Infectious diseases now 54 (2024) 104936

Contents lists available at ScienceDirect

Infectious Diseases Now

journal homepage: www.sciencedirect.com/journal/infectious-diseases-now

Pooled sample testing strategy for *Aspergillus* IgG-IgM serology in Uganda: A proof-of-concept and cost-effectiveness analysis

To The Editor,

Pulmonary tuberculosis (PTB)-related chronic pulmonary aspergillosis (CPA) is an emerging global health problem, with an estimated annual incidence of about 1.8 million cases resulting in 340, 000 deaths [1]. Screening for CPA is essential and urgent in both TB and post-TB clinics. In a TB clinic, this includes patients with active, mycobacteriologically confirmed PTB who are on treatment but not showing significant clinical improvement, as well as those with a clinical or radiological PTB diagnosis. post-TB clinic, screening for CPA is vital for patients in whom PTB relapse is suspected.

Aspergillus antibodies are elevated in over 90 % of patients with CPA and are considered the cornerstone for CPA diagnosis [2,3]. The LDBio *Aspergillus* IgG-IgM immunochromatographic test (ICT) is the only commercially available assay with 88.9–91.6 % sensitivity and 96.3–98 % specificity comparable to the ELISA tests presently in use [2,3]. Point of care *Aspergillus*-specific IgG ICT, also known as lateral flow assay, has revolutionized screening and diagnosis of CPA in resource-limited settings [4].

An estimated 87,360 Ugandans develop TB every year, including 27,840 HIV- positive cases, entailing TB mortality of 12,000 including 5,952 PLWH cases, with 43 % involving clinically diagnosed TB [5]. From these data, we estimated that 26,765 CPA cases appear yearly, with, while over a 5-year-period, 63,574 cases appear [6].

In Uganda, three previous studies indicated elevated *Aspergillus*-specific IgG levels in approximately 5 to 9 % of cases in the active and previously treated PTB populations [7–9]. With this relatively low positivity rate, approximately 9 out of 10 tests are expected to yield negative results. To optimise effective use of this vital screening test, we conducted a proof-of-concept pooled sample testing strategy, along with a cost-effectiveness analysis for the LDBio *Aspergillus* IgG-IgM testing.

In February and March 2024, we enrolled the first 100 participants into a multi-centre, prospective cohort study (the CPA_OPTIONS_Study) approved by the Gulu University Research Ethics Committee (#GUREC-2023–717). All participants were 18 years or older, with current or previously treated PTB, and they provided written informed consent.

The LDBio ICT was performed as follows: 15 µL of serum sample followed by four drops of the elution solution were aliquoted into the cassette sample well. The results were read over 30 min, and time to positivity was read after adding the elution solution. The result was considered positive if a grey or black line was visible under the "T" marker; otherwise, the sample was considered negative. The test result was considered invalid if the blue "C" line did not appear.

Individual testing for qualitative *Aspergillus* IgG-IgM detection was conducted for all 100 samples according to the manufacturer's instructions. In parallel, we carried out pooling of samples, by aliquoting 15 µL of the individually tested serum samples in a 1.5-mL microcentrifuge tubes in batches to form the different pools, which were thoroughly mixed by vortexing.

Cost-effectiveness analysis was carried out by calculating the number of tests saved by pooling of samples and economic evaluation using the cost-effectiveness ratio and cost per pooled sample testing. We estimate the cost of performing one LD Bio *Aspergillus* IgG-IgM ICT in Uganda at $15, including the cost of the kit, consumables, and human resources.

Out of the 100 samples, 7 (7.0 %) were positive for *Aspergillus* IgG-IgM. Using the Dorfman's formula to estimate the pool size of an ideal test [10], n (pool size) $= 1/\sqrt{[Prevalence]}$, a pool size of 4 was envisaged; however, since the sensitivity of the kit is less than 100 %, we opted for a pool size of 5. Five pools of five unique samples each (one positive and four negative) all yielded a positive test, with strong lines: see Fig. 1. A trial with a pool size of 10 samples (one positive and nine negative) also yielded a positive test with strong lines. A pool of 10 negative serum samples yielded a negative test (negative control).

For 100 samples, 20 pools of 5 samples each are required, and with seven positive individually tested samples, a maximum of seven pools would be positive. Therefore, for a pool size of 5, 55 kits (20 kits for the 20 pools + additional 35 (7 x5) for the individual testing of samples from the positive pools) are to be used. We would allow a saving of 45 kits. Meanwhile, for a pool size of 10, 80 kits (10 kits for the 10 pools + additional 70 (7*10) for the individual testing of samples from the positive pools) would be used, thereby saving 20 kits.

