EPIDEMIOLOGY, RISK FACTORS, INVESTIGATIONS, MANAGEMENT AND PREVENTION OF HEPATITIS C VIRUS INFECTION

INTIKHAB ALAM

Department of Medicine,
Postgraduate Medical Institute,
Lady Reading Hospital, Peshawar.

INTRODUCTION

In 1970s hepatitis A virus (HAV) and hepatitis B virus (HBV) were identified. However there remained quite a number of cases of viral hepatitis which were not due to hepatitis A, B, cytomegalo or Ebstien-Barr viruses and the term non-A non-B hepatitis was used to describe such cases. The disorder accounted for 75-90% of transfusion induced hepatitis but it also included enterically transmitted, sporadic, endemic and community acquired disease.[1] In 1989, by using molecular biological techniques, the 20-years search for the baffling agent responsible for this disease culminated in the identification of the hepatitis C virus (HCV).[2] HCV is a member of the flaviviridae family, which includes yellow fever and Dengue viruses.[3] It has a positive, single stranded RNA genome which encodes a nucleocapsid protein (C), one or two envelope (E1 or E2) proteins and four or five nonstructural (NS) proteins.[2] Nucleotide sequence variation and cloning of RNA has confirmed the heterogeneity of HCV[4] leading to its classification into genotypes. The variability of HCV is such as to suggest a two-tier classification into "types" and "subtypes".[5,6] The known types are numbered from 1 and the subtypes a, b, and c in order of discovery. The current system of nomenclature includes 6 major genetic types and upto 40 different recognized subtypes.[5] Types 1a, 1b, 2a, 2b and 3 a are common in western Europe and USA. Type 1b, 2 and 2b in China and Japan, type 3 in Thailand, Singapore, Bangladesh and India, type 4 in Middle East and centra Africa and type 5 in South Africa.[6]

EPIDEMIOLOGY

Although the exact incidence in unknown, it has been estimated that HCV affects at least 200 million people[7] with an overall prevalence ranging from 0.5% to 8.0% in blood donors worldwide and is more prevalent than HIV or HBV in Europe and Japan.[8] HCV infection is particularly worrisome since it carries 70 to 80% chance of chronicity[9] and 20 to 50% risk of developing significant liver injury or cirrhosis.[10] This is in marked contrast to acute HAV infection which does produce liver cirrhosis and not an HBV infection which results in chronic hepatitis in <5% of the cases. HCV infection is usually and indolent and progressive disease leading to cirrhosis within 20 years in 20% of the cases[11] and hepato-cellular carcinoma within 30 years time in 10% of the cirrhotics.[8,12]

RISK FACTORS

Since the risk of transfusion-associated HCV infection has been greatly reduced by

screening, most HCV infections are community acquired and are prevalent among adolescents and young adults. However there is no evidence that HCV can be transmitted by feaco-oral route analogous to hepatitis A.[13] Because most of the times the course of the illness is subclinical, the natural history of the community acquired infection remains obscured[14] and that is why 40 to 50%[9,15,16] of infected patients have none of the following identifiable risk factors:

A. Blood transfusion:[17] Before screening of the blood donors was not compulsory, 1991 when HCV blood and blood products transfusion was responsible for 75% to 90% of transfusion induced hepatitis.[1,18] Screening of the donors led to a marked reduction in the incidence of HCV infection from blood transfusion or administration of blood products from 1 in 200[19] to 1 in 103,000 units transfused.[20]

B. Injectable drug abuse:[13] Smoking is the main way of consuming cannabis and heroine but addiction and the injudicious and unnecessary use of injections by quacks and doctors without taking necessary precautions may tantamount to the same grave consequences.

