HCV infection: pathogenesis, clinical manifestations and therapy

A. Antonelli[1], C. Ferri[2], M. Galeazzi[3], C. Giannitti[3], D. Manno[4], G. Mieli-Vergani[5], E. Menegatti[6], I. Olivieri[7], M. Puoti[4], C. Palazzi[8], D. Roccatello[6], D. Vergani[6], P. Sarzi-Puttini[9], F. Atzeni[9]

[1]Department of Internal Medicine, University of Pisa School of Medicine, Pisa, Italy; [2]Department of Internal Medicine, Rheumatology Unit, University of Modena & Reggio Emilia School of Medicine, Modena, Italy; [3]Rheumatology Section, Department of Clinical Medicine and Immunological Sciences, University of Siena, Italy; [4]Department of Infectious Diseases, University of Brescia, Italy; [5]Institute of Liver Studies, King's College London School of Medicine at King's College Hospital, London, UK; [6]Multidisciplinary Centre of Immunopathological Research and Documentation of Rare Diseases, S.G. Bosco Hospital, and Clinical Pathology Section, Department of Medicine and Experimental Oncology, University of Turin, Italy; [7]Rheumatology Department of Lucania, S. Carlo Hospital of Potenza and Madonna delle Grazie Hospital of Matera, Italy; [8]Division of Rheumatology, Villa Pini Clinic, Chieti, Italy; [9]Rheumatology Unit, L. Sacco Hospital, University of Milan, Italy.

Alessandro Antonelli, Clodoveo Ferri, Mauro Galeazzi, Chiara Giannitti, Daniela Manno, Giorgina Mieli-Vergani, Elisa Menegatti, Ignazio Olivieri, Massimo Puoti, C. Palazzi, Dario Roccatello, Diego Vergani, Piercarlo Sarzi-Puttini, Fabiola Atzeni.

Please address correspondence to: Fabiola Atzeni, MD, PhD, Rheumatology Unit, L. Sacco University Hospital, Via G.B. Grassi 74, 20157 Milano, Italy. E-mail: atzenifabiola@hotmail.com

Received and accepted on January 18, 2008.

Key words: Hepatitis C virus, mixed cryoglobulinemia, autoimmune endocrine diseases, chronic arthritis, rituximab.

Competing interests: none declared.

ABSTRACT

Chronic hepatitis C virus (HCV) infection is a worldwide public health problem with a global prevalence of 2-3%. It is believed that about 170 million people are currently infected (about 3% of the world's population), and a further 3-4 million are infected each year. HCV is the main reason for liver transplantation in the developed world, and the main cause of liver-related morbidity and mortality in a number of countries, including Italy. It is not only a frequent cause of chronic liver diseases such as hepatitis, cirrhosis and hepatocellular carcinoma, but is also involved in the pathogenesis of various autoimmune and rheumatic disorders (arthritis, vasculitis, sicca syndrome, porphyria cutanea tarda, lichen planus, nephropathies, thyroid diseases, and lung fibrosis), as well as in the development of B-cell lymphoproliferative diseases. Furthermore, patients suffering from C hepatitis tend to produce rheumatoid factor, cryoglobulins and a large series of autoantibodies (ANA, anti-SSA/SSB, SAM, ATG, aCL). The use of glucocorticoids or immunosuppressant agents in HCV infected individuals, which are needed to treat autoimmune and rheumatic disorders, leads to a risk of worsening the clinical outcome of HCV. Under these conditions, the viral infection often needs to be treated with antiviral agents, mainly pegylated interferon combined with ribavirin. However, cyclosporine A seems to be safe and effective in patients with autoimmune disease (AD) and concomitant chronic HCV infection as is documented by the reduction in viremia and transaminases, particularly in patients with high baseline levels. Finally, HCV is the main trigger of mixed cryoglobulinemia. An attempt at viral eradication is therefore indicated in most patients, and is particularly effective in the case of mild or moderate manifestations. In severe cases, rituximab is an apparently safe and effective alternative to conventional immunosuppression and, specifically, it controls B-cell proliferation.

HCV: virology and epidemiology

Hepatitis C virus (HCV) was first recognised as a cause of hepatitis in 1975, but proved to be hard to identify until advances in recombinant DNA technology finally enabled Choo *et al*. to identify it in 1989. HCV is a single-stranded RNA virus whose genome consists of an RNA strand of approximately 9,600 nucleotides, coding for 3011 amino acids, encased in a nucleocapsid (1).

It is believed that about 170 million people are infected with HCV (about 3% of the world's population), and a further 3-4 million are infected each year. HCV is the main reason for liver transplantation in the developed world, and the main cause of liver-related morbidity and mortality in a number of countries, including Italy (1).

The virus is mainly spread by direct contact with human blood, although it can be detected in other body fluids (1). The higher the viral load of the vector host, the greater the risk of infection, and so the low probability of infection via other body fluids may be due to their very low viral load. Approximately 90% of newly acquired HCV infections are associated with specific exposure. The at-risk groups in whom HCV screening is mandatory are intravenous drug users, the recipients of blood or blood products, the recipients of transplantations from unscreened donors, and the children of HCV RNA-positive mothers (1).

Six mayor genotypes (1-6) and about 30 subtypes have so far been identified:

genotypes 1b and 2 are the major genotypes in Europe and Northern America; genotype 3 is mainly encountered in South-east Asia and among intravenous drug users in Europe; and genotype 4 is primarily found in the Middle East, Egypt and sub-Saharan Africa. A number of clinical characteristics have been associated with specific genotypes, including sensitivity to antiviral treatment (2).

HCV: pathogenesis of viral persistence

HCV is not cytopathic, but the host's immune response in an attempt to fight the virus is the cause of liver damage due to long-lasting inflammation. Acute self-limiting HCV infection is associated with a strong and consistent multi-specific $CD4^+$ and $C8^+$ T cell responses against the epitopes of viral proteins, whereas the probable reasons for the persistence of HCV despite such responses are: 1) extensive mutations during HCV replication, leading to multiple viral species in a single patient; 2) mutations that prevent antigen presentation; 3) the inhibition of intracellular interferon (IFN) signalling; 4) the functional impairment of $CD8^+$ T lymphocyte responses; and 5) viral inhibition of host defences (3).

