Journal of Hepatology 1999; **31**: 369–376
Printed in Denmark · All rights reserved
Munksgaard · Copenhagen

Journal of Hepatology
ISSN 0168-8278

Review

Extrahepatic manifestations of HCV infection: facts and controversies

Anna Linda Zignego[1] and Christian Bréchot[2]

[1]*Department of Internal Medicine, University of Florence, Florence, Italy and* [2]*Inserm U 370, CHU Necker-Enfants Malades, Paris, France*

HCV Infection and Lymphoproliferative Disorders

Mixed cryoglobulinaemia

The most documented extrahepatic manifestation of HCV infection is essential mixed cryoglobulinaemia (EMC). This topic has been the object of a previous, exhaustive, review by Lunel & Musset (1). Briefly, EMC is characterised by the presence of temperature-sensitive protein complexes: in type II EMC, cryoglobulins are composed of a monoclonal rheumatoid factor (RF) (usually IgMK) against polyclonal IgG. In type III EMC, all components are polyclonal. Since mono- or polyclonal B lymphocyte expansion is responsible for cryoglobulin production, EMC may be considered a lymphoproliferative disorder (LPD). Due to the fact that EMC frequently coexists with bone marrow aspects of B-cell non-Hodgkin's lymphoma (NHL) and may evolve, in about 5–8% of cases, into a frank B-cell malignancy, it may be considered a "borderline" (benign/malignant) LPD.

Evidence in favour of a pathogenetic link between EMC and HCV derives from a series of data including, first, the very high prevalence of HCV markers in EMC patients (1–8) with detection of HCV sequences in peripheral and bone marrow mononuclear cells in most patients, also in the absence of viraemia (9). The characteristic occurrence of EMC in patients with a longer history of HCV infection and/or elevated age suggests that EMC development depends on the length of infection (1,10,11). In the single patient, however, inherited or acquired factors predisposing to LPDs may accelerate the occurrence of cryoglobulinaemia and worsen corresponding clinical manifestations, as also suggested by observations made in patients receiving a liver graft for HCV-related cirrhosis (12).

Correspondence: Anna Linda Zignego, Department of Internal Medicine, University of Florence, School of Medicine, Viale Morgagni 85, 50134, Florence, Italy.
Tel: 39 55 416635. Fax: 39 55 417123.
e-mail: a.zignego@dfc.unifi.it

With respect to viral factors possibly involved in the pathogenesis of EMC/HCV, the majority of studies agree that no HCV genotype is specifically related to EMC, and that the more prevalent genotype observed in HCV-related EMC is the one most prevalent in the general HCV-positive population of the same geographical area (1,11,13). However, a high prevalence of genotype 2 in EMC patients was observed in Italy (11,13–15). In some cases, this viral type was only observed in lymphatic cells (11). Therefore, it is still debatable whether or not different viral types might exert a lesser or more important influence in favouring the appearance of LPDs. Indeed, it is interesting to note the intense stimulation of the lymphatic system reported during HCV type 2 infection (16,17) as well as the recently observed high frequency of this type in different HCV-positive B-cell LPDs (18,19). Differences in the geographical distribution of this subtype may account, at least in part, for the discrepancies among available data on the prevalence of HCV genotypes in EMC patients.

Results of interferon treatment in EMC patients represent an indirect proof for the pathogenetic link between EMC and HCV infection (1). Controlled trials have now defined the efficacy of IFN alpha in the treatment of EMC. Response to IFN can be achieved in more than 50% of patients and includes improvement of cutaneous vasculitis and renal function, reduction in hepatitis C viraemia, serum cryoglobulin concentration, and IgM RF synthesis (5,20). However, almost 80% of responders eventually have a clinical and biochemical relapse. Additional studies are therefore required to improve the outcome and extension of this therapy.

Lymphoma

The association between HCV infection and B-cell non-Hodgkin's lymphoma (NHL) has been reported both in patients with HCV-related type II EMC (EMC/NHL) (13,21–23) and in patients without EMC (non-EMC-associated or "idiopathic" NHL) (24–37). As in EMC-associated NHL, "idiopathic" NHL also ap-

pears after long-lasting HCV infection (29). Many of these studies have been, so far, performed in Italy and there are some discordant reports from other geographical areas (38,39). With respect to the possible association between HCV infection and different LPDs, a common observation is the lack of an association between HCV and non-B-LPDs (i.e., Hodgkin's lymphoma or T-NHL) (32,37). The relationship between HCV infection and other B-cell LPDs, such as chronic lymphocytic leukaemia, Waldenström's macroglobulinaemia, multiple myeloma, and different monoclonal gammopathies, has been suggested and remains open to discussion (19,25,37,40).

Taken together, the available data suggest the hypothesis that HCV infection is associated with B cell LPDs of different clinico-pathological characteristics, probably representing a predisposing factor that acts in a remote stage of their pathogenesis.

Other Extrahepatic Diseases

There are a series of disorders for which an association with HCV infection has been suggested, frequently without sufficient confirmation. These include idiopathic pulmonary fibrosis, lichen planus, Sjögren syndrome, Mooren corneal ulcers, autoimmune thyroiditis, and porphyria cutanea tarda. A pathogenetic link between HCV infection and some of these disorders has been suggested also on the basis of the response to antiviral therapy (41). Furthermore, in some cases, anti-viral treatment with interferon has been suggested actually to be responsible for the association. This may be the case in autoimmune phenomena involving the thyroid.

