ANTIBODIES AGAINST NON-STRUCTURAL c100/3 AND STRUCTURAL CORE ANTIGEN OF HEPATITIS C VIRUS (HCV) IN HEMODIALYSIS PATIENTS

C.F.T. YOSHIDA (1), Y. TAKAHASHI (2), B.O.M. VANDERBORGHT (3),
C.D. ROUZERE (3), M.S. FRANÇA (1), C. TAKAHASHI (4), A. TAKAMIZAWA (2),
I. YOSHIDA (2) & H.G. SCHATZMAYR (1)

SUMMARY

Two groups of patients undergoing hemodialysis (HD) maintenance were evaluated for their antibody response to non-structural c100/3 protein and structural core protein of hepatitis C virus (HCV). Forty-six patients (Group 1) never presented liver abnormalities during HD treatment, while 52 patients (Group 2) had either current or prior liver enzyme elevations. Prevalence rates of 32.6% and 41.3% were found for anti-c100/3 and anti-HCV core antibodies, respectively, in patients with silent infections (Group 1). The rate of anti-c100/3 in patients of Group 2 was 71.15% and reached 86.5% for anti-HCV core antibodies. The recognition of anti-c100/3 and anti-core antibodies was significantly higher in Group 2 than in Group 1. A line immunoassay composed of structural and non-structural peptides was used as a confirmation assay. HBV infection, measured by the presence of anti-HBc antibodies, was observed in 39.8% of the patients. Six were HBsAg chronic carriers and 13 had naturally acquired anti-HBs antibodies. The duration of HD treatment was correlated with anti-HCV positivity. A high prevalence of 96.7% (Group 2) was found in patients who underwent more than 5 years of treatment. Our results suggest that anti-HCV core ELISA is more accurate for detecting HCV infection than anti-c100/3. Although the risk associated with the duration of HD treatment and blood transfusion was high, additional factors such as a significant non-transfusional spread of HCV seems to play a role as well. The identification of infective patients by more sensitive methods for HCV genome detection should help to control the transmission of HCV in the unit under study.

KEY WORDS: HCV; Anti-c100/3; anti-HCV core; Hemodialysis; Aminotransferases; Anti-HBc.

INTRODUCTION

The acquisition of blood-transmitted infections remains an important risk in the course of hemodialysis treatment. Following the control of hepatitis B infection through the screening of hepatitis B surface antigen (HBsAg) and the establishment of supplementary measures to prevent the spread of hepatitis B virus (HBV) in dialysis units, the importance of what was then designated non-A, non-B hepatitis (NANBH) became apparent[5,20,22,23]. Important recent advances made in the characterization of the causative agent of NANBH[4], provide good evidence that most NANBH cases associated with transfusion are caused by hepatitis C virus (HCV)[7,26].

The development of a recombinant-based immunoassay for the detection of antibodies against c100/3[13], related to parts of the NS3 and NS4 genomic regions of HCV, has led to many clinical and epidemiological investigations of HCV

(1) Departamento de Virologia, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.
(2) The Research Foundation for Microbial Disease of Osaka University, Kanonji, Japan.
(3) Innogenetics, Industriepark, Ghent, Belgium.
(4) Clínica de Doenças Renais, Rio de Janeiro, Brazil.
Address for correspondence: Clara Fumiko Tachibana Yoshida, Departamento de Virologia, Fundação Oswaldo Cruz, Av. Brasil, 4365, 21040-360. Rio de Janeiro, RJ. Brazil.

infection[1,6,14]. Worldwide application of this assay demonstrated different prevalence rates of anti-c100/3 in hemodialysis (HD) patients: rates of 1% in the United Kingdom, 10% in Germany, 18% in Italy, and 20% in Spain were recorded in Europe, and ranged from 12 to 22% in the USA and Japan[6,8,11,16,19,29].

The complete primary structure and genetic organization of HCV isolated from human carriers was recently described in Japan, showing 77% of nucleic acid homology with the USA isolates[12,24]. Fragments of cDNA corresponding to the structural core region were cloned and molecularly expressed in cultured mammalian cells and in the baculovirus system[3,9,25]. Using this protein in immunoassays, early kinetics of antibody development were determined allowing detection of infection in patients who could not be diagnosed by the c100/3 system[3,10,27].