Natural history of hepatic HCV infection

Patients with acute hepatitis C are usually asymptomatic. Most studies have reported high (77-85%) rates of progression from acute to chronic hepatitis C, but the transition from acute disease to cirrhosis is usually symptom-free, and occurs over a period of 20-40 years in approxmately 5-25% of HCV infected patients. Hepatocellular carcinoma may develop in as many as 1-4% of patients with established cirrhosis per year. The main pathway of cirrhosis development is progressive liver fibrosis, the stage of which is the main prognostic factor in the natural history of chronic hepatitis C. Patients with persistently high aminotransferase levels and necro-inflammatory activity at liver biopsy are likely to develop more fibrosis. The progression of fibrosis is faster in males and in Afro-Americans, as well as in subjects consuming large amounts of alcohol, diabetics, and those with pathological lipid metabolism, HIV and HBV co-infections or other co-factors (4).

Extra-hepatic manifestations of HCV infection

About 40-74% of HCV patients develop at least one extra-hepatic manifestation (EHM) and, as many patients do not show any hepatic symptoms of chronic HCV infection, EHMs may be the first signs of the disease. B-cell lymphoproliferative diseases (LPDs), of which the prototype is mixed cryoglobulinemia (MC), are the best-known and most closely related EHMs of HCV (see list in Table I) (5).

HCV and lymphoma

Approximately 8-10% of the patients with type II MC develop non-Hodgkin lymphoma (NHL) over many years of infection, and more than 90% of patients developing NHL suffer from type II MC. The risk of developing lymphoma is about 35 times higher in MC patients than in the general population. Comparisons of European and North American cohorts are unequivocal, although there is a clear south-north gradient of prevalence (reflecting the prevalence of HCV infections), which suggests the existence of further environmental and/or genetic factors (6).

HCV and diabetes

Insulin resistance is the main characteristic of the metabolic syndrome, and depends on insulin secretion and insulin sensitivity. Type 2 diabetes mellitus and insulin resistance is more frequent in HCV patients than in the general population or in patients with other liver diseases. HCV infection promotes insulin resistance by increasing the production of TNF-α and *suppressor of cytokine* (SOC-3), a process that also involves Th1 cells, interleukin (IL)-6 and INF-γ. Insulin resistance can be provoked in mice as a result of TNF-α production induced by HCV core protein.

Insulin resistance is associated with increased steatosis and fibrosis, depending on the HCV genotype (3). In patients with genotype 1, the process from insulin resistance to increased fibrosis is mediated by steatosis, hyperleptinemia, increased TNF-α production, and impaired PPAR-γ receptor expression; at the same time, HCV directly causes liver fat deposition.

Insulin resistance in HCV patients leads to lower response rates to INF therapy, and improves in patients in whom HCV has been eliminated by antiviral therapy (7).

HCV and thyroid diseases

Although thyroid disease (usually hypothyroidism) is more frequent in HCV patients than in the general population, the exact connection between HCV infection and thyroid disease is not clear. About 13% of HCV patients show signs of hypothyroidism, and thyroid antibodies can be detected in up to 25%.

Thyroid dysfunction can be induced by INF therapy or unmask an autoimmune

Table I. Extra-hepatic manifestations of hepatitis C.

Association defined on the basis of pathogenesis and high prevalence
- Mixed cryoglobulinemia

Associations defined on the basis of higher prevalence than in controls
- Non-Hodgkin lymphoma.
- Monoclonal gammopathy
- Porphyria cutanea tarda
- Lichen planus.

Associations to be confirmed
- Autoimmune thyroiditis
- Thyroid cancer
- HCV-associated neuropsychological symptoms
- Pseudo-Sjögren and sicca syndrome.
- Idiopathic pulmonary fibrosis
- Diabetes mellitus
- Non-cryoglobulinemic nephropathy
- Atherosclerosis of the aorta

Anecdotal observations
- Psoriasis
- Peripheral/central neuropathy
- Chronic polyarthritis
- Rheumatoid arthritis
- Polyarthritis nodosa
- Behçet's syndrome
- Polymyositis/dermatomyositis
- Fibromyalgia
- Chronic urticaria
- Chronic pruritus
- Kaposi's pseudo-sarcoma
- Vitiligo
- Cardiomyopathies
- Mooren's ulcer
- Erectile dysfunction
- Necrolytic acral erythema

disease (Graves' disease, Hashimoto thyroiditis). INF may lead to the production of thyroid antibodies and, consequently, hypo- or hyperthyroidism, although hypothyroidism seems to be more frequent (62%); monitoring thyroid parameters is therefore essential during INF therapy. About 50% of the patients who develop hypothyroidism while receiving antiviral agents recover once the therapy has been discontinued. Some studies have also reported a high prevalence of papillary thyroid cancer (8).

The role of molecular mimicry in autoimmunity

The relationship between hepatropic viruses and autoimmunity is well known. Ever since the discovery of its causative agent in 1989, HCV infection has been linked to a variety of immunopathological manifestations, including cryoglobulinaemia, autoantibody production and B cell lymphoma, which are due to the presence on B lymphocytes of the main HCV receptor CD81 (9), a tetraspanin whose natural ligand is the envelope 2 (E2) protein of HCV (10). The B lymphocyte-specific proteins CD21 and CD19 are closely associated with CD81 (10): the first is the receptor for the complement C3d fragment, and the second transduces activation signals into the cell. The engagement of HCV (possibly coated by C3d) with CD81 lowers the B cell threshold for polyclonal activation, which leads to the production of autoantibodies and cryoglobulins; however, other factors must also be involved because a large proportion of patients with autoantibodies do not have cryoglobulins.

A different mechanism involving intracellular B lymphocyte infection by HCV seems to underlie HCV-associated monoclonal gammopathy and non-Hodgkin B-cell lymphoma.

Non-organ-specific autoantibodies have been consistently described during the course of chronic HCV infection, with a prevalence as high as 70% (11). The most frequently encountered is smooth muscle antibody (SMA), followed by anti-nuclear antibody (ANA), and, in a minority of cases, anti-liver kidney microsomal type 1 (anti-LKM1). Unlike in autoimmune hepatitis (AIH) type 1, SMA in HCV infection does not conform to the immunofluorescence 'actin' pattern, and the ANAs are usually not homogenous, wheraes the immunofluorescence pattern of anti-LKM1 is indistinguishable from that of AIH type 2. The molecular targets of ANA and SMA are unknown, but that of anti-LKM1 has been identified as cytochrome P4502D6 (CYP2D6).

