Vol. **97** (2003) 736–738
doi:10.1053/rmed.2003.1489, available online at http://www.sciencedirect.com

Detection of HCV-RNA in bronchoalveolar lavage from a woman with pulmonary fibrosis ☆

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INTRODUCTION

Hepatitis C virus (HCV) produces a chronic stimulation to the immune system because of its marked lymphotropism and it has been suggested to be an etiological agent of idiopathic pulmonary fibrosis (IPF) (1). We report, for the first time, the detection of HCV RNA in the BAL of a patient affected by pulmonary fibrosis.

CASE REPORT

An 81-year-old woman, non-smoker, complaining of a 5-year history of exertional dyspnea and a moderate dry cough, was admitted to our respiratory unit for worsening clinical status and in particular for dyspnea at rest, widespread muscle weakness, morning stiffness and pain in the large joints. She had been a waitress and then housewife, she has one sister affected by chronic HCV hepatitis. She never suffered from bronchopulmonary diseases and she did not receive fibrosis inducing therapy.

Physical examination

Poor nutritional status. The skin examination revealed purpuric lesions and peripheral edema on the legs. The large joints (hands, wrists, knees) were swollen, hot and painful, but no gross joint deformities were evident, except for a modest radial deviation at the wrist. Neither finger clubbing nor subcutaneous nodules were noted. Thoracic auscultation revealed crackles at both lung bases.

The blood tests showed a peripheral leukocytosis (white blood cells/mmc=18.5×10^3) and increased levels of the acute phase reactants.

A type II mixed cryoglobulinemia (Cryocrit 1%, IgM-delta binding polyclonal IgG) with hypocomplementemia (C4=18 mg%) and positive Waaler-Rose (WR) assay was detected. All the liver functional parameters were in the normal range and the autoimmune panel (ANA, ENA, ANCA) was negative.

The chest X-ray and the high-resolution computed tomography demonstrated a bilateral and peripheral thickening of bronchovasal interstitium more evident in upper and lower lobes of the right lung (reticular pattern) with subpleural honeycombing (Fig. 1). The hands X-ray did not show cartilage erosion.

The spirometry pointed out a worse restrictive pattern: VC (L) =1.18 (69.4%); RV (L)=0 .78 (65.4%); FEV_1(L)= 1.12 (100%); FEV_1/VC: 95%. Blood gas analysis (breathing room air): pO_2=63.2 mmHg; pCO_2=34.2 mmHg; pH=7.43.

The 6-min walking test showed a reduced walking distance (234 m −57%); BORG max. 5; $SatO_2$ min.: 87% after the test.

The patient underwent a bronchoscopy (Pentax EB-1830T2) with bronchoalveolar lavage in the middle lobe. The bronchoscope was introduced by mouth, avoiding suction. No endobronchial lesions were detected. The procedure did not provoke bleeding of bronchial mucosa.

The recovered fluid (45 ml) was clear. Cytologic analysis of BAL showed a lymphocytic-neutrophilic alveolitis (cells/ml: 37×10^4; macrophages: 56.3%; lymphocytes: 23%; neutrophils: 19.2%; eosinophils: 0.7%) (Fig. 2). The study of the lymphocyte subsets (Facscan, Becton Dickinson) showed a high $T_{helper}/T_{suppressor}$ ratio (CD3+: 53.1%; CD3+CD4+: 36.1%; CD3+CD8+: 11.8%; CD4+/CD8+: 3.06, CD19+: 3.2%) and increased expression of activated phenotypes (CD3+HLADR+: 82%; CD3+CD25+: 7.3%). The patient had a subpopulation of double positive T cells (CD3+CD4+CD8+) in both the peripheral blood and the BAL fluid (respectively, 6.2 and 3.2%). The BAL culture was negative.

