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LTA4H Prevalence and Mortality in Adult Zambians with Tuberculous Meningitis

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ana.26240

Summary for Social Media If Published

1. Twitter handles: @o_siddiqi, @BirbeckGretchen, @helen_ayles, @IgorKoralnik, @ZINCAREZambia
2. There has been no comprehensive study on LTA4H genotype prevalence among tuberculous meningitis (TBM) patients in sub-Saharan Africa.
3. This study looked at the prevalence of LTA4H genotypes in adult patients with TBM in Zambia to see if there was an association with mortality.
4. This study demonstrated that C/C genotype is most common among Zambians. Dexamethasone therapy, currently recommended and widely used in sub-Sahran Africa for treatment in TBM, may not benefit the most common LTA4H genotype in Zambia.
5. This study questions the broad recommendation of dexamethasone use across all TBM populations as it has only been shown to reduce mortality among HIV-negative patients in Vietnam. A separate trial of dexamethasone in TBM patients in sub-Saharan Africa is warranted.

We conducted a prospective cohort study to determine the prevalence of leukotriene A4 hydrolase (*LTA4H*) polymorphisms in Zambian adults with tuberculous meningitis (TBM) and its association with mortality. We completed genotype testing on 101 definite cases of TBM and 119 consecutive non-TBM controls. The distribution of genotypes among TBM patients was as follows: C/C (0.83), C/T (0.14), T/T (0.03). There was no significant difference in genotype distribution between TBM and non-TBM patients. We found no relationship between *LTA4H* polymorphism and survival. Prospective studies are needed to determine the benefit of adjuvant steroids in TBM based upon population LTA4H genotype.

Introduction:

Tuberculous meningitis is a devastating infection of the central nervous system often leading to severe neurological impairment and death [1]. Its effect is even more profound in resource-limited settings such as Zambia where 11.3% of the general population is HIV-infected [2]. The optimum treatment regimen, particularly the use of adjuvant steroids to diminish neurological complications in HIV-positive patients, remains unclear [3]. The current practice in numerous HIV-endemic settings is to place all patients with TBM on steroid treatment regardless of the HIV status and degree of immunosuppression. This is based on data from Vietnam that included predominantly HIV-negative patients [4].

In humans with TBM, a single nucleotide polymorphism at the LTA4H promoter site, rs17525495 C/T, has been associated with varying mortality depending on the host genotype. A study of Vietnamese patients with TBM suggest a heterozygous advantage (C/T – mild

inflammation) in the LTA4H promoter gene and implicate both insufficient (C/C) and excessive inflammation (T/T) of homozygotes in the pathogenesis of TBM [5]. In contrast, a study in Indonesia showed no survival benefit of LTA4H genotype in a cohort of 427 HIV-negative patients with confirmed TBM [6].

Current treatment guidelines for TBM recommend the use of steroids in addition to anti-tubercular treatment (ATT) in order to reduce mortality [7]. The benefit of steroids is thought to be from the reduction in inflammation that occurs in patients initiating ATT [4]. However, Tobin et al demonstrated host responsiveness to steroids may depend on LTA4H genotype. They showed that survival was highest in Vietnamese TBM patients with the TT genotype treated with dexamethasone and lowest in patients with C/C genotype treated with dexamethasone [5]. A separate Vietnam study, showed that the LTA4H promoter has no effect on survival in TBM patients with HIV [8].

The association of TBM morbidity and mortality with LTA4H genotype has not yet been studied in Africa or in a high prevalence HIV population like Zambia. The C/C genotype is the most common among African populations [9]. The initiation of adjunctive dexamethasone therapy in severely immunosuppressed HIV patients may be detrimental rather than beneficial depending on their LTA4H genotype. We assessed the association between LTA4H genotypes and survival in a prospective cohort of predominantly HIV-infected adults Zambians with TBM.

