Incidence of hepatitis C virus infection in Italian patients with idiopathic pulmonary fibrosis

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Abstract

Background – A viral cause of idiopathic pulmonary fibrosis (IPF) was recently suggested by a Japanese study in which a high prevalence of anti-hepatitis C virus (HCV) antibodies was detected. A subsequent British study failed to confirm these results.

Methods – Antibodies to HCV were evaluated in 60 patients with IPF, 130 patients with non-interstitial lung disease, and in 4614 blood donors. HCV-RNA and HCV genotypes were evaluated in the anti-HCV positive patients with IPF. Anti-HCV antibodies were evaluated by ELISA and confirmed by recombinant immunoblotting assay (RIBA). HCV-RNA and genotypes were detected by reverse transcriptase polymerase chain reaction (PCR).

Results – Eight patients with IPF had anti-HCV antibodies detected by ELISA (13.3%). In the blood donor control group the prevalence of HCV antibodies was lower (0.3%). In patients with non-interstitial lung disease HCV antibody prevalence was 6.1%. In all eight patients with IPF found to be anti-HCV positive by ELISA, HCV antibodies were also detected by RIBA. Furthermore, all were HCV-RNA positive by PCR assay. HCV genotypes were identified in four of these eight patients. In all four genotype II was present and in two it was associated with genotype III and/or genotype IV. In the remaining four cases the genotype was not identified.

Conclusion – Italian patients with IPF show an increased prevalence (~13%) of HCV infection and viral replication, but the prevalence of anti-HCV antibodies does not differ from other lung diseases.

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Keywords: idiopathic pulmonary fibrosis, hepatitis C virus, genotypes.

Idiopathic pulmonary fibrosis (IPF) is a chronic disease of the lung interstitium. Two different histological patterns may be observed either simultaneously or consecutively – a desquamative or cellular pattern and an interstitial or fibrotic one.¹ In recent years major advances have been made in understanding the pathogenesis of the inflammatory and fibrotic mechanisms at work in IPF¹ but its aetiology remains unresolved. A viral trigger to the immunopathogenic mechanism has long been sought.

Recently Prieto *et al*² reported the efficacy of the inhaled antiviral agent ribavirin in a patient with IPF, but Agusti *et al* found no benefit with this therapy.³ Ueda *et al*⁴ found an increased prevalence of antibodies to hepatitis C virus (HCV) in Japanese patients with IPF, but Irving *et al*⁵ failed to confirm these findings in a British series of patients with IPF. We have performed a retrospective study on the prevalence of HCV infection in a series of Italian patients with IPF.

Methods

Serum samples were obtained from 60 patients with IPF (43 men) of mean age 61 years (range 30-82). The diagnosis was made on clinical, radiological, physiological, and histological grounds. The criteria used included: history of dyspnoea and cough, fine crackles on physical examination, compatible findings on the chest radiograph (diffuse basal reticulonodular shadowing), restrictive pattern of pulmonary function, and a reduced diffusing capacity. In addition, no associated connective tissue disease was present nor was there a history of occupational exposure or hypersensitivity. Histological confirmation was obtained in 70% of cases (transbronchial biopsy in 60% and open lung biopsy in 10%). In the remaining patients high resolution computed tomographic scanning of the lungs (HRCT) was performed.⁶

All but three patients had been seen consecutively at the Chest Department of our University Hospital during the last 10 years. Serum samples were obtained on first admission and stored at -70°C. Control subjects consisted of 4614 healthy blood donors who were screened for anti-HCV antibodies by the Blood Transfusion Service at our hospital during 1992. In addition, 130 patients (84 men) of mean age 63 years (range 21-85) consecutively admitted during 1994 for non-interstitial lung diseases (chronic obstructive lung disease 93, neuromuscular chest wall diseases 13, pulmonary vascular diseases 11, bronchiectasis 7, chronic pleural diseases 4, tuberculosis 2) were also screened for anti-HCV antibodies. The HCV results in these patients were collected by checking their clinical records.

