RESEARCH PAPER

Endotracheal Aspirate Microscopy, Cultures and Endotracheal Tube Tip Cultures for Early Prediction of Ventilator Associated Pneumonia in Neonates

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Received: January 31, 2016;
Initial review: June 06, 2016;
Accepted: December 27,2016.

Objective**:* To evaluate the utility of endotracheal aspirate microscopy, culture and endotracheal tube tip culture for early diagnosis of ventilator-associated pneumonia in neonates. ***Methods: Inborn ventilated neonates were followed-up for ventilator-associated pneumonia using Center for Disease Control and Prevention (CDC) criteria. Endotracheal aspirate microscopy, culture and endotracheal tube tip cultures were performed. ***Results**:* Ventilator-associated pneumonia occurred in 28/68 (41%) neonates as per CDC criteria. Endotracheal aspirate microscopy (≥5 polymorphonuclear cells per high power field) and endotracheal aspirate culture had 78.6% and 75% sensitivity, 87.5% and 90% specificity, positive predictive value of 81.5% and 84%, and negative predictive value of 85.4% and 83.72%, respectively. Mean (SD) time of result of microscopy and endotracheal aspirate culture was 55.7 (4.3) h and 108.3 (19.7) h, respectively in comparison to diagnosis made at 143.5 (23.3) h, as per CDC criteria. ***Conclusion**:* Endotracheal aspirate microscopic examination and culture can be supportive in objective diagnosis of ventilator-associated pneumonia with an added advantage of earlier prediction.

Key words: *Complications, Diagnosis, Intensive care, Nosocomial sepsis*.

Published online: February 02, 2017. PII:S097475591600036

Ventilator support is essential part of care in neonatal intensive care unit (NICU), but ventilator-associated pneumonia (VAP) can occur as a serious complication. National Nosocomial Infections Surveillance system (NNIS) data (2004) from USA reported pooled mean VAP rate of 1.4-3.5/1000 ventilator days [1]. In developing countries, the reported rates of VAP are significantly higher, ranging from 16.1 to 89 episodes per 1,000 ventilator days [2,3].

Center for Disease Control and Prevention (CDC) criteria for diagnosis of VAP includes multiple parameters which are observer dependent, and does not include cultures, which are important for appropriate antibiotic therapy [4]. Patients with inadequate antibiotic therapy may have a poor prognosis if a change in regimen is delayed while awaiting microbiological results [5]. Bronchoalveolar lavage (BAL) and protected specimen brush (PSB) have been reported to have high sensitivity and specificity for the diagnosis of VAP [6,7], but are invasive and difficult to perform in neonates due to small endotracheal tube (ET) size. Endotracheal aspirate (ETA) is relatively noninvasive method that can be easily performed through small ET in NICU. We aimed to study the utility of ETA microscopy and cultures for diagnosis of VAP.

METHODS

This study was conducted in a tertiary care NICU over a period of one year (April 2014 to March 2015). All inborn infants admitted in the NICU requiring invasive ventilation for more than 48 hour were included after obtaining parental consent. Infants with suspected or diagnosed congenital pneumonia, critical congenital cardiac disease, life-threatening congenital and chromosomal anomalies or pulmonary haemorrhage were excluded.

Infants who required ventilatory support were intubated by oro-tracheal route in NICU or labour room, and then put on Neonatal Ventilators (Maquet servo-i, Avea standard or Schiller graph NET advance). Disposable ventilator circuits (RT 126 dual limb infant ventilator circuits, Fisher and Paykel) were connected on ventilator through servo-controlled humidifier (Fisher and Paykel MR850). The disposable tubings were not changed till extubation or until visible soiling. Humidifier was filled with sterile water by a closed system. All ventilated babies were nursed in the supine position, and routine care as per NICU protocol was provided. Suctioning and collection of sample for ETA was done by

an open suction using Hagedorn method [10], when first suction was required for the presence of secretions. Suctioning was performed by using sterile feeding tube or suction catheter and syringe. If the yield was <1 mL, the procedure was repeated by instilling 0.5 mL of normal saline (drawn from a freshly opened ampoule) into the endotracheal tube. Sample collected in the syringe was sent for quantitative culture and microscopic analysis along with sample of saline taken for suction. Studied infants were carefully followed up for signs of VAP. This included, apart from clinical examination, regular recording of body temperature, ventilator setting (peak inspiratory pressure, positive end expiratory pressure and fractional oxygen (FiO_2)), arterial blood gas, leukocyte count, serial C-reactive protein, blood culture and chest radiographs. Blood culture was done as a part of the sepsis work-up profile. No infant received steroid, local or oral antibiotic, H_2 blocker or proton pump inhibitor.

