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Absolute eosinophil count correlates with temperature and CD4 count independently of HIV infection among tuberculosis patients

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Abstract

OBJECTIVE To determine clinical correlates of the peripheral absolute eosinophil count (AEC) among bacteriologically confirmed TB patients in Uganda.

MATERIALS AND METHODS We evaluated data of bacteriologically confirmed adult TB patients who had a peripheral blood AEC measurement at the National TB Treatment Center in Uganda during a cross-sectional study. We performed linear regression analysis for correlates of log-transformed AEC.

RESULTS We included 235 patients in this analysis with a median (interquartile range, IQR) age of 31 (24–39) years. 60.4% were male, and 33.6% had TB/HIV co-infection. In a multivariable linear regression model that controlled for age, residence type, HIV status, weight loss, anorexia, body mass index, CD8+ T-cell count, haemoglobin level and TB bacillary load, males had a 47.0% higher AEC than females (adjusted coefficient (R^2) = 0.385, 95% confidence interval (CI) 0.012–0.759 P = 0.043). Also, a 1 °C raise in temperature resulted in an 11.5% decrease in the AEC (R^2 = −0.122 95% CI (−0.233 to −0.011) P = 0.031) while a 1 cell/mm^3 increase in the CD4+ T-cell count resulted in a 0.10% increase in the AEC (R^2 = 0.001 95% CI (0.000–0.001) P = 0.032).

CONCLUSION The AEC was higher among males than females, consistent with the normal population distribution of AEC among Ugandans. The AEC was weakly but positively correlated with the CD4 count and negatively correlated with temperature.

keywords eosinophil count, CD4, tuberculosis, HIV, Uganda, symptoms, temperature, eosinophilia

Sustainable Development Goals (SDGs): SDG 3 (good health and well-being), SDG 5 (gender equity)

Introduction

Tuberculosis (TB) is the leading cause of mortality from a single infectious agent globally [1]. Host immune responses against *Mycobacterium tuberculosis* (Mtb) involve a complex interplay of effector functions of airway epithelial cells, macrophages, neutrophils, dendritic cells, natural killer cells, mast cells, CD4+ and CD8+ T lymphocytes [2]. The role of eosinophils in the host's immune response to Mtb is not well characterised in humans. In animal models and from *in vivo* studies, mycobacterial lung infection is associated with a chemotactic effect on eosinophils, which have been observed to phagocytise *Mycobacterium* in the lungs [3–5]. There is evidence to suggest that eosinophils promote severe mycobacterial disease. Eosinophils may contribute to severe disease by rapid accumulation and degranulation in broncheoalveolar lavage and granulomas after primary Mtb infection [6]. In *Mycobacterium bovis BCG-infected* interferon-gamma-deficient mice, a disease-promoting Th2-cytokine profile is observed that is characterised by increased production of interleukin 5, eosinophil infiltration of granulomas and elevated levels of IgE [7]. Moreover, the eosinophilia observed in peripheral blood and in the lungs after mycobacterial infection is associated with higher Mtb bacterial counts [8]. An evaluation of resected Mtb-infected lung tissue revealed that eosinophils were enriched in TB consolidations and walls of cavities [9].

In humans, evaluation of eosinophil counts among patients with Mtb infection has been mostly limited to case reports [10–12]. Larger studies have provided

conflicting results on the effect of the absolute eosinophil count (AEC) on Mtb infection. One prospective study involving an HIV cohort found that baseline eosinophilia was associated with incident TB (risk ratio of 2.65) although only half of the cases were bacteriologically confirmed [13]. Conversely, a study comparing TB patients and healthy controls found a lower peripheral AEC among TB patients (216 ± 134 vs. 320 ± 155 cells/μl) [14]. A low AEC also predicts Mtb culture positivity among sputum negative TB patients [15]. It is unclear whether the AEC modulates clinical manifestations of TB among bacteriologically confirmed cases. We aimed to determine the clinical correlates of the peripheral blood AEC among bacteriologically confirmed TB patients at the National TB Treatment Center in Uganda.

Materials and methods

Study population and settings

We analysed data from a cross-sectional study that enrolled bacteriologically confirmed TB patients at the National Tuberculosis Treatment Center (NTTC) in Uganda between August 2017 and March 2018 [16]. In this analysis, the inclusion criteria were adults (≥18 years) with bacteriologically confirmed TB who had AEC measurement. No participant that met the inclusion criterion was excluded. TB was confirmed by either a positive nucleic acid amplification test (Xpert MTB/RIF®) among 80% of participants or an auramine/Ziehl–Neelsen sputum smear (20%). The NTTC is a centre of excellence for diagnosis and management of drug sensitive and drug resistant TB in Uganda. It is a unit of the Mulago National Referral Hospital (MNRH) located in Kampala city, the capital city of Uganda.

