INTRODUCTION

Opportunistic infections of the lungs frequently occur in the immunosuppressed individuals and are a major cause of morbidity and mortality. Infections reported worldwide in such patients include Pneumocystis pneumonia, cytomegalovirus (CMV) pneumonia, tuberculosis, cryptococcosis, Aspergillosis and Candidiasis. Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) fluid examination and is reported to be the favoured diagnostic...
procedure to aid in the rapid and accurate diagnosis of these infections. Although many reports have been published in the Western literature demonstrating the utility of BAL in diagnosis of Pneumocystis pneumonia, relatively fewer studies are available from India. One of the reasons suggested is that BAL fluid examination is not available in most centres in India. The present study highlights the role of BAL fluid examination in the detection of PCP, along with the clinical spectrum and morphological features of Pneumocystis pneumonia from a tertiary care centre where BAL fluid examination is routinely carried out in all patients with interstitial lung disease or suspected lung infection.

MATERIAL AND METHODS

Over a five-and-a-half year period from January 1998 to June 2003, 13 case records of patients in whom a diagnosis of PCP was made from BAL fluid examination at the Cytopathology Laboratory, All India Institute of Medical Sciences, New Delhi, were retrospectively studied. Cytospin preparations had been made from BAL fluid and stained with Papanicolaou (n=13) and May Grunwald Giemsa Stain (n=3). Silver methanamine (SM) stained smears and transbronchial lung biopsy (TBLB) specimens were available in 11 and 4 cases, respectively. Clinical details of these cases were obtained from the records. Cytology smears were reviewed, and diagnostic criteria propounded by Strigle et al were used for diagnosing Pneumocystis pneumonia. These criteria include presence of three-dimensional configuration of exudative masses with a coarsely granular, “foamy or bubbly honeycombed” appearance; shadowed outline of the cyst walls within the masses with occasional minute intracystic bodies (sporozoites); and the size and shape of the exudative masses looking like distended alveolar sacs (alveolar “cysts”). Numbers of such foamy alveolar casts (FACs) were graded from + to +++; and the type of inflammatory cells accompanying identified. Correlation between BAL fluid examination and TBLB findings was also studied.

RESULTS

Clinical Features
Mean age of the patients was 41.2 years (range 25-57 years); all of them were males. One patient had human immunodeficiency virus (HIV) infection, while there were 10 renal transplant recipients on immunosuppressive therapy. In remaining two cases HIV status was not known, and both were receiving antituberculosis treatment. Common presenting symptoms were fever (11/13), cough (10/13) and shortness of breath (8/13) (Table). Chest radiograph had shown parenchymal infiltrates in 11 cases and a radiological diagnosis of Pneumocystis pneumonia was rendered in only one case (Table).

BAL Findings
Foamy alveolar casts (FACs) were the characteristic feature and were seen in all cases although their numbers were variable. Number of FAC’s were 1-2 (+) in four cases, numerous (+++) in two cases and in moderate numbers (++) in rest of the cases (Table). Papanicolaou stain showed FACs in all the cases while May-Grunwald-Giems (MGG) staining (n=3) showed the presence of these casts in two of the three cases. The casts had a foamy to bubbly appearance due to the lack of the staining of cyst (Figure 1). Both the sporozoites and trophozoites stained faint blue appearing as dot like structure in the centre of these foamy areas.

Figure 1. Photomicrograph showing foamy alveolar casts along with alveolar macrophages in a bronchoalveolar lavage specimen (Papanicolaou × 400).
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Symptom Duration (days)</th>
<th>Cough</th>
<th>Expectoration</th>
<th>Fever</th>
<th>Dyspnoea</th>
<th>Chest Radiograph/CT-scan of the Chest</th>
<th>BAL Findings</th>
<th>FAC Density</th>
<th>Inflammatory Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>Male</td>
<td>30</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>B/L basal haziness</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>Male</td>
<td>10</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>B/L lung infiltrates</td>
<td>+++</td>
<td>P++</td>
<td>E ±</td>
</tr>
<tr>
<td>3</td>
<td>57*</td>
<td>Male</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Right lower lobe non-resolving pneumonia</td>
<td>++</td>
<td>P+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>43*</td>
<td>Male</td>
<td>07</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>? PCP</td>
<td>++</td>
<td>L+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>45*</td>
<td>Male</td>
<td>07</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Right sided diffuse haziness</td>
<td>++</td>
<td>P++</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>53*</td>
<td>Male</td>
<td>04</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>++</td>
<td>P++</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40*</td>
<td>Male</td>
<td>07</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>B/L middle and lower zone haziness, more on the right side</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>26*</td>
<td>Male</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Opacity in the upper segment of the lower lobe on the right side</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36*</td>
<td>Male</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>B/L middle lobe infiltrates</td>
<td>++</td>
<td>P+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>32*</td>
<td>Male</td>
<td>07</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B/L perihilar shadows</td>
<td>++</td>
<td>P+</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>54*</td>
<td>Male</td>
<td>07</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>B/L lower zone haziness</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>35*</td>
<td>Male</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>B/L lower zone infiltrates</td>
<td>++</td>
<td>P++</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>25†</td>
<td>Male</td>
<td>28</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Peribronchoarterial alveolar opacities</td>
<td>+++</td>
<td>L+</td>
<td></td>
</tr>
</tbody>
</table>

