Diagnostics to support mycetoma management – Development of two Target Product Profiles

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Sustainable Development Goal: Good Health and Wellbeing

Abstract

Objective: Mycetoma is a neglected tropical disease caused by more than 70 different micro-organisms and identified by WHO as one of the high-priority diseases for developing diagnostic tests. To ensure production of diagnostic assays for use by clinical staff in endemic regions, Target Product Profiles (TPP) were designed.

Methods: We describe the development of two TPPs: one for a diagnostic test able to identify the causative agent of mycetoma and another which would determine when treatment could be stopped. The TPPs were developed by considering product use, design, performance, product configuration and costs.

Results: Version 1.0 TPPs for two uses were posted by WHO for a one-month online public consultation on 25 October 2021 and the final TPP was posted online on 05 May 2022.

Conclusion: A major difficulty encountered in developing both TPPs was the large number of agents able to cause mycetoma and the lack of specific biomarkers for most of them.

Keywords: Target Product Profile; WHO; mycetoma; actinomycetoma; eumycetoma; point of care test

Introduction

Mycetoma is a chronic granulomatous infection which causes subcutaneous tumour-like lesions (1). In most cases, the foot is the affected body part, followed by the hand, legs and back (1, 2). Mycetoma can be caused by at least 70 micro-organisms of fungal or bacterial origin (2). Fungal mycetoma, or eumycetoma, is most often caused by *Madurella mycetomatis* followed by *Scedosporium boydii* and *Falciformispora senegalensis* (2). Bacterial mycetoma or actinomycetoma, due to aerobic actinomycetes, is most often caused by *Actinomadura madurae, Actinomadura pelletieri, Nocardia asteroides, Nocardia brasiliensis* and *Streptomyces somaliensis* (2). Mycetoma is reported in 102 countries, and the aetiology differs by region (2). *M. mycetomatis, S. somaliensis* and *A. pelletieri* are highly prevalent in Africa and Asia but rarely encountered in Latin America. *N. brasiliensis* is by far the most common causative agent in Latin America. However, this species is very rarely encountered in the rest of the world. Only *A. madurae* is globally prevalent (2).

A hallmark of mycetoma is that the causative agent organises itself in granules called grains which can be secreted through sinuses (1). The colour of the grain is dependent on the causative agent. Eumycetoma causative agents generally form black (*M. mycetomatis, F. senegalensis*) or pale (*S. boydii*) grains (1), while actinomycetoma causative agents can cause white (*Nocardia* spp, *Actinomadura madurae*), yellow (*Streptomyces* spp) or red (*Actinomadura pelletieri*) grains (1).

Although mycetoma is divided into actinomycetoma and eumycetoma based on the causative agent, the clinical presentation is virtually identical with only minor differences. In both cases, the infection starts with a small painless nodule (1, 3). This is usually at the site where the micro-organism was introduced months earlier into the subcutaneous tissue via a minor trauma such as a thorn prick. With time, this painless nodule will grow into a larger subcutaneous mass. Eventually, sinuses which discharge grains, purulent or seropurulent material will develop (1). In advanced lesions, the micro-organism will also invade the bone (1). In general, actinomycetoma can be more aggressive and destructive and invades the bone earlier than eumycetoma.

Treatment of mycetoma is dependent on the causative agent. Actinomycetoma is usually treated with a combination of antibiotics, most often trimethoprim/sulfamethoxazole (TMP/SMX) plus amikacin, but other drug combinations are also in use (1). In general, actinomycetoma caused by *N. brasiliensis* seems to respond better to these drugs than actinomycetoma caused by *A. madurae* (4). Eumycetoma is treated with a combination of antifungals and surgery. Itraconazole is used most often, however in centres were itraconazole is not available terbinafine is used (5). Surgery ranges from small local excision to amputation of the infected limb. Amputation is necessary to reach final cure in a subset of patients.

