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Differences between TB cases infected with *M. africanum*, West-African type 2, relative to Euro-American *M. tuberculosis*- an update

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Abstract

M. africanum is a common cause of human pulmonary TB in West Africa. We previously described phenotypic differences between *M. africanum* and *M. tuberculosis* among 290 patients. In the present analysis we compared 692 TB patients infected with the two most common lineages within the *M. tuberculosis* complex found in the Gambia, namely *M. africanum* West African type 2 (39% prevalence) and Euro American *M. tuberculosis* (55% prevalence). We identified additional phenotypic differences between infections with these two organisms. *M. africanum* patients were more likely to be of older age and HIV infected. In addition, they had worse disease on chest x-ray, despite complaining of cough for equal duration, and were more likely severely malnourished. In this cohort the prevalence of *M. africanum* did not change significantly over a seven year period.

Keywords

Mycobacterium africanum; *Mycobacterium tuberculosis*; Gambia; Phenotypic differences; HIV

Introduction

M. africanum (MAF), a common cause of TB in West-Africa, was first recognized as a distinct sub-species of the *M. tuberculosis* complex (MTBC) in 1968 in Senegal, due to the different results it generated on biochemical characterization [1]. The biochemical characteristics of MAF can vary [2], and molecular genotyping techniques have since suggested a revised classification that includes MAF West-African type 1 and MAF West-African type 2 as distinct sub-species within the MTBC [3,4]. Moreover, several phylogenetically distinct lineages have been identified within *M. tuberculosis* (MTB) sensu stricto, with the East African Indian lineage classified as “ancient” MTB and the Euro American, East Asian and Central Asian lineages classified as “modern” MTB. Within these lineages, sublineages can be distinguished based on Regions of Differences.

We previously described clinical characteristics of TB patients infected with MAF West-African type 2 (MAF2) compared with those infected with MTB on 290 patients from the Gambia, where MAF2 causes 38% of smear positive pulmonary tuberculosis [5]. In that analysis we identified a tendency towards worse findings on chest x-rays in MAF2 infected

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patients, and an association with HIV infection. Given that most Gambian MTB isolates belong to the Euro American lineage, we performed clinical comparisons between MAF2 and the Euro American lineage of MTB (including the subset described earlier), and assessed the prevalence of MAF2 in the Gambia over a 7-year period.

Materials and Methods

In the context of a TB case contact study in the Gambia, consecutive sputum smear positive TB cases were enrolled after informed consent [6]. The sputum samples were investigated by smear and culture, after which DNA was extracted and genotyped using spoligotype analysis [7]. Classification in lineage MAF2 and in lineages within MTB (Euro American, East Asian, Central Asian, and East Asian Indian lineages) was based on our previous identification of their signature spoligotype patterns [4,8,9].

Logistic regression was used to assess the relationship between genotype and demographic-, clinical- and diagnostic variables. Results were reported as unadjusted ORs and their 95% CIs and variables associated with MAF2 ($p<0.20$) were adjusted for age and gender. To investigate if the proportion of severely malnourished study participants was significantly different between the two groups, MAF2 infected and the Euro American lineage of MTB infected patients, we calculated the BMI-for-age z-scores for participants who were younger than 20 years of age using the WHO AnthroPlus software (WHO Anthro for personal computers, version 3, 2009: Software for assessing growth and development of the world's children. Geneva: WHO, 2009, http://www.who.int/childgrowth/software/en/). For participants aged 20 years or more the BMI was calculated. Participants with z-scores less than -3 or a BMI below 16 were defined to be severely malnourished. The resulting binary variable was included in a logistic regression model and the crude and age-, gender- and HIV- adjusted OR was calculated. We used a test for linear trend to assess if MAF prevalence increased over the 7-year study period. Analyses were performed using Stata version 10.0 (Stata corporation, Texas, USA).

