

Hepatitis G virus infection in lymphoma and in blood donors

Jane Minton, Adil Iqbal, Ayesha Eskitürk, William Irving, John Davies

Abstract

Aims—To determine whether the recently described flavivirus, hepatitis G virus (HGV), might contribute to the pathogenesis of lymphoma by testing for the presence of HGV RNA in sera from patients attending lymphoma clinics; to compare the incidence of HGV RNA in lymphoma patients with that in normal blood donors; and to look for potential risk factors for HGV infection and for evidence of hepatic disease in the HGV positive patients.

Methods—Sera were examined from 76 patients with lymphoma and 100 blood donors for the presence of HGV RNA using reverse transcriptase polymerase chain reaction (RT-PCR).

Results—HGV RNA was detected in 10% of patients' sera, but only in 1% of blood donor samples. HGV infection was found in patients with various different types of lymphoma, including Hodgkin's disease and non-Hodgkin's lymphoma. The majority (75%) of patients who were HGV PCR positive had undergone transfusion, but only 30% of those who were HGV PCR negative had received blood products. In addition, the number of donor exposures per HGV positive patient was approximately twice that of the HGV negative group.

Conclusions—The data suggest (1) that HGV is present in the normal blood donor population; (2) that patients with lymphoma are at risk of acquiring HGV because of their exposure to blood products; and (3) that persistent HGV infection does not appear to cause serious liver disease in these patients.

(*J Clin Pathol* 1998;51:676-678)

Keywords: viral hepatitis; lymphoma; blood transfusion

Recently, a novel RNA virus designated hepatitis G virus (HGV) was identified in the blood of a patient with chronic hepatitis C virus (HCV) infection. HGV has been cloned and sequenced, and has been shown to be a flavivirus with 95% identity at the amino acid level with GBV-C virus, previously described in the blood of patients with acute hepatitis; and to be distantly related to HCV.^{1,2} HGV appears to be transmitted parenterally as it occurs more often in subjects who have been multiply transfused, including those with haematological malignancy, and in intravenous drug abusers.³⁻⁵

So far, the clinical significance of HGV is not well understood. Preliminary evidence suggests

that it causes persistent infection as it has been detected in the blood of patients at least nine years after initial infection.¹ However, several studies have failed to demonstrate serious liver disease in patients with HGV viraemia.⁶⁻⁸

Other persistent viral infections, for example infection with human lymphotropic retrovirus (HTLV1) and Epstein-Barr virus, have been implicated in the pathogenesis of lymphoma.⁹⁻¹¹ While flaviviruses have yet to be shown to have oncogenic potential, the related flavivirus HCV is associated with hepatocellular carcinoma. There is some evidence that HCV infects lymphocytes in addition to hepatocytes^{12,13}; HCV is also associated with cryoglobulinaemia and possibly Waldenstrom's macroglobulinaemia, and has been suggested as a cause of lymphoma.¹⁴⁻¹⁶ Recently, it was suggested that HGV might also infect peripheral blood mononuclear cells.¹⁷ We asked whether HGV might contribute to the pathogenesis of lymphoma by testing for the presence of HGV RNA in sera from patients attending lymphoma clinics with neoplastic disorders. We chose to look for presence of viraemia rather than for evidence of previous infection, since viral oncogenesis is usually a feature of persistent rather than transient infection, and because some of our subjects are immunosuppressed, with potential impairment of their ability to generate antibody responses. In addition we looked for potential risk factors for HGV infection, particularly blood donor exposures, and for evidence of hepatic disease. We also compared the incidence of HGV RNA in our patients with that in normal blood donors.

Methods

Subjects were consecutive patients attending the lymphoma clinic at Queen's Medical Centre, Nottingham, during the months of July and August 1996. Informed verbal consent for the study was obtained. Serum alanine aminotransferase (ALT) was measured in each patient. The number of donor exposures for each patient with haematological malignancy was calculated from blood bank records. Controls consisted of 100 consecutive donors attending National Blood Authority (NBA) sessions in Trent on a single day in August, 1996. All blood donor samples had previously been tested for evidence of infection with HIV, hepatitis B virus (HBV), HCV, and syphilis, following the NBA protocols. Samples from each donor were anonymised, according to NBA recommendations.

