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Efficacy of donor screening for hepatitis C antibodies in preventing hepatitis C infection in multiply transfused patients

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SUMMARY. Patients undergoing therapy for haematological malignancies, who received blood products from United Kingdom (U.K.) donors who were unscreened for hepatitis C antibodies (anti-HCV), have previously been shown to be at risk of acquiring HCV (Brink *et al.*, 1993). Screening for anti-HCV has recently been introduced into the U.K. and, in order to determine the efficacy of this in preventing transfusion-acquired HCV infection, we monitored a group of patients for possible acute HCV infection for 1 year after the introduction of donor screening in the U.K. We identified no new cases of HCV infection that were acquired in the U.K. during this period, thus demonstrating the efficacy of the currently available anti-HCV assays in preventing the majority of transfusion-acquired HCV infections.

Key words: efficacy, donor screening, hepatitis C, passively acquired antibody.

Hepatitis C (HCV) is now known to be the most important cause of parenterally transmitted non-A, non-B hepatitis. Patients receiving intensive chemotherapy for haematological malignancies will inevitably receive blood components from multiple donors and are therefore at risk of acquiring HCV infection from a blood donor population that is not screened for HCV antibodies (anti-HCV). In the 9 months prior to the introduction of blood donor screening for anti-HCV in the U.K., we investigated a group of patients undergoing therapy for haematological malignancies. Thirty-two out of 115 patients developed abnormal liver function tests, and we demonstrated that eight (25%) of these patients had evidence of an acute HCV infection which contributed significantly to morbidity (Brink *et al.*, 1993).

Serological assays to detect anti-HCV are now commercially available in most countries. However, as seroconversion may be delayed for several months, one major disadvantage of these assays is their inability to identify the recently infected donor. This, amongst other reasons, has led to some controversy over their efficacy as screening tests for blood donors. Blood donor screening for anti-HCV was introduced into the U.K. on 1 September 1991. In this study we have sought to determine the efficacy of this in the prevention of HCV infection using multiply transfused patients receiving chemotherapy for haematological malignancies as a sentinel group. We compare the incidence of acute HCV infection in this group of patients with that of a similar group investigated prior to the introduction of blood donor screening (Brink *et al.*, 1993). In addition, we highlight the need to consider the possibility of passively acquired anti-HCV in patients who receive intravenous immunoglobulin prepared from a donor population who are not screened for anti-HCV.

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METHODS

Patients

Between 1 September 1991 and 31 September 1992, 140 patients with haematological malignancies received chemotherapy as in-patients at University College Hospital, London. All treatment entailed a period of pancytopaenia requiring haematological support with blood and platelet transfusions. In

addition, three patients received high titre CMV immunoglobulin (Venoglobulin, Alpha Therapeutic U.K. Ltd) as part of their therapy for CMV pneumonitis. From this group of 140, 32 patients were identified with raised serum transaminases (i.e. AST above 60 IU/l {normal range 20–55 IU/l}) and were prospectively investigated for an acute HCV infection.

Serology

Serum from all 32 patients with raised serum transaminases was stored at −20°C before testing. In addition, monthly serial samples taken from one of the recipients of high titre CMV immunoglobulin and a vial from the batch of CMV immunoglobulin were also stored for testing.

Anti-HCV was detected using two commercial enzyme linked immunosorbent assays (Ortho HCV ELISA Test System, Second Generation, Raritan, NJ, U.S.A.; Wellcozyme anti-HCV, Murex Diagnostics Ltd, U.K.). The specificity of the serological reactivity was confirmed with recombinant immunoblot assay (Chiron RIBA HCV Test System, Second Generation, Raritan, NJ, U.S.A.) and sera were classified as reactive, indeterminate or non-reactive according to the manufacturer's criteria.

HCV-RNA detection

Two hundred microlitres of serum obtained from all 32 patients with elevated transaminases were tested for the presence of HCV-RNA using a 'nested' PCR procedure as previously described (Garson *et al.*, 1990) but using an acid guanidinium thiocyanate extraction method and primers derived from the highly conserved 5′ non-coding region of the viral genome (Garson *et al.*, 1991). In addition, a 200 μl aliquot of the high titre CMV immunoglobulin was similarly tested for the presence of HCV-RNA.

RESULTS (see Table 1)

Thirty-two of the 140 patients had elevated transaminases and were investigated for HCV infection. The AST levels ranged from 63 to 971 IU/l. All patients had received blood products; the median number of units of blood transfused was 36 (range 5–96) and of platelets was 125 (range 35–455). Two patients had received multiple blood transfusions outside the U.K. and had recently been referred to University College Hospital for bone-marrow transplantation. Both were shown to be HCV-infected on admission to the U.K. A further three patients who had undergone bone marrow transplantation (one syngeneic, two allogeneic) developed a CMV pneumonitis, and received both ganciclovir and commercial high-titre CMV intravenous immunoglobulin as therapy. The remaining 27 patients had only received blood or blood products from U.K. donors screened for anti-HCV.

Table 1. Serology and PCR results

Number of patients	HCV-RNA	HCV antibody
2	+*	−
3	−	+†
27	−	−

*Infected on arrival in the U.K.
†Passively acquired HCV antibody.