C. Needle stick exposure: Accidental prick to health care workers from injected needle anti HCV positive patients caries a risk of transmission from 3% to 10%.[21]

D. Sexual transmission: Transmission through monogamous sexual relationship is low with a life time risk of 10%[18] is proportional to the duration of the marriage[22] but it and may be as high as 32%.[23,24] Sexual transmission may be more important in prostitutes, individuals with promiscuous sexual activity and possibly in HCV partners coinfected with HIV or other sexually transmitted disease.[13]

E. Vertical transmission: It is an uncommon mode of transmission with a risk of 0 to 13% from HCV infected (HIV negative) mothers[25] but it is directly proportional to the mother's titre of HCV RNA[26] and to the degree of liver damage.[27]

F. Use of Intravenous Immune Globulin (IVIG): Several outbreaks of non-A non-B hepatitis associated with IVIG administration have been reported[28,29] which on retrospective analysis proved to be HCV infections. HCV RNA may be present even in preparations which have been produced from second-generation anti-HCV-screened plasma and therefore viral inactivation and removal steps are needed to ensure the safety of IVIG products.[30]

G. Ear piercing, tattooing[31] have also been associated with HCV infection.

H. Low socioeconomic status, overcrowding, history of imprisonment and living in a drug using environment all increase the risk of HCV infection.[9,13,31]

COURSE OF THE ILLNESS[32]

HCV RNA can be detected within 1-2 weeks of intravenous inoculation with infected blood followed by symptomatic acute hepatitis within 5-10 weeks in only 20% of the cases. Seroconversion usually occurs after 11-12 weeks but may not be demonstrable for upto one year. Therefore a negative antibody test does not rule out acute hepatitis C virus infection. Only 30% of the patients achieve rapid biochemical resolution with gradual disappearance of antibodies to the virus (mean time to disappearance is 5 years). Unfortunately 50-70% become long term carriers[9] with persistent viraemia in about 95% of these despite the antibody response and a risk of significant liver injury leading to cirrhosis in 20-50% of the cases.[10]

DIAGNOSTIC TEST FOR HCV AND EVALUATION OF HCV-POSITIVE PATIENT

Hepatitis C virus infection is difficult to recognize because it has a mild or subclinical course, clinical signs are often absent and the transition from the acute to the chronic stage is silent.[33,34] Moreover even serum markers for liver disease (e.g ALT) may be normal in healthy people whose serum contains antibodies to hepatitis C virus. Besides, the serum enzyme levels may fluctuate widely and may even be consistently normal in the face of histologically proven Chronic hepatitis and cirrhosis.[35] Therefore the provisional diagnosis relies on assays for specific antibodies to hepatitis C.[36] These assays detect the presence of antibodies to the viral antigens present on the solid phase of an enzyme-linked immunosorbent assay (ELISA). Initially only one antigen (C100-3) was used to detect the antibody (ELISA-I). This test proved to be of immense value in identifying patients exposed to the virus and in screening for potentially contaminated blood.[37] However it had several limitations e.g, it might remain negative until one year after acute infection; there were false positive results, especially in patients with hypergammaglobulinemia and autoimmune chronic active hepatitis; and false negative results in some patients with proven HCV on polymerase chain reaction (PCR).[38] To increase the sensitivity and specificity, second-generation antibody test (ELISA-II) was introduced which included three recombinant antigens, C100-3, C33c and dC22.[39] But still there were significant false positive results[18,40] and most importantly there was failure to assess the extent of viral replication and disease activity.[41] Therefore ELISA positive sera are further tested in confirmatory recombinant-immunoblot-assays (RIBA) which show the reactivity of the test serum with each different HCV antigens blotted onto different strips.[42] True positive sera react with two or more antigen bands while ELISA-positive sera reacting with a single band are termed indeterminate and by using this technique it has been ascertained that only 20 to 33% of ELISA-positive sera are true positive and quite a significant number are indeterminate.[18] To increase the sensitivity of ELISA test even further NS5 protein is also being added in third generation tests.[43] In instances where serologic testing does not lead to a secure diagnosis of hepatitis C infection it may be necessary to actually measure circulating HCV RNA by PCR.[44] However even PCR may not be perfect as it may be false positive as well due to nonspecific amplification or amplification of contaminating DNA. The most frequent source of contamination is aerosolized droplets of previously amplified DNA released into the environment when post-PCR reaction vials are opened.[45] Another method of detecting HCV RNA is Branched-DNA (bDNA) technology which can be applied directly to the samples, does not depend on amplification of target (making it easy to perform and is not subject to sample contamination) and is highly reproducible and sensitive.[39,46]