Methods:

Study design and patient population: From 4 April 2014 to 31 August 2017, we enrolled adult Zambian inpatients who provided CSF as part of a prospective cohort study of TBM diagnostics at the University Teaching Hospital (UTH) in Lusaka as previously described [10]. We excluded

patients with cryptococcal meningitis or bacterial meningitis from enrollment. The study categorized a patient as definite TBM in the setting of a positive Mycobacteria Growth Indicator Tube (MGIT) culture with subsequent confirmation via Capilia TB MPT64 Ag assay (Tauns Laboratories, Japan). A study nurse obtained informed consent from all patients or the health care proxy. All patients provided a peripheral blood sample for genotype analysis. Laboratory staff immediately froze these samples at -80° C upon receipt for use at a later date.

All patients received a structured examination from a neurologist. We employed the Medical Research Council (MRC) severity scale to classify the grade of meningitis: grade I (GCS score, 15; no focal neurological signs), grade II (GCS score 11 to 14, or GCS score 15 with focal neurological signs), or grade III (GCS score < 10) [11]. A study nurse followed inpatients daily until discharge from the hospital or death. Study staff called patients or health care proxies quarterly to establish one-year mortality. This study was approved by the University of Zambia School of Medicine Biomedical Research Ethics Committee and Beth Israel Deaconess Medical Center Institutional Review Board.

LTA4H Testing: Genomic DNA was purified from peripheral blood using the QIAamp DNA blood kit (Qiagen). The LTA4H promoter region single nucleotide polymorphism (SNP) rs17525495 was identified with a TaqMan SNP Genotyping Assay C_25593629_10 (LifeTechnologies) on a Rotorgene 6000 (Corbett Life Sciences, Sydney, Australia) real-time polymerase chain reaction (PCR) unit. Genotype testing was completed on all definite cases of TBM. Genotype testing was completed on 119 consecutive non-TBM meningitis patients in the study for a comparison group of LTA4H allele frequency.

Statistical analyses: Descriptive measures of median, interquartile range, frequencies, and percentages were used to summarize the data. Wilcoxon rank-sum and Fisher exact tests were used to compare continuous and categorical study variables, respectively, between groups. The primary goal of the analyses was to estimate the prevalence of LTA4H genotype among TBM patients and the overall population. Secondary goals of our analyses included assessing the relationship between LTA4H genotype and 1-year TBM mortality. Univariate and multivariate logistic regression models were utilized to assess the clinical predictors of 1-year mortality. A significance level of < 0.10 was used to select variables for the multivariate analyses. All *P* values were 2-sided and considered statistically significant if < 0.05.

Results:

Five hundred and forty-five patients with suspected TBM were recruited into the study with an HIV prevalence of 85.6%. One hundred and one (18.5%) were found to have definite TBM based on positive CSF MGIT culture.

Table-1 displays the demographic data for those patients tested for LTA4H genotype stratified by TBM and HIV status. HIV-positive patients with TBM were significantly older than HIV-negative patients with TBM (*P* = 0.009). There was no significant difference in CD4 count between HIV-positive and HIV-negative patients with TBM. Thirty-six percent of HIV-infected TBM patients were diagnosed with HIV within a week of enrollment compared with 17% of non-TBM HIV-infected patients (*P* < 0.0001).

Table-2 demonstrates the overall prevalence of LTA4H genotypes and stratification by TBM and HIV status. There was no significant difference in prevalence of allele frequency from the overall group in comparison to each subgroup.

Figure-1 demonstrates risk factors for one-year mortality in patients with confirmed TBM. Due to the relative infrequency of the T/T genotype, we combined this group with the C/T genotype group for purposes of the analysis. The presence of the T allele is associated with a more inflammatory phenotype. Thus, the LTA4H analysis was composed of those without the T allele (C/C) vs. those with the T allele (C/T + T/T). LTA4H genotype was not associated with one-year mortality. Higher MRC stage and shorter duration of HIV diagnosis were significantly associated with higher odds of mortality.

Discussion:

The role of adjuvant steroids among HIV-infected and HIV-uninfected patients in sub-Saharan Africa remains unclear. Prior work has shown that the effect of steroids is partly mediated by the LTA4H gene with its greatest benefit in HIV-negative patients with the T/T genotype [8]. In this study of Zambian adults, TBM predominantly appears in HIV-positive patients with the C/C genotype. The T/T genotype appeared in 4 (4%) of TBM patients overall, one of who was HIV negative.