HCV infection

Acute HCV infection was diagnosed in the two patients who were recipients of multiple transfusions outside the U.K. by demonstrating HCV-RNA in the absence of anti-HCV.

Sera from three patients contained anti-HCV by ELISA that was confirmed by recombinant immunoblot assay. However, none of these patients had detectable HCV-RNA in their sera. All three had received high titre CMV immunoglobulin as part of their therapy for CMV pneumonitis. Serial samples taken at monthly intervals from one of the patients demonstrated a clear decline in antibody reactivity, which eventually became undetectable 8 months after the last dose of an intravenous immunoglobulin preparation. The intravenous CMV immunoglobulin given to these patients was shown to contain very high levels of antibody to HCV on screening ELISA which was shown to be reactive against both structural and non-structural viral proteins in recombinant immunoblot assay. It did not, however, contain detectable HCV-RNA.

DISCUSSION

Repeatedly abnormal serum transaminases are common in patients undergoing therapy for haematological malignancies. This may be due to a variety of causes including drugs (in particular cytotoxic chemotherapy), infections (including bacterial, viral and fungal infections), graft-versus-host disease, veno-occlusive disease or infiltration of the liver with malignant disease itself. In addition, in a study performed in our haematology unit, prior to the introduction of blood donor screening in the U.K., we showed that eight out of 32 patients investigated with raised serum

transaminases had evidence of an acute HCV infection. This contributed significantly to the morbidity of these patients in the short-term and influenced their management. The apparent severity of symptoms in our HCV-infected patients was unexpected. We felt that the enhanced liver disease was in part the result of their immunosuppressed state allowing high levels of viral replication. The diagnosis of an acute HCV infection also affected the further management of some of the patients. One patient developed fulminant hepatic failure which prejudiced subsequent chemotherapy and the management of a CMV viraemia with ganciclovir in another patient was complicated (Brink *et al.*, 1993).

We were interested in determining the efficacy of anti-HCV screening in the prevention of HCV infection, particularly in view of the uncertainty over the proficiency of the currently available serological assays. We therefore elected to continue to investigate our multiply transfused patients for acute HCV infection for a further year after the introduction of blood donor screening. Two of the 32 patients that we investigated in the current study had evidence of an acute HCV infection acquired prior to admission to our hospital, neither of whom were infected in the U.K. In contrast to our previous finding of eight infected patients among 32 with raised AST levels during the study period prior to the introduction of blood donor screening, we identified no new cases of HCV infection acquired in the U.K. during the year after the introduction of blood donor screening. This difference is statistically significant ($P < 0{\cdot}05$, Fisher's exact test) and demonstrates that anti-HCV assays, based on both structural (core) and non-structural (NS3 and NS4) proteins used in the U.K., have indeed been able to prevent the majority of HCV infections. Similar findings have been reported from recent studies from Japan and the U.S.A. (Japanese Red Cross non-A, non-B Hepatitis ResearchGroup, 1991: Donahue *et al.*, 1992). However, only patients with abnormal liver function tests were included in this study, so the overall incidence of acute HCV infection in the total in-patient population receiving therapy for haematological malignancies may have been slightly underestimated.

This study also highlights the need for caution in diagnosing HCV infection in patients who have recently received pooled blood products. Three of our patients who had intravenous immunoglobulin therapy developed detectable serum anti-HCV by both screening and supplementary testing. Their serum, however, never contained HCV-RNA. Testing of the immunoglobulin product showed that it contained anti-HCV but no HCV-RNA. The most likely explanation was that these patients had passively acquired anti-HCV and this was conclusively demonstrated in one patient in whom we showed a decrease and subsequent disappearance of anti-HCV. Extreme care should therefore be taken before a diagnosis of hepatitis C infection is made in patients who have received blood products, although in practice the recent requirement to use only antibody-screened donors for these products should reduce this possibility.

Despite the impressive safety record of intramuscular immunoglobulin, transmission of non-A, non-B hepatitis by intravenous immunoglobulin has been described (Williams *et al.*, 1989). These occasional reports stimulated the development of solvent/detergent or heat inactivation steps capable of inactivating a broad range of viruses. It is therefore of interest that in our three cases, the pooled immunoglobulin used did not transmit infection despite being prepared by cold-ethanol fractionation with no additional specific inactivation steps (personal communication—Alpha Therapeutic U.K.).

In summary no HCV infection was demonstrated in 30 patients representing the recipients of some 1204 blood and 5253 platelet donations, all of which was from anti-HCV screened donors. This represents a similar exposure to blood and blood components as seen in our previous study. The currently available serological assays, based on structural (score) and non-structural (NS3 and NS4) proteins, appear, therefore, to be able to prevent the vast majority of HCV infections. However, these assays will not identify the recently infected donor. In addition the identification of other serologically important proteins, such as those derived from the NS5 region of the viral genome, and the inclusion of these in the currently available serological assays will further reduce the risk of transfusion-acquired HCV infections. Multiply transfused patients therefore remain an important sentinel group in which to monitor the efficacy of blood donor screening.

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