Relationship between Polymorphonuclear Leukocyte Count in Bronchoalveolar Lavage Fluid and Bacterial Content in Gram's Stain and Bacterial Content in Final Microbiological Report

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A B S T R A C T

Eighty samples of bronchoalveolar lavage fluid (BALF) were obtained from the total of 48 patients (22 females and 26 males) and analyzed. Eighteen of those patients were organ transplant recipients. The relationship between polymorphonuclear leukocyte (PMN) count in direct sample and semi quantitative Gram-positive and Gram-negative bacterial content were analyzed in BALF samples. PMN count in direct sample and Gram-positive and Gram-negative bacterial content of the final microbiological report was compared as well. On the total number of samples PMN count in direct samples of BALF was statistically significant regarding the presence of Gram-positive bacteria in the same sample; it was nearly significant regarding the presence of Gram-negative bacteria; and it was statistically significant for the total bacterial content. If BALF samples are divided into those obtained from organ-transplant and those obtained from non-organ-transplant patients, positive, statistically significant relationship is found in the organ-transplant group, more specifically for the relationship between PMNs and total bacterial content. When PMN count in direct microbiological sample was compared with the results of the final microbiological report, statistically significant relationship was found neither with respect to all BALF samples, nor after dividing them into »organ-transplant« and »non-organ-transplant« group. We did not find differences caused by gender.

Key words*: bronchoalveolar lavage, polymorphonuclear leukocytes*

Introduction

BAL with BAL fluid cytology has been established as a reliable technique for the diagnosis of pulmonary infection in the immunocompromised host[1–3] including organ transplant recipients and patients with allo-or autologous bone marrow transplantation due to hematologic malignancy[2,4–6]. BALF analysis is also a valuable technique for other, and particularly for critically ill patients[7].

Significantly higher neutrophil count in BALF is considered to be good predictor of bacterial infection[1,8].

The male gender has been found to be an independent risk for pneumonia, including ventilator-associated pneumonia (VAP). The effect of the hormonal influence on cell-mediated immunity has been postulated as a possible cause of the decreased incidence in women[9].

Materials and Methods

Eighty samples of bronchoalveolar lavage fluid (BALF) obtained from a total of 48 patients (22 female and 26 male) were analyzed. BALF sample was collected repeatedly in some patients. The patients were hospitalized in University Hospital »Merkur« in the past year. Fifteen of them were hospitalized in the surgical intensive care unit, and the rest of them in the department of medicine, mostly in the intensive care unit (25) or on various medical wards (8). There were 18 organ transplant recepients: 4 patients with allo- or autologous bone marrow transplantation due to hematologic malignancy, while the rest of the patients were organ transplant recepients (8 patients with liver, 4 with kidney and 2 with pancreas-kidney transplant). Bronchoscopy with BAL was performed via endotracheal tube or tracheostomy and through the nose or mouth. In some patients BALF sample was collected several times during the hospitalization.

The relationship between polymorphonuclear leukocyte (PMN) count in direct sample and semi quantitative Gram-positive and Gram-negative bacteria content in the same sample was analyzed in BALF samples. PMN count in direct sample and Gram-positive and Gram- -negative bacterial content of the final microbiological report was compared as well. The data were analyzed on the total number of samples and after dividing them into »organ-transplant« and »non-organ- -transplant« samples.

Sample structure was described by relative frequency (percentage). To describe the results distributed on the minimum interval level, arithmetic mean was used as a measure of central tendency, and standard deviation and minumum and maximum result as a measure of spread. For categorical variables statistical significance of difference was tested using χ^2-test and 95% (p<0.05) level of statistical significance was applied.

Results

Forty-eight participants took part in the study, 26 (54%) being male and 22 (46%) female. Mean age of the participants in the sample was $\overline{X}$=60.8 years, SD=16.2 (Table 1). There was a total of 80 samples, 30 (37%) from organ transplant patients and 50 (63%) from other, non- -organ-transplant patients. Forty-five BALF samples (56%) were obtained from intubated patients, and 35 (44%) from others. Eighteen (22%) samples were obtai-

ned from the intubated organ-transplant patients (Table 2).

