Nigerian Journal of Paediatrics 2008; 35 (3&4): 52-58

Review Article

Hepatitis C Virus Infection in Children

AM Yakubu[a], HM Muktar[b*], T Raji[c**]

Abstract

Yakubu AM, Muktar HM, Raji T. Hepatitis C Virus Infection in Children. *Nigerian Journal of Paediatrics.* 2008; 35: 52.

Hepatitis C virus (HCV) was first labeled non-A non-B hepatitis virus associated with blood transfusion in 1975. It was not until 1989 that the viral genome was cloned, sequenced and thereafter named hepatitis C virus. It is the third hepatotrophic virus of public health importance, affecting more than 170 million people worldwide. It is a single-stranded RNA virus of approximately 9.6 kb. In children, vertical transmission is the most prevalent mode of transmission and is said to occur in 0 to 36 percent of infants born to viremic mothers, with the highest rates occurring in mothers who are co-infected with HIV. The triad of pathological features in the liver comprises portal aggregates of mononuclear cells, steatosis and bile duct epithelial cell damage. Acute HCV infection is rare largely because it is clinically inapparent and therefore goes unrecognized. The diagnosis of HCV infection is based on the detection of antibodies to HCV antigens or the detection of viral RNA. Although pegylated interferon and ribavirin are widely used in the treatment of HCV infection, their efficacy is still far from being universally accepted. Infection in early childhood is associated with long term sequelae in adulthood and for this reason, there has been an explosion of studies in adults and not so much in children. This paper reviews the existing knowledge on the clinical features, natural history, immunopathogenesis and treatment of HCV infection in children.

Introduction

HEPATITIS C virus (HCV) is recognized as the third hepatotropic virus of major public health problem. Seroprevalence studies of this virus after its discovery in 1989 reveal wide geographical variations and distributions in different age groups. Before 1992, a good number of children acquired HCV infection through transfusions of blood and its products. Infection with HCV in early childhood is associated with long term sequelae in adulthood and for this reason, there has been an explosion of studies in adults and not so much in children ostensibly because it remains undetected as it is largely asymptomatic. Even when it becomes chronic, its symptoms such as fatigue or vague abdominal pain are usually vague and non-specific. The natural history of this infection has therefore remained scanty. The clinical course of HCV infection in children is influenced by several factors including the mode of infection and premorbid status of the child. To date, it is widely believed that mother-to-infant transmission constitutes the major mode of infection in the paediatric population with a prevalence of four percent to 36 percent.[1,2]

Ahmadu Bello University Teaching Hospital, Zaria

Department of Paediatrics
[a]Professor/Consultant
[b*]Senior Registrar
Department of Haematology
[c**]Senior Lecturer/Consultant Haematologist

Correspondence: Prof. AM Yakubu
E-mail: alhassanmaria@yahoo.com

Virology of HCV

Hepatitis C virus infection was first suspected in 1975 when it was labeled non-A non-B hepatitis associated with blood transfusion or transfusion associated hepatitis. This non-A non-B hepatitis agent remained a virological enigma until 1989 when the viral genome was cloned, sequenced and thereafter named HCV.[3] Hepatitis C virus is a single stranded RNA virus of the Flaviridae family with many genotypes, currently nine, with over 50 subtypes having wide geographical variations in their distributions.[4] It is approximately 9.6 kb, consisting of a five non-translated region (NTR) and a single uninterrupted open frame (ORF) encoding the viral protein. The envelope glycoprotein E1 and E2 are the functional subunits embedded in the lipid envelope of the virion.[5] E2 contains hypervariable regions called HVR 1 and

HVR 2 containing amino acids sequence 390- 410 and 474–480, respectively. Mutations occur in the HVR1 during the course of infection.[1] Individuals are usually infected with multiple genotypes and subtypes at the same time. This genetic diversity and the virus mutations contribute greatly to the inability of the host to mount effective immune response coupled with the propensity of the virus to evade immune response, thereby causing chronic infection. Although HCV is hepatotrophic, it does replicate in the spleen, the pancreas, adrenal glands, lymph nodes and thyroid tissues.[5,6]

