ABSTRACT

- **Objective:** To assess the diagnostic accuracy of endotracheal aspiration (ETA) and mini-bronchoalveolar lavage (mini-BAL) in the established clinical diagnosis of Ventilator associated pneumonia (VAP).

- **Material and Method:** This prospective study was performed in who were hospitalized beyond 48h and had possessed VAP clinical criteria according to Centers for Disease Control (CDC), using ETA and mini-BAL samples collected within 24h after diagnosis of VAP. Diagnostic threshold was considered ≥10⁵ cfu/ml for ETA and ≥10³ cfu/ml for mini-BAL.

- **Results:** Forty three clinically suspected VAP episodes were investigated in 31 patients. Quantitative cultures of ETA and mini-BAL cultures yielded negative 23,2% and 25,5% of VAP diagnosed patients according to clinical criteria, respectively. There was substantial agreement between two microbiological methods (p:0,005, κ:0,804).

Presence of leukocytes and bacteria in ETA and mini-BAL samples were correlated with significant culture results of each samples, respectively (p:0,001; κ:0,39; p:0,003, κ:0,460).

- **Conclusion:** Clinical and radiological diagnosis of VAP without microbiological cultures may be misleading. Due to the fact that quantitative cultures of ETA and mini-BAL were in agreement in our study, quantitative culture of ETA may be adequate for routine diagnosis of VAP. Gram staining of ETA and mini-BAL samples could provide important clues for early and appropriate antimicrobial treatment.

- **Key Words:** Ventilator associated pneumonia, endotracheal aspirate, mini-BAL, quantitative culture Nobel Med 2010; 6(2): 68-74
ÖZET

ENDOTRAKEAL ASİRAT VE MINİ-BAL KÜLTÜRLERİNİN VENTILATÖR İLİKLİ PNÖMONİ TANISINDAKİ DEĞERİ

- **Amaç:** Çalışmamızda klinik olarak ventilatör ilâklili pnömoni (VIP) tanısı alınmış olan hastalarda endotrakeal aspirat (ETA) ve mini-bronkoalveolar lavaj kültürlerinin tanı değerlerini ortaya koymak amaçlanmıştır.

- **Materyal ve Metod:** Bu prospektif çalışma, yatış 48 saatten daha uzun olan ve CDC kriterlerine göre klinik olarak VIP tanısı alan tüm hastalara kabul edilerek yapılmıştır. Tanı eşği ETA kültür için ≥ 10⁵ cfu/ml, mini-BAL için ≥ 10⁶ cfu/ml esas alınmıştır.

- **Bulgular:** Çalışmamızda 31 hasta ait 43 VIP atağı değerlendirilmiştir. Klinik VIP tanısı alan hastalara ait ETA kültürlerinin %23,2 sinde, mini-BAL kültürlerinin ise %25,5'inde üreme olmuştur. ETA ve mini-BAL kültür sonuçlarının tamama yakını bir birey ile uyumlu bulunmuştur (p:0,005; 0,804). ETA ve mini-BAL örneklerindeki bakteri ve lökosit varlığı ile bu örneklere ait üreme sonuçları arasında korelasyon tespit edilmiştir (p:0,001; 0,39; p:0,003, 0,460).

- **Sonuç:** Mikrobiyolojik kültürlerle desteklenmeden klinik ve radyolojik veriler ve ETA'nın tanısını koymak yanılıtıcı olabilmektedir. Çalışmamızda ETA ve mini-BAL kantitatif kültür sonuçlarının benzeri bulunması, ETA'nın VIP tanısında rutin olarak kullanılabileceği kanaatini oluşturmaktadır. Örneklerin Gram boyama incelemelerindeki lökosit ve bakteri varlığının erken ve uygun antibioterapinin sağlanmasında etkili ip uçları olabileceğini düşünülmektedir.

