

## LETTER TO THE EDITOR

## Diagnostic accuracy of three morning sputum versus standard sputum smears for pulmonary tuberculosis

Sir,

In 2015, there were an estimated 10.4 million new tuberculosis (TB) cases worldwide, 1.4 million TB deaths, and 480,000 new multidrug-resistant TB cases, according to the WHO report.<sup>1</sup> Early diagnosis and immediate initiation of treatment are essential for an effective TB control program. Delay in diagnosis is significant to both disease prognosis at the individual level and transmission within the community.<sup>2</sup> To date, several new rapid TB tests (such as Xpert MTB/RIF Ultra, Real time-PCR) have been developed.<sup>3</sup> However, sputum smear remains the only readily available TB diagnostic test throughout most of the low-income and middle-income countries.

The National Institute for Health and Care Excellence guideline on TB recommends that multiple sputum samples (at least three, with one early morning sample) were collected for TB microscopy for patients with suspected pulmonary TB (PTB).<sup>4</sup> Similar recommendation was made in China.<sup>5</sup> However, in Brazil, two sputum samples, including one morning sputum sample, was recommended.<sup>6</sup> Remarkably, these recommendations did not contain specific implications on performing multiple smears in 1 day or on different days for PTB. The Shandong Provincial Chest Hospital (SPCH) is a provincial TB referral hospital of approximately 800 beds. Each year, about 1000 patients with TB were diagnosed, with positive results on acid-fast bacilli (AFB) sputum smear examination. The SPCH TB

reference laboratory had been accredited by ISO15189 in 2010 and is participating in an external quality control program conducted by the National Center for Clinical Laboratories of China. The retrospective study aimed to conduct a comparison of the diagnostic value of three sputum smear microscopy in 1 day or on different days for the diagnosis of PTB.

Smear microscopy examination was performed using auramine O staining and culture using Lowenstein-Jensen solid media. All smear microscopies were confirmed to be of satisfactory quality and were read using national guidelines for quality control.<sup>7</sup> The differences in the sensitivities between the two groups were examined using the  $X^2$  test, and a  $p$  value  $<0.05$  was considered significant. Between January 2016 and July 2017, 1106 consecutive patients with TB who met the entry criteria (three sputum samples for AFB smear within 7 days, TB culture confirmed) were enrolled. Then, these patients were divided into two groups according to sputum collection method: (1) standard collection (941 cases), three sputum samples including one morning sputum were collected in 1 day for AFB smear; and (2) non-standard collection (165 cases), three each morning sputum samples were collected on consecutive or non-consecutive separate days (within 7 days). Written informed consent was waived because this was a retrospective study. All patients were deidentified at entry into the study and patient unique ID was used for data tracking and collection.

The characteristics of patients and the performance of two collection methods are detailed in table 1. For the group of standard collection, the mean age was  $43.9 \pm 19.7$  years old, men comprised 68.1% ( $n=641$ ) and the sensitivity of AFB smear was 37.4% (34.4%, 40.5%). For the

group of non-standard collection, the mean age was  $45.0 \pm 20.9$  years old, men comprised 70.9% ( $n=117$ ), and the sensitivities of AFB smear were 49.7% (42.2%, 57.3%) in total, 51.6% (41.7%, 61.4%) in consecutive collection method and 47.1% (35.9%, 58.7%) in non-consecutive collection method, respectively; of a total of 82 AFB-positive suspects, 53 (32.1%) were found on the first smear, a further 19 (11.5%) on the second smear and 10 (6.1%) additional cases were identified on the third smear. The statistical analysis showed that (1) there was significant difference in the sensitivity of AFB smear between standard and non-standard collection groups ( $p < 0.005$ ); (2) there was no significant difference in the sensitivity of AFB smear between consecutive and non-consecutive sputum collection methods ( $p > 0.05$ ); and (3) although the third sputum adds incremental diagnostic value to the performance of AFB smear, there was no significant difference in the sensitivity of AFB smear between the first two and three specimens ( $p > 0.05$ ).