Mean (SD) intubation-to-collection time of ETA sample was 53.2 h (3.9) which was same for ET aspirate microscopy and ET aspirate culture**.** ET tip sample was taken either at 1st ET replacement or during extubation whichever was earlier. ET tube tip (1 cm) was cut by sterile surgical blade and taken into a culture tube directly and was sent for culture at mean (SD) 115.2 (40.8) hrs. The diagnosis of VAP was made on the CDC criteria for infants less than or equal to 1 year of age [4].

A smear was prepared from ETA for Gram staining to determine polymorphonuclear (PMNL) cells and the type of organism. Microscopy was done at high magnification (x400) by using VISION 2000 LED microscope and recorded as: PMNL count: >10/HPF; 5-10/HPF or <5/ HPF or Nil /HPF. Smear of ETA was scored as per Bartlett scoring system [11] and bacteria were classified as gram positive or negative; cocci or bacilli. Culture of ETA and ET Tip was done on Blood and Mac Conkey agar. Antibiotic sensitivity was done on Muller Hinton agar and results were expressed as colony forming units/ mL. With quantitative analysis of ETA and ET tube tip, the threshold for diagnosing VAP in this study was considered as 10^5CFU/mL [12].

Data analysis was done by calculating mean and standard deviation for continuous data and using unpaired t test/ Mann whitney U test for statistical significance. Pearson's chi-square test or fisher's exact test was used for categorical data. P value <0.05 was considered as statistically significant. Statistical analysis was done using the software SPSS version 17.0 for windows

RESULTS

A total of 326 neonates were screened out of which 68 were included in the study (***Fig.* 1**). The baseline demographic data of the study population were comparable (***Table* I**). Twenty-eight (41.2%) neonates developed VAP. Mean (SD) time of ET aspirate sample collection was 53.2 (2.9) h for (SD) microscopy and culture, and of ET tube tip sample was 115.2 (40.8) h. The mean (SD) time of result of endotracheal aspirate microscopy and endotracheal aspirate culture was 55.7 (4.3) h and 108.3 (19.7) h, respectively in comparison to diagnosis made at 143.5 (23.3) h as per CDC criteria (P<0.001).

The sensitivity, specificity, negative predictive value

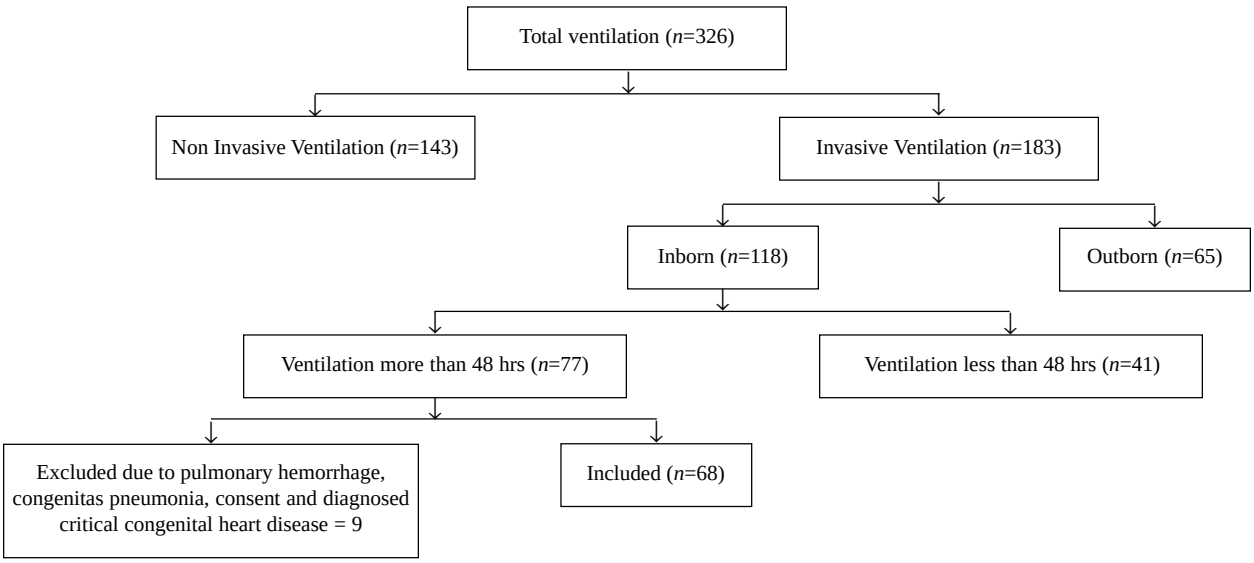

FIG.1 *Flow of patients in the study.*

WHAT THIS STUDY ADDS?

