Med Clin (Barc). 2017;xxx(xx):xxx–xxx

MEDICINA CLINICA

www.elsevier.es/medicinaclinica

Original article

Usefulness of bronchoalveolar lavage and flow cytometry in patients with hematological malignancies and respiratory failure☆

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ARTICLE INFO

Article history:
Received 27 July 2016
Accepted 20 October 2016
Available online xxx

Keywords:
Bronchoalveolar lavage
Flow cytometry
Respiratory failure
Hematological malignancies

ABSTRACT

Background and objectives: Strategies to improve the efficiency of bronchoalveolar lavage (BAL) are needed. We conducted a study to establish the diagnostic value of BAL in patients with hematological malignancies and pulmonary infiltrates.
Patients and methods: The correlation of cytologic and flow cytometric study of BAL with the microbiological findings and the clinical evolution was determined.
Results: Seventy BAL were performed and flow cytometric study was analyzed in 23 of them. Fifty-three patients did not present any adverse event attributable to BAL. Anti-infectious therapy was modified in 64 (91%) patients. T lymphocyte count >0.3 × 10^9/L in peripheral blood was associated with longer OS at 3 years (53 vs. 22%, $p = 0.009$). Higher CD4 (>20/μL) and CD8 (>35/μL) lymphocyte counts in the BAL were associated with a longer OS at 3 years: 82 vs. 21% ($p = 0.030$) and 80 vs. 23% ($p = 0.059$).
Conclusions: Our study confirms the clinical value of BAL for treatment decision making in patients with hematological malignancies and acute respiratory failure.

Utilidad del lavado broncoalveolar y la citometría de flujo en pacientes con hemopatías malignas e insuficiencia respiratoria

Palabras clave:
Lavado broncoalveolar
Citometría de flujo
Insuficiencia respiratoria
Hemopatías malignas

RESUMEN

Antecedentes y objetivo: Para mejorar la eficiencia del lavado broncoalveolar (LBA) son necesarias nuevas estrategias. Con esta finalidad se desarrolló un estudio para establecer el valor diagnóstico del LBA en pacientes con hemopatías malignas e infiltrados pulmonares.
Pacientes y método: Se analizó la correlación del estudio citológico y la citometría de flujo del LBA con los hallazgos microbiológicos y la evolución clínica.
Resultados: Se analizaron setenta LBA y se realizó estudio de citometría de flujo en 23 de ellos. Cincuenta y tres pacientes no presentaron ningún efecto adverso atribuible al LBA. Se modificó el tratamiento antiinfeccioso en 64 (un 91%) de los pacientes. La cifra de linfocitos T >0,3 × 10^9/L en sangre periférica se asoció a una mayor supervivencia global a los 3 años (el 53 vs. 22%, $p = 0{,}009$). Una cifra más elevada de linfocitos T CD4 (>20/μL) y CD8 (>35/μL) en el LBA se asoció a una mayor supervivencia global a los 3 años: el 82 vs. 21% ($p = 0{,}030$) y el 80 vs. 23% ($p = 0{,}059$).
Conclusiones: Nuestro estudio confirma el valor clínico del LBA en la estrategia terapéutica de pacientes con hemopatías malignas e insuficiencia respiratoria.

☆ Please cite this article as: Ferrà C, Xicoy B, Castillo N, Morgades M, Juncà J, Andreo F, et al. Utilidad del lavado broncoalveolar y la citometría de flujo en pacientes con hemopatías malignas e insuficiencia respiratoria. Med Clin (Barc). 2017. http://dx.doi.org/10.1016/j.medcli.2016.10.045
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Introduction

Patients with hematological malignancies (HM) and particularly patients undergoing hematopoietic stem cell transplantation (HSCT) are prone to develop infections due to their immunosuppressed status. Around 13% to 60% of patients with HM develop pulmonary infiltrates, often associated with acute respiratory failure (ARF).[1,2] It is crucial to assess the etiology of ARF as soon as possible in order to improve the survival of these patients; however, the cause of the pulmonary damage frequently remains unknown. Non-invasive tests such as sputum culture, antigen detection in blood or urine, specific polymerase chain reaction (PCR) test for some viruses and fungi in blood samples, chest-X ray and high-resolution CT scan do not always allow the identification of the causal pathogen of the process. Empirical antimicrobial treatment is usually started at the detection of a pulmonary infiltrate, being modified if a specific microorganism is identified to optimize treatment and avoid unnecessary toxicity.[3,4] Fiber-optic bronchoscopy with bronchoalveolar lavage (BAL) is helpful in identifying the etiology of ARF in about 30–50% of immunocompromised patients, but it may be associated with complications such as hypoxia worsening or hemoptysis.[5,6] Galactomannan antigen (GMN) and PCR detection of virus in BAL may increase the diagnostic rate of this procedure.[7]

Flow cytometry (FC) of BAL specimens has been used more precisely to detect neoplastic infiltration,[8] to investigate the cause of interstitial lung disease[9] and to complement the study of patients with sarcoidosis or hypersensitivity pneumonitis, among others[10]; however, little is known about its usefulness and prognostic significance in patients with HM and ARF.

