Safety and yield of sputum induction for diagnosis of pulmonary tuberculosis in children in a tertiary hospital in Ghana

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Background. Induced sputum (IS) is a sampling technique for obtaining lower airway samples for microbial investigations, including GeneXpert and culture for microbiological confirmation of *Mycobacterium tuberculosis*.

Objectives. To investigate the safety and yield of IS in children admitted to a tertiary hospital in Ghana with presumed pulmonary tuberculosis (PTB).

Methods. A prospective cross-sectional study was carried out in children aged 3 months - 14 years at Komfo Anokye Teaching Hospital in Kumasi, Ghana, over the 6-month period January - June 2022. All children with breathing difficulty and other signs of respiratory distress were given respiratory support, and IS samples were obtained when respiratory distress had resolved. One or two IS samples were collected from each child within 48 hours of admission by a trained nurse after at least 4 hours of fasting. Children were monitored during and for 30 minutes after the procedure, with recording of respiratory rate, oxygen saturation, temperature and pulse rate. They were also monitored for any adverse events such as vomiting, wheezing and nosebleeds.

Results. A total of 144 children were sampled, with approximately two-thirds sampled a second time. Nearly half of the participants were aged <2 years (49.3%; *n*=71/144), and the median (interquartile range (IQR)) age was 2.5 (0.9 - 6.8) years. Ninety-eight children (68.1%) tested positive for PTB by Xpert Ultra, with 19/98 (19.4%) being rifampicin resistant; 47/102 (46.1%) were positive by Ziehl-Neelsen staining, and 57/102 (55.9%) were positive by Auramine O staining. Three children (2.1%) had an episode of epistaxis following the procedure. No other adverse events were observed. Measurements before and 30 minutes to 1 hour after the procedure (median (IQR)) were similar: temperature 36.5°C (36.5 - 37.5°C) v. 36.5°C (36.2 - 37.1°C), oxygen saturation 98% (92 - 99%) v. 98% (93 - 99%), pulse rate 120 (106 - 139) v. 125 (112 - 142) bpm, and respiratory rate 38 (30 - 48) v. 33 (30 - 45) cycles per minute.

Conclusion. We found sputum induction to be a safe and well-tolerated procedure in the paediatric population, with minimal clinical risk and a high microbiological yield for PTB.

Keywords. Children, tuberculosis, induced sputum.

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Study synopsis

What the study adds. This study is the first to provide information on the performance and safety of induced sputum (IS) in Ghanaian children. It shows that IS can be performed safely in this population, despite safety concerns that resulted in its late introduction in the country. In addition, it shows that IS procedures can provide quality sputum samples to improve bacteriological confirmation of pulmonary tuberculosis (PTB) in children with presumed tuberculosis. Lastly, it adds to the existing body of literature showing that with requisite training, sputum induction can be performed in low-income settings.

Implications of the findings. The study shows that the IS procedure can be used in Ghana to help shift from traditional ways of obtaining sputum samples in children, such as gastric lavage and routine methods of obtaining sputum in older children without induction, to improve bacteriological confirmation when PTB is suspected. The findings indicate that roll-out to other health facilities in Ghana is possible.

Tuberculosis (TB) is one of the leading causes of death and morbidity in young children, with a recent Global Tuberculosis Report showing that 12% of TB cases were in children in 2023.[1] Children with TB may progress to severe disease and death, but the majority of cases are undiagnosed. One key factor resulting in poor childhood TB case ascertainment is the difficulty of microbial confirmation in children. The main challenge is obtaining suitable respiratory samples, particularly sputum, as young children do not expectorate easily. In the few cases where expectorated sputum samples are obtained, an additional problem is that samples may be of poor quality and low in volume, with bacillary concentrations below the detection threshold of the diagnostic test.[2]

Induced sputum (IS) is the method of obtaining samples that provides the highest sensitivity for the detection of *Mycobacterium tuberculosis (MTB)* using new rapid polymerase chain reaction methods, especially Xpert MTB/RIF Ultra.[3]

IS has been shown to be feasible and effective in several low- to middle-income countries (LMICs).[4-6] It involves a minimum of 4 hours' fasting, nebulisation with 3 - 5% hypertonic saline, and inhaled salbutamol. Children are then encouraged to cough sputum up, or sputum is suctioned through the nasopharynx to obtain samples if they are unable to expectorate. Data show that the procedure is generally well tolerated and safe.[4,5] Reported side-effects include epistaxis, vomiting, wheezing, oxygen desaturation and cough.[6-8] The procedure can be performed by trained healthcare workers and is suitable for low-resource facilities. An additional advantage of IS is that, unlike gastric lavage, it does not require an overnight fast or hospitalisation, so it can be used more easily in high-burden LMICs.[7-9]

Despite the documented advantages of IS, there are no reports on the safety and usefulness of the procedure in Ghanaian children.[8,9] This study aimed to investigate the safety and yield of IS in children presenting to hospital with suspected TB disease.

