

Chronic hepatitis C virus infection and haematological diseases: how established is the link?

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Abstract. The hepatitis C virus (HCV) is a well known cause of chronic liver disease, liver cirrhosis and hepatocellular carcinoma worldwide. Among the most exciting areas in basic research is the interaction between HCV and the immune system as well as the involvement of the virus in the induction of autoimmunity. HCV is a hepatotropic as well as a lymphotropic virus and becomes involved in an increasing number of non-hepatic diseases most of which are believed to have an autoimmune background. A wide number of extrahepatic manifestations have been associated with HCV and among them haematological manifestations are not rare. There are conflicting reports for most of these associations, but the most established disease considered definitely linked to HCV, is the syndrome of mixed cryoglobulinaemia. Chronic HCV infection is the main cause of essential mixed cryoglobulinaemia type II and III which is frequently associated with clinical or biochemical evidence of liver damage. Alpha-interferon (α -IFN) is currently the drug of choice to treat cryoglobulinaemia reducing the symptoms and cryoglobulin production. On the other hand, various malignant B cell lymphoproliferative diseases (either associated with cryoglobulinaemia or not) are considered to be also related to HCV. Moreover various, especially immune mediated, cytopenias (immune thrombocytopenic purpura, autoimmune haemolytic anaemia, a.o.) seem to be associated with HCV or can be triggered by the treatment with α -IFN for chronic HCV infection. The present review tries to update the actual knowledge about HCV associated haematological diseases reminding the clinical haematologist to search for HCV infection in certain conditions.

Key words: hepatitis C virus • HCV • hematological disease

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INTRODUCTION

More than 100 million people worldwide are infected by hepatitis C virus (HCV)¹. HCV is responsible for chronic liver disease, with 5-20% of the patients infected progressing to cirrhosis and possible development of hepatocellular carcinoma².

Hepatitis C virus (HCV) is a small single stranded

RNA virus identified in 1989³, which belongs to the family of flaviviridae⁴. The HCV expresses its genetic information in the form of a single large polyprotein of about 3000 aminoacids encoded by an open reading frame (ORF) that extends most of its genomic RNA. Proteolytic cleavage of the ORF product into multiple structural and nonstructural peptides is essential for the virogenesis and the production of the viral progeny.

HCV is characterized by viral persistence. The vi-

Received: March 27, 2004; Accepted: April 6, 2004

rus is capable to escape from the host protective immune response, either by changing image, (there is a high rate of genetic variations with the production of escape mutants during HCV viral replication) or by hiding in privileged sites (infecting cells of the immune system itself that cannot be reached by the virus specific T cell response).

Involvement of lymphoid cells in HCV infection (HCV lymphotropism) can provide insight into the pathobiology of extrahepatic dissemination of HCV (peripheral blood mononuclear cells,⁵ lymph nodes, major salivary glands, kidneys, a.o.) Extrahepatic sites of HCV may also act as a source of continuous infection with HCV and reinfection of hepatocytes.

Numerous extrahepatic disease manifestations, especially autoimmune disorders have been reported in patients with HCV infection⁶ (essential mixed cryoglobulinaemia, porphyria cutanea tarda, membranous glomerulonephritis, Sjögren syndrome, autoimmune thyroid disease⁷ a.o.).

Regarding haematological diseases related to HCV infection a large number of data encourages the "haematology-hepatology linkage".

In this review we describe the spectrum of HCV related haematologic diseases pointing out especially the lymphoproliferative diseases^{8,9} (mixed cryoglobulinaemia and non-Hodgkin lymphoma) and the HCV related cytopenias¹⁰ (autoimmune thrombocytopenic purpura, aplastic anaemia, autoimmune haemolytic anaemia, severe neutropenia, refractory sideroblastic anaemia and pure red cell aplasia). The possible treatment of these diseases with antiviral therapy is also discussed but, on the other hand, therapy induced haematological complications are also mentioned.

HCV RELATED LYMPHOPROLIFERATIVE DISEASES

Shortly after the HCV was identified, a relationship was established between the virus and mixed cryoglobulinaemia (MC)¹¹⁻¹³.

