

ELEVATED ALANINE AMINOTRANSFERASE (ALT) IN BLOOD DONORS : AN ASSESSMENT OF THE MAIN ASSOCIATED CONDITIONS AND ITS RELATIONSHIP TO THE DEVELOPMENT OF HEPATITIS C

Fernando Lopes GONÇALES Jr. (1), Raquel Silveira Bello STUCCHI (1), Priscila Maria Oliveira PAPAORDANOU (1), Maria Helena Postal PAVAN (1), Neiva Sellan Lopes GONÇALES (2) & João Renato Rebello PINHO(3).

SUMMARY

The determination of aminotransferases levels is very useful in the diagnosis of hepatopathies. In recent years, an elevated serum ALT level in blood donors has been associated with an increased risk of post-transfusion hepatitis (PTH). The purpose of the study was to research the factors associated with elevated ALT levels in a cohort of voluntary blood donors and to evaluate the relationship between increased ALT levels and the development of hepatitis C (HCV) infection. 166 volunteer blood donors with elevated ALT at the time of their first donation were studied. All of the donors were questioned about previous hepatopathies, exposure to hepatitis, exposure to chemicals, use of medication or drugs, sexual behaviour, contact with blood or secretions and their intake of alcohol. Every three months, the serum levels of AST, ALT, alkaline phosphatase, gamma glutamyl transpeptidase, cholesterol, triglyceride and glycemia are assessed over a two year follow-up. The serum thyroid hormone levels as well as the presence of auto-antibodies were also measured. Abdominal ultrasound was performed in all patients with persistently elevated ALT or AST levels. A needle biopsy of liver was performed in 9 donors without definite diagnostic after medical investigation. The presence of anti-HCV antibodies in 116 donors were assayed again the first clinical evaluation. At the end of follow-up period (2 years later) 71 donors were tested again for the presence of anti-HCV antibodies. None of donors resulted positive for hepatitis B or hepatitis C markers during the follow-up. Of the 116 donors, 101 (87%) had persistently elevated ALT serum levels during the follow-up. Obesity and alcoholism were the principal conditions related to elevated ALT serum levels in 91/101 (90.1%) donors. Hypertriglyceridemia, hypercholesterolemia, hypothyroidism and diabetes mellitus also were associated with increased ALT levels. Only 1/101 (0.9%) had mild chronic active non A-G viral hepatitis and 3/101 (2.9%) had liver biopsy with non-specific reactive hepatitis. The determination of ALT levels was not useful to detect donors infected with HCV at donation in Brazil, including the initial seronegative anti-HCV phase.

KEYWORDS: Blood donors; ALT; Pos transfusion hepatitis; anti-HCV

INTRODUCTION

The determination of transaminase levels is very useful in the diagnosis of hepatic diseases. Alanine aminotransferase (ALT) is present in great quantities in liver cells and an increase in the serum levels of this enzyme is observed in inflammatory liver damage. ALT levels are elevated in acute and chronic hepatitis as well as in non-infectious conditions such as obesity, diabetes, alcohol abuse, reactions to hepatotoxic drugs and in rare cases of enzyme deficiencies¹⁸. In recent years, an elevated serum ALT level in blood donors has been associated with an increased risk of post-transfusion hepatitis (PTH)^{1,2}. For this reason, it was proposed that the ALT levels of blood donors should be determined during routine screening^{1,2,21}. In general, transfusion services have introduced such screening for two reasons, namely as a surrogate marker for non-A, non-B hepatitis and as a diagnostic criterion for PTH²⁸.

Since the cloning of hepatitis C virus (HCV) in 1989, diagnostic tests to identify HCV-infected individuals have been developed⁹ and the number of cases of transfusion-associated hepatitis has declined significantly^{10,33}. It is very important now the evaluation of the usefulness of surrogate markers in the screening of blood donors for hepatitis C because the anti-HCV-EIA test is very sensitive and specific and is widely used. In Brazil, as in other countries, HCV is the most prevalent viral cause of post-transfusion hepatitis¹⁶ and ALT determination is a compulsory routine test to exclude HCV infected blood donors.

This study assesses the factors associated with elevated ALT levels in a cohort of voluntary blood donors rejected by a university hospital blood bank and evaluates the relationship between raised ALT levels and the development of HCV infection among them.

(1) Disciplina de Moléstias Infecciosas da Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil.

