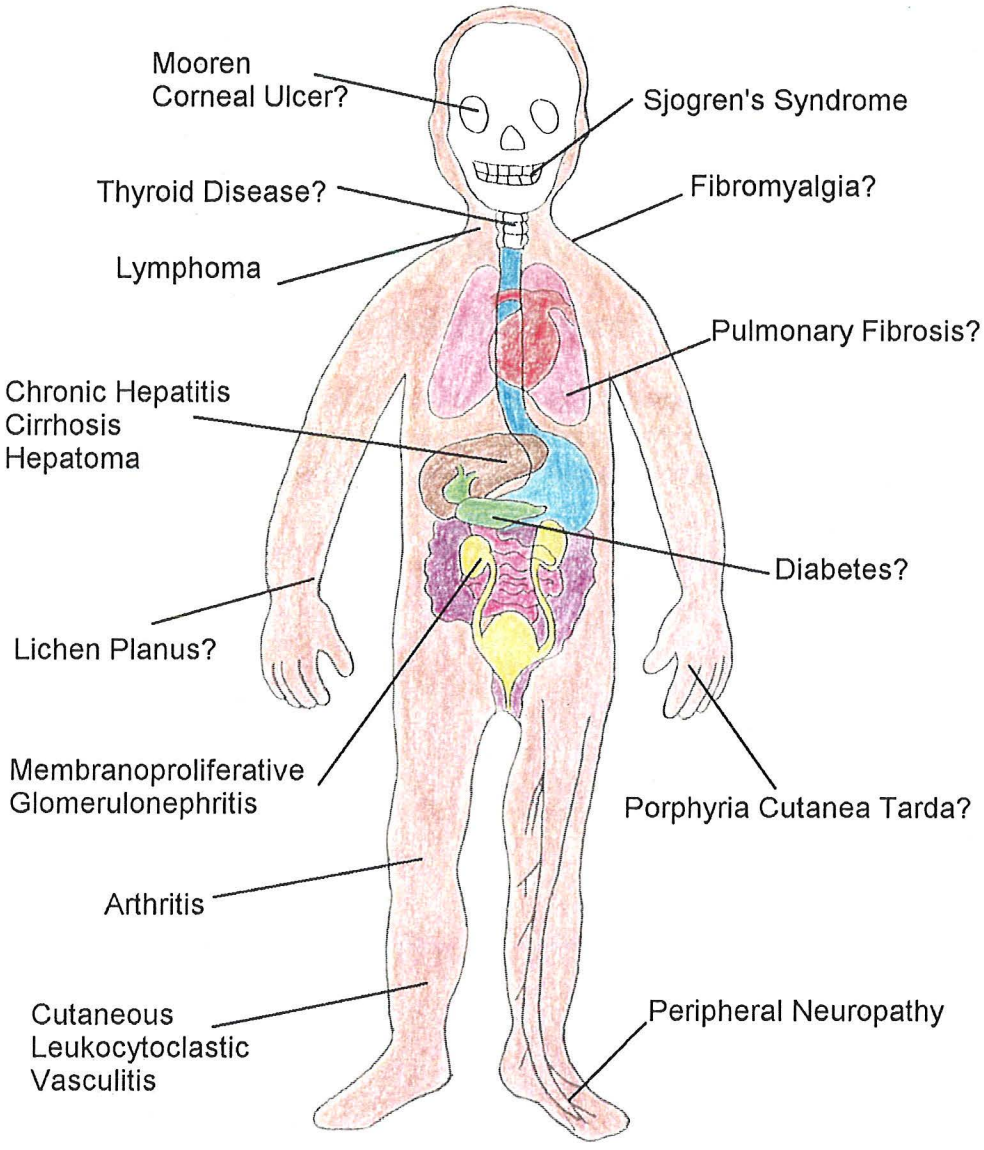
BEYOND THE LIVER: EXTRAHEPATIC MANIFESTATIONS OF CHRONIC HEPATITIS C INFECTION



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Introduction

The prevalence of extrahepatic manifestations is higher in patients with chronic hepatitis C virus (HCV) infection than in patients with any other known infectious liver disease. Approximately 38% of patients with HCV manifest symptoms of at least one extrahepatic manifestation of HCV(1), and the percentages are even higher if asymptomatic abnormalities are sought. Because Hepatitis C affects 1.8% of the general US population (2) (and the frequencies are higher in specialized populations such as veterans, substance abusers, and prisoners), the general internist will undoubtedly encounter extrahepatic manifestations of the hepatitis C during his/her career.

Within the last decade, numerous clinical syndromes have been reported to be associated with HCV infection. Some have been well proven and others remain speculative. Based upon the quality of evidence present to support the association, the major syndromes reported to be associated with HCV infection can be grouped into high, intermediate, and low probability.

HIGH	INTERMEDIATE	LOW
Cryoglobulinemic syndrome (Cutaneous leukocytoclastic vascultitis, arthritis, weakness)	Porphyria Cutanea Tarda	Thyroid disease
Renal Disease	Diabetes	Corneal Ulcers
Neuropathy		Lichen Planus
Lymphoma		Pulmonary Fibrosis
Sjögren's syndrome		Fibromyalgia

Lymphoma, renal disease, neuropathy, Sjögren's manifest incomplete overlap with the cryoglobulinemic syndrome. Many patients with HCV and these diseases will have detectable cryoglobulins in the serum even in the absence of rash, weakness, or arthralgias. A few, however, will not have any cryoglobulins.

The most common extrahepatic manifestations of chronic HCV infection are immunologic in nature. Viruses have always been considered as potential triggers of autoimmune diseases, particularly when the virus infects lymphocytes, such as EBV or HIV. The replicative form (minus strand RNA by PCR) of HCV has been demonstrated in lymphocytes, although inconsistently and at a lower level than in hepatocytes (3). Children rarely get extrahepatic manifestations of HCV(Carithers, personal communication), and neither do patients with acute hepatitis C. It appears that long-term chronic infection is necessary for the development of these associated conditions, and thus they could be considered as "complications" of chronic HCV. The molecular study of the unique way in which the HCV virus interacts with the human immune system is beginning to provide plausible explanations of the pathogenic role of HCV in some of these syndromes, but many pathogenetic links remain completely obscure.

Cryoglobulinemia

Cryoglobulinemia is defined as the presence of circulating immunoglobulins (Ig) that reversibly precipitate at <= 37°C. Cyroglobulins were classified by Brouet in 1974 into three types (4), which are still useful because they offer a good correlation with associated diseases and clinical manifestations.

