Benign and Malignant Hematological Manifestations of Chronic Hepatitis C Virus Infection

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ABSTRACT

Chronic hepatitis C virus (HCV) infection, that affects 3% of world's population, is associated with several hematological manifestations mainly benign cytopenias, coagulopathy and lymphoproliferative diseases. Immune or non-immune-mediated thrombocytopenia is a major challenge in chronic HCV infected patients especially in the setting of an advanced liver disease, with average prevalence of nearly 24%. Although several treatment modalities such as steroids, intravenous immunoglobulin, splenectomy and immunosuppresants have been tried with some success, their efficacy is not impressive and can result in an increase in viral load or other thrombotic complications. Even though a recent phase 2 study has shown promising role of a platelet growth factor, eltrombopag, in boosting platelets counts prior to antiviral treatment, its use in pre-operative setting had unexpected complications. Unlike thrombocytopenia, anemia and neutropenia are more frequently seen in treated patients and are often the result of antiviral therapy. HCV infection also pre-disposes to lymphoproliferative diseases, mainly non-Hodking's lymphomas, likely as a result of chronic antigenic stimulation and mutation of several genes involved in carcinogenesis. Understanding of the role of HCV infection in these conditions has therapeutic implications. Whereas antiviral therapy has shown therapeutic role in HCV-associated indolent lymphomas, monitoring of hepatic function and viral load is important in the management of diffuse large B-cell lymphoma in HCV-infected patients. Although our knowledge about the HCV infection and hematological manifestations has substantially grown in last few decades, further studies are important to advance our therapeutic approach.

Keywords: Anemia, bone marrow abnormality, hepatitis C virus, lymphoproliferative disorders, neutropenia, thrombocytopenia

Review Article

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a blood borne infection that has affected nearly 3.2 million people in the United

States[1] and 3% of world's population.[2] The incidence of liver disease from chronic HCV infection has only been increasing and is one of the most common causes of liver transplant in the United States.[2] In addition to liver involvement, chronic HCV infection can interestingly result in several extra-hepatic conditions with hematological problems being one of them. Hematological manifestations in HCV infection can range from benign cytopenias to malignant lymphoproliferative disorders.[3-6] The spectrum of the benign hematological diseases is large, ranging from thrombocytopenia, autoimmune hemolytic anemia (AIHA), aplastic anemia, pure red cell aplasia, neutropenia and sideroblastic anemia[4-6] [Table 1 and 2]. These hematological manifestations are elaborated below. We reviewed English language literature relevant to HCV infection and hematologic manifestations available in PubMed. Major search terms included HCV, anemia, thrombocytopenia, neutropenia, pancytopenia, bone marrow disorder, lymphoproliferative disorders and lymphoma. Available references were reviewed for additional reports.

Table 1: Benign hematological manifestations of chronic hepatitis C infection

Hematological manifestation	Prevalence among HCV-infected patients
Thrombocytopenia	
Immune	24% (0.2-45%)[7] with HR 1.8 compared to HCV-noninfected patients[8]
Non-immune (hypersplenism, bone marrow suppression, antiviral therapy, decreased production of thrombopoietin and endothelial dysfunction)	
Anemia	
Secondary to antiviral therapy (ribavarin>>peg-interferon)	11.4/100000 person-years with HR 2.8 compared to HCV-noninfected patients[8]
Auto-immune hemolytic anemia	
?Aplastic anemia	
Pure red cell aplasia	
Neutropenia	
Secondary to antiviral therapy (peg-interferon)	>10% among patients treated with peg-interferon[9]
Hypersplenism and autoimmune neutropenia	
?Bone marrow involvement or neutrophil apoptosis	