- **Anahtar Kelimeler:** Ventilatör ilâklili pnömoni, endotrakeal aspirat, bronkoalveolar lavaj, kantitatif kültür Nobel Med 2010; 6(2): 68-74

INTRODUCTION

Ventilator associated pneumonia (VAP), which is defined as inflammation of lung parenchyma by nosocomial pathogens subsequent to mechanic ventilation support via, is a common complication with increased mortality, morbidity and costs in intensive care units (ICU). Eighty-six percent of nosocomial pneumonias are associated with mechanical ventilation and are termed VAP. Between 250,000 and 300,000 cases per year is diagnosed in the United States alone, which has an incidence rate of 5 to 10 cases per 1,000 hospital admissions. The attributed VAP associated mortality has been reported between 0 and 50%. Beyond the mortality, the economics of VAP have been estimated at between $5,000 and $20,000 per diagnosis due to increased ICU lengths of stays (from 4 to 13 days), and incremental treatment costs. Early diagnosis and treatment is vital for lowering the high mortality rates between 33% to 71%. In a study which effects of the adequate and inadequate initial antimicrobial treatment regimens in VAP patients confirmed by the following antimicrobial susceptibility tests obtained from bronchoalveolar lavage (BAL) samples were compared, the mortality rate was observed as 38% in adequate group versus 91% in inadequate group, even though treatment was changed immediately upon recognition of the susceptibilities. Unfortunately, the accurate diagnosis of VAP remains a challenge for the clinicians due to the fact that clinical, radiological and microbiological findings have low sensitivity and specificity. Quantitative culture of endotracheal aspiration, which is generally used for microbiological diagnosis as a practicable method in centers not using bronchoscopic methods, has low specificity and high false positive rates due to upper respiratory way contamination. Mini-non-bronchoscopic, protected BAL, protected specimen brush (mini-BAL, PSB) has low contamination probability so it could provide higher sensitive and specific results. Both of them have been issued as alternatives to invasive methods such as lung histopathologic examination or bronchoscopy in VAP diagnosis.

The objective of this study was to assess the diagnostic accuracy of endotracheal aspiration (ETA) and mini-BAL cultures in the diagnosis of VAP.

MATERIAL and METHOD

This prospective study was conducted between August 2006 and May 2007 in our hospital. All patients that mechanic ventilation was used beyond 48 hours were included this study; patients were excluded if they had HIV seropositivity, immuno compromising and oncologic diseases. Patients whose parents signed an informed consent form which was seen and accepted by Internal Ethic Board were enrolled in this study. This study was permitted by Internal Ethics Board. Patients were examined for fever, leukocyte counts, quantity and characteristics of tracheobronchial secretions daily and chest radiography every other day. Clinical findings of VAP suspected patients were examined according to Acute Physiologic Assessment and Control Health Evaluation (APACHE II) rating system. The duration of mechanic ventilation and...
antibiotic usage were recorded before microbiological cultures were taken. ETA, mini-BAL, urine and blood samples were collected and cultivated.

Results of urine and blood cultures were not interpreted in this study due to the fact that ETA and mini-BAL were essential for objective of study and blood culture and urine cultures have low sensitivity and specificity about VAP diagnosis. Clinical diagnosis of VAP was defined according to Center for Disease Control (CDC) as two or more serial chest radiographs with at least one of the following (In patients without underlying pulmonary or cardiac disease, one definitive chest radiograph is acceptable):

1. New or progressive and persistent infiltrate
2. Consolidation
3. Cavitation and at least one of the following:
   a. Fever (>38.5°C) or hypothermia (<36°C)
   b. Leukocytosis (>12000/μL) or leukopenia (<4000/μL)

For adults ≥70 years of age, altered mental status with no other recognized cause and at least two of following:
1. New onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements
2. New onset or worsening cough, or dyspnea, or tachypnea
3. Rales or bronchial breath sounds
4. Worsening gas exchange (e.g., O2 desaturations [e.g., PaO2-FiO2 ≤240], increased oxygen requirements, or increased ventilation demand) \(^{10,14,19-21}\)

If VAP occurred in four days after entubation, it was described as an early onset VAP, if not described as an late onset VAP.

**ETA sampling:** 14 F sterile aspiration catheter was pushed forward through the endotracheal tube two centimeters (cm) beyond the endotracheal tube. Tip of aspiration tube was pushed into Luken’s trap, the other tip was pushed into aspiration machine and 5-10 ml ETA was obtained. \(^{18}\)

**Mini-BAL sampling:** Mini-BAL catheter (Protected Teleskoping Catheter, PTC, Combicath, Plastimed) was pushed forward through the endotracheal tube to end-point randomly. Outer catheter was pulled back, inner catheter was pushed forward 2-3 cm. When it reached the end-point in the lung, saline (20 ml) was injected from proximal port of inner catheter and 1-3 ml mini-BAL was obtained after pulling back inner catheter. \(^{15}\)

**Microbiological procedures:** 1 ml of ETA was mixed with 1 ml saline and vortexed, afterwards 1/10 dilution of these mixed samples was accomplished by adding 0.1 ml of the sample into 0.9 ml sterile saline. These diluted samples (1/20 dilution in total) were spread onto 5% sheep-blood agar, chocolate agar, and MacConkey agar (Salubris, Turkey) using loops calibrated to 0.01 ml. The same procedures were implemented exactly to mini-BAL samples, as well.