TABLE 2
PERCENTAGE OF PARTICIPATANS WHO WERE TRANSPLANTED AND INTUBATED

		Transplanted		Total
		No	Yes	
ET tubus	Non-intubated	30.4%	13.9%	44.3%
	Intubated	32.9%	22.8%	55.7%
Total		63.3%	36.7%	100.0%

Comparison of PMN count with semi quantitative bacterial count in the direct microscopic sample after Gram staining on the total number of samples

PMNs, as well as bacteria, were observed in 3 categories (»<10, 10–25, >25« and »none, little, quite a lot« respectively). First, cross tabulation was performed for leukocyte count categories and Gram-positive bacterial content, then for leukocyte count categories and Gram- -negative bacterial content, and in the end for leukocyte count categories and total bacterial (Gram-positive and Gram-negative) content. χ^2-test found statistically significant difference in leukocyte count depending on Gram- -positive bacterial content (χ^2=10.795, df=4, p= 0.027), which means that more PMNs were found in direct sample; at the same time higher Gram-positive bacterial content was also found in direct Gram's stain. Regarding the relationship between PMN count and Gram-negative bacterial content in direct sample, nearly significant difference was found ((χ^2=9.011, df=4, p= 0.059). When PMN count was compared in relation to the total (Gram- -positive and Gram-negative) bacterial content χ^2-test found statistically significant difference (χ^2=14.664, df=4, p=0.005) (Table 3, Figure1).

Comparison of PMN count with bacterial and fungal content in the final microbiological report on the total number of samples

PMN count in direct microscopic sample was compared with total bacterial content (none, <10^4, ≥10^4) obtained upon completion of the microbiological report. χ^2-test exact test did not find statistically significant difference in leukocyte count in relation to the Gram- -positive (χ^2=8.059, df=4, p=0.087) or total (Gram-positive

TABLE 1
ARITHMETIC MEAN, STANDARD DEVIATION, MEDIAN AND MINIMUM AND MAXIMUM AGE OF PARTICIPANTS

	$\overline{X}$	SD	Median	Minimum	Maximum	N
Male	66.2	13.8	63.0	32	97	26
Female	54.6	17.8	56.0	22	86	22
Total	60.8	16.2	61.0	22	97	48

N – number

TABLE 3

OBSERVED FREQUENCY OF PMNS IN RELATION TO THE TOTAL SEMIQUATITATIVE BACTERIAL CONTENT IN DIRECT GRAM'S STAIN ON THE TOTAL NUMBER OF BALF SAMPLES

		Bacteria			Total
		None	Little	Quite a lot	
Polymorphonuclear leukocytes (PMNs)	<10	20	17	1	38
PMNs	10–25	4	5	1	10
PMNs	>25	7	11	10	28
Total		31	33	12	76

TABLE 4

OBSERVED FREQUENCY OF PMNS IN RELATION TO THE TOTAL BACTERIAL CONTENT IN THE FINAL MICROBIOLOGICAL REPORT

		Bacteria (total)			Total
		None	$<10^4$	$\geq 10^4$	
Polymorphonuclear leukocytes (PMNs)	<10	19	8	11	38
PMNs	10–25	7	0	4	11
PMNs	>25	11	9	11	31
Total		37	17	26	80

and Gram-negative) bacterial content (χ^2=5.116, df=4, p=0.285) (Table 4, Figure 2).
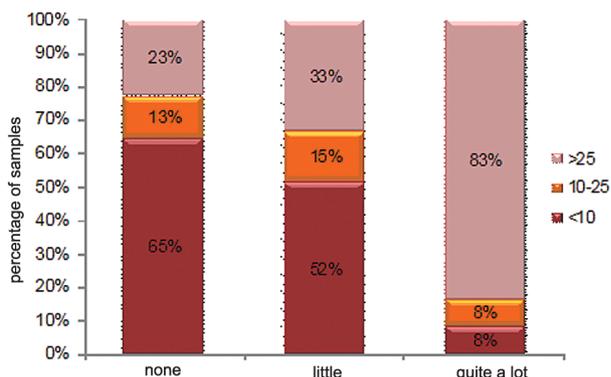

Fig. 1. Percentage of PMN samples in relation to the bacterial content in direct Gram's stain on the total number of BALF samples.
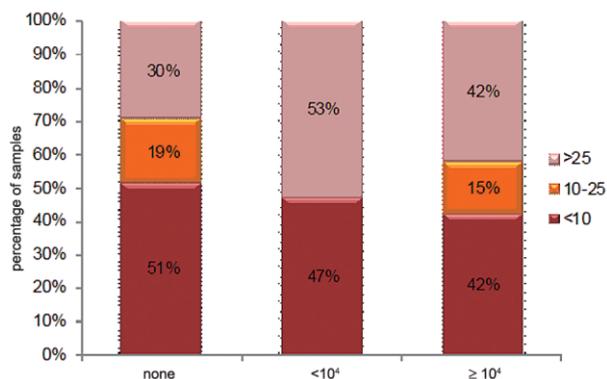

Fig. 2. Percentage of PMN samples in relation to the total bacterial content in the final microbiological report.