Type	Rheumatoid Factor	Disease Association
I IgM monoclonal IgG monoclonal	negative	Multiple myeloma, Waldenstrom's macroglobulinemia
II (mixed) IgM monoclonal + IgG polyclonal	positive, monoclonal	Chronic infection (HBV,HCV,HIV), autoimmune disorders (SLE,SS),
III (mixed) IgM polyclonal + IgG polyclonal	positive, polyclonal	lymphoma, essential

The term "mixed cryoglobulins" was coined to differentiate types II and III, which contained a mixture of two different kinds of antibodies with rheumatoid activity from type I which contained just a single monoclonal antibody. Cryoglobulins may precipitate in vivo in small blood vessels (veinules, capillaries, arterioles) causing a vasculitis syndrome. The presence of cryoglobulins in the serum, however, is not always associated with symptoms; thus the term "cryoglobulinemic syndrome" is used for patients that present with clinical manifestations. The link between HCV virus and MC is strong and is supported by epidemiological, molecular, and virological studies.

Prevalence

Prior to the discovery of the hepatitis C virus, essential MC represented the largest subgroup of cryoglobulinemic patients. In the early 1990's, multiple investigators simultaneously reported that 50%-90% of patients with essential MC had HCV infection (5-8). Hepatitis B, on the other hand, causes MC in less than 5% of individuals (9). HCV now accounts for 73% of all forms of cryoglobulinemia (10). The percentage of MC caused by HCV varies somewhat with the background prevalence of HCV infection, which is greatest in southern Italy, where up to12.6% of persons are chronically infected with HCV (11). The percentage of patients with HCV that have circulating cryoglobulins has been reported between 0%-59%.

Country	HCV N=	Cryo +	RF+
Sweden (12)	21	0%	ND
Israel (13)	90	11%	44%
Germany (14)	132	28%	42%
France (15)	58	36%	70%
France (1)	321	56%	38%
Korea (16)	49	59%	14%
Dallas, Texas			31%

The variation in reported prevalences, with much higher frequencies in parts of Southern Europe than in Northern Europe or North America, may be related somewhat to differences in technique in determining the presence of cryoglobulins. This is supported by the fact that 1) better diagnostic techniques have shown increasing prevalence rates in the same area over time 2) studies with reported prevalences of over 50% have used the most sensitive methods 3) studies with prevalences less than 50% are able to detect rheumatoid factor in an additional percentage of patients that tested negative for cryoglobulins.

For routine determination of high levels of cryoglobulins, blood is drawn into syringe warmed to 37 °C and maintained at 37 °C until coagulation is complete. Serum is isolated by warm centrifugation and stored with an antisceptic at 4°C. The serum is centrifuged in a graduated Wintrobe tube (named after the first to describe the reversible cryopreciptate in a patient with multiple myeloma in1933), which enables the amount of precipitate to be expressed as a percentage of total serum (cryocrit). MC are often present at low concentrations (<1%) and are therefore not detected with this methodology. For low serum levels, serum should be isolated by centrifugation at 37 °C and stored with an antisceptic at 4 °C for 8 days (enough for late precipitating or low concentrations). The precipitate is then repeatedly centrifuged at 4 °C and washed with 4 °C sterile NaCl 0.15M. An aliquot of purified precipitate is then used to measure total protein concentration by UV absorption (17).

The latter test is more time consuming and is not offered by most hospitals, including Parkland Memorial. The IgM component of the cryoprecipitate causes high levels of rheumatoid factor activity because it binds avidly to IgG and forms immune complexes. In one study of HCV100% of patients with symptomatic cryoglobulinemia had rheumatoid factor activity, whereas none of the HCV patients without MC had RF activity in the serum. In situations where the sensitivity of the cryoglobulin test is known to be low, RF may be used as a "poor man's cryoglobulin" (D. Thiele) test. In a published series of 408 HCV-infected patients from UTSW, the sensitivity of RF for cutaneous leukocytoclastic vasculitis was 100%, whereas the sensitivity of the cryoglobulin test was only 40%. (18). In situations where the sensitivity of the cryoblobulin test is high, the rheumatoid factor test does not detect any additional cases of mixed cryoglobulinema and, in fact, will miss some. The prevalence of RF+ in the Parkland population of HCV antibody positive donors with active hepatitis is 31%. (R, Magnum, M.D. and T. Geppert, M.D., unpublished data, 1994), suggesting that our local rate of HCV-related cryoglobulinemia is similar to what has been reported around the world.

Symptoms

Purpura, arthralgias, and weakness is the original triad used to define patients with MC syndrome in 1966, but this definition is no longer adequate because additional symptoms are now known to be related to MC.

Skin Disease	22%
Joint Disease	16%
Weakness	65%
Renal Disease	25-30%
Raynaud's	3-50%
Neuropathy	8-25%
Sicca syndrome	20%
Lymphadenopathy	3%
Fever	3%

Many persons with detectable cryoglobulins are asymptomatic. The classical skin rash is a palpable purpura that histologically resembles a leukocytoclastic vascultitis. Rarely the vasculitic process can progress to necrotizing skin lesions. Severe systemic vasculitis is rarely seen. MC-related arthritis is usually an intermittent mono- or oligoarticular nondestructive arthritis affecting most often the proximal interphalangial joints, metacarpophalangeal joints, and knees. In some cases pain and joint stiffness occurs after cold exposure (19). Arthralgias are relatively common in HCV, but arthritis not related to MC is not (20). Weakness is present in the majority of HCV related MC, although it is not clear if this is the HCV or the MC (21). Renal involvement is common and is usually due to membranoproliferative glomerular nephritis. Peripheral neuropathies and Raynaud's phenomenon are also much more common than was previously recognized. Small numbers of patients with fever and lymphadenopathy are consistently reported and probably represent the progression of the MC syndrome to frank non-Hodgin's lymphoma.

As compared to patients with non-HCV-related cryoglobulinemia, HCV patients have the same frequency of cutaneous involvement, renal involvement, and peripheral neuropathy, but they less frequently manifest fever, lymphadenopathy, articular involvement, or Raynaud's phenomenon. ANA and RF are common in both groups, but patients with HCV-negative MC are more likely to have ANA and those with HCV-related MC are more likely to have RF (10).

Circulating immune complexes activate the complement system, and thus C4, C3, and CH50 levels are typically seen in the sera of patients with mixed cryoglobulinemia (MC). In fact, C4 and CH50 levels are significantly lower in HCV-related MC than in other essential MC. Higher cryocrits are associated with more symptoms (10), but why some individuals make higher cryocrits is not completely understood.

Pathogenesis

The symptoms of MC syndrome are caused by vascular deposition of the cryoprecipitate, which contains HCV RNA, LDL, IgG, and a highly restricted monoclonal IgM that has RF activity. The pathogenic role of HCV in the cryoprecipitate has been demonstrated by selective concentration of HCV and RF in the vasculitic lesions (5,22). Virus and antibody concentration are 10 and 1000 (respectively) fold higher in cryoprecipitate than in serum (23-25).