We investigated the presence of antibodies to a structural core recombinant protein of HCV and compared its frequency with that of antibodies against HCV non-structural c100/3. As confirmation assay, a line immunoassay composed of structural and non-structural synthetic peptides coated on nylon strips was used. Association with hepatitis B virus infection was studied by means of an anti-HBc assay. The influence of the duration of HD treatment and the number of blood transfusions received was also correlated with the risk for HCV infection.

PATIENTS AND METHODS

Characterization of Patients

Ninety-eight patients (44 females, 54 males) undergoing HD maintenance at the Clínica de Doenças Renais, Rio de Janeiro, Brazil, were investigated for a period of six months. Three serum samples were collected from each patient at 3-month intervals starting in June 1990. The 44 female patients had a mean age of 46 ± 16 years (range 5-72), while the 54 males averaged 51 ± 16 years (range 12-83). The patients were divided into two groups. A first group (Group 1) consisted of 46 patients and included those with normal levels of liver enzyme activity. These patients underwent HD treatment for an average period of 25.8 ± 22.7 months (range 1-107 months) without presenting any elevations in liver enzymes. Alanine

aminotransferase (ALT) and aspartate aminotransferase (AST) were tested once a month, with an upper assay limit of 28 U/ml for uremic patients. Eleven of these patients had never received blood transfusions at that time and the remaining 35 patients had already been transfused with an average of 8.17 ± 9.09 units of blood.

A second group (Group 2) was composed of 52 patients with current or previously elevated levels of liver enzymes at least 2.5 times greater than the upper normal limit. The average period of HD treatment was 72.8 ± 42.6 months (range 1-185 months). The average of blood units transfused in this group was 13.72 ± 13.87 and 5 patients had never received blood transfusion.

Dialysis Unit

This unit is a satellite unit with a separate room for HBsAg-positive patients. All hollow fiber dialysers were reprocessed and sterilized with 1% formaldehyde at 38°C. HBsAg-positive dialysers were processed in the HBsAg-positive room. All dialysis equipment was disinfected at the end of the day with 1% hypochlorite solution. On weekends the unit was disinfected with 5 to 8% acetic acid. HBsAg was monitored and ALT and AST levels were measured monthly. Precautions for the prevention of hepatitis B infection and active immunization were implemented from 1979 and 1982, respectively.

Serological Assays

HBsAg and antibodies to HBsAg (anti-HBs) were tested by ELISA (Bio-manguinhos/FIOCRUZ). Antibodies to hepatitis B core antigen (anti-HBc) were detected by an in-house direct ELISA, using recombinant HBc antigen. The plasmid was a gift of Dr. W.H. Gerlich from the Hygiene Institute of Goettingen University, Germany.

Three tests were used to detect antibodies to HCV: Anti-non-structural c100/3 was detected using the ORTHO HCV first generation ELISA (ORTHO DIAGNOSTIC, USA) according to the manufacturer's instruction. Anti-HCV structural core was detected by a direct home-made ELISA using microplates coated with purified recombinant core antigen. This antigen with molecular

size of 15 kDa, encoded by the region 369-704 of HCV genome[24] was produced at the Research Foundation for Microbial Disease of Osaka University, Japan and was kindly supplied to perform the tests. Briefly, polyvinyl microplates (Hemobag, Campinas, Brazil) were coated with purified antigen and kept overnight at 4°C. The plates were washed once and incubated one hour at 37°C with blocking buffer. After washing, 100 μl of diluted sample (1/20) were dispensed into the wells. After one hour of incubation at 37°C, the plates were washed and anti-human IgG peroxidaselabeled conjugate (Bio-manguinhos, FIOCRUZ) was added. After one hour of incubation at 37°C, the plates were washed again and hydrogen peroxide in chromogenic solution (TMB) was added. The reaction was stopped after 20 min and the plates were read at 450 nm using a Multiskan microplate reader. The determination of cut-off points was made after testing 100 different negative controls. The mean and standard deviation (SD) were calculated and the frequency distribution was constructed. A cut-off value was chosen which allowed 98% specificity. This value was equal to the mean + 3 SD. Optical densities (OD) equal to or greater than the cut-off value were considered reactive. Lower values, were considered non-reactive. In order to confirm reactive samples for anti-c100/3 and anti-HCV core antibodies were used the anti-HCV INNO-LIA test. This single strip line immunoassay (INNO-LIA HCV Ab, INNOGENETICS, Belgium) is based on antigens representing the immunodominant epitopes of several HCV regions (core, NS4, and NS5) which permit differential detection and confirmation of different antibodies to HCV. The assay was performed according the manufacturer's instructions.