The presence of autoantibodies in infected individuals is associated with evidence of liver damage. During the screening of an unselected population of 7,000 subjects, 226 were found to be positive for HCV markers, of whom 25% had non-organ-specific autoantibodies, a much higher prevalence than that observed in 226 demographically matched uninfected individuals or in the 78 subjects in the same study group who were positive for hepatitis B surface antigen (HBsAg) (12). The presence of autoantibodies in the HCV positive cohort was significantly associated with clinical and biochemical evidence of liver disease, thus suggesting a connection between the two.

Furthermore, there have been a number of reports of adverse reactions to INF treatment in autoantibody-positive HCV patients (13, 14), although the autoantibody concerned was invariably anti-LKM1.

Up to 10% of patients with chronic HCV infection show the presence of anti-LKM1, which seems to increase the severity of the disease process (13). Interestingly, the HCV patients with anti-LKM1 tend to have HLA *DRB1*0701*, the allele predisposing to AIH type 2.

One possible mechanism by which CYP2D6 becomes a target of anti-LKM1 in patients with chronic HCV infection was first suggested by Manns *et al*. (15), who noted that the $CYP2D6_{252\text{-}271}$ sequence shares amino acid homology with sequences of the non-structural 5B and the envelope 1 (E1) protein of the HCV polyprotein, and suggested that molecular mimicry might lead to cross-reactivity and the production of anti-LKM1. This hypothesis was later supported by experimental data demonstrating humoral cross-reactivity between HCV and CYP2D6 (16). In the same vein, the $CYP2D6_{313–332}$ sequence sharing extensive homology with $HCV_{794–801}$ is recognised by CD4 T-cells in patients with chronic HCV infection (17).

The presence of anti-LKM1 in HCV-positive patients has important clinical implications because those treated with IFN may experience increased transaminase levels (13, 14), which may sometimes be such as to warrant its discontinuation (13, 14), or may occasionally progress to LKM1-positive autoimmune hepatitis (14). Furthermore, anti-LKM1/HCV-positive patients treated with INF are 14 times more likely to develop autoimmune thyroiditis (18), an event that is possibly facilitated by sequence similarities between CYP2D6 and thyroid peroxidase, a key target in autoimmune thyroid disease. Anti-LKM1 levels should therefore be measured in all patients with HCV infection, and be closely monitored in those undergoing INF treatment. Whether, and at what stage, INF treatment should be replaced by immunosuppression in anti-LKM1/HCV-positive patients is a matter of clinical judgment, but a liver biopsy showing florid interface hepatitis would tilt the balance in favour of immunosuppression.

HCV-associated mixed cryoglobulinemia

The clinical symptoms of MC range from mild palpable purpura, arthralgias and fatigue, to severe vasculitis with skin necrosis and the involvement of peripheral nerves, central nervous system, gastrointestinal tract, lungs and myocardium. Furthermore, the kidneys are frequently affected, and glomerulonephritis (GN) is a key prognostic factor.

The pathogenetic involvement of HCV in the formation, transport, and removal from circulation of cryoprecipitable immune complexes (ICs) in MC has been extensively studied over the last few years. ICs are formed by HCV, anti-HCV polyclonal immunoglobulin (Ig)G and monoclonal IgM, all of which have rheumatoid activity, which is critical for both cryoglobulin production and renal deposition. The IgM component means that these cryoprecipitable ICs escape the erythrocyte transport system (19)

and have a direct impact on hepatic and splenic macrophages, which are unable to process them due to abnormalities in the biogenesis of lysosomal enzymes.

It is currently believed that HCV infects B lymphocytes while infecting hepatocytes because of their common expression of CD81 receptors. The lymphocytes that are chronically stimulated by HCV are assigned to widespread autoantibody production related to the HCV-induced lowering of the cell activation threshold. This favours widespread autoantibody production and the development of a number of the immune manifestations associated with HCV infection, which variably make up clinical pictures that are collectively known as the "HCV syndrome" (20); this syndrome includes manifestations that are apparently distal to the characteristic picture of MC, such as thyroiditis, sicca syndrome, thrombocytopenia, pulmonary fibrosis. The B cells are protected from apoptosis by an HCV-dependent gene translocation, and develop oligoclonal monotype lymphoproliferations (21), and it is also to detect distinct lymphoid infiltrates, with cells expressing oligo- or monoclonal rheumatoid factor, in the portal tracts, spleen and bone marrow (sometimes evolving towards overt B-cell non-Hodgkin lymphoma). The most important pathogenetic aspects of the disease are: therefore the abnormal kinetics and easy deposition of HCV-containing ICs due to the IgM component, chronic stimulation by HCV infection sustaining the synthesis of cryoprecipitating IgM rheumatoid factor, and a subclinical smouldering lymphoproliferative disorder.

Therapy

No optimal strategy for HCV-associated MC nephritis has yet been defined, although the discovery of the association and its possible pathogenetic implications have prompted researchers to develop new approaches to disease control by eradicating the infection. IFN-α treatment has been increasingly used over the last ten years, but the results have been conflicting because of frequent relapses upon discontinuation combined with the reappearance of viral RNA, occasionally worsening skin ulcers (possibly due to the antiangiogenic effects of IFN-α), the precipitation of renal failure and nephrotic syndrome, psychiatric manifestations, peripheral neuropathy and autoimmune hepatitis. However, some studies have found that it has beneficial effects on urinary abnormalities and renal outcome (22), and a single study found an improvement in renal histology in a few patients with biopsy-proven GN who underwent combined antiviral therapy with IFN-α and ribavirin (23).

Further progress in terms of therapeutic response can be expected from the widespread use of pegylated interferon (peg-IFN), which has improved pharmacokinetic characteristics. Peg-IFN alone or combined with ribavirin has proved to be more effective than IFN-α alone or combined with ribavirin in patients with HCV infection, particularly those infected with genotype 1b, and patients with HCV-related MC also seem to benefit from this new combination therapy (22). However, during acute immunological flare-ups, antiviral treatment is usually insufficient to control the renal disease despite its ability to reduce viremia, and may even be detrimental. In such cases, steroids and immunosuppressants (usually cyclophosphamide), and sometimes plasmapheresis, are advocated, but they may increase viremia levels and thus exacerbate the chronic hepatitis C.