University Hospital,
Queen's Medical
Centre, Nottingham,
UK:

Department of
Microbiology and
Infectious Diseases

J Minton
W Irving

Department of
Haematology

A Iqbal
J Davies

Marmara University,
Istanbul, Turkey:

Department of
Microbiology
A Eskitürk

Correspondence to:
Dr J Minton, Department of
Infectious Diseases, Royal
Hallamshire Hospital,
Glossop Road, Sheffield
S10 2JF, UK.

Accepted for publication
13 May 1998

SERUM SAMPLES

Serum was collected from each subject, divided into aliquots, and stored at -80°C . Positive control serum samples were obtained from patients with HCV infection who had been found to have HGV viraemia in a previous study. Negative controls consisted of DEPC treated water processed at the same time as the serum samples.

REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION FOR HGV

RNA was extracted from 100 ml serum from each patient as well as from positive and negative controls using the acid guanidinium thiocyanate-phenol-chloroform method of Chomczynski and Saachi.¹⁸ RNA pellets were resuspended in 20 ml DEPC treated water. Eight microlitres of RNA per patient served as a template for first strand cDNA synthesis, using the Pharmacia First Strand cDNA synthesis kit according to the manufacturer's instructions (Pharmacia Biotech, Uppsala, Sweden). Two microlitres of the cDNA product were used as a template for first round polymerase chain reaction (PCR), with the primers GS1 (AGGTGGTGGATGGGT-GAT) and GAS1 (TGCCACCCGCCCT-CACCCGAA) directed at the conserved 5' non-coding region of the HGV/GBV-C genome. One microlitre of the first round product was used as template for second round PCR, with the primers GS2 (TGGTAG-GTCGTAAATCCCGGT) and GAS2 (GGR-GCTGGGTGGCCYCATGCWT), to give a final product of 343 base pairs.⁵ Products were detected by ethidium stained gel electrophoresis. Great care was taken to prevent false positive results from carry over of PCR products, according to the recommendations of Kwok and Higuchi.¹⁹

DETECTION OF HCV RNA

Sera from patients positive for HGV were tested for HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) followed by hybridisation using a specific oligonucleotide probe (Roche Amplicor hepatitis C virus (HCV) test; Roche Products, Welwyn Garden City, UK), according to the manufacturer's instructions. Testing was carried out in duplicate.

STATISTICAL ANALYSIS

Data were analysed using the Fisher exact test. We calculated relative risks from the crude odds ratios together with 95% confidence intervals (CI) to demonstrate the likelihood of HGV viraemia in the blood donor group compared with the lymphoma group.

Results

We found HGV RNA in the serum of seven of 76 patients with haematological malignancy (9.2%), but only in one of 100 normal blood donors (1%) (table 1; $p < 0.03$, relative risk = 2.1, 95% CI 1.6 to 2.9). The characteristics of the HGV positive patients with lymphoma are presented in table 2. The diagnoses of these patients included both non-Hodgkin's lymph-

Table 1 HGV infection in normal blood donors and patients with lymphoma

Subjects	HGV positive	HGV negative	Total
	No of subjects (% of group)		
Blood donors	1 (1.0)	99 (99.0)	100
Lymphoma patients	7 (9.2)	69 (90.8)	76

Table 2 Characteristics of patients with lymphoma shown to be HGV positive

Patient	Diagnosis	Age (years)	ALT
1	Diffuse large B cell lymphoma	71	Normal
2	Large granular lymphocytic leukaemia, NK cell type	73	Normal
3	Follicular lymphoma	39	Normal
4	High grade B Cell lymphoma, Burkitt-like	64	Abnormal
5	Hodgkin's disease, nodular sclerosing	34	Normal
6	Hodgkin's disease, lymphocyte predominance	38	Normal
7	Hodgkin's disease, nodular sclerosing	37	Normal

ALT, alanine transaminase.