The main objective of this study was to establish the diagnostic value of BAL in patients with HM and pulmonary infiltrates, and to correlate the cytologic and immunophenotypic results (CD4+ and T CD8+ lymphocyte count) of BAL samples with the microbiological findings and the clinical outcome of the patients.

Patients and methods

We reviewed the bronchoscopic procedures with BAL performed at our institution in patients with HM from 2008 to 2012, and a prospective study of these procedures from 2013 to 2014 was performed. In the prospective part of the study a FC analysis of BAL specimens was performed. All these patients with HM had received chemotherapy and/or radiotherapy and/or HSCT.

The following variables at the time of BAL were recorded: age, gender, co morbidities, underlying disease, disease status, lines of treatment received, type of HSCT and conditioning regimen, immunosuppressive therapy, anti-infectious therapy, peripheral blood cell counts, liver and renal function tests, oxygen saturation, radiologic pattern, results of microbiologic studies, results of transbronchial lung biopsies (if done), complications after BAL, clinical evolution and survival of the patients. Regarding the BAL procedure, the number of lymphocytes determined by conventional cytological examination and CD4 and CD8 lymphocyte counts by FC were also recorded.

According to the clinical practice in our institution, anti-infectious prophylaxis consisted of levofloxacin and acyclovir when absolute neutrophil counts were under 0.5×10^9/L. Patients under HSCT received trimethoprim sulfamethoxazole twice a day, 2 days weekly, for *Pneumocystis jirovecii* prophylaxis. Prophylaxis with levofloxacin and posaconazol was administered in neutropenic patients with acute leukemia. Supportive care with transfusions was given when hemoglobin levels were less than 75 g/L or the platelet count was less than 10×10^9/L. Platelet transfusions were intensified to achieve platelet counts around 50×10^9/L before the BAL procedure.

The institutional review board approved the study and all enrolled patients signed informed consent before the initiation of therapy and before bronchoscopic exploration.

Patients underwent BAL if they met the following criteria: (1) fever greater than or equal to 38 °C and/or (2) pulmonary infiltrates in radiologic tests and/or (3) ARF. BAL was performed by a pneumologist using a fiber-optic bronchoscope. Warmed normal sterile saline serum was instilled in 4–6 aliquots of 10–20 mL, which was suctimed and sent for cytologic and microbiologic study. The BAL procedure was considered diagnostic if a microorganism was identified.

Flow cytometry studies were prospectively performed in BAL samples from October 2013 to October 2014, when the cell count in BAL fluid was greater than 200 cells/μL. The FC protocol was as follows: briefly, 200 μL of BAL were mixed in a polypropylene tube with anti-CD4 FITC, CD8PE, CD3ECD, CD19 Pcy5 and CD56Pcy7 (5 μL each), all purchased from Beckman Coulter (Hialeah, FL, USA). After incubating for 15 min, red blood cells were lysed with Optilyse (Beckman Coulter), and then further incubated for 10 min, and centrifuged, washed once with phosphate buffered saline (PBS), centrifuged again, decanted, and resuspended in 0.5 mL PBS. The sample was then ready for acquisition in a Cytomics FC500 cytometer (Beckman Coulter) equipped with an argon laser at 488 nm. The analysis was performed using the program incorporated in the

Table 1
Clinical characteristics of the 70 patients included in the study.

	All patients, n (%)
Age, years (median, range)	53 (18–78)
Sex (male/female)	45/25
Diagnosis	
Acute myeloid leukemia	19 (27)
Acute lymphoblastic leukemia	8 (12)
Myelodysplastic syndromes	7 (10)
Non-Hodgkin's lymphoma	23 (33)
Hodgkin's lymphoma	5 (7)
Multiple myeloma	3 (4)
Severe aplastic anemia	2 (3)
Chronic myelomonocytic leukemia	3 (4)
Hematopoietic stem-cell transplantation	36
Autologous	10 (28)
Matched related donor	9 (25)
Matched unrelated donor	10 (28)
Haploidentical related donor	1 (2)
Unrelated cord blood	6 (17)
Conditioning regimen, including total body irradiation	
Yes	6 (17)
No	30 (83)
Lines of treatment	
0–1	48 (69)
≥2	22 (31)
Disease status	
Complete remission	39 (56)
Stable disease	20 (28)
Progression or resistance	7 (10)
Newly diagnosed (induction treatment)	4 (6)
Systemic immunosuppression	
Yes	33 (47)
No	37 (53)
Neutrophil count ($\times 10^9$/L), median (range) (n = 62)	2.1 (0–12.8)
Neutropenia (<0.5 $\times 10^9$/L)	16/62 (26)
Lymphocyte count ($\times 10^9$/L), median (range) (n = 66)	0.5 (0–6.7)
Platelet count ($\times 10^9$/L), median (range) (n = 69)	40 (3–1489)
Oxygen saturation, %, median (range) (n = 66)	93.5 (77–100)
Serum creatinine (mg/dl), median (range) (n = 70)	0.97 (0.21–4.3)
Serum bilirubin (mg/dl), median (range) (n = 68)	0.9 (0.26–14)