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Original Research Article

Tracheal aspirate in the diagnosis of neonatal pneumonia soon after birth: A prospective observational study

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Abstract: Background: In early onset sepsis (EOS) blood culture is often sterile due to antibiotics. Since tracheal aspirate (TA) is sterile after birth and antibiotics reach lung fluid late it may be a good fluid to isolate bacteria. **Methods:** Neonates admitted with Respiratory Distress (RD) and maternal risk factors for sepsis were evaluated for pneumonia. Sepsis work-up, TA smear and culture were done. Diagnostic accuracy of TA for pneumonia and correlation with markers of EOS evaluated. **Results**: Of 148 neonates enrolled pneumonia was diagnosed in 70 of which blood culture was positive in 10, TA Gram smear and culture in 40 and 36 respectively. Association between TA Gram smear and pneumonia was OR=0.209, 95% CI 0.102-0.427.Sensitivity, specificity, PPV and NPV were 40%, 58.3%, 8.2%, 91.3% respectively. Correlation with sepsis screen was OR 3.11; 95% CI 1.52-6.3. **Conclusions**: In pneumonia at birth TA smear increases the pathogen yield more than threefold and helps rule out pneumonia.
Keywords: Blood culture, early onset sepsis, neonate, pneumonia, tracheal aspirate.

INTRODUCTION

Congenital or perinatal infection results from transplacental transfer of pathogens or from inhalation of infected amniotic fluid or vaginal secretions. The clinical diagnosis of congenital pneumonia is usually made on the basis of clinical picture, hematological tests, radiographs, and bacteriological cultures.

Early onset respiratory distress (RD) is a frequently faced problem in a neonatal unit and contributes to 30-40% of admissions in NICU (Mathai, S. S. *et al.*, 2007). It occurs in 2.2 % of all newborns and 60 % of the infants below 1000gm (Bonafæ, L., & Rubaltelli, F. F. 1996). Such neonates are often treated with antibiotics, often empirically and unnecessarily.

Remington and Klein (1995) suggested that direct laryngoscopy and tracheal aspiration could be used to identify bacterial etiology in neonatal pneumonitis. They assumed that pulmonary secretions are sterile at birth and for 8 hours thereafter.

Sherman MP stated the usefulness of TA in the early diagnosis of congenital pneumonia (Sherman, M. *et al.*, 1980). They found microscopic evaluation of tracheal aspirate was remarkably specific and accurate in identifying newborns with congenital pulmonary infection.

So this study was done to evaluate the role of TA in the evaluation of pneumonia soon afterbirth.

MATERIAL AND METHODS:

This was a prospective observational study done in a public sector tertiary level neonatal unit in a teaching hospital of North India. The study was done over a period of 1 year after taking ethical approval from Ethics Committee of the university. The aim of the study was toevaluate the diagnostic accuracy of TA Gram smear in the diagnosis of pneumonia soon after birth. Also to find out the correlation between TA Gram smear with other markers of EOS. All consecutive neonates (term as well as preterm) admitted in the unit with RD within 8 hours of birth, either not intubated or intubated for less than half an hour and having one or more perinatal maternal risk factors for infection, formed the study population. Maternal risk factors for

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EOS were maternal fever> 100.4 ˚F, foul smelling liquor, prolonged rupture of membrane > 24 hours (NNPD report 2002-03). Neonates with congenital malformation, intubated for more than 30 minutes before enrolment and whose parents did not give consent were excluded.

The criterion for diagnosis of RD was presence of any 2 of the following: respiratory rate > 60/min, subcostal or intercostal recession, expiratory grunt and groaning (NNPD report 2002-03).

In a neonate with RD, pneumonia was diagnosed by the presence of a positive blood culture or if any two one of the following were present (NNPD report 2002-03).

- Existing or predisposing factors: maternal fever, foul smelling liquor, prolonged rupture of membranes or gastric polymorphs more than 5 per high power field
- Clinical picture of septicaemia (poor feeding, lethargy, poor reflexes, hypothermia, hyperthermia, abdominal distension)
- X-ray picture suggestive of pneumonia
- Positive sepsis screen

The following investigations were done soon after admission: TA smear and culture, sepsis screen, blood culture, and Xray chest.