Table 3 Frequency of transfusion in lymphoma patients

Blood products	HGV positive	HGV negative
	No of subjects (% of group)	
Yes	5 (71.4)	21 (30.4)
No	2 (28.6)	48 (69.6)
Total	7	69

oma and Hodgkin's disease. In addition the stage of disease varied from advanced lymphoma to patients in remission for many years.

Only one of these patients had abnormal liver function tests, and in this case the patient had other potential causes for hepatic dysfunction, including aggressive disease with probable liver involvement and methotrexate treatment. None of our HGV positive patients had evidence of concurrent HCV infection.

Analysis of transfusion histories (table 3) showed that patients with HGV infection were more likely to have undergone blood transfusion than the uninfected patients ($p < 0.05$, relative risk = 4.8, 95% CI 1.0 to 23.1). In addition, the number of donor exposures per HGV infected patient was approximately twice that of the uninfected patients.

Discussion

Our data show that patients with lymphoma have an increased incidence of HGV viraemia compared with normal blood donors. Our finding that approximately 10% of such patients are HGV positive is lower than the incidence (47%) observed by Neilson and colleagues in a study of long term survivors of haematological malignancy in Birmingham.³ However, over half the patients examined by Neilson *et al* had undergone bone marrow transplantation, while in our study only two patients (one HGV PCR positive and one negative) had undergone this procedure. In addition, some of our patients were still undergoing treatment, while the Birmingham patients were considered to be "probably cured."

Subjects receiving blood products—such as multiply transfused patients, haemophiliacs, intravenous drug users, and those on maintenance haemodialysis—are much more often infected with HGV, suggesting that the virus is transmitted parenterally.^{1–5} Our patients with lymphoma had had multiple blood donor exposures, so they were at risk of infection with HGV. Those patients with HGV infection were significantly more likely to have received blood products than the HGV negative patients. Thus transfusion is a probable explanation for the increased incidence of HGV in the lymphoma patients compared with the normal blood donor controls. It is also possible that immunosuppression may be a risk factor for HGV viraemia in some of the patients, either because they are more susceptible to initial infection or because they have high titres of circulating virus which can be more easily detected by RT-PCR.

Our results do not suggest that HGV has a role in the pathogenesis of lymphoma or the subsequent outcome. However, this cannot be absolutely excluded because transfusion may have had a confounding effect on our results, and because our series included patients with several different forms of lymphoma and both aggressive and quiescent disease. A larger study of patients sampled before they receive blood products will be necessary to determine whether HGV is associated with a particular haematological diagnosis, or whether it has any influence on disease outcome.

HGV has been suggested as a cause of both fulminant and chronic hepatitis.^{20–21} However, when careful transfusion histories are obtained, it has been shown that HGV infection occurs only after the onset of fulminant hepatitis and after the patient has received blood products.²² Where prospective studies of post-transfusion HGV infection have been carried out, HGV can cause a mild hepatitis with ALT values usually less than 200 U/litre, though there may often be no rise in liver enzymes.^{7–23} Most patients with persistent HGV infection do not have evidence of liver disease, and coinfection with HCV does not appear to influence the course of chronic HCV infection.^{6–8, 24–25} In addition, most studies—including our own—suggest that HGV RNA is present in the sera of approximately 1–2% of apparently healthy blood donors.^{1–5, 7} Thus there is little evidence that HGV causes significant liver disease and the clinical importance of the virus remains unclear.

We are grateful to Dr R Sokol, National Blood Authority Trent Centre, for providing us with anonymised blood donor samples.