Table 1. Demographic and anthropometric characteristics of the study participants (*N*=144)

Characteristic	*n* (%)*
Age (years)	
Median (IQR)	2.5 (0.9 - 6.8)
<2	71 (49.3)
2 - 5	35 (24.3)
6 - 10	27 (18.8)
>10	11 (7.6)
Gender	
Female	59 (41.0)
BMI	
0 - 5 years (*n*=98)	
Severe acute malnutrition	34 (34.7)
Moderate acute malnutrition	15 (15.3)
Normal	44 (44.3)
Overweight	2 (2.0)
Obese	3 (3.1)
6 - 19 years (*n*=40)	
Severe thinness	15 (37.5)
Thinness	5 (12.5)
Normal	15 (37.5)
Overweight	2 (5)
Obesity	3 (7.5)

IQR = interquartile range; BMI = body mass index.
*Except where otherwise indicated.

Methods

A prospective cross-sectional study was conducted over the 6-month period January - June 2022 at Komfo Anokye Teaching Hospital (KATH), a tertiary hospital and major referral centre in Kumasi, the capital city of the Ashanti region of Ghana.

Study participants

The study participants were children in the paediatrics department at KATH aged <14 years who were being investigated for presumed pulmonary TB (PTB) based on World Health Organization (WHO) criteria such as cough, especially if persistent and not resolving, prolonged fever with or without night sweats, not eating well or anorexia, weight loss or failure to thrive, unusual fatigue, reduced playfulness or decreased activity.[10]

A medical history was obtained, and physical examination was performed. We excluded children with severe hypoxia (oxygen saturation <92% on supplemental oxygen), severe bronchospasm, seizures or inability to protect their airways, and those who tested positive for COVID-19.

Children who had breathing difficulty, central cyanosis and/or wheezing on admission were given respiratory support, and all other necessary emergency procedures were initiated. They were then monitored closely until their signs of respiratory distress improved or resolved, after which they were assessed for fitness to undergo the IS procedure.

The parents or legal guardians were given information on the study procedure, and consent was obtained. Assent was also obtained from children aged >8 years. The nutritional status of the child was estimated as the weight-for-height z-score using sex, date of birth, weight and height. In addition, the WHO 2017 classification of nutritional status of infants and children was used to derive the body mass index. Weight and height were measured with a Seca scale, model no. 813, and a stadiometer, model no. 213 (Seca, Germany).

Safety assessment

Sputum induction was performed in a dedicated sputum induction room after the patient had fasted for 4 hours. Oxygen saturation and pulse rate were monitored with a Rad 4 pulse oximeter (Masimo, USA) before and 30 minutes to 1 hour after the procedure. The respiratory rate was also measured over a minute before and after the procedure and documented.

Children were nebulised with 2.5 - 5 mg salbutamol and 1 - 2 mL sterile 3% hypertonic saline attached to a nebuliser for 5 minutes. Sputum was then suctioned through the nasopharynx with a sterile mucus extractor with a catheter, size 6 or 8. Older children who were able to expectorate were encouraged to do so after nebulisation. A

Table 2. Clinical parameters on arrival and before and after the IS procedure (*N*=144)

Clinical parameter	On arrival, *n* (%)*	Before procedure, *n* (%)*	After procedure, *n* (%)*	*p*-value
Fast breathing	49 (34.0)	0	0	
Central cyanosis	3 (2.1)	0	0	
Chest wall retractions	55 (38.2)	0	0	
Pallor	42 (29.2)	0	0	
Stridor	3 (2.1)	0	0	
Wheezing	27 (18.8)	0	0	
Epistaxis	0	0	3 (2.1)	
Temperature (oC), median (IQR)	36.9 (36.4 - 37.8)	36.5 (36.5 -37.5)	36.5 (36.2 - 37.1)	0.618
Respiratory rate (cpm), median (IQR)	39 (30 - 50)	38 (30 - 48)	33 (30 - 42)	0.871
Pulse rate (bpm), median (IQR)	135 (115 - 154)	120 (106 - 139)	125 (112 - 142)	0.210
Oxygen saturation in room air (%), median (IQR)	97 (90 - 99)	98 (92 - 99)	98 (93 - 99)	0.087