It is now well established that type II MC is not a low-grade malignancy, as was previously thought, but rather the result of an antigen-driven process directed in most cases by the hepatitis C virus. 80% of the monoclonal IgMs found in HCV-MC patients share a major complementarity region named WA. This WA cross idiotype (named after the patient WA in whom it was initially found) has been recognized for many years to be associated with a high degree of rheumatoid activity. These antibodies almost invariably express a Vκ light chain derived from a single germinal gene, the human KV 325 (3). The antibody repertoire of IgM RFs in HCV is thus extremely limited. They are encoded by just a few genes, probably in response to very close antigenic stimuli.

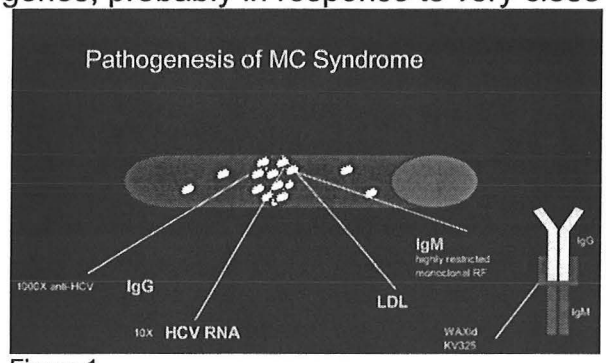

Figure 1.

The predominant IgM κ RF present in type II MC is generated mainly in the liver, and also in the bone marrow (26). The formation of lymphoid follicles in the liver is a characteristic histologic feature of chronic hepatitis C infection (27). Immunophenotyping of the mononuclear cells found in liver biopsies of patients with HCV and MC shows that they are mostly B cells expressing IgM (28,29). Clonal and phenotypic analysis of the B cells in the liver and bone marrow has shown that the monoclonal IgM k infiltrating the liver are CD5+, a phenotype that has previously been associated with RF production, whereas the monoclonal IgMκ B cells in the periphery are CD5- (30,31).

The mechanism by which HCV infection leads to specific and exuberant stimulation of B cells is beginning to be understood. Hepatitis C is lymphotropic and it may replicate in lymphocytes, although to a lesser degree than in hepatocytes (32).The second portion of the HCV envelope (E2 protein) binds to CD81(33). CD81 is a tetraspanin receptor that is present on all nucleated cells; its varied functions depend upon the cell type and associated molecules. On B cells, CD81 is associated with CD19 and CR2 and also with MCH class II. Ligation of CD81 on B cells activates this complex, which lowers the antigen threshold necessary for antibody stimulation, thus rendering the B cell hyperresponsive. EBV, which binds to the CD21 component of this complex, stimulates lymphoproliferation in a similar manner. By itself, this process could lead to a type III (polyclonal) MC, but a dominant B cell clone must emerge in order to produce a type II (monoclonal) MC. Type III MC, therefore, may be a precursor to type II MC in some patients. In one Korean study, 80% of patients with HCV and MC were type III, and progression to type II has been documented in some cases (34).

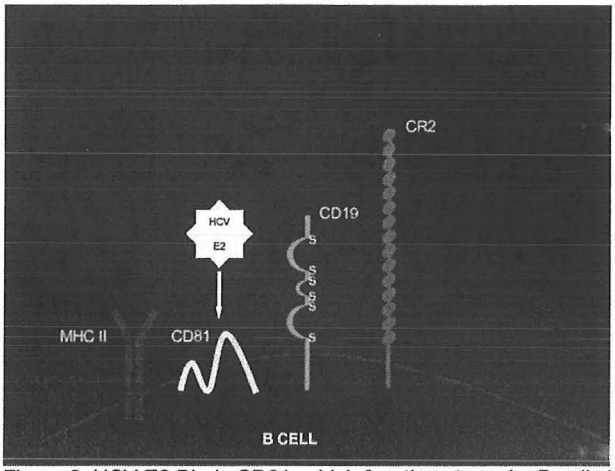

Figure 2. HCV E2 Binds CD81, which functions to make B cells hyperresponsive.

The emergence of a dominant B cell clone capable of producing monoclonal IgM WA probably occurs when one of the B cells undergoes a genetic alteration that enhances B cell survival. For example, translocation of Bcl-2 gene from chromosome 18 to chromosome 14 (next to Ig heavy chain) [t(14;18)translocation] results in overexpression of the anti-apoptotic gene bcl-2 (35). 88% of patients with HCV and cryoglobulinemia, as compared to 8% of patients with HCV and 2% of patients with other liver diseases and 3% of persons with other rheumatoid disorders demonstrate this genetic mutation (36).

Exactly which subset of patients with chronic hepatitis C infection will develop MC cannot be predicted at this time. Three general potential risk factors include 1) specific qualities of the virus, 2) host genetic predisposition, and 3) duration of viral infection. Originally, some investigators reported high prevalence of genotype 2 in some patients (27,37), but most studies have found no such association (38-40). There is a slight female preponderance (60-70%) and HCV-MC patients often older (21). One prospective study of HLA class II alleles was performed in a cohort of 158 HCV patients with and without MC. By univariate and multivariate analysis, HLA-DRB1*11 (DR11) was the only positive predictor (41.1% versus 17.1%, odds ratio 2.58) and HLA-DR7 was protective(13.2% vs 30.5%, OR 0.34) for the development of MC (41). In almost all studies, length of infection appears to be an important risk factor for the development of MC. In one French study of 127 patients, the apparent duration of infection was 14.2 +/- 13.7 years for Type II MC, 7.6+/-7.7 for Type III MC, and 5.5 +/-4.3 for patients without cryoglobulinemia (15)

Another interesting question is why some patients with HCV-MC are symptomatic and others are not. Both the level of the cryocrit and the degree of RF activity of the cryoglobulin appear to be important. A discrete-sized D region in WA-positive IgM is strictly required for the RF activity (42). Specific D segments confer a high degree of RF activity and are associated with the clinical syndrome. In one study 9/9 of patients with the cryoglobulinemia syndrome used D21 or D2 genes in their Ig with RF and 8/8 who had detectable RF but no history of cryoglobulinemia syndrome did not (43). Thus, an individual person's antibody repertoire may be a risk factor for the development of MC.

Treatment

Prior to the discovery of HCV as the major cause of MC, symptomatic flares were usually treated with plasmapheresis to remove circulating cryoglobulins and steroids and/or cyclophosphamide to decrease production of cryoglobulins and suppress inflammatory injury to the vessel wall. These treatments were effective at improving vasculitic manifestations and preventing deposition in the glomeruli and vessel walls, but the immunosuppression enhanced viral replication and no definitive long-term benefit could be demonstrated.