Having discussed all this, it will be prudent to enunciate at this point that there are no clinical or laboratory markers, including ALT, which are reliable predictives of liver damage in PCR-positive patients which makes liver biopsy an essential part of the management of such patients.[47] However liver biopsies of PCR-negative donors are almost always normal, while converse is true of PCR-positive donors.[18] Therefore, if one is trying to avoid liver biopsy in an anti-HCV-positive patient who is under consideration for interferon treatment, it is advisable to go for PCR to detect HCV RNA. However liver biopsy is useful for assessing and staging the disease and ruling out other causes of liver damage such as autoimmune liver disease, Wilson's disease alcohol or iron overload and therefore may clinicians prefer liver biopsy to demonstrate the extent of liver disease before initiating the treatment.[13]

MANAGEMENT

Treatment of chronic HCV infection is challenging, prolonged, expensive and most of all the results of the treatment are not very encouraging. Under this setting diagnosing HCV infection in a person, healthy enough to be considered for blood donation, is a dilemma both for the patient and the doctor. However, as chronic HCV infection is a common cause of liver cirrhosis and hepatocellular carcinoma worldwide[7] and as several studies have confirmed the efficacy of interferon alfa[48,49,50] alone or in combination with Ribavarine[51,52] all seropositive patients for HCV should be considered for the treatment after having done their PCR (for HCV RNA) and ideally a liver biopsy. Especially suitable candidates for interferon treatment are those who have elevated levels of ALT and chronic active hepatitis on liver histology.[53] Interferon is the only drug approved for HCV infection in a dose of 3 million units three times a week for 24 weeks (in USA) to 48 weeks (in Europe).[54] The initial trials were conducted before HCV RNA was isolated and quantitated and the response rate was defined as normalization of serum ALT. The results of the initial trial showed that 50% respond, 25% partially respond while 25% do not respond at all. However relapse occurred in 51% of the responders within 6 months of stopping the treatment[49] resulting in an overall cure rate of 20-25% only.[18,55] Patients who do not normalize their ALT within 12 weeks of starting the treatment are unlikely to have sustained response and their treatment may be stopped before completion of the course.[54] Besides, serum HCV RNA may still be positive in upto 40% of the patients with sustained ALT normalization after interferon therapy and late relapse may occur.[56,57] Therefore virologic end points are also important in defining the response. Loss of HCV RNA from serum during therapy and its continued absence after therapy is associated with long term improvement.[58] However, response rate falls to only 10-15% to a 6 month and 25-30% to a 12-18 months course of interferon treatment if the loss of HCV RNA is included in the definition of a response.[59] Moreover, an undetectable HCV RNA at the end of treatment does not preclude relapse.[53] Despite all these shortcomings it should be stressed that eradication of circulating HCV RNA is the goal of the therapy and that instead of a 6 months course, a 12-18 months course should be considered in all patients.[50] The treatment of chronic HCV infection should be considered successful in patients who have normal serum ALT levels and are negative for HCV RNA a year after discontinuing interferon treatment with histologically improved disease activity and perhaps a normal serum level of procollagen III peptide.[53] Successfully treated patents should be followed by obtaining serial serum ALT levels over 12-18 months period after cessation of therapy.[13] No studies of hepatitis C have included the critical end points of improved survival and prevention of cirrhosis.

TABLE – I

PREDICTORS OF LONG TERM RESPONSE TO INTERFERON ALFA TREATMENT

1. Age <45 years.
2. Female sex.
3. Body weight <70 Kg.
4. Shorter duration of illness.
5. Patients without cirrhosis or minimal hepatic fibrosis.
6. Low level of viremia (<350,000 HCV RNA eq/ml).
7. Infection with genotype 2 or 3.
8. Total interferon dose of >600 million units.

PREDICTORS OF NON-RESPONSE

1. Body weight >70 Kg.
2. Presence of cirrhosis on liver biopsy.
3. Infection with genotype 1b.

Nevertheless, the nagging questions still remain. Is interferon alfa effective in inducing permanent remissions? Does therapy change the natural history of chronic viral hepatitis? and Does it improve survival?[59]

Unfortunately, responsiveness to alfa interferon remains somewhat unpredictable. However, meticulous analysis of several pretreatment parameters has revealed several factors predicting favorable response to interferon treatment[13,53,60] Table-I.