Patients 1, 2, 9 and 13 underwent transbronchial lung biopsy

* = renal transplant recipient; † = human immunodeficiency virus positive

+ = present; - = absent; B/L = bilateral; P = polymorphs; L = lymphocytes; E = eosinophils; FAC = foamy alveolar casts; BAL = bronchoalveolar lavage
These features helped to distinguish casts from mucous plugs or artifacts.

Silver methanamine stain showed impregnation of the cyst wall and enhanced the rounded, helmet or cleft forms of intracystic bodies (sporozoites), and thus, confirmed the diagnosis in all 11 cases (Figure 2), where SM was available.

![Figure 2. Photomicrograph showing impregnation of cyst wall and the intracystic bodies (Silver methanamine × 400).](image)

Polymorphs were the predominant constituent of inflammatory cell infiltrate (7/13 cases) whereas lymphocytes (2/13 cases) and eosinophils (1/13 cases) were less commonly seen (Table). Co-infection with CMV was observed in one case (Case No. 3).

Bronchial Biopsy

Transbronchial lung biopsies were available in four cases (Table). However, all the four cases showed non-specific features with the absence of organisms. This was due to the fact that the biopsies obtained were very tiny and it was difficult to detect the organisms on routine stains alone. Although interstitial polymorphonuclear and mononuclear infiltrates were observed, intact alveoli were lacking, in the biopsies. Special stains could not be performed due to inadequate tissue in the biopsies.

DISCUSSION

Though life threatening, Pneumocystis pneumonia is a treatable infection and therefore, a rapid and accurate diagnosis is mandatory. Clinically these patients present with respiratory symptoms and/or signs suggestive of pulmonary infection like cough, dyspnoea, fever or an abnormal chest radiograph. These signs and symptoms may also be observed in other opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS) or other immunocompromised states. Pneumocystis pneumonia was diagnosed to be the cause of respiratory symptoms in 40.5% (79/195), 46.9% (420/894) and 86% (3642) cases of AIDS in some of the published series. Selwyn et al studied the clinical predictors of Pneumocystis pneumonia thereby distinguishing it from other opportunistic infections and observed that exertional dyspnoea and interstitial infiltrates in lung have a specificity of 92 percent. We observed the combination of these two features in only around 50% of our cases.

Boiselle et al had reinforced that the diagnosis of Pneumocystis pneumonia can be correctly made on the chest radiograph in 75% of cases. However, in the present study only one case could be diagnosed correctly as Pneumocystis pneumonia on the chest radiograph.

Various methods including evaluation of sputum, tracheal aspirate, endobronchial brush biopsy and percutaneous needle aspiration have been employed to identify P. jiroveci. Currently, BAL fluid examination is the procedure of choice. The excellent sensitivity of BAL for diagnosis of Pneumocystis pneumonia may be related to the extensive bilateral pulmonary involvement in immunocompromised hosts.

