



Predictive Values of Quantitative VS Qualitative Culture of Broncholaveolar Lavage in Diagnosis of Ventilator Associated Pneumonia in Patients on Mechanical Ventilation

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Abstract

Purpose: Ventilator associated pneumonia (VAP) is one of the most common hospital-acquired infection in patients hospitalized in intensive care unit (ICU). Aim of this study was to evaluate predictive values of quantitative and qualitative culture of broncholaveolar lavage (BAL) in the diagnosis of VAP comparing with clinical pulmonary infection score (CPIS), and to determine positive and negative predictive values of the tests in patients on mechanical ventilation. **Methodology:** 209 samples were prospectively taken from the patients hospitalized in ICU on mechanical ventilation; along with the clinical pulmonary infection score (CPIS). After sampling, quantitative and qualitative culture of BAL was done. As the cut off value of quantitative culture 104 CFU/mL was taken, according CDC recommendations. **Results:** In our study, sensitivity of the quantitative culture of BAL was 91%, specificity 70%, positive predictive value 80% and negative predictive value was 85%. Sensitivity of the qualitative culture of BAL was 93%, specificity 36%, positive predictive value 70,2% and negative predictive value was 76%. **Conclusion:** Quantitative culture of BAL has better predictive values in VAP diagnosis in patients on mechanical ventilation, helping in the discrimination between colonization and the infection. Qualitative culture of BAL has higher sensitivity, but lower specificity than quantitative culture.

Keywords: broncholaveolar lavage (BAL), ventilator associated pneumonia (VAP), quantitative culture, qualitative culture, intensive care unit (ICU)

Introduction

Ventilator associated pneumonia (VAP) is one of the most common hospital-acquired infection in patients hospitalized in intensive care unit (ICU). VAP is a form of hospital-acquired pneumonia that develops 48 hours after mechanical ventilation has been initiated. It has been estimated that approximately 27% all patients hospitalized in ICU develop this complication.^[1] Outside ICU, on the other wards, the incidence has been estimated on 5 till 10 cases on 1000 hospitalized.^[2] The mortality associated with VAP can be as high as 50%.^[3,4] The mortality depended of main

diagnosis, since higher mortality have 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 the adequate antimicrobial treatment in the first 48 hours after admission.^[5] VAP have also an important economic impact, since they can lead to increasing of hospitalization duration more than 7 days, and significantly increasing hospitalization costs.^[5,6]

VAP diagnosis starts with clinical observation and findings, RTG diagnostic and microbiological analysis of respiratory tract samples.^[2,4] It is important, however, that in ICU the multiresistant

pathogens are common finding 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 colleagues were developed the system of clinical score named Clinical Pulmonary Infection Score (CPIS), which has been determined on 6 variables: temperature, leukocyte count, the volume and appearance of tracheal secretion, oxygenation, pulmonary RTG and quantitative culture of tracheal aspirate.^[8] CPIS score range from 0 till 12. Predictive value of CPIS ≥ 6 has the sensitivity of 93% and specificity of 100% in clinical diagnosis VAP. In order to improve specificity of VAP diagnosis and to avoid unnecessary antibiotic usage, numerous study of utility of quantitative cultures of the respiratory tract samples were conducted.^[9,10] Till today, there is not the strong consensus if quantitative culture of BAL improves the diagnosis of VAP comparing to qualitative culture.^[8,9,10]

Qualitative culture gives the data about the species isolated and antibiotic sensitivity without quantification. It is easy to perform and most routine microbiology laboratories perform this test in VAP diagnosis. In contrary, quantitative culture gives the data about the quantity of the isolated organism in CFU/mL, along with species isolated and antibiotic sensitivity data. The cut off value for the qualitative culture of the BAL was 104 CFU/mL, according CDC recommendations.^[7] These cut off values were settled in accordance with studies in infected lung tissue. The published studies were shown that in patients with pneumonia, bacteria were present in quantity of 105 CFU/mL and more. If the number was 103 CFU/mL and lower, majority of cases were colonization.^[11,12,13] The sampling and the quantitative procedure were described in details in a study conducted by Berton et al.^[12]

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

This study was conducted with the aim to estimate positive and negative predictive value of the BAL quantitative and qualitative culture in patients with VAP in our hospital. Some of the previously published studies and reviews have shown that qualitative culture of BAL have relatively small positive predictive value.^[13,15] However, qualitative culture is still the most prevalent method for the VAP diagnosis in majority of routine microbiology laboratories in our country. That is why we decided to estimate positive and negative predictive value of quantitative and qualitative culture in diagnosis of VAP in our settings.