Currently, most mycetoma cases are diagnosed based on their clinical characteristics. The identification of the causative agent is most often done by a combination of histology and culturing of the grains (6). For this, a deep-seated biopsy is recommended, as the grains secreted from open sinuses are often non-viable (3). With histology, the grain can be easily seen inside the infected tissue and actinomycetoma and eumycetoma can be differentiated. However, identification to the species level is not possible (6). With culturing of grains, the isolate can be grown, and species can be identified based on both macroscopic and microscopic morphology. However, a positive culture can take up to six weeks and both contamination of the culture and misidentifications are common (7). Molecular diagnostic tests such as conventional PCR, qPCR and isothermal amplification techniques are commonly used in research settings but rarely in primary care settings in endemic regions (6). Furthermore, almost all molecular assays were developed for *M. mycetomatis,* the most common causative agent. Species-specific molecular assays are not available for the majority of the other causative agents (6).

In 2016 mycetoma was added to WHO's list of Neglected Tropical Diseases (NTD) and included in the 2021-2030 roadmap for NTDs (8). For mycetoma, the core strategic intervention planned for the period 2021-2030 is case management by developing differential rapid diagnostic tests and effective treatment, establishing surveillance for case detection and reporting, developing a standardised field manual for diagnosis and treatment, ensuring proper training of health care workers and providing access to affordable diagnosis and treatment (8). Since case management is heavily dependent on accurate diagnosis, the WHO Diagnostic Technical Advisory Group (DTAG) for NTDs identified mycetoma as one of the priority NTDs to be addressed. To ensure that mycetoma diagnostic assays needed by clinical staff in endemic regions will be made, DTAG recommended the development of Target Product Profiles (TPP) to guide their development.

As indicated in the 2021-2030 roadmap for NTDs and by experts in the field, mycetoma urgently requires point-of-care diagnostic tests to improve early detection at primary health care level. Such an assay should not only detect mycetoma but also identify the causative agent to species level to allow initiation of an appropriate therapy. Furthermore, since it is not easy to determine when treatment can be stopped, a point-of-care test of cure is also needed. In this paper we describe the development of these two TPPs for mycetoma.

Methods

Following the recommendation of the DTAG, WHO formed a group of skin NTD experts, end-users and other stakeholders. For each specific skin-associated NTD, a different subgroup was formed, including one focused on mycetoma. The mycetoma subgroup, which consists of the authors of this paper, met from January 2021 to April 2021 to agree on priority uses for the TPPs and the process for the developments of the TPPs. The two priority uses for mycetoma were: (i) to identify the causative agent to species level so that appropriate treatment can be initiated; and (ii) a test of cure to stop treatment. Two expert subgroups were formed, one to determine the attributes required for each use (use characteristics) and another to review diagnostic assays. TPPs were intended to facilitate expeditious development of missing diagnostic assays addressing prioritized public health needs. Using the WHO core TPP development process (Figure 1), the expert subgroups for mycetoma convened online three times to discuss and determine attributes required for each use.

TPPs for each use considered the following parameters: product use, design, performance, product configuration and cost, and access and equity. Initial Draft 0 requirements in each TPP were selected based on review analyses, use needs analysis and expert consensus on the diagnostic performance through a consultative process coordinated by WHO's Department of the Control of Neglected Tropical Diseases. For certain elements in each use, parameters were defined at the outset, and assumptions were made to move forward with sensitivity and specificity estimates. The mycetoma subgroup critically reviewed and modified Draft 0 where warranted. Draft 0 was sent to the DTAG for review and comments.

After revising based on the comments from the DTAG, the mycetoma subgroup finalized the TPP details, and draft 0.1 TPPs were posted on the WHO website for public comment from October to November 2021. Comments received were shared with the experts of the mycetoma subgroup, and TPPs were revised accordingly to generate version 1.0 TPPs.