Clinical features

Acute HCV infection in children is rarely observed except in very special circumstances such as transfusion associated epidemics, while fulminant hepatic failure from HCV infection is also rare. Those who develop chronic infection may take as long as eight years or more, before the manifestations become obvious.[6,7] Differences in the chronicity rates of infection in different paediatric cohort groups are determined by several factors including definition of clearance of HCV, duration of follow-up, population or size of the patients studied, mode of acquisition, age at acquisition and co-morbid factors. Manifestations of persistent infection with HCV include chronic active hepatitis, cirrhosis and hepatocellular carcinoma.[7] Extrahepatic manifestations include kerato-conjunctivitis secca, glomerulonephritis and autoimmune phenomena such as autoimmune arthritis and serum sickness-like illness.[4-6,8,9] Spontaneous remission of HCV in children defined as normalization of serum alanine aminotransferase lasting more than one year, disappearance of HCV RNA in the blood and decreased serum titre of anti-HCV core antigen, do occur. However, intrahepatic HCV RNA assessment which is necessary to confirm complete remission is not available in many places.[10]

In general, hepatic manifestations of HCV infection include elevated alanine aminotransferase and jaundice in about 25 percent of cases. Vague symptoms such as abdominal pain and fatigue also occur in the older child.

Modes of acquisition

Perinatally acquired HCV transmission is claimed to be the major route of HCV maternal-infant transmission. In this group, the liver disease may be mild or aggressive resulting in end-stage liver disease.[7] Infants born to mothers infected with HCV may become HCV-RNA positive while others remain negative.[10-12] In the case of transfusion-associated HCV infection, the underlying pre-transfusion pathological states play a crucial role in determining the outcome. Cancers, thalassaemia, sickle cell anaemia, and immunodeficiency have adverse effects on patients with HCV infection.[7] Hepatitis C viral infections in transfused patients with malignancies and immunodeficiency states (both primary and secondary) are associated with higher percentage of chronic infections and worse clinical outcome compared with the usually milder outcome associated with HCV infections in otherwise healthy children.[13,14] Poly transfused thalassaemic patients may have more severe hepatic injury and less response to therapy, as a result of secondary haemochromatosis.[14]

The modes of transmission of HCV in the general population include occupational exposure such as needle prick among health workers, percutaneous exposure in other settings associated with commercial barbering, tattooing, ear piercing, sharing of toothbrush, traditional scarifications and circumcisions by traditional surgeons.[4,7] Other modes include diagnostic and therapeutic procedures like endoscopy, venepuncture, urinary catheterization and blood transfusions. Commercial sex workers and homosexuals are at higher risk of infection with HCV and of co-infection with HBV and HIV.

Transmission and risk factors

Before 1992, blood transfusion was considered to be the sole mode of transmission of HCV. However, when blood screening for HBV, HCV, and HIV became available, the rate of new HCV infection declined by more than 50 percent, lowering the risk of HCV seroconversion to 1.54 percent in the USA. By 1999, transfusion related HCV infection was reduced to zero.[1,2]

Intrafamilial transmission has been reported in Egypt[2] among children, where infected parents have been implicated. The Egyptian study showed that children whose parents had anti-HCV were at greater risk for HCV infection than those children whose parents did not. The association was higher with mothers than with fathers and when both parents had HCV RNA. Sequencing the viral isolates from some of the families with parents and children who had HCV RNA showed the viruses to be genetically identical.[2] Mother-infant vertical transmission rates range from 0 to 36 percent with the highest rates occurring in mothers who are co-infected with HIV. Factors influencing mother–infant transmission include the presence or absence of HCV RNA, viral load, HIV co-infection in the mother, mode of delivery (vaginal vs caesarian section), bottle vs breast feeding, and HCV subtypes.[15] Vertical transmission has not so far been reported in infants of mothers

who are HCV RNA negative,[15] but babies born to asymptomatic mothers who are HCV RNA positive and develop infection are frequently associated with high maternal viral load greater than 5.0×10^6. The rate of HCV infection in babies of HCV infected mothers has been reported to be higher in those delivered vaginally than by caesarian section.[16] This is yet to be corroborated in larger studies.