Material and Methods

It was prospective study taking 6 months, started 1.6.2018, finished 31.12.2018. We obtained the samples and clinical data from 209 adult patients hospitalized in intensive care units of University Clinical Centre of Republika Srpska (UKC RS), who were at mechanical ventilation. Sample was bronchoalveolar lavage (BAL) at least 5-10 mL. From each sample quantitative and the qualitative culture was made along with Gram stain.

1. Sampling

Bronchoalveolar lavage was taken through fiber optical bronchoscope and the method was described previously.^[10] Shortly, saline in quantity of 50-100 mL was injected through system and aspirated immediately to recover microorganisms from lower respiratory tract. If classical BAL could not be obtained, than the procedure of mini-BAL was taken (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 fraction 2 and 3 are representative. All the samples were transported in short period (less than 30 minutes) to the microbiology laboratory in order to preserve leukocyte morphology in the samples.

2. Checking the sample quality

The presence more than 1% of epithelial cells or 10 cells on low magnitude (x100) in the samples indicate the contamination of the samples with oropharyngeal flora, so those samples were rejected because they were not representing for lower respiratory tract.

3. Quantitative culture- method description

All the samples were homogenized by gentle shaking on vortex. Using the micropipette with the sterile tips, 50 μ L of the sample were transferred on blood, McConkey and chocolate agar. Inoculated samples were smeared on the agar using the sterile glass stick. After the inoculation, the agars were left on the room temperature 10 till 15 minutes to drying the top. After that period, the *Staphylococcus aureus* ATCC 25923 streak were taken over the blood and chocolate agar to obtain the conditions for potential growing *Haemophilus* spp. Incubation was done 24 hours on 35-37°C in microaerophilic atmosphere. Reincubation: If there were no visible growing, incubation was prolonged 24 hours more on 35-37°C.

The final finding consist from the data about the species and subspecies of the isolated microorganism, quantity stated in Colony Forming Units (CFU)/mL, antimicrobial susceptibility test and remarks if they were needed.

3.1. Quantitative culture calculation

In physiological state, there is usually around 1 ml of secrets in the lungs. BAL was diluted in 10 till 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 till 1:100. Since the fact that we have used quantity of 50 μ L for the inoculation of the agar, it is needed to multiply the colony number with 20 to obtain the number in 1 mL and then to multiply with dilution factor (e.g. x100 if 100 mL saline was used in BAL sampling). The cut off value for the quantitative culture was 104 CFU/mL.^[7]

4. Qualitative culture- method description

All the samples were homogenized by gentle shaking on vortex. Using the inoculation loop, sample was transferred on blood agar plate, McConkey and Chocolate agar. Inoculated samples were smeared on the agar using the loop. After the inoculation, the agars were left on the room temperature 10 till 15 minutes to drying the top. After that period, the *Staphylococcus aureus* ATCC 25923 streak was taken over the blood and chocolate agar to obtain the conditions for potential growing *Haemophilus* spp. Incubation was done 24 hours on 35-37°C in microaerophilic atmosphere.

Reincubation: If there were no visible growing, incubation was prolonged 24 hours more on 35-37°C. The final finding consists from the data about the species and subspecies of the isolated microorganism, antimicrobial susceptibility test and remarks if they were needed.

5. Clinical data

For the each patient the findings about leukocyte number, CRP level, procalcitonine level and CPIS score calculated on the day of sampling. For the each patient the data about antibiotic administration were collected.