Comparison of the PMN count with the semi quantitative bacterial content in direct Gram's stain on BALF samples in relation to organ-transplant status

χ^2-test did not find statistically significant difference in leukocyte count in relation to the Gram-positive bacteria neither in organ-transplant (χ^2=7.415, df=4, p=0.118), nor in non-organ-transplant (χ^2=5.466, df=4, p=0.245) patients. This applies to Gram-negative bacteria also: organ-transplant (χ^2=5.715, df=4, p=0.216) and non-organ-transplant (χ^2=4.245, df=4, p=0.340) patients, respectively. If the total (Gram-positive and Gram-negative) bacterial content was taken into account, statistical significance in organ transplant (χ^2=14.710, df=4, p=0.006), but not in non-organ-transplant (χ^2=4.615, df=4, p=0.359) group was established.

Comparison of PMN count with bacterial content in the final microbiological report in relation to organ-transplant status

Statistically significant difference was not found in any of the groups (Gram-positive, Gram-negative, or total bacteria) (Tables 5, 6 and 7).

Sexual differences

In total there were 34 samples received from female and 46 samples from male patients. The difference between male and female samples in the number of PMN was not statistically significant (χ^2=0.037, df=2, p=0.982), neither it was significant in the case of considering Gram positive (χ^2=1.679, df=2, p=0.432) and Gram negative bacteria (χ^2=0.328, df=2, p=0.849) in direct Gram's stain. Difference between male and female samples was not statistically significant neither in the num-

TABLE 5
STATISTICAL SIGNIFICANCE OF THE COMPARISON OF THE PMN COUNT WITH GRAM- POSITIVE BACTERIA CONTENT IN THE FINAL MICROBIOLOGICAL REPORT

Organ- transplant		Value	df	Asymp. Sig. (2–sided)	Exact Sig. (2–sided)
No	Pearson χ^2	3.520(a)	4	0.475	0.515
Yes	Pearson χ^2	7.254(c)	4	0.123	0.124

TABLE 6
STATISTICAL SIGNIFICANCE OF THE COMPARISON OF THE PMN COUNT WITH THE GRAM- NEGATIVE BACTERIA CONTENT IN THE FINAL MICROBIOLOGICAL REPORT

Organ- transplant		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
No	Pearson χ^2	2.828(a)	4	0.587	0.704
Yes	Pearson χ^2	3.018(c)	4	0.555	0.627

TABLE 7
STATISTICAL SIGNIFICANCE OF THE COMPARISON OF THE PMN COUNT WITH BACTERIAL CONTENT IN THE FINAL MICROBIOLOGICAL REPORT (GRAM- POSITIVE AND GRAM- NEGATIVE)

Organ- transplant		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
No	Pearson χ^2	3.844(a)	4	0.428	0.466
Yes	Pearson χ^2	8.068(c)	4	0.089	0.087

TABLE 8
DIFFERENCES BETWEEN MALE AND FEMALE SAMPLES WITHIN TRANPLANT PATIENTS

	χ^2	df	p
PMN	0.196	2	0.906
Gram positive – direct Gram's stain	1.845	2	0.398
Gram negative – direct Gram's stain	4.510	2	0.105
Gram positive – final microbiological report	0.008	2	0.996
Gram negative – final microbiological report	1.130	2	0.568

ber of bacteria in the final microbiological report – Gram positive (χ^2=0.354, df=2, p=0.838), as well as Gram negative (χ^2=4.873, df=2, p=0.087). Additionally, there were no statistically significant differences between male and female transplant patients (Table 8) as well as within group of intubated patients (Table 9).

Discussion

It can be concluded that on the total number of samples PMN count in direct microbiological samples of BALF was statistically significant regarding the presence of Gram-positive bacteria in the same sample; it was nearly significant regarding the presence of Gram-negative bacteria; and it was statistically significant when it is being observed for the total bacterial content. If BALF samples are divided into those obtained from organ--transplant and those obtained from non-organ-trans-

plant patients, positive, statistically significant relationship is found in the organ-transplant group, more specifically for the relationship between PMNs and total bacterial content.

