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Perspective

Sputum smear microscopy in tuberculosis: Is it still relevant?

Tuberculosis (TB) is a leading cause of morbidity and death worldwide, with approximately two billion people infected and approximately two million annual deaths attributable to it. In 2010, there were an estimated 8.8 million incident cases of TB (range, 8.5-9.2 million) globally, equivalent to 128 cases per 100 000 population, and an estimated 12.0 million prevalent cases (range, 11.0-14.0 million) of TB. This is equivalent to 178 cases per 100,000 population. Thus, approximately 1.4 million people (range, 1.2-1.5 million) died of TB in 2010[1]. The current guidelines of the World Health Organization[1] and the International Union Against Tuberculosis and Lung Disease (The Union)[2] specify that the essential step in the investigation of patients who are suspected of having pulmonary tuberculosis should be the microscopic examination of their sputum samples. Standard 2 of the International Standards for Tuberculosis Care categorically states that all patients (adults, adolescents, and children who are capable of producing sputum) suspected of having pulmonary tuberculosis should have at least two, and preferably three, sputum specimens obtained for microscopic examination[3]. However, in the current era of molecular diagnostics, where does sputum smear microscopy stand? It is important to consider the role of smear microscopy, particularly in view of the recent WHO endorsement of the new rapid, automated nucleic acid amplification test, Xpert MTB/RIF[2].

Sputum smear microscopy has been the primary method for diagnosis of pulmonary tuberculosis in low and middle income countries[3], which is where nearly 95 per cent of TB cases and 98 per cent of deaths due to TB occur. It is a simple, rapid and inexpensive technique which is highly specific in areas with a very high prevalence of tuberculosis[3]. It also identifies the most infectious patients and is widely applicable in various populations with different socio-economic levels[3-5]. Hence, it has been an integral part of the global strategy for TB control. However, sputum smear microscopy has significant limitations in its performance. The sensitivity is grossly compromised when the bacterial load is less than 10,000 organisms/ml sputum sample. It also has a poor track record in extra-pulmonary tuberculosis, paediatric tuberculosis and in patients co-infected with HIV and tuberculosis[6,7]. Due to the requirement of serial sputum examinations, some patients who do not come back for repeated sputum examinations become "diagnostic defaulters"[8]. Some do not come back for results, and are lost to treatment and follow up. A personal observation showed that limited resources, large numbers of samples, all combined together often reduce the observation time per slide to less than 60 seconds, and this also contributes to reduction in the sensitivity of the test. Therefore, techniques for optimization of smear microscopy are under active investigation. There has been an attempt to reduce diagnostic defaulting by assessing the feasibility of diagnosing pulmonary tuberculosis by collecting two sputum samples on a single day (1-day protocol), and comparing this protocol with the national policy of collecting samples on consecutive days (2-day protocol). It was felt that since the 2-day protocol did not show a statistically significant difference in diagnostic performance compared with the 1-day protocol, the latter may be adopted as an alternative protocol, particularly for patients who are more likely to default[9].

Fluorescence microscopy was introduced in the 1930s, in an attempt to improve outcomes of smear microscopy. Fluorochrome dyes are used to stain the smear. A halogen or high-pressure mercury vapour lamp is traditionally used to excite the dye, and make it flouresce. A meta analysis of studies comparing fluorescent and conventional microscopy found that the

sensitivity of fluorescent microscopy was 10 per cent higher than that of conventional microscopy, and that it remains high even after concentration of the samples[3]. Sensitivity was found to be higher particularly in low grade smear positive sputum. Specificity estimates, however, were similar to conventional microscopy, though turnaround times were shorter. This meta analysis concluded that the successful and widespread implementation of fluorescence microscopy might be expected to improve case finding through an expected increase in sensitivity and decrease in time spent on microscopic examination. Although fluorescence microscopy increases the sensitivity of sputum smear microscopy, additional data on specificity and on the clinical consequences associated with false-positive results are needed to guide implementation of this technology in high HIV prevalence settings[10]. Cost constraints are major issues with fluorescent microscopy. This may be circumvented by the use of light-emitting diodes (LEDs) which cost less than 10 per cent of a mercury vapour lamp. With a life >50,000 h, it can run on batteries and thus has been used in peripheral areas with definite operational advantages[11].

Rapid culture based methods for diagnosis of tuberculosis include rapid automated liquid culture, where results may be available in a few weeks[12]; thin layer agar culture, which has an average turnaround time of 11.5 days[13], and Microscopic Observation Drug-Susceptibility Assay (MODS), which can provide results in an average of 9.2 days[13]. Phage based assays give results in 2 days[14]. While performance indicators of these techniques might be better than that of smear microscopy, turnaround times are longer. There are also requirements in terms of investment in infrastructure and equipment, leading to higher costs per test.

Nucleic acid amplification tests (NAATs) attempt to provide accurate and rapid diagnosis of TB using a technology that provides improved sensitivity and specificity functions as compared to sputum smear microscopy. Unfortunately, NAATs have infrastructure and investment requirements that are often beyond the scope of most diagnostic facilities that offer TB diagnostics to communities, particularly in developing countries. While most NAATs are unable to match the accessibility of sputum smear microscopy, Loop-mediated Isothermal Amplification (LAMP) is one NAAT, which has the potential to be accessible and cost-effective. LAMP is being evaluated as a point of care test for the diagnosis of pulmonary tuberculosis. The overall performance characteristics of LAMP and fluorescence smear microscopy appear to be broadly similar. However, the performance of LAMP in smear negative samples was not found to be completely acceptable[15]. In addition, culture and drug sensitivity testing will still be required to monitor progress of the disease.

As ground realities go, it will take a fair amount of time before the new NAAT on the block, Xpert MTB/RIF, can be decentralized sufficiently to replace smear microscopy as a diagnostic test. This is particularly true in geographical areas with high prevalence of multi-drug-resistant TB or HIV/TB co-infections, because most of these areas are in poorly developed zones of low income group countries, with irregular availability of electricity and water, as well as a poorly developed infrastructure for uninterrupted supply of consumables and their storage. Ironically, these are the areas where Xpert has the potential to make the maximum impact. Therefore, it must also be kept in mind that Xpert MTB/RIF technology does not eliminate the need for conventional microscopy, culture and drug sensitivity testing, which are required to monitor treatment progress and to detect resistance to drugs other than rifampicin. In addition, cost considerations tilt in favour of smear microscopy as the initial diagnostic and screening tool for tuberculosis.

Talking hypothetically, a rapid and universally accessible test that is not affected by HIV status, with a sensitivity of 85 per cent, and a specificity of 97 per cent, has the potential to save 392,000 adjusted lives annually, or 22 per cent of the global TB deaths[16]. Ideally, in order to be able to efficiently diagnose tuberculosis, a test should be available for use in peripheral centres where there are limited resources. This test should require no electricity, refrigeration, or access to clean water. It should be widely available, user friendly, with a requirement of minimal, or even no training. The results should be available within an hour, and it should have high sensitivity, specificity and positive and negative predictive values. The technology should be robust and stand the test of time. The diagnostic test should face up to the challenge of making effective TB diagnosis available to populations that need it most, but can afford the least.

Currently, such a diagnostic test does not exist. However, as of now, the closest we have to this is sputum smear microscopy. Till such an effective, point of care diagnostic test is available; perhaps, sputum smear microscopy is here to stay.

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