6. Statistical analysis

Statistical analysis was done by program package SSPS version 16.0. for Windows (SSPS Inc. Chikago, USA). In the analysis, the standard methods of descriptive statistical analysis were used, and

for the variance analysis ANOVA was applied. For statistically significant result, the value of <0,05 was taken.

Results

The samples taken from 209 patients hospitalized at ICU with clinical suspicion for developing ventilator associated pneumonia were taken. There were 127 males and 82 females, average age 58 ±17,2 for the adults.

For all the patients the Clinical Pulmonary Infection Score (CPIS) was recorded. As clinical relevant, the value of CPIS ≥6 was taken. From 209 patients, 121 had CPIS score ≥6. All the other parameters, including leukocyte count, CRP serum level, procalcitonine serum level in the two groups of patients according CPIS findings were shown in the Table 1.

Table 1: The leukocyte count, CRP serum level, procalcitonine serum level in the two groups of patients separated according CPIS findings

Groups	Leucocyte	CRP serum level (mg/L)	Procalcitonine (ng/mL)
Patients with CPIS <6 N=88	<11 000/mm ³ 86 (97,7%)	0-20 51 (57,9%)	Not done
	>11 000/mm ³ 2 (2,3%)	CRP level 21-50 28 patients (31,8%) >51 9 (10,3%)	
Patients with CPIS ≥6 N=121	<11 000/mm ³ 28 (23,1%)	0-20 0 (0%)	Not done 78 (66,7%)
	>11 000/mm ³ 93 (76,9%)	CRP level 21-50 31 patients (25,6%) >51 90 (74,4%)	PCT level >0,5 43 (33,3%)

Quantitative BAL culture

The results of quantitative culture have shown that from all the patients, BAL culture was positive in 142 (68%), whereas 67 patients were culture negative (32%). Value of 10⁴CFU/mL was taken as cut off (7). From 142 positive, the most common isolates were Acinetobacter baumannii in 49 (34,5%) patients, Klebisella pneumoniae in 31 (21,8%), Pseudomonas aeruginosa in 14 (9,9%), Staphylococcus aureus in 8 (5,6%), Serratia marcescens in 7 (4,9%), Escherichia coli in 4 (2,8%), other Enterobacteriaceae in 5 (3,5%), Pseudomonas spp in 3 (2,2%), Candida albicans in 2 (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 (±1826,3), whereas the average value in the group with CPIS ≥6 was 298661,9 CFU/mL (±415323,4).

Staphylococcus aureus in 10 (5,84%), Serratia marcescens in 9 (5,3%), Escherichia coli in 6 (3,5%), other Enterobacteriaceae in 3 (1,75 %), Pseudomonas spp in 2 (1,16 %), Candida albicans in 2 (1,2%), whereas mixed infection was present in 10 patients (5,84 %).

The predictive values of the tests

According CPIS score and BAL quantitative and qualitative culture results, the results were putted into the contingency tables for calculation of sensitivity and specificity of the tests, along with the calculation of positive and negative predictive value of the tests. The results have shown in Table 2 and 3.

Table 2: Contingency table- quantitative culture

The result of BAL quantitative culture	CPIS>6	CPIS<6	Total
> 10 ⁴	110 (RP)	26 (FP)	136
< 10 ⁴	11 (FN)	62 (RN)	73
Total:	121	88	209

Abbreviations: 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%)

Variance analysis have shown that there is statistically important difference among the quantitative culture results among these two groups (CPIS <6 and CPIS ≥6) at the level p<0,0001 (95% CI: 362198.24 -236579.76).

Qualitative BAL culture

From all the patients, qualitative BAL culture was positive in 171 (81,8%), whereas 38 patients were culture negative (18,2%). From 171 positive, the most common isolates were Acinetobacter baumannii in 63 (36,84%) patients, Klebisella pneumoniae in 42 (24,56%), Pseudomonas aeruginosa in 24 (14,03%),

Table 3: Contingency table- qualitative culture

The result of BAL qualitative culture	CPIS>6	CPIS<6	Total
Positive	120 (RP)	51 (FP)	171
Negative	9 (FN)	29 (RN)	38
Total:	129	80	209

Abbreviations: RP really positive, FP-false positive, FN-false negative, RN-really negative.

