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Prophylaxis of Hepatitis C with Intramuscular Immunoglobulin

Clinical and Economic Appraisal

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Abstract

Hepatitis C virus (HCV) affects millions of individuals worldwide. In most cases, HCV infection progresses to chronic liver disease and, subsequently, to liver cirrhosis and hepatocellular carcinoma. HCV is transmitted by the parenteral route, for example by transfusion of blood or blood products, injection during drug abuse, etc., and by the inapparent parenteral route (penetration of the virus through difficult-to-identify microlesions present on the skin or mucosae), for example, sexual exposure or household exposure to infected contacts, etc. The cost of chronic hepatitis C and its sequelae is high in both financial and human terms.

At present, only anti-HCV screening of blood/organ/tissue donors and universal precautions for the prevention of blood-borne infections are recommended for HCV prevention. Before the discovery of the main aetiological agent of non-A, non-B hepatitis (HCV), several randomised controlled clinical trials demon-

strated that standard intramuscular immunoglobulin exerted a preventive effect on post-transfusional and sexual and /or horizontal transmission of non-A, non-B hepatitis. When serological tests for HCV infection became available, bimonthly inoculation of standard unscreened intramuscular immunoglobulin (prepared from plasma pools containing about 2% of anti-HCV–positive units) was demonstrated to significantly prevent sexually transmitted HCV infection. The immunoglobulin used contained high titres of anti-HCV neutralising antibodies (anti-E2 neutralisation of binding assay), whereas currently available commercial screened immunoglobulin (prepared from anti-HCV–negative blood units) did not. This finding suggested that anti-HCV neutralising antibodies are concentrated only in anti-HCV–positive units (which are currently discarded). Thus, anti-HCV hyperimmune globulin (HCIg) can be produced only from anti-HCV–positive units. The neutralising titre can be increased by the exclusive use of units with higher titres of neutralising antibodies. Unlike other hyperimmune globulins, which are produced from a limited number of selected donors, HCIg should be produced from a large number of units so as to contain neutralising antibodies to the different HCV strains. HCIg will have a number of advantages: (i) it is easy to produce and inexpensive; (ii) it has a long half-life, allowing infrequent administration; (iii) new additional viral inactivation procedures have been introduced to eradicate transmission of infection, and (iv) it may be possible to neutralise all the emerging HCV strains. HCIg could be used in all individuals at risk of HCV infection (sexual partners, haemodialysis patients, etc), in preventing reinfection of transplanted livers, and perhaps also in the treatment of chronic hepatitis C, alone or associated with other drugs.

1. Public Health Significance of Hepatitis C Virus (HCV) Infection

HCV infection is recognised as one of the world's major public health problems. About 300 million people are estimated to be infected with HCV worldwide.[1-3] The infectivity rates vary from 0.3 to 14.5%; the prevalence of antibody to HCV (anti-HCV) is much lower in most developed countries (US, Canada, the northern European countries) than in the countries of South East Asia, Africa, Arabia, Eastern Europe and the Mediterranean basin.[4] HCV infection is responsible for chronic liver disease in about 80% of infected patients; as many as 20% of patients with chronic C hepatitis develop liver cirrhosis; lastly, hepatocellular carcinoma occurs in about 1 to 4% of cirrhotic patients per year.[1,2,5-8] Hepatitis C is the leading reason for liver transplantation in most countries, including the US.[2]

HCV is transmitted by the parenteral route. Risk factors for acquiring HCV infection include transfusion of blood or blood products (especially before implementation of blood screening for anti-HCV), shared use of needles among intravenous drug addicts, transplantation of organs from HCV-infected donors and haemodialysis.[1-8] Like hepatitis B virus (HBV), HCV is also transmitted through the inapparent parenteral route,[1-5] which represents the most frequent route of HCV transmission. The inapparent parenteral route is the penetration of virus through difficult to identify microlesions present on the skin or the mucosae;[9,10] this route also includes sexual and household exposure to infected contacts and perinatal exposure.[11,12]

The sexual transmission of HCV has long been debated: in previous retrospective studies the prevalence of anti-HCV positivity in the sexual partners of HCV-infected patients varied from very low or absent up to 30%.[3,11-34] In addition, there were no studies that quantified the risk of sexual transmission of HCV to a partner of an HCV-infected indi-

vidual. Only very recently did a prospective randomised controlled trial unequivocally demonstrate sexual transmission of HCV and quantify the risk of infection (12.00/1000 person-years).[35] The rate of sexual transmission was found to be low (each year about 1% of the partners became infected), particularly when compared with the rates of sexual transmission of HBV or HIV. However, in countries with an intermediate or high prevalence of anti-HCV in the general population, and thus with an equivalent number of sexually active HCV-infected patients, a relatively high number of individuals are potentially exposed to sexually transmitted HCV infection. This event can also occur during a steady monogamous sexual relationship.[35]