Comparison of the data relating to a robust sample of patients with MC-associated GN (20) and those relating to the only comparable cohort described ten years before (24) clearly shows that, although overall 10-year survival has improved (80% *vs*. 50%), cardiovascular deaths have replaced deaths due to infections and hepatic failure as the major cause of mortality. The reasons for this have been extensively discussed elsewhere (20), but include the suspicion that the better overall survival was due to time-limited aggressive treatment of acute renal involvement together with the long-term antiviral therapy used to control the moderate manifestations of renal and extra-renal involvement.

The efficacy of antiviral therapy with peg-IFN after rituximab-induced immunosuppression could be investigated in future trials, but it is even more critical to validate alternative therapeutic options to standard steroid and immunosuppressive treatment that could further improve survival by reducing cardiovascular risks. Rituximab has raised hopes for a new therapeutic approach to patients with active cryoglobulinemic nephropathy and systemic signs of severe vasculitis. It is a humanised mouse monoclonal antibody against CD20, a B cell-specific membrane protein with four transmembrane spanning domains, which are members of a family that includes Fc gamma R chains (probably critical for T cell depletion). In addition to individual case reports, Pub-Med includes six cohort studies of 59 patients, including 22 cases of nephritis and seven of post-kidney transplantation cryonephritis.(25-30). These generally used the lymphoma protocol consisting of four weekly infusions of 375 mg/m^2, although Roccatello added a further two doses at 1-month intervals (27); no additional therapy was given in two studies (27, 28) that included half of the nephritis patients. Twenty of the 22 nephritis patients achieved disease remission, and side effects were limited in the non-transplanted patients. Unexpectedly, viral load did not substantially increase. Relapses were frequent after a mean of six months (usually after 6-15 months), but seemed to be delayed (12-36 months) in the study using Roccatello's "4+2" protocol.

The use of rituximab treatment in MC is based on the selective depletion of IgM-producing B cells because, in addition to the classical pathogenetic pathway of the glomerular deposition of megacomplexes consisting of HCV antigens, anti-HCV IgG and IgM-k RF (31), it has been suggested that the IgM-k produced by a permanent clone of B cells may share a strong affinity for the glomerular matrix and thus be deposited in the glomerulus together with the anti-HCV IgG that was previously bound in the circulation, or subsequently fixed by means of an *in situ* binding mechanism.

MC-associated nephritis is a unique example of an immune-mediated disorder

in which rituximab specifically targets the nephrotoxic Ig-producing cells, and is thus effective in the relatively long run even given alone.

HCV infection and autoimmune endocrine diseases

The most frequent and clinically important endocrine manifestations of chronic HCV infection are autoimmune thyroid disorders (AITD), type 2 diabetes mellitus, and gonadal dysfunction (32, 33).

Table II. Pooled thyroid autoimmunity (AITD) and hypothyroidism (Hypothyr.) data in HCV+ patients (with and without chronic hepatitis) and controls (healthy subjects and HBV+ patients).

	No. of HCV+ patients	AITD HCV+	No. of controls	AITD controls	Odds ratio
Thyroid autoimmunity 95% confidence interval	2305	367 (15.9%)	3711	382 (10.2%)	1.65 1.41-1.92
	No. of HCV+ patients	Hypothyr. HCV+	No. of controls	Hypothyr. controls	Odds ratio
Hypothyroidism 95% confidence interval	1604	126 (7.8%)	1515	48 (3.1%)	2.60 1.85-3.65

HCV and autoimmune thyroid disorders

Many studies have evaluated the prevalence of thyroid autoimmunity in HCV+ patients, but the results are conflicting; however, a meta-analysis of the published literature has found that there is a significant association between HCV infection and AITD (34). Furthermore, pooling data relating to HCV+ patients and controls (healthy subjects and HBV+ patients) (3) and our own unpublished data suggests that HCV+ patients have a significantly increased risk of AITD or hypothyroidism (Table II).

The pattern of HCV-related thyroid disorders is characterised by increased levels of anti-thyroid peroxidase (AbTPO) antibodies, and an increased risk of hypothyroidism in AbTPO-positive subjects (34-36). It is generally agreed that being female is a risk factor for the development of AITD, and that the major risk factors for hypothyroidism are female gender and the presence of AbTPO (34-36).

A number of studies have shown an increased expression of IFN-γ and IFN-γ inducible chemokines (particularly CXCL10) in the hepatocytes and lymphocytes of HCV-infected patients (34), which directly correlates with the degree of inflammation and an increase in the circulating levels of IFN-γ and CXCL10. It has also been shown that NS5A and core proteins, alone or as a result of the synergistic effect of cytokines (IFN-γ and TNF-α), are capable of upregulating CXCL10 and MIG gene expression and secretion in cultured human hepatocyte-derived cells (34). This suggests that CXCL10, which is produced by HCV-infected hepatocytes, could play a key role in regulating T cell trafficking to a Th1-type inflammatory site because liver tissue recruits Th1 lymphocytes that secrete IFN-γ and TNF-α during chronic HCV infection, thus inducing CXCL10 release by hepatocytes and perpetuating the immune cascade (34).

It has also recently been shown that CXCL10 levels are high in patients with AITD, especially those with hypothyroidism (37), and that Th1 immune responses are involved in inducing AITD, Graves' disease and Graves' ophthalmopathy (37). Our preliminary data show that IFN-γ and CXCL10 are also involved in patients with HCV infection and MC (34), in the presence of AITD and hypothyroidism. The presence of HCV in the thyroid of chronically infected patients has recently been demonstrated (34), but its possible consequences on thyrocyte function, vitality and immunogenicity remain to be clarified. Given all of this, it can be speculated that HCV thyroid infection may act by upregulating CXCL10 gene expression and secretion in thyrocytes, thus leading to the appearance of AITD in genetically predisposed subjects as has been previously shown in the case of human hepatocytes (34).

There seems to be a high prevalence of papillary thyroid cancer patients with chronic HCV chronic infection and MC (34-36, 38), particularly in those with AITD. These results need to be confirmed, seem to be sufficient to suggest a need for careful thyroid ultrasonography monitoring during follow-up.

HCV and type 2 diabetes

A possible link between HCV infection and diabetes has been investigated since 1994, but most of the early studies included HCV patients with and without cirrhosis, and it is well known that cirrhosis of any etiology is a risk factor for type 2 diabetes (T2D). The association between HCV infection and T2D in patients without cirrhosis was first studied by us (39,40), and one population study (the National Health and Nutrition Examination Survey-NHANES III 1988–1994) that found an adjusted odds ratio of 3.8 for T2D in HCV+ subjects aged > 40 years, and an increased incidence of T2D. Chronic HCV infection can therefore be considered a risk factor for developing T2D.