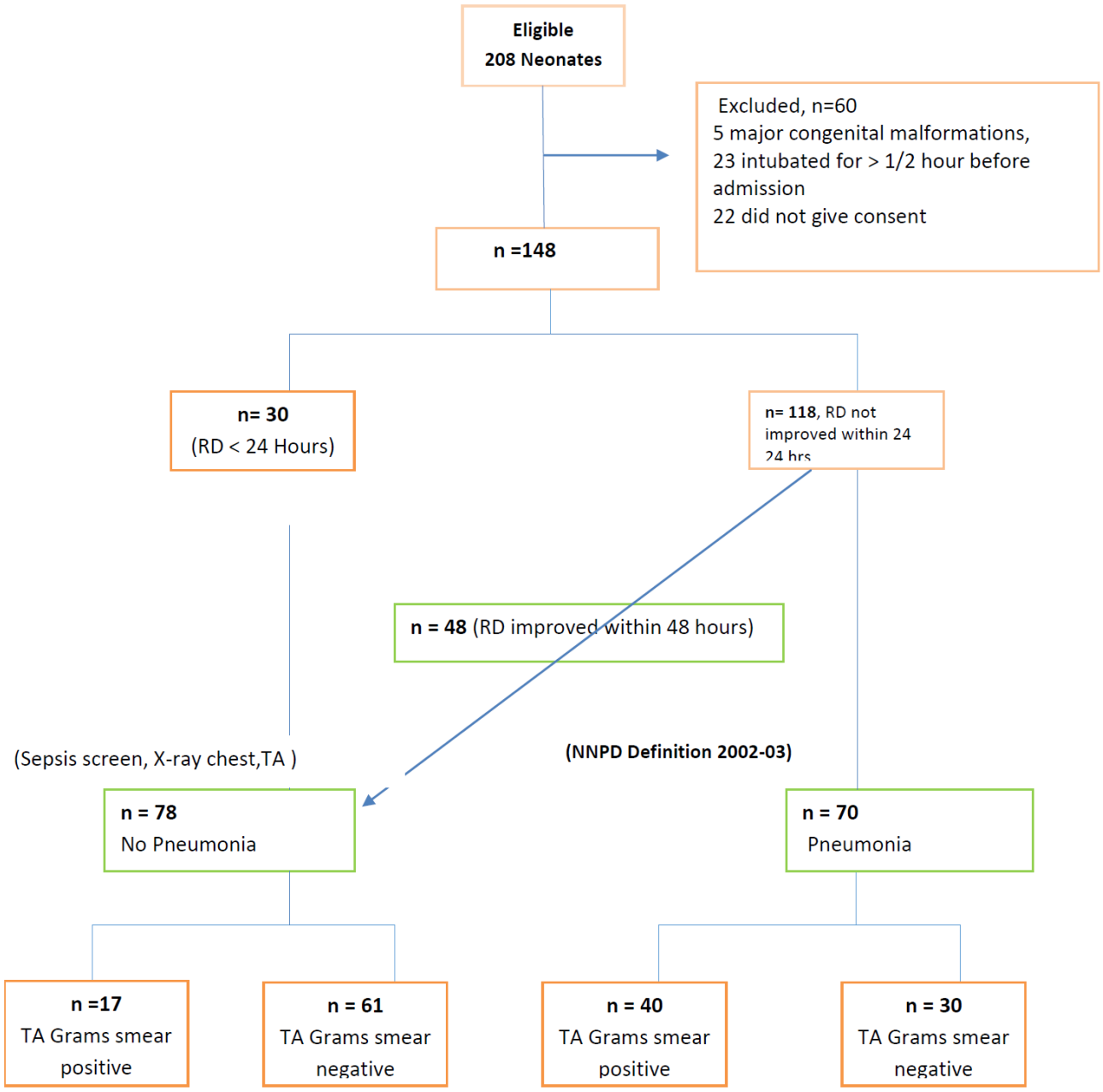

Figure: 1 Flow chart of the study

Tracheal Aspirate Collection:

Keeping the neonate in sniffing position the epiglottis was lifted using a straight blade laryngoscope till larynx was visualized. Tracheal aspirate was obtained by passing 10 F suction catheter attached to a 5 ml sterilesyringe (or mucus extractor) into trachea avoiding oro-pharyngeal contamination. To increase yield of TA Grams smear 0.5ml saline was pushed into trachea then aspirated with the same syringe. The neonates were monitored by a pulse oximeter during the procedure. TA smears were stained within 2 hours of collection and examined for bacteria and polymorphonuclear cells. A culture sample was transported to laboratory and culture was performed using blood, chocolate and MacConkey's agar plates and thioglycolate broth.

Patients were managed according to the unit protocol. Empirical antibiotics were given according to the unit policy (based upon previous antibiotic audit) till the results of cultures were available.The clinical course and final outcome of the patient was noted.

