ORIGINAL ARTICLE

# Comparative analysis of case screening with varying cough duration and sputum samples for diagnosis of tuberculosis in patients attending the OPD at a tertiary care hospital at Srinagar, India

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## Abstract

**Research Question:** Can we minimize cough duration and number of sputum samples in chest symptomatic patients for screening of TB?

**Objective:** To evaluate cough of 3 weeks versus 2 weeks duration using two sputum samples versus three samples in chest symptomatic patients attending the OPD.

**Study Design:** Hospital-based cross-sectional study.

**Materials and Methods:** Outpatients (2810) with H/O cough of 3 weeks and 2 weeks duration were screened by subjecting them to sputum microscopy for tuberculosis using two sputum samples as well as three samples following standard procedure for sputum collection, staining and acid-fast bacillus (AFB) identification. Those on drugs were not included.

**Results:** Using ≥2 weeks cough, sputum positivity rate was 12%, nearly as high as the sputum positivity among patients with ≥3 weeks cough, i.e. 14%. First sputum smear alone on an average could detect 91.8% cases, while the first two sputum smears could detect on an average 96% cases. The study showed that maximum number of cases was diagnosed by only two sputum smears and added diagnostic value of third specimen was small, i.e. 4%.High sputum positivity rate using ≥2 weeks cough with two sputum samples was seen.

**Conclusion:** The sensitivity analysis of the study showed that using ≥2 weeks cough with two sputum samples gives almost similar values as ≥3 weeks cough with three sputum samples, but this needs further confirmatory results of culture sensitivity. Hence, using ≥2 weeks cough with two sputum samples as the diagnostic criteria for screening of cough patients for TB should be recommended as one way of improving efficient use of scarce resources.

**Key words:** Cough duration, India, OPD patients, sputum samples, Srinagar, tuberculosis

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## Introduction

Most standard laboratory tests[1] and guidelines[2,3] for mycobacteriology laboratories recommend that at least three sputum specimens, preferably collected on successive days, be submitted to the laboratory for acid-fast bacillus (AFB) smear and culture for patients suspected to have tuberculosis (TB). Unfortunately, there has been a paucity of published data analyzing the validity of this recommendation.[4]

Recently, the number of sputum specimens contributed

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has become a matter of debate to assess the benefit and to reduce hospital expenses in the diagnosis of pulmonary TB (Nelson *et al.*, 1998; Finch and Beaty, 1997; Cascina *et al.*, 2000; Craft *et al.*, 2000; Harvell *et al.*, 2000).[5] Studies have shown that examination of two consecutive specimens (on the spot and overnight sputum) is sufficient to detect a large number of infectious cases in the community.[6] Moreover, the possibility of improving the detection of smear-positive cases by using cough of ≥2 weeks instead of ≥3 weeks as the criterion for screening patients for sputum microscopy has not been explored.[7] With this background, this study was done to see the yield of screening out-patients with cough of ≥3 weeks versus ≥2 weeks duration and using two sputum samples versus three samples in different combinations and the practical utility of such a finding if any.

### Objectives of the study

To evaluate cough of 3 weeks versus 2 weeks duration using two sputum samples versus three samples in chest symptomatic patients attending the OPD.

To find the best possible combination of cough duration and sputum sample number in diagnosis of TB.

## Materials and Methods

### Study design

Hospital-based cross-sectional study was carried out using a standardized protocol in Chest Disease Hospital, Srinagar.

### Setting

The present study was carried out in Chest Disease Hospital, Srinagar, a tertiary care hospital, in order to evaluate screening of out-patients with cough of 3 weeks duration versus cough of 2 weeks duration and for diagnosing TB using two sputum samples versus three samples in chest symptomatic patients.

### Instruments

Specially designed proforma

A total of 2810 subjects (those with ≥2 weeks cough and ≥3 weeks cough) were included in the study.

### Study methodology

The study was conducted for a period of 1½ years starting from December 2006 and was carried out in two phases.

