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Sputum conversion at the end of 8 weeks among category 1 tuberculosis patients: How reliable are the peripheral laboratory results?

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ABSTRACT

Objective: To assess the quality of week 8 sputum smear AFB microscopy performed by peripheral TB laboratories in Nigeria.
Method: A cross-sectional review was performed of all week 8 tuberculosis sputum smear slides reported for the first quarter of 2009 by peripheral laboratories in five States of Nigeria. Each slide was reviewed by two independent external slide readers as external quality check and also crosschecked with fluorescent microscopy.
Results: In Akwa Ibom, Anambra, Enugu, Kogi and Ogun States, a total of 415, 315, 231, 206 and 428 week 8 slides respectively were studied (a grand total of 1595 slides studied). The wide range of conversion rates between the different States as reported by peripheral labs (83.8% in Anambra State to 98.1% in Kogi State) was also observed by the external quality check (68.4% in Kogi State to 88.0% in Akwa Ibom State). In all the States, the studied sputum conversion rates reported by the peripheral labs were significantly higher than values obtained from external quality check and fluorescent microscopy ($P = 0.000$).
Conclusion/recommendation: There is a wide range of sputum conversion rates between States, but the conversion rate in each State is significantly higher than those of external quality check possibly indicating many false negative reports by peripheral labs. It is recommended that training and re-training of laboratory persons be continued. Internal and external quality checks should also continue to be practiced in the national TB program.

Introduction

Tuberculosis (TB) is one of the major causes of morbidity and mortality in developing countries. Its incidence has increased significantly since the onset of HIV and indeed TB is one of the major killers in patients with HIV. Nigeria is one of the countries with the highest TB and HIV burden and was ranked fourth among the 22 countries with the highest burden of

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TB. Each year in Nigeria, 220,000 positive cases of pulmonary tuberculosis (PTB) cases occur, of which approximately 50% are smear-positive [1]. To combat this Nigeria adopted the WHO recommended "TB Control Strategy". One of the components of this strategy is diagnosis through a quality assured network of TB sputum microscopy services.

Sputum smear microscopy is an important diagnostic procedure for PTB in the National Tuberculosis and Leprosy Control Program (NTBLCP) in Nigeria [2]. Suspects diagnosed as smear positive are started on anti-TB drugs as soon as possible. A patient is normally treated for 8 months (32 weeks) according to the national guideline using the four fixed dose combination (4FDC) treatment regimen [3,4]. A patient started on treatment, at the end of 8 weeks, submits a sputum sample for assessment of treatment [5].

Most patients convert to a negative smear result, but a reasonable number of patients do not convert to a negative smear result at the end of 8 weeks [6,7]. A report of the sputum conversion rate at 8 weeks in the NTBLCP in 2007 showed a very wide range of 51–93%. Many questions come to mind: can inefficient TB microscopy be responsible? What is the actual sputum conversion rate at the end of 8 weeks in Nigeria? There have been various reports on sputum conversion rates done in some countries of the world, but as far as this research is concerned, there has not been any research to establish reliable conversion rates in Nigeria. The dangers of reporting false negative/positive results in TB control cannot be overemphasized.

The aim of this study was to evaluate previous week 8 smear results of new TB patients reported by peripheral labs in various regions and States in Nigeria.

Materials and methods

Study area

The study site covers all the Directly Observed Treatment Short course (DOTS) microscopy centers in five selected States covering the North-central, South-east, South-west and South-south geopolitical zones in Nigeria.

Study population

The study was conducted in five States sampled from the four geopolitical zones mentioned above. Kogi State was selected from the North-Central zone while Enugu and Anambra States were selected in the south-east of Nigeria. Ogun State was selected from the south-west zone. Akwa Ibom State was selected from the South-south zone.

Study design

The study design is a descriptive cross-sectional study involving a review of smear microscopy of sputum samples at week 8.

Sample size estimation

The number of patients to be sampled was guided by the upper limit required to give a 95% level of confidence at an expected conversion of about 85.0% [8] using the precise prevalence formula: Sample size (N) = Z^2P $(100 - P)/D^2$ where Z is a constant given as 1.96, P is expected conversion 85%, and D is acceptable error (5%) [9]. When the above formula is used, a sample size of 195.9 is obtained. To make up for errors in data completion, all complete slides (i.e., two week 8 slides per patient) for the first quarter of 2009 from these selected States were studied.