Statistical Analysis:

Expecting 40% prevalence for congenital pneumonia derived from previous study by Sherman *et al.*, (1980), sample size was calculated to be 143.

- N =Z 2 (p (1-p)/d2/ prevalence
- (Z=1.72 at 90% confidence limit and 80% power, p =0.82 (sensitivity)
- d= precision =0.10 or 10%, prevalence= 40% for congenital pneumonia)

The results were presented as frequency and percentages for categorical data and mean ±S.D for parametric data. Analyses were performed on SPSS software. For analyzing association with various categorical parameters Chi-square test and for analyzing data in which any value <5, Fisher exact test was used. Independent sample "t" test was used to analyze difference between two groups for any parametric variables. The data was taken significant if p value obtained was less than 0.05. The confidence interval of the study was kept at 95%.

RESULTS:

Table 1: Baseline Characteristics of neonates with respiratory distress at birth

	Total	Pneumonia (n=70)		NoPneumonia (n=78)		Statistical significance	
		No.	%	No.	%	χ^2 (OR:95%CI)	P
Gestation							
Term	102	47	67.14	55	70.51	0.196 (0.85: 0.426-1.715)	0.658
Preterm	46	23	32.86	23	29.49		
Gender							
Male	71	29	41.43	42	53.85	2.279 (0.61: 0.316-1.163)	0.131
Female	77	41	58.57	36	46.15		
Birth weight							
ELBW	2	2	2.86	0	0.00	4.434	0.218
VLBW	35	17	24.29	18	23.08		
LBW	57	30	42.86	27	34.62		
NBW	54	21	30.00	33	42.31		
Residence							
Rural	67	32	45.71	35	44.87	0.011 (1.03: 0.541-1.978)	0.918
Urban	81	38	54.29	43	55.13		
Mode of delivery							
LSCS	79	45	64.29	34	43.59	6.350 (2.33: 1.201-4.520)	**0.01**
VD	69	25	35.71	44	56.41		
Place of Birth							
Inborn	74	40	57.14	34	43.59	2.711 (1.73; 0.899-3.310)	0.100
Outborn	74	30	42.86	44	56.41		

NBW= Normal birth weight; VLBW=very low birth weight; ELBW= extremely low birth weight; LSCS=lower segment caesarean section; NVD=normal vaginal delivery

Table: 2 Diagnostic efficacy of TA Gram smear for diagnosis of pneumonia:

	Blood culture Positive (n=10)	Blood culture Negative (n=108)	Total (n=118)
TA Gram stain Positive	4	45	49
TA Gram stain Negative	6	63	69
	10	108	118
Sensitivity 40%, specificity 58.3%,NPV 91.3%,PPV 8.2%, Diagnostic Accuracy 56%			

During the study period, 208 neonates with RD and having at least 1 maternal risk factor for sepsiswere admitted within 8 hours of birth. Sixty of these neonates did not fulfill the inclusion criteria (5 had major congenital malformations, 23were intubated for > 1/2 hour before admission and 22 did not give consent). So, finally 148 neonates were enrolled. Out of the enrolled neonates three groups emerged: neonates in whom RD improved within 24 hrs (n=30), neonates in whom RD persisted but diagnosis was other than pneumonia (n=48) and neonates in whom RD persisted and diagnosis of pneumonia was made (n=70). The neonates in the two groups without pneumonia were clubbed (48+30=78) and compared with neonates diagnosed as pneumonia (n=70).

As shown in table: 1, baseline characteristics of the study neonates were comparable in both the groups except, mode of delivery. In the pneumonia group, 45/70(64.3%) neonates were born by LSCS as compared to 34/70 (48.5%) in the no pneumonia group (OR=2.33; CI=1.201-4.520, p=0.01).

Fifty seven (38.5%) of 148 study neonates had positive TA Gram smear. Out of 70 neonates with pneumonia TA smear was positive in 40 (57.1%). While in 78 neonates with no pneumonia only 17(21.7%) were positive.There was a statistically significant association between TA Gram smear positivity and pneumonia as diagnosed by NNPD criteria (OR=0.209 with 95% CI 0.102-0.427, p<0.001). All positive TA Grams smears had polymorphs on microscopic examination. They were scanty (4-5/hpf) in 75.4% (43/57) and moderate (10-20/hpf) in the rest. Blood culture was positive in 10 of the neonates with pneumonia.

No trauma was apparent during or after the procedure of TA Hypoxemia was brief during suctioning, and no cardio respiratory deterioration occurred secondary to the TA. As shown in table-2 using a positive blood culture as gold standard for diagnosing pneumonia the sensitivity, specificity, PPV and NPV of TA Grams smear were 40%, 58.3%, 8.2%, 91.3% respectively.