Therefore, individual testing of 100 serum samples would cost about $1,500 (100 * $15 per sample) and pooled testing would cost $825 (55 * $15 per sample), with a 45 % cost reduction (1-825/1500) for pools of size 5 and $1,200 (80 * $15 per sample), with a 20 % cost reduction (1-1, 200/1500) for pools of size 10, with a cost-effectiveness ratio of $8.25 per test, and 12.5$ per test, respectively.

Therefore, pooled testing strategy for the detection of *Aspergillus*-specific IgG may be effective and cost-effective; if widely validated, it would significantly expand CPA surveillance in TB clinics particularly in low-and middle-income countries with the most unmet needs for *Aspergillus* serology. Determination of baseline *Aspergillus* IgG-IgM positivity rate in an area is essential to establish to the establishment of pool sizes sufficient to guide large-scale pooled serological screening for CPA in the clinical laboratories.

1. Availability of data and materials

All relevant data are within the article and its supporting information files. Data are available upon reasonable request from the first author.

https://doi.org/10.1016/j.idnow.2024.104936
Received 30 April 2024; Accepted 3 June 2024
Available online 7 June 2024

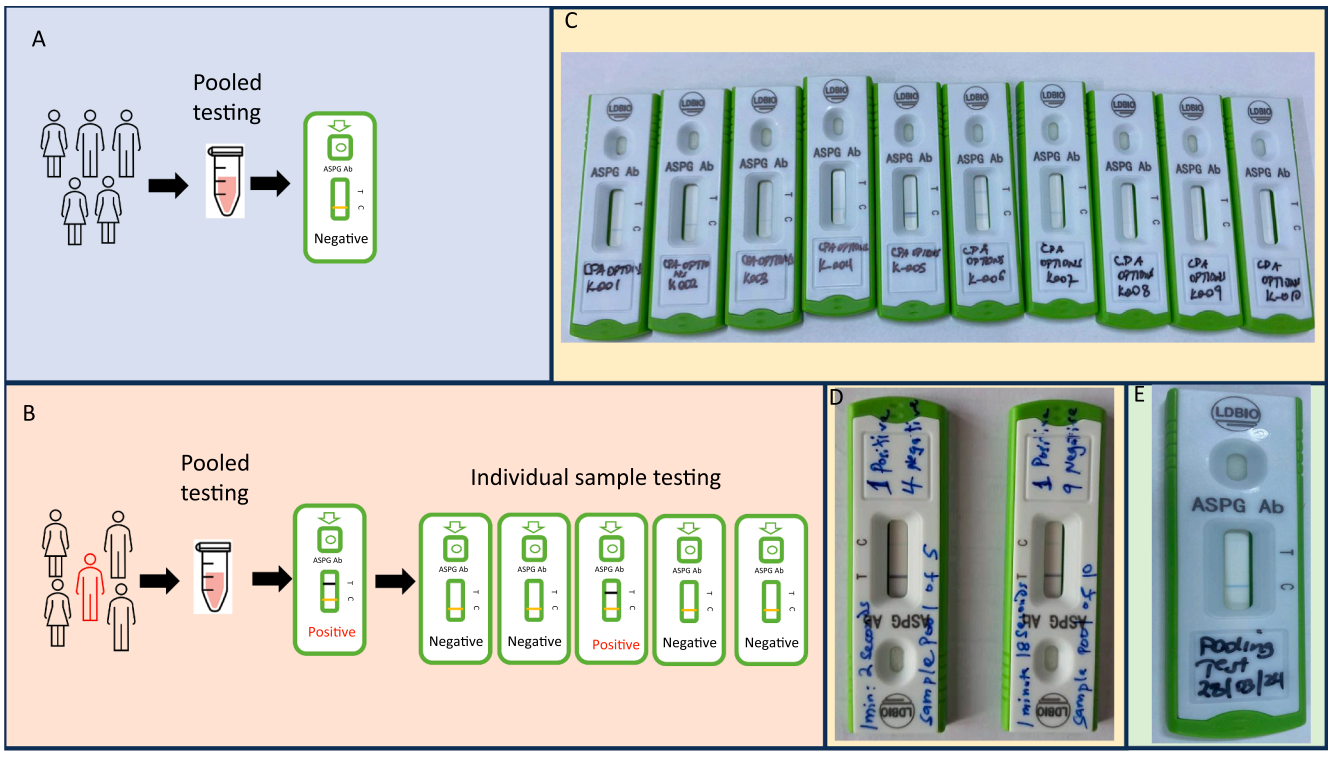

Fig. 1. Illustration of pooled sampling strategies consisting of pool size of five with negative *Aspergillus* IgG-IgM ICT (A) following pooling of five sera from five participants. For a positive test after pooling, all sera are to be individually tested (B). A panel of 10 individually tested samples from a single center with one positive result –Koo6 (C). An example of a pooled test results of pool sizes 5 and 10, both with strong positive lines (D), a negative test after pooling 10 negative sera (E).