Phase I was based on sensitization, utilization, and willingness of the doctors and laboratory staff of the Chest Disease Hospital to participate in the study.

The doctors and the laboratory staff were sensitized and motivated toward the modality of identifying and referring patients with cough of ≥2 weeks duration to the TB laboratory for sputum examination, besides routinely sending those with ≥3 weeks cough duration. The approach that was used to identify AFB in the sputum samples of the chest symptomatic patients in hospital was Z-N direct staining method.

Data collection: The laboratory staff was sensitized regarding the collection of sputum samples from these out-patients (with ≥2 weeks and ≥3 weeks cough duration). The laboratory staff was also supposed to maintain the complete record of these out-patients, including age, gender, residential address, H/O contact, family H/O TB, number of family members, type of family, smoking habits, education, income, and occupation.

Phase II was based on the monthly collection of sputum sample results [two and three sputum sample results from the cough patients with cough of varying duration (≥2 weeks and ≥3 weeks)]. Sputum samples of all cough patients with cough of varying duration were collected and sent for Z-N staining and sputum microscopy. Patients on treatment attending the hospital for follow-up were excluded. The sputum specimen was collected on the spot from each symptomatic patient. The patient was then instructed to return on the following day for the examination of an overnight sputum specimen and to provide a second spot specimen. The smears were then processed and read by trained laboratory technicians. Data were collected as in phase I.

### Analysis and interpretation

The data so collected were subjected to statistical analysis for obtaining the results of screening of patients with varying cough duration and diagnosis using multiple sputum samples in terms of sensitivity, specificity, predictive values, and positivity rates. The statistical significance analysis was performed using chi-square test. Two-tailed P-value ≤0.05 was considered significant.

## Results

The total number of new adult patients who attended the OPD during the study period was 70,000, and the total suspects subjected to sputum microscopy were 2810, which accounted for 4% of the total OPD attendees [Table 1].

The sputum positivity rate of 12.4% was seen among patients with 3 weeks cough having three sputum samples positive, while sputum positivity rate of 11.9% was seen in patients with 2 weeks cough with two sputum samples positive.

It was seen that among the 367 sputum smear-positive cases, all the three sputum smears were positive in 86.6% (318) cases. 88.9% (184) cases had ≥2 weeks cough, but all three sputum smears were positive [Table 2].

Taking the first sample alone, the case yield was 91.8% [Table 3]; second smear took the yield to 96.2%, showing a percentage increase as 4.4%.

Taking two samples together and using different combinations, the highest incremental increase was seen with first and third samples (6.8%).

By adding the third sample to the first and second, incremental increase in case yield was 3.8%. Taking the first sample alone, the case yield was 92.8% in ≥2 weeks compared to 90.6% in ≥3 weeks; second smear took the yield to 96.6% in ≥2 weeks compared to 95.6% in ≥3 weeks cough. The percentage increase by adding second smear to the first was 3.8% in ≥2 weeks and 5% in ≥3 weeks [Tables 4 and 5].

Taking two samples together and using different combinations, highest incremental increase was seen with the first and third samples (6.7% in ≥2 weeks and 6.9% in ≥3 weeks).

By adding the third sample to first and second, incremental increase was 3.4% in ≥2 weeks cough group compared to 4.4% in ≥3 weeks cough group.

The sensitivity and specificity of using two sputum samples for diagnosis of TB in patients with 2 weeks cough was found to be 56.5% and 37.4%, respectively. Using two sputum samples for diagnosis of TB in patients with >3 weeks cough, the sensitivity and specificity were 43.6% and 62.6%, respectively [Tables 4 and 5].

## Discussion

The present study looks at permutations of using combinations of two or three sputum smears with ≥2 weeks cough and ≥3 weeks cough duration.