Statistical Analysis

Fisher's exact test and the chi-squared test applied with Yate's correction were used for analysis of the data; $\alpha = 0.05$.

RESULTS

The analysis of the three sequential samples collected from each patient during a six-month period defined the HCV status. Of the 98 patients, only one showed evidence of seroconversion. Two tests were used to evaluate the prevalence of anti-

HCV antibodies: anti-c100/3 and anti-HCV core antibodies (Table 1). Fifty patients were positive with both tests and these results were subsequently confirmed by the line immunoassay (INNO-LIA). Nineteen showed reactivity in only one of the two ELISAs (2 for anti-c100/3, 17 for anti-HCV core), 16 of which were confirmed with the INNO-LIA (2 for anti-c100/3, 14 for anti-HCV core). There was 100% (52/52) correlation between anti-c100/3 and INNO-LIA reactivity. Concordant results between anti-HCV core and INNO-LIA were found in 95.53% (64/67), and one serum was INNO-LIA positive and negative for other tests.

TABLE 1
Correlation Between c100/3 and HCV Core Antibodies in Hemodialysis Patients Samples

	anti-c100/3 (+)	LIA (+)	anti-c100/3 (-)	LIA (+)	Total	LIA (+)
anti-HCV core (+)	50	50	17	14	67	64
anti-HCV core (-)	2	2	29	1	31	3
Total	52	52	46	15	98	67

anti-c100/3: ORTHO/HCV, 1st generation, USA
anti-HCV core: in-house EIA, using recombinant antigen, Japan
LIA: line immunoassay, INNOGENETICS, Belgium

HCV infection was considered to be present when at least one of the antigens was recognized. The prevalence of anti-HCV in patients of Group 1, which never presented alterations of liver enzymes, was 32.6% for anti-c100/3 and 41.3% for anti-HCV core antibodies. In Group 2, 71.1% and 86.5% of patients with liver enzyme alterations were positive for anti-c100/3 and anti-HCV core antibodies, respectively (Table 2).

TABLE 2
Prevalence of Anti-HCV Antibodies in Two Groups of Hemodialysis Patients

anti-HCV antibodies	Group 1 +/total (%)	Group 2 +/total (%)
Non-structural c100/3	15/46 (32.6)	37/52 (71.1)
Structural core	19/46 (41.3)	45/52 (86.5)

Group 1: patients with normal liver enzyme activity
Group 2: patients with previous or currently elevated liver enzyme activity.

The prevalence of anti-c100/3 and anti-HCV core antibodies in Group 2 was significantly higher than that in Group 1. There was no statistically significant difference between the prevalence of anti-c100/3 and core antibodies in each of the groups (Group 1: $\chi^2 = 0.42$; Group 2: $\chi^2 = 2.78$).

Regarding hepatitis B infection, anti-HBc antibodies were found in 39.8% (39/98) of HD patients. Six patients were chronic carriers of HBsAg and 13 had naturally acquired anti-HBs antibodies. The prevalence of anti-HBc was 2.56 times higher in Group 2 patients than in those of Group 1. Elevated transaminases levels were much more related to HCV than to HBV infection. Both HCV and HBV markers of infection were present in 48.1% (25/52) of the Group 2 patients in contrast to 13.0% (5/46) of the patients of Group 1. Absence of both HCV antibodies was observed in 45.7% (21/46) of Group 1 patients versus 5.8% (3.52) of Group 2 (Table 3).

TABLE 3
Correlation Between HBV and HCV Infection by Means of the Presence of Anti-HBc and Anti-HCV Antibodies

Infection	Group 1 total	%	Group 2 total	%
HBV + /HCV +	6	13.0	25	48.1
HBV + /HCV −	4	8.7	3	5.8
HBV − /HCV +	15	32.6	21	40.4
HBV − /HCV −	21	45.7	3	5.8
	46		52	

Group 1: patients with normal liver enzyme activity
Group 2: patients with previous or currently elevated liver enzyme activity.

Table 4 shows the positive correlation between the duration of HD treatment and HCV infection. Of 15 patients who underwent less than one year of HD treatment, 12 belonged to Group 1. In contrast, of 35 patients with more than 5 years of HD treatment, 31 belonged to Group 2; 30 of these, developed anti-HCV antibodies. It seems that duration of HD treatment is correlated to anti-HCV positivity.