2. Preclinical and Clinical Evaluation of HCV Prophylaxis with Intramuscular Immunoglobulin

As yet, there are no codified recommendations for HCV immune prophylaxis for exposed individuals. Screening of blood/organ/tissue donors for anti-HCV and universal precautions for prevention of blood-borne infections are the only measures adopted for HCV prevention.[36,37] Individuals with multiple sexual partners are advised to practice safe sex, i.e. use of the latex condom and reducing the number of partners.[12,36,37] HCV-infected patients with steady monogamous sexual partners are not advised to change sexual practices but should discuss the risk, which is low but not absent, with the partner and might decide to use barrier precautions.[37] In any case, this strategy does not completely protect the at-risk partner from exposure to biological fluids such as saliva or to blood-contaminated items (e.g. razors or hairbrush).

In the past, 6 prospective randomised controlled trials (PRCT) evaluated the role of immunoglobulin in preventing post-transfusional non-A, non-B hepatitis.[38-43] Five of these trials, all employing early administration of immunoglobulin found at least an arithmetical trend for, if not a statistically significant, benefit. Only one of these trials did not find any benefit, but in this case the first inoculation of immunoglobulin was given according to the protocol at least 4 days after exposure.[44] Hyperimmune globulins against HBV (HBIg) also protected patients undergoing blood transfusion[45] or dialysis[46] from non-A, non-B hepatitis. The same protective efficacy was achieved in a PRCT on prophylaxis of sexual and/or horizontal non-A, non-B hepatitis in more than 100 000 US soldiers in Korea.[47] Unfortunately, all such studies were undertaken before the discovery of the major aetiological agent of non-A, non-B hepatitis and before serological tests for non-A, non-B (i.e. HCV) infection became available. Moreover, obviously, at that time it was not possible to verify whether or not immunoglobulin contained neutralising antibodies against HCV.

After the discovery of HCV, a prospective randomised controlled trial was carried out on steady heterosexual partners of HCV-infected patients. 1102 partners were enrolled and randomly assigned to receive 4ml of standard intramuscular immunoglobulin prepared from unscreened donors (n = 450) or placebo (n = 449) every 2 months for a mean of 13.5 months. Tests for HCV infection (anti-HCV and detection of HCVRNA by polymerase chain reaction in serum samples tested positive for anti-HCV) were done every 4 months. Seven partners became infected with HCV: 6 in the placebo group [incidence density 12.00/1000 person-years; 95% confidence interval (CI) 3.0 to 21.61] and 1 in the immunoglobulin group (incidence density 1.98/1000 person-years; 95% CI 0 to 5.86). The risk of infection was significantly higher in placebo- versus immunoglobulin-treated patients ($p = 0.03$).[35]

The application of immunoglobulin in HCV prevention has been questioned, and several studies suggested that HCV does not elicit protective immunity in the host. Indeed, there is a high rate of chronicity and persistent viraemia in humans,[48] and frequent reinfection and/or reactivation of HCV genotypes in multitransfused haemophili-

acs[49] and in thalassaemic children.[50] In addition, recovered chimpanzees rechallenged with the same or different strains of HCV showed reappearance of viraemia.[51] All these data seemed to show that HCV does not elicit a protective response and consequently did not support the use of immunoglobulin prophylaxis.

Only in 1994 was it demonstrated that neutralising antibodies can be elicited by HCV. Chimpanzees with high titres of homologous antibodies against HCV envelope glycoproteins gpE1 and gpE2 are protected from infection.[52] In other experimental studies on chimpanzees, infection was prevented by antibody-mediated *in vitro* neutralisation of HCV with serum from a chronically infected patient[53] or with hyperimmune rabbit serum.[54]

Periodic administration of immunoglobulin significantly reduces the risk of transmission of HCV in at-risk sexual partners.[35] In addition, we first demonstrated that all the lots of immunoglobulin used in our study, which were prepared from unscreened blood, contained high titres of neutralising antibodies against HCV (anti-E2),[55] and that the neutralising activity of these lots was very high when tested with the neutralisation of binding (NOB) assay (table I).[35] It seems reasonable to postulate that neutralising antibodies present in the immunoglobulin neutralised the very small amount of virus transmitted by the sexual route. Recently a molecule (CD81) which binds the E2 protein present on the HCV envelope has been identified on the surface of human hepatocytes (and other human cells, e.g. B lymphocytes).[56] In addition anti-E2 neutralising antibodies, which neutralise HCV infection in chimpanzees, can inhibit the binding of HCV to CD81 *in vitro*.[56]

