Quantitative Culture of Bronchoalveolar Lavage in Diagnosis of Ventilator-Associated Pneumonia

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Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most common hospital-acquired infections in patients hospitalized in intensive care unit (ICU). The aims of this study were to evaluate quantitative culture of bronchoalveolar lavage (BAL) in the diagnosis of VAP comparing with clinical pulmonary infection score (CPIS), and to determine positive and negative predictive values of the test.

Methods: A total of 209 samples were taken from the patients hospitalized in ICU with clinical suspicion on VAP, along with the CPIS. The cut-off value of test, quantity of $10^6$ colony-forming unit (CFU)/mL, was taken, according to Centers for Disease Control and Prevention (CDC) recommendations.

Results: Positive culture has been found in 142 patients (68%). The most common isolates were Acinetobacter baumanii in 49 (34.5%) patients, Klebsiella pneumoniae in 31 (21.8%), Pseudomonas aeruginosa in 14 (9.9%), Staphylococcus aureus in eight (5.6%), Serratia marcescens in seven (4.9%), Escherichia coli in four (2.8%), other Enterobacteriaceae in five (3.5%), Pseudomonas spp in three (2.2%), Candida albicans in two (1.4%), and mixed infection in 19 patients (13.4%). In our study, sensitivity of the quantitative culture of BAL was 91%, specificity was 70%, positive predictive value was 80% and negative predictive value was 85%.

Conclusions: Quantitative culture of BAL can be useful in VAP diagnosis in patients hospitalized in the ICU, helping in the discrimination between colonization and the infection.

Keywords: Bronchoalveolar lavage; Ventilator-associated pneumonia; Quantitative culture; Intensive care unit

Introduction

Ventilator-associated pneumonia (VAP) is one of the most common hospital-acquired infections in patients hospitalized in intensive care unit (ICU). VAP is a form of hospital-acquired pneumonia that develops 48 h after patient has been intubated and mechanical ventilation has been initiated. It has been estimated that approximately 27% of all patients hospitalized in ICU develop this complication [1]. In the other units, the incidence has been estimated at 5 - 10 cases per 1,000 hospitalized [2]. The mortality associated with VAP can be as high as 50% [3, 4]. The mortality depends on main diagnosis, since higher mortality has been observed in patients with acute trauma, patients with acute distress syndrome (ARDS), and ICU patients admitted due to different diagnosis. The most serious influence on outcome has introduced the adequate antimicrobial treatment in the first 48 h after admission [5]. VAP also has an important economic impact, since it can lead to increasing of hospitalization duration from 4 to 13 days, and average hospitalization costs can be as high as 5 to 20 thousand dollars [5, 6].

VAP diagnosis starts with clinical observation and findings, and radiography (RTG) diagnostic and microbiological analysis of respiratory tract samples [2, 4]. It is important, however, that in ICU the multi-resistant pathogens are common findings and that the treatment of these patients is complicated due to limited spectrum of antimicrobial drugs which can be used [4, 6, 7].

Due to low specificity of clinical findings in VAP diagnosis, Pugin et al developed the system of clinical score named Clinical Pulmonary Infection Score (CPIS), which has been determined on six variables: temperature, leukocyte count, the volume and appearance of tracheal secretion, oxygenation, pulmonary RTG and quantitative culture of tracheal aspirate [8]. CPIS score ranges from 0 to 12. Predictive value of CPIS ≥ 6 has the sensitivity of 93% and specificity of 100% in clinical diagnosis of VAP. In order to improve specificity of VAP diagnosis and to avoid unnecessary antibiotic usage, numerous studies about the utility of quantitative cultures of the respiratory tract samples were conducted [9, 10].

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The cut-off value for the qualitative culture of tracheal aspirate was 10^3 colony-forming unit (CFU)/mL, and for qualitative culture of the bronchoalveolar lavage (BAL) was 10^4 CFU/mL, according to Centers for Disease Control and Prevention (CDC) recommendations [7]. These cut-off values were settled in accordance with studies in infected lung tissue. The published studies show that in patients with pneumonia, bacteria were present in quantity of 10^5 CFU/mL and higher. If the number was in accordance with studies in infected lung tissue. The published (CDC) recommendations [7]. These cut-off values were settled in details in a study conducted by Berton et al [12].

Numerous factors can influence the result of quantitative culture, including timing of sampling, education and professional skills of the person who takes the samples, adequacy of the samples, technical characteristics including transportation to the microbiology lab, delay in transportation, temperature of transportation, presence of the other diseases in the patient, including chronic obstructive lung disease (in this condition a large number of bacteria can be present without pneumonia) and introducing antimicrobial therapy [14, 15].

