

AFB YIELD OF GASTRIC ASPIRATE AFTER STANDARDIZATION OF GASTRIC ASPIRATE TECHNIQUE

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ABSTRACT:

Objectives of the Study: The general objective is to compare the AFB yield of gastric aspirate smears in children with suspected TB following standardized technique with those using non-standardized method. Specific objectives were to correlate the gastric aspirate AFB smear results with the signs and symptoms suggestive of TB and with other laboratory results.

Methodology: A randomized control study was done at Philippine Children's Medical Center from July-October 2001 in children up to 18 years old suspected of having TB based on the criteria of the National Consensus on Childhood TB (1997) who were not able to expectorate sputum. In group 1, standardized protocol was followed in collection of gastric aspirates. In group 2, aspirates were collected using non-standardized method.

Results: There were 54 patients enrolled in the study, 27 in group 1 and 27 in group 2. Ten in group 1 and 9 in group 2 had TB disease: 14 PTB, 3 TB meningitis, 2 congenital TB. Three (30%) patients in group 1 yielded AFB with 1 positive culture. All 3 with positive AFB had poor weight gain, 2 had TB exposure, 2 were non-reactive to PPD, no CXR suggested TB. Group 2 yielded no AFB.

Conclusion: Although there is sparsity of data, there seems to be a better yield of AFB in the standardized protocol. Using the Fischer's exact test, there is no correlation of the smear results with signs and symptoms and other laboratory results. Studies using larger sample size and other possibilities of increasing the AFB yield of gastric aspirates should be conducted.

INTRODUCTION

TUBERCULOSIS (TB) is a common disease worldwide, especially in the developing countries. In the Philippines, TB ranked as the 6th leading cause of morbidity and mortality in 1998¹. TB disease will develop in up to 50% of infants within 3-9 months of infection, 25% in children 1-5 years of age within 1-2 years and 15% in adolescents^{2,3,4,5}.

Despite advances in mycobacteriology, it remains challenging to establish the diagnosis of TB in children. Even in centers with experienced microbiology laboratories, *Mycobacterium tuberculosis* can be detected in samples from fewer than 50% of children with TB. Smears of sputum are positive in 50-70% of adults with pulmonary TB. In children suspected of having pulmonary TB, the best specimen for smear and culture is the early morning gastric aspirate obtained before the child arises and peristalsis empties the stomach of the respiratory secretion swallowed overnight⁶.

Three consecutive morning gastric aspirates yield *M tuberculosis* in up to 70% of infants and 30-50% of children with pulmonary TB⁶. However, in our hospital the AFB yield of gastric aspirate is low. In the year 2000, out of 153 gastric aspirates sent for AFB smears of children suspected of having TB, only 5 smears (3.27% taken in 3 patients) were positive. Institutional guidelines for gastric aspirate collection were not available.

An important factor which may influence the yield of gastric aspirate smear is the manner by which the gastric aspirate procedure is done. A study done by Pomputius III, et. al. showed that after institution of a protocol, gastric aspirate yielded positive culture in 50% of cases with pulmonary tuberculosis compared to only 8% in historical controls⁷.

General Objective:

This study aims to compare the AFB yield of the gastric aspirate smears in children suspected of having TB following a standardized gastric aspirate technique with the AFB yield of gastric aspirate smears using a non-standardized method.

Specific Objectives:

1. To correlate the gastric aspirate AFB smear results with the signs and symptoms suggestive of TB.
2. To correlate the gastric aspirate AFB smear results with other laboratory results, such as gastric

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aspirate TB culture, CXR, PPD size, CSF analysis, cranial ultrasound, cranial CT scan, MRI, thoracic ultrasound, thoracic CT scan.

MATERIALS AND METHODS

A randomized control study was done at the Philippine Children's Medical Center (PCMC). Included in the study were children, male and female, from birth to 18 years old, admitted from July-October 2001 who were suspected of having tuberculosis based on the criteria of the National Consensus on Childhood Tuberculosis⁵. Since these patients were not able to expectorate sputum, AFB smear of gastric aspirates were done as part of the TB work-up. Excluded in the study were patients who were on anti-Koch's medication/s for at least 2 weeks at the time of the study, patients who were not able to be on fasting state for 8 hours or more, such as neonates without intravenous line.