Sampling method used for the States

The thirty-six States in Nigeria were stratified to correspond to low, medium and high ranges of sputum conversion rates at week 8 based on the statistical report for 2007, published by the NTBLCP. The 2009 sputum conversion rate report has since been released and it showed a similar wide range of sputum conversion rates for new smear positive (Category 1) PTB cases. However, the 2009 report is incomplete for some States and so cannot be used for the study. Two States were selected from each stratum using a simple random method. The States are: for low range (51% to <75%) – Kogi; for medium range value (75% to <85%) – Ogun and Enugu; for high range values (85–93%) – Anambra and Akwa Ibom.

Only the slides that were complete (i.e., two week 8 slides per patient) in all the five States for the first quarter of 2009 were selected. All slides used were stored for not more than four months; they are slides stored for the existing quarterly external quality assurance (EQA) of the NTBLCP in the year.

Ethical consideration and inclusion/exclusion criteria

Approval was obtained from the Ethics Committee of the University of Nigeria Teaching Hospital and any other relevant regulatory agency. Inclusion criteria were: the patient must have been AFB smear positive before the start of treatment and the patient must be a new case, i.e., Category 1. Those excluded were: all transferred cases; all Retreatment and Category 2 cases; months 3, 5 and 7 cases; smear negative at diagnosis and cases with incomplete slides.

Data collection

All week 8 slides were picked across TB smear microscopy labs in each State, for rechecking using fluorescent microscopy and the light microscopy. The slides were picked by the State Laboratory focal persons (SLFP) in the five States. The SLFP sent the slides to Irrua Specialist Teaching Hospital by courier. The re-checking was done by two independent readers (to minimize error due to variation inherent in multiple readers). A third reader read any discordant slides arising from the two independent readers. Slides selected from all the States were re-checked at Irrua Specialist Teaching Hospital. All slides picked from this study area for re-checking were de-oiled using xylene and dried. The slides were re-stained using Ziehl–Neelsen as well as Fluorescence method.

Quality check

For quality check the slides are read by two independent readers; any discordance is re-checked by a third person, who serves as a gold standard.

Quality assurance

The laboratory and the staff are part of the NTLCP; the hospital laboratory is part of the network of peripheral labs that perform TB smear microscopy in Nigeria and they are part of an ongoing external quality assurance.

Recording of results

Semi-quantitative results were recorded according to the National TB Programme (NTP) guidelines. If one or both slides collected from a patient is positive, the result is recorded as positive (as in NTLCP). Also, the higher value of the two readings is recorded as the result, e.g. where one slide is + and the second slide is ++ then the recorded result is ++.

Data analysis

The data generated were entered and analyzed in a Statistical Package for Social Sciences (SPSS). Chi-square test was used to compare the previous readings to present readings.

Results

In Akwa Ibom (South-south region) 415 second-month slides were studied. The sputum conversion rate recorded by

Table 1 – Comparison between week 8 AFB peripheral microscopy results and EQC results for Akwa Ibom State.

Akwa Ibom State – week 8 AFB microscopy results comparison			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	394 (94.9)	365 (88.0)	322 (77.6)
Positive	21 (5.1)	50 (12.0)	93 (22.4)
Total	415 (100.0)	415 (100.0)	415 (100.0)
Comments: conversion rate	94.9%	88.0%	77.6%

χ^2 and *P* value.
1. Between peripheral lab and EQC light microscopy results = 12.95 (*P* value = 0.000) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 52.71 (*P* value = 0.000) Significant.

Table 2 – Comparison between week 8 AFB peripheral microscopy results and EQC results for Anambra State.

Anambra State – week 8 AFB microscopy results comparison			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	264 (83.8)	238 (75.6)	238 (75.6)
Positive	51 (16.2)	77 (24.4)	77 (24.4)
Total	315 (100.0)	315 (100.0)	315 (100.0)
Comments: conversion rate	83.8%	75.6%	75.6%

χ^2 and *P* value.
1. Between peripheral lab and EQC light microscopy results = 6.63 (*P* value = 0.01) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 6.63 (*P* value = 0.01) Significant.

Table 3 – Comparison between week 8 AFB peripheral microscopy results and EQC results for Enugu State.