**Aims of study**

Aims of the study were to determine the correlation between the results of quantitative culture of BAL with clinical parameters (CPIS) in patients hospitalized in ICU in our hospital and to determine if there was statistically significant difference between the results of quantitative culture in patients with CPIS < 6 and those with CPIS ≥ 6. Also, we wanted to determine the positive and negative predictive values of the test in our settings in the VAP diagnosis.

**Materials and Methods**

The study was conducted from January 4, 2017 to January 10, 2017 (6 months). All the microbiology tests were conducted in the Department of Clinical Microbiology, University Clinical Center of the Republic of Srpska. We obtained the samples and clinical data from 209 patients hospitalized in ICUs UKC RS (intensive care of surgical and non-surgical patients, and pediatric intensive care). Sample was BAL of at least 5 - 10 mL. From each sample quantitative culture was performed along with Gram staining.

**Sampling**

BAL was taken through fiber optical bronchoscope as described previously [11]. Shortly, saline in quantity of 50 - 100 mL was injected through system and aspirated immediately to recover micro-organisms from lower respiratory tract. If classical BAL could not be obtained, the procedure of mini-BAL was performed (with less than 50 mL saline).

The selection of the sampling place was done on basis of the location of infiltrates on RTG or CT imaging. The first fraction of BAL was not representative for lower parts of the respiratory tract, whereas the fractions 2 and 3 are representative. All the samples were transported in a short period (less than 30 min) to the microbiology laboratory in order to preserve leukocyte morphology in the samples.

**Checking the sample quality**

The presence of more than 1% of epithelial cells or 10 cells on low magnitude (× 100) in the samples indicates the contamination of the samples with oropharyngeal flora. So those samples were rejected because they were not representing for lower respiratory tract.

**Quantitative culture-method description**

All the samples were homogenized by gentle shaking on vortex. The samples were inoculated as soon as they were received. Using the micropipette with the sterile tips, 50 µL of the sample was transferred on blood, endo and chocolate agar. Inoculated samples were smeared on the agar using the sterile glass stick. After the inoculation, the agars were left at the room temperature for 10 to 15 min to dry the top. After that, the staphylococcus streak was moved over the blood and chocolate agar to obtain the conditions for growing *Haemophilus spp*. Incubation was performed for 24 h at 35 - 37 °C in microaerophilic atmosphere. If there was no visible growing, incubation was extended for another 24 h at 35 - 37 °C.

The final findings consist of the data about the species and subspecies of the isolated micro-organism, quantity stated in CFU/mL, antimicrobial susceptibility test and remarks if they were needed.

**Quantitative culture calculation**

In physiological state, there is usually around 1 mL of secretes in the lungs. BAL was diluted in 10 to 100 mL of saline. Regarding of quantity of the saline used for sampling procedure (different quantity for each patient noted by the clinician taking the sampling), dilution factor was 1:10 to 1:100. Because of the fact that we used quantity of 50 µL for the inoculation of the agar, it is necessary to multiply the colony number by 20 to obtain the number in 1 mL and then to multiply by dilution factor (e.g. × 100 if 100 mL saline was used in BAL sampling). The cut-off value for the quantitative culture was 10^4 CFU/mL [7].

As for the clinical data, for each patient, the findings about leukocyte number, C-reactive protein (CRP) level, procalcitonin level and CPIS score were calculated on the day of sampling. For each of them, the data about antibiotic administration were collected.

**Statistical analysis**

Statistical analysis was conducted by program package SSPS...
version 16.0 for Windows (SSPS Inc., Chicago, USA). In the analysis, the standard methods of descriptive statistical analysis were used, and for the analysis of variance (ANOVA) was applied. For statistically significant result, the value of < 0.05 was taken.

Results

The samples from 209 patients hospitalized at three ICUs with clinical suspicion for developing VAP were taken. There were 127 males and 82 females, with an average age of 58 ± 17.2 for the adults, and 1.2 ± 1.1 for pediatric patients.

For all the patients the CPIS was recorded. As for clinical relevance, the value of CPIS ≥ 6 was taken. From 209 patients, 121 had a CPIS score of ≥ 6. All the other parameters, including leucocyte count, CRP serum level, and procalcitonin serum level in the two groups of patients according to CPIS findings were shown in Table 1.

For all the patients, BAL culture was positive in 142 (68%), whereas 67 patients were culture negative (32%). Value of 10^4 CFU/mL was taken as cut-off [7].