Written consent was obtained from the parent or guardian of the patient. Patients were randomly assigned into two groups. In group 1, (standardized group) gastric aspiration was done by the principal investigator using the following steps:

- 1) Patient was placed on an overnight fast for 8 hours⁸.
- 2) A Fr. 8 nasogastric tube was inserted on the 8th hour of fasting, preferably before the child arises and the gastric contents will be aspirated. The aspirated was then placed in a sterile specimen bottle with label (name of the patient, date of collection, type of specimen).
- 3) 20 ml of sterile water was passed down the tube, allowed to stay for 2-3 minutes, aspirated and then added to the first collection⁹.
- 4) Additional 5-10 ml of sterile water aliquots were passed down the tube and aspirated until a minimum of 20 ml of total aspirate will be obtained.
- 5) No more than 50-70 ml sterile water was injected into the tube.
- 6) The specimen, with the accomplished laboratory request, was sent immediately to the laboratory.

In group 2, (non-standardized group) gastric aspirates were collected by the resident on duty using variable hours of fasting and without doing gastric

lavage. The specimen, with the accomplished laboratory request, was sent to the laboratory.

Gastric aspirate smears were performed by the investigator or the medical technologist-in-charge of the microbiology section. The modified Kinyoun method of staining was used, which is the standard procedure used in the hospital laboratory. Two horizontal lines (300 fields) of each smear were read under oil immersion. Presence of acid-fast bacillus was reported as positive smear.¹⁰

Others laboratory examinations were taken, such as chest radiograph, gastric aspirate TB culture, PPD, CSF analysis, cranial ultrasound, abdominal or hepatobiliary ultrasound, thoracic ultrasound, MRI on as needed basis. Results were gathered and analyzed using frequency, Fischer's exact test, unpaired t-test.

RESULTS

There were 54 patients enrolled in the study, 27 (50%) patients in group 1 (standardized group) and 27 (50%) in group 2 (non-standardized group). Twenty nine patients (54%) were male, 14 in group 1, 15 in group 2 and 25 (46%) were female, 13 in group 1 and 12 in group 2. The age ranged from 1 day to 13 years old. Of the 54 patients, 19 had TB disease.

Of the 19 patients with TB disease, 10 (53%) were in group 1 (standardized group) and 9 (47%) in group 2 (non-standardized group). Twelve (63%) had known exposure to adult PTB patients. Eighteen (95%) patients had signs and symptoms suggestive of TB; 9 had chest radiograph suggestive of tuberculosis; 9 (47%) had positive PPD reaction. The positive PPD reaction in group 1 ranged from 10-18 mm (mean 14.5 \pm 3.3 mm). In group 2, the positive PPD measured 10-16 mm (mean 13.0 \pm 2.8 mm). Difference in the measurements was not statistically significant. (Table 1)

Fourteen patients (74%) of the 19 TB disease patients had pulmonary tuberculosis disease, 8 (80%) in group 1, 6 (67%) in group 2; 3 (16%) had tuberculosis meningitis, 1 (10%) in group 1, 2 (22%) in group 2; 2 (11%) patients had congenital tuberculosis, 1 (10%) in group 1 and 1 in group 2 (11%).

Table 2. Patients with Positive Acid-fast Bacilli on Gastric Aspirate Smears from Group 1 (Standardized Group) (n=3)

Patient Age/Sex	TB Exp	S/s Sug of TB	CXR Sug of TB	PPD	GA TB culture	Diagnosis	BCG	Measles Vac.	Past illness
4/12 M	-	+ cough >2 weeks, poor wt gain, failure to return to N wt.	-	-	+	PTB dse	+	-	BPN
16/12 M	+	+ poor wt gain	-	-	ND	PTB dse	+	-	measles
1 1/2 Mo F	+	+ poor wt gain	-	15 mm	ND	Congenital TB	-	-	-

Of the 19 patients with TB disease 13 (68%) patients had BCG immunizations, 7 (70%) in group 1 and 6 (67%) in group 2. Signs and symptoms suggestive of tuberculosis found in 18 out of the 19 patients with TB disease were: poor weight gain in 14 (77%) patients; 6 (33%) had fever of >2 weeks duration, 6 (33%) had cough or wheezing of >2 weeks duration. One patient born to a tuberculosis mother was worked-up for tuberculosis upon delivery. (Table 3)

Table 3. Signs and symptoms suggestive of tuberculosis in patients with TB disease (n=18)

Signs & symptoms	(+) Gastric aspirate AFB	(-) Gastric aspirate AFB
Poor weight gain	3	11
Fever > 2 weeks	0	6
Cough/wheezing > 2 weeks	1	5
Painless cervical and/or other lymphadenopathy	0	5
Failure to respond to appropriate antibiotic therapy	0	5
Seizures	0	4
Failure to return to normal health after an infection	1	2
Sinus in neck	0	0
Angle deformity of the spine	0	0
Unexplained abdominal mass/ascites	0	0

All patients with TB disease had chest x-rays done. Four of the 19 (21%) patients with TB disease,

showed hilar fullness or enlarged lymph nodes. Of the 3 patients with positive AFB smears from group 1 (standardized group), the chest radiographs were not suggestive of TB. All patients with TB disease had PPD test using 5 TU. PPD indurations ranged from 10-18 mm in 9 (47%) patients, 4 (40%) patients in group 1 and 5 (55%) patients in group 2. Both PTB disease patients with positive smears were non-reactive to PPD, while the congenital TB case had an induration of 15 mm. (Tables 4 and 5).