Nevertheless, there is still debate about the mechanisms involved. It has been speculated that insulin resistance (due to hepatic steatosis, which is present in about 50% of HCV+ subjects) and/or high TNF-α expression (which closely correlated with the severity of liver disease and the level of insulin resistance) may lead to the development of T2D (40), and other authors have recently demonstrated that HCV has a direct cytopathic effect on islet cells.

However, the diabetes shown by patients with chronic HCV infection is not the classical T2D. Various studies have reported (39, 40) that the HCV+ patients with T2D are leaner than T2D controls, and have significantly lower LDL-cholesterol, ands systolic and diastolic blood pressure levels; furthermore, the MC-HCV+ patients with T2D more frequently had non-organ-specific autoantibodies than non-diabetic MC-HCV+ patients (34% *vs*. 18%) (39). It has been postulated that the diabetes of MC-HCV+ patients may be due to an immune-mediated mechanism (39),

and a similar pathogenesis may be true in the case of HCV+ patients (40).
As HCV+ patients do not have a greater prevalence of classic beta-cell autoimmune markers, other immune phenomena may be involved. Our preliminary data suggest that HCV infection of beta cells may act by upregulating CXCL10 gene expression and secretion (as previously shown in human hepatocytes) and recruiting Th1 lymphocytes that secrete IFN-γ and TNF-α and induce CXCL10 secretion by beta cells, thus perpetuating the immune cascade leading to the appearance of beta cell dysfunction.

HCV and gonadal dysfunction
Sex hormone alterations have been observed in HCV-positive patients with MC, and erectile dysfunction has been anecdotally reported in patients with chronic HCV-related hepatitis undergoing INF-α treatment. The possible role of HCV infection in gonadal dysfunction is suggested by the findings of a preliminary study of a large series of male HCV+ patients and controls, although it is interesting to note that erectile dysfunction and low levels of total and free testosterone were not related to the severity of the liver damage (41). The reduced endogenous immunosuppressive activity caused by low levels of adrenal gonadal androgens could amplify the autoreactive lymphocyte proliferation triggered by HCV infection (32, 33).
The above findings further support the role of host (genetic and hormonal) factors in the pathogenesis of different HCV-driven autoimmune disorders.

HCV infection and chronic arthritis
Joint involvement is the most common EHM of HCV infection (42, 43). Three mechanisms are usually indicated to explain the development of articular inflammation due to viral agents (direct invasion of joint tissue, the formation of immune complexes, and immune dysregulation leading to active autoaggressive processes), all of which may come together in patients with HCV-related arthritis (HCVrA), although their exact pathogenetic roles have yet to be clarified (44, 45).
On the basis of data reported by experienced groups, HCVrA affects 4-5% of patients with chronic hepatitis, but slight involvement is much more frequent. In France, Cacoub *et al*. (5) observed generic arthralgias in 23% of 1,614 patients with chronic hepatitis C, and the Korean group of Lee reported that 35% of their HCV-positive subjects suffered from arthralgia or arthritis. Iagnocco *et al*. (46) ultrasonographically studied the knees, hips and shoulders of 29 HCV+ patients without any rheumatic symptoms and found that 96% showed inflammatory joint alterations, a significantly higher percentage than among the healthy controls.
Since the introduction of tests for the detection of HCV, a number of studies and case reports have allowed the identification of two clinical subsets of HCVrA: the most frequent is polyarticular, resembles rheumatoid arthritis (RA), and chronically involves small joints; the other is mono-oligoarticular and involves medium-sized and large leg joints (especially the ankles) (45). The second often has an intermittent course and seems to be strictly associated with the presence of serum cryoglobulins and the appearance of cryoglobulinemic orthostatic purpura (43).
The differential diagnosis between RA-like HCVrA and true RA can be very difficult because their clinical pictures can be very similar. The American Rheumatism Association (ARA) classification criteria for RA can be easily fulfilled by this form of HCVrA, although the ESR can be normal and there are no rheumatoid nodules; prolonged morning stiffness and positive rheumatoid factor are common to both disorders. Fortunately, the clinical course of RA-like HCVrA is much less aggressive than that of RA, although some authors have described joint erosions in 20-30% of patients with HCV infection and polyarthritis (reviewed in refs. 2 and 4); in these cases, a fortuitous association between HCV and true RA cannot be ruled out. Recently marketed anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific for RA and can help in differentially diagnosing RA and other kinds of polyarthritides, including RA-like HCVrA (43).
The less common, intermittent, mono-oligoarticular form of HCVrA should be differentiated from other arthritides that usually involve the lower limbs, such as crystal-related arthritis, spondyloarthropathies and septic arthritis (43). The appearance of HCVrA together with cutaneous cryoglobulinemic vasculitis is very helpful in making a correct diagnosis.
The treatment of RA-like HCVrA is often based on the administration of coxibs, NSAIDs, low doses of oral corticosteroids and hydroxychloroquine, although specific experiences are scarce (47). These drugs are fairly effective. The risks of possible negative consequences on the liver disease have greatly limited the use of other DMARDs in patients with HCVrA, but some studies have found that penicillamine is partially effective, and that methotrexate is safe and effective (48). More recent studies carried out in Japan have documented the anti-HCV properties of cyclosporine, and allowed it to be proposed as a candidate for arthritis treatment in HCV patients (49).
Few data are available concerning the use of anti-TNF agents in HVCrA because of its non-aggressive evolution, as these modern drugs are usually used in serious forms of RA or spondyloarthropathies. In any case, etanercept seems to be less effective in HCVrA than in RA (49).
Israeli authors have suggested administering INF-α and ribavirine together with NSAIDs, hydroxychloroquine or low doses of corticosteroids to HCVrA patients without any contraindications to the use of antiviral therapy (51). Unfortunately, INF can induce the appearance or worsening of other autoimmune manifestations, such as neuritis, nephropathies and vasculitis. Furthermore, a recent French study has reported the worsening of rheumatic manifestations caused by the use of INF (48).