Results

Of 802 study cases, 746 (93%) had TB DNA available for genotyping, of which 737 (92%) gave interpretable spoligotype results. Seven isolates repeatedly failed to amplify and 2 isolates had results suggestive of mixed infection. The group with interpretable results did not significantly differ from the one without by age and sex. We identified two isolates (0.27%) of MAF West-African type 1 (MAF1), 289 isolates (39.2%) of MAF West-African type 2 (MAF2), and 446 (60.5%) isolates of MTB *sensu stricto*. MTB isolates included 403 (90%) of the Euro American lineage, 23 (5.2%) of the East Asian ("Beijing") lineage, 8 (1.8%) of the Central Asian lineage, and 12 (2.7%) of the East Asian Indian lineage. On univariable analysis comparing sex, ethnicity, BMI, prevalence of HIV co-infection, maximum smear grade, radiographical findings, and presence of a BCG scar between the four different MTB lineages, no significant differences were identified, although there were significant differences in age and in the proportion with a positive Mantoux between these lineages (data not shown). The low numbers of non-Euro American MTB cases however precluded further analysis of these differences. On univariable analysis comparing these same variables between different sublineages within the Euro American MTB lineage no significant differences were identified (data not shown), and for the remainder of the analysis we compared MAF2 (n=289) with the Euro American lineage of MTB (EAMTB, n=403). The odds of MAF2 infection were significantly increased in patients with increasing age and in those with HIV infection (Table). EAMTB was significantly more common in people of Wollof ethnicity. In addition, MAF2 patients had worse abnormalities on chest x-ray, despite reporting a similar duration of cough preceding TB diagnosis. MAF2 patients

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were moreover significantly more likely to be severely malnourished after adjusting for age, gender and HIV infection (OR 2.62, 95% CI 1.13–6.08, p=0.024). MAF2 infected patients were significantly less likely to have a positive ESAT-6/CFP-10 ELISPOT test result compared to EAMTB patients (OR 0.31, 95% CI 0.17–0.58, p<0.0001) There was no significant difference in prevalence of MAF2 over the seven year period, ranging from 35.4% in 2005 to 50.8% in 2008 (p=0.22).

Discussion

In addition to the association of MAF2 with HIV infection and with negative ELISPOT results, we also found MAF2 to be more common in older people, in malnourished people, and in those with worse x-ray abnormalities, without reporting a significant difference in duration of cough. The predilection of MAF2 for causing disease in HIV infected patients suggests the presence of a larger reservoir of latent TB infection (LTBI) with MAF2 relative to its prevalence among patients with active disease. A larger reservoir of LTBI with MAF2 in the Gambia could explain the association of MAF2 and HIV in the Gambia [10], in contrast to Ghana where no such association was identified [11]. Alternatively, significant polymorphisms could exist between Gambian and Ghanaian lineages of MAF2, as suggested by SNP analysis of a few such isolates [12]. Older age of MAF2 patients may be related to the lower prevalence of MAF2 relative to EAMTB, or to the lower progression to disease in MAF2 exposed contacts during the first 2 years after exposure [13].

While the association with HIV-infected hosts suggests attenuation of MAF2, the chest radiographs of MAF2 patients showed worse findings than of EAMTB patients and MAF2 patients were significantly more likely to be severely underweight. Perhaps attenuation of MAF2 results in a lower degree of immunosuppression of the host, resulting in a stronger inflammatory response and associated weight loss.

The lack of a clear association between MAF2 and ethnicity suggests that MAF2 does not select for hosts of a certain genetic background, unless this is a shared background between the different ethnicities present in the Gambia. The higher prevalence among people of Manjago ethnicity, who originate from Guinea Bissau, is probably explained by the 51% prevalence of MAF2 in Guinea Bissau [14]. Similarly, the lower prevalence in people of Wollof ethnicity correlates with a lower prevalence of MAF2 in Senegal (around 20% [15]), where the Wollofs originate.

The stable prevalence of MAF2 in this cohort, albeit over a short time span in evolutionary terms, suggests that MAF2 is not being outcompeted rapidly by EAMTB, as was described in Cameroon for MAF1 [16].

Conclusion

MAF, which causes 39% of TB in the Gambia, is more common among older patients and among HIV co-infected TB patients. Furthermore, MAF infection is associated with severe malnutrition, negative ELISPOT results, and with worse chest x-ray abnormalities. The prevalence of MAF has not significantly changed over the past seven years.

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Table

Differences between TB cases infected with *M. africanum* West African type 2 and Euro American *M. tuberculosis sensu stricto*.