Virological findings

The following virological analysis were performed: HCV antibodies determination by enzyme immuno assay (EIA), qualitative HCV RNA analysis by reverse transcription polymerase chain reaction (RT-PCR) (2),

Received 20 August 2002, accepted in revised form 8 November 2002
☆ For the IRCCS Policlinico San Matteo this work was partially supported by Ricerca Finalizzata 2000 (820/RFM/00/01) and Ricerca Corrente 1998 (Grant 80221)
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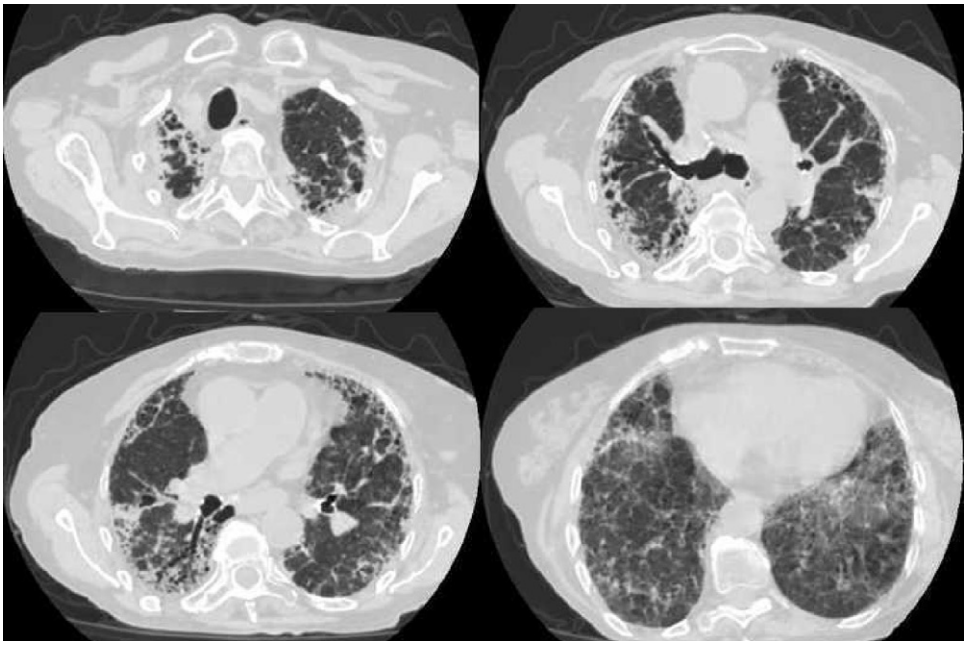

Fig. 1. HRCT showing diffuse reticular fibrosis with peripheral honeycombing.

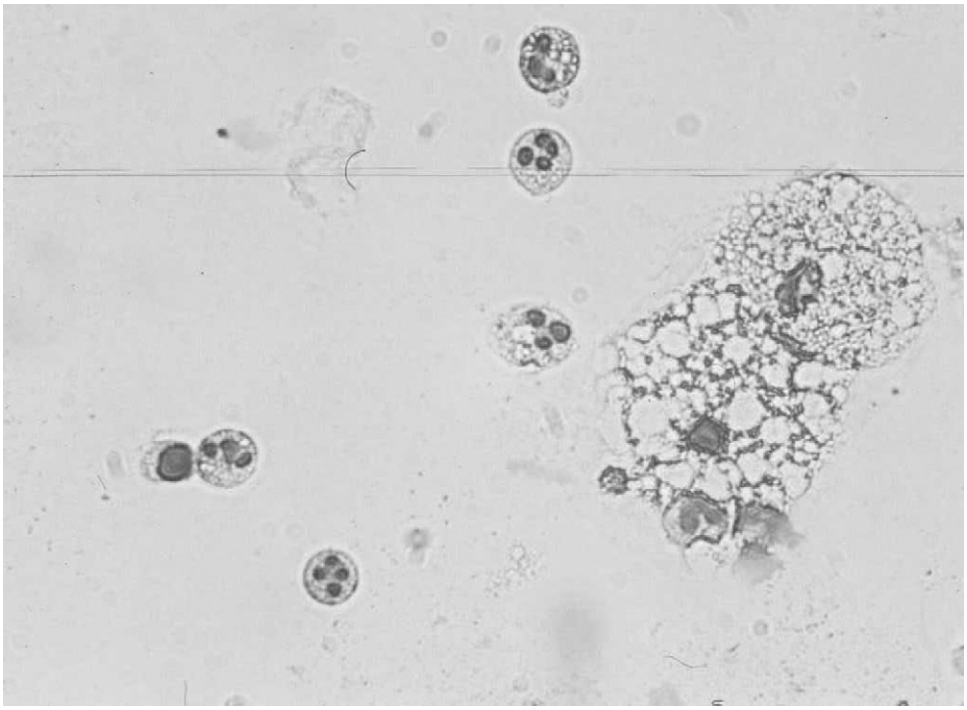

Fig. 2. Neutrophilic alveolitis in the BAL from the presented case. Magnification 40 ×.

quantitative HCV RNA analysis by b-DNA assay (Quantiplex Versant 3.0 , Bayer, Eraigny, France), HCV genotyping by HCV II Innolipa (Bayer, Eraigny, France).