Table 4. Chest Radiograph Findings in Patients with TB (n=19)

Variable	(+) AFB smear (n=3)	(-) AFB smear (n=16)	Total (n=19)
CXR suggestive of TB	0	9	9 (47%)
Hilar fullness/Enlarged LN	0	4	4 (21%)
Bronchopneumonic infiltrates R post basal lobe	0	1	1 (5%)
Hilar LN, pneumothorax pneumomediastinum	0	1	1 (5%)
Neonatal Pneumonia, T/C Koch's etiology	0	1	1 (5%)
Pleural effusion	0	1	1 (5%)
Pneumonia/atelectasis Ant segment of R lower lobe, hilar LA	0	1	1 (5%)

Table 5. Clinical Manifestations and Results of Diagnostic Tests among Patients with (+) AFB Smears and (-) AFB Smears (n=19)

Variable	(+) GA AFB YIELD (n=3)	(-) GA AFB YIELD (n=16)	p value
Signs/symptoms suggestive of TB	3	15	NS
Known TB exposure	2	10	NS
PPD reaction >5 mm	1	8	NS
CXR suggestive of TB	0	9	NS
GA TB culture	1	1	NS
CSF analysis suggestive of TB	0	3	NS
CUTZ suggestive of TB	ND	1	-
Cranial CT scan suggestive of TB	ND	1	-
MRI suggestive of TB	ND	1	-
HBT UTZ	0	0	NS

ND = not done
 NS = not significant
 CUTZ = cranial ultrasound
 HBT UTZ = hepatobiliary tree ultrasound

Fourteen out of 19 patients had pulmonary TB disease. Diagnosis of pulmonary TB disease in 11 patients was made using the National Consensus on Childhood TB⁵. The most common criteria present in these patients (4 out of 11 patients) were: known TB exposure, clinical manifestations suggestive of TB and chest radiograph suggestive of TB. Three patients were still signed out as pulmonary TB disease with only 2 criteria present: 2 with clinical manifestations suggestive of TB and with radiographic findings suggestive of TB; 1 patient with signs and symptoms suggestive of TB and a positive PPD reaction. (Table 6).

Sixteen (84%) of the 19 patients with negative AFB smear, 12 (75%) had pulmonary TB disease, 3 (19%) had TBM, and 1 (6%) had congenital TB. Ten (62%) had known TB exposure, 15 (94%) had signs and symptoms suggestive of TB. The chest radiographs were suggestive of TB in 9 (56%), while 8 (50%) had PPD induration of >5 mm. One gastric aspirate was cultured and was positive for *M. tuberculosis*. (Table 5).

Table 6. Clinical Manifestations and Diagnostic Tests Done in Patients with Pulmonary Tuberculosis Disease (n=14)

Patient's Age/Sex	Gastric aspirate AFB +	TB exposure	S/s sug. of TB	CXR sug. of TB	PPD diameter (mm)	Gastric aspirate TB culture	Comment
Group 1							
1 3/12 F		+	+	+			
2 10/12 M		+	+	+			
2 6/12 M		+	+	+			
2/12 M		+	+	+			
4/12 M	+		+			+	
1 6/12 M	+	+	+	+			
8/12 M			+	+	10mm		ARDS
1 4/12 F		+	+		15 mm		
Group 2							
1 3/12 M			+	+			
3 F			+	+			
9/12 M			+		10 mm		
10/12 F		+	+			+	
8/12 M		+	+	+	10 mm		
1 8/12 M			+	+	16 mm		Lung biopsy: caseation necrosis thoracic UTZ-granulation tissue

HAMA - Home against medical advice
 ARDS - Acute respiratory distress syndrome

Three patients were diagnosed to have tuberculous meningitis on clinical grounds and by lumbar tap. Two patients were born to tuberculous mothers.

DISCUSSION

Direct examination of the smear is relatively easy to do and cheap, and is thus useful in developing countries. However, since most children cannot expectorate sputum, gastric aspirate is considered the best specimen for smear and culture⁶. Because of the low yield, gastric aspirate smear/culture it is not often requested in our hospital.