Treating patients with HCV infection and autoimmune disorders (ADs)
Given the relatively high prevalence of HCV infection (about 3%) and ADs (about 10%) in Western countries, it is not rare to encounter patients with both in everyday clinical practice. Although needed to treat ADs, the use of glucocorticoids or immunosuppressants in

HCV+ subjects leads to a risk of worsening the outcome of HCV infection, with the occurrence of hepatic diseases such as chronic active hepatitis (CAH), cirrhosis and hepatocellular carcinoma. As experience in transplanted patients clearly shows that a potent immunosuppressive regimen prevents allograft rejection but also favours viral replication and facilitates viral-mediated graft injury, rheumatologists have often refrained from using immunosuppressants to treat AD in the presence of HCV RNA.

Under these conditions, it is often necessary to add antiviral agents to treat the viral infection, mainly peg-IFN combined with ribavirin.

The role of cyclosporine A (CsA) in patients with AD and concomitant HCV infection has been recently reviewed (52). CsA is an immunosuppressant that is used to treat a wide range of autoimmune disorders, including rheumatoid arthritis, but a large body of published evidence suggests that standard therapeutic doses also inhibit HCV replication. This is highly specific, and it has been shown that it is due to the inhibition of cyclophilin-B and not to the calcineurin inhibition that is responsible for CsA's immunosuppressive effect. The anti-HCV effect of CsA has been demonstrated *in vitro* and *in vivo*, in liver transplanted patients at lower rejection risk (53), and in patients with CAH, in combination with antiviral agents such IFN-α. Furthermore, it seems that CSA suppresses hepatic stellate cell growth and collagen production *in vitro*, thus suggesting an anti-fibrogenetic effect. These findings have opened up new means of improving the treatment and prognosis of patients with HCV-related liver diseases and those with AD and concomitant HCV infection (52).

Anti-TNF-α blockers are immunosuppressants used to treat severe forms of RA, but post-marketing analyses have clearly demonstrated that patients receiving anti-TNF treatment are more susceptible to infections (54) because TNF-α is a key cytokine in the integrated host system of defence against infectious diseases. Furthermore, as TNF-α blockers have to be given in combination with methotrexate or other immunosuppressant agents, rheumatologists have refrained from using them in patients with AD and concomitant chronic HCV infection. It has been found that high TNF-α levels are involved in refractoriness to IFN therapy in HCV patients. The role of TNF-α and the imbalance in Th1/Th2 lymphocyte response in the development of chronic HCV infection and refractoriness to antiviral therapies has been recently reviewed by Calabrese (55). It has been shown that circulating Th cells predominantly produce IFN-γ in acute self-limiting HCV infection, thus suggesting a Th1-like response. Similarly, the patients who can spontaneously clear acute HCV infections have a strong Th1 response without any detectable Th2 response, whereas those who become chronically infected have a dominant Th2 response. Furthermore, preliminary data concerning the use of the existing anti-TNF agents in patients with chronic HCV infection and coexisting RA show that they are safe (56-61).

Personal experience

In order to evaluate the safety and antiviral efficacy of CsA in patients with AD and concomitant HCV infection, we conducted a pilot study of 32 patients, three of whom were affected by mixed cryoglobulinemia, two by psoriatic arthritis, one each by Sjögren's syndrome and undifferentiated connective tissue disease, 22 by RA, and three by RA combined with MC. All of the patients were treated with CsA 3 mg/kg/day for nine months, and antiviral efficacy was assessed every three months on the basis of SGOT/SGPT and viremia levels (at the start of the study, the patients with high levels of transaminases and viremia were examined separately).

After six months, all of the parameters had considerably improved, including the clinical course. SGOT and SGPT levels had decreased by respectively 21.62% ($p = 0.0001$) and 20.6% ($p = 0.001$), and viremia by 60.79% ($p = 0.0013$). In eleven patients monitored for nine months, the viremia decreased by 43.74% ($p = 0.0099$). Similar decreases were observed in the patients with high initial levels which had normalised by the end of the study. Moreover, the viremia had decreased by 64.17% in 21 patients examined after six months ($p = 0.0004$) and by 46.55% ($p = 0.0175$) in eight patients examined after nine months.

Given their large number, the patients with RA were analysed separately in order to verify whether they showed any particular features. The 6-month findings in the global sample were confirmed, with SGOT being reduced by 19.26%($p = 0.0001$), SGPT by 16.14% ($p = 0.0222$) and viremia by 66.98% ($p = 0.0028$), and the eight patients monitored for nine months showed a decrease of 40.08% in viremia ($p = 0.1426$), which was not statistically significant because of the small size of the sample. It is also worth noting that six of the RA patients were treated with CsA combined with anti-TNF-α and, after six months, there was a decrease of 39.94% in SGOT, 26.48% in SGPT, and 64.32% in viremia (again not significant because of the small sample size).

Temporary side effects were observed in eight patients (arterial hypertension, hypercreatininemia), but none of them had to be withdrawn from the study.

In conclusion, CsA therapy seemed to be safe and effective in our patients with AD and concomitant chronic HCV infection, as documented by the reduction in both viremia and transaminase levels; this was particularly true in the patients with high baseline levels. However, further studies are needed to confirm our findings in larger groups of patients possibly treated for longer periods.

Conclusions

HCV is not only a frequent cause of chronic liver diseases such as hepatitis, cirrhosis and hepatocellular carcinoma, but is also involved in the pathogenesis of a number of autoimmune and rheumatic disorders (arthritis, vasculitis, sicca syndrome, porphyria cutanea tarda, lichen planus, nephropathies, thyroid diseases, and lung fibrosis), as well as in the development of B-cell lymphoproliferative diseases. Although they are needed to treat autoimmune and rheumatic disorders, the use of glucocorticoids or immunosuppressants in HCV+ subjects may worsen

the clinical outcome of HCV, and it is often necessary to add antiviral agents (mainly pegylated IFN combined with ribavirin) to treat the viral infection. However, CsA therapy seems to be safe and effective in chronic HCV+ patients with concomitant autoimmune and rheumatic diseases, as documented by the reduction in both viremia and transaminase levels. In severe cases of MC, rituximab is an apparently safe and effective alternative to conventional immunosuppression.