(2) Laboratório de Sorologia, Centro de Hematologia e Hemoterapia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil.

(3) Serviço de Virologia, Instituto Adolfo Lutz, Secretaria Estadual da Saúde, São Paulo, Brasil.

Correspondence to: Fernando Lopes Gonçalves Junior. Av. Dr. Romeu Tortima 725. 13084-520 Campinas, SP, Brasil. E-mail: flopes@correionet.com.br.

MATERIALS AND METHODS

One-hundred and sixteen volunteer blood donors who were rejected by a university hospital blood bank because of elevated serum ALT levels found at the time of their first donation, were studied. All of the donors tested negative for hepatitis B surface antigen (Abbott Auzyme Monoclonal, Abbott Laboratories, North Chicago, IL, USA), anti-HCV (Abbott 2nd. generation HCV EIA, Abbott Laboratories, North Chicago, IL, USA), anti-HIV 1/2 (Abbott 3rd. generation, anti-HIV), syphilis and Chagas' diseases. They also showed no clinical symptoms before donation. In the presence of an abnormal level of ALT (>40 IU/L), the donors were sent to the ambulatory for diagnostic elucidation.

All of the donors were questioned about previous hepatopathies, exposure to hepatitis, exposure to chemicals, use of medication or drugs, sexual behavior, contact with blood or secretions and their intake of alcohol. Those who consumed more than 30 grams of alcohol daily were considered alcoholic, especially if their γ -glutamyl transpeptidase levels were elevated. Each of the subjects was examined physically for the presence of icterus and enlargement of the liver and spleen. Based on their body weight and the National Health and Nutrition Examination Survey tables (NHANES) and Body Mass Index [BMI = body weight (Kg)/height (m)] they were classified as overweight¹³. Every three months, the serum levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase, γ -glutamyl transpeptidase, bilirubin, cholesterol, triglycerides, glycemia and the electrophoretic profile of plasma proteins were assessed over a one to two-year follow-up. Donors with elevated ALT levels only at the time of blood donation were placed in Group I while those with persistently elevated levels during the follow-up were placed in Group II. ALT values higher than 40 IU/L were labeled as elevated. The serum thyroid hormone levels as well as the presence of auto-antibodies (anti-nuclear, anti-smooth muscle, anti-mitochondrial, anti-DNA) were measured. Abdominal ultrasound was used to check for hepatomegaly in all patients with persistently elevated ALT or AST levels. A needle biopsy of the liver using the Menghini technique³⁰ was performed in nine concordant individuals with no defined etiology after clinical and laboratorial evaluation.

Anti-HCV antibody levels in the 116 donors were assayed at the time of blood donation and then at the first clinical evaluation. During the follow-up (about two years), 10 donors from Group I and 61 donors from Group II were tested again for anti-HCV antibodies in order to eliminate a possible delayed HCV seroconversion. One patient with a histological diagnosis of chronic hepatitis and 3 others with non-specific reactive hepatitis underwent serological tests to check for hepatitis A (IgM and IgG anti-HAV), hepatitis B (HBsAg and anti-HBc), hepatitis delta (IgM and IgG anti-HDV), cytomegalovirus and E-B virus infection. The test for HCV RNA also was carried out in these 3 patients with non-specific reactive hepatitis and in the patient with chronic hepatitis using a simple, quick method for reverse transcription (RT)-PCR amplification that did not require the RNA extraction step³⁶. Amplification was performed according to a

nested primers protocol¹⁴ with two sets of primers situated in the 5' untranslated region (UTR) of the viral genome.

Hepatitis G virus (HGV) and hepatitis GB virus C (HGBV-C) were screened in the patient who had chronic hepatitis. The detection of HGV/HGBV-C was carried out as follows: the RNA was extracted from serum using the guanidine isothiocyanate method⁸, reverse transcribed with MMLV-RT and random primers, and amplified with nested PCR using primers 3.2-a2 and 3.2-s1³⁸ in the first round and primers gbvc-s1 and gbvc-al²⁶ in the second round.