HCV=Hepatitis C virus, HR=Hazard ratio

HEPATITIS C AND THROMBOCYTOPENIA

Epidemiology

Thrombocytopenia is a major problem in HCV-infected patients and the most common hematological manifestation. A recent systematic review of 27 studies reported a 24% prevalence of thrombocytopenia in chronic HCV-infected patients in more than half of the studies with a wide range of 0.2-45% depending on the definition of thrombocytopenia.[7] In another study, out of 250 patients who were diagnosed with chronic immune thrombocytopenia, 30% (n = 76) were found to be positive for HCV infection.[15] HCV infection is associated with an increased risk of idiopathic thrombocytopenic purpura (ITP) (hazard ratio [HR] of 1.8; 95% confidence interval [CI]: 1.4-2.3) compared with HCV-noninfected patients; the risk being elevated among both untreated and treated HCV-infected persons.[8] No specific HCV genotype is associated with thrombocytopenia.[16] The degree of thrombocytopenia reported in HCV infection is greater than other forms of liver disease.[17] HCV ribonucleic acid (RNA) was detected in platelets with a higher frequency in thrombocytopenic patients compared to non-thrombocytopenic patients.[16] Furthermore, the relationship between the infectious agent and the development of thrombocytopenia is also clearly demonstrated by the improvements in platelet counts after successful treatment of HCV infection.[18] These results indicate that HCV infection is casually associated with thrombocytopenia. In another study, the prevalence of thrombocytopenia increased with

Table 2: Malignant hematological manifestations of chronic hepatitis C infection

Lymphoproliferative diseases	Prevalence among HCV-infected patients
Lymphoproliferative diseases	
Mixed cryoglobulinemia	Variable based on regional differences[10-13] (with odds ratio as high as 5.7)[14]
Waldenstrom's macroglobulinemia	
Marginal zone lymphoma	
Diffuse large B-cell lymphoma	

HCV=Hepatitis C virus

the severity of liver disease and correlated to hepatocellular damage and hepatic fibrosis.[19]

Pathobiology

Numerous mechanisms have been proposed to explain thrombocytopenia in HCV-infected patients. Immune mechanism involves the formation of platelet antibodies, which lead to platelet destruction. In one study, platelet specific antibodies were identified in 86% of HCV-infected patients and there was an inverse correlation between platelet count and the levels of platelet glycoprotein specific antibodies.[20] Thrombocytopenia is observed in HCV-infected patients without evidence of cirrhosis and splenomegaly suggesting that immune mechanism played an important role in its pathogenesis.[17,21,22] Presence of other antibodies such as anticardiolipin antibodies and cryoglobulins were seen in higher rates in HCV infected patients (62% and 90%, respectively) than non-infected ITP (15 and 7%, respectively),[18] thus suggesting the presence of auto-immunity in these patients. On the other hand, other studies have shown the presence of platelet antibodies without any association with thrombocytopenia, thus questioning their etiological role.[23] Non-immune mechanisms include HCV-mediated bone marrow suppression,[24,25] sequestration of platelets in the enlarged spleen secondary to portal hypertension (hypersplenism),[26] inadequate production of thrombopoietin[24,27,28] and endothelial dysfunction[29] which is especially seen with advanced liver fibrosis. Finally, peg-interferon and ribavarin used in the treatment of HCV infection can also cause thrombocytopenia.[30] A study showed an inverse correlation between platelet count and spleen size and a direct correlation with spleen size and portal hypertension, thus demonstrating the role of portal hypertension and hypersplenism in thrombocytopenia. Thrombocytopenia correlated to the grade of fibrosis among patient without splenomegaly. Furthermore, thrombopoietin level was inversely related to grade of fibrosis. Thus, in addition to portal hypertension and splenomegaly, advanced cirrhosis causes thrombocytopenia by reduced thrombopoietin production.[27] Another study showed that soluble thrombomodulin and von Willebrand antigen (vWF) were found to be significantly increased in patients with cirrhosis and inversely correlated with platelet count. A positive correlation was noted between thrombomodulin and vWF. In the absence of elevated C reactive protein (thus suggesting lack of inflammation) and no correlation between ADAMTS13 activity and vWF (thus suggesting an increase in vWF independent of decrease in ADAMTS13 with advanced liver cirrhosis), this suggest that the increase in thrombomodulin and vWF reflect endothelial dysfunction. Thus, HCV infection-related thrombocytopenia is related to vascular endothelial dysfunction.[29]

Clinical features

Thrombocytopenia secondary to HCV infection share clinical features with ITP.[15,31] Although these patients are less symptomatic, they tend to have major bleeding more frequently than HCV-uninfected ITP.[15] Mild thrombocytopenia, defined by platelet <1,50,000/mm^3, was present in 40-50% of HCV infected patients and severe thrombocytopenia, defined by platelet <50,000/mm^3 was present in 9%.[17,21,32,33] Peripheral blood smear shows large platelets and bone marrow biopsy reveals normal to increased megakaryocytes[31] [Figure 1].

