Bronchoalveolar Lavage Cellular Patterns in Monoclonal Antibody-Induced Lung Disease

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Key Words

Monoclonal antibody · Interstitial lung disease · Bronchoalveolar lavage

Abstract

Background: The increase in the use of monoclonal antibodies (mAB) as a targeted therapy for a variety of diseases has been accompanied by an increase in reports of interstitial lung abnormalities in treated patients. **Objective:** Bronchoalveolar lavage (BAL) is routinely performed in these patients to rule out infection, so we sought to determine the BAL cellular pattern in individuals with mAB-induced lung disease (mAB-ILD). **Methods:** We utilized a case-control study design. Among patients treated with mAB, cases were defined as those with otherwise-unexplained interstitial lung abnormalities, which resolved after cessation of treatment, while controls were defined as those with interstitial abnormalities clearly explained by other etiologies. **Results:** From 2000 to 2012, we identified 9 cases and 7 controls. The mean age of the cases was 62.6 ± 26 years and 6 were female. The most common radiographic finding was diffuse ground-glass opacities. The most common BAL cellular pattern was mixed inflammation with moderate lymphocytic and mild neutrophilic alveolitis. The cases had a higher

mean lymphocyte count than the controls (40.1 ± 32.6 vs. 13.1 ± 25.5 , $p = 0.008$). The rest of the BAL cellular analyses were similar between the 2 groups. The median CD4:CD8 ratio in 7 patients with $>15\%$ lymphocytes was 0.9 (0.6–3). There was no significant difference in the CD4:CD8 ratio between the 2 groups. **Conclusions:** Mixed inflammation with moderate lymphocytic and mild neutrophilic alveolitis is the most common BAL cellular pattern in patients with mAB-ILD. Such findings may be useful for the early identification of mAB-ILD.

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Introduction

Monoclonal antibodies (mAB) directed against various components of the immune system have gained in importance in the treatment of autoimmune disorders and neoplasms in recent years [1]. With the increasing use of mAB, there have been more case reports and case series describing adverse effects which range from as-

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Table 1. Patient characteristics in the mAB-ILD group

No.	Age/sex	Race	Smoker	mAB agent	Underlying disease	Presenting complaints	CT findings
1	82/M	Caucasian	No	cetuximab	metastatic colon cancer	dyspnea, fever	patchy GGOs, predominantly in upper lobes
2	53/F	Caucasian	Quit	infliximab	inflammatory bowel disease	dyspnea, fever, cough	diffuse GGOs, worse at bases
3	56/F	Caucasian	No	rituximab	non-Hodgkin lymphoma	dyspnea, fever, cough	diffuse GGOs
4	68/F	Caucasian	No	rituximab	non-Hodgkin lymphoma	dyspnea	minimal bilateral GGOs
5	66/F	Caucasian	No	bevacizumab	metastatic breast cancer	dyspnea	diffuse GGOs and consolidation
6	35/F	Caucasian	No	trastuzumab	invasive breast cancer	dyspnea	diffuse GGOs
7	67/M	Caucasian	No	rituximab	chronic lymphocytic leukemia	cough	diffuse GGOs
8	66/M	Hispanic	No	rituximab	non-Hodgkin lymphoma	dyspnea, hemoptysis	diffuse GGOs
9	71/M	Afro-American	Quit	rituximab	Factor VIII deficiency	dyspnea	diffuse GGOs

ymptomatic increases in autoantibody titers [2] to life-threatening systemic complications.

The first case of mAB-induced lung disease (mAB-ILD) was reported in 2003 with rituximab [3]. Since then, over 150 cases have been reported in the literature. In 2006, a multicenter project called BIOGEAS was created to collect data on the use of mAB in adult patients with systemic autoimmune diseases, and to study their adverse effects. Data on 118 patients with mAB-ILD-related side effects were reported. The most common mAB agents implicated were rituximab and infliximab [1].

Bronchoalveolar lavage (BAL) is widely used in the evaluation of interstitial lung disease (ILD), and recent guidelines from the American Thoracic Society highlighted the utility of BAL cellular patterns in the diagnostic evaluation of this disease [4]. BAL has been used in the evaluation of patients with suspected drug-induced lung disease, as it helps to support clinical-pathological patterns and exclude other causes such as infections or neoplasms [5]. However, among patients with mAB-ILD, the description of BAL abnormalities has been limited to case reports and small case series, with no systematic evaluation having been performed.

In this study, we describe the BAL cellular profiles in patients with mAB-ILD, and compare them to patients with lung disease due to other etiologies. We also describe the demographic characteristics of patients with mAB-ILD.

