Pulmonary toxicity of chemotherapy and G/GM-CSF: a report of five cases

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Introduction
Granulocyte and granulocyte-macrophage colony stimulating factors (G-CSF and GM-CSF) are haematopoietic growth factors. They stimulate haematopoietic progenitor cells to proliferate and differentiate into granulocytes and macrophages and are used prophylactically during the period of bone-marrow suppression after chemotherapy (1).

Anecdotal reports have suggested an increased risk of pneumonitis in patients treated with cytotoxic chemotherapy and G/GM-CSF (2–7). We report our experience in five patients with pneumonitis related to administration of cytotoxic drugs and G/GM-CSF and review the earlier literature, including our preliminary report (8).

Methods
BRONCHOALVEOLAR LAVAGE
Bronchoalveolar lavage was performed under local anaesthesia. Four aliquots of 50 ml sterile saline solution were instilled. The fluid was recovered by gentle aspiration into sterile siliconized jars. T-lymphocyte subsets were counted by indirect immunofluorescence using monoclonal antibodies (OKT3, OKT4 and OKT8; Ortho Diagnostics, Raritan, New Jersey, U.S.A.).

CHEST RADIOLOGICAL IMAGING
High resolution chest computed tomography (HRCT) scans were performed on an Elscint model (Elite+ CT scan: Elscint, Haifa, Israel).

PATIENTS
We performed a retrospective study of five cases observed in the Department of Pulmonary Medicine of Foch Hospital over 3 years. During this period, 57 patients with lymphoma received a comparable chemotherapy regimen in the Division of Haematology of Necker Hospital, and 21 in the Department of Haematology/Oncology of Foch Hospital.

Case Reports
All five patients (three men, two women) were non-smokers. The median age was 43 years (range 33–70). All patients had aggressive non-Hodgkin’s lymphoma (four diffuse large B cell, and one with diffuse large T cell). Three patients had mediastinal adenopathy, and one (patient 4) had histologically demonstrated lymphomatous pulmonary involvement. All were seronegative for Human Immuno-deficiency Virus and Human T-cell Leukaemia Virus type I. The Karnofsky performance status was 90% for four patients and 80% in one case (patient no. 2). All had an unremarkable pulmonary history and normal echocardiograms. The chemotherapy protocol was identical in all patients. Each 3-weekly course consisted of doxorubicin 75 mg m⁻² (day 1), cyclophosphamide 1200 mg m⁻² (day 1), vindesine 2 mg m⁻² (day 1 and day 5), bleomycin sulfate 10 mg daily (day 1 and day 5), methylprednisolone 60 mg m⁻² (day 1 to day 5) and intrathecal methotrexate 15 mg (day 1). In addition each course was followed prophylactically by a dose of non-glycosylated G-CSF 5 µg kg⁻¹ day⁻¹ s.c (patients 1, 3 and 5) or GM-CSF 300 µg day⁻¹ s.c. (patients 2 and 4) from day 6 to day 13.

After the second (patients 1 and 4), third (patient 3), and the fourth (patients 2 and 5) cycles of chemotherapy, the course was marked by the abrupt onset (within 24–48 h) of very rapidly increasing dyspnoea and fever above 39°C. These symptoms occurred on average 10 days (range 8–16 days) after beginning the chemotherapy cycle. No patient developed clinical fluid retention. Chest radiograph and thoracic CT scan in all five patients showed bilateral diffuse interstitial infiltrates without pleural effusion. HRCT scan identified interstitial pneumonitis in two cases (patients 3 and 5). White-blood-cell count was normal (4700–10,000 mm⁻³) as was the platelet count (mean = 250 × 10³ mm⁻³). Initial arterial blood gases on room air
showed hypoxaemia (mean $P_aO_2$, 60 mmHg; range 41–76), and hypcapnia (mean $P_aCO_2$, 33 mmHg; range 25–47). All five patients underwent fiberoptic bronchoscopic examination. No endobronchial abnormalities were observed. Specific stains and cultures for bacteria, mycobacteria, fungi, cytomegalovirus, adenovirus, and respiratory syncytial virus were negative. The presence of human herpesvirus 6 was not studied. The characteristics of cells recovered by bronchoalveolar lavage are summarized in Table 1. Four patients had a lymphocytic alveolitis with a lymphocyte proportion of more than 30% of the total alveolar cells. Alveolar lymphocytes were morphologically normal. Pneumonitis occurred, while the non-Hodgkin's lymphoma responded well to treatment, in all five cases, four patients showed complete response and one patient (no. 3) showed only partial response.