Interferon is given subcutaneously at bedtime with 500 mg of Paracetamol one hour before its administration to minimize the flu-like symptoms like myalgia, fever and chills. Its long term side effects include psychological adverse effects like anxiety, irritability, marked mood swings and depression to the extent of suicidal tendencies.[48,61] There may be leuco and thrombocytopenia necessitating a temporary reduction in the dose of interferon, alopecia and reversible autoantibody reactions in the form of hyper or hypothyroidism, vasculitis and lupus like syndrome.

Various treatment strategies have been tried to help initial nonresponders and partial responders like increasing the dose of interferon to 5-10 million units 3 times a week with very little benefits[62] or combining it with another viral agent like Ribavarin with modest success.[51,52] Increased interferon responsiveness by reducing the iron content of the liver by repeated phlebotomies[63] needs confirmatory trials, similarly the role of maintenance interferon therapy to prevent relapse awaits the results of ongoing treatment trials. Finally, liver transplantation is the only answer in a stable cirrhotic patient. However viraemia is present in almost all patients within the first few months after transplantation, leading to significant liver injury in about 50% of the patients.[64]

PREVENTION OF HCV INFECTION

Fraught with all these grave consequences, with its natural history still remaining enigmatic and especially with no active or passive immunization yet on the cards, it becomes of paramount importance to take utmost precautions to avoid exposure to this dangerous organism. Screening of the blood donors was the first step in this direction which definitely resulted in the fall of blood and its products transfusion associated HCV infection. To further reduce the risk of transmitting the infection the transfusion of autologous bloody (by collecting patients own blood before elective surgery), along with intraoperative hemodilution and perioperative blood salvage is the safest method for the patients.[65] One should still keep in mind this advice from 21 years ago: "Blood transfusion is like marriage; it should not be entered upon lightly, unadvisedly or wantonly, or more often than is absolutely necessary".[66] HCV is present in low titres in blood; approximately 10-100 virus particles/ml virus particles/ml.[43] Therefore compared to HBV, HCV is less transmissible at a single exposure in medical personals after needle stick accidents.[67] However one must not get complacent in avoiding these accidents which may still cause infection in 3-10% of the cases.[21] Patient to patient transmission has been reported in patients admitted in hospitals.[68,69] Treatment that involves frequent parenteral procedures, tissues transfers, and often immunosuppression as well, provides numerous opportunities for patient-to-patient transmission of blood borne viruses. Whenever health care workers fail to comply with recommendations about hand washing which is generally considered the single most important procedure for preventing nosocomial infections[70] patient to patient transmissions are likely to occur. Environmental and instrumental contamination may heighten this risk as well.[71] Here lies the importance of isolating patents with HCV infection and that they should be

examined in the last during ward rounds immediately followed by hand washing to prevent inadvertent spread to others and to ourselves. Having discussed all this, the importance of curbing the quackery and injudicious use of injectable and "drips" in our medical practice cannot be underestimated. The best strategy in this regard would be a well publicized and widespread public health education program by the medical personals and by the government on the electronic media against this "injectable drug abuse" and its hazards in spreading blood-borne viral disease like HCV, HBV and HIV. The role of HCV in causing human sufferings is frightening as the list of diseases possibly caused by HCV is increasing. Apart from hepatitis, it may be a pathogenic factor in diseases like Cryoglobulinemia, Autoimmune thyroiditis, Glomerulonephritis, Lymphocytic sialoadenitis and sporadic porphyria cutanea tarda.[72]

PROSPECTS FOR A HEPATITIS C VACCINE

Seven years after the discovery of HCV, a vaccine against the virus still seems a long way off. Patients who have HCV infection are already positive for antibodies against a variety of viral proteins including nucleocapsid, nonstructural proteins and even the surface glycoproteins of the virion and whether this positivity against viral proteins will protect the individual from future challenge is still not known especially when studies have shown that repeated acute infections may occur in one individual.[73] Secondly the amino terminus of the surface glycoprotein (gp70) is highly variable and therefore variants may emerge following changes in this region that may escape neutralization by the antibody.[8] Thirdly without an efficient and reliable cell-culture system for HCV, an inactivated, whole virus vaccine is not yet possible. The most straightforward approach would be to develop a vaccine based on HCV proteins expressed by recombinant DNA technology and a hepatitis C vaccine that protects against chronic infection might be better than no vaccine, even if a mild and anicteric acute infection ensues in the event of the exposure. Finally, until a vaccine is available that is not only safe and effective but also cheap enough for widespread application, precautions for preventing parenteral spread are likely to be the only way of limiting transmission of HCV.