Funding

Dr Felix Bongomin is a PhD student at the University of Manchester, United Kingdom. His work is supported by the Carigest SA Conny Naeva Charitable Foundation as part of the "Chronic Pulmonary Aspergillosis: Optimization of Therapy, Immunogenetic Screening, and Diagnosis in Uganda [CPA_OPTIONS_Uganda]" project. CARIGEST SA did not play any role in the design, implementation, and analysis of the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to thank Tobius Odongo and Paul Sempiira for their contributions in data collection and laboratory analysis, as well as the different study participants.

Ethical statement

The study protocol was approved by the Gulu University Research Ethics Committee (#GUREC-2023-717). All study participants provided written informed consent.

References

[1] Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis 2024;S1473–3099:00692–8. https://doi.org/10.1016/S1473-3099(23)00692-8.

[2] Stucky Hunter E, Richardson MD, Denning DW. Evaluation of LDBio Aspergillus ICT Lateral Flow Assay for IgG and IgM antibody detection in chronic pulmonary aspergillosis. J Clin Microbiol 2019;57:e00538–619. https://doi.org/10.1128/JCM.00538-19.

[3] Piarroux RP, Romain T, Martin A, Vainqueur D, Vitte J, Lachaud L, et al. Multi-center evaluation of a novel immunochromatographic test for anti-aspergillus IgG detection. Front Cell Infect Microbiol 2019;9:1–7. https://doi.org/10.3389/fcimb.2019.00012.

[4] Kwizera R, Katende A, Teu A, Apolot D, Worodria W, Kirenga BJ, et al. Algorithm-aided diagnosis of chronic pulmonary aspergillosis in low- and middle-income countries by use of a lateral flow device. Eur J Clin Microbiol Infect Dis 2020;39. https://doi.org/10.1007/s10096-019-03782-x.

[5] World Health Organization. Uganda Tuberculosis Profile 2021. www.who.int/tb/data (accessed April 20, 2024).

[6] Bongomin F, Kwizera R, Namusobya M, van Rhijn N, Andia-Biraro I, Kirenga BJ, et al. Re-estimation of the burden of serious fungal diseases in Uganda. Ther Adv Infect Dis 2024;11:1–12. https://doi.org/10.1177/20499361241228345.

[7] Kwizera R, Parkes-Ratanshi R, Page ID, Sekaggya-Wiltshire C, Musaazi J, Fehr J, et al. Elevated Aspergillus-specific antibody levels among HIV infected Ugandans with pulmonary tuberculosis. BMC Pulm Med 2017;17:149. https://doi.org/10.1186/s12890-017-0500-9.

[8] Namusobya M, Bongomin F, Mukisa J, Olwit WK, Batte C, Mukashyaka C, et al. Chronic pulmonary aspergillosis in patients with active pulmonary tuberculosis with persisting symptoms in Uganda. Mycoses 2022;65:625–34. https://doi.org/10.1111/myc.13444.

[9] Page ID, Byanyima R, Hosmane S, Onyachi N, Opira C, Richardson M, et al. Chronic pulmonary aspergillosis commonly complicates treated pulmonary tuberculosis with residual cavitation. Eur Respir J 2019;53:1801184. https://doi.org/10.1183/13993003.01184-2018.

[10] Dorfman R. The detection of defective members of large populations. Ann Math Stat 1943;14:436–40. https://doi.org/10.1214/aoms/1177731363.

Felix Bongomin[a,b,c,*], Quinto Ogwang[c], Shamim Katusabe[d], Claudine Mukashyaka[e], Joseph Baruch Baluku[d], Martha Namusobya[e], Norman van Rhijn[a], David W. Denning[a]

[a] *Manchester Fungal Infection Group, Division of Evolution, Infection and Genomics, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom*

[b] *Department of Medical Microbiology and Immunology, Faculty of Medicine, Gulu University, Gulu, Uganda*

[c] *Gulu Regional Referral Hospital, Gulu, Uganda*

[d] *Division of Pulmonology, Kiruddu National Referral Hospital, Kampala, Uganda*

[e] *Clinical Epidemiology Unit, College of Health Sciences, Makerere University, Kampala, Uganda*

[*] Corresponding author at: Department of Medical Microbiology and Immunology, Faculty of Medicine, Gulu University, P. O. Box 166, Gulu, Uganda.
E-mail address: drbongomin@gmail.com (F. Bongomin).