Treatment

As various mechanisms cause thrombocytopenia in HCV-infected patients, a well-defined treatment has not been established and there is no approved treatment for these patients. Steroid has been used

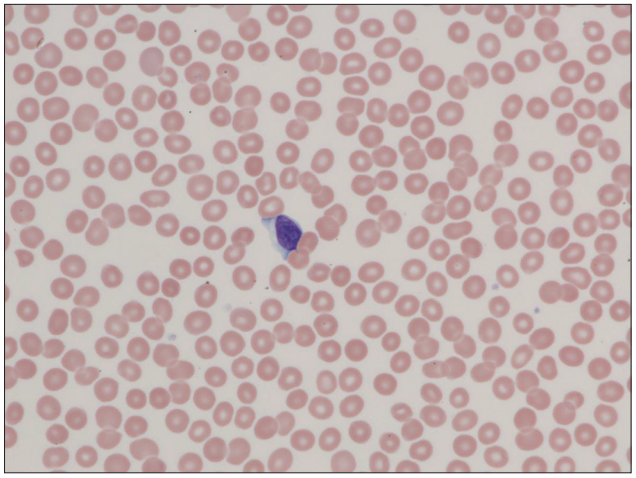

Figure 1: Peripheral blood smear (Wright-Giemsa stain, magnification 600X) with thrombocytopenia and occasional reactive lymphocytes in a hepatitis-C patient with mild splenomegaly who had to discontinue antiviral therapy due to cytopenias

with some success[22,31] but steroid use can result in an increase in viral load and transaminases as well as clinical deterioration, particularly when used for a long period of time.[15,31] Antiviral therapy with interferon alpha have shown promising role in the management of HCV-associated thrombocytopenia.[31,34,35] Other agents which have been shown to have partial efficacy include intravenous immunoglobulin,[22,35] splenectomy,[26,36] rituximab and cyclophosphamide.[35,37] Patients with hypersplenism can have good hematological response after splenectomy,[26,36] which has allowed successful antiviral treatment and successful viral remission in up to a third of patients.[36] However, most such studies are from a single center; and complications with splanchnic thrombosis is seen in more than 34% patients,[38] hence surgical treatment should be carefully considered in select patients. A phase 2 placebo-controlled clinical trial ($n = 74$) has shown that eltrombopag, a thrombopoietin receptor agonist, is safe and effective in increasing the platelet count in HCV-associated thrombocytopenia;[39] confirmation of these findings in larger phase 3 trials can change the way we manage this condition. Another randomized study,[40] has shown a reduced need for platelet transfusions with the use of eltrombopag for those undergoing invasive procedures (n=292), however there was a six-fold increased risk of thrombosis in the portal circulation with such usage. Hence, dosing and timing and patient selection need further improvement before such drugs take center role.

Previously, recombinant human interleukin (IL)-11 (Oprelvekin), approved for use in chemotherapy-related thrombocytopenia,[41] has been shown to improve platelet counts in HCV-infected patients.[42] In a pilot study ($n = 20$), the use of IL-11 resulted in a significant increase in platelet counts, which however started declining with the discontinuation of IL-11.[42] Furthermore, fluid retention and leg edema seems to be a common problem seen in all patients. Though this responded to diuretics in the majority of the patients, one patient required drug discontinuation within 6 weeks.[42]

HEPATITIS C AND ANEMIA

Anemia associated with HCV infection is often related to peg-interferon and ribavarin use in the treatment of HCV infection,[30,43] however, it has also been described in treatment-naïve patients.[5] Two-third of patients undergoing treatment can develop anemia and dose reductions can impair virologic response.[43] In a large retrospective study, the incidence of AIHA among HCV-infected patients ($n = 1,20,691$) versus matched HCV-uninfected patients ($n = 4,54,905$) was 11.4 versus 5.0/1,00,000 person-years respectively; HCV infection increased risk of AIHA (HR, 2.8; 95% CI, 1.8-4.2) however the incidence of AIHA was increased only among treated patients.[8] Ribavarin use leads to the depletion of adenosine triphosphate inside RBC and predisposes to oxidative damage and extravascular hemolysis.[30] Although ribavirin is the more common cause of AIHA in treated patients, peg-interferon can also be the culprit in few cases of AIHA.[44] Furthermore, peg-interferon can also cause bone marrow suppression contributing to anemia.[30] Management options include ribavarin dose reduction and use of erythropoiesis stimulating agents.[30,43]