The role of breast feeding in the transmission of HCV is still debatable. Kumar[17] in United Arab Emirates reported that both anti-HCV antibody and HCV RNA were detected in colostrums albeit at significantly lower levels ($P < 0.0001$). However, symptomatic mothers with anti-HCV titres of the order of 1:45,000 to 1:90,000 and HCV RNA estimate between 2.5 and 4.5×10^9, infected their offsprings who developed symptoms at the age of three months. In this group, hepatitis C genotype 3a was concordant within each pair. All the infants were delivered by caesarian section at term, breastfed and the mothers had no apparent nipple trauma. The authors concluded that breastfeeding among HCV asymptomatic mothers was safe but symptomatic mothers with high viral load should avoid breastfeeding their babies. This recommendation need to be substantiated particularly where the possibility of estimating the viral load is not possible.

At the moment, there is no satisfactory diagnostic tool for perinatal infection with HCV. Many studies theorized about optimal time for determining infection status of infants.[15-17] Both anti-HCV and HCV RNA have proved unreliable markers of HCV infection in the perinatal period up to the age of two years. Anti-HCV which are detectable in neonates, decreases from four months to 18 months and HCV RNA positivity occurs at birth in some babies and disappears at six months and vice versa.[18] All the same, detection of HCV viraemia at birth in those infants who develop chronic HCV disease is highly suggestive of intrauterine infection.[15]

Hepatitis C virus subtypes 1a and 3a have been reported with high frequency among Italian pregnant mothers with HIV co-infection,[15] accounting for up to 30 percent of vertical transmission. Six main HCV genotypes have been identified to date and these are important because of their significance in relation to geographical occurrence, severity, and response to treatment. Both current and past maternal intravenous drug abuse are also considered risk factors for paediatric infections with HCV.

Pathology

Immunopathogenesis

Acute HCV infection is rare largely because it is clinically inapparent and therefore goes unrecognized. Farci *et al* [19] followed a group of 12 patients with acute infection and observed that those who cleared the acute infections had vigorous T cell response to the HCV that later resulted in the formation of quasispecies. The development of viral quasispecies correlated well with the development of chronicity and viral mutation.[20,21] Failure to clear the HCV led to chronic infection. The normal immune response for viral clearance occurs through the activation of natural killer cells, processing of viral antigens by immature dentritic cells which on maturity activate $CD4^+$ and natural killer T cells. $CD4^+$ cells normally produce cytokines which induce cytotoxic T lymphocytes. Cytotoxic T lymphocytes control the replication of the virus through lysis of infected cells or through production of cytokines which inhibit replications of the virus.[21]

How does the HCV escape all these processes and result in persistent infection? This could be as a result of insufficient quantity of the virus specific immune response or insufficient qualitative immune response due to either low level of viral expression on the surface of the host cells or incomplete activation of the virus specific cytotoxic T lymphocytes.[21] Secondly, viral evasion of these immune responses could occur through viral sequence variation or the virus may directly interfere somehow with antigen processing.[21,22] It has been observed that T lymphocytes subpopulations in the liver and peripheral circulation differ profoundly.[21,22] The liver contains unconventional lymphocytes that are not found in the peripheral blood.[5] These include $CD4^-$ and $CD8^-$ double negative T cells, $CD4^+$ and $CD8^+$ double positive T cells that have specific receptors and furthermore, the liver may contain natural killer cell marker CD56 and T cell marker CD3 all of which have distinct functional characteristic ability to recognize nonspecific antigens presented to them by non-classical MHC molecules.[22] The liver has a high percentage of truly resident lymphocytes. These lymphocytes are distinct in their ability to express genes such as recombinant activation genes 1 and 2 otherwise expressed by immature thymocytes undergoing gene rearrangement.[23]

The HCV circulate as populations of quasispecies making immune escape possible. Some of the viral variants act as antagonist for induction of T cells. Hepatitis C virus is able to control CD4+ T cells by mutating its immnodominant epitomes that down regulate antiviral T helper cells (Th1) response thereby up regulating T helper 2 cytokines which foster host tolerance to HCV, even though infection with other pathogens produce protective antibodies through this pathway.[24]

Two kinds of autoantibodies associated with autoimmune phenomenon in HCV infection are

organ and non-organ specific autoantibodies. The organ specific autoantibodies are those of smooth muscles, gastric parietal cell, antinuclear antibodies and liver–kidney antibodies.[7] The prevalence of liver–kidney and gastric parietal cell antibodies have been reported to be higher in children than adults and are common in the course of natural infection by HCV in children. These do not seem to be influenced by interferon therapy.[8,9]

