Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial

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Summary

Background **In several settings, women with suspected tuberculosis are less likely to test smear positive than are men. Submission of poor-quality sputum specimens by women might be one reason for the difference between the sexes. We did a pragmatic randomised controlled trial to assess the effect of sputum-submission instructions on female patients.**

Methods **1494 women and 1561 men with suspected tuberculosis attending the Federal Tuberculosis Centre in Rawalpindi, Pakistan, were randomly assigned between May and July, 2005 either to receive sputum-submission guidance before specimen submission or to submit specimens without specific guidance, according to prevailing practice. Of enrolled patients, 133 (4%) declined to participate. The primary outcome measure was the proportion of instructed and non-instructed women testing smear positive. Intention-to-treat analysis was undertaken on the basis of treatment allocation. This study is registered with the International Standard Randomised Controlled Trial number 34123170.**

Findings **Instructed women were more likely to test smear positive than were controls (Risk ratio 1·63 [95% CI 1·19–2·22]). Instructions were associated with a higher rate of smear-positive case detection (58 [8%] in controls *vs* 95 [13%] in the intervention group; p=0·002), a decrease in spot-saliva submission (p=0·003), and an increase in the number of women returning with an early-morning specimen (p=0·02). In men, instructions did not have a significant effect on the proportion testing smear positive or specimen quality.**

Interpretation **In the Federal Tuberculosis Centre in Rawalpindi, lower smear positivity in women than in men was mainly a function of poor-quality specimen submission. Smear positivity in women was increased substantially by provision of brief instructions. Sputum-submission guidance might be a highly cost-effective intervention to improve smear-positive case detection and reduce the disparity between the sexes in tuberculosis control in low-income countries.**

Lancet 2007; 369: 1955–60

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Introduction

In 2004, there were 8·9 million new cases of tuberculosis and 1·7 million deaths. Tuberculosis control remains a public health priority, as indicated by the Millennium Development Goals and by the World Health Assembly, whose targets included detection of 70% of new smear-positive cases by 2005. Although there has been progress in the geographical expansion of WHO's directly observed treatment short-course (DOTS) programme, estimates are that only 53% of the predicted smear-positive cases were notified under DOTS in 2004.[1,2] This low percentage shows that a great proportion of smear-positive cases are undetected within DOTS areas, and recent studies indicated that women, in particular, are at risk of underdetection. In developing countries, more men than women are diagnosed with tubercolosis.[3] Studies in several settings, including Malawi, South Africa, and Bangladesh have shown that women attending tuberculosis diagnostic centres are less likely to test smear positive than are men.[4–7] In a recent study in Pakistan, we showed that, although there was little discrepancy in hospital attendance and specimen submission between the sexes, the rates of smear positivity of specimens submitted by men and women were 12% versus 6%.[8] Whether sex differences in smear-positive detection occur because women have paucibacillary disease or because they face more barriers to diagnosis is unclear. Previous studies have suggested that cultural inhibitions about producing deep sputum, particularly in public places, and lack of knowledge about tuberculosis diagnosis in low-income countries might affect the quality of specimens submitted by women.[9] However, provision of specific sputum-submission guidance is often not emphasised, and most diagnostic facilities do not provide such guidance. In Pakistan, which ranks seventh among the high-burden countries, about 26% of avoidable adult deaths are caused by tuberculosis.[10] Pakistan faces many of the same hurdles in the control of this disease as other low-income countries. Although DOTS coverage increased rapidly from 9% in 2000 to 79% in 2004, only 27% of estimated smear-positive cases were detected with DOTS, which is

far below the World Health Assembly target of 70%. The number of smear-positive case notifications in adult women (aged 15 years and over) is 10% lower than that in adult men; 15 275 women versus 16 790 men were notified in 2004.[1]

Analysis of records from the Federal Tuberculosis Centre, one of the main tuberculosis hospitals in Pakistan, showed that women were more likely to be notified as smear negative and men more likely to be notified as smear positive. Non-participant observations at the Federal Tuberculosis Centre indicated that women, in particular, received inadequate guidance on how to produce good-quality sputum specimens. In-depth interviews revealed that most women were not aware that sputum rather than saliva has to be submitted for tuberculosis diagnosis.[7] Therefore, one reason for lower smear positivity in women at the Federal Tuberculosis Centre might be that they are unaware of how to submit a good-quality sputum specimen. We tested this hypothesis in a pragmatic randomised controlled trial[11] to assess the effect of sputum-submission guidance on female patients testing smear positive.