In a study among treatment naïve patients ($n = 35$), compared with thrombocytopenia ($n = 16$), AIHA ($n = 17$) was more commonly associated with other immunological conditions such as hypocomplementemia, cryoglobulinemia and the presence of autoimmune antibodies such as rheumatoid factor and antinuclear antibodies. AIHA patients responded well with steroids, had cirrhosis more frequently and had overall poorer prognosis.[5] Hypersplenism can contribute to anemia and these patients can have good hematological response after splenectomy.[26] The role of HCV infection in the causation of aplastic anemia is not well-established. Rare cases reports of aplastic anemia have been described in patients with established HCV infection[5] or after treatment with interferon alpha 2a.[45] However, larger studies looking at the association between HCV infection and aplastic anemia have failed to establish an association between the two including among patients with "hepatitis-associated aplastic anemia" (aplastic anemia seen following an attack of acute hepatitis).[46-50] A study detected HCV viremia in 21% of hepatitis-associated aplastic anemia compared with 26% of patients with aplastic anemia of other causes, thus concluding that this likely reflected transfusion-associated HCV infection.[47] The frequency of HCV viremia in these patients has been shown to increase with the number of blood

product transfused prior to sampling and the time interval between diagnosis and sample collection, thus supporting the idea that HCV viremia in these patients are likely due to blood transfusion prior to the introduction of routine HCV screening of donor blood.[49] Cases of pure red cell aplasia have also been reported in treatment-naïve HCV-infected patients[5] and as a result of erythropoietin antibodies in HCV-infected patients receiving epoetin for the treatment of anemia related to antiviral therapy.[51]

HEPATITIS C AND NEUTROPENIA

Neutropenia is common in HCV-infected patients who are receiving antiviral therapy and can result in dose reduction or discontinuation of peg-interferon therapy.[30] In one study, grade 4 neutropenia (<500/μL) occurred in >10% of the patients treated with peg-interferon with or without ribavirin.[9] On the contrary, neutropenia is very uncommon in treatment naïve patients.[5,52] Interestingly, a large study (n = 16,196) based on patients enrolled in the Third National Health and Nutrition Examination Survey conducted by the United States National Center for Health Statistics for the Centers for Disease Control and Prevention revealed that neutrophil count <2100/μL was more frequently found in HCV-infected patients compared to patients without HCV infection (9% vs. 3%, $P < 0.0001$). Although 2% of HCV-infected patients had counts <1000/μL, none had counts <500/μL.[53] However, the paper is criticized because it did not take into consideration whether patients received any antiviral therapy or not. The participants of this study were enrolled during 1990s when anti-HCV testing became available and a large number of patients might have been started on antiviral therapy during that time.[54] This seems likely since subsequent studies have uncommonly shown significant neutropenia in treatment naïve patients.[5,55] Apart from being the side-effect of antiviral therapy,[30] leucopenia or neutropenia could be the result of hypersplenism,[26] autoimmune neutropenia,[5,56,57] direct bone marrow involvement[58,59] and activation of caspase 10 and increased neutrophil apoptosis.[60] In addition to bone marrow,[58,59] peripheral blood neutrophils[61] have also been shown to be the replication site for HCV, however, its potential role in causing neutropenia is unclear. Neutropenia is not associated with a serious infection in this setting and responds to dose reduction or discontinuation of antiviral therapy as well as the use of growth factors such as filgrastim.[30,52,62] In fact, filgrastim is considered the first line agent for neutropenia in general as well.[57] Patients with hypersplenism can have good hematological response after splenectomy.[26] Interestingly, a case report also suggests that autoimmune neutropenia may respond to antiviral therapy.[56]

HEPATITIS C AND BONE MARROW ABNORMALITIES

Cytopenias in HCV infection is often thought to be related to autoimmune destruction, hypersplenism, antiviral therapy and decreased thrombopoietin level, hence these patients often do not get marrow evaluation. However, one study among 47 HCV-infected patients showed a spectrum of bone marrow findings. Although dyserythropoeisis was the most common findings [Figure 2], patients were also found to have acute leukemia and clonal disorders. These findings were independent of the degree of liver fibrosis, stage of cirrhosis, splenomegaly and antiviral therapy.[63] Although it is debatable whether HCV infection is associated with these bone marrow changes or not, the study attempts to highlights the importance of considering bone marrow biopsy in HCV infected patients, especially with severe or sudden pancytopenia [Figure 3].