The seminal paper to demonstrate the benefit of adjuvant steroids in TBM from Vietnam occurred in a largely immunocompetent patient population [4]. The study was not powered to adequately demonstrate an effect on HIV-infected patients. Despite this, many countries recommend the use of steroids regardless of HIV status or stage of disease [12, 13].

HIV-associated TBM represents a different disease entity in comparison to HIV-uninfected TBM. HIV-associated TBM patients have a global increase in CSF cytokine levels [8]. The CSF cytokine and inflammatory profile appears to normalize at a CD4 count $\geq$ 150 cells/μL. At this CD4 cutoff, LTA4H genotype may play a role in TBM steroid responsiveness. This may have the

most relevance in HIV patients undergoing immune reconstitution. This represent 29% of our cohort with known CD4 counts. We did find low CD4 counts in the HIV-negative TBM which was likely due to *Mycobacterium tuberculosis* itself rather than underlying immunodeficiency [14].

The most common LTA4H profile in Africa is the C/C genotype [9]. This is associated with an inadequate immune response. It has been theorized that steroids may actually be harmful in this patient population [8]. We did not demonstrate any association between LTA4H genotype status and mortality. The infrequency of the T allele in this population and sample size was underpowered to detect the observed effect size but argues for consideration of modifying steroid treatment protocols in Zambia and similar settings.

The largest limitation of our study is the absence of data to indicate who received steroid treatment. While the national guidelines recommend steroid initiation for all patients with confirmed TBM it is certainly possible that some did not which could confound the outcome of mortality. In addition, the use of CSF culture as a gold standard is widely considered to be imperfect since it can be negative in TBM cases with a low bacilliary burden. We did not look at CSF inflammatory markers to confirm prior correlations of HIV and LTA4H status with cytokine levels.

The role of adjuvant steroids and the LTA4H genotype in the African population remains an open question. African-based prospective studies need to be undertaken to determine whether current guidelines regarding adjuvant steroids for TBM need to be reconsidered or modified in this population based upon HIV status, HIV severity and LTA4H profiles.

Acknowledgments: The authors thank the UTH microbiology lab for technical assistance and the patients and their families. This work was supported by the National Institutes of Health (grant numbers K23NS084054 to O. K. S. and P30AI 060354 to M.G.).

Author contributions:

OKS, GLB, HA, MA and IJK contributed to the conception and design of the study; OKS, MG, KM, XD, EM, SL, CB, BK, HA, MA, and IJK contributed to acquisition and analysis of data, XD, EM, SL, CB, BK, HA, MA, and IJK contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest:

Nothing to report.