From all ETA and mini-BAL samples smears were prepared and they were stained with Gram stain. ETA samples were rated by Q score system as 0, +1, +2, +3. \(^{24}\) ETA samples with Q score +2 or +3 were included in the study. MacConkey and 5% sheep blood agars were incubated in ambient air at 35.5°C, chocolate agars were incubated in 5-10% CO2 at 35.5°C, all agars were incubated for 48 hours if there was no growth observed. Quantitative measurement was calculated as Colony Forming Unit X Concentration Rate X 10. Diagnostic threshold for ETA was considered ≥ 10\(^5\) cfu/ml and for mini-BAL ≥10\(^7\)/ml according to published former studies. \(^{7,14,25,26}\)

Microorganisms were identified with Mini Api (bioMérieux, France) system. Corynebacterium spp., viridans group streptococci, Neisseriae spp. were not consented VAP causes due to the fact that they are members of upper respiratory flora and minority among cultivated microorganisms. Atypic microorganisms, viral or fungal causes were not investigated in the study due to the fact that atypic microorganisms need specific culture medium or serologic studies, fungal diagnosis requires pathological examination and viruse culture was not implemented in our laboratory and their scanning tests have not high sensitivity and specificity.

**Statistics:** Statistical assesment was done with SPSS 13.0 programme. Data which are presented as the mean (±SD) and percent (%), were analysed in 95% confidence interval. ETA and mini-BAL culture results were compared for existence of leukocytes and bacteria in Gram stain, and also culture results of both methods (ETA and mini-BAL) were compared by chi-square with Yate’s correction and Kappa statistic. Kappa values greater than 0.75 are arbitrarily considered to indicate excellent agreement, values between 0.4 and 0.75 indicate moderate agreement, and values less than 0.4 indicate poor agreement. P values less than 0.05 were considered significant.

**RESULTS**

Fouaty three attacks were examined by ETA and mini-BAL in 31 patients suspected with VAP. Mean age, male/female ratio, mean APACHE II score and mean mechanic ventilation duration were 56±26, 18/12, 27.1±7.9, 22±20, respectively (Table 1). Increasing
or changing character of secretions, fever (>38.5°C) and leukocytosis rates were 53.4%, 83.7% and 69.7% in patients with suspected VAP, respectively (Table 2). Twelve of 31 patients had prior antibiotic coverage.

Both ETA and mini-BAL cultures yielded *P. aeruginosa* in the same 37.2% of samples. Similarly, for other microorganisms ETA and mini-BAL culture results were as follows; *Acinetobacter baumannii* in 6.9% vs. 6.9%; Meticillin Resistant *Staphylococcus aureus* (MRSA), Meticillin Resistant *Staphylococcus epidermidis* (MRSE), Meticillin Sensitive *Staphylococcus aureus* (MSSA) in 6.9% vs. 6.9%; *E. coli* in 6.9% vs. 4.6% *Enterobacter* spp. (*Enterobacter amnigenus* and *Enterobacter cloacae*) in 4.6 vs. 4.6%; *K. pneumoniae* in 4.6% vs. 4.6%; *Enterococcus faecalis* in 2.3% vs. 2.3%; *K. oxytoca* in 2.3% vs. 2.3%; *P. mirabilis* in 2.3% vs. 2.3%; *S. pneumoniae* in 2.3% vs. 2.3%, mixed respiratory flora (*Viridans group streptococci, Corynebacterium spp., Neisseriae spp.*) 11.6% vs. 0%; and negative cultures in 23.2% vs. 25.5%, respectively (Table 3,4). Four VAP suspected patients with negative cultures had prior antibiotic coverage. ETA and mini-BAL results were not different (p>0.05) and found similar in VAP suspected patients (p: 0.005, K: 0.804). Existence of leukocytes and bacteria in ETA samples were correlated with significant cultivation results of these samples (p: 0.01, K: 0.395) and in mini-BAL samples, as well (p: 0.003, K: 0.46). Contamination with upper respiratory flora observed in 9.3% of all ETA samples, whereas none of the mini-BAL samples yielded growth of contaminant microorganisms.