In the present study, majority of the cases were renal transplant recipients who were on immunosuppressive therapy. In contrast, Sternberg et al observed CMV to be the commonest infectious cause of pneumonitis (8/48) in renal transplant recipients, whereas Pneumocystis pneumonia was found only in three patients and that too as a co-existent infection with CMV. Similarly Huerlin et al found CMV as the predominant cause of...
infectious pneumonia in post renal transplant patients. Menon et al. did not observe a single case of *Pneumocystis* pneumonia in 16 post-renal transplant cases and 14 patients on dialysis. Cytomegalovirus was diagnosed in two of the 16 post transplant cases. However, they observed *Pneumocystis* pneumonia in four of the eight HIV positive cases. Findings of the present study differ from reported cases as CMV was observed in only one of the 10 post transplant cases. Our laboratory had studied BAL from 15 renal transplant patients with fever and pulmonary infiltrates previously (1996-1997) and did not observe a single case of CMV or PCP (unpublished observations).

Despite a high prevalence of HIV/AIDS cases in India, *Pneumocystis* pneumonia remains less common here. This could be because BAL is not routinely done in most of the centres in India. Therefore, the published literature from India with regards to *Pneumocystis* pneumonia is relatively limited. Singh et al. reported the first few cases of *Pneumocystis* pneumonia in AIDS patients from India. The clinical and radiological profile of *Pneumocystis* pneumonia simulating tuberculosis has been reported by Arora et al. Mathew et al. reported *Pneumocystis* pneumonia in 5/15 (33%) AIDS patients and have stressed upon the utilisation of sensitive diagnostic tests. A case of combined tuberculosis and *Pneumocystis* pneumonia presenting as a cavitary lesion has been reported by Jindal et al. in a post-renal transplant patient. *Pneumocystis* pneumonia was demonstrated in 9/32 AIDS patients using induced sputum samples and indirect immunofluorescence technique by Usha et al. Menon et al. have emphasised upon the greater sensitivity of cytological examination of BAL in diagnosing *Pneumocystis* pneumonia over culture and biopsy. The latter found four cases of *Pneumocystis* pneumonia out of 38 BAL specimens from immunosuppressed hosts. Bijur et al. studied BAL and TBLB specimens in five HIV patients and demonstrated *Pneumocystis* pneumonia in three cases. They attribute the lower proportion of *Pneumocystis* pneumonia infection as opposed to other opportunistic infections in developing countries to the lack of use of sensitive diagnostic methods like BAL cytology. Deshmukh et al. have reported five cases of *Pneumocystis* pneumonia among 34 autopsies in patients with AIDS and have emphasised the occurrence of other co-existing opportunistic infections, like Cryptococcosis, tuberculosis, etc. Lanjewar et al. observed *Pneumocystis* pneumonia in seven out of 143 adult lung specimens from HIV-positive patients at autopsy, while Santosh et al. observed one case of *Pneumocystis* pneumonia in autopsy/biopsy series of 10 HIV-positive patients.

The standard method of establishing the diagnosis of *Pneumocystis* pneumonia remains tinctorial staining which can be divided into two groups. The first includes cyst wall stains such as toluidine blue and SM and the other category includes MGG and Papanicolaou techniques, which stain the intracystic sporozoites as well as trophozoites. Both the types of stains were used in the present study for the demonstration of the organisms.

Few authors have tried to observe the clinical significance of cellular infiltrates with prognosis in immunocompromised patients with *Pneumocystis* pneumonia and concluded that accumulation of polymorphs in BAL is associated with more severe respiratory compromise. Unfortunately, follow up is not available in the present cases and we cannot comment upon significance of cell type associated with *Pneumocystis* pneumonia. Sternberg et al. observed that the percentage of polymorphs in the lavage fluid was significantly lower in post renal transplant recipients with *Pneumocystis* pneumonia as compared to other infections. Results of the present study do not support this finding.

Although BAL cytology has been traditionally used for evaluation of interstitial lung diseases, it also serves as an effective and easy technique to diagnose *Pneumocystis* pneumonia. A careful cytological examination for foamy alveolar casts along with the use of special stains confirms the diagnosis. In this regard, BAL fluid specimen which may also be termed as a “liquid biopsy”, is considerably superior over a tissue biopsy as abundant material is
available for performing special stains. Furthermore, the complications associated with TBLB such as pneumothorax, bleeding, can be avoided. A cytopathologist should always be vigilant towards the presence of *Pneumocystis* infection besides other opportunistic infections in all immunosuppressed patients. Characteristic cytomorphological features on routine and special stains offers a sensitive tool for diagnosing this pathogen. Studies employing BAL cytology in immunosuppressed patients with a large sample size are required define the epidemiology of *Pneumocystis* pneumonia in India.

REFERENCES


FOR AUTHORS

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