Materials and Methods

Study Design and Data Collection

A case-control study was conducted on all patients receiving mAB therapy for autoimmune or neoplastic diseases who developed pulmonary symptoms associated with chest radiograph abnormalities and underwent bronchoscopy with BAL. To be included in the study, a portion of the BAL had to be sent to the clinical

bronchoalveolar lavage laboratory at the Indiana University. The rest of the BAL was sent to the microbiology laboratory at Indiana University Health for bacterial, fungal and viral studies. We included patients from the Methodist, Wishard and Indiana University Hospitals from January 2000 to December 2012. Patients who were not on mAB or did not have complete records were excluded. BAL was processed as previously described [6]. Cell counts and differentials were determined. CD4 and CD8 T cell subsets were quantified using flow cytometry in BAL samples with >15% lymphocytes [6]. The study was approved by the Indiana University Institutional Review Board.

Definition of mAB-ILD

For patients presenting with any radiographic pattern of ILD, the definition of mAB-ILD for the purpose of our study was according to the diagnosis of mAB-ILD made by the treating pulmonologist. The diagnosis was made after review of clinical data and lung imaging, and after excluding causes such as infections, neoplasms, hemorrhage and adult respiratory distress syndrome. Patients on mAB with radiographic abnormalities clearly explained by other etiologies were used as controls.

Statistical Analysis

Nonparametric analysis was used. Results were expressed as median and interquartile range (IQR). The continuous variables were analyzed by means of the χ^2 test. For noncontinuous variables, the Mann-Whitney rank sum test was conducted. Statistical significance was defined as a p value <0.05. Analysis was performed using SPSS software, version 9.

Results

A total of 38 patient records were reviewed. Sixteen patients were excluded because they were not on mAB and another 6 due to the unavailability of complete medical records. A total of 9 cases and 7 controls were identified. Of the 7 controls, 4 had pneumonia and 1 patient each had lymphangitic spread of an underlying breast cancer, graft-versus-host disease and sarcoidosis.

Baseline Characteristics

In patients with mAB-ILD, the mean age was 62.6 (± 13) years. Six (66.7%) were female and 7 (77.8%) were Caucasian. None of the patients were active smokers. The most common mAB used was rituximab in 5 (55.5%) patients. Cetuximab, infliximab, bevacizumab and trastuzumab were used in 1 patient each. The most common indication for use was malignancy ($n = 7$; 77.8%). The baseline characteristics of these patients are shown in table 1. With regard to age, sex, race, smoking status and the mAB used, patients were similar to controls (table 2).

Clinical Presentation and Management

The most common presenting symptom in the cases was dyspnea in 8 (88.9%) patients. Cough and fever were present in 3 (33.3%) patients. The most common radiographic finding was diffuse ground-glass opacities (GGOs; table 1). Chest computed tomography and bronchoscopy with cellular and microbiologic analysis of the BAL fluid were obtained for all patients in both groups. Lung biopsies were obtained for 3 patients with mAB-ILD (transbronchial biopsy in 2 and open-lung biopsy in 1) and 4 controls (transbronchial biopsies in 2, transbronchial and open-lung biopsy in 1 and open-lung biopsy and autopsy in 1). In the mAB-ILD group, mAB was discontinued in all patients. All patients received corticosteroids, though variable doses and regimens were used. With the above management, all patients in the mAB group experienced a clinical and radiographic improvement. One of the patients was rechallenged and redeveloped similar symptoms. Discontinuation of mAB and corticosteroids resulted in complete recovery.

BAL Cellular Analysis

The most common BAL cellular pattern in patients with mAB-ILD was mixed inflammation with moderate lymphocytic and mild neutrophilic alveolitis. The median cell counts in the mAB group were 38% macrophages (IQR 30–46), 42% lymphocytes (IQR 40–52), 17% neutrophils (IQR 14–22) and 1% eosinophils (IQR 0–4). The median CD4:CD8 ratio in 7 patients with >15% lymphocytes was 0.9 (IQR 0.6–3). The median lymphocyte count in BAL was higher in the patients with mAB-ILD than in controls (42 vs. 10%, $p = 0.007$; fig. 1). The rest of the BAL cellular analysis in both the groups was not significantly different. There was no significant difference in the CD4:CD8 ratio between the groups (table 3).

Table 2. Patient characteristics

	mAB-ILD (n = 9)	Other etiologies (n = 7)	p value
Median age, years (IQR)	66 (56–68)	55 (47–60)	0.06
Females, n	6 (67%)	4 (57%)	0.7
Caucasians, n	7 (78%)	6 (86%)	0.7
Nonsmokers, n	7 (78%)	6 (86%)	0.7
On rituximab, n	5 (56%)	2 (29%)	0.3
On other mAB, n	4 (44%)	5 (71%)

Fig. 1. Percentage of lymphocytes in BAL in mAB-ILD patients and in patients with lung disease due to other etiologies.