Anti-HCV antibodies were detected by the second generation immunoassays ELISA (Ortho Diagnostic System, Raritan, New Jersey, USA) and RIBA (Chiron Corporation, Emeryville, California, USA). Viral replication was evaluated by reverse transcriptase (RT)/PCR using "nested" PCR primers following

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Clinical and virological data from the eight anti-HCV positive patients with idiopathic pulmonary fibrosis

Patients	Sex	Age (years)	Disease duration (months)	T	Abnormal liver function tests*	Anti-HCV ELISA (OD)	Anti-HCV RIBA				HCV-RNA	HCV genotype
							5.1.1.	c100.3	c33.c	c22.3		
1	M	57	10	Yes	No	>3.0	+	—	+	+++++	+	II, III, IV
2	M	61	10	No	Yes	>3.0	++++	++++	++++	+++++	+	Ind
3	M	80	NA	No	Yes	>3.0	+	+	++++	+++++	+	II, IV
4**	M	68	24	No	Yes	>3.0	++++	++++	++++	+++++	+	II
5	F	62	33	No	No	>3.0	+	+++	++++	+++++	+	II
6	F	64	27	Yes	No	>3.0	—	—	++++	+++++	+	Ind
7	M	50	18	Yes	Yes	>3.0	—	+++	++++	—	+	Ind
8	F	54	36	Yes	Yes	>3.0	—	—	++++	+++++	+	Ind

* At the time of virological investigations.

** Patient with biopsy proven chronic active hepatitis.

T=immunosuppressive treatment at the time of virological investigations; NA=not available; OD=optical density; Ind=indeterminate.

the method of Ravaggi and coworkers⁷ with a minor modification: before the PCR procedure serum samples were subjected to solid phase RNA extraction with Exdna Biomek 100 (Beckman Analytical, Milan, Italy).

HCV genotypes were evaluated using the method described by Okamoto *et al*⁸ for genotypes I, II, IV, V and the method described by Silini *et al*⁹ for genotype III.

Major risk factors for HCV infection included multiple transfusions and intravenous drug abuse. Minor risk factors were tattooing, working in health care institutions, living in institutions and surgical procedures.

The prevalence of anti-HCV antibodies in the IPF and control groups was compared using the χ^2 test.

Results

Eight of the 60 patients with IPF were positive for anti-HCV as detected by ELISA (13.3%), a significantly higher prevalence than that obtained in the blood donor group (14/4614, 0.3%, $p<0.0001$). Anti-HCV antibodies were found in eight of 130 patients (6.1%) with non-interstitial lung disease. No clustering of the anti-HCV positive patients in a single diagnostic group was observed. This prevalence is significantly higher than that of blood donors ($p<0.0001$) but is not significantly different from that of the patients with IPF. While no risk factor for HCV infection was present in patients with IPF, five of these eight anti-HCV positive patients had major risk factors (four had received multiple transfusions and one was an intravenous drug abuser) and two had a history of surgical treatment.

All eight anti-HCV positive patients with IPF were also positive for two or more antigens by the RIBA (table), and all had circulating HCV-RNA detected by RT/PCR.

HCV genotypes were identified in four patients. All were type II positive and two were also infected with genotypes III and IV. Genotypes were unidentified in the remaining four cases (table).

At the time of the viral investigations five of the eight patients had mild biochemical signs of liver disease which fluctuated both before and after the viral studies. Three other patients did not show elevated transaminase values either before or after HCV-RNA detection. Liver biopsy was performed in only one patient and showed chronic active hepatitis.