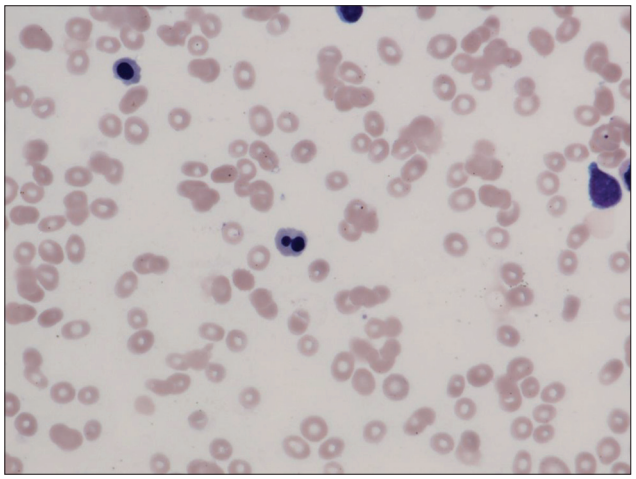

Figure 2: Peripheral blood smear (Wright-Giemsa stain, magnification 600X) showing dyserythropiesis in the form of a nuclear bleb in a hepatitis C patient diagnosed with refractory cytopenia with multilineage dysplasia

In another study, HCV RNA was detected in bone marrow in more than half of the patients ($n = 16/30$); patients with HCV RNA in marrow, compared with those with a negative test, were found to have a higher level of viremia, immune complex deposition in marrow, morphological changes in the marrow (both hypo- and hyper-cellularity as well as the presence of inflammatory cells) and peripheral cytopenias, thus suggesting the association between viral replication in marrow and alteration of the marrow microenvironment with the hematological manifestations.[58]

HEPATITIS C AND HEMOSTATIC CHANGES

Hepatitis C infection creates a myriad of hemostatic changes. Coagulopathy due to thrombocytopenia and prolonged prothrombin time are well-known. Thrombophilia due to complex changes in procoagulants and anticoagulants are well-described in a recent review.[64] VWF can increase due to endothelial damage resulting from hepatitis C infection, as previously explained.[29] Simplistically a balance exists between thrombophilic features by virtue of increase in VWF, factor VIII and decreased levels of ADAMTS-13, protein S, protein C, antithrombin III, heparin cofactor II and plasminogen and coagulopathic risks in the form of thrombocytopenia, platelet function defect, enhanced production of prostacyclin, nitrous oxide, tissue plasminogen activator levels, reduced production of clotting factor II, V, VII, IX, X, XI and XIII, alpha 2 antiplasmin and dysfibrinogenemia. Though most of these occur with advanced liver disease,[64] acquired inhibitors to factor VIII with interferon treatment has also been describe in patients with hepatitis C.[65] Hence when evaluating a hepatitis C patient with bleeding, a wide range of complex coagulation disorders should be considered. Similarly, the thrombophilic state, as described above, may be responsible for increased risk of splanchnic thrombosis associated with hepatitis C or its treatment.[38,40]

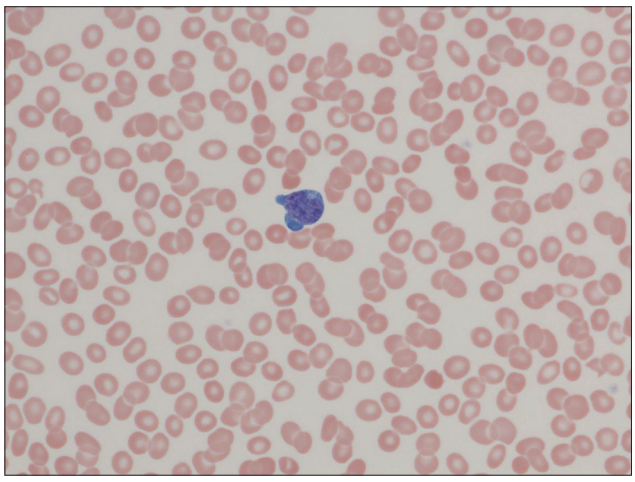

Figure 3: Peripheral blood smear (Wright-Giemsa stain, magnification 600X) with rare atypical lymphocytes in a hepatitis C patient with persistent monoclonal B-cell lymphocytosis

HEPATITIS C AND LYMPHOPROLIFERATIVE DISEASES

Epidemiology

Studies have shown that the HCV associated lymphoproliferative disease is more frequently seen in female and patients aged ≥50 years[66,67] [Figure 4]. Several epidemiological studies have demonstrated regional differences in the prevalence of HCV infection and its association with lymphoproliferative diseases. The association between these two conditions was found to be significant in areas such as Italy, Japan and Southwestern region of United States where the prevalence of HCV infection is high.[10,11] However, studies done in areas with low