Study measurements

We extracted participant data from the study data set pertaining socio-demographics, medical history, symptoms and signs, Mtb bacillary load, haemoglobin levels, HIV test result, CD4+ and CD8+ T-cell counts and the AEC. Socio-demographic data, medical history and TB symptoms were obtained through a face-to-face interview from TB patients by trained research assistants. Axillary temperature was measured using a digital thermometer (Royal Care® Model: MT 1027, SOJOY ELECTRONICS, China). The Mtb bacillary load was extracted from the laboratory result slip issued by a peripheral TB diagnostic laboratory and standardised as described in the primary study [16]. Full haemogram parameters were determined on peripheral blood using an automated haemoanalyser (Sysmex® XN series – XN 1000) at MNRH haematology laboratory. The AEC was calculated by multiplying the total white cell count by the percentage of eosinophils. The CD4+ and CD8+ T cell were determined by flow cytometry using a flow cytometer (BD FACSCalibur™) according to manufacturer's instructions [17]. HIV testing was conducted according to the Uganda national HIV testing guidelines [18]. Study methods of the primary study are described in detail elsewhere [16].

Statistical analysis

Data were analysed with STATA 15.1 (StataCorp, College Station, TX, USA). Categorical variables were summarised as counts and frequencies while continuous variables are presented as medians with the corresponding interquartile ranges (IQR). We performed log transformation of the AEC due to a skewed distribution. For categorical variables, we performed ANOVA test to determine the variance of the mean log-transformed AEC across sub-groups. For continuous variables, we performed simple linear regression analysis with the log-transformed AEC as the outcome variable. To determine the correlates of the AEC, we included all variables with $P \leq 0.2$ at bivariate analysis to fit the final multivariate linear regression model. Statistical significance was set at $P < 0.05$ at the 95% confidence interval (CI). Effect sizes of the predictor variables are reported as percentages changes in the AEC by converting the adjusted coefficient (R^2) using the formula: Percentage change = (exponent of $R^2 - 1) \times 100$ [19].

Ethical approvals and consent to participate

The study was approved by the School of Medicine Research and Ethics Committee of Makerere University College of Health Sciences (REC REF 2017-087). Participants provided written informed consent to participate in the primary study including the use of their data for secondary analyses.

Results

In this analysis, we included 235 bacteriologically confirmed TB patients who met the inclusion criteria.

Characteristics of study participants

The median (IQR) age of participants was 31 (24–39) years, and 66.9% were <35 years of age. Males were 142 (60.4%), and study participants were predominantly from urban areas (73.2%). The proportion of patients

with TB/HIV co-infection was 33.6% (79/235), of which 36 (45.6%) were taking anti-retroviral therapy (ART). Of the 188 participants with Xpert MTB/RIF® results, 28 (14.9%) had rifampicin resistance. History of previous TB treatment was reported by 44 (18.7%) participants, of whom 32 (72.7%) indicated that the treatment was within the preceding 2 years. The median (IQR) number of symptoms reported by study participants was 4 (3–5). The median (IQR) body mass index (BMI) was 18.7 (17.1–20.5), and 47.7% had a BMI < 18.5. The median (IQR) temperature was 36 °C (35.3–36.8 °C). Malaria co-infection was found in 6 (2.5%) participants. The median (IQR) haemoglobin level was 12.3 (10.5–13.9) g/dl while the median (IQR) CD4 T-cell count was 468 (265–741) cells/mm^3. Other characteristics of the study participants are shown in Table 1.

Clinical correlates of absolute eosinophil count

The median (IQR) AEC was 70 (20–160) cells/μL. In a multivariable linear regression model that controlled for age, residence type, HIV status, weight loss, anorexia, body mass index, CD8+ T-cell count, haemoglobin level and TB bacillary load, males had a 47.0% higher AEC than females (adjusted coefficient (R^2) = 0.385, 95% confidence interval (CI) 0.012–0.759 P = 0.043). Also, a 1 °C raise in temperature resulted in an 11.5% decrease in the AEC (R^2 = −0.122 95% CI (−0.233 to −0.011) P = 0.031) while a 1 cell/mm^3 increase in the CD4+ T-cell count resulted in a 0.10% increase in the AEC (R^2 = 0.001 95% CI (0.000–0.001) P = 0.032). The bacillary load grade did not significantly correlate with the AEC (Figure 1). The multivariable linear regression model is shown in Table 2.