The efficacy of HCV neutralising antibody was demonstrated in 3 experimental studies in the chimpanzee. In the first experimental study,[57] 3 chimpanzees were injected with a relatively high dose of HCV; 1 hour later, 1 chimpanzee was injected with intravenous immunoglobulin prepared from unscreened blood, 1 was injected with intravenous immunoglobulin prepared from screened blood and the third was the control. The infection progressed as usual in the chimpanzee treated with screened immunoglobulin and in the control. Only the chimpanzee treated with immunoglobulin prepared from unscreened blood showed a markedly prolonged incubation period, a limited intrahepatic spread of the infection (for more than 60 days) and a delayed appearance of acute hepatitis. Massive intrahepatic necrosis and related events appeared 90 days later than in the 2 other chimpanzees and only after the neutralising antibody titre had markedly decreased. It is plausible that the results would have been different if high concentrations of neutralising antibodies had been maintained by periodic injection of immunoglobulin.

In the second experimental study,[58] 1 chimpanzee was given an intravenous infusion of plasma containing a high dose of HCV that had been incubated *in vitro* with experimental anti-HCV–positive intravenous immunoglobulin (which also contains anti-E2 neutralising antibody); 1 chimpanzee was given an intravenous infusion of plasma containing a high dose of HCV that had been incubated *in vitro* with commercial anti-HCV–negative intravenous immunoglobulin (devoid of anti-E2 neutralising antibody) and another chimpanzee was inoculated with HCV incubated with human albumin. Both the latter control animals developed

Table I. Neutralising antibodies against hepatitis C virus and neutralisation of binding assay in intramuscular immunoglobulin preparations[35]

Lot number	Anti-gpE1/gpE2 ELISA titre	50% gpE2 NOB titre (mg/ml)
11 151 Berna[a]	1168	0.4
11 763 Berna[a]	3592	0.01
11 947 Berna[a]	118	1
12 069 Berna[a]	338	0.2
130 590 101 Berna[b]	0	0
932 001 Biagini[b]	0	0

a Prepared from donors not screened for anti-HCV.

b Prepared from donors screened for anti-HCV.

ELISA = enzyme-linked immunosorbent assay; **gpE** = HCV envelope glycoprotein; **NOB** = neutralisation of binding.

evidence of HCV infection (HCV RNA and aminotransferase elevation), whereas the animal receiving HCV mixed with anti-HCV–positive immunoglobulin has been followed for more than 1 year without evidence of HCV infection. Clearly, anti-E2 neutralising antibody present in anti-HCV–positive immunoglobulin neutralised HCV *in vitro* and prevented the infection.

In the third experiment,[59] 2 pairs of chimpanzees were challenged intravenously with high doses of HCV and subsequently received multiple infusions of intravenous immunoglobulin prepared from anti-HCV–positive blood units for 13 weeks (this immunoglobulin was HCV-RNA negative). Control chimpanzees received multiple infusions of intravenous immunoglobulin prepared from anti-HCV–negative units. During infusion of anti-HCV–positive immunoglobulin there was no evidence of liver pathology and HCV viraemia lasted only 39 to 42 days. In contrast, in control chimpanzees, HCV viraemia was prolonged (73 to 105 days) and acute hepatitis C occurred. In animals administered anti-HCV–positive immunoglobulin, HCV viraemia followed by acute hepatitis recurred 72 days after immunoglobulin discontinuation. The investigators concluded that multiple infusions of anti-HCV–positive immunoglobulin shortened HCV viraemia and prevented acute hepatitis. HCV viraemia recurred only after the disappearance of passively transferred anti-HCV E2 present in the immunoglobulin preparation.

A retrospective clinical trial on the prophylaxis of HBV infection with hyperimmune globulin against HBV (HBIg) after liver transplantation showed that HBIg prepared from unscreened donors, which presumably contained anti-HCV prevented HCV infection transmitted by transplantation.[60]

428 patients (more than half were anti-HCV positive) who underwent liver transplantation in the period 1984-1994 were studied. HBIg significantly ($p < 0.001$) prevented reinfection of the transplanted liver (absence of HCV-RNA one year after transplantation). After march 1990, the date on which the production of HBIg only from anti-HCV-negative blood units became mandatory in France, this protective effect dramatically decreased.[60] These findings strongly suggest that the protective effect of HBIg was due to the presence of anti-HCV in HBIg; unfortunately these batches were destroyed and could not be tested retrospectively for neutralising antibodies.[60]

Lastly, in patients with chronic hepatitis C, the development and persistence (for more than 3 months) of high serum titres of anti-E2 NOB antibodies coincided with viral clearance (loss of serum HCV-RNA) and clinical resolution of hepatitis. In contrast, very low titres, or a lack of anti-E2 NOB antibodies were found in patients without clinical resolution.[61] The available data indicates that anti-E2 NOB is the 'true' neutralising antibody. However, we cannot completely rule out the existence of other types of neutralising antibodies,[35] which may be present in immunoglobulin.