REFERENCES

1. Alter HJ, Purcell RH, Holland PV, et al. Clinical and serological analysis of transfusion associated hepatitis. Lancet 1975; 2: 838.
2. Choo QL, Kuo G, Weiner AJ, et al. Isolation of cDNA clone derived from a blood borne non-A non-B hepatitis genome. Science 1989; 244: 359.
3. Houghton M, Weiner A, Han J, et al. Molecular biology of hepatitis C viruses: Implications for diagnosis, development and control of viral disease. Hepatology 1991; 14: 381.
4. Ulrich PP, Romeo JM, Lane PK, et al. Detection, semiquantitation and genetic variation in hepatitis C virus sequences amplified from the plasma of blood donors with elevated alanine aminotransferase. J Clin Invest 1990; 86: 1609.
5. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol 1993; 74: 2391.
6. Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genomes. Hepatology 1994; 19: 1321.
7. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion induced hepatitis C. N Eng J Med 1995; 332: 1463.
8. Dusheiko GM, Harrison TJ. Genetic diversity of hepatitis C virus: implications for pathogenesis, treatment, and prevention. Lancet 1995; 345: 562.

9. Alter M. Epidemiology of hepatitis C in the West. Semin Liver Dis 1995; 15: 5.

10. Choo QL, Weiner AJ, Overby LR, et al. Hepatitis C virus. The major causitive agent of viral non-A non-B hepatitis. Br Med Bull 1990; 46: 423.

11. Tong MJ, El Farra NS, Reiks AR, et al. Clinical outcomes after transfusion associated hepatitis C. N Eng J Med 1995; 332: 1463.

12. Kiyosawa K, Sodeyamma T, Tanaka E, et al. Interrelationship of blood transfusion, non-A non-B hepatitis and hepatocellular carcinoma analysis by detection of antibody to hepatitis C virus. Hepatology 1990; 12: 671.

13. Sorrell MF. A 47 years old man with a symptomatic hepatitis C infection. JAMA 1996; 275(13): 1023.

14. Alter MJ, Margolis HS, Krawezynski K, et al. The natural history of community acquired hepatitis C in the United States. N Eng J Med 1992; 327: 1899.

15. Alter MJ, Hadler SC, Judson FN, et al. Risk factors for acute non-A non-B hepatitis in the United States and association with hepatitis C virus infection. JAMA 1990; 264: 2231.

16. Shapiro CN. Transmission of hepatitis viruses. Ann Intern Med 1994; 120: 82.

17. Aach RD, Stevens CE, Hollinger FB, et al. Hepatitis C virus infection in post transfusion hepatitis; an analysis with first and second generation assays. N Eng J Med 1991; 325: 1325.

18. Irvig WL. Hepatitis C virus infection and liver disease in blood donors. Br J of Hos Med 1994; 51(11): 567.

19. Donahue Jg, Munoz A, Ness PM, et al. The declining risk of post transfusion hepatitis C virus infection. N Eng J Med 1992; 327: 369.

20. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion transmitted viral infections. N Eng J Med 1996; 3334: 1685.

21. Mitsui T, Iwano K, Masuko K, et al. Hepatitis C virus infection in medical personal after needle stick accident. Hepatology 1992; 16: 1109.

22. Akahane Y, Kokima M, Sugai Y, et al. Hepatitis C virus infection in spouses of patients with type C chronic liver disease. Ann Intern Med 1994; 120: 748.

23. Osmond DH, Padian Ns, Sheppard HW, et al. Risk factors for hepatitis C virus seropostivity in heterosexual couples. JAMA 1993; 269: 361.

24. Kao JH, Chen PJ, Yang PM, et al. Intrafamilial transmission of hepatitis C virus the important role of infections between spouses. J Infect Dis 1992; 166: 900.