From 142 culture-positive patients, the most common isolates were Acinetobacter baumanii in 49 (34.5%) patients, Klebsiella pneumoniae in 31 (21.8%), Pseudomonas aeruginosa in 14 (9.9%), Staphylococcus aureus in eight (5.6%), Serratia marcesens in seven (4.9%), Escherichia coli in four (2.8%), other Enterobacteriaceae in five (3.5%), Pseudomonas spp in three (2.2%), Candida albicans in two (1.4%), whereas mixed infection was present in 19 patients (13.4%).

The average value of quantitative culture BAL in the group with CPIS < 6 was 610.9 CFU/mL (± 1,826.3), whereas the average value in the group with CPIS ≥ 6 was 298,661.9 CFU/mL (± 415,323.4).

ANOVA has shown that there is statistically important difference in the quantitative culture results between these two groups (CPIS < 6 and CPIS ≥ 6) at the level of P < 0.0001 (95% CI: 362,198.24 - 236,579.76).

The predictive value of the test

According to CPIS score and BAL quantitative culture results, the results were put into the contingency table for calculation of sensitivity and specificity of the test, along with the calculation of positive and negative predictive values of the test. The results are shown in Table 2.

As it can be seen, there were 110 patients with CPIS ≥ 6 with the results of BAL quantitative culture > 10^4 CFU/mL. They were understood as “really positive”. The number of patients with CPIS ≥ 6, but with quantitative culture lower than 10^4 CFU/mL was 11, who were “false negative”. The number of patients with CPIS < 6 and with quantitative results above 10^4 was 26, who were “false positive”. The number of patients with CPIS < 6 with negative quantitative culture results was 62, who were “really negative”.

In our study sensitivity of BAL quantitative culture was 91%, specificity was 70%, positive predictive value was 80%,

Table 1. The Leucocyte Count, CRP Serum Level, and Procalcitonin Serum Level in the Two Groups of Patients Separated According to CPIS Findings

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leucocyte</th>
<th>CRP serum level (mg/L)</th>
<th>Procalcitonin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CPIS &lt; 6, N = 88</td>
<td>&lt; 11,000/mm³</td>
<td>0 - 20</td>
<td>Not done</td>
</tr>
<tr>
<td></td>
<td>86 (97.7%)</td>
<td>51 (57.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 11,000/mm³</td>
<td>21 - 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (2.3%)</td>
<td>28 (31.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 51</td>
<td>9 (10.3%)</td>
<td></td>
</tr>
<tr>
<td>Patients with CPIS ≥ 6, N = 121</td>
<td>&lt; 11,000/mm³</td>
<td>0 - 20</td>
<td>Not done</td>
</tr>
<tr>
<td></td>
<td>28 (23.1%)</td>
<td>0 (0%)</td>
<td>78 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 11,000/mm³</td>
<td>21 - 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>93 (76.9%)</td>
<td>31 (25.6%)</td>
<td>PCT level &gt; 0.5</td>
</tr>
<tr>
<td></td>
<td>&gt; 51</td>
<td>90 (74.4%)</td>
<td>43 (33.3%)</td>
</tr>
</tbody>
</table>

Table 2. Contingency Table

<table>
<thead>
<tr>
<th>The result of BAL quantitative culture</th>
<th>CPIS &gt; 6</th>
<th>CPIS &lt; 6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 10^4</td>
<td>110 (RP)</td>
<td>26 (FP)</td>
<td>136</td>
</tr>
<tr>
<td>&lt; 10^4</td>
<td>11 (FN)</td>
<td>62 (RN)</td>
<td>73</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>88</td>
<td>209</td>
</tr>
</tbody>
</table>

RP: really positive; FP: false positive; FN: false negative; RN: really negative. Test sensitivity = RP/RP + FN = 110/121= 0.91 (91%); Test specificity = RN/RN + FP = 62/88 = 0.7 (70%); Positive predictive value (PPV) = RP/RP + FP = 110/136 = 0.8 (80%); Negative predictive value (NPV) = RN/RN + FN = 62/73 = 0.85 (85%).
Discussion

This study was conducted with the aim to estimate positive and negative predictive values of the BAL quantitative culture in our hospital. Because of the fact that qualitative culture of BAL has a relatively small positive predictive value [13, 15], we decided to estimate positive and negative predictive values of quantitative culture in diagnosis of VAP in our hospital.