3. Economic Evaluation of HCV Prophylaxis with Intramuscular Immunoglobulin

Although the precise economic impact of HCV is difficult to assess, cases of chronic hepatitis C are undoubtedly costly. The financial burden on healthcare systems worldwide is not known. In the US it has been estimated that approximately 10 000 American people died of HCV infection in 1997 alone, and that this annual number will triple in the next 20 years unless a way is found to treat the disease more effectively and/or prevent it.[1] The economic data available are incomplete: medical and indirect (employment-related) costs from hepatitis C, exclusive of liver transplantation, are estimated at $US500 million.[1] The costs of liver transplantation are very high (about $US200 000). Currently, HCV is considered an important indication for liver transplantation in some patients and it represents a major financial burden for the US healthcare system.[2,62] In addition, it must be considered that HCV reinfection is almost always the

rule in the transplanted liver and it progresses to chronic hepatitis in about 50% of cases.[1]

Several theoretical models have been devised in an attempt to calculate the direct medical costs of HCV infection, e.g. the costs of interferon therapy, of the follow-up of an infected patient, of hospitalisation for sequelae of chronic liver disease and for liver transplantation.[63-66] It has been calculated that the marginal cost-effectiveness per year of life gained in a patient treated with interferon for 12 months can vary between $US2300 and $US53 000, depending on patient age.[64] For all medical intervention, including interferon therapy, the marginal cost-effectiveness per gain of 1 year of life has been deemed acceptable up to $US50 000 to $US100 000.[64]

In our trial,[35] the prevention of 1 case of infection per year obtained by administering 6 injections of immunoglobulin to 100 potential at-risk partners assumes great significance, particularly because the prophylaxis is simple, well tolerated and inexpensive. At the time of the trial, the direct cost of 600 immunoglobulin injections was about L6 million ($US5000). Thus, it cost only $US5000 not to gain 1 year of life in an infected patient, but to prevent the infection and its associated costs. In addition, prophylaxis spares an individual all of the psychological and physical distress related to chronic hepatitis C and can ameliorate the quality of life of both partners in an at-risk sexual relationship.

It is important to note that compliance with immunoglobulin prophylaxis was high; in our experience, only a few patients (1.6%) withdrew from the study. Withdrawal was not due to the adverse effects of treatment, but to lack of compliance, cessation of the sexual relationship or moving to another city.[35]

Intramuscular immunoglobulin is prepared according to the Cohn fractionation process, which separates the fraction containing antibodies that neutralise various infectious agents. Other manufacturing procedures do not ensure the same safety.[67] Over the last 50 years, many millions of individual worldwide have received intramuscular immunoglobulin. Intramuscular immunoglobulin prepared according to the Cohn process has been proclaimed safe by the Centers for Disease Control (Atlanta, Georgia, USA)[68,69] and the World Health Organization.[70] Recently, concern was aroused when 50% of immunoglobulin, both standard and hyperimmune, tested positive for HCVRNA.[67] We provided the first direct evidence that HCV infection is not transmitted by intramuscular HCV-RNA–positive immunoglobulin; in fact, in our trial a total of 3260 doses were administered, about 50% of which were HCV-RNA positive, and none of the immunoglobulin recipients monitored developed HCV infection.[67,71]

The safety of HCV-RNA–positive intramuscular immunoglobulin can be attributed to several factors: (i) partitioning of viruses away from the immunoglobulin; (ii) inactivation of viruses by the fractionation process; and (iii) a high concentration of neutralising antibodies.[67] The problem of the infectivity of immunoglobulin has been widely discussed.[67] It appears that intramuscular immunoglobulins have never transmitted HCV infection. Some intravenous immunoglobulin products used before 1994 caused a few cases of HCV infection, whereas intravenous immunoglobulin prepared after 1994, although many millions of grams are used each year, has not.[67] In fact, since 1994, in addition to the Cohn method, new additional stringent procedures of viral inactivation have been introduced to ensure that immunoglobulin is not infectious.[67,72,73]

The treatment is well tolerated and periodic injection over many years does not have any adverse effects. In our noncontrolled study, 78 patients were injected every 2 months for almost 7 years without untoward effects, and no sexual partners became HCV-RNA-positive.[35,67]

4. Perspectives for the Production of Hyperimmune Globulin Against HCV

There is compelling evidence that prophylaxis with the old standard immunoglobulin, prepared

from unscreened plasma pools also containing anti-HCV–positive units, significantly reduced the risk of HCV infection.