Despite corticosteroid therapy with high dose methylprednisolone (240 mg day$^{-1}$), two patients died 12 and 15 days after onset of clinical signs. Post-mortem lung examination of patients 1 and 4 showed diffuse and extensive interstitial pulmonary fibrosis with lymphocytic interstitial infiltration and diffuse alveolar damage. No vasculitis, tumour infiltration or pathogens were found. Moreover, no residual lymphomatous involvement was found in other organs. Patient 2 remained stable with oral corticosteroids. In patient 3, pneumonitis followed a favourable course without corticosteroids, and the patient was able to receive two courses of aracytine without haematopoietic growth factor. Patient 5 improved dramatically within 2 days of prednisone treatment (60 mg day$^{-1}$).

**Discussion**

In these five patients treated with the same chemotherapy regimen for non-Hodgkin's lymphoma, we observed the development of acute febrile pneumonitis. The characteristics and course of the pneumonopathy, and the role of the cytotoxic drugs call for comment.

The five patients had febrile interstitial pneumonitis, characterized by abrupt onset within less than 48 h, lymphocytic alveolitis in four of the five cases with an increased number of CD8$^+$ cells in the three patients studied, occurrence after two to four cycles of chemotherapy, and a highly unfavourable course as two out of the five patients died of respiratory insufficiency.

A relationship between G/GM-CSF administration and the occurrence of pneumonitis has been previously suggested. Studies using G-CSF show conflicting results: eight cases of drug-induced pneumonia have been reported in 40 patients with lymphoma who received G-CSF vs. nine in 35 patients treated with a similar chemotherapy regimen without G-CSF (6). A retrospective study reported four cases of drug-related pneumonitis in 12 patients treated with chemotherapy and G-CSF for lymphoma vs. only one out of 24 patients treated with a similar chemotherapy regimen but without G-CSF (7). Matthews (3) reported pulmonary toxicity in four out of five patients with Hodgkin's disease treated with ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) and G-CSF. However, no difference in pulmonary toxicity has been detected between two groups of patients within non-Hodgkin's lymphoma treated with G-CSF, (nine cases of pulmonary toxicity out of 139 patients) or with placebo, (seven of 139 patients) (9) and in patients with advanced-stage germ cell tumours receiving bleomycin either with (10 pneumonitis cases out of 29 patients) or without (10 of 37 patients) G-CSF (10).

Cytotoxic drugs may contribute to the development of pneumonitis. Bleomycin has been the drug most frequently implicated as a cause of pneumopathy. Bleomycin has a high rate of pulmonary complication in patients with non-Hodgkin's lymphoma. Bleomycin-associated pneumonitis is mainly chronic pulmonary fibrosis occurring over a critical dose, approximately 450–500 mg (11). The cumulative dose of bleomycin in our patients and in reported patients with Hodgkin's disease and CSF-related pneumonitis was lower, <100 mg (3). However acute bleomycin-induced hypersensitivity pneumonitis after doses as low as 50 mg has been described (11). Most patients with acute pulmonary damage associated with bleomycin responded favourably to corticosteroids (11). Our five cases differ from this acute pulmonary-associated bleomycin toxicity because two of our patients and nine of 27 reported patients suffering from CSF-related pneumonitis died of respiratory failure. It is notable that none of our patients received thoracic radiation or suffered renal function impairment, which are known risk factors for increasing bleomycin pulmonary toxicity (11).