References

1. WORLD HEALTH ORGANISATION: Hepatitis C fact sheet No. 164
2. SIMMONDS P: Genetic diversity and evolution of hepatitis C virus – 15 years on. *J Gen Virol* 2004; 85: 3173-88.
3. GREMION C, CERRY A: Hepatitis C virus and the immune system: a concise review. *Rev Med Virol* 2005; 15: 235-68.
4. HOOFNAGLE JH: Course and outcome of hepatitis C. *Hepatology* 2002; 36 (Suppl. 1): S21-S29.
5. CACOUB P, POYNARD T, GHILLANI P *et al*: Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999; 42: 2204-12.
6. ZIGNEGO AL, GIANNINI C, FERRI C: Hepatitis C virus-related lymphoproliferative disorders: an overview. *World J Gastroenterol* 2007; 13: 2467-78.
7. LECUBE A, HERNÁNDEZ C, GENESCÀ J, SIMÓ R: Glucose abnormalities in patients with hepatitis C virus infection: Epidemiology and pathogenesis. *Diabetes Care* 2006; 29: 1140-9.
8. ANTONELLI A, FERRI C, PAMPANA A *et al*.: Thyroid disorders in chronic hepatitis C. *Am J Med* 2004; 117: 10-3.
9. PILERI P, UEMATSU Y, CAMPAGNOLI S, GALLI G *et al*.: Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938-41.
10. LEVY S, SHOHAM T: The tetraspanin web modulates immune-signalling complexes. *Nat Rev Immunol* 2005; 5: 136-48.
11. DICKSON RC: Clinical manifestations of hepatitis C. *Clin Liver Dis* 1997; 1: 569-85.
12. LENZI M, BELLENTANI S, SACCOCCIO G *et al*.: Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: a nested case-control study of the Dionysos cohort. *Gut* 1999; 45: 435-41.
13. GIOSTRA F, MANZIN A, LENZI M *et al*.: Low hepatitis C viremia levels in patients with anti-liver/kidney microsomal antibody type 1 positive chronic hepatitis. *J Hepatol* 1996; 25: 433-8.
14. MURATORI L, LENZI M, CATALETA M *et al*.: Interferon therapy in liver/kidney microsomal antibody type 1-positive patients with chronic hepatitis C. *J Hepatol*. 1994; 21: 199-203.
15. MANNS MP, GRIFFIN KJ, SULLIVAN KF, JOHNSON EF: LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. *J Clin Invest* 1991; 88: 1370-8.
16. BOGDANOS D, LENZI M, OKAMOTO M *et al*.: Multiple viral/self immunological cross-reactivity in liver kidney microsomal antibody positive hepatitis C virus infected patients is associated with the possession of HLA b51. *Int J Immunopathol Pharmacol* 2004; 17: 83-92.
17. MA Y, BOGDANOS DP, HUSSAIN MJ *et al*.: Polyclonal T-cell responses to cytochrome P450IID6 are associated with disease activity in autoimmune hepatitis type 2. *Gastroenterology* 2006; 130: 868-82.
18. MURATORI L, BOGDANOS DP, MURATORI P *et al*.: Susceptibility to thyroid disorders in hepatitis C. *Clin Gastroenterol Hepatol* 2005; 3: 595-603.
19. ROCCATELLO D, MORSICA G, PICCIOTTO G *et al*.: Impaired hepatosplenic elimination of circulating cryoglobulins in patients with essential mixed cryoglobulinemia and hepatitis C virus (HCV) infection. *Clin Exp Immunol* 1997; 110: 9-14.
20. ROCCATELLO D, FORNASIERI A, GIACHINO O *et al*.: Multicenter study on HCV-related cryoglobulinemic glomerulonephritis. *Am J Kidney Dis* 2007; 49: 69-82.
21. FERRI C, PILERI S, ZIGNEGO AL: Hepatitis C virus, B-cell disorders, and non-Hodgkin lymphoma. *In* GOEDERT J.J. (Ed.) *Infectious causes of cancer. Targets for intervention*. Totowa, New Jersey; Humana Press; 2000: 349-68.
22. DELLA ROSSA A, TAVONI A, BALDINI C, BOMBARDIERI S: Treatment of chronic hepatitis C infection with cryoglobulinemia. *Curr Opin Rheum* 2002; 13: 231-37.
23. ROSSI P, BERTANI T, BAIO P *et al*.: Hepatitis C virus-related cryoglobulinemic glomerulonephritis: long-term remission after antiviral therapy. *Kidney Int* 2003; 63: 2236-41.
24. TARANTINO A, CAMPISE M, BANFI G *et al*.: Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. *Kidney Int* 1995; 47: 618-23.
25. SANSONNO D, DE RE V, LAURETTA G *et al*.: Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon *alpha* with an anti-CD20. *Blood* 2003; 101: 3818-26.
26. ZAJA F, DE VITA S, MAZZARO C *et al*.: Efficacy and safety of Rituximab in type II mixed cryoglobulinemia. *Blood* 2003; 101: 3827-34.
27. ROCCATELLO D, BALDOVINO S, ROSSI D *et al*.: Long term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinemic glomerulonephritis. *Nephrol Dial Transplant* 2004; 19: 3054-61.
28. QUARTUCCIO L, SOARDO G, ROMANO G *et al*.: Rituximab treatment for glomerulonephritis in HCV-associated mixed cryoglobulinaemia: efficacy and safety in the absence of steroids. *Rheumatology* 2006; 45: 842-6.
29. BASSE G, RIBES D, KAMAR N *et al*.: Rituximab therapy for *de novo* mixed cryoglobulinaemia in renal transplant patients. *Transplantation* 2005; 80: 1560-4.
30. VISENTINI M, GRANATA M, VENEZIANO ML *et al*.: Efficacy of low-dose rituximab for mixed cryoglobulinemia. *Clin Immunol* 2007; 125: 30-3.
31. ZAJA F, RUSSO D, FUGA G *et al*.: Rituximab for the treatment of type II mixed cryoglobulinemia. *Haematologica* 1999; 84: 1157-9.
32. FERRI C, ANTONELLI A, MASCIA MT *et al*.: B-cells and mixed cryoglobulinemia. *Autoimmun Rev* 2007; 7: 114-20.
33. FERRI C, ANTONELLI A, MASCIA MT *et al*.: HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Digest Liver Dis* 2007; 39 (Suppl. 1): S13 S21.
34. ANTONELLI A, FERRI C, FALLAHI P *et al*.: Thyroid disorders in hepatitis C virus chronic infection. *Thyroid* 2006; 16: 563-72.
35. ANTONELLI A, FERRI C, PAMPANA A *et al*.: Thyroid disorders in chronic hepatitis C. *Am J Med* 2004; 117: 10-13.
36. ANTONELLI A, FERRI C, FALLAHI P *et al*.: Thyroid involvement in patients with overt HCV-related mixed cryoglobulinaemia. *QJM* 2004; 97: 499-506.
37. ANTONELLI A, ROTONDI M, FERRARI SM *et al*.: Interferon-γ-inducible α-chemokine CXCL10 involvement in Graves' ophthalmopathy: modulation by peroxisome proliferator-activated receptor-γ agonists. *J Clin Endocrinol Metab* 2006; 9: 614-20.
38. ANTONELLI A, FERRI C, FALLAHI P *et al*.: Thyroid cancer in HCV-related chronic hepatitis patients: A case-control study. *Thyroid* 2007; 17: 447-51.
39. ANTONELLI A, FERRI C, FALLAHI P *et al*.: Type 2 diabetes in hepatitis C-related mixed cryoglobulinaemia patients. *Rheumatology* 2004; 43: 238-40.
40. ANTONELLI A, FERRI C, FALLAHI P *et al*.: Hepatitis C virus infection: Evidence for an association with type 2 diabetes. *Diabetes Care* 2005; 28: 2548-50.
41. FERRI C, BERTOZZI MA, ZIGNEGO AL: Erectile dysfunction and hepatitis C virus infection. *JAMA* 2002; 288: 698-9.
42. MAYO MJ: Extrahepatic manifestations of hepatitis C infection. *Am J Med Sci* 2003; 325: 135-48.
43. PALAZZI C, OLIVIERI I, D'AMICO E, CACCIATORE P, PENNESE E: Difficulties in the differential diagnosis between primitive rheumatic diseases and hepatitis C virus-related disorders. *Clin Exp Rheumatol* 2005; 23: 2-6.
44. LU MC, HSIEH SC, LAI NS, LI KJ, WU CH, YU CL: Comparison of anti-agalactosyl IgG antibodies, rheumatoid factors, and anti-cyclic citrullinated peptide antibodies in the differential diagnosis of rheumatoid arthritis and its mimics. *Clin Exp Rheumatol* 2007; 25: 716-21.
45. OLIVIERI I, PALAZZI C, PADULA A: Hepatitis C virus infection and arthritis. *Rheum Dis Clin North Am* 2003; 29: 111-22.
46. IAGNOCCO A, COARI G, MAMMARELLA A *et al*.: Joint sonography in asymptomatic patients with HCV correlated hepatitis. *Clin Exp Rheumatol* 2004; 22: 43-8.
47. PALAZZI C, OLIVIERI I, D'AMICO E, CACCIATORE P, PENNESE E: Treatment of HCV-related arthritis. *Expert Opin Pharmacother* 2005; 6: 27-34.
48. NISSEN MJ, FONTANGES E, ALLAM Y, ZOULIM F, TREPO C, MIOSSEC P: Rheumatological manifestations of hepatitis C: incidence in a rheumatology and non-rheumatology setting and the effect of methotrexate and interferon.