More than 90% of patients with TB live in low-income and middle-income countries, where the diagnosis of TB relies primarily on identification of AFB on sputum smears. Reviews showed that current sputum collection methods can be optimized to generate higher than usual yields.<sup>8</sup> The study showed that examining three morning sputum samples within 7 days was superior to standard sputum collection method in the diagnosis of suspected PTB. This may be due in part to the accumulation of sputum in the lungs overnight, resulting in a concentration of bacilli in the morning samples. In contrast, patients may be more active during the day and may shed bacilli intermittently, thus reducing the yield of bacilli in spot sputum samples.<sup>9</sup> Two sputum samples may be sufficient for TB testing, because no statistical difference occurred in

**Table 1** Comparison of sputum collection methods in detection of pulmonary tuberculosis using acid-fast bacilli smear

Sputum collection method	n	Age (years)	Sex, male (%)	Sensitivity (%)			
				Total	First smear	Second smear	Third smear
Non-standard collection							
Consecutive	95	46.0 $\pm$ 21.9	65 (68.4)	51.6 (41.7, 61.4)	36.8 (27.8, 46.9)	10.5 (5.8, 18.3)	4.2 (1.7, 10.3)
Non-consecutive	70	43.6 $\pm$ 19.4	52 (74.3)	47.1 (35.9, 58.7)	25.7 (17.0, 37.0)	12.9 (6.9, 22.7)	8.6 (4.0, 17.5)
Total	165	45.0 $\pm$ 20.9	117 (70.9)	49.7 (42.2, 57.3)	32.1 (25.5, 39.6)	11.5 (7.5, 17.3)	6.1 (3.3, 10.8)
Standard collection							
	941	43.9 $\pm$ 19.7	641 (68.1)	37.4 (34.4, 40.5)			

the sensitivity of AFB smear between the first two and three specimens. Similar result was reported by Mase *et al.*<sup>10</sup> In addition, to reduce the risk of patients being lost to follow-up, the latest WHO guidelines recommend that two instead of three spot sputum samples are collected during 1 day.<sup>11 12</sup> However, considering the incremental value of the third sputum (increasing by 6.1%), we still recommended that the third sputum should be collected in practice. Remarkably, a recent meta-analysis comparing morning with spot sputum collection showed no significant difference in diagnostic performance<sup>13</sup>; it means spot sputum would be an alternative to morning sputum. However, because of retrospective nature, we did not evaluate the performance of spot sputum in AFB smear and compare it with morning sputum. Considering the retrospective nature of the study, the presence of a selection bias cannot be excluded. However, since consecutive cases were enrolled, the present data represent a close picture of the actual population of local subjects with suspected PTB.

In conclusion, we recommended that examining three morning sputum samples within 7 days would improve the diagnosis of suspected PTB. Moreover, data from this analysis have informed revision of these guidelines.

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**Contributors** Y-NW and J-LW (3th) collected the data and did the analysis. CH supervised the data collection and analysis, and revised the manuscript. M-SW and J-LW (5th) designed the study and wrote the manuscript.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent** Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

**Ethics approval** The ethics committee of Shandong Provincial Chest Hospital has approved the study and written informed consent was waived, according to article 39.1 of the ethics guidelines enacted by the National Health and Family Planning Commission of the PRC (<http://www.nhfpc.gov.cn/fzs/s3576/201610/84b33b81d8e747eaaf048f68b174f829.shtml>).

**Provenance and peer review** Not commissioned; externally peer reviewed.

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Y-NW and CH contributed equally.



**To cite** Wang Y-N, Han C, Wang J-L, *et al.* *J Investig Med* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jim-2018-000724

Accepted 17 February 2018

*J Investig Med* 2018;0:1–2.  
doi:10.1136/jim-2018-000724

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