IS = induced sputum; IQR = interquartile range; cpm = cycles per minute.
*Except where otherwise indicated.

second IS specimen was taken within 48 hours after the first sample had been obtained. No second sample was obtained if the child had been discharged, had died or was noted to be critically ill. Nebulising masks were used once for each child and disposed of after the procedure. Suction tubing was also disposed of after a single use. The procedure was terminated if the child's oxygen saturation dropped during the procedure.

All sputum specimens were transported immediately to the Department of Microbiology laboratory at KATH for storage in a 4°C fridge. Batches of specimens were transported twice weekly in a cooler box to the Kumasi Centre for Collaborative Research for Xpert MTB/RIF Ultra and microscopy testing (Ziehl-Neelsen and Auramine O staining). Mycobacterial cultures were not performed, as culture media were not available countrywide.

Ethical considerations

Ethical approval was obtained from the Committee on Human Research Ethics of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology (ref. no. CHRPE/AP/588/21) and the Institutional Review Board at KATH (ref. no. KATH/IRB/AP/137/21).

Statistical analysis

Data were entered into a Research Electronic Data Capture (REDCap) online database and exported to Stata SE 17.0 statistical software (StataCorp, USA) for analysis after data cleaning. Summary descriptive statistics were used for all baseline characteristics. The paired t-test was used to test the mean difference in the vital signs before and after the IS procedure. The proportions of positive IS samples on GeneXpert testing, Ziehl-Neelsen staining and Auramine O staining for first and second samples were also presented.

Results

From January to June 2022, 144 children, of whom 85 (59.0%) were male, were enrolled in the study. Nearly half of the participants were aged <2 years (49.3%), and the median (interquartile range (IQR)) age was 2.5 (0.9 - 6.8) years (Table 1). Of the 144 participants, 49 (34.0%) had breathing difficulty on admission, and 55 (38.2%) had lower chest wall retractions. The median (IQR) oxygen saturation on admission was 97% (94 - 99%), the median pulse rate 135 (115 - 154) bpm, and the median temperature 36.9°C (36.4 - 37.8°C). Three participants (2.1%) presented with stridor, and 3 (2.1%) with central cyanosis (Table 2).

Sputum induction was successfully performed in all 144 participants a day or 2 days after the patient's condition had normalised or improved, with approximately two-thirds of the children sampled twice. Epistaxis was the main adverse effect observed, occurring in 3 participants (2.1%). It was intermittent and ceased within 30 minutes after the procedure. Overall, for all the children sampled there were no clinically significant changes observed in temperature, respiratory rate, pulse rate or oxygen saturation before and after the procedure. The clinical parameters before and after IS are shown in Tables 2 and 3, and the adverse effects in Table 2.

Cumulatively, 98 of the children (68.1%) tested positive for PTB on Xpert Ultra, comprising 86/144 first samples (59.7%) and 73/88 second samples (83.0%) (Table 4). Of the 98 samples that were positive on Xpert Ultra, 19 (19.4%) were rifampicin resistant (drug resistant).

In addition, 102 of the first sputum samples and 89 of the second samples were tested for the presence of MTB using older methods, Auramine O and Ziehl-Neelsen stains on microscopy. Cumulatively, Auramine O stains were positive in 57 samples (55.9%) and Ziehl-Neelsen stains were positive in 47 (46.1%) (Table 4). All the children who tested positive on Xpert Ultra were successfully initiated on anti-TB medications.