TABLE 4
Prevalence of anti-HCV Antibodies in HD Patients and Correlation with Duration of HD Treatment and Blood Transfusion.

HD Treatment	Anti-HCV + (%)	Blood Transfusions No	Yes (x Units ± SD)
< 1 year			
Group 1	4/12 (33.3)	1	3 (9.0 ± 7.7)
Group 2	1/3 (33.3)	0	1 (5.0)
1-5 years			
Group 1	13/30 (43.3)	2	11 (11.6 ± 10.2)
Group 2	15/18 (83.3)	3	12 (11.2 ± 12.9)
> 5 years			
Group 1	2/4 (50.0)	0	2 (4.5 ± 0.5)
Group 2	30/31 (96.7)	2	28 (19.9 ± 15.7)
Total	65/98 (66.3)	8	57 (13.6 ± 13.9)

Group 1: patients with normal liver enzyme activity
Group 2: patients which previous or currently elevated liver enzyme activity

Although blood transfusion was considered an important risk factor for acquisition of HCV infection, the number of transfused blood units did not clearly influence the rate of infection. Out of the 65 anti-HCV positive patients, 57 (89.0%) were transfused with a mean of 13.6 units. But of the 34 HCV-negative patients, 26 (76.5%) were transfused with a mean of 7.3 units. Both Groups 1 and 2 presented anti-HCV antibodies. Prolonged HD treatment and transfusions of a large number of blood units in Group 2 patients account for a high percentage of HCV infection (96.7%). It should also be noted that in the group of patients who never received a transfusion, anti-HCV antibodies were detected in 8 such patients, of which 3 belonged to Group 1 and 5 to Group 2. They represent 25% and 100% respectively of the non-transfused patients in those groups.

DISCUSSION

While prophylactic measures for the control of hepatitis B have been established since 1979 in our hemodialysis unit[23], the impact of NANBH has become increasingly apparent, as seen by the elevation of serum transaminases levels, the absence of serological markers for hepatitis B virus, hepatitis A virus, cytomegalovirus, Epstein-Barr virus, and the exclusion of other factors that could

induce liver enzyme alterations. A NANBH prevalence rate of 10% was observed in 1988[22]. In 1989, we collected blood from 16 of those patients who had survived dialysis treatment and from new cases which had appeared during these years. Patients were tested for HCV infection by the ORTHO c-100/3 test. All patients were positive with this test indicating that HCV was the causative agent for the observed NANBH[30].

In the present study, we used two different tests to detect antibodies against proteins encoded by different parts of the HCV genome. Structural core and non-structural c-100/3 were used. No differences in reactivity patterns against these two antigens were observed during the 6 months of follow-up, except for 1 seroconverting patient. A previous evaluation of the prevalence of anti-HCV antibodies in a multicenter study performed in Rio de Janeiro revealed that approximately 50% of HD patients had contact with the virus[unpublished data,21]. In comparison with European countries, the USA, and Japan, this prevalence can be considered as being high[6,8,11,16,19,29].

Both ELISAs used in this study showed a good predicability in this high-risk group, confirmed by line immunoassay, although it is likely that false positives would be found in a blood donors population[2,28].

The study of two groups of patients in the same HD unit showed that patients who never presented signs of liver injury during HD treatment had been exposed to HCV. The same observation was made in multitransfused hemophiliac patients[18]. However, the strong correlation between the group of patients with elevated ALT levels and anti-HCV reactivity clearly showed that HCV infection is a major cause of liver disease in HD patients[15,29]. Only 3 out of 52 patients with elevated ALT levels showed results suggestive of causes other than HBV or HCV infection.

Forty-eight percent (25/52) of Group 2 patients had markers for both HBV and HCV infection. In addition, 21 patients were anti-HCV positive without any marker for HBV, which indicates chronic infection caused by HCV. Although the presence of anti-HCV core antibodies seems to be associated with the presence of the viral genome, it would be appropriate to detect cDNA by means of PCR in order to establish whether the liver is actively infected or if these

findings are due to either silent infection or to long-lasting antibodies in resolved hepatitis.