Several investigators have studied the effect of interferon on MC, and all have found a remarkable improvement in symptoms in 2/3-3/4 of patients (44-47). More than 50% with clinical features respond to interferon (decreased HCV RNA, cryocrit, RF level, and symptoms), but only 10% achieve a long term remission. almost all relapse shortly after treatment is stopped (48,49). Misiani studied 53 patients with HCV and cryoglobulins and cutaneous vasculitis and renal disease. Symptoms improved in the 60% that had disappearance of RNA, but all relapsed (48). The chance of achieving a sustained virologic response is the same, whether the patient has MC or not. The largest studies were done in the early 1990's, when interferon monotherapy was the only treatment available and the sustained response rate was only about 15%, regardless of MC status. It is interesting to note that interferon had historically been tried to treat patients with mixed cryoglobulinemia because of its anti-proliferative effects long before the association with HCV was made. These early trials demonstrated complete response rates of up to 50%, which is actually better than the complete response rates of more recent interferon trials (46,47,50). This greater efficacy may be due to the fact that higher doses of interferon (3MU daily or every other day) as compared to the standard regimens used to treat hepatitis (3MU TIW) were used. We now recognize (after numerous hepatitis trials) that higher doses of interferon are more effective at treating HCV, but also more poorly tolerated. The predictors of a complete response to treatment are the same in patients with or without MC, namely genotype 2 or 3 or lower pre-treatment viral load (51).

Long term interferon therapy is effective in controlling symptoms in patients who are partial virological responders, particularly those with cutaneous vasculitis. Lowering the viral load is associated with, but not absolutely necessary for, an improvement in symptoms (45,52-54). In addition, viral loads measured using standard PCR procedures are unreliable in patients with cryoglobulinemia because varying amounts of viral RNA may cryoprecipitate out of the serum. It is well documented that after the serum viral load becomes undetectable by routine serum PCR assay, the cryoglobulins may persist at a low level (<1%), and these cryoglobulins harbor the virus. Therefore new treatment endpoints need to be developed for patients with HCV and cryoglobulinemia. Agnello has proposed that disappearance of the B cell clonal expansion and undetectable HCV in B cells is a the most appropriate treatment endpoint for patients with HCV-MC.

Long term suppressive therapy with combination steroids and interferon alpha does not improve the clinical response rate beyond what is achieved with long term interferon monotherapy (53% combination versus 53% interferon only,

versus 17% prednisone only, or 7% untreated). However, initial responses were slightly earlier, and relapse times were delayed by a few months in the combination group. Viral load increases about 40% in pts on prednisone alone, but not in the combination group (49). However, combined antiviral and immunosuppressive therapy may be treatment of choice in patients with severe renal disease (24,55,56).

The combination of interferon and ribavirin has been tested in a small number of patients who failed interferon monotherapy (N=9). In this trial, 2/9 subjects became HCV -. All patients experienced improvement of MC symptoms within 10 weeks. Arthritis resolved in 7/9 and improved in 1/9. 24 hr proteinuria improved substantially in 3/3. Serum Cr improved, but renal failure was not reversed. Peripheral neuropathies were resistant to therapy; 1/4 experienced slight improvement after 4 months. All subjects relapsed off treatment and symptoms correlated well with the cryocrit (57).

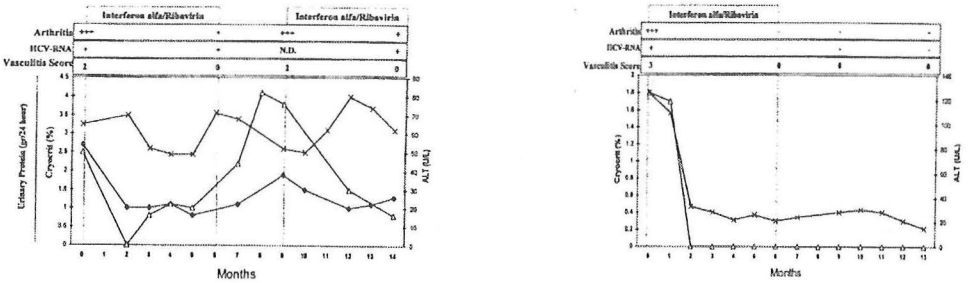

Figure 3. Symptoms of cryoglobulinemic syndrome improve during treatment but relapse after treatment if HCV is not eradicated. J Rheum 2000;27:2172-8.

Ribavirin monotherapy failed as a potential cure for HCV liver disease because it only improved ALT without achieving sustained viral clearance. In one small study (n=5), ribavirin monotherapy markedly improved MC symptoms for 10-36 months. HCV RNA remained detectable in all patients, but the level was decreased in three. When therapy was discontinued, symptoms recurred. Thus, ribavirin may have some efficacy in patients who cannot tolerate interferon, but its role is not defined (58).

Treatment of hepatitis C liver disease has improved substantially over the past 15 years, largely due to the strong support of the US pharmaceutical industry, which has sponsored large multi-center trials using standardized protocols and reasonable control populations. Unfortunately, clinical manifestations of the cryoglobulinemic syndrome are less frequent and often unrecognized throughout most of the world (with the exception of southern Italy). Most studies involve small populations without control groups. Experts do not agree on what the appropriate end point is for treating these patients. No data has been published regarding the effects of combined pegylated interferon and ribavirin, which is now the state of the art therapy for active liver disease due to HCV. Pegylated interferon is the long-acting formulation of interferon. Compared to regular alpha-interferon, it has better viral suppression and improved rates of sustained viral clearance. Therefore it is logical to extrapolate these finding to predict that it will become the state of the art therapy for HCV-MC symptoms as well.

Cutaneous vasculitis

The most frequent cutaneous manifestation of the MC syndrome is palpable purpura of the lower extremities, although upper extremities, abdomen, and/or buttocks are sometimes also involved. Petichiae, papulae, livedo, and ulceration may also be present. Biopsy shows a leukocytoclastic vaculitis, an immune complex vasculitis involving small vessels with a mixed inflammatory infiltrate composed of predominantly mononuclear cells within the walls of the vessel. Some patients have fibrinoid necrosis of the arteriolar walls and infiltration of monocytes in and around the wall and vascular thrombi (59). HCV antigens are demonstrated in skin lesions. Cutaneous vasculitis is the MC manifestation which responds the best to antiviral therapy. Skin lesions disappear in those that become HCV RNA negative and improve significantly in the rest. Improvement is often seen within the first month of therapy (44,52).

Renal Disease

Prevalence

The most common renal manifestation of HCV is MPGN (membranoproliferative glomerulonephritis). Less common are proliferative or membranous GN and focal segmental glomerular sclerosis (24,60). HCV infection accounts for a significant proportion of MPGN cases. 10%-20% of MPGN in the US is associated with HCV infection (61). In Japan, as many as 60% of adult MPGN may be HCV-related (62). 81% of MC-associated MPGN is HCV RNA+ versus 25% of non-cryoglobulinemia GN (8). Most, although not all, HCV-related renal disease is related to MC.