Autoimmune thyroiditis

A high prevalence of thyroid dysfunction and anti-thyroid antibodies including thyroid microsomal, thyreoglobulin and thyroid peroxidase antibodies, has been found in up to 30% of chronic HCV infections. However, some investigations were unable to find either a higher prevalence of HCV infection in patients with Hashimoto's thyroiditis (42), or an increased prevalence of thyroid dysfunction and antithyroid peroxidase antibodies in blood donors with HCV infection in comparison to controls (43). These patients may be particularly susceptible to the development of Hashimoto's thyroiditis or Grave's disease following therapy with alpha interferon and to the direct reduction by r-IFN-alpha of the intrathyroidal organification of iodine (44). In fact, thyroid dysfunction is not infrequently observed in patients receiving r-IFN-alpha therapy because of chronic active hepatitis of different aetiologies, and IFN-alpha therapy may induce, reveal or exacerbate various autoimmune-related disorders. In light of these data it is possible that cooperation between IFN and HCV takes place in treated patients and that HCV infection plays a contributory role in the pathogenesis of such disorders.

Other associations may, at least in part, overlap with the already-described extrahepatic manifestations of HCV infection. This is the case, for example, in the suggested link between HCV infection and Sjögren's syndrome (SS) or idiopathic pulmonary fibrosis (IPF).

Sjögren's syndrome

Different viruses (i.e., herpes virus and retrovirus) have been suspected to play a role in triggering lymphoid proliferation, which, like EMC, may culminate in the development of a malignant lymphoma. A close relationship between SS and HCV has been shown by epidemiological studies (45,46) and the coexistence of EMC in patients with HCV-associated SS has been suggested (46). However, the pathogenetic role of HCV infection in the development of SS, as well as the characteristics distinguishing classical SS from the HCV-associated syndrome, have been an object of debate (47). More recently, observations of the presence of a typical autoimmune sialadenitis in HCV-positive subjects, similar to that described in primary SS (48,49), as well as experimental data, suggest that HCV might effectively be involved in the pathogenesis of this syndrome. In this respect, transgenic mice carrying the HCV envelope genes have recently been shown to develop an exocrinopathy involving salivary and lacrymal glands, which strongly resembles SS (50). In consideration of the frequent association of SS with EMC in HCV-positive patients, as well as of the possible evolution of SS into a B-cell NHL (35,36,51,52), the syndrome observed in infected patients may be interpreted as one of the possible clinical manifestations of the HCV-related lymphoproliferative disorder.

Idiopathic pulmonary fibrosis

A pathogenetic link between HCV infection and IPF has been suggested because of the higher frequency of HCV markers in IPF patients than in normal controls (53,54) and the observation that some patients with HCV-positive chronic hepatitis treated with IFN-alpha developed pulmonary fibrosis. Moreover, there was an increased count of lymphocytes and neutrophils in bronchoalveolar lavage fluid in patients with HCV chronic infection (55), suggesting that HCV infection may trigger alveolitis (55). However, there are conflicting epidemiological data, mainly from the UK, making this association an object of debate (56). The fact that EMC may be complicated by interstitial lung involve-

ment suggests that the association between HCV and IPF may be indirect, at least in some cases, and due to surrounding HCV-related EMC. In a study we recently performed in a group of HCV chronically infected patients with IPF, interstitial lung involvement appeared medially 4.5±3.2 S.D. years after the clinical onset of chronic hepatitis and was associated with some rheumatic symptoms and serum cryoglobulins and/or autoantibodies (57).

Dermatological manifestations

Apart from the palpable purpura due to leukocytoclastic vasculitis, which is the most frequently observed dermatological manifestation of HCV-related EMC, HCV infection has also been associated with a series of different cutaneous disorders, including sporadic porphyria cutanea tarda (PCT) and cutaneous and/or mucosal lichen planus (LP).

A strong association between sporadic PCT and HCV infection has been proposed on the basis of the observation of a high prevalence (>50%) of HCV markers in those patients, mostly in studies from southern Europe (58–61). In several studies, no alterations in porphyrin metabolism were demonstrated in HCV-positive patients without PCT (62,63), suggesting that HCV infection probably acts as a triggering factor in genetically predisposed subjects. In contrast, preliminary data suggest that HCV infection induces a decreased concentration of uroporphyrinogen decarboxylase (URO-D), the enzyme responsible for PCT manifestations, also in patients with no evidence of PCT (S. Fargion, personal communication). In addition, a significantly higher frequency of immunological alterations in HCV-positive patients with PCT, including anti-GOR antibodies, ANA, ASMA, anti-LKM1, RF and mixed cryoglobulins than in healthy subjects and patients with different immunological diseases was observed, suggesting the pathogenetic importance of HCV-related autoimmunity in inducing the precipitation of this metabolic disorder (64).

Lichen planus is another skin disorder which is thought to be associated with HCV infection on the basis of the observation of an increased frequency of HCV infection among LP patients, in particular in Italy and Japan (65–67). However, these results have not been confirmed by other reports from other geographical areas and this association remains controversial. In addition, IFN treatment has been shown to trigger LP in some HCV-positive cases (68). The wide geographical variations reported for prevalence rates of HCV antibodies in LP patients are in agreement with the hypothesis of the prevalent role of host factors in the pathogenesis of LP (68).

Extrahepatic Infection by HCV: Is There a Link with Extrahepatic Pathological Manifestations?

