42)	3 (50)
3	64 (20)	29 (22)	60 (20)	26 (21)	4 (21)	3 (50)
	OR, 0.86; CI, 0.6–1.3; ns		OR, 0.94; CI, 0.6–1.4; ns			
Cavities						
Severity score						
0	17 (5)	4 (3)	15 (5)	4 (3)	2 (11)	0 (0)
1	135 (43)	54 (42)	124 (42)	51 (41)	11 (58)	3 (50)
2	118 (37)	52 (40)	113 (38)	49 (40)	5 (26)	3 (50)
3	45 (14)	19 (15)	44 (15)	19 (15)	1 (5)	0 (0)
	OR, 0.91; CI, 0.6–1.4; ns		OR, 0.92; CI, 0.6–1.4; ns			
Exclusive lower-lobe disease	10 (3.2)	5 (3.9)	7 (2.4)	5 (4.1)	3 (16.7)	0 (0)
	OR, 0.79; CI, 0.3–2.5; ns		OR, 0.50; CI, 0.1–1.7; ns			
Shrinkage						
Severity score						
0	71 (23)	21 (16)	63 (21)	20 (16)	8 (42)	1 (17)
1	159 (50)	69 (53)	151 (51)	65 (53)	8 (42)	4 (67)
2	72 (23)	37 (29)	70 (24)	36 (29)	2 (11)	1 (17)
3	13 (4)	2 (2)	12 (4)	2 (2)	1 (5)	0 (0)
	OR, 1.00; CI, 0.7–1.5; ns		OR, 1.04; CI, 0.7–1.6; ns			
Pleural thickening						
Severity score						
0	80 (25)	38 (29)	71 (24)	34 (28)	9 (47)	4 (67)
1	146 (46)	51 (40)	138 (47)	51 (41)	8 (42)	0 (0)
2	68 (22)	31 (24)	68 (23)	29 (24)	0 (0)	2 (33)
3	21 (7)	9 (7)	19 (6)	9 (7)	2 (11)	0 (0)
	OR, 1.19; CI, 0.8–1.8; ns		OR, 1.17; CI, 0.8–1.8; ns			

Odds ratios (OR) and 95% confidence intervals (CI) were calculated by ordinal logistic regression and adjusted for age, sex, ethnicity, recruitment center and duration of cough, the latter because it was found correlated with the radiographic signs. Due to the small numbers of HIV-positive patients infected with either *M. africanum* West African 1 ($n = 19$) or *M. africanum* West African 2 ($n = 6$), valid statistical analyses were not feasible.

disease and pleural thickening of HIV-positives;[14,15] and (iii) the association of exclusive lower-lobe disease with HIV positivity and not with clustering as an indicator of recent infections.[16] Since immunologic parameters were not assessed in our study, factual immunosuppression of our HIV-positive patients was not demonstrated but the consistency of our data with previous descriptions of TB in AIDS suggests that the vast majority of our HIV-positive patients were indeed immunocompromised.[19–21] This is corroborated by the finding that HIV-positive persons are >4-fold overrepresented in our patient group compared to the general population (15% vs 3.6%, respectively, [Ref. 22]), which is in agreement with previous studies on TB in Africa.[23,24]

The proportion of approximately 30% *M. africanum* infections compared to 70% of *M. tuberculosis* in our patient group is in agreement with previous reports from West Africa.[1,25] The prevalence data show some correlation to the proportions of isolates found in clusters (21% vs 79%).

From our clinical and radiographic findings, the different prevalences of the two species appear not to be determined by striking differences in elements of human pathology, which could favor transmission. The frequencies of self-reported hemoptyses or radiographic evidence of cavities, which might reflect the transmission potential, were similar in the two infections. In fact, all major clinical and radiographic findings of cases associated with *M. africanum* closely resembled those seen associated with *M. tuberculosis*. The only exception we found was that the particular radiographic sign of exclusive lower-lobe disease was substantially more frequent among *M. tuberculosis* infections, an association, which, on stratification, was found in HIV-negative patients only.

Studying 19 HIV-positive and 228 HIV-negative patients from The Gambia, de Jong and co-workers reported that *M. africanum* preferentially infects HIV-positives and concluded that *M. africanum* might be considered to have properties of an opportunistic pathogen.[6] As we found in our study group of 1627 HIV-negative and 279 HIV-positive patients very similar rates of *M. africanum* infections, we cannot confirm this observation. Recently, a study of 301 patients from The Gambia yielded evidence that radiographic findings in pulmonary TB associated with *M. africanum* might be more severe than in cases of *M. tuberculosis*, suggesting that *M. africanum* might be more virulent.[5] From our data, we cannot confirm this finding either. As mentioned above, we found a subtle piece of evidence for a higher virulence, assessed by the severity of radiological presentation, of *M. tuberculosis*, i.e. that *M. tuberculosis* caused exclusive lower-lobe disease more frequently than *M. africanum*. As exclusive lower-lobe disease may reflect reduced immunocompetence of the host (Ref. 16 and our data on HIV coinfection), the difference between *M. tuberculosis* and *M. africanum* suggests that there are more patients who have a reduced immunocompetence towards *M. tuberculosis* than do so towards *M. africanum*. If this is confirmed, it could be regarded as circumstantial evidence suggesting that, in the population under study, *M. tuberculosis* is discretely more virulent than *M. africanum*, which would merit further investigation as a possible cause for the higher prevalence of *M. tuberculosis* in this population. As, on stratification, the difference in virulence was found in HIV-negative patients only, it appears not to have any bearing on HIV coinfections.

Our failure to see any difference in the clinico-radiological presentation between infections with *M. africanum*, West African 1, and *M. africanum*, West African 2, suggests that the genetic differences found between the two clades[4,9] do not translate into differences in virulence, although the number of cases in our study was not sufficient to draw any final conclusions.

In summary, studying nearly 2000 patients we found highly similar clinical and radiographic findings in *M. africanum* and *M. tuberculosis* infections, which did not differ either in HIV coinfections. Circumstantial evidence might indicate a slightly higher virulence of *M. tuberculosis* in HIV-negative patients of the population under study.

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