Enugu State – week 8 AFB microscopy results comparison			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	214 (92.6)	186 (80.5)	186 (80.5)
Positive	17 (7.4)	45 (19.5)	45 (19.5)
Total	231 (100.0)	231 (100.0)	231 (100.0)
Comments: conversion rate	92.6%	80.5%	80.5%

χ^2 and *P* value.
1. Between peripheral lab and EQC light microscopy results = 14.61 (*P* value = 0.000) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 14.61 (*P* value = 0.000) Significant.

peripheral laboratories was 94.9%. This was significantly higher than the conversion rate of 88.0% obtained by external quality check (EQC) light microscopy ($\chi^2 = 12.95$; P value = 0.000) and 77.6% by fluorescent microscopy ($\chi^2 = 52.71$; P value = 0.000). Similar high conversion rates were also obtained in peripheral laboratories in Anambra and Enugu States (both in South-east region). In Anambra State 315 s-month slides were studied. A peripheral laboratory result recorded 83.8% as against 75.6% recorded by EQC light microscopy and fluorescent microscopy ($\chi^2 = 6.63$; P value = 0.01). In Enugu 231 slides from peripheral laboratories had a conversion rate of 92.6%, while EQC microscopy laboratories and fluorescent microscopy had 80.5% ($\chi^2 = 14.61$; P value = 0.000) (Tables 1–3).

In Kogi State (North-central region) 206 second-month slides reported a conversion rate of 98.1% by peripheral laboratory. This was significantly higher than 68.4% and 60.7% conversion rates recorded by EQC ($\chi^2 = 64.78$; P value = 0.000) and fluorescent microscopy ($\chi^2 = 84.32$; P value = 0.000) respectively. In Ogun State (South-south region) 428 slides collected showed conversion rates of 90.2% by peripheral laboratories. This again was significantly higher than the conversion rate of 76.2% obtained by EQC and fluorescent microscopy ($\chi^2 = 37.45$; P value = 0.000) (Tables 4–6).

Table 4 – Comparison between week 8 AFB peripheral microscopy results and EQC results for Kogi State.

Kogi State – week 8 AFB microscopy results comparison			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	202 (98.1)	141 (68.4)	99 (60.7)
Positive	4 (0.9)	65 (31.6)	64 (39.3)
Total	206 (100.0)	206 (100.0)	163 (100.0)
Comments: conversion rate	98.1%	68.4%	60.7%

χ^2 and P value.
1. Between peripheral lab and EQC light microscopy results = 64.78 (P value = 0.000) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 84.32 (P value = 0.000) Significant.

Table 5 – Comparison between Week 8 AFB peripheral microscopy results and EQC results for Ogun State.

Week 8 AFB microscopy results comparison			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	386 (90.2)	326 (76.2)	326 (76.2)
Positive	42 (0.8)	113 (23.8)	113 (23.8)
Total	428 (100.0)	428 (100.0)	428 (100.0)
Comments: conversion rate	90.2%	76.2%	76.2%

χ^2 and P value.
1. Between peripheral lab and EQC light microscopy results = 37.45 (P value = 0.000) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 37.45 (P value = 0.000) Significant.

Table 6 – Comparison between week 8 AFB microscopy results and EQC results for the 5 States.

Week 8 AFB microscopy results comparison for the 5 States			
	Peripheral lab (microscopy)	External quality check (EQC) result (light microscopy)	External quality check (EQC) result (fluorescent microscopy)
AFB test result	Number (%)	Number (%)	Number (%)
Negative	1460 (91.5)	1256 (70.9)	1171 (75.5)
Positive	135 (8.5)	339 (21.3)	381 (24.5)
Total	1595 (100.0)	1595 (100.0)	1552 (100.0)
Comments: conversion rate	91.5%	70.9%	75.5%

χ^2 and P value.
1. Between peripheral lab and EQC light microscopy results = 103.12 (P value = 0.000) Significant.
2. Between peripheral lab and EQC fluorescent microscopy results = 148.46 (P value = 0.000) Significant.

Range (conversion rate):
Peripheral laboratory = 83.8% (Anambra State) to 98.1% (Kogi State).
EQC (light microscopy) = 68.4% (Kogi State) to 88.0% (Akwa Ibom State).

Discussion

The quality of sputum smear results is a critical element in the management and control of TB (DOTS strategies). This is of major concern especially in the developing countries that still largely depend on sputum smear microscopy for diagnosis. A false positive smear result at 8 weeks will be interpreted as non-conversion and will lead to extending the intensive phase treatment for an extra month while a false negative result would mean wrongly converting a patient to the continuation phase treatment when he/she should have been given an extended 4 weeks of intensive phase drugs. This false negative result is particularly worrisome because it could lead to drug resistant strains of TB (MDR-TB or XDR-TB).

Results in the present study reveal two particularly interesting issues. First, there is a wide range of sputum conversions reported by peripheral labs in various regions of the country: in Anambra State (South-east region) 83.8% was reported; while in Kogi State (North-central region) 98.1% conversion rate was reported. Secondly, in all the regions studied, the peripheral labs report significantly higher rates of conversion at the end of the 8 weeks of intensive treatment for TB when compared with results from both external quality checks and fluorescent microscopy.