Discussion

The determination of anti-HCV antibodies in the serum samples of our Italian patients with IPF confirms the results obtained in Japan.³ Furthermore, all anti-HCV positive patients were also positive for HCV-RNA, indicating active HCV replication in 13.3% of patients with IPF. Irving *et al* did not find a significant increase in anti-HCV antibodies in their series and suggested either geographical differences in the aetiology of IPF or false positive tests in the Japanese study. Possible false positives were ascribed to the poor specificity of first generation screening ELISA for anti-HCV and the interference of high levels of IgG in patients with IPF. We used a second generation ELISA and the results obtained with RT/PCR stressed the specificity of our anti-HCV antibody tests. There is a discrepancy between our results and the negative British study although the two patient populations appeared to be clinically similar. Geographical difference may be a factor. Indeed, the prevalence of HCV infection is high in Japan and Mediterranean countries and low in Northern Europe. The prevalence of anti-HCV antibodies in our series was higher than in the blood donor control group, but these subjects do not represent the most appropriate control group as they were younger and had a selection bias. People who volunteer as blood donors are generally not at risk for hepatitis viruses; however, the prevalence of HCV has recently been documented in the general population of Northern Italy at 3.2% which is lower than that found in patients with IPF.¹⁰

The high prevalence of HCV infection found in patients with IPF is similar to that in the group with other lung diseases. It should be noted that we did not determine HCV-RNA in this group and, among the eight positive cases, five had major and two had minor risk factors for hepatitis C whereas none of the HCV positive patients with IPF had risk factors. A common feature in the two groups (IPF and other lung diseases) is a history of multiple hospital admissions which could be an additional risk factor for HCV infection.¹¹

The study of HCV genotypes provided three points. Firstly, in four of eight patients the genotype was not identified, perhaps due to as yet unclassified genotypes, but the prevalence of indeterminate genotypes is very low in patients who have not had multiple transfusions¹². Secondly, all four patients in whom

genotypes were identified had genotype II alone or in combination with other types, and the high frequency of type II genotype is in keeping with the prevalence in Italy.¹³ Thus, HCV infection in patients with IPF is not associated with specific genotypes. Thirdly, in two patients a combination of two and three genotypes was present, a rare association in a single patient and almost exclusively confined to cases who have received multiple transfusions.¹²

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Use of tracheal auscultation for the assessment of bronchial responsiveness in asthmatic children

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Abstract

Background – It can be difficult to assess bronchial responsiveness in children because of their inability to perform spirometric tests reliably. In bronchial challenges lung sounds could be used to detect the required 20% fall in the forced expiratory volume in one second (FEV₁). A study was undertaken to determine whether a change in lung sounds corresponded with a 20% fall in FEV₁ after methacholine challenge, and whether the occurrence of wheeze was the most important change.

Methods – Fifteen children with asthma (eight boys) of mean age 10.8 years (range 8–15) were studied. All had normal chest auscultation before the methacholine challenge test. Lung sounds were recorded over the trachea for one minute and stored on tape. They were analysed directly and also scored blindly from the tape recording by a second investigator. Wheeze, cough, increase in respiratory rate, and prolonged expiration were assessed.

Results – The total cumulative methacholine dose causing a fall in FEV₁ of 20% or more (PD₂₀) was detected in 12 children by a change in lung sounds – in four by wheeze and in eight by cough, increased

respiratory rate, and/or prolonged expiration. In two subjects altered lung sounds were detectable one dose step before PD₂₀ was reached. In three cases in whom no fall in FEV₁ occurred, no change in lung sounds could be detected at the highest methacholine dose.

Conclusion – Changes in lung sounds correspond well with a 20% fall in FEV₁ after methacholine challenge. Wheeze is an insensitive indicator for assessing bronchial responsiveness. Cough, increase in respiratory rate, and prolonged expiration occurs more frequently.

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Keywords: bronchial responsiveness, lung sounds, children.

Measurement of bronchial responsiveness to methacholine has been useful in the diagnosis, assessment of severity, and follow up of asthma, but some children are not able to perform spirometric tests reliably.¹ A method based on detecting audible wheeze over the trachea and requiring passive cooperation only has been described for bronchial challenge with methacholine in children.² In older children a close correlation has been observed between the 20% fall in forced expiratory volume in one second

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