As shown in table 3, While correlating the TA Gram smear with other sepsis markers, TA Gram smear had a good correlation with the sepsis screen (OR 3.11; 1.52-6.3,p 0.012) specially the IT ratio (OR 19;2.38-154.1, p< 0.01), x-ray chest (OR 8.3;3.41-20.3, p< 0.01) and TA culture (OR 276; 35-2171, p<.01). It did not have correlation with blood culture (OR 1.07; 0.29-3.97, p=0.3).

Of 70 neonates with pneumonia, 36 (51%) had positive TA culture, 10 (14.2%) had positive blood culture and only 3 had positive CSF culture (4.2%). Of the TA isolates more than 90% were Gram negative. (Acinetobacter 48.8 %, E coli 16.2 %, Enterobacter 11.6 %, Staph aureus, Klebsiella , Pseudomonas aeruginosa 6.9 % each and Enterococcus fecalis and CONS 2.3 % each.) One TA was considered contaminated as two organisms were isolated from it. One TA grew bacteria on culture even though the Grams smear was negative. Only 10 (6.75%) bacteria were isolated from concomitant blood cultures. Fifty percent organisms isolated from blood were Gram negative (Acinetobacter in 30%, Staph aureus and CONS each 20%, and E coli, Klebsiella, Enterococcus fecalis each 10 %.) In 2 neonates the same organism was isolated from both TA and blood. Of the 3 positive CSF culture 2(66.7%) were Acinetobacter, and 1 (33.3%) was E coli.

Neonates with pneumonia who had a positive TA culture were more likely to be discharged as compared to those with a negative culture (OR 0.3, 95% CI = 0.8 – 1.3,p< 0.001,).In our study mortality was 13.7% (4/ 29) for neonates with a positive TA culture and 8/24 (33.3%) without positive TA culture. (Excluding those who left against medical advice).

DISCUSSION:

During study period, 1351 neonates were admitted in the unit. Of these 208 consecutive neonates with RD and at least one maternal risk factor for sepsis were considered for enrolment. Sixty did not meet inclusion criteria, so 148 were included. These neonates were potential candidates for having pneumonia. Of these RD was transient in 30 while out of 118 with persistent RD pneumonia was diagnosed in 70 (NNPD criteria). So three groups emerged. One with pneumonia (n=70), the second with persistent RD but no pneumonia (n=48) having either MAS, HIE or RDS and a third whose RD settled within 24 hours with supportive care (n=30). Groups 2 and 3 were combined to make 2 broad groups, one with pneumonia (n=70) and the other with no pneumonia (n= 48+30=78). The baseline characteristics of these two groups were similar except that more neonates in the pneumonia group were born by caesarean section. This may be due to the fact that these neonates had more fetal distress and had to be delivered surgically.

In the 70 neonates with pneumonia TA Gram stain was positive in 40, TA culture in 36, while blood culture grew pathogenic organism in 10. This may be because antenatal antibiotics reach the blood faster than the lung fluid (Sherman, M. *et al.*, 1980) TA and blood culture were similar in 2 neonatesand grew Acinetobacter. In none of the neonates different organisms were isolated from blood and TA.

On the whole 57 (38.5%) TA was positive on Gram stain out of 148 neonates tested. These 57 included 17 neonates with no pneumonia. These TA organisms were probably of maternal origin but did not cause pneumonia in the neonate. All positive TA Grams

smear had polymorphs on microscopic examination though they were scanty (4-5/hpf) in 75.4% (43/57). This may be because we instilled saline to collect the TA so quantification of cells could be inaccurate. Sherman MP also reported presence of polymorphs in all positive TA Gram smears (Sherman, M. *et al.*, 1980). Booth GR however reported white blood cells to be present in only 67% of smears from culture positive TA. In their study Gram smear was positive in only 44.4% of culture positive TA (Booth, G. R. *et al.*, 2008). So TA culture positivity was higher than Gram stain smear. On the contrary we found TA Gram smear to be positive in more babies than culture (40 vs 37). This is probably because not all stained bacteria on smear were viable.

TA Grams smear positivity was maximum in neonates with pneumonia (57.1%) and minimum in those with no pneumonia (26.6%). The overall positivity of TA Grams smear in neonates with RD was 38.5%. Sherman MP reported 7.8% TA Grams smear positivity in neonates at risk for infection presenting with respiratory distress in first 8 hours of life (Sherman, M. *et al.*, 1980). Booth GR found TA culture to be positive in 6.5% neonates intubated for any cause in first 12 hours of life (Booth, G. R. *et al.*, 2008). As compared to these studies from developed countries we had five times greater TA Grams smear positivity in our unit.