In the first issue, one wonders if such is truly the case, and, if yes, why is there such a wide range of conversions at 8 weeks in these regions within the same country? Incidentally, the wide range of conversion rates recorded by peripheral labs is also reported by the external quality checks and fluorescent microscopy. Conversion rates have been documented by some studies to be determined by some factors like age of patient, pre-treatment bacillary load, rate of default, presence or absence of resistant strains, etc. A Cameroonian study observed that the factors of age 40 years and above and a bacillary load of 3+ on pre-treatment sputum were significantly associated with non-conversion of sputum smears at the end of two months of treatment [10]. Another study established that under field conditions even with DOTs, new smear positive patients with a heavy bacillary load showed statistically significant poor sputum conversion rates at two and three months and higher failure rates as compared with patients with a lesser bacillary load [11]. Similarly, in Rwanda, the smear conversion rate was 82%, and this varied significantly from facility to facility depending on location of the facility, i.e., rural or urban [12]. Two separate studies in India also reported two widely different conversion rates at the end of 8 weeks of treatment: one reported 58.0% [13] while another reported 84.0% [14].

In the second issue, there were significantly higher conversion rates from all the reports from peripheral labs in all the regions studied compared with external quality checks and fluorescent microscopy. Interestingly also, a 2007 report on some States showed a widely different conversion rate compared with that of a 2009 report, e.g., Kogi State conversion rate for 2007 was 58% while in 2009 it was 98.1%. Could such a "remarkable improvement" in conversion rates within the same State mean that there is an equally remarkable improvement in TB case management or false reporting by the TB lab persons who may not be diligent enough to examine the slides properly?

Diligence in studying the TB slides greatly affects the interpretation of results. Indeed, the sensitivity of sputum smear microscopy has been reported to vary (range, 20–80%), often depending on the diligence with which specimens are collected, smears are made, and stained smears are examined [15]. Hence, those lab staff who lack diligence in slide examination hastily conclude that the slide is negative when they are actually positive, thus leading to a higher conversion rate.

The very high conversion rates obtained from peripheral labs are suspicious because they are significantly different from external quality checks and fluorescent microscopy results done on the same slides and also do not agree with most findings from other parts of the world, especially in countries with high HIV/TB co-infection like Nigeria. A study in Oman obtained a sputum smear conversion rate of 78.6% [16]. In another study done in India the conversion rate was 58%, 61% and 62% in patients with PTB alone, PTB plus type 2 diabetes and PTB with HIV infection, respectively [13].

Attempts have been made to improve the quality of TB microscopy. One method used to improve the quality of TB microscopy was by integrating malaria microscopy quality assessment into the existing TB microscopy QA system. When such a program was implemented, it resulted in an increase in the specificity of both TB and malaria microscopy results. At the final assessment, 100% specificity was achieved for TB microscopy results [1]. However, this needs to be further tested in various other settings.

Conclusion and recommendation

A wide range of sputum TB microscopy conversion rates were observed after 8 weeks of treatment from region to region as reported by sputum smear microscopy and confirmed by External Quality Check and fluorescent microscopy. However, the conversion rates reported by the peripheral labs in all regions of the country are higher than what was observed from the quality control checks and fluorescent microscopy indicating that some of the peripheral results may be false negative for AFB. It is therefore recommended that training and re-training of lab persons be carried out. Internal and external quality checks should also routinely be conducted.

Study limitations

Niger State was originally selected in the research work to make it a total of six States, but unfortunately could not produce slides at the end of the intensive phase because such slides were not stored or produced for rechecking. The National TB control program requests a quarterly report from the peripheral laboratories; hence, it is not certain if some of the collected slides are second or third month AFB results. This, however, did not affect the study since the external quality checks were done on the same slide irrespective of month of collection.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

M.C.A., J.N.C., D.C.O., E.N.A. and I.N.J. conceived the study. The study protocol was designed by M.C.A., D.C.O., I.N.J., J.N.C., C.C.N. and E.N.A. M.C.A., I.N.J. and S.B.O. carried out the laboratory study and oversaw data collection. E.N.A. performed data entry and carried out the data analysis. D.C.O., M.C.A., E.N.A., A.O.M., N.O.M., C.O. and N.E. did the data interpretation and conceptualization of the manuscript outline. E.N.A., M.C.A. and D.C.O. drafted the manuscript, while D.C.O., M.C.A., E.N.A., J.N.C., C.C.N., A.O.M., N.O.M., C.O. and N.E. critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. D.C.O. is guarantor of the paper.

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