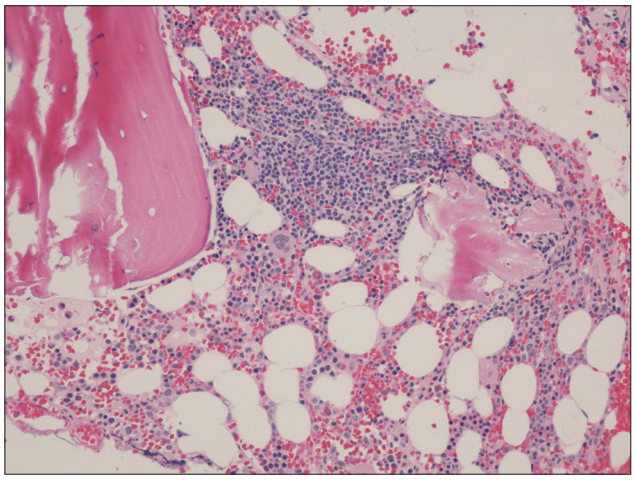

Figure 4: Bone marrow biopsy (Hematoxylin and eosin, magnification 200X) with an atypical paratrabecular lymphoid aggregate composed predominantly of B-cells with scattered T-cells in a hepatitis C patient with thrombocytopenia

prevalence of HCV infection failed to show such association.[12,13] Several meta-analyses have shown high prevalence of HCV infection among patients with non-Hodgkin's lymphomas (NHL) and strong association between the two. A meta-analysis of 48 studies showed that the mean prevalence of HCV infection among 5, 542 patients with B-cell lymphoma was 13%.[68] Another meta-analysis of case control studies (n = 4049 NHL patients) found a strong association between HCV seropositivity and the development of NHL with odds ratio (OR) of 5.70 (95% CI, 4.09-7.96, $P < 0.001$).[14] Studies have shown an association of HCV infection with certain types of NHL. The subgroup analysis done in the above-mentioned meta-analysis showed a similar trend for B-cell (OR = 5.04, 95% CI: 3.59-7.06) and T-cell NHL (OR = 2.51, 95% CI: 1.39-4.56).[14] The International Lymphoma Epidemiology Consortium (Interlymph) based on Europe, North America and Australia performed a pooled case control study to obtain a robust estimate of the risk to develop specific NHL subtypes after HCV infection. Among 4784 cases of NHL and 6269 controls, HCV infection was detected in 172 NHL cases (3.6%) and in 169 controls (2.7%). In a subtype specific analysis, OR was increased for occurrence of diffuse large B-cell lymphoma (DLBCL) (OR = 2.24), marginal zone lymphoma (MZL) (OR = 2.47) and lymphoplasmacytic lymphoma (OR = 2.57), whereas risk for follicular lymphoma (FL) (OR = 1.02) was not increased.[69] Few studies have attempted to explore the association between HCV infection and lymphoid and myeloid malignancies other than B-cell NHL. A large retrospective cohort study (HCV-infected cohort = 1,46,394; HCV-non-infected cohort = 5,72,293), among US veterans showed significant association between HCV infection and NHL, Waldenstrom's Macroglobulinemia (WM) and cryoglobulinemia but failed to show any association with Hodgkin's lymphoma (HD) or multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).[70] Another large case control study failed to show a higher prevalence of HCV infection in patients with HD or MM compared with the controls. Although the prevalence was higher in patients with T-cell NHL, CLL, ALL, AML and CML, the number of patients in these groups was small and the result was not statistically significant.[71] In another study, the association between HCV infection and AML/ALL/refractory anemia with excess blast was found to be weak and statistically non-significant.[72] Another study failed to show an association between HCV infection and myeloid malignancy.[73] Thus, the association between HCV infection and hematological malignancies has only been shown to be true for certain NHLs. Furthermore, HCV infection is likely casually related to the development of NHL. This is supported by following findings: The presence of clonal B lymphocytes in the peripheral blood and liver[74] as well as the presence of chromosomal translocation t (18;14) and over-expression of bcl-2 oncogene in peripheral blood mononuclear cells among HCV infected patients;[75] most of the NHL cells in HCV infected patients are typical of germinal center and post germinal center B cells[76] suggestive of the antigenic stimulation by the virus; and successful antiviral therapy against HCV has been shown to cause the disappearance of t (18;14) translocation[77] as well as regression of certain lymphomas in HCV infected patients.[78,79]