**Table 1: General description**

| Characteristics | | n | % |
|---|---|---|---|
| Total Number of new OPD attendees during study period | | 70,000 | 100 |
| Total screened population for sputum positivity | | 2810 | 4.0 |
| Sputum positive | | 367 | 13.1 |
| Duration of cough | ≥2 Weeks | 1736 | 61.8 |
| | ≥3 Weeks | 1074 | 38.2 |
| Sputum positive in ≥2 weeks cough | Present | 207 | 11.9 |
| | Absent | 1529 | 88.1 |
| Sputum positive in ≥3 weeks cough | Present | 160 | 14.9 |
| | Absent | 914 | 85.1 |

**Table 2: Pattern of sputum smear positivity with duration of cough**

| Pattern of smear positivity | 2 Weeks | | ≥3 Weeks | | Total | | *P*-value |
|---|---|---|---|---|---|---|---|
| | n | % | N | % | N | % | |
| All three + + + | 184 | 88.9 | 134 | 83.8 | 318 | 86.6 | 0.127 (NS) |
| Any two sputum +ve | 23 | 11.1 | 26 | 16.3 | 49 | 13.4 | 0.058 (NS) |
| + + − | 1 | 0.5 | 4 | 2.5 | 5 | 1.4 | 0.054 (NS) |
| + − + | 7 | 3.4 | 7 | 4.4 | 14 | 3.8 | 0.063 (NS) |
| − + + | 15 | 7.2 | 15 | 9.4 | 30 | 8.2 | 0.182 (NS) |
| Overall two smear +ve | 207 | 11.9 | 160 | 14.9 | 367 | 13.1 | 0.023 (Sig) |
| Only first smear +ve | 192 | 92.8 | 145 | 90.6 | 337 | 91.8 | 0.053 (NS) |
| Only second smear +ve | 200 | 96.6 | 153 | 95.6 | 353 | 96.2 | 0.034 (Sig) |
| Overall third smear +ve | 206 | 99.5 | 156 | 97.5 | 362 | 98.6 | 0.041 (Sig) |

**Table 3: Overall incremental increase in number of sputum +ve cases using different combinations of sputum +ve samples in all cough symptomatic patients**

| Samples +ve | No. | % | Incremental increase | |
|---|---|---|---|---|
| | | | No. | % |
| I Sample positive | 337 | 91.8 | 337 | 91.8 |
| I and II | 353 | 96.2 | 16 | 4.4 |
| II and III | 362 | 98.6 | 9 | 2.4 |
| I and III | 362 | 98.6 | 25 | 6.8 |
| I and II + III | 367 | 100 | 14 | 3.8 |

**Table 4: Sensitivity and specificity of sputum smear examination in patients with ≥2 weeks cough for diagnosis of TB**

| | | Overall sputum +ve | | Result | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yes | No | Sensitivity | Specificity | PPV | s | 95% CI | *P* value |
| Duration of cough | ≥2 weeks | 207 | 1529 | 56.4% | 37.4% | 11.92% | s | 0.109–0.129 | 0.023 (Sig) |
| | ≥3 weeks | 160 | 914 | | | | | | |

Sensitivity and specificity of sputum smear examination in patients with ≥3 weeks cough for diagnosis of TB

| | | Overall sputum +ve | | Result | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yes | No | Sensitivity | Specificity | PPV | NPV | 95% CI | *P*-value |
| Duration of cough | ≥3 weeks | 160 | 914 | 43.6% | 62.6% | 14.8% | 88% | 0.133–0.166 | 0.023 (Sig) |
| | ≥2 weeks | 207 | 1529 | | | | | | |

**Table 5: Analysis showing sensitivity and specificity using two sputum specimens for diagnosis of TB in patients with ≥2 weeks cough and in patients with ≥3 weeks cough**

| | | 2 Sputum positive | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Yes | No | Sensitivity | Specificity | PPV | NPV | *P*-value |
| Duration of cough | ≥2 Weeks | 207 | 1529 | 56.5% | 37.4% | 11.9% | 85.1% | 0.023 |
| | ≥3 Weeks | 160 | 914 | 43.6 % | 62.6 % | 14.9% | 88.1% | 0.023 |