Methods

Participants

The trial was done at the Federal Tuberculosis Centre, a specialised outpatient tuberculosis hospital in Rawalpindi, Pakistan. This centre was chosen because it has one of the highest patient loads and the greatest ethnic and geographic diversity of patients of any such facility in Pakistan. On average, 100–300 new patients undergo diagnostic screening every day, with an additional 50–100 patients attending for routine follow-up treatment. An estimated 25% of the patients come to the centre from the cities of Rawalpindi and Islamabad, 60% from other urban, semiurban, and rural settlements within the Punjab province, and the remaining 15% from other provinces.

Smear-positive tuberculosis diagnosis is made on the basis of two specimens at this centre: a spot specimen provided on the day of screening and a second early-morning specimen brought in the next day. A third specimen is not routinely obtained because few patients are able to return for a third day of testing, owing to the costs of travelling to or renting accommodation close to the hospital. This is a common occurrence in urban tuberculosis centres where many patients travel long distances.

Patients were screened by doctors, and those meeting National Tuberculosis Programme (NTP) criteria (history of cough for longer than 3 weeks or fever for 1 month, or both, blood in sputum, night sweating, weight loss and loss of appetite, and age between 14 and 75 years) were referred for initial sputum testing. These patients were our study population. Exclusion criteria were history of tuberculosis diagnosis and treatment or intake of oral steroids in the 3 months before presentation, or both.

This pragmatic randomised controlled trial[11] was done to assess the effect of sputum-submission guidance on female patients testing smear positive (primary outcome), male patients testing smear positive, smear-positive sputum specimens submitted by men and women, and inadequate sputum specimens (ie, saliva specimens) submitted by men and women (secondary outcomes).

Procedures

20 unmarked opaque envelopes, ten containing control cards and ten containing instruction cards, were placed in a bag by the researcher and given to the clinical trial officer. Patients referred for sputum testing by doctors were sent to the clinical trial officer, who discussed the information and consent sheet with the eligible patients. If written consent was obtained, the clinical trial officer listed the patient's hospital identification number on a register and drew one envelope from the bag. The response on the card was recorded and determined allocation of each patient to the control or intervention group. When all 20 envelopes had been used, and at the start of each day, new envelopes were provided by the researcher and the randomisation process was restarted. To confirm that the clinical trial officer was closely following the randomisation protocol, frequent, unannounced checks were made by the researcher and by the members of the NTP unaffiliated with the trial. No deviation from the protocol was recorded during any of the checks. Randomisation was not stratified because the large sample size was expected to yield comparable intervention groups.[12]

Patients in the intervention group were referred to a designated room where they received guidance as to how to produce a good sputum sample from a female health worker who had been trained by the researcher and a senior tuberculosis control officer as to how to provide sputum samples. The health worker was not involved in recruitment or randomisation. The guidance session began by emphasising the importance of submitting sputum rather than saliva, and by providing a description of the visual difference between them. The technique that should be used to expectorate a good sputum specimen[13] (take three deep breaths, followed by a deep cough to bring up sputum from your lungs) was then demonstrated. Finally, patients were told to aim to fill at least a quarter of the container (5 mL, shown by pointing out the required level on a demonstration container), and to provide one spot specimen and return the next day with an early-morning specimen, which should be expectorated on awakening. After these instructions, which lasted about 2 min, patients in the intervention group were directed to the laboratory to obtain sputum containers. Controls were processed according to the standard protocol in practice at the tuberculosis centre. They were sent directly by the clinical trial officer to the laboratory to obtain sputum

containers without receiving instructions from a dedicated instructor. Patients in both groups returned specimens in identical containers to the laboratory for testing. Laboratory staff were unaware of the group from which a specimen originated.

Outcome measurement

Slides prepared from the specimens were stained with the Ziehl-Nielson method and microscopically examined for the presence of acid-fast bacilli. According to WHO guidelines, patients who submitted two specimens containing acid-fast bacilli were classified as smear positive. Specimen quality was assessed visually and by microscopic examination of Ziehl-Nielson-stained smears, with a modification of the rating system of Bartlett.[14,15] Specimens with a purulent, mucoid or blood-stained visual appearance or containing polymorphoneutrophils on microscopic inspection were designated sputum. Specimens with a clear and watery appearance containing squamous epithelial cells, but no polymorphoneutrophils, were designated saliva.

For the purpose of this study, all specimens were analysed microscopically for acid-fast bacilli and polymorphoneutrophils, irrespective of whether they were classified as saliva or sputum visually.

Technicians taking part in the study were highly trained and had over 5 years of experience. Every week during the study period, the head of NTP laboratories, unaware of the original result assessed by the technicians, took a random selection of stained slides and assessed their sputum quality and acid-fast bacilli status. She then checked her results against the technicians' results to confirm a high level of accuracy. No batch of slides failed this quality assurance procedure and none needed to be re-examined.