Pathobiology

The definite underlying pathogenesis of lymphoproliferative diseases in HCV infection is unclear, but several theories have been proposed. It has been proposed that the chronic antigenic stimulation of the immune system by the virus leads to clonal B-cell expansion. This is supported by the following findings: Most of the NHL cells in HCV infected patients is typical of germinal center and post germinal center B cells[76] and immunoglobulin variable region genes expressed by B-Cell NHL from HCV positive patients shows certain somatic mutations, which indicates antigenic selection process.[80-82] The antigenic stimulation is induced by a viral envelop protein, known as E2 which can bind to a specific receptor, CD81 present on the hepatocytes as well as the T-and B-lymphocytes. CD81 along with CD19 and CD21 present on the B-cell provides stimulatory signals that lower the threshold required for B-cell to respond to antigen.[83,84]

The other theories propose that HCV infection enhances deoxyribonucleic acid (DNA) damage

and gene mutations as well as inhibits apoptosis of the infected lymphocytes. The viral core and NS3 proteins activate the gene for inducible nitric oxide synthase and hence stimulate production of nitric oxide, which can cause double-stranded DNA breaks and DNA mutations.[85] In fact, HCV infection has been shown to induce error-prone DNA polymerase and activation-induced cytidine deaminase. These enzymatic alterations result in the formation of double-stranded DNA breaks and an increase in the mutation of immunoglobulin heavy chains as well as tumor-suppressor genes and proto-oncogenes such as myc, bcl-6, p-53 and beta-catenin genes in HCV-infected B cell lines. The mutated proto-oncogenes are found to be amplified in HCV-associated lymphomas. In addition, the mutation of immunoglobulin heavy chains may reduce the immune response to the viral infection.[86] HCV infected lymphocytes also have chromosomal translocation t (18;14) resulting in over-expression of bcl-2 oncogene, which inhibit apoptosis.[75] The inhibition of immune response towards viral infection, mutation of tumor suppressor genes, amplification of proto-oncogenes and inhibition of apoptosis together can contribute to the development of B-cell lymphoma.

Finally, not all HCV infection causes lymphocyte abnormality, which indicates that the interaction of environmental and genetic factors may influence the manifestation of various HCV-related B-cell lymphoproliferative diseases.[87]

Specific lymphoproliferative diseases

Certain lymphoproliferative diseases such as mixed cryoglobulinemia (MC), MZL, WM and DLBCL are commonly associated with HCV infection whereas FL and small lymphocytic lymphoma are rarely associated.[79] We will now discuss briefly about some of the important HCV-associated lymphoproliferative diseases focusing on their unique aspects.

HCV-associated MC

MC, a lymphoproliferative disease characterized by variable levels of serum cryoglobulins,[88] is associated with HCV infection in 80% or more cases.[89] About 50% of HCV-infected patients have the presence of circulating cryoglobulins but the clinical manifestation (such as purpura, arthralgia, or glomerulonephritis) are seen in only 5% of patients.[90] The risk of developing NHL in symptomatic MC is much higher than the general population, with risk as high as 35 times.[91] HCV-associated MC patients are shown to have translocation t (14;18) and bcl-2 gene rearrangement,[92] as well as may harbor occult low-grade NHL.[93] In fact, the presence of cryogloglobulins may be an early marker of HCV-associated lymphoproliferative disease.[91] Treatment of HCV-associated MC should target the HCV infection along with the anti-B-cell proliferation. Therefore, antiviral therapy with pegylated interferon and ribavirin should be combined with monoclonal antibody against CD20 (rituximab) for a better response.[94,95]