Discussion

This study has shown that the IS procedure is a safe and effective diagnostic tool, with minimal adverse effects and well tolerated in young children, and with a high microbiological yield. Despite the late introduction of IS in Ghana in 2022 owing to concerns about patient safety and lack of expertise in performing the procedure, the

Table 3. Clinical parameters before and after the IS procedure, according to Xpert Ultra status (*N*=144)

Variable, median (IQR)	Xpert Ultra status		*p*-value
	Negative	Positive	
Examination before IS			
Temperature (°C)	36.5 (36.4 - 37.5)	36.6 (36.2 - 37.2)	0.743
Respiratory rate (cpm)	34 (24 - 50)	32 (28 - 42)	0.911
Pulse rate (bpm)	114 (105 - 130)	120 (106 - 140)	0.149
Oxygen saturation (%)	98 (96 - 99)	99 (96 - 99)	0.633
Examination 30 minutes to 1 hour after IS			
Temperature (°C)	36.5 (36.3 - 36.8)	36.5 (36.2 - 37.2)	0.618
Respiratory rate (cpm)	34 (28 - 48)	32 (30 - 42)	0.871
Pulse rate (bpm)	126 (15 - 142)	120 (110 - 140)	0.210
Oxygen saturation (%)	99 (95 - 100)	98 (95 - 99)	0.087

IS = induced sputum; IQR = interquartile range; cpm = cycles per minute.

Table 4. Positivity of GeneXpert MTB/RIF Ultra, Zeihl-Neelsen staining and Auramine O staining in detecting *Mycobacterium tuberculosis*

Test	First samples, *n* (%)	Second samples, *n* (%)
GeneXpert		
Positive	86 (60)	73 (83.0)
Negative	58 (40)	15 (17.1)
Total	144 (100)	88 (100)
Ziehl-Neelsen		
Positive	38 (37.3)	18 (20.2)
Negative	64 (62.7)	71 (79.8)
Total	102 (100)	89 (100)
Auramine O		
Positive	40 (39.2)	34 (38.2)
Negative	62 (60.8)	55 (61.8)
Total	102 (100)	89 (100)

study supports reports on IS in many LMICs, such as South Africa, Thailand, Zambia, Mali, The Gambia and Senegal, that found the procedure to be safe and well tolerated.[6-8,11] We have shown that IS can be performed in a low-resource setting, as it requires minimal equipment and expertise to perform with the requisite training.

The IS procedure was successfully performed in all cases, with no life-threatening complications. Epistaxis was the main adverse effect observed, and no specialised intervention was required, as all cases resolved in <30 minutes after the procedure. Vital signs recorded before and after the procedure were similar and within the normal range, with no statistically significant changes. Our findings are similar to those of other studies that also reported limited occurrence of episodes of vomiting, wheezing and epistaxis in a small number of children undergoing the IS procedure.[7,12,13] The implications of our findings and findings reported by others are that the IS procedure is a safe and well-tolerated way to obtain samples for testing for TB in children from whom a sample would otherwise not be available. In addition, we have shown that the procedure does not require high-level expertise and can be performed by well-trained nurses. Similar studies have reported on the feasibility and ease of the procedure performed by healthcare workers.[8,14]

Furthermore, we have shown that IS can help to confirm the diagnosis of TB by providing appropriate sputum samples. It can provide samples to which a newer diagnostic method, GeneXpert, and older methods such as Auramine O and Ziehl-Neelsen staining using microscopy, can both be applied.

The most important advantage of the IS procedure is that it can help increase the number of confirmed diagnoses of PTB, as has been reported in other centres.[11,12] IS therefore provides an added advantage to the clinical diagnosis of PTB that has generally been used in children because of the lack of quality sputum samples for testing, particularly in young children. Children with presumed TB therefore have a better chance of a confirmed diagnosis.

As well as supporting microbial confirmation, IS makes it possible to identify children with drug resistance, as we noted in this study. When only a clinical diagnosis of TB is made, and there is no sputum sample, microbial confirmation and confirmation of drug sensitivity or resistance are difficult to document. An additional long-term benefit of IS is that it helps to promote follow-up of patients with a positive GeneXpert test with more confidence.

The limitation of the present study is that it was carried out in one centre, and the results therefore cannot be generalised to all healthcare settings. However, there is potential to expand training in the IS procedure to other healthcare facilities, and use of the technique in children with presumed TB across Ghana remains very promising.

Conclusion

The IS procedure is safe and well tolerated in the paediatric population, and it can increase the yield for microbial confirmation. GeneXpert testing of IS samples provides information on the sensitivity of MTB

to rifampicin, which is of major importance when a diagnosis of TB is made in a child in an LMIC setting.

Data availability. The datasets generated and analysed during the present study are available from the corresponding author (SKO) on reasonable request.

Declaration. The research for this study was done in partial fulfilment of the requirements for SKO's MPhil degree at Kwame Nkrumah University of Science and Technology, Ghana.

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