Approaches to the diagnosis of viral pneumonias in the immunocompromised host: the importance of assaying cytopathogenic viral effects in bronchoalveolar lavage cells


ABSTRACT: Pneumonopathies are frequently found in immunocompromised patients (IH). Sixty-two pneumonopathic episodes in 53 IH patients were examined by BAL, for viral cytopathic effects (CPE) in isolated cells, with appropriate viral culture techniques. Viral culture was positive in 17 of the 27 episodes in renal allograft recipients and AIDS patients as against eight of the forty-four episodes in other causes of IH (p < 0.001). CPE was found thirteen times; in seven cases it was characteristic of cytomegalovirus. Positive viral culture and CPE were shown simultaneously during thirteen episodes in eleven patients. Ten patients died (autopsies performed in three cases confirmed viral presence). Positive viral culture with absence of CPE was observed in twelve cases. There were only four fatalities in this group (the autopsies performed in three cases did not establish the presence of a virus in the pulmonary parenchyma). The percentage of lymphocytes was high in both groups of patients (18.6 ± 2.8%). CPE is a simple and rapid examination for the diagnosis of viral pneumonopathology in the IH. Prognosis at present is gloomy; more complex examinations such as viral cultures and/or identification of the virus by immunofluorescence will be indicated only when effective antiviral agents become available.

Original version in French.

The causes of pathological lesions in the lung in the immunocompromised host (IH) are numerous and often interrelated [1–3].

Viruses are frequently involved [4]. Their presence is often regarded as serious but their true pathogenic effect remains to be defined. Given the progress of antiviral agents [5], it is possible to envisage effective treatment for viral pneumonias. It is therefore useful to make a rapid and accurate virological diagnosis of pneumonias in the IH. Bronchoalveolar lavage by endoscopy (BAL) is easy to perform, rapid and well-tolerated [6] and suitable for these patients.

The aim of this study was to compare the value of demonstrating a cytopathogenic viral effect (CPE) upon BAL cells, with a positive viral culture from the BAL fluid for the diagnosis of viral pneumonia in the IH.

Patients

The following were defined as immunocompromised hosts: a) patients with AIDS and b) patients treated with immunosuppressive drugs for malignant blood disorders, solid tumours, systemic illnesses after renal or bone marrow transplantation. Diagnosis of pneumonia was established by a radiological infiltrate, diffuse or otherwise, associated with hypoxaemia. None of the immunocompromised patients received any antiviral treatment.

Methods

The BAL was performed either at the site of the infiltrate if localized, or in the middle lobe with three or four aliquots of 50 ml of salted isotonic serum at room temperature.

After cytocentrifugation of the BAL fluid (2000 G x 10 min), three slides (about 20,000 cells per slide), were stained using the Harris-Schore technique for cytological examination, together with assessment of the cellular populations and a search for aspecific or specific viral CPEs of cytomegaloviruses (CMV) (fig. 1) [7]. A CPE was regarded as established when found on more than four occasions per slide. Further cytological analysis and search for micro-organisms was performed after staining with haematoxylin-
The cellular culture was incubated for sterile phosphate sorption observed by microscopy. Shown by haemadsorption in monkey kidney cells.

at ambient temperature and fixed for immuno-fluorescence examined daily for eosin, Gram, periodic acid-Schiff base, acetone in guinea-pig erythrocytes. The culture was rinsed with PBS; 0.5 ml PBS, 20 μl were placed on slides for IF, dried at ambient temperature and fixed for 20 min in acetone at -20°C.

CMV was identified by indirect IF using monoclonal murine antibodies (kindly provided by Prof. A. Boue, INSERM U73) and murine anti-IgG conjugated with fluorescein isothiocyanate (FITC) (Pasteur Institute).

Herpes simplex virus Type I (HSV I) was found by direct IF using specific monoclonal antibodies conjugated with FITC (Bio-Mérieux).