References

1. Woldeamanuel YW, Girma B. A 43-year systematic review and meta-analysis: case-fatality and risk of death among adults with tuberculous meningitis in Africa. J Neurol. 2014 May;261(5):851-65.
2. UNAIDS. Zambia. 2018 [cited 2020 August 16]; Available from: https://www.unaids.org/en/regionscountries/countries/zambia.
3. Prasad K, Singh MB, Ryan H. Corticosteroids for managing tuberculous meningitis. Cochrane Database Syst Rev. 2016 Apr 28;4:CD002244.
4. Thwaites GE, Nguyen DB, Nguyen HD, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. N Engl J Med. 2004 Oct 21;351(17):1741-51.
5. Tobin DM, Roca FJ, Oh SF, et al. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell. 2012 Feb 3;148(3):434-46.
6. van Laarhoven A, Dian S, Ruesen C, et al. Clinical Parameters, Routine Inflammatory Markers, and LTA4H Genotype as Predictors of Mortality Among 608 Patients With Tuberculous Meningitis in Indonesia. J Infect Dis. 2017 Apr 1;215(7):1029-39.
7. WHO. Treatment of Tuberculosis. 2017 [cited 2020 August 19th]; Available from: https://apps.who.int/iris/bitstream/handle/10665/255052/9789241550000-eng.pdf?sequence=1.
8. Thuong NTT, Heemskerk D, Tram TTB, et al. Leukotriene A4 Hydrolase Genotype and HIV Infection Influence Intracerebral Inflammation and Survival From Tuberculous Meningitis. J Infect Dis. 2017 Apr 1;215(7):1020-8.
9. Genomes Project C, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012 Nov 1;491(7422):56-65.
10. Siddiqi OK, Birbeck GL, Ghebremichael M, et al. Prospective Cohort Study on Performance of Cerebrospinal Fluid (CSF) Xpert MTB/RIF, CSF Lipoarabinomannan (LAM) Lateral Flow Assay (LFA), and Urine LAM LFA for Diagnosis of Tuberculous Meningitis in Zambia. J Clin Microbiol. 2019 Aug;57(8).
11. Wilkinson RJ, Rohlwink U, Misra UK, et al. Tuberculous meningitis. Nat Rev Neurol. 2017 Oct;13(10):581-98.
12. Africa DoHRoS. National Tuberculosis Management Guidelines 2014. 2014 [cited 2020 August 20th]; Available from: https://health-e.org.za/wp-content/uploads/2014/06/NTCP_Adult_TB-Guidelines-27.5.2014.pdf.
13. WHO. Republic of Botswana Ministry of Health National Tuberculosis Programme Manual. 2007; 6th:[Available from: https://www.who.int/hiv/pub/guidelines/botswana_tb.pdf?ua=1.
14. Skogmar S, Schon T, Balcha TT, et al. CD4 cell levels during treatment for tuberculosis (TB) in Ethiopian adults and clinical markers associated with CD4 lymphocytopenia. PLoS One. 2013;8(12):e83270.

Figure 1 - Risk factors for one-year mortality

Table 1 - Demographic Data

Demographic	HIV-positive			HIV-negative		
	TB CSF culture positive (n=94)	TB CSF culture negative (n=387)	*P* value	TB CSF culture positive (n=7)	TB CSF culture negative (n=52)	*P* value
Age y, median (IQR)	35 (30-41)	36 (30-43)	0.26	25 (24-27)	42 (28-55)	< 0.0001
Gender, male (%)	62 (66%)	189 (48%)	0.003	3 (43%)	28 (54%)	0.88
CD4+ T cells/µL, median (IQR)	102 (45-165)	129 (42-331)	0.04	165 (142-264)	587 (593-851)	0.61
Prior TB diagnosis (%)	19 (21%)	168 (43%)	< 0.0001	0 (0%)	5 (10%)	0.46
Days since HIV Diagnosis, median days (IQR)	21 (4-240)	348 (30 - 1825)	< 0.0001	-	-	-
Taking ART (%)	45 (49%)	282 (73%)	< 0.0001	-	-	-
Duration of ART, median days (IQR)	90 (14-730)	730 (60-2099)	0.004	-	-	-
MRC TBM severity grade (%)						
Grade 1	13 (14%)	-		0 (0%)	-	-
Grade 2	41 (44%)	-		3 (42%)	-	
Grade 3	32 (34%)	-		2 (29%)	-	
Unknown*	8 (8%)	-		2 (29%)	-	
Inpatient Mortality (%)	41 (44%)	89 (23%)	0.0001	2 (29%)	14 (27%)	0.99
One-year Morality (%)	55 (59%)	165 (43%)	0.006	2 (29%)	24 (46%)	0.64

Table 2 - LTA4H genotype distribution

LTA4H genotype	Overall	HIV-positive		HIV-negative	
		TBM culture positive (n=94)	TBM culture negative (n=91)	TBM culture positive (n=7)	TBM culture negative (n=18)
C/C (%)	171 (81%)	78 (83%)	72 (79%)	6 (86%)	15 (83%)
C/T (%)	33 (16%)	13 (14%)	18 (20 %)	0 (0%)	2 (11%)
T/T (%)	6 (3%)	3 (3%)	1 (1%)	1 (14%)	1 (6%)