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High rate of chronic pulmonary aspergillosis in active TB patients in Indonesia with GenXpert positive

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Objectives: Chronic pulmonary aspergillosis (CPA) commonly affects post-tuberculosis (TB) patients. However, the incidence of this slowly destructive disease is also increasingly being reported in patients with active TB. This study aimed to identify the clinical, radiological, and serological characteristics of probable CPA patients both with proven TB with GenXpert positive and clinically diagnosed TB (GenXpert negative) in early TB therapy.

Methods: All patients were on antituberculosis therapy in the early phase (6-2 months). Clinical profiles and radiology findings were collected from hospitals in Jakarta and Depok, Indonesia. Aspergillus antibodies were measured using indirect ELISA assay. Specimen was tested with GenXpert MTBDR same.

Results: A total of 70 patients with a median age of 37 years were studied. Overall, 29% (n=20) met the criteria for proven or probable CPA. The rate of CPA in GenXpert positive patients was 78%, whereas it was only 14% in GenXpert negative patients (P = 0.01). Most of CPA patients had GenXpert result intermediates (n=7, 13%), 4 of them (8%) had a high-level of TB resistance by GenXpert. Cough was the most common symptom in GenXpert positive groups (n = 11, 39%). Fatigue was frequently found in GenXpert negative groups (n = 6, 13%). The proportion of patients with positive test results was significantly higher in CPA patients. The median score of Aspergillus antibody in non-CPA patients was lower (77, range 3.1-13.2) ng/mL compared with CPA patients (119, range 4.5-144 ng/mL) (P = 0.02)

Conclusion: Aspergillus gallopodi is a fungal pathogen that might cause co-infection in active TB patients. The measurement of Aspergillus antibody is a hallmark for the diagnosis of CPA. The difference of CPA in active TB patients is underdiagnosed.
Figure 2. Analytical performance of IMMY anti-Sporothrix antibodies LFA to the clinical form and cross-reactivity analysis

| Clinical form          | Sensitivity | 95% CI          |
|------------------------|-------------|-----------------|
| Lymphocutaneous (n=59) | 83.05%      | 71.03%-91.56%   |
| Fixed cutaneous (n=27) | 77.78%      | 57.74%-91.38%   |
| Ocular (n=13)          | 92.31%      | 63.97%-99.81%   |
| Mixed form (n=1)       | 100.00%     | 2.50%-100.00%   |

Cross-reactivity analysis:

| Disease                  | Number of samples | % positive |
|--------------------------|-------------------|------------|
| Healthy volunteers        | 100               | 26.0% (26/100) |
| Cryptococcosis            | 32                | 6.3% (2/32)  |
| Candidemia                | 27                | 14.8% (4/27) |
| Paracoccidioides         | 14                | 14.3% (2/14) |
| Aspergilosis             | 10                | 0% (0/10)   |
| Histoplasmosis           | 9                 | 20% (2/10)  |
| Fusariosis               | 4                 | 0% (0/4)    |
| Lobomycosis              | 1                 | 0% (0/1)    |
| Chromoblastomycosis      | 1                 | 0% (0/1)    |
| Trichosporonosis         | 1                 | 100% (1/1)  |
| Mucormycosis             | 1                 | 0% (0/1)    |

**Methods:**
1. A total of 8 isolates of Microsporum gypseum, 29 isolates of Trichophyton spp., 3 isolates of Nannizzia gypsea, 4 isolates of non-dermatophyte filamentous fungi, Malassezia spp., and Candida spp. were included in the study.
2. Primer Express Software (V3.0) was used to design specific primers and TaqMan probe for qRT-PCR assay. The specificity of each system was validated using the above fungal isolates. The standard curve of each system was constructed by using the DNA of the standard substance of fungal isolates to find the minimum detection.
3. The clinical specimens from confirmed and suspected patients with tinea capitis were collected. Fungal DNA was extracted from clinical samples and detected by a two-step qRT-PCR system. The results of qRT-PCR were analyzed comprehensively and compared with a fungal microscopic and fungal culture.
4. As a supplementary interpretation, the next generation sequencing targeted amplicon was conducted in 14 clinical samples that generated objective results between fungal culture and qRT-PCR.

**Results:**
1. The molecular diagnostic system for tinea capitis herein consists of seven single-tube qRT-PCR assays designed on the complex and species level, which include the group of M. canis complex, T. rubrum complex, T. mentagrophytes complex, T. tonsurans, T. schoenleinii, T. verrucosum, and N. gypsea. The analytical specificity of each group meets the design expectation.
2. The minimum detection limit of the M. canis complex group, T. rubrum complex group, T. schoenleinii group, and N. gypsea group was 100 Colony Forming Units (CFUs), the counterpart of which for T. mentagrophytes complex group, T. tonsurans and T. verrucosum were 10 CFUs.
3. A total of 535 clinical specimens were collected, including 231 cases confirmed of tinea capitis, 100 suspected of tinea capitis, and 20 with non-tinea capitis. Positive fungal microscopy and/or fungal culture were the gold criteria for diagnosis. Compared with the diagnostic gold standard, the sensitivity and efficacy of qRT-PCR, the combination of qRT-PCR and fungal microscopy, the combination of qRT-PCR and fungal culture were 95.1% and 93.6%, 100% and 100%, and 96.1% and 96.4%, respectively. The diagnostic specificity for cases of non-tinea capitis was 100%. The coincidence rate between qRT-PCR and fungal culture was 95.14%. The positive rate of qRT-PCR in suspected cases was 68%.
4. Amplification sequencing results confirmed that dermatophytes existed in 13 of 14 samples. Consistent with qRT-PCR, there were two species of dermatophytes mixed infection in 4 samples.

**Conclusions:** The seven single-tube qRT-PCR assays validated in this study can rapidly detect a variety of pathogenic fungi causing tinea capitis, with a high level of sensitivity and specificity. The combination of qRT-PCR and traditional mycological identification methods can further improve the diagnostic efficacy of tinea capitis.