The possibility that HCV also infects extrahepatic cells has been widely discussed. A series of cell types possibly supporting viral replication has been proposed, including biliary cells (Loriot et al., submitted) and salivary gland epithelium (69). However, lymphatic cells represent the most studied cellular type. *In vivo*, HCV RNA sequences have been detected in fresh peripheral blood mononuclear cell (PBMC) preparations from HCV-infected patients (70–73). However, such an approach does not allow distinction between absorption of serum particles and true infection of PBMC in viraemic patients. Still, several observations support this hypothesis, including: (i) short-term cultures of PBMC which yield a significant increase in the amount of viral RNA on stimulation by PHA and PMA (70); (ii) *in situ* hybridization showed viral RNA in a limited percentage (around 1%) of circulating PBMC and lymph nodes (74), (iii) PBMC from normal individuals can be infected by HCV (73,75); (iv) there is evidence that Epstein Barr virus-immortalised B cells and T-cell clones from HCV carriers show long-term persistence of HCV genomes (Bronowicki, personal communication and 73) and (v) we recently presented strong evidence for the persistence of HCV RNA in PBMC obtained from HCV-positive subjects and injected into SCID mice (severe combined immunodeficient mice). Artefacts due to contamination with serum particles were excluded by this approach which showed infection of these mononuclear cells (76).

A second issue concerns the detection of replicative forms of HCV RNA in PBMC. This point has been discussed with regard to the specificity of negative HCV RNA detection. Thus, although such negative strands are clearly detected in some patients and on stimulation with mitogens, the real prevalence of this phenomenon remains to be determined. In a recent study, Lerat et al., using a strand-specific RT-PCR strategy, showed the presence of HCV RNA sequences in PBMC from chronically infected patients, with a detection rate ranging from 15% to 100% and from 8% to 83.3% for the positive- and negative-strand RNA respectively, independently from viral load (77). Interestingly, this study suggests that HCV type 1 might show a distinct lymphotropism, in comparison to other frequent HCV types. Also, using a highly strand-specific method of RT-PCR, Shimizu et al. were able to demonstrate HCV RNA of negative polarity in PBMC samples from infected chimpanzees. In addition, they found that the capacity to infect PBMC and/or liver cells in chimpanzees, as well as human lymphocyte cell lines infected *in vitro*, varied among different HCV

strains, suggesting the existence of lymphotropic HCV strains (78). The possibility of specific cell subsets harbouring HCV sequences has also been discussed. HCV negative-strand RNA, possibly reflecting viral replication, was found in all cell subsets in some studies (70,73) and in specific cell subpopulations in others (77).

Finally, the possibility of PBMC infection by HCV has recently been confirmed in studies indicating that HCV infection of lymphoid cells may favour selection of distinctive viral variants. In a recent *in vitro* study, Kato et al. (79) observed that after infection of different cellular systems of lymphoid or hepatic origin, the predominant HVR1 populations were different. Similarly in *in vivo* studies different quasispecies between serum and PBMC have been shown both in chronic hepatitis C patients and in HCV-positive patients undergoing liver transplantation (80–82 and Feray et al., personal communication). In particular, Maggi et al. recently observed considerable quasispecies differences between the liver and PBMC in all patients they analysed involving variant numbers, relative quantities and relative electrophoretic mobilities. Genomic variants present in the liver and/or PBMC were not detected in the corresponding plasma samples, while certain HCV variants were present only in plasma. The differences in quasispecies composition between these tissues may arise from factors in the liver and PBMC which favour the growth of certain variants over others (82).

Taken together, the available data indicate that hematopoietic cells may support HCV replication, although generally at low levels. Due to the fact that most extrahepatic manifestations of HCV infection are of lymphoproliferative and/or autoimmune nature, it is tempting to speculate that one important factor influencing their development may be viral lymphotropism (70,73,83). In this light, HCV infection of hematopoietic cells may represent a common "remote" pathogenesis, inducing similar mechanisms which may result in different clinical manifestations because of different individual, constitutional and/or environmental factors (84). It has also been suggested that direct infection of organ-specific cell populations by HCV may play a role in determining certain clinical manifestations, as in disorders of the salivary glands and kidney (35).

The hypothesis concerning the critical importance of HCV lymphotropism in the pathogenesis of extrahepatic manifestations of HCV infection was initially only supported by observation of the high frequency of HCV infection of peripheral or bone marrow lymphatic cells in most of these patients (9,83). The recent observations by Mecchia and co-workers offer a hypothetical role for the infection of lymphatic cells by HCV in determining autoimmune disorders. These authors analysed patients with HCV-related EMC and demonstrated the existence of disease-characteristic IgM specific for a cryptic epitope of the CD4-like LAG-3 protein. A similar phenomenon has been demonstrated in HIV infection, where it is possible to observe an autoimmune response against a cryptic epitope of CD4 protein due to an abnormal processing of this protein by infected T cells. It is thus plausible that cryptic epitopes of LAG-3 may stem from an abnormal processing of this protein by infected lymphocytes (85). The results by Lohr and co-workers are also interesting (86). They cultured PBMCs from patients with chronic hepatitis C and found that only cells infected by HCV were able to release anti-HCV antibodies in the supernatant, whereas non-infected cells were anti-HCV negative *in vitro*. These data suggest that HCV infection in mononuclear cells stimulates anti-HCV secretion by B cells *in vitro*. In addition, using the particular model represented by intra-peritoneal injection of lymphoid cells (PBMC or bone marrow cells) from chronically HCV-infected patients into SCID mice, we provided further evidence concerning the *in vivo* infection of lymphatic cells by showing long-term persistence of HCV RNA in human cells (mean follow-up: 2 months) and the possibility of successful serial passages of HCV-positive cells. On the other hand, preliminary results obtained by comparing the behaviour of lymphatic cells derived from patients with and without LPDs (lymphoma and leukaemia) showed that following the injection of PBMC from patients with LPDs, PBMC and sera yielded the strongest HCV RNA signals (plus and minus strand), and greater rapidity of lymphoproliferative tumour emergence was observed (76).