Statistical analysis

Intention-to-treat analysis was undertaken on the basis of treatment allocation. The primary analysis compared the detection rate for smear-positive tuberculosis in women with and without instructions. An additional analysis compared the rates in men.

Previous analysis of the Federal Tuberculosis Centre registers showed that the baseline smear-positivity rate in female patients was 10%. On this basis, the target sample size was 1450 female patients (725 in each group), chosen to give 80% power to detect a 50% difference in the proportion of specimens from women testing smear-positive, with an allowance of 5% for type I error. We expected that a similar number of men would be recruited and that the effect of the intervention might be smaller so the additional analysis would have less power to detect a difference.

Data were analysed with Stata version 9.1. The χ^2 test was used to compare outcomes between control and intervention women and between control and intervention men. Point estimates for the differences in these proportions between treatment groups with their respective 95% CI were calculated. All p values reported are two-sided.

The cost per extra case detected by the intervention was calculated by dividing the additional cost incurred to implement the intervention by additional cases detected, where additional cost is the cost of employing a health worker for the duration of the trial (because Ziehl-Nielson staining was done according to standard tuberculosis centre procedure, no additional laboratory staff were needed for this procedure), and additional cases detected are the number of extra male and female smear-positive cases detected in the intervention group compared with controls.

The trial procedures were approved by the ethics committees of the Pakistan Medical Research Council (Pakistan) and the London School of Hygiene and Tropical Medicine (UK). This trial is registered with the International Standard Randomised Controlled Trial number 34123170.

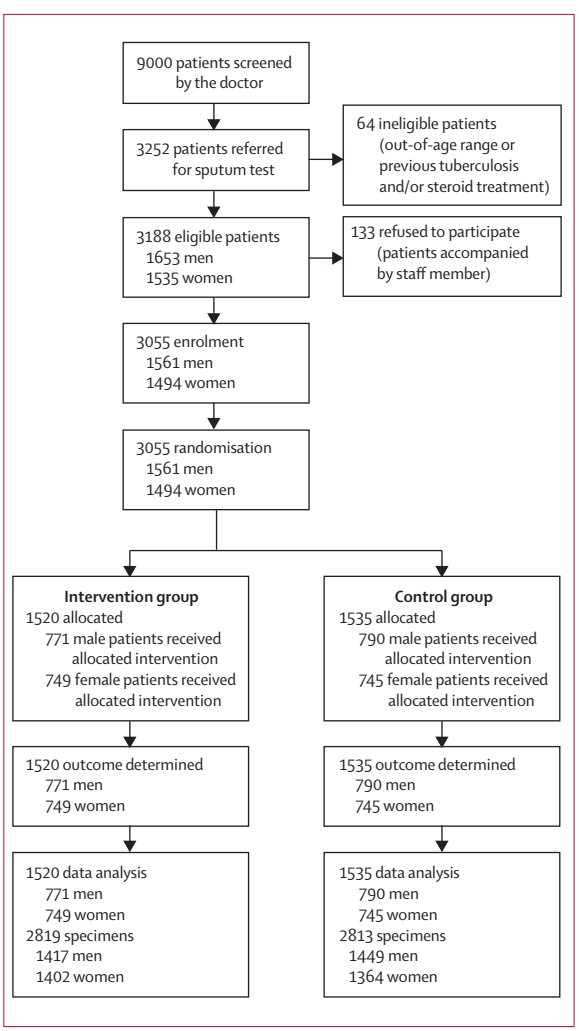

Figure: **Trial profile**

Role of the funding source

The study sponsor had no role in study design, data collection, data analysis, data interpretation, or in writing the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

	Control (n=745)	Instructed (n=749)	Risk ratio instructed *vs* control (95% CI)	Risk difference: instructed minus control (95% CI)	p*
Women					
Age (years)					
Mean (SD)	35·0 (15·5)	34·8 (15·7)	..	..	..
Median (25%–75% centiles)	35 (22–45)	30 (20–47)	..	..	..
Patients positive†	58 (8%)	95 (13%)	1·63 (1·19 to 2·22)	4·9 (1·8 to 8·0)	0·002
Men					
Age (years)					
Mean (SD)	35·5 (15·8)	35·5 (16·6)	..	..	..
Median (25%–75% centiles)	31·5 (22–47)	30 (20–50)	..	..	..
Patients positive†	78 (9·9)	90 (11·7)	1·18 (0·89 to 1·57)	1·8 (−1·3 to 4·9)	0·25

*χ^2 test. †WHO definition: two specimens smear positive.