25. Lma PJ, McOmish F, Burns SM, et al. Infrequent vertical transmission of hepatitis C virus. J Infect Dis 1993; 167: 572.

26. Ohto H, Terazawa S, Sasaki N, et al. Transmission of hepatitis C virus from mother to infants. N Eng J Med 1994; 330: 744.

27. Kato N, Yukosuka O, Omato M, et al. Quantification of hepatitis C virus by competitive reverse transcription polymerase chain reaction: increase of the virus in advanced liver disease. Hepatology 1991; 14: 67.

28. Weiland O, Mattson L, Glauman H. Non-A non-B hepatitis after intravenous immunoglobulin. Lancet 1986; 1: 976.

29. Williams PE, Yap PL, Gillon J, et al. Non-A non-B hepatitis transmission by intravenous immunoglobulin. Lancet 1988; 2: 501.

30. Bresee JS, Mast EE, Coleman PJ, et al. Hepatitis C virus infection associated with administration of intravenous immune globulin a cohort study. JAMA 1996; 276: 1563.

31. Holsen DS, Harthug S, Myrmei H. Prevalence of antibodies to hepatitis C virus and association with drug abuse and tatooing in a national prison in Norway. Eur J Clin Microbiol Infect Dis 1993; 12: 673.

32. Levi S, Foster C. Chronic liver disease due to hepatitis C difficult to diagnose and treat. Br Med J 1993; 306: 1054.

33. Ryan KE, Maclennan S, Barbara JAJ, Hewet PE. Following of blood donors positive for antibodies to hepatitis C virus. BMJ 1994; 308: 696.

34. Bruno S, Rossi S, Letizia PM, et al. Normal aminotransferase concentrations in patients with antibodies to hepatitis C virus. BMJ 1994; 308: 697.

35. Van Rhiel DH, Caraceni P, Molloy PJ, et al. Chronic hepatitis C in patients with normal or near normal alanine aminotransferase levels the role of interferon alpha 2b therapy. J Hepatol 1995; 23: 503.

36. Kuo G, Choo QL, Alter Hj, et al. An essay for circulating antibodies to major etiologic virus of human non-A non-B hepatitis. Science 1989; 244: 362.

37. Alter Hj, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A non-B hepatitis. N Eng J Med 1989; 321: 1494.

38. Alter HJ. New kit on the block. Evaluation of second generation assays for detection of antibody to the hepatitis C virus. Hepatology 1992; 15: 350.

39. Craxi Am, Fiorentini G, Di Marco V, et al. Second generation tests in diagnosis of chronic hepatitis C. Lancet 1991; 337: 1354.

40. De Medina M, Schiff ER. Hepatitis C. Diagnostic assays. Semin Liver Dis 1995; 15: 33.

41. Aceti A, Taliant G, De Bac C, Sebastiani A. Anti HCV False positivity in malaria. Lancet 1990; 336: 1442.

42. Kleinmans S, Alter H, Busch M, et al. Increased detection of hepatitis C virus-infected blood donors by a multiple antigen HCV enzyme immunoassay. Transfusion 1992; 32: 805.

43. Thomas HC. Clinical features of viral hepatitis, in Oxford Textbook of Medicine, 3rd ed, Weatherall DJ, Ledingham JGG and Warrell DA (eds). Oxford University press, New York, 1996; 2063.

44. Inchauspe G, Abe K, Zebedee S, et al. Use of conserved sequences from hepatitis C virus for the detection of viral RNA in infected wera by PCR. Hepatology 1991; 14: 595.

45. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989; 346: 425.

46. Lau JYN, Simmonds P, Yedea MS. Implications of variations of conserved regions of hepatitis C virus genome. Lancet 1995; 346: 425.

47. Irving WL, Neal KR, Underwood JCE, et al. Trent Regional HCV Study Group. Chronic hepatitis in UK blood donors infected with hepatitis C virus. BMJ 1994; 308: 695.