Numerous studies have shown that quantitative culture is superior to qualitative culture in VAP diagnosis [9, 12-14]. In the study presented here, the BAL samples from 209 patients hospitalized in the ICU in UKC RS were taken and quantitative BAL culture has been done. For all the patients, BAL culture was positive in 142 (68%), whereas 67 patients were culture negative (32%). The value of 10^4 CFU/mL was settled as cut-off, according to CDC recommendations [7]. For 142 culture-positive patients, the most common isolates were Acinetobacter baumannii in 49 (34.5%) patients, Klebsiella pneumoniae in 31 (21.8%), Pseudomonas aeruginosa in 14 (9.9%), Staphylococcus aureus in eight (5.6%), Serratia marcesens in seven (4.9%), Escherichia coli in four (2.8%), other Enterobacteriaceae in five (3.5%), Pseudomonas spp in three (2.2%), and Candida albicans in two (1.4%), whereas mixed infection was present in 19 patients (13.4%). These results were comparable with the results of the similar studies from Europe and USA [3, 6, 13], only with the difference in the number of multidrug-resistant isolates Acinetobacter baumannii, which is more prevalent in our hospital. The mixed infection (two different species of bacteria and/or fungi) in quantitative BAL culture was diagnosed in 19 patients. All these patients were on mechanical ventilation for more than 7 days, so there was the possibility that during sampling, the bacteria-forming biofilm on plastic devices has been sampled.

According to CDC recommendations [7], the result of quantitative culture should be noted in CFU/mL without interpretation as “positive” or “negative”. In the present study, average value of quantitative culture in patients with CPIS < 6 was 6 × 10^5 CFU/mL, whereas average value of quantitative culture in patients with CPIS ≥ 6 was 3 × 10^5 CFU/mL.

ANOVA has shown that there is a statistically significant difference in the result of quantitative culture of BAL between patients with CPIS < 6 and CPIS ≥ 6 at the level of P < 0.0001. In our study, from the BAL samples Gram-negative bacteria were isolated in 113 patients and that was dominant comparing to Gram-positive bacteria that have been found just in eight patients. As stated in CDC recommendations, empirical antimicrobial therapy in the cases of highly suspected Gram-negative infection should include the combination of ceftazidime or cefepime with an aminoglycoside antibiotic or the combination of carbapenem with aminoglycoside [7]. In our hospital the combination of carbapenem and aminoglycoside was most often prescribed as empiric therapy.

However, it is important to note that the most prevalent isolate from BAL in the patients with VAP hospitalized in ICU in our hospital was Acinetobacter baumannii (extensively drug-resistant, XDR). In this finding, the initial therapy with combination of carbapenem and aminoglycoside was not effective and should be corrected according to antimicrobial susceptibility findings. In our setting that Acinetobacter baumannii was isolated, in 94% cases it was XDR strain, and the only choice of the therapy was colistin (polimiksin E). However, resistance to colistin is an important issue, since emerging of mcr-1 gene has been observed in several bacterial species in six continents [16]. Gram-positive bacteria from BAL were found in eight (5.6%) patients. All the isolates were Staphylococcus aureus, and methicillin-resistant Staphylococcus aureus (MRSA) was isolated in five cases (62.5%).

In our study, the sensitivity of quantitative culture was 91%, whereas specificity was 70%. Positive predictive value was 80% and negative predictive value was 85%. Our results are very similar and corresponding to the results of the other study about predictive value of quantitative culture of BAL in diagnosis of VAP [11, 17, 18].

It is interesting that numerous factors can influence quantitative culture result, including timing of sampling, skills of the specialist who is taking the sample, portion of the sample (the first one corresponding to trachea, or the third one corresponding to alveolae), adequate and fast transportation to microbiology laboratory, delay in culturing, etc. Some patient conditions, such as chronic obstructive lung disease, can lead to finding numerous bacteria in the sample without the presence of pneumonia. The most important factor that can lead to the false-negative results is administration of antibiotic therapy [11, 17, 19].

Till today there are no strong evidences that quantitative culture of BAL improves the clinical outcome, but it was shown to be superior to qualitative culture in the VAP diagnosis [12, 13, 18, 20].

However, it is important to note that CPIS ≥ 6 can be present in various conditions without the presence of VAP, such as sepsis, thrombembolia, and other comorbidities in patients hospitalized in ICU [13, 19, 20], so it is not specific for VAP diagnosis. Therefore there is a need for using quantitative BAL method to support this diagnosis, along with the clinical findings.

Unnecessary antimicrobial drug usage can lead to presence of numerous side effects in patients hospitalized in ICU, increasing cost and duration of hospitalization, as well the selection of multi-resistant bacteria [21, 22]. Quantitative culture of BAL can decrease in some instances unnecessary antimicrobial usage as well side effect of that therapy in patients who often have other comorbidities [22]. Importantly, it can also decrease the cost of treatment for the patients hospitalized in ICU [23]. The results of our study have confirmed that quantitative culture is important and valuable in management of patients with VAP.

References

1. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and health-