RESULTS

Of the 116 blood donors, 111 (95.7%) were whites and 5 (4.3%) were blacks, 111 (95.7%) were men and 5 (4.3%) were women with ages varied from 19 to 55 years old with an average age of 34 years. Fifteen (13%) donors showed elevated ALT levels only at the time of their initial blood donation (Group I) with 13 (86.7%) of these having more than twice the normal ALT level. There were 5 (33%) overweight individuals in Group I, of whom 1 had hypercholesterolemia, 1 had hypertriglyceridemia and 1 had steatosis. Five (33%) alcoholics showed an increase in their γ -glutamyl transpeptidase serum levels: of these, 1 had marked hyperlipidemia, 1 was using a tricyclic antidepressant (clomipramine hydrochloride) and had ALT levels two fold above than normal and the other 3 had no clinical or laboratory conditions which could have resulted in an elevation in ALT levels (Table 1). Upon ultrasound examination only two overweight individuals showed mild hepatomegaly. Approximately six months after the first test, serum samples from ten individuals in this group were retested for the presence of anti-HCV antibody using a second generation enzyme immunoassay (HCV EIA, Abbott). In all cases the results were negative and remained so in all subsequent tests until two years after.

In Group II, 101 (87%) donors had persistently high serum ALT levels at all tests performed throughout the follow-up period. 82 donors had ALT levels two fold above normal. In this group, there were 48 (47.5%) overweight individuals. Of these, 3 were associated with hyperglycemia and hyperlipidemia; 3 with hypertriglyceridemia, 9 with hypercholesterolemia; 11 with steatosis, 4 with hyperlipidemia and 3 were continuously exposed to industrial solvents.

There were 24 (23.8%) alcoholics in this group. Twenty-two of these (91%) had high levels of γ -glutamyl transpeptidase while 2 (9%) heavy drinkers had normal levels of this enzyme. Nine of the alcoholics had high levels of cholesterol and triglycerides, 3 had steatosis and 1 was frequently exposed to industrial solvents.

Nineteen donors (18.9%) in Group II were classified as overweight, alcoholic and having high levels of γ -glutamyl transpeptidase. Of these, 8 showed high levels of cholesterol and triglycerides and 4 had steatosis. Three (2.9%) donors were exposed daily through their occupation to hepatotoxic industrial solvents and paints. In this group there also was 1 (0.9%) subject

TABLE 1
Conditions associated with elevated ALT levels in blood donors

Conditions	ALT levels elevated only at first blood donation (n=15)		ALT levels elevated during follow-up (n=101)		All donors with elevated ALT* (n=116)	
	n	(%)	n	(%)	n	(%)
Obesity	5	33	48	47.5	53	45.6
Alcohol abuse	5	33	24	23.8	29	25.0
Obesity and alcohol abuse	-	-	19	18.9	19	16.3
Exposition to solvents/paints	-	-	3	2.9	3	2.6
Hyperlipidemia	1	7.0	1	1.0	2	1.7
Use of hepatotoxic drugs	1	7.0	-	-	1	0.9
Hypothyroidism	-	-	1	1.0	1	0.9
Diabetes mellitus	-	-	1	1.0	1	0.9
Reactive liver	-	-	3	2.9	3	2.6
Chronic hepatitis	-	-	1	1.0	1	0.9
Unknow	3	20	-	-	3	2.6

* ALT normal = 40 IU/L

who had severe probably familial hyperlipidemia; 1 (0.9%) with hypothyroidism (Hashimoto's thyroiditis) and 1 (0.9%) with severe diabetes mellitus. In 3 (2.6%) donors the liver biopsy showed mild non-specific reactive hepatitis and in 1 individual (0.9%) there was mild, chronic, active hepatitis with a negative serology for the principal viruses (non-A, non-B, non-C, non-D hepatitis) and also negative for CMV and EB virus infection (Table 1). Thirty of the subjects in this group (29.7%) showed hepatomegaly.

Sixty-one donors in Group II underwent the anti-HCV EIA test about 6 months after the initial negative serological tests and the results again showed no reactivity to this antibody until 2 years follow-up period. None of 71 blood donors in our 2 groups, underwent seroconversion during the 2 year follow-up. Repeated assays of the sera of 3 donors classified as having non-specific reactive hepatitis and of 1 having chronic hepatitis did not detect the HCV-RNA. In the patient with chronic hepatitis, the test for HGV/HGBV-C was negative, too.