Test sensitivity= $RP/RP+FN=120/129=0,93$ (93%)

Test specificity= $RN/RN+FP=29/80=0,26$ (36%)

Positive predictive value (PPV)= $RP/RP+FP=120/171=0,70$ (70,17%)

Negative predictive value (NPV)= $RN/RN+FN=29/38=0,76$ (76%)

In our study sensitivity of BAL quantitative culture was 91%, specificity 70%, positive predictive value was 80%, whereas negative predictive value was 85%.

In our study, the sensitivity of BAL qualitative culture was 93%, whereas specificity was 36%. Positive predictive value was 70,2% and negative predictive value was 76%.

Discussion

Numerous studies have been done to estimate usefulness of quantitative culture comparing to qualitative in VAP diagnosis.^[9,12,13,14] Till today there are no strong evidences that quantitative culture of BAL improves the clinical outcome, but there are strong evidences that are more superior in VAP diagnosis comparing qualitative culture.^[12,13,19,21]

In the study presented here, the BAL samples from 209 patients hospitalized in the ICU in UKC RS were taken and qualitative and quantitative BAL culture has been done. From all the patients, quantitative BAL culture was positive in 142 (68%), whereas 67 patients were culture negative (32%). Value of 104 CFU/mL was settled as cut off, according CDC recommendations.^[7] These results were comparable with the results of the similar studies from Europe and USA.^[13,15,16,17] There is only the difference in number of multidrug resistant isolates *Acinetobacter baumannii*, which is more prevalent in our hospital. The mixed infection (two different species of bacteria and/or fungus) in quantitative BAL culture was diagnosed in 19 patients. All these patients were on mechanical ventilation longer than 7 days, so there was the possibility that during sampling, the bacteria forming biofilm on plastically devices have been sampled. 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 of predictive value of quantitative culture BAL in diagnosis VAP.^[11,18,19]

Qualitative culture was positive in 171 (81,8%), that is statistically significant higher than in quantitative culture (only 68%) at level $p<0,05$. In our study, the sensitivity of qualitative culture was 93%, whereas specificity was only 36%. Positive predictive value was 70,2% and negative predictive value was 76%. The results of our study have shown that despite higher sensitivity of qualitative BAL culture, its specificity is lower than quantitative culture.

Our results have shown that there is statistically significant difference in the result of quantitative culture BAL between patients with $CPIS<6$ and $CPIS \geq 6$ at the level $p<0,0001$. It is interesting that numerous factors can influence on 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 transport to microbiology laboratory, delay in culturing etc. Some patient conditions, as chronic obstructive lung disease can lead in finding the 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.^[11,18,20]

However, it is important to note that $CPIS \geq 6$ can be present in various conditions without the presence of VAP, as sepsis, embolia, and other morbidities in patients hospitalized in ICU^[13,19,21], so it is not specific for VAP diagnosis. Therefore there is need for using quantitative BAL method to give an addition to this diagnosis, along with the clinical findings. As stated in CDC recommendations, empirical antimicrobial therapy in the cases of highly suspected Gram negative infection should include the combination of ceftazidim or cefepime with an aminoglycoside antibiotic or the combination of carbapenem and aminoglycoside.^[7] In our hospital the combination of carbapenem and aminoglycoside was the most often prescribed as empiric therapy. The one more reason for potential introducing quantitative culture of BAL is avoiding unnecessary antimicrobial treatment in patients with colonization.

Unnecessary antimicrobial drug usage can lead to appearance of numerous side effects in patients hospitalized in ICU, increasing cost and duration of hospitalization, as well the selection of multiresistant bacteria.^[22,23] Quantitative culture of BAL can decrease in some instances unnecessary antimicrobial usage as well side effect of that therapy in patients that often have other conditions.^[23] Important issue is also decreasing the cost of treatment the patients hospitalized in ICU.^[24] The results of our study have shown that quantitative culture is superior than qualitative and that is important and valuable in management of patients with VAP.

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