	***M. africanum* West African 2**		**Euro American *M. tuberculosis***		**Unadjusted**		**Adjusted**	
	n	**(%)**	**n**	**(%)**	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**
N	289	(41.8)	403	(58.2)				
Age								
<20	25	(8.6)	47	(11.7)	0.98 (0.57–1.68)	0.94	1.04‡ (0.60–1.80)	0.89
20–29	99	(34.3)	182	(45.2)	1	-	1	-
30–39	76	(26.3)	90	(22.3)	1.55 (1.05–2.30)	0.028	1.56‡ (1.06–2.32)	0.025
≥40	89	(30.8)	84	(20.8)	1.94 (1.32–2.86)	0.001	1.95‡ (1.32–2.87)	0.001
median, (IQR)	32, (24–43)		28, (22–37)					
Gender								
Female	76	(26.3)	135	(33.5)	1	-	1	-
Male	213	(73.7)	268	(66.5)	1.41 (1.01–1.97)	0.043	1.35# (0.96–1.89)	0.082
Ethnicity	279		394					
Mandinka	106	(38.0)	140	(35.5)	1	-	1	-
Wollof	24	(8.6)	59	(15.0)	0.54 (0.31–0.92)	0.023	0.52* (0.30–0.89)	0.018
Fula	36	(12.9)	47	(11.9)	1.01 (0.61–1.67)	0.96	0.99* (0.60–1.65)	0.98
Jola	61	(21.8)	86	(21.8)	0.94 (0.62–1.42)	0.76	0.91* (0.60–1.39)	0.68
Serere	18	(6.5)	18	(4.6)	1.32 (0.66–2.66)	0.44	1.12* (0.55–2.28)	0.76
Manjago	20	(7.2)	15	(3.8)	1.76 (0.86–3.60)	0.10	1.62* (0.78–3.34)	0.20
Other	14	(5.0)	29	(7.4)	0.64 (0.32–1.27)	0.20	0.62* (0.31–1.24)	0.17
HIV positive	32/241	(13.3)	24/369	(6.5)	2.20 (1.26–3.83)	0.005	2.13* (1.20–3.79)	0.010
Cough ≥ 4 wks	76/111	(68.5)	21/160	(75.6)	0.70 (0.41–1.20)	0.19	0.69* (0.40–1.19)	0.18
Severe malnutrition**	21/84	(25.0)	16/130	(12.3)	2.38 (1.16–4.88)	0.018	2.56* (1.09–5.27)	0.012

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	M. africanum West African 2		Euro American *M. tuberculosis*		Unadjusted		Adjusted	
	n	**(%)**	**n**	**(%)**	**OR (95%CI)**	**p**	**OR (95%CI)**	**p**
BCG scar present	91/243	(37.5)	133/338	(39.4)	0.92 (0.66–1.30)	0.64	1.03* (0.73–1.46)	0.87
Maximum smear grade	277		392					
Grade 1	13	(4.7)	15	(3.8)	1.24 (0.58–2.66)	0.58	1.30* (0.60–2.82)	0.51
Grade 2	59	(21.3)	84	(21.4)	1.00 (0.69–1.46)	0.98	1.01* (0.68–1.47)	0.99
Grade 3	205	(74.0)	293	(74.7)	1	-	1	-
EC elispot positive	91/127	(71.7)	172/193	(89.1)	0.31 (0.17–0.56)	<0.0001	0.32* (0.18–0.59)	<0.0001
Mantoux	117/151	(77.5)	180/224	(80.4)	0.84 (0.51–1.39)	0.50	0.84* (0.50–1.39)	0.50
Over half of CXR zones involved	145/276	(52.5)	160/393	(40.7)	1.61 (1.18–2.20)	0.003	1.56* (1.14–2.14)	0.005
Cavities on CXR	160/275	(58.2)	208/393	(52.9)	1.24 (0.91–1.69)	0.18	1.22* (0.89–1.67)	0.23

‡adjusted for gender

#adjusted for age

*adjusted for age and gender

**Severe malnutrition was indicated by BMI-for-age z-scores lower than -3 for study participants under the age of 20 or a BMI of less than 16 kg/m^2 for 20 years old and older study participants.

BCG= Bacille Calmet Guerin vaccine

EC= ESAT6/CFP10

CXR= Chest x-ray