A total of 43 episodes were studied in 31 patients clinically suspected with VAP. These episodes contain relaps or reinfections which occured in patients in different times and after cleanup of preceding infection.

**DISCUSSION**

VAP diagnosis has been a challenge for patients under mechanic ventilation support since definitive methods or criteria have not been established yet. Radiologic abscess image in the lung and the cultivation of the abscess material were approved as VAP criteria according to VAP agreement announcement in 1992.33-35 Appearance of a new or increased infiltration in chest radiogram, positive histopathologic findings or positive culture of purulent secretion obtained by bronchoscopic or non-bronchoscopic methods (ETA, BAL, PSB or mini -BAL) without upper respiratory way contamination have been determined as probable diagnosis criteria.33-36 In a postmortem study by Fabregas et al., where findings of histological analysis and culture results of lung samples obtained immediately after death were used as clinical and laboratory references, the sensitivity and specificity for establishing the diagnosis of VAP were found as 69% and 75%, respectively.36 When all three clinical variables (fever, leukocytosis, new or increased purulent secretion) were required for the diagnosis, the sensitivity declined further (23%); the use of a single variable resulted in a decrease in specificity (33%).

ETA and mini-BAL are the most common procedures for diagnosis VAP. Sensitivity and specificity of ETA culture were found as 76% and 66.7% in a study of Fangio et al. that compared ETA with mini-BAL.37

| Table 1: Characteristics of patients (n=31) |
|-----------------|-----------------|
| Age (years)     | 56±26           |
| Male: female    | 18:13           |
| Apache II score | 27±7.9          |
| Duration of mechanic ventilation (days) | 22±20 |

<table>
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<tr>
<th>Table 2: Clinical and laboratory findings of patients percent (%)</th>
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<tr>
<td>Radiographic infiltration at admission</td>
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<td>New or increased tracheobronchial secretion in patients</td>
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<tr>
<td>Fever &gt;38.5°C</td>
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<tr>
<td>Hypothermia &lt;36.0°C</td>
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<tr>
<td>Leukocytosis (&gt;12,000/µL)</td>
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<td>Leukopenia (&lt;4000/µL)</td>
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<th>Table 3: ETA and mini-BAL culture results</th>
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<td>Microorganism (is 43 VAP suspected attacks)</td>
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<tr>
<td>ETA (n-%)</td>
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<tr>
<td>Mini-BAL (n-%)</td>
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<tr>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
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<tr>
<td>MRSA, MRSE, MSSE</td>
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<tr>
<td><em>E. coli</em></td>
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<tr>
<td><em>K. pneumoniae</em></td>
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<tr>
<td><em>Enterobacter</em> spp.</td>
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<tr>
<td><em>K. oxytoca</em></td>
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<td><em>P. mirabilis</em></td>
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<tr>
<td><em>Enterococcus faecalis</em></td>
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<tr>
<td><em>S. pneumoniae</em></td>
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<tr>
<td>MIE*</td>
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<td>Culture negative</td>
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* MIE Respiratory flora includes *Corynebacterium spp., Neisseriae spp., Viridans group streptococi in insignificant numbers.
In a study of Elatrus et al., thresholds of ETA quantitative cultures were compared, sensitivity yielded 92% for 10^4 cfu/ml whereas 84% was obtained for 10^5 cfu/ml, but specificity was 95% for both. On the other hand, in a study of Salata et al., specificity of ETA cultures that belong to patients without VAP in mechanic ventilation, was found between 29% and 50%. However, ETA which tends to contamination with upper respiratory way is used as a common method in hospitals where bronchoscopy could be not used. ETA sample cultures were positive per 86-100 of patients who has VAP to histopathologic examination in studies which used the threshold as e10^5 cfu/ml. A few recently published studies highlighted the fact that ETA quantitative cultures could be as effective as invasive methods. In our study, although ETA cultures included five mixed respiratory flora contamination, the culture results obtained with both methods were found to be similar statistically. ETA quantitative culture also could provide less expense and time per patient compared to mini-BAL quantitative culture in VAP diagnosis, as well. Mini-BAL has been used especially in France instead of bronchoscopic methods based on the fact that it is not an invasive method and its low contamination rates and high specificity. Sensitivity and specificity of mini-BAL were cited between 60%-100%. Sensitivity and specificity were not measured in our study due to the fact that VAP was diagnosed by clinical and radiological findings without histopathologic examination.