Chickenpox varicella zoster virus (VZV) was identified by indirect IF using human serum showing a high concentration of anti-VZV antibodies (negative for other viruses at the strength used), to which was added human anti-IgG conjugated with FITC. Respiratory syncytial virus (RSV), myxoviruses, paramyxoviruses and adenoviruses were sought using indirect IF with polyclonal chicken or bovine antibodies and the corresponding conjugated anti-IgGs (Wellcome).

**Statistical analysis**

Comparisons of quantitative data were made by a Student's t-test or Wilcoxon test. Qualitative data were analysed by Chi-squared test or exact Fischer test. The level of significance was fixed at 5%.

**Results**

Between January, 1985 and March, 1986, 62 pneumopathological episodes were studied in 53 IHs: 26 males and 27 females, aged 18-80 yrs, (average 45 yrs). There were five cases of AIDS, 27 of malignant blood disorders, ten of solid tumour, two of systemic disease (disseminated lupus erythematosus, Churg-Strauss's disease), six renal allografts, and three bone marrow transplantations. There was no incident or accident in any phase of endoscopy or BAL.

Viral cultures were positive in 25 episodes (40.3%) with 26 viruses being isolated: fourteen CMV, four HSV I, four RSV, four parainfluenzae-3 (PIV 3) and one HSV I-RSV association. Associated or isolated positive viral culture was found in seventeen of the eighteen episodes in transplantation or AIDS patients and in eight of the forty-four episodes occurring in other patients (p < 0.001). In eight episodes (12.9%), including four with blood disorders, positive viral culture was the only aetiology found. In BAL cells CPE was found in thirteen cases and characteristic of CMV in seven of these. Direct IF on BAL cells was performed in all transplantation and AIDS patients (n = 18). It proved positive in three cases (3 AIDS) and was confirmed by culture (two CMV and one PIV 3).

Three groups of pneumonopathic episodes were distinguished. Group I (n = 13) (table 1) was characterized by the presence of CPE and positive viral culture. In group II (n = 12) (table 2) there was only positive viral culture (without CPE). Group III showed neither CPE nor positive viral culture.

BAL lymphocytosis was higher with positive viral culture (group I and II) (18.6% SEM 2.8 vs 11.4% SEM 2.2; p < 0.05) with no difference between groups I and II. Cell counts for alveolar macrophages and neutrophil polynucleocytes were the same for all three
Table 1. - Evolution of patients in group I

<table>
<thead>
<tr>
<th>n</th>
<th>Sex</th>
<th>Age</th>
<th>Initial pathology</th>
<th>Virus isolated</th>
<th>Associated pathology</th>
<th>Outcome</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>50</td>
<td>Renal tr.</td>
<td>CMV</td>
<td>Kaposi</td>
<td>DCD</td>
<td>CMV</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>39</td>
<td>Renal tr.</td>
<td>CMV</td>
<td>H. influenzae</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>41</td>
<td>Bone marrow tr.</td>
<td>CMV</td>
<td>P. pyocyanea</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>41</td>
<td>Bone marrow tr.</td>
<td>CMV</td>
<td>P. pyocyanea</td>
<td>DCD</td>
<td>NP</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>37</td>
<td>Bone marrow tr.</td>
<td>CMV</td>
<td></td>
<td>DCD</td>
<td>NP</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>34</td>
<td>AIDS</td>
<td>CMV</td>
<td>Pneumocystis</td>
<td>DCD</td>
<td>NP</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>50</td>
<td>AIDS</td>
<td>CMV</td>
<td></td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>7b</td>
<td>M</td>
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<td>AIDS</td>
<td>CMV</td>
<td>Staphylococcus</td>
<td>DCD</td>
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</tr>
<tr>
<td>8</td>
<td>M</td>
<td>33</td>
<td>AIDS</td>
<td>CMV</td>
<td>Pneumocystis</td>
<td>DCD</td>
<td>NP</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>67</td>
<td>AL</td>
<td>RSV</td>
<td></td>
<td>DCD</td>
<td>NP</td>
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<tr>
<td>10</td>
<td>F</td>
<td>60</td>
<td>AL</td>
<td>RSV</td>
<td></td>
<td>DCD</td>
<td>NP</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>71</td>
<td>AL</td>
<td>HSV I</td>
<td></td>
<td>DCD</td>
<td>NP</td>
</tr>
</tbody>
</table>