DISCUSSION

In recent years, HCV has been responsible for most cases of non-A, non-B hepatitis in many countries^{5,24} and in the USA it is the most probable cause of chronic liver diseases⁶. Before the introduction in 1993 of routine blood tests for anti-HCV in the Brazilian blood banks¹⁷, this virus was responsible for about 90% of PTH cases. The frequency of anti-HCV in blood donors is about 2.6%-2.7%^{15,40}. Screening donors for surrogate markers reduced the risk of HCV transmission prior to the introduction of a systematic anti-HCV screening policy in France in 1990²⁰. A prospective study of blood receivers carried out before the compulsory testing of anti-HCV in Brazil, showed that testing for anti-HBc in blood donors was useful as it could prevent 56% of the PTH cases in those receiving blood transfusions¹⁶.

In the present study, 13 % of the donors (Group I) had elevated ALT levels which when the subjects were instructed not to use drugs, hepatotoxic substances or alcohol, always returned to normal ALT values by later check ups (Table 1). Thus, in an individual with elevated ALT levels, who used clomipramine hydrochloride, a tricyclic antidepressant with hepatotoxic activity²⁵, the activity of this enzymes returned to normal when use of the drug was stopped.

The rejection of the donors in Group I because of their elevated ALT levels is not justifiable based in the above results. This situation is aggravated in blood banks by the high number of anti-HBc Ag positives donors rejected which accounts for about 11% of the donations¹⁵. It has been argued that the predictive value and the cost-effectiveness of surrogate markers have declined dramatically with the introduction of anti-HCV assays. For this reason, the discontinuation of screening for ALT levels has been recommended in various countries^{7,19}. A recent panel of experts at the US National Institutes of Health (NIH), concluded that ALT testing as a surrogate marker for blood donors likely to transmit PTH non-A, non-B hepatitis infection should be discontinued since specific hepatitis C antibody testing eliminates more than 85 % of these cases. The panel recommended, however, that anti-HBc screening be continued as it prevents some cases of post-transfusion hepatitis B³³.

Ninety-two of the 101 donors in Group II suffered from obesity, alcoholism and hyperlipidemia. In previous studies, obesity and alcoholism were also frequently associated with elevated ALT levels in blood donors^{12,34,37}. According to some authors, HCV is a likely cause for high ALT levels in thin people who do not drink regularly, but an unlikely cause in obese people and in those who drink regularly²². In our subject, ALT levels were elevated in 5% of the blood donors because of occupational exposure to solvents and paints and, rarer, hypothyroidism (Hashimoto's disease) and asymptomatic diabetes mellitus (Table 1). We

believe that the rejection of the majority of donors in the two groups with elevated ALT levels is not justifiable in preventing PTH. Three of the nine donors who underwent hepatic biopsy had non-specific reactive hepatitis³⁵ and two had hepatic steatosis and alcoholism that could explain the elevated serum ALT levels. Four other alcoholics had intense hepatic steatosis thus considerably reducing the possibility of a viral etiology. Another donor, who underwent a histological examination, showed hepatic cirrhosis.

Only one out of nine donors who underwent a hepatic biopsy showed histological alterations compatible with mild, chronic active hepatitis of a viral etiology. He was a 21 year-old black male donor that showed a moderate and persistent increase (3-4 folds above normal) in ALT levels during the two-year follow-up. This patient was seronegative for HAV, HBV, HCV and HDV. The absence of cases of epidemic hepatitis E in the region of Campinas eliminates the possibility of any association with this virus, and also, this illness does not cause chronic liver disease or persistent viremia²³. The serum examinations of this individual also discarded other viruses such as cytomegalovirus and EB virus. During the two year follow-up the auto-antibodies tests were negative. The RT-PCR test did not detect HCV-RNA nor HGV/HGBV-C.

The observation that delayed seroconversion to anti-HCV did not occur in donors with elevated ALT levels indicates that this test does not detect donors infected with HCV and does not prevent the transmission of HCV and that the systematic screening for anti-HCV by blood banks may be of limited value. Some consider that the retention of ALT testing ultimately depends on the existence of another clinically significant human hepatitis agent such as HFV³, a new enterically transmitted virus^{4,39}. The virus previously identified as GB-C virus³⁸ but now termed hepatitis G virus (HGV) is distantly related to HCV and is associated with acute and chronic hepatitis²⁷. Using RT-PCR, HGV sequences have been identified in the plasma patients with non-A-E hepatitis in the USA and in Australia and in two cases of PTH from South America and Europe²⁷. In Japan, none of 16 patients with detectable HGV/HGBV-C RNA in their serum and who were maintained on hemodialysis had elevated serum aminotransferase activities. In these cases, HGV infection resulted in minimal or no clinical liver disease²⁹. In France, 57.5% of patients on hemodialysis have detectable serum HGV/HGBV-C RNA and only 6.5 % had an elevated serum ALT activity¹¹. HGV has been found also in Pakistan³¹ and in Australia, where 4% of the blood donors examined tested positive for HGV RNA by RT-PCR and only 5% had elevated ALT levels³².