K. Berean, et. al. did a 23-year review of acid-fast stains in unconcentrated gastric aspirate specimens. One hundred one gastric aspirate specimens from 64 patients were reported positive for AFB on the basis of smear and/or culture. Thirty-two specimens from 23 patients had smears positive for AFB. All but 2 of the specimens were culture positive for *M. tuberculosis* giving a specificity of 99% and a sensitivity of 30%. The 2 smear-positive/culture-negative were confirmed positive for *M. tuberculosis* by sputum culture. The acid-fast stained gastric aspirate smear was not a sensitive method of diagnosis as only 30 of 101 culture positive specimens had positive smear. However, a positive smear is valuable as it makes a diagnosis 1 to 2 weeks before the cultures are positive¹². A retrospective study by A. Bahamman and Long showed similar results¹³. Out of 1155 gastric aspirates done, 13 smears were positive for AFB (19%). All 13 gastric aspirates were culture-positive for *M. tuberculosis*. There were no false positive smears giving a specificity of 100%¹³. The low rate of smear-positive disease among children may be explained by the fact that children generally have primary disease without extensive cavitation¹⁴.

In a study done by Pompotius, et. al., standardization of gastric aspirate technique resulted to higher yield of *M. tuberculosis* in culture when compared to historical controls. Retrospective survey of gastric aspirate results from 1979 to 1994 revealed that only 1 of 13 cases (8%) of pediatric TB were confirmed following a non-standardized technique. During a 12-month period after institution of a protocol, gastric aspirates yielded positive cultures in 4 of 8 (50%) with PTB⁷. In our study, the standardized gastric aspirate procedure yielded 30% (3 out of 10) AFB (+) smear, while in the non-standardized procedure, no AFB was seen. This falls within the reported yield of 3-morning gastric aspirates, 30-70% and compares

favorably with the 3.27% yield in the year 2000. Identification of TB disease within 24 hours from the time of specimen collection was done and thus, early institution of antiKoch's medications. However, because of the cost, only 1 had culture of the gastric aspirate for tuberculosis, which was positive for *M. tuberculosis*.

There are variety of factors that may affect the AFB yield of gastric aspirate. Concentration of the specimen by centrifugation, may affect the yield of the gastric aspirate. In a retrospective study of over 14,500 gastric aspirate specimens, the sensitivity of acid-fast smears and cultures was found to improve when the speed of centrifugation was increased (relative centrifugal force or RCF)¹². In this study, as with the laboratory procedure in the past, no centrifugation was done.

Variations in the gastric aspirated technique itself may affect the yield. In this study, gastric lavage was done on Group 1 patients but not in Group 2. In the study by Pompotius, et. al., gastric lavage was not done. Relative contribution of gastric lavage to the results is not known⁷. In a study done by Vallejo, et. al, lavage was done during gastric aspirate procedure, which yielded *M. tuberculosis* from 75% of infants with pulmonary disease¹⁵.

Another factor, apart from the gastric aspirate procedure itself, which may contribute to the acid-fast yield, is the delay in the processing of the specimen. Except for the specimens sent to Lung Center of the Phils. for TB culture, all gastric aspirate specimens were sent to the hospital laboratory immediately and processed as soon as possible. No neutralization was done (such as addition of 10% sodium carbonate) nor refrigeration. Addition of a buffer is recommended when there is delay of at least 4 hours in the processing of the specimen. This is to maintain survival of the organism in the acidic gastric washings.

Inadequately decolorized smears can cause false-negative interpretations. Smears that are too thick can reduce the likelihood of detecting AFB. Also, smears that are too thick might flake off the slide. Inadequate examination of the smear by observing too few fields can result in failing to observe acid-fast bacilli that are present¹⁶.

To facilitate diagnosis of TB, scoring systems have been resorted to. Most system consider the following: history of contact with an adult with PTB, presence of symptoms, especially cough; tuberculin test and chest radiograph results; and response to anti-TB

therapy⁶. In the Philippines, the National Consensus on Childhood Tuberculosis was published in 1997 to help physicians establish the diagnosis of PTB.

Exposure implies that the child has had recent and substantial contact with an adult or adolescent who has suspected or confirmed contagious pulmonary TB (a case source)¹⁷. Up to 70%^{8,9} of children are identified as having exposure to a case source. In the study, exposure was revealed in only 63%. This may not reflect the true value, since parents or guardians usually deny the disease. TB infection is diagnosed by a (+) Mantoux 5 TU test reaction, read after 48-72 hours. Significant cervical lymphadenopathy in this study was 3 or more measuring at least 1 cm.