tr.: transplantation; AL: acute leukaemia; DCD: deceased; S: satisfactory; NP: not performed; CMV: cytomegalovirus; RSV: respiratory syncytial virus; HSV I: herpes simplex virus I.

Table 2. - Evolution of patients in group II

<table>
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<tr>
<th>n</th>
<th>Sex</th>
<th>Age</th>
<th>Initial pathology</th>
<th>Virus isolated</th>
<th>Associated pathology</th>
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<th>Autopsy</th>
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</thead>
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<td>S</td>
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<td>S</td>
<td>-</td>
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<td>M</td>
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<td>-</td>
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<tr>
<td>4</td>
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<td>39</td>
<td>Renal tr.</td>
<td>HSV I</td>
<td>H.I.P</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
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<td>M</td>
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<td>P. carinii</td>
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<tr>
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<td>27</td>
<td>AIDS</td>
<td>P13</td>
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<td>DCD</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>10</td>
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</tbody>
</table>

tr.: transplantation; NHL: non-Hodgkin's lymphoma; AL: acute leukaemia; DCD: deceased; S: satisfactory; NP: not performed; S: satisfactory; HSV I: Herpes simplex virus; RSV: respiratory syncytial virus; CMV: cytomegalovirus; PIV 3: para-influenza virus 3.

groups. In group I, ten episodes were fatal, three progressed satisfactorily. Only one of these three cases did not suffer relapse, the two others showed new viral episodes (positive viral culture and CPE) which proved fatal. The three autopsies performed on this group confirmed the diagnosis of viral pneumonia [8] (table 1).

In group II, eight episodes showed satisfactory progress and four were fatal. Autopsies were performed on three (two AIDS, one malignant melanoma) which confirmed the initial diagnoses (pneumocystis and klebsiella-associated pneumonia, cerebral toxoplasmosis and pleuro-parenchymatous localization of the melanoma respectively) and excluded viral pathology. The fourth of these patients (Churg-Strauss's disease) showed Pseudomonas pyocyanea septicemia (table 2).

In group III, the diagnoses were as follows: fourteen bacterial respiratory infections, fourteen neoplastic infiltrations, eight intra-pulmonary haemorrhages and one mycosis. In thirteen cases no aetiological diagnosis could be made. Fifteen patients died, six autopsies were performed which excluded viral pneumonia (six neoplastic infiltrations).

Groups I and II show significant differences in the development of pneumonopathic episodes (ten deaths out of thirteen episodes against four deaths out of twelve; p < 0.001) and in the progress of IHs (ten deaths out of eleven patients against four deaths in ten; p < 0.001).

There were significant differences in the rate of development of pathology between groups I and III (pneumonopathic episodes and IHs) (p < 0.02). There
were no significant differences in progress between groups II and III.

Discussion

Analysis of BAL fluid for diagnosis of pulmonary pathology in the IH is important because it is very effective, well-tolerated, and suitable for repeated use. Surgical biopsy, the method of reference, no longer seems the first option [3, 9, 10]. In our series, tolerance of endoscopy and BAL was satisfactory. Analysis of BAL fluid allowed aetiological diagnosis in 68% of cases [11].

Group II raises the question of the significance of an isolated positive viral culture [2]. The absence of CPE may be explained by the small number of infected cells. The progress of episodes in group II is mostly satisfactory (four deaths in ten patients) with no antiviral treatment and is no different from that of group III. Isolated positive viral culture does not appear to indicate unfavourable prognosis.