Clinical Features

The typical patient with HCV-related renal disease is recognized when he/she presents with nephrotic syndrome (71%). They often (72%) have serum albumin <3 g/dl with mild renal insufficiency. A few progress to dialysis (24).

Lab abnormalities in 34 patients with HCV-associated glomerulonephritis (61)

Creatinine clearance	31-76 ml/min
Urinary protein	0.4-17 g/day
Anti-HCV	100%
HCV RNA	100%
Cryoglobulins	62%

All have detectable viremia and antibody to HCV. Most have cryoglobulinemia (59% at first visit, 85% overall), but only 44% have extrarenal manifestations of cryoglobulinemia. Liver disease may be occult; 82% have no clinical manifestations of liver disease, while 44% have completely normal liver tests. However, 88% will have abnormal liver biopsy consistent with a chronic active hepatitis, often with cirrhosis (50%) (24). Complement levels are also depressed in majority (8,25).

Pathogenesis

Renal biopsy reveals distinct morphological features consistent with immune complex disease: endocapillary proliferation, monocytic infiltration, double contour membranes. Tubular and annular crystalline structures are also found in the cryoglobulins. Glomerular deposits are mainly IgG, IgM and C3. IgM in glomerular deposits has RF activity and corresponds to monoclonal RF in cryoglobulins (61,63). Demonstration of HCV RNA in affected glomeruli has been inconsistent. The HCV antigens are often difficult to demonstrate using antibody probes due to RF-induced artifact. One group found HCV proteins in 2/3 of patients with MPGN and type II MC after liver transplant (64), but others cannot confirm this finding (65). RF from patients with type II MC has high affinity for cellular fibronectin, which is richly represented in the glomerular mesangium, so some have speculated that RF causes deposition of Ig in glomerulus without the presence of HCV (66).

Treatment

The largest trial clinical trial of HCV-related MPGN treated14 patients with interferon monotherapy (24). In that study, proteinuria improved, but Cr did not. All patients relapsed when therapy was stopped.

	HCV RNA- during ifn		HCV RNA + during ifn	
	Before	After	Before	After
Creatinine (mg/dl)	1.8 +/- 0.5	1.5 +/- 0.7	2.0 +/- 0.8	1.8 +/- 0.8
Urine protein (gday)	6.1 +/- 4.6	1.3 +/- 1.5 (to 0 in 4 pts)	6.1 +/- 3.3	4.2 +/- 4.2

Established renal disease may regress if the HCV eradicated. One patient with HCV infection, cryoglobulinemia, nephrotic syndrome, and MPGN was treated with high dose interferon and cleared the virus. After one year, the patient was still negative for HCV RNA and had histologic improvement on renal biopsy (62).

Results from large numbers of patients treated with interferon/ribavirin are lacking, but positive results a few cases have been reported (55-57,67).

Pulse steroid therapy is effective as a temporizing measure, but long-term immunosuppression has many adverse side effects, including enhanced viral replication. Combined antiviral and immunosuppressive therapy is feasible and may be treatment of choice in patients with severe MPGN or disease that is refractory to interferon (24,68)

Neuropathy

Most HCV-related peripheral neuropathies due to type II MC, although some are due to polyarteritis nodosa, and a few are related to neither (69). HCV-related MC may also cause encephalopathies or optic neuropathy, but peripheral neuropathy is by far the most common neurological symptom (69,70).

Prevalence

The prevalence of neuropathies in HCV patients is unknown, but peripheral neuropathies due to MC are frequently unrecognized. In one series from southern Italy, 13% of chronic idiopathic sensory polyneuropathies were found to have cryoglobulins in the serum, even in the absence of skin lesions or other indications that vasculitis may have been present (71).

Clinical Features

MC-associated neuropathy is classically a moderate axonal sensory polyneuropathy. It may involve bilateral nerves symmetrically or multiple isolated nerves (polyneuritis or multiple mononeuropathies). They are often painful for months or years before motor deficits develop. Pure motor polyneuropathies have never been described. The diagnosis is best made by nerve biopsy, which may show loss of myelinated fibers in the axons, and mononuclear cell infiltrate around vessels. It is important to remember that involvement is patchy and the lesion may be missed on biopsy. Only a few cases have demonstrated the typical deposits of IgM, IgG, C3, C1q, C4 complement components and fibrin, and HCV is inconsistently detected in lesions (72,73).

HCV is sometimes implicated in the pathogenesis of PAN. Hepatitis B, in contrast, is commonly associated with PAN. In retrospective studies of PAN, the prevalence of HCV antibodies in 5%-12% (74,75).

The clinical presentation of PAN is different from MC-associated neuropathy. Lesions are typically asymmetrical (multifocal mononeuropathies versus symmetrical distal, with necrotizing vasculitis of medium sized arteries found on biopsy. Motor involvement is more prominent, and life-threatening systemic vasculitis involvement of abdominal vessels more frequently occurs (76,77).

PAN	MC
Life threatening systemic	
Severe multifocal sensorimotor	Distal moderate sensory
Malignant HTN	
Cerebral angiitis	
Ischemic abdominal pain	
Kidney and liver microaneurisms	
Increased ESR, C-reactive protein	
Renal insufficiency	Renal insufficiency
Medium size artery involvement	Small-medium size artery involvement
Necrotizing vasculitis (occlusion of lumen, fibrinoid necrosis)	Mononuclear cell infiltrate in perivascular areas
Good response to interferon + prednisone + plasma exchange	Poor response to interferon

Numerous cases of peripheral neuropathy in individuals without MC or PAN have been reported. Their response to treatment is highly variable. (8, 70,78).

Treatment

In general, the response of peripheral neuropathies to anti-viral therapy has been discouraging. To date, most clinical trials of MC-related neuropathies using viral suppression with interferon therapy have reported either no response or continued progression of neurological symptoms (44,79). In one study of non-MC, HCV-related peripheral neuropathy, 3/4 cases improved with interferon therapy, as opposed to 4/4 control cases of HCV-MC-related neuropathy that all worsened (79).

Of patients who can tolerate long term (more than 24 months) of anti-viral therapy, gradual improvement may be seen (52,54,57,80) so the poor response of neuropathies as compared to skin lesions in short term trials may just represent the difference in the speed of regeneration of the target organ. Of concern, however, are several cases of dramatic worsening of symptoms from interferon therapy. The combination of plasmapheresis and long-term, high-dose alpha interferon is currently under investigation (45).

PAN can be a dramatic and devastating disease. Combination therapy given in sequential fashion has been demonstrated to be efficacious against PAN associated with HCV. In one of the largest French studies, patients were initially treated corticosteroid therapy, followed by plasma exchange, followed by α-interferon. Then maintenance therapy with low dose prednisone and α-interferon was given for 8 months. They had complete recovery in all but one pt without significant side effects (81).