During the later years it became more evident that HCV may have tropism for other cells except of hepatocytes, particularly lymphocytes¹⁴. This lymphotropism is considered to be one of the causes of MC and seems to be also responsible for the development of other lymphoproliferative disorders in patients with HCV infection.

MC is a systemic immune complex-mediated "borderline" (benign/malignant) lymphoproliferative di-

sorder characterized by polyclonal or monoclonal proliferation of B lymphocytes that is associated with the appearance of a monoclonal gammopathy producing serum rheumatoid factor and cryoglobulins. Cryoglobulinaemia is driven by the clonal expansion of B cells¹⁵. This clonal expansion is the result of chronic antigenic stimulation of lymphocytes which have undergone genetic alterations which enhances survival. In many patients with cryoglobulinaemia translocation of the bcl-2 gene was found, with overexpression of bcl-2 which blocks the programmed cell death (apoptosis)¹⁶. The consequence of this genetic alteration is a clonal proliferation of the B cells that produce monoclonal IgM rheumatoid factor (possible directed against anti-HCV?).

Clinical symptoms of MC include purpura, arthritis and glomerulonephritis. During the chronic HCV infection nearly 80% have asymptotically positive rheumatoid factor and about 40%^{17,18} of the patients may develop positive cryoglobulins in serum. The full blown clinical syndrome of MC associated with HCV can rarely be observed. However, patients with HCV and MC have more aggressive liver disease with higher incidence of cirrhosis¹⁹ (40% vs 17%²⁰) and higher fibrosis scores than patients without MC. The serum levels of HCV-RNA in these patients are usually lower despite aggressive liver disease due to significant binding of viral quantities to the cryoprecipitate. No clear association with HCV genotypes was found²¹.

More than 50% of patients with clinical features of cryoglobulinaemia respond to interferon therapy²². Unfortunately most of them (80%) relapse shortly after discontinuation of treatment.

MC is associated with a wide variety of other chronic disorders, including autoimmune²³ and infectious diseases but especially haematologic malignancies. The HCV associated B lymphocyte clonal expansion has been proved to potential evolve from cryoglobulinaemia into overt B-cell non-Hodgkin lymphoma (NHL)²⁴ in 4-6% of the patients²⁵.

A large number of studies have generated conflicting results on this issue, a positive association being found in some of them but not in others. This discrepancy seems to be the result of restrictions to several geographical areas. The incidence of malignant transformation varies in certain areas of the world between 7.4% and 37%. In a large study in France the prevalence of HCV infection in patients with B-cell Non-Hodgkin lymphoma was low 1.83%²⁶, HCV was not considered to play an important role in lymphoma-

genesis. Another study in the neighbouring Italy found a 3.1 times higher incidence of HCV infection in patients with B-cell Non-Hodgkin lymphoma than in controls confirming a positive association²⁷. However, the same group of researcher found also a positive association between HCV and other lymphoid and myeloid malignancies²⁸ comparing with control groups.

The wide spectrum of HCV related lymphomas include:

- 1) the low grade lymphomas preceded by long standing symptomatic MC type II and
- 2) the "idiopathic" non-cryoglobulinaemic intermediate to high grade lymphomas.

The intimate pathogenetical mechanism involved in HCV-lymphomas remains unknown. HCV is not an oncogenic virus but may exert oncogenic potential via two possible mechanisms: a) an indirect mechanism which does not necessary implicates infection of target cells and b) a direct one which hypothesizes that lymphoma is the result of the direct infection of the cells.

For the first presumed mechanism the key event seems to be the chronic antigenic stimulation of B cells by HCV proteins. The most important protein seems to be the HCV-E2 protein²⁹ which was found to activate B lymphocytes which are considered the origin of HCV associated lymphomas³⁰. On the other hand, immunoglobulin variable region genes expressed by B-NHL cells from HCV positive patients, have been shown to exhibit features of ongoing somatic mutations (indicative of antigen selection), as well as the use of a restricted set of variable region genes, indicative of the presence of a common antigen³¹. Recently, the CD81 protein expressed on the surface of various cells including lymphocytes was found to represent one of the HCV receptors. This protein is implicated in B cell activation³².