Analysis showing sensitivity and specificity of three sputum specimens for diagnosis of TB in patients with ≥2 weeks cough and in patients with ≥3 weeks cough

| | | 3 Sputum positive | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Yes | No | Sensitivity | Specificity | PPV | NPV | *P*-value |
| | | n | n | | | | | |
| Duration of cough | ≥2 Weeks | 184 | 1552 | 57.9% | 37.7% | 10.6% | 87.5% | 0.127 |
| | ≥3 Week | 134 | 940 | 42.1% | 61.8% | 12.5% | 89.4% | 0.127 |

## ≥2 Weeks cough versus ≥3 weeks cough

The study shows that performing sputum microscopy among out-patients with ≥2 weeks cough can improve the detection of smear-positive TB cases. Sputum positivity among patients with ≥2 weeks cough was 12%, nearly as high as the sputum positivity among patients with ≥3 weeks cough (14%). Using ≥2 weeks instead of ≥3 weeks cough as the criterion for screening patients for sputum microscopy, there was a 61% increase in the number of cough symptomatic cases (from 1074 to 1736) and a substantial increase in the detection of smear-positive cases (from 160 to 207). The findings are consistent with the studies conducted by Bailey et al.[8] and Aleyamna Thomas.[9] Thus, the advantage of bringing down screening criteria to ≥2 weeks cough among cough symptomatic cases may reduce delays in diagnosis or shifting to a private practitioner. It has been observed by Santha et al,[7] in their study that two-thirds of cough symptomatic patients seek care within 2 weeks of onset of cough and about 50% of patients with 2–3 weeks of cough would not return to government health facility, hence a policy of screening patients with ≥2 weeks cough could potentially lead to an additional yield of smear-positive patients.

Two sputum versus three sputum samples: Our study also shows that maximum number, i.e. 96%, of cases were diagnosed by only two sputum smears with the third smear adding only a small number of cases of cases, i.e. only 4% cases were further diagnosed. In a study by Mase et al.,[10] added diagnostic value of third smear was also found to be small.

Thus, under routine conditions, two sputum smears can be recommended in place of three smears for screening chest symptomatic patients.

## Diagnostic incremental yield

The practice of repeating successive sputum examinations after second sample has remained a debatable subject. Recommendations for the most appropriate number of serial-diagnostic examinations have ranged from two[11,12] to three.[13,14] The present study shows that first sputum smear sample alone could detect overall 91.8% cases, and in chest symptomatic patients with ≥2 weeks cough, it could detect 92.8% cases compared to 90.6% cases in chest symptomatic patients with ≥3 weeks cough. These results are consistent with the studies by Cascina et al.,[15] Mathew et al.,[16] and Nelson et al.[17] First two sputum smears could detect on an average 96% cases. Similar results are replicated in the studies by Craft et al.[18] and Cambanis[19] Addition of third sputum smear sample to first two smear samples increased the diagnostic yield by only 3.8% in all cough symptomatic patients in our study, and if we take separately those with ≥2 weeks cough and ≥3 weeks cough, in them it increased

by 3.4% and 4.4%, respectively. Wu et al.[20] showed increase in diagnostic yield of 3.2% by adding third smear to the first two, and Yilmaz et al.[21] observed the additional diagnostic yield by third smear to be 4.2%. The two unique studies also contribute data suggesting that the number or proportion of smear-negative cases identified is not significantly reduced by using a two-specimen rather than a three-specimen strategy, although workload and cost are significantly reduced.[8,19]

Comparison of different combinations of two smears (first spot and morning, first and second spots, morning and second spot)