Persistence of HCV infection

From the above, it would be seen that once chronic infection is established, the HCV specific immune response could exert some degree of control over the viral load but it seems incapable of terminating totally either persistent infection and/or resolve chronic hepatitis. The intensity of immune response depends on cytopathic effects of the virus, antigenic load, co-stimulatory signals, and the cytokine profile of T helper cells; each of these do not seem to be optimal in acute HCV infection.[3–5] It seems that HCV has evolved several strategies to actively suppress efficient immune system. The high genetic variability of HCV may be the overriding factor of viral persistence. The high propensity of the virus to mutate due to high replication rate with an estimated half life of circulating HCV of three hours make elimination of the virus through therapy with antiviral drugs very difficult.[10] The development of effective vaccines lies in a careful analysis of these factors to map out the basic strategic points of action.

Hepatic injury

Hepatitis C virus is not cytopathic like HBV.[10] However, while the role of antigen specific T cells in liver injury is well established in HBV and other hepatotropic virus infections, the mechanism of liver injury in HCV infection is still inconclusive. In primary HCV infection, the liver cell damage coincides with the development of host immune response and not with viral replication.[11] Secondly, chronic viral replication has been observed in humans without evidence of liver cell damage.[12] It has also been observed that immune suppression of patients with chronic HCV infection is associated with transient normalization of transaminase and upsurge in viraemia, suggesting immunologically mediated liver cell damage and control of viral replication.[13] Evidence from histological examination of liver biopsy in patients with HCV infection shows that liver cell damage is associated with inflammatory infiltrates in the liver by HCV immune effector cells.[5] Both humoral and cellular mechanisms are involved in the pathogenesis of liver injury in HCV infection.[6,14]

Humoral immune response is targeted against epitomes with the HCV proteins, but whether this is done through humoral antibody binding to HCV and preventing viral entry or that of opsonisation for elimination by macrophage for onward transmission to secondary lymphatic organs to induce cellular immune responses not yet settled. Hepatitis C virus antibodies in human sera are detectable between seven and 31 weeks after infection,[15] a period considered too late compared with humoral response to other viruses.[16] Evidence for a protective role of HCV-specific antibodies has been derived from chimpanzees' infections where HCV has been neutralized *in vitro* by inoculation with antibodies. The hyper variable region (HVR1) of the HCV E2 protein is identified as the target for neutralizing antibodies.[17]

$CD4^+$ T helper cells, $CD8^+$ T cells and B cells are found in the portal areas of the liver.[18] This lymphoid triad form a characteristic histological finding in HCV infection. Intrahepatic T helper cells response is focused on HCV N54 protein.[19] Their response is mediated by T cells that express selective T cell receptors which are not found on the peripheral blood components of the $CD4^+$ and $CD8^+$ T cells. Immune mediated liver disease is therefore initiated by HCV-specific liver infiltrating T cells. Presentation of HCV antigens on infected hepatocytes, recognition by cytotoxic T lymphocytes and induction of liver injury seem to be induced by HCV which in turn, result in the expression of HLA-A, B and C as well as intercellular adhesion molecules inclusive of CD8, macrophages and monocytes.[2] The end result is that these activated cells mediate enhanced antigen presentation and inflammatory process.[2] HCV infected hepatocytes are killed by HCV specific cytotoxic T lymphocytes clonus via tumour necrosis factor (TNF) predominantly produced by macrophages and are released and expressed on the surface of cytotoxic T lymphocytes.[3] After exerting their effector functions, many of these liver infiltrating HCV specific T cells undergo programmed cell death.[14]

Histopathology

Reported histological features of hepatic lesions due to HCV infection in children are rather few and have largely come from multi-centre studies in Japanese, European and North American children.[12,18] Given the geographic variation in the distribution of HCV genotype, the histological findings are those of a wide range of morphological features.[6,19,20] These include portal fibrosis, portal central bridging, sinusoidal lymphocytosis, bile duct epithelial damage and steatosis. Cirrhosis was reported in eight to 14

percent of cases studied from these centres. Necroinflammatory changes ranged from mild to severe and seem to correlate well with fibrosis and elevated serum alanine aminotransferase, while steatosis correlates with body mass index as well as alanine aminotransferase.[5,6] Overweight children are frequently noted to have more fibrosis than those who are not. In general, the triad of pathological features is portal aggregates of mononuclear cells, steatosis and bile duct epithelial cell damage.[7]