Using a positive blood culture as gold standard for diagnosing pneumonia the sensitivity, specificity, PPV and NPV of TA Grams smear were 40%, 58.3%, 8.2%, 91.3% respectively. Thus we found that TA Grams smear is not a good test to screen neonates for pneumonia at birth however, it would be a good test to rule out pneumonia as NPV is high. Sankar found sensitivity of 93 to 100%, specificity of 83%, positive and negative predictive value of 27% and 100% respectively for the sepsis screen for diagnosing sepsis which is much higher than that for TA Gramsmear in our study for pneumonia (Shankar, M.J. *et al.*, 2008). However same sepsis screen used by Nandy (2007) was not found to be of much use in the diagnosis of sepsis (Nandy, M. *et al.*, 2007).

The second part of our study was regarding the correlation of TA smear with blood culture and sepsis screen. We found that it had a good correlation with the sepsis screen ($p=0.02$) specially the IT ratio ($p< 0.0003$), x-ray chest ($p=0.001$) and TA culture ($p<0.001$) Like us Sherman MP found raised IT ratio and decreased ANC in neonates with positive TA (Sherman, M. *et al.*, 1980). In the study of Booth GR maternal fever and clinical chorioamnionitis were found to be significantly associated with pathogenic TA culture results (Booth, G. R. *et al.*, 2009). In our study we had all neonates with maternal risk factor for EONS as it was one of the inclusion criteria.

Of the TA culture isolates more than 90% were Gram negative. Acinetobacter were 48.8 %, E coli 16.2%, Enterobacter 11.6 %, Staph aureus, Klebsiella and Pseudomonas aeruginosa each 6.9 % and Enterococcus fecalis and CONS each 2.3 %. Only 10 (6.75%) bacteria were isolated from concomitant blood cultures probably because of antibiotic pretreatment of mothers. The antibiotics take longer to reach effective levels in the lung fluid so more TA culture grew organisms than blood culture. Sherman MP 1980 reported a blood culture positivity of 5.3% while Booth GR 2009 reported a positivity of only 1.4 % (Sherman, M. *et al.*, 1980; Booth, G. R. *et al.*, 2009).

In blood about half the organisms isolated was Gram negative. A cinetobacter in 30%, Staph aureus and CONS each 20% and E coli, Klebsiella, Enterococcus fecalis each 10%. In 2 neonates the same organism was isolated from TA and blood. Though Acenitobacter is usually an organism that causes nosocomial infection in the NICU but it has been reported as the leading cause of early onset sepsis in several studies from India (De, A.S. *et al.*, 2013; Rathi, M.R. *et al.*, 2009; and Begum, M. *et al.*, 2013).

Mahale R 2009 from PGI Chandigarh found culture positive EOS in 28/125 (22%) (Mahale, R. *et al.*, 2010). In our study blood culture was positive in only 14.2% (10/70) neonates presenting with Pneumonia probably due to antibiotic exposure in mothers and neonates prior to admission. None of the neonates with No Pneumonia group (78) grew organism in blood culture.

In our study if TA was not done etiological diagnosis could have been made only in 13/70 (18.5%) neonates with pneumonia (10 by blood and 3 by CSF culture).TA cultures enabled us to isolate 32 more pathogens thus raising the culture positivity rate more than threefold from 18.5 % to 64.2%. We found that neonates with pneumonia who had a positive TA culture were more likely to be discharged as compared to those with a negative culture. (OR 0.3, 95% CI = 0.8 – 1.3, $p< 0.001$,) This can be explained on the basis of appropriate antibiotic use according to sensitivity report. Sherman MP 1980 reported a mortality of 24% (6/25) for neonates with positive TA cultures (Sherman, M. *et al.*, 1980). In our study mortality was lower at 13.7% (4 /29) for neonates with a positive TA culture. This may be as the neonates who left against medical advice were excluded.

From our study we conclude that though TA examination is not a desirable test to screen for pneumonia presenting soon after birth, as it has a low sensitivity (40%), its potential to provide an etiological diagnosis is noteworthy. It increases culture yield by more than threefold when added to blood and CSF culture. Our study indicates that it is a very useful test

to rule out pneumonia presenting soon after birth as it has a high NPV of 91.3%. Moreover it is obtained easily by a noninvasive procedure without endotracheal intubation, is inexpensive, safe and can give prompt results. It is surprising that its potential has not been tapped adequately by neonatologists.

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