Group I (pneumonopathic episodes with positive CPE and viral culture) were mostly AIDS, renal or bone marrow transplantation patients [2, 12, 13]. Progress is almost always unfavourable (ten deaths in eleven patients) and significantly different from that of groups II and III. Hence CPE always indicates an unfavourable prognosis.

Presence of CPE showing intracelullar viral multiplication appears, therefore, in this group of IHs, to demonstrate that the virus is responsible for the respiratory disorder. However, an isolated viral culture showing persistence of the virus (or viraemia) due to the intensity of immunosuppression does not necessarily indicate a diagnosis of viral pneumonia [14].

Although statistically insignificant, results from autopsies seem to support this hypothesis. Thus, in group I, all three autopsies confirmed the diagnosis of viral pneumonia, in two cases Kaposi's sarcoma and pneumocystis pneumonia. Due to pathological associations it is impossible to know precisely to what extent the virus is responsible for the severity of clinical and radiological symptomatology.

There is good correlation between CPE in BAL cells and positive viral culture (p<0.001) especially since the CPE is characteristic of CMV (p<0.00002). Presence of CPE therefore renders viral culture unnecessary. We accept that only the presence of CPE shows that the virus causes pathology. There was no difference in alveolar lymphocytosis between groups I and II. It does not therefore appear to indicate a diagnosis of viral lung pathology especially since it is higher in pathologies causing immunosuppression [15] or possible associated pathologies [16, 17].

In conclusion, presence of CPE on cytological analysis of BAL seems to be a necessary and sufficient condition for diagnosis of viral pneumonopathy in the IH. Possible viral identification will then be made by culture. Direct IF seems a sensitive method for the diagnosis of viral pneumonopathy, since the intensity of IF correlates with the number of cells showing CPE [18]. However, direct IF remains an onerous process and cytological analysis of BAL, which is easily available in all centres, seems to us more appropriate for the diagnosis of viral pneumonopathy in the IH.

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SAMENSTELLING: Inhalatiorcapules 200 μg en 400 μg salbutamol als sulfaat. INDERZIES: Besluiten van astma bronchiale, chronische bronchitis en emfyseem. CONTRA-INDERZIES: Belonende overgevoeligheid. WAARSCHUWINGEN EN VOORZORGEN. Voorzichtig bij hypertensie, myocard insufficiëntie en thyroideos. BIJWERKINGEN: Geringe stijging der polsfrequentie en tremor. VERPAKKINGSVORM: Ventolin Rotacaps a 200 en 400 microgram worden in verpakkingen van 120 stuks geleverd.


RÉSUMÉ: Les pneumopathies chez l’immunodéprimé (IH) sont fréquentes et souvent graves. Un diagnostic rapide est essentiel et s’obtient grâce au lavage broncho-alvéolaire (BAL). Soixante-deux épisodes de pneumopathies, chez cinquante-trois malades immunodéprimés, ont été examinés par BAL avec les techniques de culture virale appropriées afin de déceler un éventuel effet viral cytopathogénique (CPE) dans les cellules isolées. La culture virale était positive dans dix-sept épisodes sur dix-huit dans les cas de greffes du rein et de SIDA par rapport à huit épisodes sur quarante-quatre pour les autres causes d’immunodéficience (<0.001). Un CPE a été décelé treize fois; dans sept des cas, il était caractéristique du cytomegalovirus. La culture virale positive et le CPE ont été constatés simultanément au cours de treize épisodes chez onze patients. Dix d’entre eux sont morts (des autopsies faites dans trois des cas ont confirmé une présence virale). La culture virale positive avec absence de CPE a été constatée dans douze cas. Il n’y avait que quatre décès dans ce groupe (les autopsies faites dans trois cas n’ont pas établi la présence d’un virus dans le parenchyme pulmonaire). Le pourcentage de lymphocytes était élevé dans les deux groupes de malades (18,6 ± 2,8%).