Proposal for a revision of the case definition of “Sputum Smear-Positive Tuberculosis”

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Introduction

A workshop of experts held in Antwerp proposed a change in the definition of “sputum smear-positive tuberculosis”, a proposal subsequently re-confirmed and endorsed by another workshop of technical experts held in The Hague from April 2-3, 2007. Both groups recommended STAG and WHO to adapt the following definition of a new sputum smear-positive case of tuberculosis:

“A sputum smear-positive case of tuberculosis is defined as a tuberculosis suspect with at least 1 acid-fast bacillus in at least 1 sputum smear examination”

This revised definition explicitly excludes the microscopic definition of treatment failure.

Background

The World Health Organization in its first “Framework for Effective Tuberculosis Control” defined sputum smear-positive tuberculosis as follows:

“Tuberculosis in a patient with at least two initial sputum smear examinations (direct smear microscopy) positive for acid fast bacilli (AFB+)”,

Or: Tuberculosis in a patient with one sputum examination positive for AFB+ and radiographic abnormalities consistent with active pulmonary tuberculosis as determined by the treating medical officer,

Or: Tuberculosis in a patient with one sputum specimen positive for AFB+ and culture positive for AFB+.”

In the “Revised International Definitions in Tuberculosis Control” the World Health Organization, the International Union Against Tuberculosis and Lung Disease and the KNCV Tuberculosis Foundation retained the gist of the previous definition:

“A definite tuberculosis cases: a patient with culture positive for the Mycobacterium tuberculosis complex (in countries where culture is not routinely available, a patient with two sputum smears positive for acid-fast bacilli (AFB) is also considered a ‘definite’ case).”

and:

“Pulmonary tuberculosis – Sputum smear-positive:

1 two or more initial sputum smear examinations positive for AFB, or
2 one sputum smear examination positive for AFB plus radiographic abnormalities consistent with active pulmonary tuberculosis as determined by a clinician, or
3 one sputum smear positive for AFB plus sputum culture positive for M. tuberculosis.”
Specificity of acid-fast microscopy

For the determination of the specificity of acid-fast bacilli, culture is frequently taken as the referent. Best known among these studies is Kubica’s large review on behalf of the Union’s Committee on Bacteriology and Immunology, where he states “…more than 2 AFB in a Ziehl-Neelsen-stained smear was enough to call the smear positive, and these results confirmed what I had learned and taught others…”. What is usually not explicitly stated because it is not mentioned in the article is that the data were entirely from the United States where 300 oil immersion fields have to be examined if none or fewer than 3 AFB are found. In other words the “more than 2” are equivalent to “at least 1 per 100 oil immersion fields”.

Culture is an imperfect tool to determine the specificity of acid-fast microscopy in its extreme low range as even the most diligent and “mild” culture technique must take recourse to a decontamination procedure that by necessity also results in some killing of bacilli that might have been seen as AFB.

A more sturdy assessment of the specificity of AFB microscopy would only ascertain that AFB are actually mycobacteria, without distinction of the species (an accepted limitation to begin with) nor regard for viability. In a diagnostic specimen finding AFB will virtually always indicate the presence of viable bacilli while in specimens taken during treatment they very frequently do not, depending to some extent on the treatment regimen (and thus require different criteria). A more correct study than using culture as the gold standard to ascertain the specificity of acid-fast bacilli reports was conducted in the late 1970ies in East Africa.

In that study, artificial specimens were created to resemble sputum specimens but that could not have contained acid-fast bacilli. They were then given to laboratories in East Africa for examination with fluorescence microscopy (Table 1). The overall specificity was very high with 98.6%, with individual laboratories ranging from 96.8% to 99.9%. Seemingly the specificity was everywhere very high, but was influenced by the individual technician’s ability (see Kampala versus the other two laboratories). This concerned reference laboratories, fluorescence microscopy and artificial specimens, and was thus not representative of a routine situation.

The definitions above take into account one of the most frequent sources of error in clinical laboratories, i.e. mislabeling and cross-contamination of specimens, leading to the requirement for another similar result for validation (even in case the first positive is a high positive). Surprisingly, the rationale has not been applied to culture results, where the risk of cross-contamination is far higher while that of mislabeling stays the same. Considerable frequencies of false positive cultures have been reported repeatedly from laboratories in industrialized countries.

Reproducibility of AFB findings in quality-assured routine work

A database from a laboratory network subject to continuous external quality assessment (EQA) by means of blinded re-checking of slides was utilized to determine the frequency by which smears reported as having at least 1 AFB could not be confirmed. As there is usually less difficulty to confirm AFB graded as 1+ positive or higher, the emphasis of the evaluation was on scanty positive smears. Among the 43,140 smears with at least 1 AFB, 4,389 (10.2%) contained 1 to 9 AFB per 100 fields. Of all suspects in whom the first result was scanty, in only 10.1% was it not possible to find again 1 or more AFB in the second and/or third examination. Expectedly, failure to confirm a scanty result was most frequent among those suspects who had only 1 AFB in 100 fields in the first examination (Figure 1). Overall, all results with at least 1 AFB considered, only 1.5% could not be confirmed.

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Results of routine rechecking EQA of acid-fast smears also suggest this very low frequency of false positives in such projects, at least when retesting of discordant identified is performed prior to a second control reading, as recommended[10].