Diagnosis

The diagnosis of HCV infection is based on serological and virological tests. Serum alanine aminotransaminase is elevated even in asymptomatic patients with HCV infection. Serial estimation may be necessary to evaluate the course of the illness since the behaviour of this parameter is known to be variable. Maternal-neonatal HCV transmission is associated with early transient elevation of serum transaminase levels for a few years returning to normal regardless of the liver histology.[8] Levels of liver–kidney microsomal antibodies should be estimated where facilities are available. Virological studies should include HCV viral load, HCV RNA and viral genotypes and subtypes. Although liver biopsy for histology is not necessary for the diagnosis of HCV infection, it still remains the gold standard for assessment of the severity of hepatic injury and for identifying and quantifying any cofactor of hepatic damage such as steatosis and coinfection.[9] Ultrasound examination is reported to be useful in picking steatosis in about 20 percent of patients.[10]

Indications for liver biopsy: Although liver biopsy for histology has been an important tool in the management of paediatric patients with HBV, it is a painful and an invasive procedure. It is subject to sampling errors despite revised criteria for sample adequacy.[10,11,12] The problems of sampling errors have been extensively studied in adults but not in children.[4] These reasons have made liver biopsy in children highly debatable. However, the following can be considered reasonable indications for liver biopsy for histology:[13]

1. Evaluation of hepatic damage associated with autoimmune markers particularly liver–kidney autoantibodies
2. Exclusion of liver cirrhosis.
3. Staging of liver disease.
4. Evaluation of cofactors e.g., steatosis

Treatment

As indicated from the foregoing discourse, there is still a lot of unclear areas which frustrate physicians as far as treatment and prevention of HCV are concerned. The lack of universally acceptable immune interaction between HCV and the host, the multiplicity of HCV genotypes and subtypes having widespread geographical variation with different behaviour of these viral variants in human disease have all frustrated the formulation of effective therapeutic policy for the treatment of HCV infection. Furthermore, the fact that infection with HCV could be asymptomatic makes it difficult to formulate indication for therapy apart from the fact that there is yet to be developed, drugs that are effective against the HCV. Although commonly asymptomatic, the possibility of progressing to significant liver disease including cirrhosis, end-stage liver disease and hepatoma, increases with time. The indications for treatment include persistence of HCV RNA beyond 6–12 months usually with a viral load in the millions, and chronic inflammation of the liver at biopsy and histology, with or without hypertransaminasemia. In addition, interferon alpha or pegylated interferon plus ribavirin are now well established in the treatment of HCV infection.[7,14]

Some authorities have recommended that persistent viral replication which are known in some cases to progress to end-stage liver disease in the perinatally exposed group, maternal intravenous drug use and infection with HCV genotype 1a and 3a, should be considered indications for early treatment with pegylated interferon and ribavirin.[12]

In a previous study, children with histologically proven chronic hepatitis C, otherwise healthy but with elevated transaminase, positive antihepatitis C virus antibodies and HCV RNA were recruited for treatment with interferon and ribavirin. Alanine aminotransferase levels normalized in 45 percent without relapse at 30 months follow-up and HCV-RNA disappeared in 29 percent. Biopsy specimen in treated patients showed improvements; the proportion was however not stated.[13]

Although interferon–alpha (IFN á) and ribavirin are widely used in the treatment of HCV infection, their efficacy is still far from being universally accepted. All the same, treatment of children with HCV infection is still being advocated. Rapicetta *et al*[14] recommended the use of IFN-alpha in children in the treatment of HCV infection. The possible explanations for initial good response in children treated with IFN include the shorter time of infection in children compared to adults and that most of the cases in children were classified as mild.

Conclusion

Hepatitis C virus, considered as the third hepatotrophic viral infection in human subjects, is a

major public health problem because of its protean clinical presentations, wide range of genotypes with wide geographical distributions and its propensity to evade immune response resulting in ineffective therapeutic and preventive interventions. It poses a serious public health problem in childhood.

Acknowledgements

We wish to thank all the authors whose publications are cited in this article.