Another important mechanism that involves HCV in lymphomagenesis is the association with specific chromosomal mutations. Of particular interest is the translocation of the *bcl-2* gene to form a fusion gene with the Ig heavy chain region t(14;18). This mutation has been associated with the transformation of benign follicular hyperplasia to malignant lymphoma³³, and was also found in patients with HCV associated mixed cryoglobulinaemia and benign lymphocytosis who evolved into lymphoma³⁴. However, a recent review of 854 lymphomas in patients with HCV infection⁶, although confirming the predominance of B-NHL with a higher prevalence among patients with cryoglobuli-

naemia, could not document the expected high frequency of follicular lymphoma which is well known associated with t(14;18). The most common HCV associated lymphomas were immunoplasmaeytic lymphomas, extranodal marginal zone cell lymphomas (liver, salivary gland) and Waldenström macroglobulinaemia.

The second proposed mechanism implicates HCV included in the target cell which is supposed to undergo transformation. HCV sequences have been found in lymph node biopsies from patients with B-NHL^{35,36} and HCV-associated proteins were found in lymphoma cells³⁷. In one of the 9 primary liver lymphomas in HCV of infected patients the virus has been detected in the lymphoma cells and not in the surrounding hepatocytes with in situ hybridization assay³⁸. Moreover, a line of transgenic mice that express the HCV transgene has been found to develop malignant lymphoma with a high frequency within 20 months³⁹. HCV core mRNA was found in the enlarged lymph nodes of these animals.

However, at present, although the association of HCV with "cryoglobulinaemic" or "non-cryoglobulinaemic" lymphoma seems to become clearer, the possible pathophysiological mechanisms are still unknown and misty.

Regarding therapy, HCV was not found to have significant impact on response to chemotherapy in lymphoma patients. On the other hand, whether to treat low grade lymphomas with anti-viral therapy is debatable. The only well documented study which encourage the role of antiviral treatment in HCV associated lymphoma has been done in patients with splenic lymphoma in whom lymphoma regression occur parallel with the decline of viraemia⁴⁰.

HCV AND PERIPHERAL BLOOD CELL CYTOPENIA

Evidence of HCV replication has been reported in peripheral blood cells⁴¹ and abnormal blood counts have been noted in patients with HCV infection⁴². There are several reports of neutropenia, thrombocytopenia or pancytopenia associated with HCV infection. Cirrhosis, and hypersplenism⁴³ are the most common causes of cytopenia but in several cases bone marrow suppression and autoimmune destruction of blood cells may also be present. Moreover coexisting viral infections such as HIV coinfection may play a major role in the pathogenesis of blood count abnor-

malities in HCV patients. Often multiple mechanisms were found to be responsible.

HCV Infection and Autoimmune Haemolytic Anaemia (AHA)

AHA in patients with chronic HCV infection has been rarely reported in anecdotal cases^{44,45}. In one report 17 cases of clinical relevant AHA are described. Those patients had a higher prevalence of associated autoimmune diseases (71%) cryoglobulins (67%) and cirrhosis (59%), had a good response to corticosteroids but a poor prognosis (47% mortality).

Moreover in some patients AHA may develop during interferon-alpha therapy^{46,47}. A proposed mechanism may be similar with AHA due to methyl-dopa revealing a drug induced altering of the red cell membrane in susceptible patients⁴⁸.

HCV Related Thrombocytopenia

Thrombocytopenia is common in chronic liver disease and is attributed usually to hypersplenism. Nevertheless rare cases of immune thrombocytopenia associated with positive anti-platelet antibodies (PAIgG) have been described in association especially with chronic HCV infection. On the other hand, in ITP patients a higher frequency of HCV positivity has been documented than in general population^{49,50}.

HCV related thrombocytopenia is usually mild, with platelet count $>5 \times 10^3$ platelets/ μ l but in rare cases severe thrombocytopenia with clinical relevant bleeding tendencies has been reported.