Results

The draft TPPs for two uses were published by WHO on 25 October 2021 (https://www.who.int/news-room/articles-

detail/public-consultation-tpps-for-mycetoma-diagnostics). The final TPPs were published by WHO on 5 May 2022 (https://www.who.int/publications/i/item/9789240047075). Select TPP features, and their associated requirements are presented in Table 1 and Table 2.

Table 1. Select TPP characteristics of needed test to differentiate actinomycetoma from eumycetoma

Feature	Ideal requirement	Minimum requirement
Intended use	An *in vitro* point-of-care test that detects mycetoma analyte(s) for the purpose of identifying the causative agent to the species level so that appropriate treatment can be initiated.	An *in vitro* laboratory-based test that detects mycetoma analyte(s) for the purpose of diagnosing the type of mycetoma (fungal or bacterial) so that appropriate treatment can be initiated.
Target analyte	Biomarker(s) specific for eumycetoma and actinomycetoma. Markers that can permit the differentiation to the species level of the most common causative agents. Ideally, biomarkers should also be able to tell if the infection is caused by *Nocardia* or *Madurella.*	Biomarker(s) specific for eumycetoma OR actinomycetoma.
Diagnostic/clinical sensitivity	>99%	>95%
Diagnostic/clinical specificity	>90%	>75%

Table 2. Select TPP characteristics of needed test for stop treatment

Feature	Ideal requirement	Minimum requirement
Intended use	An *in vitro* point-of-care test that detects mycetoma analyte(s) for the purpose of deciding if a mycetoma patient on treatment is free of disease so that treatment can be stopped.	An *in vitro* laboratory-based test that detects mycetoma analyte(s) for the purpose of deciding if a mycetoma patient on treatment is free of disease so that treatment can be stopped.
Target analyte	Biomarker(s) specific for eumycetoma and actinomycetoma.	Biomarker(s) specific for eumycetoma or actinomycetoma
Diagnostic/clinical sensitivity[a]	>95%	>90%
Diagnostic/clinical specificity[b]	>90%	>75%

[a]Due to drug toxicities, unnecessary treatment must be avoided. Amikacin can cause hearing problems. The antifungal agents can damage the liver.

[b]More laxity on specificity because the follow up for mycetoma is long and patients will be seen more than once. This means if they stop treatment and there is recurrence, they will be placed back on treatment. Definition of cure: clear of disease for a 24-month period of follow-up (for eumycetoma) and for 12 months follow-up for *Nocardia*.

Discussion

Access to appropriate diagnostic tools is critical for individual patient care and for achieving the 2021-2030 programmatic goals for mycetoma management. Based on discussions within our expert panel, two uses were

considered most urgent. The first was a diagnostic test that ideally can identify the causative agent to species level but should at least differentiate actinomycetoma from eumycetoma to start appropriate therapy. The second was a test which can determine when mycetoma treatment can be stopped.

Developing a test that can identify the species of causative agent is not an easy task. There are more than 70 agents known to cause mycetoma, and since the introduction of molecular identification, an average 3-4 new causative agents are identified every year (9). In 2021 alone, four new causative agents were described (10-13). Hence efforts to identify species of causative agents have concentrated on the most common ones. Globally the fungus *M. mycetomatis* (n=2032) is most often reported, followed by the bacterium *N. brasiliensis* (n=1946). *M. mycetomatis* is predominantly found in Africa and Asia and is mostly absent in Latin-America, whereas *N. brasiliensis* is predominantly found in Latin-America and hardly in Africa and Asia (2).

To identify *M. mycetomatis* to species level, molecular identification tools ranging from classical PCR (14) to isothermal amplification techniques (15, 16) have been developed; however, molecular assays are available for hardly any of the other causative agents (6). For *N. brasiliensis* diagnostic antigens have been identified that can be used in an enzyme-linked immunosorbent assay (ELISA) (17) or a lateral flow device in future. For *M. mycetomatis* antigens have been identified, but these were not able to differentiate patients from healthy controls (18) or were not further characterized (19). No antigens are available for the other causative agents, and antigen-specific antibodies cannot be quantified in sera from patients with lateral flow assays or enzyme-linked immunosorbent assays (ELISA).