Discussion

In this study, we evaluated the clinical correlates of the AEC among bacteriologically confirmed TB patients at a tertiary TB referral facility in Uganda. We found that males had a higher AEC than females and that the AEC decreased with an increase in the patients' temperature. The AEC increased slightly with an increase in the CD4+ T-cell count regardless of HIV status. Our findings fill a knowledge gap with regard to clinical correlates of the AEC among TB patients.

Ugandan males inherently have a significantly higher AEC than females [20]. We suppose that our study finding resonates with this fact rather than a unique observation among TB patients. Similar to our study findings, the AEC has been reported to increase with CD4 T-cell count albeit among HIV-positive individuals with TB

Table 1 Other characteristics of the study population

Patient characteristics	Frequency (N = 235)	Percentage
Symptoms		
Cough	232	98.7
Median duration (IQR) days	60 (30–90)	
Fever	145	61.7
Median duration (IQR) days	14 (7–30)	
Weight loss	171	72.8
Median duration (IQR) days	30 (14–60)	
Chills	104	44.3
Median duration (IQR) days	14 (7–30)	
Night sweats	168	71.5
Median duration (IQR) days	21 (10–30)	
Headache	86	36.6
Median duration (IQR) days	7 (3–14)	
Anorexia	82	34.9
Median duration (IQR) days	14 (7–30)	
Other symptoms	19	8.1
Median duration (IQR) days	41 (7–90)	
Number of symptoms		
<4	69	29.4
≥4	166	70.6
Bacillary load†		
Very low	28	11.9
Low	55	23.4
Medium	78	33.2
Very high	67	28.5
Missing	7	3.0
CD4 count (cells/mm^3)		
<418	97	41.3
418–2015*	138	58.7
CD8 count (cells/mm^3)		
<256	50	21.3
256–1619*	185	78.7
White blood cell count‡, median (IQR)	6.74 (4.8–9.34)	
Neutrophil count‡, median (IQR)	4.5 (2.9–6.6)	
Lymphocyte count‡, median (IQR)	1.4 (1.0–1.8)	
Basophil count, median (IQR)	0.0 (0.0–0.1)	
Monocyte count‡, median (IQR)	0.6 (0.4–0.9)	
Red blood cell count§, median (IQR)	4.8 (4.2–5.5)	
Haematocrit (%), median (IQR)	38.6 (32.7–43.5)	
Mean corpuscular volume (fl), median (IQR)	80.6 (72.4–88.9)	
Mean corpuscular haemoglobin (pg), median (IQR)	25.6 (23.2–28.3)	
Platelete count‡, median (IQR)	368 (249–467)	

IQR, Interquartile range; fl, femtolitres; pf, picograms.
*Normal range for adult Ugandans [31].
†Grading of bacillary load is described in primary study [16].
‡$\times 10^3$ per microlitre.
§$\times 10^6$ per microlitre.

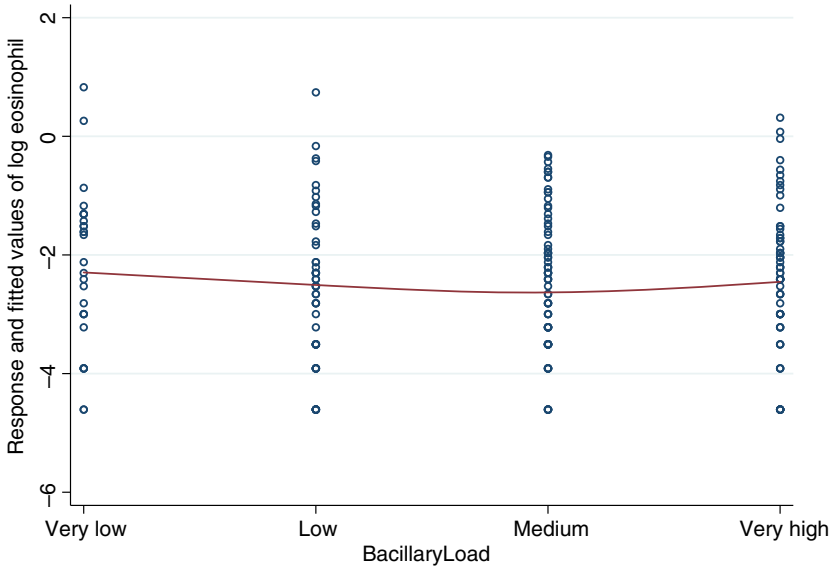

Figure 1 Multivariable linear regression analysis graph showing relationship between bacillary load grade and log of the absolute eosinophil count. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Multivariable linear regression model for correlates of the absolute eosinophil count among tuberculosis patients