About 75 % of HGV transfusion-infected receptors show no evidence of liver disease and in the few cases of hepatitis where the only agent identified is HGV, the elevation in ALT levels is small because this virus causes a benign persistent infection^{4, 29}. These observations in various countries make us believe that the ALT determinations would not detect the donors infected by HGV, since they rarely present an increase in the enzyme activity in the serum.

Currently, the screening of ALT levels does not aim to detect possible HCV-infected donors that are anti-HCV EIA negative

and does not seem to prevent the transmission of unknown hepatotropic viruses. Screening with indirect markers (ALT and anti-HBc) to eliminate cases of PTH was useful before the routine anti-HCV test was applied.

We consider, at this time, that the ALT screening is inefficient to detect possible donors infected with HCV in Brazil and with initial negative anti-HCV. Actually the ALT dosages, as we could note here, have been generally useful to detect non-infectious conditions present in blood donors.

RESUMO

Alanina aminotransferase (ALT) aumentada em doadores de sangue: avaliação das condições associadas e sua relação com o desenvolvimento de hepatites C.

A determinação dos níveis de alanina aminotransferase (ALT) tem sido útil para o diagnóstico de hepatopatias. Ultimamente, a elevação dos níveis séricos de ALT em doadores de sangue, tem sido associada a um maior risco de hepatites pós-transfusionais. Este estudo busca identificar os fatores associados com elevados níveis de ALT entre doadores voluntários de sangue e avaliar as relações entre estes aumentos de ALT e o desenvolvimento de infecção pelo vírus da hepatite C. Assim, 116 doadores voluntários de sangue com níveis de ALT elevados, quando da primeira doação, foram estudados. Todos foram questionados sobre hepatopatias prévias, exposição a hepatites, exposição a produtos químicos, uso de drogas ou medicamentos, comportamento sexual, contacto com sangue ou secreções e consumo de álcool.

A cada 3 meses foram medidos os níveis de AST, ALT, fosfatase alcalina, gama-glutamil transferase, colesterol, triglicérides e glicemia durante o período de 1-2 anos. Os níveis séricos de hormônios tireoideanos e a presença de auto-anticorpos também foram mensurados. Ultrassonografia abdominal foi realizada em todos os pacientes com elevação persistente dos níveis de AST ou ALT. Foi realizada biópsia hepática em 9 doadores sem diagnóstico definido após investigação clínica. A presença de anticorpos anti-HCV foi novamente pesquisada em 116 doadores no momento da primeira avaliação clínica. Ao final do follow-up (2 anos) 71 doadores foram re-testados para a presença do anti-HCV. Nenhum doador se tornou reagente para os marcadores dos vírus da hepatite B ou hepatite C, durante o seguimento. Dos 116 doadores, 101 (87%) mantiveram níveis séricos de ALT persistentemente aumentados. Obesidade e alcoolismo foram as principais condições associadas à elevação dos níveis séricos de ALT em 91/101 (90,1%) doadores. Hipertrigliceridemia, hipercolesterolemia, hipotireoidismo e diabetes mellitus também se associaram a níveis aumentados de ALT. Somente 1/101 (0,9%) apresentou hepatite crônica ativa não A-G e 3/101 (2,9%) apresentaram biópsia hepática com diagnóstico de hepatite reacional. A determinação rotineira dos níveis de ALT, em bancos de sangue não foi útil para detectar doadores infectados com o vírus da hepatite C no Brasil no período que antecede a soroconversão para anti-VHC.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Maria Silvia Kroll Lazarini for her excellent technical advice and Mr. Rodrigo Nogueira Angerami for his help in collecting the data analysed in this paper.