In a Japanese study thrombocytopenia ($<15 \times 10^4$ platelets/ μ l) was found in 41% out of 368 patients with hepatitis C⁵¹ with a significant difference when compared with patients with chronic hepatitis B (18.9%). Moreover, elevated titres of platelet associated immunoglobulin G (PAIgG) were found in 88.1% of the patients with hepatitis C and HCV-RNA by RT-PCR was detected in the platelets from 11 out of 14 tested patients. A positive correlation between the PAIgG titre and the severity of architectural changes in liver histology could also be documented.

In contrast with these findings another study could not find positive PAIgG in 11/13 HCV-positive thrombocytopenic (without hypersplenism) patients⁵². Nevertheless, in six out of these patients a trial of recombinant α 2b-interferon at a dose 3 MU three times a week for 6-24 months was performed, and a significant and

lasting increase in platelet count could be documented in all treated patients.

The mechanism of HCV associated not hypersplenic thrombocytopenia has not yet been clearly defined. The findings that HCV-RNA could be detected in platelets and in megacaryocytes⁵³ lead to the suspicion of a mechanism in which HCV is directly involved in a possible underproduction of platelets. The other hypothesis is the possibility of a HCV induced dysregulation of the host immune system (HCV binds to immunoglobulin⁵⁴ which leads to the peripheral destruction of the platelets. On the other hand a progressive decrease of thrombopoietin production in patients with HCV infection in parallel with the decline in liver function could also be documented⁵⁵. A decrease in thrombopoietin serum levels may partly be responsible for the thrombocytopenia in chronic hepatitis C⁵⁶. Therapy with recombinant thrombopoietin (R-hu-Tpo) in HCV associated thrombocytopenia may therefore be of important clinical and research interest but there are no reports regarding its application in these cases.

There are no guidelines for the treatment of patients with severe HCV associated immune thrombocytopenia (ITP) but patients with positive PAIgG seem to respond to immunosuppressive therapy with corticosteroids and i.v. IgG or cyclophosphamide. Although a slight increase in the level of HCV-RNA during immunosuppressive therapy is common, no progression of liver disease can be observed.

On the other hand, therapy of chronic hepatitis C with classical or Pegylated Interferon has been reported to induce thrombocytopenia in a number of patients usually via myelosuppression. In 4-19% of patients receiving IFN-alpha⁵⁷ several autoimmune disorders may occur. Among them immune thrombocytopenic purpura has been described in rare cases as evolving during therapy in susceptible patients^{58,59}.

Other HCV Related Cytopenias

Bone marrow depression associated with hepatitis has been first reported in 1955⁶⁰. Since then >200 cases of aplastic anaemia have been recognized⁶¹. The overall incidence of this rare complication is estimated at 0.1-0.2%⁶² implicating hepatitis A, B and especially non-A, non-B^{63,64}. The described patients were all young (mean age 20) rather male than female and aplastic anaemia occurred especially in cases of acute hepatitis with a mean eight weeks after the onset of symptoms.

In one patient with hepatitis C severe autoimmune neutropenia has been reported. Although associated with other viruses this is the only report of a possible association of autoimmune neutropenia with HCV⁶⁵.

In 1998 a 51 year old patient with HCV associated pure red cell aplasia has been described which did not respond to corticosteroid treatment but showed a favourable outcome with interferon treatment⁶⁶.

A possible association of HCV infection with myelodysplastic syndromes⁶⁷ has also been suggested in rare cases. A patient with refractory sideroblastic anaemia with severe thrombocytopenia and deletion of chromosome 7 has been described in a case report⁶⁸.

However the most of these associations are anecdotal and there is no direct evidence of the HCV implication in the pathogenetic mechanism of the described cytopenias. Nevertheless in unexplained cytopenias searching for HCV may be indicated.

CONCLUSION

The hepatitis C virus seems to be one of the responsible agents which bring hepatology and haematology together and it is obvious that in this area there is a great perspective of clinical and basic research investigations.

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