Table 1: **Baseline characteristics and the primary outcome of patient smear positivity**

	Control*	Instructed*	Risk difference: instructed minus control (95% CI)	p†
Women				
Number of patients	745	749		
Spot specimens submitted	736 (99%)	740 (99%)	0·01 (−1·1 to 1·1)	0·99
Early-morning specimens submitted	628 (84%)	662 (88%)	4·1 (0·6 to 7·6)	0·02
Spot specimens	736	740		
ZN-positive	60 (8%)	100 (13%)	5·3 (2·2 to 8·5)	0·001
Salivary	208 (28%)	159 (21%)	−6·8 (−11·2 to −2·4)	0·003
Sputum spot specimen	528	582		
ZN-positive	59 (11%)	99 (17%)	5·8 (1·8 to 9·9)	0·005
Early-morning specimens	628	662		
ZN-positive	70 (11%)	101 (15%)	4·2 (0·4 to 7·8)	0·03
Salivary	83 (13%)	68 (10%)	−2·9 (−6·5 to 0·6)	0·10
Sputum early-morning specimen	545	594		
ZN-positive	69‡ (13%)	101§ (17%)	4·3 (0·2 to 8·5)	0·04
Men				
Number of patients	790	771		
Spot specimens submitted	772 (98%)	758 (98%)	0·6 (−0·8 to 2·0)	0·40
Early-morning specimens submitted	677 (86%)	659 (85%)	−0·2 (−3·7 to 3·3)	0·90
Spot specimens	772	771		
ZN-positive	85 (11%)	95 (12%)	1·3 (−1·9 to 4·5)	0·36
Salivary	184 (24%)	172 (22%)	−1·5 (−5·7 to 2·8)	0·60
Sputum spot specimen	588	586		
ZN-positive	84 (14%)	95 (16%)	1·9 (−2·2 to 6·0)	0·36
Early-morning specimens	677	659		
ZN-positive	90 (13%)	100 (15%)	1·9 (−1·9 to 5·6)	0·32
Salivary	66 (10%)	63 (10%)	−0·1 (−3·4 to 3·0)	0·91
Sputum early-morning specimen	611	596		
ZN-positive	90¶ (15%)	98‖ (16%)	1·6 (−2·4 to 5·8)	0·39

ZN=Ziehl-Nielson. *Data are number (percentage). †χ^2 test. ‡Two missing. §Three missing. ¶One missing. ‖Four missing.

Table 2: **Secondary endpoints**

Results

Patients with suspected tuberculosis were enrolled in the trial between May and July, 2005 (figure). 133 individuals refused to take part; all of these patients were accompanied by a member of the hospital staff who wished to explain sputum-submission procedures. Table 1 shows the baseline characteristics and differences in smear positive cases detected. The proportion of women who tested smear positive was significantly higher in the instructed group than in controls (table 1).

The smear-positivity rate was significantly higher in instructed than in control women for both spot and early-morning specimens (table 2). Women in the instructed group were also more likely to return with an early-morning specimen and less likely to submit a spot specimen of saliva. Instructions did not have a significant effect on the proportion of women providing a spot specimen or on those submitting an early-morning specimen of saliva.

Men in the instructed group also showed increased smear positivity and specimen positivity, and improvement in specimen quality. However, the magnitude of the improvement was smaller in men than in women and was not significant (table 1). As expected, in both spot and early-morning specimens, fewer saliva samples tested smear positive than did sputum samples (table 2).

The cost of employing a health worker for the period of data collection was 6300 PKR. The number of additional male and female cases detected in the intervention group was 49. Therefore, the cost per extra case detected was 129 PKR (which is about US$2).

Discussion

Sputum-submission guidance led to a significant improvement in the rate of detection of smear-positive cases among women. Our results suggest that the low smear-positive case-detection rate previously recorded in women at the Federal Tuberculosis Centre was mainly a function of poor-quality specimen submission, and that improvement can be achieved by brief instructions about the importance and technique for producing a good quality sputum specimen.

In line with our findings, others have shown the beneficial effect on case detection of instruction of patients.[9,10,16] However, we have now shown in a randomised controlled trial how provision of instructions leads to improvement in smear-positive case detection rate in women at a primary diagnostic centre.