Reproducibility of AFB findings from routine service activities in countries without EQA program

A study of a representative sample of laboratory registers from the national microscopy networks in Moldova, Mongolia, Uganda and Zimbabwe was carried out to determine what was routinely reported in these countries (Katamba A, Int J Tuberc Lung Dis 2007;11:in press). This study also permitted to determine the frequency with which scanty and/or positive results were confirmed by a second smear examination (unpublished data presented at the TSRU meeting, The Hague, 4-6 April, 2007: Mabuza B, Lauritsen J M, Katamba A, Latieeovski D, Naranbat N, Rieder H L. Sputum smear-positive tuberculosis: empirical evidence challenges the need for confirmatory smears. TSRU Progress Report 2007:71-82).

Of the 13,577 with at least 1 AFB in any of a serial examination, 27.4% (3,716) did not have a confirmatory examination (Figure 2). However, only 9,014 of the examinees with at least 1 AFB had a complete series of three examinations. Among these, 7.6% (689) had no confirmatory result in the same series (Figure 3). The absence of regular EQA leaves some uncertainty as to the interpretation of these results: they might represent true false positive error, false negative errors because of sampling or technical deficiencies (leading to reduced reproducibility and isolated positive/scanty smears), as well as poor practice (i.e. only one smear read). However, even with poor control, the possible magnitude of false positive error remains far below that of chest X-Ray[12]. The restrictions on use of smear for case definitions thus seem far out of proportion compared to the liberal use of culture or X-Ray, particularly now that smear-negative case diagnosis is much more emphasised.

Implications

Historically, treatment for patients with sputum smear-positive tuberculosis differed from that for patients with sputum smear-negative and extrapulmonary tuberculosis. The former were allocated rifampicin-containing treatment of 8 months duration, the latter (unless they were seriously ill) a 12-month regimen not containing rifampicin. This differentiation had its rationale in the large costs of rifampicin and pyrazinamide, the limited budgets available in low-income countries, logistic problems in delivering directly observed rifampicin, and the emphasis on curative treatment as quickly as possible of the most potent sources of infection.

Meanwhile drug costs have been reduced remarkably and are similar for 8- and 12-month regimens, and the latter has proven to be inferior in patients co-infected with HIV. In the third edition of its treatment guidelines, the World Health Organization basically recommends the same treatment regimen for all previously never treated patients, irrespective of the initial bacteriological findings in sputum smear examinations. Furthermore, tuberculosis suspects negative on smear examinations, judged to have tuberculosis by a qualified medical officer, should never be denied treatment. Also patients with only one positive sputum smear are eligible for tuberculosis treatment, even when no confirmatory smears are obtained. However, in the field this is regularly not done, because of exaggerated emphasis on smear-positive cases or poor understanding of the guidelines. As a result, clear-cut cases of tuberculosis identified on one smear disappear or die before treatment can be started, reaching high numbers in some settings.
The demonstration of AFB in sputum smears should thus affect mainly the surveillance definition of a “definite” case of tuberculosis, in particular that of a sputum smear-positive case. Specificity of sputum smear microscopy is already very high, requiring confirmatory smears will decrease the sensitivity of the surveillance definition. Moreover, it is not efficient since subsequent examinations may simply not be done particularly in poorly controlled laboratories, and while reproducibility in the low range or under poor conditions is not good. Conversely, not requiring a confirmatory smear examination will barely affect the specificity, but has the potential to considerably increase the sensitivity of the surveillance definition of a definite case. And it will definitely reduce loss of diagnosed smear-positive cases before treatment.

For maximum impact, also the current threshold for action based upon a finding of AFB is too high (minimum 10 AFB/100 high power fields), and not based on any experimental basis or even good rationale. It causes a lot of confusion and frustration in the field, with again cases lost for treatment before they can be confirmed on further smears. Lowering it tenfold would greatly simplify diagnosis and treatment, particularly in areas with high HIV-prevalence or early case detection. The only condition might be the existence of a functional (though maybe not perfect) EQA system, identification of those making systematic false positive errors poses hardly a challenge.

Recommendation

Provided that microscopy EQA is in place, the finding of a single AFB in at least one examination of 100 high power field in a tuberculosis suspect should satisfy the criterion to report the patient as having “sputum smear-positive tuberculosis” and to start treatment.
Table 1. Specificity of fluorescence microscopy for acid-fast bacilli in three east African laboratories.

<table>
<thead>
<tr>
<th>Place</th>
<th>No of specimens</th>
<th>(False) positive</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dar es Salaam</td>
<td>767</td>
<td>4</td>
<td>99.5</td>
</tr>
<tr>
<td>Kampala</td>
<td>1,530</td>
<td>49</td>
<td>96.8</td>
</tr>
<tr>
<td>Nairobi</td>
<td>1,533</td>
<td>1</td>
<td>99.9</td>
</tr>
<tr>
<td>Total</td>
<td>3,830</td>
<td>54</td>
<td>98.6</td>
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</tbody>
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Figure 1. Confirmation of diagnostic smear examination results found to contain 1 to 10 AFB per 100 fields in any other smear in the same series.\footnote{8}
Figure 2. Examinees with at least 1 AFB in at least 1 examination, sputum smear microscopy laboratories, Moldova, Mongolia, Uganda, and Zimbabwe, 1999-2003. Mabaera B, et al, unpublished data.
Figure 3. Examinees with at least 1 AFB among 3 examinations, sputum smear microscopy laboratories, Moldova, Mongolia, Uganda, and Zimbabwe, 1999-2003. Mabaera B, et al, unpublished data.
References