Rheumatology (Oxford) 2005; 44: 1016-20.
49. GALEAZZI M, BELLISAI F, MANGANELLI S, MOROZZI G, SEBASTIANI GD: Cyclosporine A for the treatment of autoimmune disorders in HCV infected patients. *Autoimmunity Reviews* 2006; 5: 493-8.
50. ROSNER I MAROTTE H, FONTANGES E *et al*.: Etanercept treatment for three months is safe in patients with rheumatological manifestations associated with hepatitis C virus. *Rheumatology* (Oxford) 2007; 46: 97-9.
51. ROZENBAUM M, TOUBI E, KESSEL A, NASCHITZ JE, ZUCKERMAN E: The case for hepatitis C arthritis. *Semin Arthritis Rheum* 2004; 33: 375-87.
52. GALEAZZI M, BELLISAI F, MANGANELLI S, MOROZZI G, SEBASTIANI GD: Cyclosporine A for the treatment of autoimmune disorders in HCV infected patients. *Autoimmun Rev* 2006; 5: 493-8.
53. BERENGUER M, AGUILERA V, PRIETO M *et al*.: Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. *Liver Transpl* 2006; 12: 762-7.
54. ELLERIN T, RUBIN RH, WEINBLATT ME: Infections and anti-tumor necrosis factor alpha therapy. *Arthritis Rheum* 2003; 48: 3013-22.
55. CALABRESE L.H., ZEIN N, VASSILOPOULOS D: Safety of anti-tumour necrosis factor (anti-TNF) therapy in patients with chronic viral infections: hepatitis C, hepatitis B, and HIV infection. *Ann Rheum Dis* 2004; 63 (Suppl. 2): ii18-ii24.
56. ZEIN NN: A phase II randomised, double blind, placebo controlled study of tumor necrosis factor antagonist (Etanercept, Enbrel) as an adjuvant to interferon and ribavirin in naive patients with chronic hepatitis C (Abstract). *Hepatology* 2002; 36: 504A.
57. PETERSON JR, HSU FC, SIMKIN PA *et al*.: Effect of tumour necrosis factor alpha antagonists on serum transaminases and viraemia in patients with rheumatoid arthritis and chronic hepatitis C infection. *Ann Rheum Dis* 2003; 62: 1078-82.
58. ASLANIDIS S, VASSILIADIS T, PYRPASOPOULOU A, DOULOUMPAKAS I, ZAMBOULIS C: Inhibition of TNFalpha does not induce viral reactivation in patients with chronic hepatitis C infection: two cases. *Clin Rheumatol* 2007; 26: 283.
59. PARKE FA, REVEILLE JD: Anti-tumor necrosis factor agents for rheumatoid arthritis in the setting of chronic hepatitis C infection. *Arthritis Rheum* 2004; 51: 800-4.
60. BELLISAI F, GIANNITTI C, DONVITO A, GALEAZZI M: Combination therapy with cyclosporine A and anti-TNF alpha agents in the treatment of rheumatoid arthritis and concomitant hepatitis C virus infection. *Clin Rheumatol* 2007; 26: 1127-9
61. GALEAZZI M, BELLISAI F, GIANNITTI C, MANGANELLI S, MOROZZI G, SEBASTIANI GD: Safety of cyclosporin A in HCV-infected patients: experience with cyclosporine A in patients affected by rheumatological disorders and concomitant HCV infection. *Ann N Y Acad Sci* 2007; 1110: 544-9.