Gopi *et al*. observed that examination of two specimens – first spot and early morning spot (two visits) or early morning and second spot specimens (single visit) – yields the highest number of cases.[22] Rohit Sarin et al.[11] observed that early morning specimen (single visit) gave the best results as compared to the other two spot specimens. In our study, we found the highest incremental increase by using two spots together, whereas with the other two combinations (first spot and morning, morning and second spot), the incremental increase was less. The remarkably high diagnostic value of the two spot smear combination is surprising. The addition of morning specimen to either first spot or second spot gives surprisingly low incremental yield. The scenario many reflect poor instructions for specimens collected at home or very good instructions for the two spot specimens and might be also due to improved cough technique by the time of the third specimen. These findings were found in overall all cough symptomatic patients as well as when cough symptomatic patients with ≥2 weeks and ≥3 weeks cough were analyzed separately. On the basis of our findings, we suggest the use of two spot specimens rather than I and II, but it needs further investigations.

### Sensitivity and specificity of sputum smear examination

Computing sensitivity and specificity for sputum positivity in this study was not possible, since we did not have confirmatory findings of culture positivity available. We assessed cough symptom duration against sputum positivity for its diagnostic validity. As cough is not the diagnostic symptom of TB and it is present in many more diseases, it was expected to have lower sensitivity and specificity of this parameter in the current study. The sensitivity in this study, using combinations of varying durations of cough (≥2 weeks or ≥3 weeks cough), with either two or three sputum specimens, was found to vary between 42 and 60 and specificity in between 37 and 62. If we compare the sensitivity of using ≥2 weeks cough with two sputum samples with that of ≥3 weeks cough with two sputum samples, our study showed higher sensitivity (56.4%) in the former case than in the latter (43.6%).

Though the sensitivity in our study was almost consistent with those reported by Levy et al. (53.1%) and Githu et al. (55.3%), specificity and positive predictive value (PPV) were lower than those in these studies.[23,24] These findings are expected since these studies have used sputum positivity with sputum culture that serves as "gold standard" for confirmation of TB disease.

On the basis of our findings, it was observed that using ≥2 weeks cough duration with two sputum sample specimens is in no way different in sensitivity, specificity, overall yield results, from all other combinations and permutations of cough duration and number of sputum samples. One can, therefore, safely say that use of ≥2 weeks cough with two sputum samples can be safely recommended for screening of cases in OPD setup. In fact, not lately, WHO has also recommended on similar lines for resource-poor settings[25] and the present study findings substantiate the new guidelines.

Regarding RNTCP, in India, the National Tuberculosis Programme (NTP) has been in operation since 1962. It is essentially a permanent country-wide program integrated with the general health services. The Government of India, WHO, and World Bank together reviewed the NTP in the year 1992. Based on the findings of that review, a revised strategy for NTP was evolved.[26] The main pillars of this revised program, i.e. of RNTCP, are achievement of 85% cure rate through short course chemotherapy and detecting 70% of cases through quality sputum microscopy.[27]

## Conclusion

Till recently, under RNTCP, the guidelines for screening of pulmonary TB in chest symptomatic patients recommend that patients with ≥3 weeks cough be subjected to at least three sputum smear examinations. On the basis of our findings, bringing the screening criteria from ≥3 weeks to ≥2 weeks seems to have almost similar outcome. One would say that detection of smear-positive cases can be substantially increased by revising the screening policy for sputum microscopy among out-patients from ≥3 weeks to ≥2 weeks. However, there may be concern regarding increase in the number of sputum samples for screening adding to the overall cost and affecting the efficiency of the screening program. One way of improving efficient use of scarce resources might be to reduce the number of sputum examinations ordered per patient. The present study has also shown that just two sputum samples are adequate enough to result in a comparable overall yield as is seen while using three sputum samples for screening in a cough symptomatic patient. Based on the results of this study, combination of ≥2 weeks cough and two sputum samples can be recommended. The sensitivity analysis in this study shows that using ≥2 weeks cough with two sputum samples gives almost similar values as ≥3 weeks cough with three sputum samples, but

this needs further confirmation using confirmatory results of culture sensitivity.

Our study shows that using two spot specimens can yield better than any other possible permutation of samples used, which needs further evaluation.

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