Regarding the 6 chronic hepatitis B patients, 3 were also positive for anti-HCV (2 with a ALT pattern and one without ALT alterations). The percentage of HBsAg in anti-HCV-positive patients was much lower than the percentage of HBsAg in HCV-negative patients (4.5% vs. 9.7% respectively). The same observations have been made by other authors[17,27]. Nevertheless, further studies are needed to evaluate the interference of HCV in chronic hepatitis B patients and its influence on liver disease.

Several authors have described the correlation betweeen the duration of hemodialysis treatment and HCV infection. Furthermore, the number of blood units transfused during HD treatment has been considered as an important risk factor for acquisition of HCV infection[8,17,18]. The adoption of anti-HCV screening measures in blood banks — now gradually occurring in Rio de Janeiro — will undoubtedly decrease the incidence of HCV infection through transfusion. However, the existence of contamination deduced from HCV-infected, non-tranfused patients, indicates, that the measures adopted to prevent the spread of hepatitis within HD units may be insufficient. The correct application of control measures must be strickly enforced to prevent new infections.

RESUMO

Anticorpos contra os antígenos não estrutural c100/3 e estrutural core do vírus da hepatite C (HCV) em pacientes de hemodiálise.

Dois grupos de pacientes em tratamento hemodialítico foram avaliados quanto às respostas de anticorpos contra as proteínas não estrutural c100/3 (anti-c100/3) e estrutural core (anti-HCV core) do vírus da hepatite C (HCV). Quarenta e seis pacientes (Grupo 1) nunca apresentaram alterações dos níveis de alanina aminotransferase (ALT) durante o tratamento hemodialítico, ao passo que 52 pacientes (Grupo 2) têm ou já tiveram elevações desta enzima hepática. Em pacientes sem alterações bioquímicas evidentes (Grupo 1) foram encontradas prevalências de 32.6% e 41.3% para anti-c100/3 e anti-HCV core, respectivamente. No Grupo 2, estas prevalências foram de 71,15% e 86,5%. Um teste composto de pepti-

319

deos estruturais e não estruturais imobilizados separadamente e dispostos em bandas paralelas em fitas de nailon, foi usado como teste confirmatório. A infecção pelo HBV, avaliada pela presença de anticorpos anti-HBc foi observada em 39.8% dos pacientes; 6 eram portadores crônicos de HBsAg e 13 possuiam anti-HBs adquiridos naturalmente. O tempo de tratamento hemodialítico teve correlação com a positividade para anti-HCV. A prevalência de 96,7% (Grupo 2) foi encontrada em pacientes que tiveram mais de 5 anos de tratamento. Nossos resultados sugerem que o anti-HCV core é um marcador mais preciso para detectar a infecção pelo HCV do que o anti-c100/3. Apesar dos riscos associados com a duração do tratamento e transfusões sanguíneas, uma significante disseminação não transfusional do HCV parece ocorrer dentro da unidade. A identificação de pacientes infectantes por métodos sensíveis como a detecção do genoma viral deverá auxiliar no controle da transmissão do HCV na unidade em estudo.

ACKNOWLEDGEMENTS

REFERENCES

1. ALTER, H.J.; PURCELL, R.H.; SHIH, J.W.; MELPOLDER, J.C.; HOUGHTON, M.; CHOO, Q.L. & KUO, G. -Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. **New Engl. J. Med., 321:** 1494-1500, 1989.

2. BARBARA, J.A. & CONTRERAS, M. - Non-A, non-B hepatitis and the anti-HCV assay. **Vox Sang. (Basel), 60:** 1-7, 1991.

3. CHIBA, J.; OHBA, H.; MATSUURA, Y.; WATANABE, Y.; KATAYAMA, T.; KIKUCHI, S.; SAITO, I. & MIYAMURA, T. - Serodiagnosis of hepatitis C virus (HCV) infection with an HCV core protein molecularly expressed by a recombinant baculovirus. **Proc. nat. Acad. Sci. (Wash.), 88:** 4641-4645, 1991.

4. CHOO, Q.L.; KUO, G.; WEINER, A.J.; OVERBY, L.R.; BRADLEY, D.W. & HOUGHTON, M. - Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. **Science, 244:** 359-362, 1989.

5. DIENSTAG, J.L. & ALTER, H.J. - Non-A Non-B hepatitis: evolving epidemiological and clinical perspective. **Semin. Liver Dis., 6:** 67-81, 1986.