- 1 Linnen J, Wages J, Zhang-Keck Z-Y, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996;271:505–8.
- 2 Simons JN, Leary TP, Dawson GJ, et al. Isolation of novel virus-like sequences associated with human hepatitis. *Nature Med* 1995;1:564–9.
- 3 Neilson J, Harrison P, Milligan DW, et al. Hepatitis G virus in long-term survival of haematological malignancy. *Lancet* 1996;347:1632.
- 4 Aikiwa T, Sugai Y, Okamoto H. Hepatitis G infection in drug abusers with chronic hepatitis C. *N Engl J Med* 1996;334:195–6.
- 5 Jarvis LM, Davidson F, Hanley JP, et al. Infection with hepatitis G virus amongst recipients of plasma products. *Lancet* 1996;348:1352–5.
- 6 Alter MJ, Gallagher M, Morris TT, et al. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. *N Engl J Med* 1997;336:741–6.
- 7 Alter HJ, Nakatsuji Y, Melpolder J, et al. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N Engl J Med* 1997;336:747–54.
- 8 Miyakawa Y, Mayumi M. Hepatitis G virus—a true hepatitis virus or an accidental tourist? *N Engl J Med* 1997;336:795–6.
- 9 Wong-Staal F, Gallo RC. Human T-lymphotropic retroviruses. *Nature* 1985;317:395–403.
- 10 Zur Hausen H, Schulte-Holtehausen H, Klein G. EBV DNA in biopsies of Burkitt's tumors and anaplastic carcinomas of the nasopharynx. *Nature* 1970;228:1056–8.
- 11 Weiss LM, Movahed LA, Warnke RA. Detection of Epstein-Barr virus genomes in Reed-Sternberg cells of Hodgkin's disease. *N Engl J Med* 1989;320:502–6.
- 12 Zignego AL, Macchia D, Monti M, et al. Infection of peripheral mononuclear cells by hepatitis C virus. *J Hepatol* 1992;15:382–6.
- 13 Moldvay J, Deny P, Pol S, et al. Detection of hepatitis C virus RNA in peripheral blood mononuclear cells of infected patients by in situ hybridisation. *Blood* 1994;83:269–73.
- 14 De vita S, Sansonno D, Dolcetti R, et al. Hepatitis C virus within a malignant lymphoma lesion in the course of type II mixed cryoglobulinemia. *Blood* 1995;86:1887–92.
- 15 Pozzato G, Mazzaro C, Crovatto M, et al. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994;84:3047–53.
- 16 Silvestri F, Baccarani M. Hepatitis C virus-related lymphomas. *Br J Haematol* 1997;99:475–80.
- 17 Madejon A, Fogeda M, Bartoloma J, et al. GB virus C RNA in serum, liver, and peripheral blood mononuclear cells from patients with chronic hepatitis B, C, and D. *Gastroenterology* 1997;113:573–8.
- 18 Chomczynski P, Saachi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1992;162:156–9.
- 19 Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237–8.
- 20 Yoshida M, Okamoto H, Mishiro S. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. *Lancet* 1995;346:1131–2.
- 21 Fiordalisi G, Zanella I, Mantero G, et al. High prevalence of GB virus C infection in a group of Italian patients with hepatitis of unknown aetiology. *J Infect Dis* 1996;174:181–3.
- 22 Heringlake S, Osterkamp S, Trautwein C, et al. Association between fulminant hepatic failure and a strain of GB virus C. *Lancet* 1996;348:1626–9.
- 23 Hwang S-J, Lu R-H, Chan C-Y, et al. The role of hepatitis G virus infection in patients with acute posttransfusion hepatitis in Taiwan. *Gastroenterology* 1997;112:1260–4.
- 24 Bralet MP, Roudot-Thoraval F, Pawlowsky JM, et al. Histopathologic impact of GB virus C infection on chronic hepatitis C. *Gastroenterology* 1997;112:188–92.
- 25 Nakatsuji Y, Shih JW, Tanaka E, et al. Prevalence and disease association of hepatitis G virus infection in Japan. *J Viral Hepat* 1996;3:307–16.