The intervention aimed to improve case detection in several ways: increasing quality and volume of the first (spot) sample; increasing the likelihood of the patient returning with an early-morning sample; and increasing the quality and volume of this second sample. Improved

quality of the samples submitted by women in the intervention group was probably the main factor leading to higher case detection. The effect was most probably greater in female patients because women were less knowledgeable about the difference between sputum and saliva and the need to submit a proper sputum sample for diagnosis of tuberculosis. Men, in general, are probably physically more able and comfortable about coughing deeply to expectorate sputum, whereas women benefit from some guidance on the importance and technique for submitting a good-quality specimen. Although instructions had a noticeable effect on the smear positivity rate of both spot and early-morning specimens, the effect was greater for spot specimens. The increase in specimen positivity was so large that decreased saliva submission alone would not explain the change. The intervention caused an improvement in yield from sputum specimens submitted and a reduction in saliva specimens submitted. One reason for the improved yield might be related to an increase in the volume of specimens submitted by instructed women, because one of the main points conveyed to patients was that a minimum of 5 mL of sputum should be submitted. Studies have shown that, in the absence of specific instructions, men submit larger-volume specimens than do women,[17] and that specimens with a volume of more than 5 mL have a higher probability of testing smear positive.[18]

Women in the instructed group were significantly more likely to return with a second specimen and complete the tuberculosis diagnosis process than were controls, which also contributed to increased case finding for instructed women.

Overall, the effect of instructions on all outcomes seemed to be greater in women than in men, although this study was not sufficiently powered to show an interaction by sex (p=0·17). In the absence of a dedicated instructor, women were more likely to be neglected than were men. In many facilities, including the tuberculosis centre, laboratory staff are the main source of guidance for patients when they obtain sputum-submission containers. Non-participant observations at the tuberculosis centre indicated that sporadic, incomplete instructions were provided by overburdened laboratory staff. Male patients were more likely to receive instructions than were female patients, who often allowed people accompanying them to collect their containers.

To keep any clinical difference between control and intervention women, and control and intervention men, to a minimum we ensured that all women were examined by the same doctor and that all men were examined by another doctor. Although all doctors at the tuberculosis centre followed standard WHO protocols for symptomatic screening, because men and women were examined by different individuals, cross-sex comparisons should be interpreted with caution.

There are very few studies that have examined the effect of sputum-submission instructions on smear-positive case detection. In a study in Japan,[19] the smear-positivity rate was two times higher after provision of instructions. However, the authors admitted that the result might be due to differences in disease severity between groups. A small (n=174 patients) randomised controlled trial in Indonesia showed that instructions resulted in a 15·1% higher case-detection rate.[20] However, the effect of instructions on men and women separately was not reported in that study, and the prevalence of tuberculosis in the intervention and control groups was much higher (50·6% and 35·5%, respectively) than in most primary diagnostic settings.

As in many other settings, in the absence of specific instructions the smear-positivity rate of men at the tuberculosis centre was higher than that of women. However, with instructions, this trend was reversed and the smear positivity rate was slightly higher in women than in men (13% *vs* 12%). Higher female smear-positivity rates have also been recorded in other centres in south Asia,[6] despite theories suggesting that women are prone to develop smear-positive tuberculosis less frequently than are men.

This was a pragmatic randomised trial, conducted in real, resource-constrained conditions. Pragmatic trials balance the need for excellence in trial methods with feasibility and generalisability. The large sample size and straightforward randomisation procedures make the comparison of the two groups possible, although data other than age and sex that could have been used to assess the comparability of the two groups were not obtained. Although this study was done in one urban centre, when considering its generalisability, we need to take into account that, because the Federal Tuberculosis Centre is a well established tuberculosis facility providing free treatment, there was a broad ethnic and demographic mixture of patients in the trial. The prevalence of tuberculosis in this setting was typical of diagnostic facilities in countries with high tuberculosis incidence. We would, therefore, argue that sputum-submission guidance is probably beneficial in settings where patients, particularly women, have little knowledge about tuberculosis and where saliva submission rates are high.

Finally, we emphasise that the intervention was designed to be cheap and easily replicable in low-income countries; implementation required only a private space for instructions and a trained health worker to provide about 2 min of guidance to each patient. Training the health worker to deliver instructions took only half a day, and the cost per extra case detected was about $2, showing the cost-effectiveness of this intervention. In addition to benefiting individual patients, provision of sputum-submission instructions might increase the proportion of smear-positive cases detected within DOTS areas, which is currently lagging behind global targets. Furthermore, by improving female case detection, sputum-submission guidance might contribute towards a reduction of the disparity between men and women in tuberculosis control in developing countries.

Contributors
All authors participated in the study. MK, OD, CS and PG-F took part in the data analysis. All authors saw and approved the final version of the manuscript.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgments
The study was sponsored by the DFID TB Knowledge Program. We thank Laura Gillini of WHO and all members of the National TB Control Program (Pakistan) for their support in the completion of this study.

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