6. ESTEBAN, J.I.; ESTEBAN, R.; VILADOMIU, L.; LOPEZ TALAVERA, J.C.; GONZALEZ, A.; HERNANDEZ, J.M.; ROGET, M.; VARGAS, V.; GENESCA, J.; BUTI, M.; GUARDIA, J.; HOUGHTON, M.; CHOO, Q.L. & KUO, G. - Hepatitis C virus antibodies among risk groups in Spain. **Lancet, 2:** 294-297, 1989.

7. ESTEBAN, J.I.; GONZALEZ, A.; HERNANDEZ, J.M.; VILADOMIU, L.; SANCHEZ, C.; LOPEZ TALAVERA, J.C.; LUCEA, D.; MARTIN VEGA, C.; VIDAL, X.; ESTEBAN, R. & GUARDIA, J. - Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. **New Engl.J. Med., 323:** 1107-1112, 1990.

8. GILLI, P.; MORETTI, M.; SOFFRITTI, S.; MARCHI, N.; MALACARNE, F.; BEDANI, P.L.; DE PAOLI VITALI, E.; FIOCCHI, O. & MENINI, C. - Non-A, Non-B hepatitis and anti-HCV antibodies in dialysis patients. **Int. J. artif. Organs, 13:** 737-741, 1990.

9. HARADA, S.; WATANABE, Y.; TAKEUCHI, K.; SUZUKI, T.; KATAYAMA, T.; TAKEBE, Y; SAITO, I. & MIYAMURA, T. - Expression of processed core protein of hepatitis C virus in mammalian cells. **J. Virol., 65:** 3015-3021, 1991.

10. HOSEIN, B.; FANG, C.T.; POPOVSKY, M.A.; YE, J.; ZHANG, M. & WANG, C.Y. - Improved serodiagnosis of hepatitis C virus infection with synthetic peptide antigen from capsid protein. **Proc. nat. Acad. Sci. (Wash.), 88:** 3647-3651, 1991.

11. JEFFERS, L.J.; PEREZ, G.O.; DE MEDINA, M.D.; ORTIZ INTERIAN, C.J.; SCHIFF, E.R.; REDDY, K.R.; JIMENEZ, M.; BOURGOIGNIE, J.J.; VAAMONDE, C.A.; DUNCAN, R.; HOUGHTON, M.; CHOO, Q.L. & KUO, G. - Hepatitis C infection in two urban hemodialysis units. **Kidney Int., 38:** 320-322, 1990.

12. KATO, N.; HIJIKATA, M.; OOTSUYAMA, Y.; NAKAGAWA, M.; OHKOSHI, S.; SUGIMURA, T. & SHIMOTOHNO, K. - Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. **Proc. nat. Acad. Sci. (Wash.), 87:** 9524-9528, 1990.

13. KUO, G.; CHOO, Q.L.; ALTER, H.J.; GITNICK, G.L.; REDEKER, A.G.; PURCELL, R.H.; MIYAMURA, T.; DIENSTAG, J.L.; ALTER, M.J.; STEVENS, C.E.; TEGTMEIR, G.E.; BONINO, F.; COLOMBO, M.; LEE, W.S.; BERGER, K.; SCHUSTER, J.R.; OVERBY, L.R.; BRADLEY, D.W. & HOUGHTON, M. - An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. **Science, 244:** 362-364, 1989.

14. MATTSSON, L.; ABERG, B.; VON SYDOW, M. & WEILAND, O. - Incidence of hepatitis and seroconver-

sion to hepatitis C virus after open-heart surgery in transfused and non-transfused patients in Sweden. **Scand. J. infect. Dis., 23:** 25-29, 1991.

15. MONDELLI, M.V.; CRISTINA, G.; FILICI, C.; RONDANELLI, R.G.; PIAZZA, V. & BARBIERI, C. - Anti-HCV positive patients in dialysis units? **Lancet, 336:** 243-244, 1990.

16. MORTIMER, P.P.; COHEN, B.J.; LITTON, P.A.; VANDERVELDE, E.M.; BASSENDINE, M.F.; BRIND, A.M. & HANBLING, M.H. - Hepatitis C virus antibody. **Lancet, 2:** 798, 1989.

17. PONZ, E.; CAMPISTOL, J.M.; BARRERA, J.M.; GIL, C.; PINTO, J.; ANDREU, J. & BRUGUERA, M. - Hepatitis C virus antibodies in patients on hemodialysis and after kidney transplantation. **Transplant. Proc., 23:** 1371-1372, 1991.