Methotrexate may also be incriminated because it is known to cause interstitial pneumonitis, which is independent of both dose and route of administration (11). Analysis of bronchoalveolar lavage in methotrexate-associated pneumonitis found predominantly CD8$^+$ lymphocytes, a result also found in our cases. However, peripheral blood eosinophilia is present in 40% of the cases, and acute methotrexate pneumonitis usually has a dramatic response to corticosteroid therapy, although a fulminant course has been documented in some patients. Another possibility is a reaction to cyclophosphamide. As a rule, a prolonged course of high dose cyclophosphamide precedes the development of pneumonitis, but cases of interstitial pneumonia have been reported after only a few courses of combination chemotherapy (11). To our knowledge, when not combined with mitomycin, vindesine has not been previously indicated in pneumonitis. The same is true for doxorubicin when not in combination with radiotherapy. Thus, we believe that a causal relationship of these two cytotoxic drugs is unlikely in lung disease.

The pathogenesis of CSF-related pneumonitis is unknown. Several hypothesis may be evoked.

1. Animal models have shown that haemopoietic growth factors promote synthesis of free radicals, which may contribute to fibrosis. Subcutaneous administration of GM-CSF to rats produced fibroblast proliferation with acquired a-smooth muscle actin expression (12).
2. An immune mechanism independent from a direct CSF effect may be possible because G/GM-CSF-related
### TABLE 1. Bronchoalveolar lavage findings in five patients with G/GM-CSF-associated pneumonitis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chemotherapy regimen (n cures)</th>
<th>CSF/delay† (days)</th>
<th>Total cells (x 10^3 ml⁻¹)</th>
<th>Alveolar macrophages (%)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
<th>CD4⁺⁺ (%)</th>
<th>CD8⁺⁺ (%)</th>
<th>CD4:CD8 ratio</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>G-CSF/16</td>
<td>436</td>
<td>51</td>
<td>48</td>
<td>0</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Dead</td>
</tr>
<tr>
<td>2</td>
<td>GM-CSF/14</td>
<td>740</td>
<td>80</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>35</td>
<td>65</td>
<td>0.55</td>
<td>Alive</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>G-CSF/13</td>
<td>543</td>
<td>12</td>
<td>81</td>
<td>7</td>
<td>0</td>
<td>30</td>
<td>55</td>
<td>0.45</td>
<td>Alive</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>GM-CSF/14</td>
<td>288</td>
<td>13</td>
<td>68</td>
<td>12</td>
<td>7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Dead</td>
</tr>
<tr>
<td>5</td>
<td>G-CSF/8</td>
<td>200</td>
<td>32</td>
<td>38</td>
<td>29</td>
<td>1</td>
<td>44</td>
<td>58</td>
<td>0.75</td>
<td>Alive</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*Percentage of alveolar lymphocytes; †time from the initiation of G/GM-CSF. ND = not determined.
pneumonitis occurred at a point when the steroid components of the regimen are being withdrawn. The cytokine network disregulation induced by CSF may be implicated in the local immunopathogenesis of interstitial pneumonitis. The release of various cytokines by CSF may increase the production of TGF-β, which has an important role in the pathophysiology of bleomycin-induced lung fibrosis (13). The increased number of alveolar CD8+ lymphocytes contrasts with the neutrophilic alveolitis related to high levels of IL-8 after administration of GM-CSF (14). These findings are similar to the influx of cells into the lungs in hypersensitivity pneumonitis: early neutrophil alveolitis is observed soon after antigen challenge, followed by an increase of lymphocytes; mainly the CD8+ subsets (15).

3. No patient developed fluid retention, suggesting pulmonary oedema as reported in a few cases of acute respiratory distress syndrome occurring with G-CSF treatment of drug-induced agranulocytosis (16).

In conclusion, the prophylactic use of G/GM-CSF following cytotoxic chemotherapy may be associated with the development of acute interstitial pneumonitis in patients with lymphoma. The CSF may activate the known pulmonary toxicity of these cytotoxic drugs. The occurrence of haematopoietic growth-factor-associated pneumonitis seems unpredictable. These uncommon adverse events highlight the importance of post-marketing surveillance studies and surveys for new drug products.

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References