Characteristic	Crude coefficient 95% confidence interval (CI)	*P*-value	Adjusted coefficient (95% CI)	*P*-value
Sex				
Female	0 (ref)		0 (ref)	
Male	0.236 (−0.092–0.564)	0.157	0.385 (0.012–0.759)	0.043
Resident type				
Urban	0 (ref)		0 (ref)	
Rural	0.288 (−0.074–0.650)	0.118	0.181 (−0.172–0.533)	0.314
CD8+ T-cell count	0.001 (−0.000–0.001)	0.076	0.001 (−0.001–0.001)	0.161
CD4+ T-cell count	0.001 (0.001–0.002)	<0.001	0.001 (0.000–0.001)	**0.032**
HIV status				
Negative	0 (ref)		0 (ref)	
Positive	−0.447 (−0.783–−0.111)	0.009	−0.249 (−0.681–0.184)	0.258
Temperature	−0.156 (−0.266–−0.045)	0.006	−0.122 (−0.233–−0.011)	**0.031**
Anorexia				
Yes	0 (ref)		0 (ref)	
No	0.247 (−0.090–0.583)	0.150	0.153 (−0.184–0.491)	0.371
Body mass index	0.051 (−0.001–0.103)	0.055	0.044 (−0.011–0.099)	0.118
Bacillary load				
Very low	0 (ref)		0 (ref)	
Low	−0.433 (−1.009–0.143)	0.140	−0.283 (−0.851–0.284)	0.326
Medium	−0.294 (−0.840–0.253)	0.291	−0.054 (−0.590–0.482)	0.842
Very high	−0.172 (−0.730–0.387)	0.545	−0.096 (−0.661–0.469)	0.739
Haemoglobin level	0.078 (0.016–0.141)	0.014	0.004 (−0.070–0.079)	0.906
Weight loss				
No	0 (ref)		0 (ref)	
Yes	−0.434 (−0.792–−0.077)	0.017	−0.261 (−0.643–0.121)	0.180

Bold values indicates statistically significant.

[21]. The multivariable model in our study suggests that this relationship is independent of the HIV status. The clinical significance of the slight increment in AEC for a unit rise in CD4 T-cell count observed among TB patients in our study is unclear. Nonetheless, CD4 T cells recruit and activate eosinophils in lung infections resulting in profound pulmonary pathology [22]. It is therefore desirable to further characterise the relationship between the eosinophil count and radiological chest findings among patients with pulmonary tuberculosis.

Although eosinophilia among TB patients negatively correlates with fever as a symptom [23], we have not found a report on the variation of the AEC with temperature as observed in our study. Our study finding is expected because TB patients have a predominant Th1 immune response against Mtb that is characterised by pyrogenic cytokines as opposed to a Th2 response that engenders eosinophilia [24, 25]. Conversely, Boneberg and Hartung (2003) demonstrated a 3-fold rise in Th2 cytokines and a 15% reduction in Th1 cytokines at high temperatures (40 °C) using staphylococcal antigens [26]. The median temperature of our study population was normal, and it is not known if Mtb antigens would induce similar immune responses at high body temperatures as the LPS and staphylococcal enterotoxin B used in the aforementioned study.

We did not observe a significant relationship between the bacillary load and the AEC as was reported by Kirman *et al.* [8]. Notably, their observation of an increased bacillary load was among interferon-gamma (IFNγ)-deficient mice and thus non-comparable to our study population.

We were unable to determine the proportion of patients with disseminated forms of TB from the data available. This is important to control for since disseminated forms of TB favour Th2 immune responses [27]. Another limitation of our study is the lack of data on helminthic infection among the TB patients in our study population. Helminth co-infection among TB patients has been associated with eosinophilia and could have been a potential confounder especially in a low-income country [28]. However, the association of helminth co-infection with eosinophilia among TB patient is not consistently reported in literature with Resende *et al* reporting no significant difference in eosinophil counts between co-infected patients and those without helminths [29]. Moreover, our study population was predominantly adult urban patients who inherently have a very low prevalence of helminth infection [30]. Lastly, due to the paucity of literature about the AEC and tuberculosis, some unknown confounders may not have been accounted for. Our study has identified temperature, gender and the CD4 count as correlates, and therefore contributes to literature that can be used by subsequent studies to adjust for confounders.

Conclusion

The AEC among bacteriologically confirmed TB patients positively correlates with the CD4 count but negatively correlates with temperature. The clinical utility of the AEC needs further evaluation.

Data Availability Statement

Data sets generated and/or analysed during this study are available from the corresponding author upon reasonable request.

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