18. RUMI, M.G.; COLOMBO, M.; GRINGERI, A. & MANNUCCI, P.M. - High prevalence of antibody to hepatitis C virus in multi-transfused hemophiliacs with normal transaminase levels. **Ann. intern. Med., 112:** 379-380, 1990.

19. SCHLIPOTTER, U.; ROGGENDORF, M.; ERNST, G.; RASSHOFER, R.; DEINHARDT, F.; WEISE, A.; GLADZIWA, U.& LUZ, N. - Hepatitis C virus antibodies in hemodialysis patients. **Lancet, 335:** 1409, 1990.

20. SEAWORTH, B.J.; GARRET, L. E.; STEAD, W.W. & HAMILTON, J.D. - NonA, nonB hepatitis in chronic dialysis - Another dilemma. **Amer. J. Nephrol., 4:** 235-239, 1984.

21. STURM, J.A.; PEREIRA, C.F.A.; SUASSUNA, J.H.R.; NOGUEIRA NETO, J.F.; LOBÃO, V.I.; COELHO, C.J.B.P. & LAURIA, C. - Marcadores virais (vírus B e vírus C) da hepatite em uma unidade de transplante renal. **Rev. bras. Pat. clín., 23:** 128-135, 1990.

22. TAKAHASHI, C.; YOSHIDA, C.F.T.; GASPAR, A.M.C.; BAPTISTA, M.L.; HOETTE, M. & RUZANY, F. - Ocorrência das hepatites nãoA, nãoB em unidade de hemodiálise. **Rev. Soc. bras. Med. trop., 21:** 105-111, 1988.

23. TAKAHASHI, C.; YOSHIDA, C.F.T.; HOETTE, M.; RUZANY, F. & SCHATZMAYR, H.G. - Prevenção e controle da hepatite B em unidade de diálise. **J. bras. Nefrol., 7:** 1-8, 1985.

24. TAKAMIZAWA, A.; MORI, C.; FUKE, I.; MANABE, S.; MURAKAMI, S.; FUJITA, J.; ONISHI, E.; ANDOH, T.; YOSHIDA, I. & OKAYAMA, H. - Structure and organization of the hepatitis C virus genome isolated from human carriers. **J. Virol., 65:** 1105-1113, 1991.

25. TAKEUCHI, K.; KUBO, Y.; BOONMAR, S.; WATANABE, Y.; KATAYAMA, T.; CHOO, Q.L.; KUO, G.; HOUGHTON, M.; SAITO, I. & MIYAMURA, T. - Nucleotide sequence of core and envelope genes of the hepatitis C virus genome derived directly from human healthy carriers. **Nucleic Acids Res., 18:** 4626, 1990.

26. VAN DER POEL, C.L.; REESINK, H.W.; LELIE, P.N.; LEENTVAAR KUYPERS, A.; CHOO, Q.L.; KUO, G. & HOUGHTON, M. — Anti- hepatitis C antibodies and non-A, non-B post-transfusion hepatitis in The Netherlands. **Lancet, 2:** 297-298, 1989.

27. WATANABE, Y.; HARADA, S.; SAITO, I. & MIYAMURA, T. - Prevalence of antibody against the core protein of hepatitis C virus in patients with hepatocellular carcinoma. **Int. J. Cancer, 48:** 340-343, 1991.

28. WONG, D.C.; DIWAN, A.R.; ROSEN, L.; GERIN, J.L.; JOHNSON, R.G.; POLITO, A & PURCELL, R.H. - Non-specificity of anti-HCV test for seroepidemiological analysis. **Lancet, 336:** 750-751, 1990.

29. YAMAGUCHI, K.; NISHIMURA, C.; FURUOKA, N.; MACHIDA, J.; KUSUMOTO, Y.; FUTAMI, G.; ISHII, T. & TAKATSUKI, K. - Hepatitis C virus antibodies in hemodialysis patients. **Lancet, 335:** 1409-1410, 1990.

30. YOSHIDA, C.F.; TAKAHASHI, C.; GASPAR, A.M.; SCHATZMAYR, H.G. & RUZANY, F. - Hepatitis C virus in chronic hemodialysis patients with non-A, non-B hepatitis. **Nephron, 60:** 150-153, 1992.