Lymphoma

About 10% of Type II mixed cryoglobulinemia may evolve, after several years post diagnosis, into a malignant lymphoma (29,82). Many are older patients with longstanding HCV infection and symptomatic cryoglobulinemia (39,83,84)

Epidemiological studies form Italy, Japan, and US implicate also HCV in the pathogenesis of a subset of B cell tumors not complicating the course of MC syndrome (85-87).

Prevalence

HCV-related NHL represents a significant portion of worldwide cases of NHL, although it varies considerably (0-42%) in different studies.

Country	# NHL	% HCV + NHL	% HCV+ Controls
Canada (88)	100	0	0
France (89)	201	2%	1.1%
Italy (87)	311	9%	No controls
USA (90)	312	11.5%	No controls
Italy (91)	126	21%	1% matched
USA (86)	120	22%	5%
Italy (85)	157	22.3%	No controls
Italy (92)	91	23%	1.9%
Italy (93)	199	28.6%	2.87% p<.00000001
Italy (94)	50	34%	3% (not age matched)

The corollary to this observation is that 25-40% of HCV patients have a lymphoproliferative disorder evident on random bone marrow biopsy (6,39,84,95-97). A case control study performed in Italy found that HCV infection increased the risk for NHL of the liver or salivary glands 50-fold, which was greater than the relative risk (RR) of hepatocellular carcinoma. The RR for for NHL of other sites was increased about 4-fold (98).

Clinical Features

Follicular and lymphoplasmocytoid lesions are the most common types of NHL reported in association with HCV, but a wide range of tumor types has been reported (87). Many are low-grade, but some series report no difference in tumor stage and prognosis as compared to other NHL (85,99). Extranodal involvement is common (65%HCV-related versus 19% non-HCV), with liver and major salivary glands being significantly overrepresented (86,99). Another site reported not infrequently is the stomach, and HCV has been associated with gastric lymphoproliferation and has proposed to be another potential cause for MALT lymphomas in addition to *Helicobacter pylori* (98-101).

Pathogenesis

The preponderance of data indicate that HCV-associated lymphomas arise out of B cell proliferation that is driven by the same process that drives cryoglobulinemia production. It is well documented that HCV infection usually precedes NHL by many years (83,98,99). Sequencing of the antigen binding region of the immunoglobulin that is produced by the malignant lymphocytes demonstrates that they have a high degree of homology to both antibodies specific for E2, as well as the antibodies produced by B cells that secrete RF(43,102) Translocation of bcl-2 to form a fusion gene with the Ig heavy chain region occurs in 13/15 86% of Israeli patients with HCV and MC II, as compared to 2/12 16% of HCV patients w/o MC and 0/7 patients with other liver diseases and 0/10 healthy controls (103). A "second hit," therefore, is likely all that is needed for the development of NHL in a person with HCV-MC. In one well-documented case, the bcl-2 translocation was present w/cryoglobulinemia and benign lymphocytosis, and then progression to accelerated malignant lymphoma occurred at the time of a second mutation of the myc oncogene to the λ light chain locus (104).

Treatment

Low-grade monoclonal B-cell proliferation can regress with interferon treatment and clearance of the virus (105), but high-grade malignancies require systemic chemotherapy. Zuckerman studied the effect of anti-viral therapy on the t(14,18) translocation and Ig gene rearrangement in 15/29 patients. The IgH rearrangement became negative In 7/9 patients, versus 1/8 untreated patients, and the t(14:18) translocation became negative in 6/7 treated versus 1/6 untreated. 2 untreated patients progressed to develop overt lymphoma(106).

Sjögren's Syndrome

Prevalence

The epidemiological association between Sjögren's syndrome and HCV infection is strong. Although only 10% of HCV-infected persons will have symptomatic xeropthalmia or xerostomia, up 77% will have evidence of Sjögren's syndrome if it is sought with diagnostic tests. Note that this is an opposite relationship as compared to the general population, where complaint of dry eyes (5%) is more common than finding histological evidence (<0.5%)(107).

Prevalence of Sjögren's in HCV

Country	Prevalence	Definition
Sweden (12)	67%	Xeropthalmia on exam,* sialometry, lip biopsy
		symptoms
	9%	
	14%	Grade 3,4 Lymphocytic sialadenitis similar to Sjogren's
UK + France		
(40)		Lymphocytic capillaritis
	49%	symptoms
	2%	
Italy (108)	77%	Grade 1,2 lymphocytic sialadenitis
France	57%	>1 nodular lymphocytic focus per $4mm^2$
(109)		symptoms
	36%	
USA (1)	10%	Symptoms

*semiquantitative rose bengal stain, Schirmer, break up time

Only 5-10% of patients with Sjögren's syndrome, however, has evidence of HCV infection (110-112)

Clinical Features

Females are the predominant sex affected by HCV-associated Sjogrens. In fact, 0ne study that examined almost exclusively males with HCV failed to find any histologic evidence of Sjögren's (113). There are distinct differences between HCV-associated Sjoören's and primary Sjögren's, namely the absence of anti-Ro/SS-A and Anti-La/SS-B, RNP, Jo-1, PCNA or Scl-70 (12,111,112).

	HCV-SS (n=51)	Primary SS (n=110)	P value
ANA	49%	58%	ns
Anti-smooth muscle	43%	25%	ns
Anti-Ro/SS-A Anti-La/SS-B	14%	38%	.01
RF	55%	36%	.05
Cryoglobulins	60%	10%	.000
Hypo-complementemia	60%	8%	.000

Pathogenesis

The process begins as a lymphocytic capillaritis of the glands, which progresses to a lymphocytic sialadenitis. It is not clear whether HCV directly infects the salivary/lacrimal glands. HCV has consistently been demonstrated by PCR in saliva and is concentrated 10-fold in tears as compared to plasma (114-116), but viral proteins were not demonstrated in the glandular tissue by immunohistochemistry in the one study that examined this (12). It is possible that there is molecular mimecry to an HCV protein, or that HCV proteins may directly stimulate immune attack of the salivary/lacrimal glands. 84% of mice transgenic for HCV envelope proteins (E1,E2) develop sialadenitis, as compared to 2% of mice siblings, and 0% of HCV core protein transgenics. These mice express E1,2 in both liver and glands but have no liver disease. The severity of their histololgic disease correlates with the level of expression of E1,2 by Western blot (117). These transgenic studies indicate that HCV is directly linked to the cause, rather than an innocent bystander in the development of Sjögren's in HCV-infected patients.

Treatment

To date, there are no studies addressing the efficacy of interferon as a treatment for HCV-sialadenitis.