HCV-associated MZL

The association between HCV infection and MZL is very well established[79] with one study showing 26% HCV positivity in MZL[96] and several studies documenting lymphoma responding to antiviral therapy.[97,98] WHO has classified MZL into three subtypes: Splenic B-cell MZL, primary nodal MZL and extra-nodal MZL of mucosa associated lymphoid tissue (MALT) type.[99] Splenic B-cell MZL is a rare indolent type of NHL accounting for <2% of cases.[100] The leukemic counterpart of this rare lymphoma, also known as splenic lymphoma with villous lymphocytes (SLVL), has been well-associated with HCV infection.[78,101] The main presenting feature is symptomatic splenomegaly. SLVL associated with HCV infection is clinically similar to SLVL without HCV infection,[78] however, in HCV-positive cases; serum cryoglobulin is a consistent feature.[101] Primary nodal MZL is a rare MZL with HCV seropositivity in 20-24% cases;[102,103] immunoglobulin heavy chain variable region gene rearrangement studies have shown that it is derived from germinal-center experienced B-cells as a result of clonal selection from antigenic stimulation from common antigen, which is probably a HCV antigen epitope.[82] MALT lymphoma is another form of indolent NHL. Both gastric and non-gastric MALT lymphomas have been associated with HCV infection.[104,105] Among HCV-infected patients, non-gastric MALT lymphoma more frequently involved skin (35%), salivary gland (25%) and orbit (15%). The presence of HCV-infection did not influence outcomes and disease response to standard lymphoma therapy.[105] Studies exploring the role

of antiviral therapy (interferon and ribavirin) in the management of MZL and indolent NHL have shown a complete response in approximately half or more of the patients, with the possible association between virological and hematological response.[97,98]

HCV-associated WM

A retrospective Italian study detected HCV positivity in 15% of WM (n = 140). HCV positivity correlated with a greater degree of cytopenias, presence of cryoglobulins, autoantibodies and splenomegaly as well as higher level of LDH and beta-2 microglobulin (markers of tumor burden). However, there was no difference in clinical outcomes as well as a response to chemotherapy. Rituximab in combination with cyclophosphamide and fludarabine did not result in hepatitis or further toxicity.[106]

HCV-associated DLBCL

Studies have shown HCV positivity in as many as 15-19% of DLBCL patients.[96,107] A study showed that HCV-positive DLBCL, compared with HCV-negative patients, were more frequently transformed from low-grade lymphoma, had more frequent involvement of spleen and elevated LDH, had similar event-free survival, but poorer overall survival and greater short-term chemotherapy-related hepatotoxicity. After matching for age and prognostic factors, 2-year overall survival was 56% among HCV-positive patients compared with 80% among HCV-negative patients.[108] Another study showed that 8% of HCV-positive patients had transformed from low-grade lymphoma; spleen was the most frequently involved extranodal site and 4% patients had to discontinue chemotherapy because of hepatotoxicity. However, the addition of rituximab did not influence the occurrence of hepatotoxicity. Advanced Ann Arbor stage, co-infection with Hepatitis B virus and nodal origin were found to be adverse prognostic factors.[109] Hepatotoxicity is associated with the therapy of HCV-infected DLBCL. Case reports/series of rituximab-induced acceleration or reactivation of HCV infection have been published in the literature.[110-112] More recently, a larger study among DLBCL patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (RCHOP) or RCHOP-like chemotherapy showed that HCV infection was not associated with worse prognosis but with greater hepatotoxicity. HCV infection was a significant risk factor for hepatotoxicity with 27% (36/131) HCV-positive patients having grade 3-4 hepatotoxicity compared with 3% (13/422) HCV-negative patients. Thus, careful monitoring of hepatic function and viral load is important.[113]

Management of lymphoproliferative diseases

Given the association of HCV infection and its implication in management, patients with certain NHLs should be evaluated for the presence of HCV infection. As described above, antiviral therapy with interferon-alpha and ribavirin has therapeutic role in HCV-associated indolent NHL.[79] On the other hand, identifying HCV infection in aggressive DLBCL is important due to increased risk of hepatotoxicity and the need to closely monitor hepatic function and viral load.[113]

CONCLUSIONS

The high prevalence of the chronic HCV infection in above-mentioned hematological diseases and subsequent management implications require evaluation of HCV infection particularly in high-risk patients without any other obvious explanations. Understanding of the causative role of HCV infection in lymphoproliferative diseases have led to the successful use of antiviral therapy in HCV-associated indolent lymphomas. However, current management of several HCV-associated hematological diseases is far from optimal especially in patients with advanced liver disease. Growth factors have been used with success in the setting of antiviral therapy-related cytopenias. More recently, growth factors such as IL-11 and eltrombopag have shown some efficacy in increasing platelet counts in HCV-associated thrombocytopenia. Similar studies targeted at therapeutic development as well as studies to better understand the underlying pathophysiology and molecular mechanisms of HCV-associated hematological diseases are needed to improve the outcome in this patient population.

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