In the early 1990s, most developed countries forbade the use of anti-HCV–positive blood units for immunoglobulin products (e.g. 1990 in France, 1991 in the US, 1993 in Italy). After these dates, all blood units have been screened for anti-HCV and the units that test positive are discarded. At present, immunoglobulins are prepared only from anti-HCV–negative blood units. Unfortunately, these immunoglobulin preparations cannot replace the old unscreened immunoglobulin.[74] In fact, currently available immunoglobulin formulations prepared from screened donors are completely devoid of neutralising antibodies against HCV[55] and lack neutralising activity (anti-E2 NOB).[35] The only difference between the old and new preparations is that the unscreened immunoglobulin was prepared from a pool of plasma units containing a small percentage (about 2%) of anti-HCV–positive blood units, whereas screened immunoglobulin is prepared from anti-HCV–negative blood units. This led us to conclude that the HCV-neutralising antibodies (anti-E2 NOB) are contained in the anti-HCV–positive blood units that, according to law, are now discarded. Consequently, preparations obtained solely from anti-HCV–positive blood units should yield hyperimmune globulins against HCV (HCIg) containing a titre of neutralising antibodies about 50-fold higher than that found in standard unscreened immunoglobulin. The concentration of neutralising antibodies could be increased even further if preparations are made from only those anti-HCV–positive blood units that contain high titres of neutralising antibodies. HCIg will have the advantages of: (i) being easy to produce, because the manufacturing procedures are available; (ii) the long half-life of the drug and consequently the possibility of infrequent administration; (iii) additional viral inactivation procedures introduced to eradicate possibility of transmission of infection, and (iv) possible neutralisation of all emerging HCV strains. The drug should also be inexpensive. Unlike other hyperimmune immunoglobulins, such as HBIg which is produced from a limited number of selected donors, HCIg should be produced from a very large number of blood units (in order to contain neutralising antibodies to different strains of HCV) that are now discarded.

This prophylaxis could be applied in all situations where individuals are at risk of HCV infection (for example haemodialysis, intravenous drug users and needle stick injury) and in many other situations where appropriate preventive measures against disease transmission are not always taken (for example chiropodist or dental treatment, or colonoscopy). HCIg could also prevent reinfection of the transplanted liver in patients affected by HCV-related liver diseases: fulminant hepatitis, cirrhosis and hepatocellular carcinoma. Finally, one may envisage the use of HCIg as an option in the treatment of chronic hepatitis C[75] in combination with other antiviral drugs such as interferon and/or ribavirin.

5. Conclusions

There is now a large body of evidence that the 'old' immunoglobulin preparations protected from non-A, non-B/C virus infection patients exposed to: (i) transfusions (6 trials);[38,40-43,45] (ii) dialysis (1 trial);[46] (iii) sexual and/or horizontal transmission (1 trial).[47] In addition, a retrospective study has demonstrated that immunoglobulin significantly prevented ($p < 0.001$) the infection/reinfection of transplanted livers.[60] All these trials were undertaken before the discovery of HCV and the corresponding neutralising antibody.

A recent prospective randomised controlled trial, conducted after the discovery of HCV, demonstrated that the old standard immunoglobulin preparations significantly prevented sexual transmission of HCV.[35] The immunoglobulin lots used in this trial contained high titres of neutralising antibodies against HCV. Because these immunoglobulin preparations were produced using the same procedures as were those in the trials mentioned

above which protected from infection, we concluded that the old lots used in those trials also contained neutralising antibodies against HCV. Immunoglobulin is well tolerated and is safe to use (i.e. does not cause transmission of infection), even when administered for prolonged periods.

We have recently suggested that the production of anti-HCV hyperimmune globulins containing titres of neutralising antibodies about 50-fold higher than the old immunoglobulins is feasible.[35] The concentrations of neutralising antibodies could be increased even further if the hyperimmune globulins were prepared exclusively from anti-HCV-positive blood units characterised by particularly high concentrations of neutralising antibodies.[74] The available data indicate that hyperimmune globulin would be more effective than the early preparations, as well as safe to use and relatively inexpensive.

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