48. Fried MW, Hoofnagle JH. Therapy of hepatitis C. Semin liver dis 1995; 15: 82.

49. Davis GL, Balart LA, Schiff ER, et al. Hepatitis Interventional Study Group. treatment of chronic hepatitis C virus recombinant interferon alfa. A multicenter randomised, controlled trial. N Eng Med 1989; 321: 1501.

50. Poynard T, Bedossa P, Cevallier M, et al. A comparison of three interferon alfa-2b regimens for the long term treatment of chronic non-A non-B hepatitis. N Eng J Med 1995; 332: 1457.

51. Lai MY, Kao JH, Yang PM, et al. Long term efficacy of ribavarin plus interferon alfa in the treatment of chronic hepatitis C. Gastroenterology 1996; 111: 1307.

52. Brillanti S, Garson J, Foli M, et al. A pilot study of combination therapy with ribavarin plus interferon-alfa for interferon-alfa resistant chronic hepatitis C. Gastroenterology 1994; 107: 812.

53. Dusheiko G. treatment of chronic viral hepatitis: the end of the beginning? Br J Hos Med 1994; 52: 8.

54. Wright TL, Presoton H. Interferon therapy for hepatitis C. Lancet 1996; 348: 973.

55. Tine F, Magrin S, Craxi A, Pagllaro L. Interferon for non-A non-B chronic hepatitis. A meta analysis of randomized clinical trials. J Hepatol 1991; 13: 192.

56. Saracco G, Risina F, Abate MI, et al. Long term follow up of patients with chronic hepatitis C treated with different doses of interferon alfa 2b. Hepatology 1993; 18: 1300.

57. Lau JYN, Mizokami M, Ono T, et al. Discrepancy between biochemical and virological response to interferon alfa in chronic hepatitis C. Lancet 19993; 342: 1208.

58. Hoofnagle JH. Therapy of acute and chronic hepatitis. Adv Inter Med 1994; 39: 241.

59. Hoofnagle JH, Lau D. Chronic viral hepatitis; benefits of current therapies. N Eng J Med 1996; 334: 1407.

60. McNair ANB, Tibbs CJ, Williams R. Hepatology; recent advances. BMJ (Pakistan) 1996; 6: 507.

61. Janssen HLA, Brouwer JT, Van Der Mast R, et al. Suicide associated with alfa interferon therapy for chronic viral hepatitis. J Hepatol 1994; 21: 241.

62. Marcellin P, Pouteau M, Martiont-Pignous M, et al. Lack of benefit of escalating dosage of interferon alfa in patients with chronic hepatitis C. Gastroenterology 1995; 109: 156.

63. Van Thiel DH, Friedlander L, Fagiuoli S, et al. Response to interferon alfa therapy is influenced by the iron content of the liver. J hepatol 1994; 20: 410.

64. Wright Tl, Periera B. Liver transplantation for chronic viral hepatitis. Liver Trans Surg 1995; 1: 30.

65. Holland PV. Viral infections and the blood supply. N Eng J Med 1996; 334: 1734.

66. Beal RW. The rational use of blood. Aust N Z J Surg 1976; 46: 309.

67. Mitsui T, Iwano K, Masuko K, et al. Hepatitis C virus infection in medical personnel after needle stick accident. Hepatology 1992; 16: 1109.

68. Allander T, Medin C, Kacobson SH, et al. Hepatitis C transmission in a hemodialysis unit: molecularm evidence for spread of virus among patients not sharing equipment. J Med Virol 1994; 43: 415.

69. Allander T, Gruber A, Naghavi M, et al. Frequent patient to patient transmission of hepatitis C virus in a hematology ward. Lancet 1995; 34: 603.

70. Jarvis WR. Handwashing: the Semmelweis lessons forgotten? Lancet 1994; 334: 1311.

71. Piazza M, Borgia G, Picciotto L, et al. Detection of hepatitis C virus RNA by polymeras chain reaction in dental surgeries. J Med Virol 1995; 45: 40.

72. Friedman LS. Liver, Biliary Tract and Pancreas, in Current medical Diagnosis and treatment. Lawrence M, Tierney Jr, Stephen J, McPhee and Maxine A (eds), Lange 1996; 581.

73. Farci P, Alter HJ, Govindaragan S, et al. Lack of protective immunity against reinfection with hepatitis C virus. Science 1992; 258: 135.