References

1. Ding Yon Li, Schwarz KB. Immunopathogenesis of chronic hepatitis C virus infection. *J Pediatr Gastroenterol Nutr* 2002; 35: 260–7.
2. Mohamed MK, Magder LS, Abdel-Hamid M, *et al.* Transmission of hepatitis C virus between parents and children. http://www.ajtmh.org/cgi/content/full/75/1/16 .
3. Choo QL, Kuo G, Weiner AJ, *et al.* Isolation of a cDNA clone derived from a blood-borne non A non B viral hepatitis genome. *Science* 1989; 244: 359–62.
4. Sehgal S, Jackson Allen PL. Hepatitis C in children. *Pediatr Nurs* 2004; 30: 409–13.
5. Rehermann B. Interaction between the hepatitis C virus and the immune system. *Semin Liver Dis* 2000; 20: 127–41.
6. Laskus T, Radkowski M, Wang LF, *et al.* Search for hepatitis C virus extrahepatic replication sites in patients with acquired immunodeficiency syndrome: specific detection of negative-strand viral RNA in various tissues. *Hepatology* 1998; 28: 1398–401.
7. Rosenthal P. Hepatitis C in children: Update 2006. http://www.hcvadvocate.org/hcvp/articles/hcv_children_2006.html.
8. Fujisawa T, Komatsu I, Inui A, *et al.* Spontaneous remission of chronic hepatitis C in children. *Eur J Pediatr* 1997; 156: 773–6.
9. Gregorio GV, Pensati P, Iorio R, *et al.* Autoantibody prevalence in children with liver disease due to chronic hepatitis C virus (HCV) infection. *Clin Exp Immunol* 1998; 112: 171–6.
10. Azzari C, Resti M, Bortolotti F, *et al.* Serum levels of hepatitis C virus RNA in infants and children with chronic hepatitis C. *J Pediatr Gastroenterol Nutr* 1999; 29: 514–7.
11. Sasaki N, Matsui A, Momoi M, *et al.* Loss of circulating hepatitis C virus in children who developed a persistent carrier state after mother-to-baby transmission. *Pediatr Res* 1997; 42: 263–7.
12. Palomba E, Manzini P, Fiammengo P, *et al.* Natural history of perinatal hepatitis C virus infection. *Clin Infect Dis* 1996; 23: 47–50.
13. Zeller A, Thomas DL, Moolchitker C, *et al.* High viral load and mild liver injury in children with hemophilia compared with other children with chronic hepatitis C virus infection. *J Pediatr Gastroenterol Nutr* 1999; 29: 418–23.
14. Veruchi G, Lenzi M. Allard T, *et al.* Natural history of chronic viral HCV in children. http://www.hepatprimer.com/patient/child23.html
15. Moriya T, Sasaki T, Mizui M, *et al.* Transmission of hepatitis C virus from mothers to infants: its frequency and risk factors re-visited. *Biomed Pharmacother* 1995; 49: 59–64.
16. Zuccotti GV, Ribero ML, Giovannini M, *et al.* Effect of hepatitis C genotype on mother-to-infant transmission of virus. *J Pediatr* 1995; 124: 278–80.
17. Kumar RM, Shahul S. Role of breast feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. *J Hepatol* 1998; 29: 191–7.
18. Marco M. Epidemiology, modes of transmission and risk factors for hepatitis C virus (HCV). http://www.thebody.com/content/art1696.html
19. Farci P, Alter HJ, Shimoda A, *et al.* Hepatitis C virus-associated fulminant hepatic failure. *N Engl J Med* 1996; 335: 631–4.
20. Alter NF. Immunology and pathogenesis of hepatitis C virus. http://www.medscape.com/viewarticle/412948
21. Bendelac A, Lantz O, Quimby ME, *et al.* CD1 recognition by murine NK1 + T lymphocytes. *Science* 1995; 268: 863–5.
22. Collins C, Norris S, McEntee G, *et al.* RAG1, RAG2 and pre-T cell receptor alpha chain expression by adult human hepatic T cells: evidence for extrathymic T cell maturation. *Eur J Immunol* 1996; 26: 3214–8.
23. Eckels DD, Wang H, Bian TH *et al.* Immunobiology of hepatitis C virus (HCV) infection: the role of CD4 T cells in HCV infection. *Immunol Rev* 2000; 174: 90–7.
24. Koziel MJ. Cellular immune responses against hepatitis C virus. *Clin Inf Dis* 2000; 31: Suppl 1:S25–31.
25. Brillanti S, Foli M, Gaiani S, *et al.* Persistent hepatitis C viraemia without liver disease. *Lancet* 1993; 341: 464–5.
26. Koziel MJ, Dudley D, Wong JT, *et al.* Intrahepatic cytotoxic T lymphocytes specific for hepatitis