When PMN count in direct microbiological sample was compared with the results of the final microbiological report, statistically significant relationship was found neither with respect to all BALF samples, nor after dividing them into »organ-transplant« and »non-organ- transplant« group. Within given samples, we did not find statistically significant difference between male and female samples in PMN number, Gram positive or Gram negative bacteria in direct microscopic preparation, nor in final microbiological report.

BAL with BAL fluid cytology has been established as a reliable technique for the diagnosis of pulmonary infection in the immunocompromised host[1–3] including organ transplant recipients and patients with allo-or autolo-

TABLE 9
DIFFERENCES BETWEEN SAMPLES WITHIN INTUBATED PATIENTS

	χ^2	df	p
PMN	0.400	2	0.819
Gram positive – direct Gram's stain	2.611	2	0.271
Gram negative – direct Gram's stain	0.699	2	0.705
Gram positive – final microbiological report	2.007	2	0.367
Gram negative – final microbiological report	2.578	2	0.275

gous bone marrow transplantation due to hematologic malignancy[2,4–6]. BALF analysis is also a valuable technique for other, and particularly for critically ill patients[7]. Significantly higher neutrophil count in BALF is considered to be good predictor of bacterial infection[1,8].

Interestingly, our study did not find positive correlation between PMN count in direct microbiological sample with bacterial content of the final microbiological report, although this relationship was found with the semi quantitative bacterial content obtained by Gram staining of the same sample on the total number of samples as well as in the »organ-transplant« group. Perhaps antimicrobial therapy had influence on such a result, having been already initiated at the time-point of BALF sampling, which is common in case of critically ill patients, with bronchoscopy still being unavailable[1,10]. Despite that, we are unable to explain the relationship between PMN count and semi quantitative bacterial content in the same sample after Gram staining.

The male gender has been found to be an independent risk for pneumonia, including VAP[9]. Two recent clinical studies have shown sex- specific differences in the development of sepsis and multiple organ failure in severely injured patients. The incidence of posttraumatic sepsis and multiple organ dysfunction syndrome was significantly increased in severely injured men when compared with an equivalent female group[11–13]. In our research we have analyzed number of PMN which we consider as usual mark for inflammation in BALF on »male« and »female« samples which, according to our knowledge, has not been analyzed until now. Although, we did not find differences between »male« and »female« samples, we think that this research should be conducted over larger number of samples.

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ODNOS BROJA POLIMORFONUKLEARNIH LEUKOCITA IZ BRONHOALVEOLARNOG LAVATA S BROJEM BAKTERIJA U PREPARATU BOJENOM PO GRAMU I BROJEM BAKTERIJA U KONAČNOM MIKROBIOLOŠKOM NALAZU

S A Ž E T A K

Analzirano je 80 uzoraka bronhoalveolarnog lavata (BAL) uzetog od ukupno 48 pacijenata od kojih je 22 žene i 26 muškaraca. Osmnaest pacijenata od ukupnog broja je bilo transplantirano. Na uzorcima BAL-a analizirali smo povezanost broja polimorfonuklearnih leukocita (PMN) u direktnom preparatu sa semikvantitativnim brojem Gram pozitivnih i Gram negativnih bakterija u istom preparatu. Također smo uspoređivali broj polimorfonuklearnih leukocita u direktnom preparatu s brojem Gram pozitivih i Gram negativnih bakterija u konačnom mikrobiološkom nalazu. Na ukupnom broju uzoraka- broj PMN u direktnom mikroskopskom preparatu uzoraka BAL-a bio je statistički značajan u odnosu na pojavnost Gram pozitivnih bakterija u istom preparatu, blizu je statističke značajnosti kada se radi o Gram negativnim bakterijama, a također statistički značajan kada ga promatramo u odnosu na ukupan broj bakterija. Kada uzorke BAL-a raščlanimo na one koje su dobiveni od transplantiranih i netransplantiranih pacijenata, pozitivnu, statistički značajnu povezanost nalazimo u »transplantacijskoj« skupini i to za odnos PMN i ukupnog broja bakterija. Uspoređujući broj PMN u direktnom mikroskopskom preparatu sa rezultatima konačnog mikrobiološkog nalaza, nije nađena statistički značajna povezanost niti kada se to odnosi na sve uzorke BAL-a, niti kada ih podijelimo u »transplantacijsku« i »netransplantacijsku« skupinu. Nismo našli razlike među spolovima.