Porphyria Cutanea Tarda

Prevalence

Porphyria cutanea tarda (PCT) is the most common form of porphyria. The reported prevalences of HCV in patients with PCT ranges considerably, but averages about 45%. The association is stronger in Southern Europe and the US than in northern Europe, and it is broadly related to the level of endemicity of HCV in the general population as well as the severity of liver disease in the population studied. The association of PCT with HCV is strongest in those patients that have cirrhosis (60%-90%, 124,126)

Country	PCT cases	HCV Prevalence in sporadic PCT
NZ (118)	25	0%
Germany (119)	106	8%
Ireland (120)	20	10%
Oz (121)	27	25%
USA (Mass) (122)	70	56%
USA(Utah) (123)	108	59%
Spain (124)	34	71%
S. France (125)	13	77%
Spain (126)	100	79%
Italy (127)	74	82%

In all countries, PCT is an uncommon complication of HCV infection. A prospective study of 321 mostly non-cirrhotic patients found that the incidence of PCT did not exceed that of the general population (1%-2%)(1). Elevated

urinary uroporphyrin levels are only detected in 0.5-22% of patients with chronic HCV infection (122,128,129).

Clinical Features

The clinical manifestations of PCT are dermatological only and include increased skin fragility, bruising, vesicles and bullae that may become hemorrhagic in the sun-exposed areas. Over time, pigmentation, depigmentation, hirsutism, and sclerodermoid appearance can develop.

Pathogenesis

PCT is caused by reduced (<50%) activity of uroporphyrinogen decarboxylase (Uro-D). There are two types: type I (sporadic, common) in which the enzyme activity is reduced only in the hepatocytes, and type II (familial, less common) in which the defect is also present in other cell types. The enzymatic defect is necessary but not sufficient for clinical manifestations. The majority of persons with the familial form have a 50% decrease in Uro-D activity, but most still do not exhibit symptoms. Because a high proportion of symptomatic PCT patients have evidence of HCV, but a low percentage HCV patients have asymptomatic PCT, it is thought that HCV may be trigger for clinical expression, but that it is insufficient alone to cause porphyrin metabolic derangement. Enzyme expression is modulated by alcohol, estrogens, and iron overload. In many studies, alcohol was a co-variant with HCV, so they could not be evaluated as independent risk factors. In one large study (124) however, the prevalence of HCV in PCT was 71%, as compared to 17.5% of pure alcoholic liver disease or 0.79% for blood donors. Many cases of PCT that were previously attributed to ETOH, therefore, were probably present in patients with both a history of alcohol and HCV.

The mechanism by which HCV triggers clinical expression of the disease is not completely known, although it is probably related to HCV's specific propensity to cause increased hepatic iron accumulation. The virus has no effect on hepatic uroporphyrin decarboxylase activity (130). It is likely that a latent Uro-D defect results in an increase the uroporphyrinogen pool. Increased oxidative stress by liver injury from accumulated iron increases the likelihood that uroporphyrinogen will be oxidized to uroporphyrin. Uroporphyrin is not a substrate for uro-D, and thus these porphyrins accumulate in liver cells. Other non-porphyrin products of uroporphyrinogen oxidation can inhibit uro-D further and thereby increase the accumulation of uroporphyrinogen and uroporphyrin in hepatocytes.

Few studies have reported HCV status and iron saturation status in the same individuals, although one study (131) found iron overload in all HCV-infected patients with PCT. Some have hypothesized that HCV decompartmentalizes iron to create "free iron" which may lead to formation of active free radicals or that HCV decreases intracellular glutathione concentration (122).

Treatment

There are no published trials of anti-viral therapy specifically addressed to patients with PCT and HCV. Several case reports indicate that successful eradication of the HCV virus with interferon-based therapy may improve symptoms (132,133). The mainstay of treatment remains phlebotomy, which is effective at controlling symptoms and often improves ALT as well. It is logical that persons with chronic HCV infection should be treated with anti-viral therapy in a effort to clear the virus after elevated iron stores have been depleted through phlebotomy. The question of whether an individual with normal ALT and minimal inflammation with no fibrosis on biopsy and symptomatic PCT should be treated with anti-viral therapy has not been addressed because, unlike cryoglobulinemia, PCT is hardly ever present in the absence of overt hepatitis.

Diabetes Mellitus

Several studies have reported an association between hepatitis C and diabetes mellitus (DM). One of the problems in interpreting these studies is their frequent failure to account for other variables that predispose to diabetes and lack of matched control groups. For example, cirrhosis has been associated with abnormal glucose metabolism for over 20 years. Decreased hepatic insulin clearance leading to hyperinsulinemia and development of insulin resistance, as well as reduced hepatic uptake of glucose are all known to occur in cirrhosis and predispose the cirrhotic patient to the development of NIDDM (134). Once patients and controls are matched for cirrhosis and other variables known to predispose persons to diabetes mellitus, the association between HCV and DM is weak, but nevertheless, still present.

In the largest US retrospective study of 1117 patients with chronic viral hepatitis, the prevalence of type 2 DM was 21% in HCV patients versus 12% in HBV patients. Both groups were well matched with respect to cirrhosis. DM was strongly associated with cirrhosis. After excluding patients with conditions predisposing to DM, there was no difference in the prevalence of DM in non-cirrhotic HCV or HBV. In cirrhotic patients, however, HCV patients were significantly ($p=.04$) more likely to have DM than HBV cirrhotic patients. Unfortunately, the HBV group contained significantly more young persons, and the HCV group contained significantly more females (135). Other groups have confirmed that patients with HCV cirrhosis is associated with DM, although none have perfectly matched control groups (134,136-8)

In a large well-done study from the UK and Sicily (134), 1,332 patients with HCV cirrhosis were compared to 181 patients with HBV cirrhosis. The prevalences of diabetes were 23.6% and 9.4%, respectively ($p=.0002$). The prevalence of diabetes was higher in the HCV patients than the HBV patients at each Child-Pugh score, reaching 50% at a score of 15. In a prospective part of this study, glucose intolerance was measured in patients with chronic hepatitis B and C. HCV was not a significant predictor of glucose intolerance by multivariate analysis. Cirrhosis, on the other hand, was strongly associated with glucose

intolerance. Only 1/70 (1%) patients with non-cirrhotic chronic hepatitis had glucose intolerance, compared to 34/165 (21%) patients with cirrhosis.

Even in those patients who have liver function restored through liver transplantation, there is a higher incidence of HCV-infected recipients to develop DM than in those transplanted for other liver diseases (139).

Pathogenesis

The incidence of autoantibodies present in autoimmune insulin-dependent DM(anti-insulin, anti-Langerhans islet, anti-glutamic acid decarboxylase antibodies) is not increased (1). HCV patients with cirrhosis and DM have high insulin levels, consistent with the insulin resistance seen in cirrhosis. However, the acute insulin responsiveness is also subnormal, suggesting that in addition to insulin resistance, HCV persons may also have β-cell dysfunction (134). HCV RNA has been detected in pancreas, indicating that the virus might be able to infect the pancreas (134).