C virus in persons with chronic hepatitis. *J Immunol* 1992; 149: 3339-44.

27. Alberti A, Cavalletto D, Pontisso P, *et al*. Antibody response to pre-S2 and hepatitis B virus induced liver damage. *Lancet* 1988; 1: 1421–4.
28. Arichi T, Major M, Wedemeyer H, *et al*. A vigorous HCV helicase specific T helper response dominates in the liver of chimpanzee during acute self limiting hepatitis C. *J Virology* 1999; 73: 1118-26.
29. Simmonds P, Rose KA, Graham S, *et al*. Mapping of serotype-specific immunodominant epitopes in the NS-4 region of hepatitis C virus (HCV): use of type-specific peptides to serologically differentiate infections with HCV types1, 2, and 3. *J Clin Microbiol* 1993; 31:1493–503.
30. Kinkhabwala M, Schajpal P, Skolnik E, *et al*. A novel addition to the T cell repertory: Cell surface expression of tumor necrosis factor/ cachectin by activated normal human T cells. *J Expt Med* 1990; 171:941-6.
31. Nuti S, Rosa D, Valiante NM, *et al*. Dynamics of intra hepatic lymphocytes in chronic hepatitis C: enrichment for Valpha24+ T cells and rapid elimination of effector cells by apoptosis. *Eur Immunol* 1998; 28:3448 55.
32. Fujisawa T, Inui A, Komatsu H, *et al*. A comparative study on pathologic features of chronic hepatitis C and B in paediatric patients. *Pediatr Pathol Mol Med* 2000; 9:469-80.
33. Badizadegan K, Jonas MM, Ott MJ, *et al*. Histopathology of the liver in children with chronic hepatitis C viral infection. *Hepatology* 1998; 28:1416 23.
34. Kage M, Fujisawa T, Shiraki, *et al*. Pathology of chronic hepatitis C viral infection in children. Child Liver Study Group of Japan. *Hepatology* 1997; 26: 771-5.
35. Guido M, Bortolotti F, Jara P, *et al*. Liver steatosis in children with chronic hepatitis C. *Am J Gastroenterol* 2006; 101: 2611-5
36. Guido M, Colloredo G, Fassan M, *et al*. Clinical practice and ideal liver biopsy sampling standards: not just a matter of centimeters. *J Hepatol* 2006; 44:823-4.
37. Guido M, Rugge M. Liver biopsy sampling in chronic viral hepatitis. *Semin Liver Dis* 2004; 24: 89-97.
38. Guido M, Bortolotti F. Chronic viral hepatitis in children: any role for the pathologist? *Gut* 2008; 57: 873-7.
39. Goodman ZD, Makhlouf HR, Liu L, *et al*. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C trial. *Hepatology* 2008; 47: 836-43.
40. Comparcola D Nobili V, Sartorelli MR, *et al*. Childhood hepatitis C virus infection. *J Gastroenterol Hepatol* 2005; 20:1948-9.
41. Bortolotti F, Verucchi G, Cammà C, *et al*. Long-term course of chronic hepatitis C in children: from viral clearance to end stage liver disease. *Gastroenterology* 2008; 134:1900-7.
42. Iorio R, Pensati P, Porzio S, *et al*. Lymphoblastoid interferon alfa treatment in chronic hepatitis C. *Arch Dis Child* 1996; 74: 152-6.
43. Jonas MM. Interferon-alpha for viral hepatitis. *J Pediatr Gastroenterol Nutr* 1996; 23: 93-106.
44. Rapicetta M, SuperSarita IST. High dosage Alpha – Interferon for treatment of children and young adults with chronic hepatitis disease. *Pediatr Infect Dis J* 1997; 16:1049-53.