The most important unsolved issue is the intimate pathogenetic mechanism(s) potentially involved in HCV-related LPDs. Since HCV cannot integrate in the host genome, it is possible to hypothesise that it triggers B-cell LPDs through indirect, concurrent mechanisms, such as reactivation of other lymphotropic viruses, environmental factors, and not secondarily, a genetic-driven hosts reactivity. No evidence for a cooperative role of coinfecting lymphotropic viruses in determining LPDs in HCV-positive patients could be demonstrated (87,88).

Interestingly, HCV-related LPDs usually appear after a long-lasting period of infection. During this phase some genetic aberrations can take place. Recent data suggest that HCV infection may influence oncogenes associated with LPDs. The recent observation of a genetic aberration (bcl-2 rearrangement) in one patient with HCV/EMC during the benign phase of the disease, followed by the occurrence of additional genetic aberrations during the accelerated phase of the dis-

ease (89) is of particular interest. The Bcl-2 proto-oncogene codifies for the bcl-2 protein, which counteracts the occurrence of apoptosis and prolongs cell survival without affecting the cycling cells. This oncogene is located on chromosome 18 where its expression is subjected to complex regulating mechanisms. An increased expression of bcl-2 protein, observed in patients with HCV-positive II EMC (14), may be due to the activation of bcl-2 gene in the absence of genetic modifications. However, BCL-2 overexpression in B-cells may be a consequence of reciprocal t(14; 18) translocation. This translocation occurs during early B-cell development and juxtaposes the immunoglobulin heavy chain locus (IgH) with the bcl-2 locus, leading to bcl-2 activation (90,91). T(14; 18) translocation is known as the most constant genetic aberration in follicular, but also in some diffuse lymphomas, and appears to be one step in the progression of a normal cell to a cancer cell. It may be detected also in the absence of neoplastic transformation, following particularly strong antigenic stimulation, as suggested by its possible detection in hyperplastic lymphoid tissues (92). In light of these data, the recent observation of a high prevalence of this mutation in patients with chronic HCV infection, with and without lymphoproliferative diseases (EMC, NHL), appears interesting and deserves further analysis (93).

Given the role of bcl-2 in the negative control of apoptosis, it is tempting to speculate that an initial polyclonal lymphoproliferation may eventually result in the emergence of a clone protected from apoptosis which, following additional mutational events can evolve into a frank malignancy (94). In turn, abnormal protection against apoptosis may predispose to autoimmunity and represent one of the possible mechanisms by which HCV infection may persist in the host. The chronic stimulation of the lymphatic system by HCV, possibly amplified by the infection of lymphoid cells, together with the high viral variability, could explain the high frequency of this mutation in HCV-positive patients. The recent *in vitro* evidence for the control of c-myc expression by the HCV core (95,96) might also be relevant to this issue, and the impact of cooperation between bcl-2 and c-myc in lymphomagenesis has been well established (97). The recent identification of the CD81 protein as one of the HCV receptor candidates (98) further substantiates this hypothesis. CD81 is indeed expressed at the surface of a variety of cell types, including lymphocytes, and is implicated in B cell activation. It is therefore plausible that HCV-CD81 interactions might participate in the strong polyclonal B cell activation observed during HCV infection.

Conclusions

The apparent over-abundance of extrahepatic manifestations of HCV infection may be re-appraised when it is observed that the majority are based on lymphoproliferative and/or autoimmune mechanisms. This suggests that HCV infection leads to different clinical manifestations, probably by inducing a general tendency to develop lymphoproliferative and/or autoimmune phenomena through a primary action on the lymphatic system. On the basis of this virus-related predisposition, the final clinical manifestations would greatly vary in different subjects according to individual genetically and/or environmentally driven predisposing factors. In other words, on the basis of the general "remote" pathogenetic mechanism, for each HCV-related extrahepatic manifestation, different pathogenetic pathways, i.e. involving the production of RF, cryoglobulins, the intervention of local factors, etc., as suggested by various interesting studies (1,99), may lead to a full understanding of these disorders. Further studies will be critical in confirming or denying the many speculative pieces of this intricate and fascinating puzzle.

Acknowledgements

This work was supported by the Istituto Superiore di Sanità (ISS), Research Project on Viral Hepatitis: Etiopathogenesis and Diagnosis, the Associazione Italiana Ricerca sul Cancro (AIRC), the Italian Liver Foundation (ILF), the Fondazione Istituto di Ricerca Virologica O.B. Corsi and in France by INSERM and ARC.