- Endotracheal aspirate microscopic examination and culture can be supportive to CDC criteria in objective diagnosis of VAP with an added advantage of earlier prediction.

TABLE I BASELINE DEMOGRAPHIC AND RISK FACTORS IN THE STUDY POPULATION

Variable	*VAP (28)*	*NO VAP (40)*
Age (wks) *	36.9 (1.8)	38 (1.7)
Weight (kg) *	2.3 (0.6)	2.7 (0.5)
Male gender	15 (53.6%)	21 (52.5%)
Meconium stained amniotic fluid	17 (60.7%)	15 (37.5%)
Vaginal delivery	22 (78.6%)	30 (75%)
Resuscitation at birth		
Bag and mask ventilation	7 (25.0%)	8 (20%)
Intubation	13 (46%)	6 (15%)
APGAR at 5 minutes <7	19 (67.9%)	11 (27.5%)
No. of endotracheal tube changes*	1.82 (0.67)	1.65 (0.66)
Duration of ventilation*	14.07 (2.23)	10.18 (2.02)
Mortality (all cause)	9 (32.1%)	10 (25%)

**values in mean (SD); VAP: Ventilator-associated pneumonia.*

and positive predictive value of ETA microscopy (≥5 PMNL/HPF and >10 PMNL/HPF), ETA culture (colony count $>10^5$ CFU/mL), endotracheal tube tip culture (colony count $>10^5$ CFU/mL) are shown in ***Table* II**. In our study, organisms cultured in ETA were Acinetobactor (29.4%), Klebsella (4.4%), Pseudomonas (3%), MRSA (3%); mixed growth was seen in one case.

DISCUSSION

In this study, ETA microscopy (≥ 5PMNL/HPF) and ETA culture colony count $>10^5$ CFU/mL were found to be useful for early diagnosis of VAP with significantly lower diagnosis time. No significant complications of ETA were found in this study.

The limitations of our study were it being a single center study with a small sample size. Our trial had enrolment of mainly near term and full term infants, and the findings may not be generalizable to the preterm population. Also, we did not repeat ETA for documenting normalization.

The ETA culture sensitivity and specificity when cut off was $>10^5$ CFU/mL for diagnosis of VAP in our study was comparable to findings noted by Labenne, *et al*. [6] where samples were retrieved by BAL technique. The mean time to diagnosis of VAP as per CDC criteria in our study was comparable to that documented by Tripathi, *et al*. [3], but detection of VAP could be done earlier by us using ETA microscopy and culture.

We conclude that ETA culture colony count ($>10^5$ CFU/mL) and ETA microscopy ≥5PMNL/HPF is supportive in the objective diagnosis of VAP with added advantage of early diagnosis.

Contributors: MKG and DH conceptualized and designed the study, analyzed data and drafted the manuscript; AS: collected the data and helped in data analysis; JM: supervised patient management; SG: literature search and helped in data analysis. All authors approved the final manuscript.
Funding: none; *Competing interest*: None stated.

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TABLE II RESULTS OF MICROSCOPY AND CULTURE

Variable	*VAP (n=28)*	*No VAP (n=40)*	*Sensitivity (%)*	*Specificity(%)*	*PPV (%)*	*NPV (%)*
ETA microscopy ≥5 PMNL/HPF	22 (78.6%)	5 (12.5%)	78.6	87.5	81.5	85.4
ETA microscopy >10 PMNL/HPF	13 (46.4%)	4 (10%)	46.4	90.0	76.5	70.6
ET aspirate culture colony count $>10^5$ cfu/mL	21 (75%)	4 (10%)	75.0	90.0	84.0	83.72
ET tip culture colony count $>10^5$ cfu/mL	11 (39.3%)	3 (7.5%)	39.3	92.5	78.6	68.5
Gram Staining organism	13 (46.4%)	2 (5%)	46.4	95	86.7	71.7

VAP- ventilator associated pneumonia; PPV-positive predictive value; NPV-negative predictive value; ETA-endotracheal tube aspirate; PMNL-polymorphonuclear cells.

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