This indicates that currently we have no diagnostic markers for a point-of-care test able to identify the causative agents to species level for the majority of mycetoma cases. The TPP developed specifies the diagnostic criteria to which assays should ideally or minimally adhere. As it would be impossible to develop diagnostic tests for >70 causative agents, test developers should be aware that for a physician to prescribe the appropriate therapy, the minimal requirement is to discriminate between actinomycetoma and eumycetoma as they are managed differently. Actinomycetoma is usually treated with a combination of antibiotics, eumycetoma with a combination of antifungal therapy and surgery (5).

Even a test that can only differentiate between actinomycetoma and eumycetoma would allow health care providers to treat or refer patients early. Although not all forms of actinomycetoma seem to respond equally well to standard treatment (4), current treatment guidelines do not differentiate the recommended treatment by causative agent. However, in the future, it is plausible that it may be necessary to identify certain causative agents to species level, requiring a new or updated TPP.

One of the current assays to differentiate actinomycetoma from eumycetoma is ultrasound. Ultrasound is minimally invasive and can differentiate actinomycetoma and eumycetoma based on hyper-reflective echoes (20). With portable ultrasound machines, it can also be used in endemic villages (21). Apart from the high cost of the machines, their downside is that they cannot be operated without extensive training. More work is needed to either transfer this technique to a point-of-care technique that can be used in local villages by individuals with minimal training, or to develop a new point-of-care tool to differentiate actinomycetoma from eumycetoma. Serological markers, such as β-1,3-*D*-glucan, currently in use for other fungal infections, are not specific enough, as certain actinomycetes can cause false positives with these assays (22). This challenge might be solved by identifying alternative serological markers, developing lateral flow assays, ELISAs or spot assays or DNA markers.

Fungal and bacteria-specific DNA barcoding genes have been used to identify mycetoma causative agents. They include the internally transcribed spacer region for fungal isolates and the 16S rRNA gene and the gene encoding for

heat shock protein 65 for bacterial isolates. However, at the moment these techniques are only used on strains, and sequencing of these regions remains mandatory for identifying the species of most causative agents, as few species-specific PCRs have been developed (6).

Knowing when treatment can be stopped is equally important to reduce the exposure to drugs with toxic side effects such as itraconazole and amikacin (5). Currently, treatment is stopped when clinical cure is observed as indicated by the disappearance of the mass and sinuses; when no grains are seen by ultrasound; and when there is no microbiological evidence of mycetoma (5). At the end of treatment, identification of residual grains by ultrasonography can become more challenging and when residual grains are present it is not possible to determine whether or not they are still viable. This can only be determined after taking a biopsy which is invasive and therefore neither point-of-care nor patient-friendly.

The only causative agent response to treatment can be monitored is *N. brasilienis.* The *N. brasiliensis*-specific ELISA, which can be used diagnostically to identify patients with actinomycetoma, can also be used to monitor treatment response and possibly be turned into a lateral flow device. During treatment, antibody levels decrease, and in cured patients, antibody levels return to normal (17). In the case of the *N. brasiliensis* ELISA, this assay would adhere to the requirements of both TPPs. Although this is encouraging, there is a need for equivalent tests either for mycetoma as a whole or for the other causative agents.

Conclusion

Two TPPs with the criteria required for diagnostic tests that will aid clinicians in the clinical management of mycetoma were developed. Rapid, point-of-care diagnostic tests which can identify the causative agent to species level or at least differentiate between actinomycetoma and eumycetoma will allow early initiation of appropriate therapy. Non-invasive tools to monitor treatment response and determine the appropriate time to stop treatment will prevent unnecessary side effects and further aid in the management of mycetoma.

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Figure 1. The WHO Core TPP development process