Three patients did not fulfill the criteria for pulmonary TB but were treated because of strong consideration. Two had signs and symptoms and chest radiographs suggestive of TB, while 1 had signs and symptoms suggestive of TB and PPD induration of 10 mm. Other scoring systems used 2 out of 3 criteria: (1) Mantoux 5TU skin result >10 mm of induration, (2) other disease entities were ruled out and the subsequent clinical course was consistent with TB, and (3) an adult source case with contagious *M tuberculosis* disease was discovered¹⁸. Failure to gain weight, fever and cough >2 weeks, painless cervical lymphadenopathy and failure to respond to antibiotics were the most common signs and symptoms. This is in contrast to the survey findings of 386 physicians which showed that the most common signs and symptoms were failure to return to normal health after an infection and painless cervical and/or other lymphadenopathy.

The most common radiologic feature of hilar adenopathy is consistent with those in published data, which is seen in 21-92% of cases^{15, 18}. Other chest

radiograph findings suggestive of TB are mediastinal or subcarinal lymphadenopathy, segmental hyperinflammation, atelectasis, alveolar consolidation, interstitial densities, pleural effusion, focal mass and cavitation⁹.

A positive Mantoux test measures >5 mm based on the recent recommendation of the National Consensus on Childhood TB. Mantoux tuberculin test was reactive in 47% of the patients. This may be understated, since most patients were malnourished (74%). In local studies, sensitivity ranged from 19-41%⁵. Up to 50% of children with TB meningitis or miliary TB is initially nonreactive⁶.

TB meningitis develops more commonly in infants, occurring between 2 and 12 months after primary infection^{9, 17}. Foreign studies reported cases from 7-23%^{15, 18}. At PCMC, TB meningitis was found in 28.9% of patients with tuberculosis. Outcome correlates with the clinical stage. Chest radiograph may not be suggestive of TB as in our patients. Lumbar puncture is the decisive test. The tubercle bacillus is rarely seen in direct smear. Culture of 3 specimens of CSF taken on 3 consecutive days identifies the bacillus in 90% of cases.

Clinical signs of TB of the newborn are so scant that no conclusion can be drawn as to the symptomatology. It should not be forgotten that infants infected with TB, with only minor or no tuberculous lesions, may be born full term, seemingly robust. The most important clue to the diagnosis is maternal tuberculosis.

The sample size of this study is small but shows a higher yield from the standardized gastric aspirate procedure. Further studies should be conducted to improve the gastric aspirated collection using larger sample size.

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INSTRUCTIVE CASE

2-YEAR OLD BOY WITH SPONTANEOUS CHYLOTHORAX

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A 2-year old male, from Guadalupe, Makati was admitted at a tertiary government hospital for further management of pleural effusion. Since one month prior to admission, the patient was noted to have intermittent productive cough associated with colds. Neither consult was done nor medications taken. Three weeks prior to admission, he was noted to have low-grade fever (T37.7-38°C) and cough persisted. He was given Paracetamol 250mg/5ml ½ tsp every 4 hours affording temporary relief. Condition continued which prompted consult at a private hospital where he was assessed to have an Upper Respiratory Tract Infection. He was prescribed to take at home Amoxicillin, Ambroxol and Ascorbic Acid.

Nine days prior to admission, he was admitted to the same hospital due to difficulty of breathing. Fever, cough and vomiting accompanied the dyspnea. He was assessed to have community-acquired pneumonia. Chest x-ray revealed pleural effusion. PPD test was done and showed positive result. Patient was given Cefuroxime, Oxacillin and Amikacin. Triple anti-TB drugs were also started. Thoracentesis

was done revealing a white milky fluid. Pleural fluid analysis, serum LDH, sugar and protein showed abnormal results.

One day prior to admission, a repeat chest x-ray was done revealing persistence of pleural effusion on the right lung field. Chest tube thoracostomy was done evacuating 150 cc of non-foul smelling, white milky pleural fluid. Analysis of the pleural fluid revealed pH of 7.5, WBC of 54,000/mm³, protein ratio higher than 0.5, LDH ratio higher than 0.6, pleural fluid LDH of 565 U/L, pleural fluid protein of 48.5 g/L. (These findings based on Light's criteria define the effusion as an exudate). Immunophenotyping was done. At this time, patients relatives opted to transfer patient to a tertiary government hospital.

The past medical history was unremarkable. Family history showed that the eldest sister had pulmonary TB for which she was treated for 6 months with good compliance. The birth, maternal, immunization, nutritional, developmental milestones, personal and social histories were unremarkable.

What is your diagnosis?

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