Treatment

In one trial of interferon (open, no controls) for the treatment of non-diabetic patients with HCV, anti-viral treatment was associated with an improvement in glucose tolerance. This improvement was attributed to an increase in hepatic insulin clearance and a reduction of free fatty acids, but there was no change in first phase insulin response. (140). However, several case reports also suggest that interferon may enhance an underlying autoimmune process against beta cells and induce overt IDDM in persons who are genetically predisposed (141-143)

Fact or Fiction?

A number of additional potential extrahepatic manifestations of chronic hepatitis C have been reported. Many are epidemiological associations drawn from relatively small retrospective studies, so caution must be taken in interpreting these studies to exclude the bias of small sample size, inappropriate controls, and failure to exclude other potential explanations. With the exceptions of cryoglobulinemia, lymphoma, and Sjogren's syndrome, no pathogenic mechanism has been proposed for most of these associations. Although many may ultimately prove to be true extrahepatic manifestations of chronic HCV, more careful clinical investigation is needed to prove these associations.

Thyroid Disease

For example, thyroid disease has been frequently reported in persons with hepatitis C. Many of these reports, however, are of persons undergoing interferon treatment for their hepatitis, and alpha interferon has been previously known to unmask thyroid disease in patients with other illnesses. Approximately 3%-5% of patients with chronic HCV who are not taking interferon has biochemical evidence of hypothyroidism, which is not significantly different from controls (144). The prevalence of thyroid antibodies in patients with HCV who are

not taking interferon has been reported to be increased and ranges from 3% to 36% (145). However, this finding is usually observed in the older female patients, and the prevalence of thyroid antibodies is 15-24% in normal women over the age of 60 (146,147). These data highlight the importance of selecting the appropriate age and sex-matched controls in trying to establish a link between HCV and other diseases. Many of these patients have subclinical disease, but about one third to one half will develop symptomatic thyroid disease when given interferon (148). High titers of antithyroid antibodies before interferon therapy are significantly associated with the development of thyroid dysfunction during interferon therapy.

Eye Disease

Mooren ulcers have been reported in a handful of patients with chronic HCV, and some have even noted that the lesion resolved with interferon treatment (149,150). One study of 21 South Indian patients with Mooren ulcer and 44 controls, however, reported that none of the ulcer patients but 1 of the controls was HCV + (151).

Retinopathy frequently complicates the course of HCV patients receiving treatment with alpha-interferon (152-154), but this is a side effect of interferon that is not specific to HCV patients. One third of patients without pre-existing DM or HTN will develop a reversible retinopathy which usually develops with the first 8 weeks. Almost all patients with pre-existing DM or HTN (80%-100%) develop an interferon-associated retinopathy which may be permanent.

Lichen Planus

Lichen planus is known to be associated with chronic liver disease. The prevalence of liver disease in patients with lichen planus is reported as high as 35% (155). Several small studies have suggested that HCV may be related (155-158). A large prospective study of mostly non-cirrhotic HCV patients, however, found that the prevalence of lichen planus (1%-2%) did not exceed that of the general population (1). Although lichen planus disappeared in one patient after interferon therapy (159), severe exacerbations have been reported in several cases (160,161). Therefore, use of interferon in patients with HCV and lichen planus should be undertaken with great caution.

Idiopathic Pulmonary Fibrosis

One Japanese group reported found a high prevalence of anti-HCV in IPF patients (19/66 or 28.8%), as compared to the general population (162). However, another British group has failed to confirm this (0/62) (163).

Rheumatoid Arthritis

Rheumatoid arthritis is often misdiagnosed in patients with HCV on the basis of arthralgias and positive rheumatoid factor. A careful history and radiographs will usually disclose that the patient does not have morning stiffness or the characteristic radiographic features of rheumatoid arthritis. Rather, a history of intermittent nonerosive oligoarthritis involving large and medium sized joints is often observed.

Autoimmune Hepatitis

HCV-infected patients express a high prevalence of a variety of autoantibodies, usually in low titers. The clinical significance of most of these autoantibodies is unclear. Many are also present in autoimmune hepatitis, which may pose a diagnostic dilemma. 70% of adult and 80% of pediatric HCV-infected patients have at least 1 autoantibody and 13% have 3 or more (1,164). The most common autoantibodies are ANA (4%-41%), anti-smooth muscle (9%-22%), anti-thyroglobulin (8%-13%) and anti-cardiolipin (20%-27%). Anti-LKM1, and anti-GOR are also found in <15% (1). The reason for these high prevalences is not understood. It appears to be more than a nonspecific polyclonal activation of B lymphocytes by HCV because other autoantibodies are usually negative. One autoantibody, anti-GOR, is likely produced because these cross-reactive autoantibodies are specific for both HCV core and the host nuclear antigen, GOR. The LKM-1 Ab was previously thought to be very specific for type II autoimmune hepatitis, a childhood variant that follows an aggressive course resistant to steroids. However, it is now recognized that HCV is the most common cause of the LKM-1 Ab in the US (165). None of these HCV-related autoantibodies is associated with a particular genotype, clinical profile, or treatment outcome (164,166). Recognizing that HCV infection alone may account for autoantibodies that were previously thought to be specific for autoimmune hepatitis may prevent the erroneous diagnosis of autoimmune hepatitis in association with HCV infection.

Fibromayalgia

One study has evaluated fibromyalgia in 90 patients with HCV,128 healthy controls, and 32 patients with non-HCV cirrhosis. 16% of HCV patients versus 3% of cirrhotic controls met diagnostic criteria for fibromyalgia (167), but these findings have not yet been confirmed.

Summary: Principles of Extrahepatic Manifestations of HCV

- Extrahepatic manifestations of HCV are common (38%).
- Long-term infection is necessary for the development of most extrahepatic manifestations; many are present primarily in patients with cirrhosis.
- MC is strongly associated with HCV. Do not be misled by a negative cryoglobulin test.
- Renal disease, lymphoma, Sjogren's, and peripheral neuropathies manifest incomplete overlap with cryoglobulinemia
- Anti-viral therapy is efficacious for extrahepatic manifestations, although mostly features related to cryoglobulinemia have been studied in trials.

Summary: Treatment of HCV-MC

- The initial goal should be cure of HCV with state-of-the-art anti-viral treatment.
- Severe symptoms may be controlled with addition of plasmapheresis or immunosuppression in the acute setting.
- If HCV is not eradicated, then long term suppression with antivirals may be appropriate. Follow symptoms, cryocrit. Clearance of virus with increase in cryoglobulins should prompt an evaluation for malignant transformation.
- Patients who cannot tolerate interferon + ribavirin therapy may be treated with monotherapy.
- Recognize that neurological deficits and severe renal disease may not reverse or may progress with anti-viral therapy

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