Discussion

To our knowledge, this is the first study describing systematic BAL cellular analysis in patients with suspected mAB-ILD. Our study reveals that patients with mAB-ILD present with a mixed moderate lymphocytic and mild neutrophilic alveolitis. Compared to patients on mAB with other lung diseases, patients with mAB-ILD had a significantly higher percentage of lymphocytes in their BAL. We did not find any difference in CD4:CD8 ratio between the 2 groups.

In the evaluation of patients with ILD including suspected drug-induced lung disease, the utility of BAL has been well established. BAL cellular patterns are useful for narrowing the differential diagnosis in the appropriate clinical setting [5]. In patients with suspected mAB-

Table 3. BAL cellular analysis

	mAB-ILD (n = 9)		Other etiologies (n = 7)		p value
	median	IQR	median	IQR	
Cell count, %					
Macrophages	38	30–46	48	13–86	0.5
Lymphocytes	42	40–52	10	3–20	0.007
Neutrophils	17	14–22	10	1–39	0.8
Eosinophils	1	0–4	1	0–20	0.6
CD4	47	37–73	25	9–76	0.3
CD8	53	24–60	61	19–92	0.4
CD4:CD8 ratio	0.9	0.6–3	0.4	0.1–4	0.3
Cells/ml	9.2×10^5	9.9×10^4 to 1×10^7	1.2×10^6	1.6×10^5 to 6.8×10^6	0.8

ILD, previous case reports and case series have described abnormalities in the BAL fluid. Perez-Alvarez et al. [7] analyzed all case reports and case series of patients with suspected mAB-ILD due to anti-TNF agents for autoimmune rheumatologic diseases. In 122 reports, BAL findings were available for only 12 patients. Six of the cases had lymphocytic predominance while 4 had macrophage predominance. Ramos-Casals et al. [1] published a similar study. BAL findings were reported for only 10 cases, and these showed predominantly lymphocytic alveolitis. Liote et al. [8] published a systematic review on 45 patients with rituximab-induced lung disease. BAL cellular analysis was available for 9 patients, and was consistent with lymphocytosis (13–90%) with a predominance of CD4 lymphocytes. Arulkumaran et al. [9] described mixed neutrophilic and lymphocytic alveolitis in a case report of rituximab-induced lung disease. Sen et al. [10] reported a case of infliximab-induced interstitial pneumonitis, and they found that BAL showed CD8 lymphocyte predominance with a CD4:CD8 ratio of 0.74. Most other case reports have BAL findings of either lymphocytic or mixed inflammation [11–13]. However, all of these reports described the BAL findings only in patients with suspected mAB-ILD. A direct comparison with BAL findings in patients on mAB with lung disease due to other causes was not performed.

Our study shows that patients with mAB-ILD have an increase in the percentage of lymphocytes in the BAL fluid. The CD4:CD8 ratio in our subjects was consistent with those reported in other case series and case reports with a median CD4:CD8 ratio of 0.9.

The presence of lymphocytic alveolitis in patients with mAB-ILD suggests the presence of a pneumonitis-type, hypersensitivity response [4, 14]. Many drugs, including

hydroxyurea, imatinib and lenalidomide, are known to cause lung disease with a hypersensitivity reaction [15–18]. Consistent with this kind of reaction, all of our patients improved with discontinuation of the offending agent and administration of systemic corticosteroids. The nature of the antigenic trigger of the hypersensitivity response in these subjects is not known. Chimeric antibodies are usually made up of a murine Fab fragment which binds antigen on the target cell and a human Fc fragment which binds human immune cells for clearance of the targeted cell [19]. Either one or both components could potentially trigger the immune reaction. Detailed studies of the antigen specificity of the responding T cells are necessary to identify the triggering component of the hypersensitivity response.

Our study had certain limitations. Due to the rarity of the disease, we had a relatively small and heterogeneous group of patients. In addition, our control group consisted of patients with a variety of underlying lung diseases that could potentially affect the BAL cellular analysis. The comparison is still clinically meaningful as these diagnoses are often considered when working up patients with interstitial lung diseases. Further studies need to be conducted to establish what the ‘normal’ BAL cellular differential is in patients on mAB without any pulmonary disease.

Conclusions

Our study demonstrates that, in patients treated with mAB, mixed inflammation with moderate lymphocytic and mild neutrophilic alveolitis is the most common BAL cellular pattern in those who develop mAB-ILD.

The presence of lymphocytic alveolitis with a low-to-normal CD4:CD8 ratio in patients with GGOs on chest computed tomography and a good response to corticosteroids is highly suggestive that this ILD has a pneumo-

nitic-type hypersensitivity reaction. In these patients, discontinuation of the mAB, consideration of systemic corticosteroids and careful clinical follow-up can be helpful.

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