References

1. Lunel F, Musset L. Hepatitis C virus infection and cryoglobulinemia. J Hepatol 1998; 29: 848–55.
2. Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, et al. Antibodies against hepatitis C virus in mixed cryoglobulinemia patients. Infection 1991; 19: 417–20.
3. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 1992; 327: 1490–5.
4. Misiani R, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M, et al. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. Ann Intern Med. 1992; 117: 573–7.
5. Ferri C, Marzo E, Longombardo G, Lombardini F, La Civita L, Vanacore R, et al. Interferon-alpha in mixed cryoglobulinemia patients: a randomized, crossover-controlled trial. Blood 1993; 81: 1132–6.
6. Marcellin P, Descamps V, Martinot-Peignoux M, Larzul D, Xu L, Boyer N, et al. Cryoglobulinemia with vasculitis associated with hepatitis C virus infection. Gastroenterology 1993; 104: 272–7.
7. Cacoub P, Fabiani FL, Musset L, Perrin M, Frangeul L, Leger JM, et al. Mixed cryoglobulinemia and hepatitis C virus. Am J Med 1994; 96: 124–32.
8. Zignego AL, Ferri C, Giannini C, La Civita L, Careccia G, Longombardo G, et al. Analysis of HCV infection in mixed cryoglob-

ulinemia and B-cell non-Hodgkin's lymphoma: evidence for a pathogenetic role. Arch Virol 1997; 142: 545–55.
9. Ferri C, Monti M, La Civita L, Longombardo G, Greco F, Pasero G, et al. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. Blood 1993; 82: 3701–4.
10. Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, Andre C, et al. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. Ann Intern Med 1995; 122: 169–73.
11. Zignego AL, Ferri C, Giannini C, Monti M, La Civita L, Careccia G, et al. Hepatitis C virus genotype analysis in patients with type II mixed cryoglobulinemia. Ann Intern Med 1996; 124: 31–4.
12. Gournay J, Ferrell LD, Roberts JP, Ascher NL, Wright TL, Lake JR. Cryoglobulinemia presenting after liver transplantation Gastroenterology 1996; 110: 265–70.
13. Pozzato G, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, et al. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. Blood 1994; 84: 3047–53.
14. Monteverde A, Ballare M, Pileri S. Hepatic lymphoid aggregates in chronic hepatitis C and mixed cryoglobulinemia. Springer Semin Immunopathol 1997; 19: 99–110.
15. Zehender G, De Maddalena C, Monti G, Ballare M, Saccardo F, Piconi S, et al. HCV genotypes in bone marrow and peripheral blood mononuclear cells of patients with mixed cryoglobulinemia. Clin Exp Rheumatol 1995; 13S: S87–S90.
16. Cerino A, Silini E, Brambilla S, Asti M, Bellati G, Lisa A, et al. Serological response to HCV hypervariable region 1 are mainly cross-reactive and genotype-dependent. J Hepatol 1997; 26S1: 62.
17. Missale G, Cariani E, Lamonaca V, Ravaggi A, Rossini A, Bertoni R, et al. Effects of interferon treatment on the antiviral T-cell response in hepatitis C virus genotype 1b- and genotype 2c-infected patients. Hepatology 1997; 26: 792–7.
18. Silvestri F, Barillari G, Fanin R, Pipan C, Falasca E, Salmaso F, et al. The genotype of the hepatitis C virus in patients with HCV-related B cell non-Hodgkin's lymphoma. Leukemia 1997; 11: 2157–61.
19. Andreone P, Zignego AL, Cursaro C, Gramenzi A, Gherlinzoni F, Fiorino S, et al. Prevalence of monoclonal gammopathies in patients with hepatitis C virus infection. Ann Intern Med 1998; 129: 294–8.
20. Misiani R, Bellavita P, Fenili D, Vicari O, Marchesi D, Sironi PL, et al. Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994; 330: 751–6.
21. Ferri C, Monti M, La Civita L, Careccia G, Mazzaro C, Longombardo G, et al. Hepatitis C virus infection in non-Hodgkin's B-cell lymphoma complicating mixed cryoglobulinaemia. Eur J Clin Invest 1994; 24: 781–4.
22. La Civita L, Zignego AL, Monti M, Longombardo G, Pasero G, Ferri C. Mixed cryoglobulinemia as a possible preneoplastic disorder. Arthritis Rheum 1995; 38: 1859–60.
23. Franzin F, Efremov DG, Pozzato G, Tulissi P, Batista F, Burrone OR. Clonal B-cell expansions in peripheral blood of HCV-infected patients. Br J Haematol 1995; 90: 548–52.
24. Zignego AL, Ferri C, Giannini C, La Civita L, Careccia G, Longombardo G, et al. Hepatitis C virus infection in mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma: evidence for a pathogenetic role. Arch Virol 1997; 142: 545–55.