In our study, negative culture rates were found as 23.2% for both, ETA and mini-BAL samples of patients diagnosed as VAP by clinical findings. On account to the fact that atypic bacteria, fungi and viruses were not included in our study, negative cultures could have had one or more of them. But results of both methods were evaluated in our study basically. Furthermore it could relate with antibiotic suppression and low sensitivity and specificity of clinical and radiological findings in diagnosis of VAP. In a comparison study which depends on autopsy, clinical and radiological findings, falsely diagnosed VAP patients were found between 29%-62% and sensitivity of chest x-ray and computed tomography were found as 39% and 50%, respectively, in a study that scrutinized low sensitivity of clinical and radiological findings. Blood cultures were found to have low sensitivity compared to BAL cultures positive in patients with VAP for detecting the pathogenic microorganisms. In 1991, Clinical Pulmonary Infection Score (CPIS) was used for VAP diagnosis but subjective examinations and false scores arised a conflict in practice. Definitive clinical and radiological methods have been uncertain currently, consequently microbiological examination keeps on importance in VAP diagnosis.
study has obtained similar rates which are; P. aeruginosa 32.5%, E. coli and K. pneumoniae 26.1%, MRSA 2.3% and MRSE 2.3%. Gram negative bacteria colonisation in ICU predisposed to VAP so initial antibiotic choice and infection control measures should be against them primarily. Timsit and Souweine\textsuperscript{46} revealed that BAL and PSB quantitative cultures are effective for the diagnosis of VAP in patients with antibiotic usage beyond 72 hours and prolonged antibiotic usage increases the colonisation of resistant strains, especially in respiratory ways, and predispose to VAP. But Dotson et al.\textsuperscript{47} revealed that prior and current antibiotic therapy reduce intracellular bacteria (ICB) quantity in mini-BAL so the predictive accuracy of ICB for subsequent to positive PSB cultures could be low. However, negative prediction by ICB for subsequent negative PSB cultures was found to be successful. In contrast, ICB obtained from patients not receiving antibiotics are highly predictive of subsequent PSB culture results, both positive and negative.\textsuperscript{47} Colony quantity are not affected from antibiotic usage so we did not make any correction in colony scores in our study.

Early diagnosis, agent determination and initial appropriate antibiotic treatment are vital in VAP so Gram stained slides obtained by BAL, ETA or bronchoscopy provide important clues about VAP agent and to implement appropriate antibiotic as soon as possible. In the study of Christian et al., Gram smears of mini-BAL samples ensured accurate diagnosis and antibiotic choice for 80% of patients before quantitative culture results were obtained; moreover two thirds of the patients who have clinical findings for VAP were excluded and these patients received no antibiotics by them.\textsuperscript{47} In our study, Gram stain outcomes were correlated with quantitative cultures in mini-BAL and ETA samples, significantly (p: 0.005).

In another study from Marion Kollef’s group from Washington University, the original choice of antibiotics was not adequate, based upon susceptibilities, in 73% of ICU patients, primarily because many of these patients had multi-drug-resistant Gram-negative bacteria (GNB). And delay in antibiotics up front was the most important risk factor for death.

The most common reason for inadequate therapy was GNB resistant to third generation cephalosporins. And it’s of interest that of 23 patients who had a cephalosporin-resistant GNB, 21 of them had received cephalosporins during that same hospitalization.\textsuperscript{49} Michel et al.\textsuperscript{49} emphasized that routine ETA performed twice a week makes it possible to prescribe adequate antibiotic therapy (while waiting for BAL culture results) in 95% of the patients in whom a VAP is ultimately diagnosed by BAL culture.

Consequently, microbiological examination should be an important component in the diagnosis of VAP in patients with clinical and radiological findings which could not reveal the accurate results. Although further investigations are required to validate our results, they indicate that ETA quantitative culture, as an inexpensive method, is at least as sensitive as mini-BAL quantitative culture and could be used for VAP diagnosis in centers which have not opportunity for bronchoscopy. Gram stain of samples could provide early data to find out the plausible agent or agents so appropriate antimicrobial treatment could be initiated as soon as possible.

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