Serum, bronchoalveolar lavage and saliva of the patient were analyzed. Serum anti-HCV antibodies were detected and HCV genotype was 2a/c.

For HCV RNA qualitative analysis serum, native unconcentrated BAL and saliva were submitted to nucleic acid extraction by a commercial procedure (NucliSens Isokit, bioMérieux, France). A 5 μl volume of the extracted RNA was used for RT-PCR, as described (3).

For quantitative analysis 50 μl volume of serum and BAL were used for b-DNA assay, as reccomended by manufacturers. The viral load was expressed in International unit (IU)/ml by this quantitative test. In particular 1 IU corresponds to 5.2 copies of HCV viral genome.

HCV RNA was found in serum and BAL, but not in saliva, by RT-PCR. Quantitative analysis was performed on positive samples, only. In particular, HCV RNA load in serum was 3 061 600 IU/ml, whereas it was 6980 IU/ml in BAL, by b-DNA.

Treatment and clinical evolution

The patient started treatment with prednisone 25 mg/day and hydroxychloroquine 400 mg/day for 1 month and 200 mg/day for the next 2 months. Re-evaluation of the clinical picture after 3 months of treatment showed worsening dyspnea, increased lung fibrosis assessed by HRCT and a further decrease in lung volumes. In contrast, joint pain had improved, and the cryocrit and Waaler-Rose tests had become negative.

DISCUSSION

This is the first documented case of isolation of HCV genome in the BAL of a patient with pulmonary fibrosis, HCV infection and type II cryoglobulinemia. The main limitation of microbiological assays of BAL is contamination of the fluid by oropharyngeal secretions or blood. We, therefore, considered that it was essential to exclude the presence of HCV in the saliva, which we did by RT-PCR, and confirm that there were no traces of blood in the cytological preparations of the BAL. In the past Ferri *et al*. (2) isolated the genome of HCV in lung biopsy specimens of a patient with desquamative interstitial pneumonia, but in that case it was not possible to exclude contamination of the tissues by blood during the biopsy procedure, with consequent carriage of the virus present in the blood.

The clinical data, the HRCT changes and the cytological profile of the BAL suggest a diagnosis of "idiopathic" pulmonary fibrosis associated with a non-erosive arthritis with a low titer positive rheumatoid factor, which is commonly observed in cases of HCV-related cryoglobulinemia (4).

A pathogenic relationship between IPF and HCV infection has been supported by data from published studies reporting that there is a higher frequency of anti-HCV antibodies in patients with idiopathic pulmonary fibrosis (IPF) than in control populations (1–5), the presence of a subclinical alveolitis in patients with HCV infection without signs of lung damage (6–8) and anecdotal reports of an association between HCV, cryoglobulinemia and various forms of interstitial lung diseases (9,10). The hypothesis of a correlation between IPF and HCV infection seems to be further strengthened by the isolation, for the first time in a patient with lung fibrosis, of the viral genome in the BAL by RT-PCR. In a recent paper, Idilman et al. reported the detection of HCV in the BAL of a patient with chronic hepatitis C and subclinical alveolitis but without clinical or radiological findings of interstitial lung disease (ILD) (8). Interestingly, our patient also had a subpopulation of double positive T cells in both the peripheral blood and in the BALF. This population, characterized by a CD4+CD8+ phenotype, has been previously described in both healthy individuals and in subjects infected by EBV or other viruses (11).

There are various possible origins of viral RNA in BAL fluid: contamination by saliva or blood (both these possibilities were excluded in our case), passive leakage from the blood to the alveoli, transport via infected mononuclear cells or active replication in the alveolar microenvironment. The effect of steroid therapy should also be considered as a possible factor facilitating isolation of the virus, as hypothesized in a study by Kuwano et al. (12)

In conclusion, although it is difficult to establish whether the concomitant presence of IPF and HCV infection is coincidental (both increase with age) or the manifestation of a pathogenic link, the data from this single case report seem to be consistent with the latter hypothesis.

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