25. Izumi T, Sasaki R, Tsunoda S, Akutsu M, Okamoto H, Miura Y. B cell malignancy and hepatitis C virus infection. Leukemia 1997; S3: 516–8.
26. Satoh T, Yamada T, Nakano S, Tokunaga O, Kuramochi S, Kanai T, et al. The relationship between primary splenic malignant lymphoma and chronic liver disease associated with hepatitis C virus infection. Cancer 1997; 80: 1981–8.
27. Sikuler E, Shnaider A, Zilberman D, Hilzenrat N, Shemer-Avni Y, Neumann L, et al. Hepatitis C virus infection and extrahepatic malignancies. J Clin Gastroenterol 1997; 24: 87–9.
28. Zuckerman E, Zuckerman T, Levine AM, Douer D, Gutekunst K, Mizokami M, et al. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. Ann Intern Med 1997; 127: 423–8.
29. Ferri C, La Civita L, Monti M, Longombardo G, Pasero G, Zignego AL. Can type C hepatitis infection be complicated by malignant lymphoma? Lancet 1995; 346: 1426–7.
30. Ferri C, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G, et al. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. Br J Haematol 1994; 88: 392–4.
31. Ferri C, Caracciolo F, La Civita L, Monti M, Longombardo G, Zignego AL. Hepatitis C virus infection and B-cell lymphomas. Eur J Cancer 1994; 30A: 1591–2.
32. Zignego AL, Ferri C, Innocenti F, Giannini C, Monti M, Bellesi G, et al. Lack of preferential localization of tumoral mass in B-cell non-Hodgkin's lymphoma associated with hepatitis C virus infection. Blood 1997; 89: 3066–8.
33. Luppi M, Grazia Ferrari M, Bonaccorsi G, Longo G, Narni F, Barozzi P, et al. Hepatitis C virus infection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. Leukemia 1996; 10: 351–5.
34. Silvestri F, Pipan C, Barillari G, Zaja F, Fanin R, Infanti L, et al. Prevalence of hepatitis C virus infection in patients with lymphoproliferative disorders. Blood 1996; 87: 4296–301.
35. De Vita S, Sacco C, Sansonno D, Gloghini A, Dammacco F, Crovatto M, et al. Characterization of overt B-cell lymphomas in patients with hepatitis C virus infection. Blood 1997; 90: 776–82.
36. Ferri C, La Civita L, Caracciolo F, Bellesi G, Zignego AL. Hepatitis C virus and lymphoproliferative disorders. Blood 1997; 88: 4730.
37. De Rosa G, Gobbo ML, De Renzo A, Notaro R, Garofalo S, Grimaldi M, et al. High prevalence of hepatitis C virus infection in patients with B-cell lymphoproliferative disorders in Italy. Am J Hematol 1997; 55: 77–82.
38. Brind AM, Watson JP, Burt A, Kestevan P, Wallis J, Proctor SJ, et al. Non-Hodgkin's lymphoma and hepatitis C virus infection. Leuk Lymphoma 1996; 21: 127–30.
39. McColl MD, Singer IO, Tait RC, McNeil IR, Cumming RL, Hogg RB. The role of hepatitis C virus in the aetiology of non-Hodgkins lymphoma – a regional association? Leuk Lymphoma 1997; 26: 127–30.
40. La Civita L, Zignego AL, Monti M, Longombardo G, Greco F, Pasero G, et al. Type C hepatitis and chronic lymphocytic leukaemia. Type C hepatitis and chronic lymphocytic leukaemia. Eur J Cancer 1996; 32A: 1819–20.
41. Moazami G, Auran JD, Florakis GJ, Wilson SE, Srinivasan DB. Interferon treatment of Mooren's ulcers associated with hepatitis C. Am J Ophthalmol. 1995; 119: 365–6.
42. Wong S, Mehta AE, Faiman C, Berard L, Ibbott T, Minuk GY. Absence of serologic evidence for hepatitis C virus infection in patients with Hashimoto's thyroiditis. Hepatogastroenterology 1996; 43: 420–1.
43. Boadas J, Rodriguez-Espinosa J, Enríquez J, Miralles F, Martínez-Cerezo FJ, González P, et al. Prevalence of thyroid autoantibodies is not increased in blood donors with hepatitis C virus infection. J Hepatol 1995; 22: 611–5.
44. Roti E, Minelli R, Giuberti T, Marchelli S, Schianchi C, Gardini E, et al. Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-alpha. Am J Med 1996; 101: 482–7.
45. Aceti A, Taliani G, Sorice M, Amendolea MA. HCV and Sjögren's syndrome. Lancet 1992; 339: 1425–6.
46. Haddad J, Deny P, Munz-Gotheil C, Ambrosini JC, Trinchet JC, Pateron D, et al. Lymphocytic sialadenitis of Sjögren's syndrome associated with chronic hepatitis C virus liver disease. Lancet 1992; 339: 321–3.
47. Pirisi M, Scott C, Fabris C, Ferraccioli G, Soardo G, Ricci R, et al. Mild sialoadenitis: a common finding in patients with hepatitis C virus infection. Scand J Gastroenterol 1994; 29: 940–2.

