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Elevated Aspergillus-specific antibody levels among HIV infected Ugandans with pulmonary tuberculosis

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Background

Tuberculosis (TB) remains one of the major causes of morbidity and mortality worldwide with the highest burden found in Africa and Asia, mainly linked to the HIV epidemic [1]. The 2016 WHO report on TB revealed that there were an estimated 10 million new cases of TB in the year 2015 worldwide, with 11% having HIV. An estimated 1.8 million people died due to TB in 2015, including 0.4 million deaths due to HIV/TB co-infection. The incidence of TB among HIV patients in Uganda was estimated at 202 new cases per 100 000 population [2].

Due to the limited published data on fungal disease epidemiology in sub-Saharan Africa, a recent review attempted to estimate the burden of fungal infections in Uganda using specific populations [3]. In this review, chronic pulmonary aspergillosis (CPA) was estimated at 12-22% in TB patients with cavities and 1-4% in those without cavities. Considering post-TB data in Uganda, asymptomatic CPA was estimated at 7% with an additional 1.7% having detectable *Aspergillus*-specific IgG antibodies with cavitation.

Recent work done in Northern Uganda has validated some of these estimates, with a CPA prevalence of 8.2% and 6.7% having cavities among patients who had been successfully treated for pulmonary TB within the last 7 years [4]. More results from this work showed *Aspergillus*-specific IgG antibody levels were raised in 26% of patients with "smear negative TB" and suggested that previously unrecognized CPA might be responsible for significant mortality in patients treated for TB in Uganda [5]. Beyond this limited data, little is known about the epidemiology of fungal colonisation and sensitisation, and their contribution to TB disease progress and treatment outcomes in Uganda where pulmonary TB is very common, in part driven by the high prevalence of HIV [6].

We hypothesized that patients with pulmonary TB may get colonized with *Aspergillus* during and in the post treatment period leading to chronic lung infection and/or allergic fungal disease if the patient was pre-sensitized to Aspergillus antigens. Pulmonary cavitation is a pre-disposing factor for CPA [7] and may or may not be for *Aspergillus* sensitization

Objective of the study

We therefore aimed to establish and compare *Aspergillus*-specific antibody levels among HIV-infected Ugandans with TB, at the beginning and end of TB treatment; using ImmunoCAP® and Immulite® immunoassays.

Methods

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We retrieved and tested paired serum aliquots for 101 HIV-TB co-infected patients at the beginning and week 24 of TB treatment. We tested samples for *Aspergillus*-specific IgG and IgE using ImmunoCAP; and *Aspergillus*-specific IgG and total serum IgE using Immulite immunoassays. We compared antibody levels between baseline and week 24, relating them to selected baseline characteristics.

Summary of findings

10% of the patients had elevated *Aspergillus*-specific IgE (*Aspergillus* sensitization) and *Aspergillus*-specific IgG antibodies were elevated in 9% of the patients at the end of TB treatment. There was a significant fall in the *Aspergillus*-specific IgG antibody levels between baseline and week 24 (P=0.02). Patients with CD4 T-cell count <100 cells/µl and those who were not on anti-retroviral therapy at baseline had more elevated *Aspergillus*-specific IgG antibodies (P=0.01, P=0.03). The ImmunoCAP *Aspergillus*-specific IgG antibody titres were higher at week 24 than baseline with more positives at week 24; even though the difference in means was small. However, this difference was statistically significant (P=0.02). Pulmonary infiltrates were the commonest x-ray abnormality and only 5% of the patients had pulmonary cavities on chest x-ray at week 24.

Study limitations

1. We found a major challenge in defining which diagnostic cut-offs to use for *Aspergillus*-specific IgG antibodies in both ImmunoCAP and Immulite. Previously published cut-offs range in 10 to 50 mg/l in similar populations.

2. We did not have a control group for comparison of these antibody titers. This would possibly give more useful information.

Conclusions

In conclusion, *Aspergillus*-specific antibody levels were significantly raised in patients being managed for pulmonary tuberculosis. This colonization and/or sensitization by *Aspergillus* antigens may complicate disease progression and treatment outcomes among TB patients. Detection of *Aspergillus* antibodies is an indispensable tool in the diagnosis and management of the patients with pulmonary aspergillosis. More epidemiological studies are needed to explore and expand the utility of *Aspergillus* antibody testing in resource limited setting.

References

1. Keeler E, Perkins MD, Small P, Hanson C, Reed S, Cunningham J, Aledort JE, Hillborne L, Rafael ME, Girosi F: **Reducing the global burden of tuberculosis: the contribution of improved diagnostics.** *Nature* 2006, 444:49-57.

2. WHO: **GLOBAL TUBERCULOSIS REPORT.** 2016.

3. Parkes-Ratanshi R, Achan B, Kwizera R, Kambugu A, Meya D, Denning D: **Cryptococcal disease and the burden of other fungal diseases in Uganda; Where are the knowledge gaps and how can we fill them?** *Mycoses* 2015, 58(S5):85-93.

4. Page I, Onyachi N, Opira C, Opwonya J, Odongo-Aginya E, Mockridge A, Byrne G, Richardson M, Denning D: **Chronic pulmonary aspergillosis (CPA) is likely to be a common complication of pulmonary tuberculosis: initial results of a cross-sectional survey.** In: *19th Congress of the International Society for Human and Animal Mycology.* 2015.

5. Page ID, Worodria W, Andama A, Ayakaka I, Davis L, Huang L, Richardson M, Denning DW: **Pulmonary aspergillosis may be common in AIDS with smear negative tuberculosis**. In: *CROI.* Boston, Massachusetts; 2016.

6. Shafer LA, Biraro S, Nakiyingi-Miiro J, Kamali A, Ssematimba D, Ouma J, Ojwiya A, Hughes P, Van der Paal L, Whitworth J: **HIV prevalence and incidence are no longer falling in southwest Uganda: evidence from**

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a rural population cohort 1989–2005. *AIDS* 2008, 22(13):1641-1649.

7.Dhooria S, Kumar P, Saikia B, Aggarwal A, Gupta D, Behera D, Chakrabarti A, Agarwal R: Prevalence of ***Aspergillus*** **sensitisation in pulmonary tuberculosis-related fibrocavitary disease**. *The International Journal of Tuberculosis and Lung Disease* 2014, 18(7):850-855.

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