48. Coll J, Gambus G, Corominas J, Tomas S, Esteban JI, Guardia J. Immunohistochemistry of minor salivary gland biopsy specimens from patients with Sjögren's syndrome with and without hepatitis C virus infection. Ann Rheum Dis 1997; 56: 390–2.
49. Scott CA, Avellini C, Desinan L, Pirisi M, Ferraccioli GF, Bardus P, et al. Chronic lymphocytic sialoadenitis in HCV-related chronic liver disease: comparison of Sjögren's syndrome. Histopathology 1997; 30: 41–8.
50. Koike K, Moriya K, Ishibashi K, Yotsuyanagi H, Shintani Y, Fujie H, et al. Sialadenitis histologically resembling Sjögren syndrome in mice transgenic for hepatitis C virus envelope genes. Proc Natl Acad Sci USA 1997; 94: 233–6.
51. Tzioufas AG, Manoussakis MN, Costello R, Silis M, Papadopoulos NM, Moutsopoulos HM. Cryoglobulinemia in autoimmune rheumatic diseases. Evidence of circulating monoclonal cryoglobulins in patients with primary Sjögren's syndrome. Arthritis Rheum 1986; 29: 1098–104.
52. Luppi M, Grazia Ferrari M, Bonaccorsi G, Longo G, Narni F, Barozzi P, et al. Hepatitis C virus infection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. Leukemia 1996; 10: 351–5.
53. Ueda N, Shah SV. Apoptosis. J Lab Clin Med 1994; 124: 169–77.
54. Ohta K, Ueda T, Nagai S, Yamada K, Yamaguchi M, Nakano J, et al. Pathogenesis of idiopathic pulmonary fibrosis: is hepatitis C virus involved?. Nippon Kyobu Shikkan Gakkai Zasshi 1993; 31S: 32–5.
55. Yamaguchi S, Kubo K, Fujimoto K, Honda T, Sekiguchi M, Sodeyama T. Analysis of bronchoalveolar lavage fluid in patients with chronic hepatitis C before and after treatment with interferon alpha. Thorax 1997; 52: 33–7.
56. Irving WL, Day S, Johnston ID. Idiopathic pulmonary fibrosis and hepatitis C virus infection. Am Rev Respir Dis 1993; 148: 1683–4.
57. Ferri C, La Civita L, Fazzi P, Solfanelli S, Lombardini F, Begliomini E, et al. Interstitial lung fibrosis and rheumatic disorders in patients with hepatitis C virus infection. Br J Rheumatol 1997; 36: 360–5.
58. Piperno A, D'Alba R, Roffi L, Pozzi M, Farina A, Vecchi L, et al. Hepatitis C virus infection in patients with idiopathic hemochromatosis (IH) and porphyria cutanea tarda (PCT). Arch Virol Suppl 1992; 4: 215–6.
59. Fargion S, Piperno A, Cappellini MD, Sampietro M, Fracanzani AL, Romano R, et al. Hepatitis C virus and porphyria cutanea tarda: evidence of a strong association. Hepatology 1992; 16: 1322–6.
60. Ferri C, Baicchi U, la Civita L, Greco F, Longombardo G, Mazzoni A, et al. Hepatitis C virus-related autoimmunity in patients with porphyria cutanea tarda. Eur J Clin Invest 1993; 23: 851–5.
61. Navas S, Bosch O, Castillo I, Marriott E, Carreno V. Porphyria cutanea tarda and hepatitis C and B viruses infection: a retrospective study. Hepatology 1995; 21: 279–84.
62. Hussain I, Hepburn NC, Jones A, O'Rourke K, Hayes PC. The association of hepatitis C viral infection with porphyria cutanea tarda in the Lothian region of Scotland. Clin Exp Dermatol 1996; 21: 283–5.
63. O'Reilly FM, Darby C, Fogarty J, O'Moore R, Courtney MG, O'Connor J, et al. Porphyrin metabolism in hepatitis C infection. Photodermatol Photoimmunol Photomed 1996; 12: 31–3.
64. Ferri C, Baicchi U, la Civita L, Greco F, Longombardo G, Mazzoni A, et al. Hepatitis C virus-related autoimmunity in patients with porphyria cutanea tarda. Eur J Clin Invest 1993; 23: 851–5.
65. Nagao Y, Sata M, Tanikawa K, Itoh K, Kameyama T. Lichen planus and hepatitis C virus in the northern Kyushu region of Japan. Eur J Clin Invest 1995; 25: 910–4.
66. Tanei R, Watanabe K, Nishiyama S. Clinical and histopathologic analysis of the relationship between lichen planus and chronic hepatitis C. J Dermatol 1995; 22: 316–23.
67. Carrozzo M, Gandolfo S, Carbone M, Colombatto P, Broccoletti R, Garzino-Demo P, et al. Hepatitis C virus infection in Italian patients with oral lichen planus: a prospective case-control study. J Oral Pathol Med 1996; 25: 527–33.
68. Nagao Y, Sata M, Ide T, Suzuki H, Tanikawa K, Itoh K, et al. Development and exacerbation of oral lichen planus during and after interferon therapy for hepatitis C. Eur J Clin Invest 1996; 26: 1171–4.
69. De Vita S, Sansonno D, Dolcetti R, Ferraccioli G, Carbone A, Cornacchiulo V, et al Hepatitis C virus within a malignant lymphoma lesion in the course of type II mixed cryoglobulinemia. Blood 1995; 86: 1887–92.
70. Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, et al. Infection of peripheral mononuclear blood cells by hepatitis C virus. J Hepatol 1992; 15: 382–6.
71. Muller HM, Pfaff E, Goeser T, Kallinowski B, Solbach C, Theilmann L. Peripheral blood leukocytes serve as a possible extrahepatic site for hepatitis C virus replication. J Gen Virol 1993; 74: 669–76.
72. Willems M, Sheng L, Roskams T, Ramdani B, Doutrelepont JM, Nevens F, et al. Hepatitis C virus and its genotypes in patients suffering from chronic hepatitis C with or without a cryoglobulinemia-related syndrome. J Med Virol 1994; 44: 266–71.
73. Zignego AL, De Carli M, Monti M, Careccia G, La Villa G, Giannini C, et al. Hepatitis C virus infection of mononuclear cells from peripheral blood and liver infiltrates in chronically infected patients. J Med Virol 1995; 47: 58–64.
74. Moldvay J, Deny P, Pol S, Brechot C, Lamas E. Detection of hepatitis C virus RNA in peripheral blood mononuclear cells of infected patients by *in situ* hybridization. Blood 1994; 83: 269–73.
75. Cribier B, Schmitt C, Bingen A, Kirn A, Keller F. *In vitro* infection of peripheral blood mononuclear cells by hepatitis C virus. J Gen Virol 1995; 76: 2485–91.
76. Bronowicki JP, Loriot MA, Thiers V, Grignon Y, Zignego AL, Brechot C. Hepatitis C virus persistence in human hematopoietic cells injected into SCID mice. Hepatology 1998; 28: 211–8.
77. Lerat H, Rumin S, Habersetzer F, Berby F, Trabaud MA, Trepo C, et al. *In vivo* tropism of hepatitis C virus genomic sequences in hematopoietic cells: influence of viral load, viral genotype, and cell phenotype. Blood 1998; 91: 3841–9.
78. Shimizu YK, Igarashi H, Kanematu T, Fujiwara K, Wong DC, Purcell RH, et al. Sequence analysis of the hepatitis C virus genome recovered from serum, liver, and peripheral blood mononuclear cells of infected chimpanzees. J Virol 1997; 71: 5769–73.
79. Kato N, Ikeda M, Sugiyama K, Mizutani T, Tanaka T, Shimotohno K. Hepatitis C virus population dynamics in human lymphocytes and hepatocytes infected *in vitro*. J Gen Virol 1998; 79: 1859–69.
80. Fujii K, Hino K, Okazaki M, Okuda M, Kondoh S, Okita K. Differences in hypervariable region 1 quasispecies of hepatitis C virus between human serum and peripheral blood mononuclear cells. Biochem Biophys Res Commun 1996; 225: 771–6.
81. Saito H, Tsuchimoto K, Miyaguchi S, Tada S, Sawaguchi K, Komatsu K, et al. Multicenter trial in high-dose interferon-alpha 2b treatment for chronic hepatitis C. Keio Interferon-alpha 2b Study Group. J Med 1996; 45: 161–7.
82. Maggi F, Fornai C, Vatteroni ML, Giorgi M, Morrica A, Pistello M, et al. Differences in hepatitis C virus quasispecies composition between liver, peripheral blood mononuclear cells and plasma. J Gen Virol 1997; 78: 1521–5.
83. Zignego AL, Ferri C, Monti M, LaCivita L, Giannini C, Careccia G, et al. Hepatitis C virus as a lymphotropic agent: evidence and pathogenetic implications. Clin Exp Rheumatol 1995; 13S: 33–7.
84. Lenzi M, Frisoni M, Mantovani V, Ricci P, Muratori L, Francesconi R, et al. Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C virus-related mixed cryoglobulinemia. Blood 1998; 91: 2062–6.
85. Mecchia M, Casato M, Tafi R, Filocamo G, Bonomo L, Fiorilli M, et al. Nonrheumatoid IgM in human hepatitis C virus-associated type II cryoglobulinemia recognize mimotopes of the CD4-like LAG-3 protein. J Immunol 1996; 157: 3727–36.

86. Lohr HF, Goergen B, Meyer zum Büschenfelde KH, Gerken G. HCV replication in mononuclear cells stimulates anti-HCV-secreting B cells and reflects nonresponsiveness to interferon-alpha. J Med Virol 1995; 46: 314–20.
87. Ferri C, Lo Jacono L, Monti M, Caracciolo F, La Civita L, Barsanti LA, et al. Lymphotropic virus infection of peripheral blood mononuclear cells in B-cell non-Hodgkin's lymphoma. Acta Haematol 1997; 98: 89–94.
88. Zignego AL, Giannini C, Bellesi G, Gentilini P, Hadzyiannis S, Ferri C. May HGV infection be implicated in lymphomagenesis? Br J Haematol 1997; 98: 778–9.
89. Ellis M, Rathaus M, Amiel A, Manor Y, Klein A, Lishner M. Monoclonal lymphocyte proliferation and bcl-2 rearrangement in essential mixed cryoglobulinaemia. Eur J Clin Invest 1995; 25: 833–7.
90. Jaeger U, Karth GD, Knapp S, Friedl J, Laczika K, Kusec R. Molecular mechanism of the t(14; 18) – a model for lymphoid-specific chromosomal translocations. Leuk Lymphoma 1994; 14: 197–202.
91. Ueda N, Shah SV Apoptosis. J Lab Clin Med 1994; 124: 169–77.
92. Poetsch M, Weber-Matthiesen K, Plendl HJ, Grote W, Schlegelberger B. Detection of the t(14;18) chromosomal translocation by interphase cytogenetics with yeast-artificial-chromosome probes in follicular lymphoma and nonneoplastic lymphoproliferation. J Clin Oncol 1996; 14: 963–9.
93. Zignego AL, Giannelli F, Marrocchi ME, Giannini C, Gentilini P, Ferri C. Frequency of bcl-2 rearrangement in patients with mixed cryoglobulinemia and HCV-positive liver diseases. Clin Exp Rheumatol 1997; 15: 711–2.
94. McDonnell TJ, Korsmeyer SJ. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). Nature 1991; 349: 254–6.
95. Ray RB, Lagging LM, Meyer K, Steele R, Ray R. Transcriptional regulation of cellular and viral promoters by the hepatitis C virus core protein. Virus Res 1995; 37: 209–20.
96. Ray RB, Meyer K, Ray R. Suppression of apoptotic cells death by hepatitis C virus core protein. Virology 1996; 226: 176–82.
97. Fanidi A, Harrington EA, Evan GI. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature 1992; 359: 554–6.
98. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. Science 1998; 282: 938–41.
99. Agnello V. The etiology and pathophysiology of mixed cryoglobulinemia secondary to hepatitis C virus infection. Springer Semin Immunopathol 1997; 19: 111–29.