REFERENCES

1. AACH, R.D.; SZMUNESS, W.; MOSLEY, J.W. et al. - Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients. *New Engl. J. Med.*, 304: 989-994, 1981.
2. ALTER, H.J.; PURCELL, R.H.; HOLLAND, P.V.; ALLING, D.W. & KOZIOL, D.E. - Donors transaminase and recipient hepatitis. Impact on blood transfusion services. *J. Amer. med. Ass.*, 246: 630-634, 1981.
3. ALTER, H.J. - The cloning and clinical implications of HGV and HGBV-C. *New Engl. J. Med.*, 334: 1536-1537, 1996.
4. ALTER, H.J. - Transfusion transmitted hepatitis C and non-A, non-B, non-C. *Vox Sang. (Basel)*, 67: 19-24, 1994.
5. ALTER, M.J.; HADLER, S.C.; JUDSON, F.N. et al. - Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. *J. Amer. med. Ass.*, 264: 2231-2235, 1990.
6. ALTER, M.J.; MARGOLIS, H.S.; KRAWCZYNSKI, K. et al. - The natural history of community-acquired hepatitis C in the United States. *New Engl. J. Med.*, 327: 1899-1905, 1992.
7. BUSCH, M.P.; KORELITZ, J.J.; KLEINMAN, S.H. et al. - Declining value of alanine aminotransferase in screening of blood donors to prevent posttransfusion hepatitis B and C virus infection. The Retrovirus epidemiology donor study. *Transfusion*, 35: 903-910, 1995.
8. CHOMCZYNSKI, P. & SACCHI, N. - Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.*, 162: 156-159, 1987.
9. CHOO, Q.L.; KUO, G.; WEINER, A.J. et al. - Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, 244: 359-362, 1989.
10. CHOUDHURY, N.; RAMESH, V.; SARASWAT, S. & NAIK, S. - Effectiveness of mandatory transmissible diseases screening in Indian blood donors. *Indian J. med. Res.*, 101: 229-232, 1995.
11. DE LAMBALLERIE, X.; CHARREL, R.N. & BUSSOL, B. - Hepatitis GB virus C in patients on hemodialysis. *New Engl. J. Med.*, 335: 1549, 1996.
12. FRIEDMAN, L.S.; DIENSTAG, J.L.; WALKINS, E. et al. - Evaluation of blood donors with elevated serum alanine aminotransferase levels. *Ann. intern. Med.*, 107: 137-144, 1987.
13. GARROW, J.S. - *Obesity and related diseases*. New York, Churchill Livingstone, 1988.
14. GARSON, J.A.; TEDDER, R.S.; BRIGGS, M. et al. - Detection of hepatitis C viral sequences in blood donations by "nested" polymerase chain reaction and prediction of infectivity. *Lancet*, 335: 1419-1422, 1990.
15. GONÇALES Jr., F.L.; BOCCATO, R.S.B.S.; PEDRO, R.J. et al. - Prevalências do HBsAg, do anti-HBc e do anti-HCV na população de candidatos a doadores de sangue do Hemocentro-Campinas. *Rev. Inst. Med. trop. S. Paulo*, 35: 45-51, 1993.
16. GONÇALES Jr., F.L.; PEDRO, R.J.; SILVA, L.J. et al. - Hepatites pós-transfusionais na cidade de Campinas, SP, Brasil. II. Presença dos anticorpos anti-HBc e anti-HCV em candidatos a doadores de sangue e ocorrência de hepatites pós-transfusionais pelo vírus C nos receptores de sangue ou derivados. *Rev. Inst. Med. trop. S. Paulo*, 35: 63-71, 1993.
17. GONÇALES Jr., F.L.; PEDRO, R.J.; SILVA, L.J. et al. - Hepatites pós-transfusionais na cidade de Campinas, SP, Brasil. I. Incidência, agentes etiológicos e aspectos clínico-epidemiológicos da hepatite por vírus C. *Rev. Inst. Med. trop. S. Paulo*, 35: 53-62, 1993.
18. HULTCRANTZ, R.; GLAUMANN, H.; LINDBERG, G. & NILSSON, L.H. - Liver investigation in 149 asymptomatic patients with moderately elevated activities of serum aminotransferases. *Scand. J. Gastroent.*, 21: 109, 1986.
19. ISMAY, S.L.; THOMAS, S.; FELLOWS, A. et al. - Post-transfusion hepatitis revisited. *Med. J. Aust.*, 163: 74-77, 1995.
20. JULLIEN, A.M.; COUROUCE, A.M.; MASSARI, V. et al. - Impact of screening donor blood for alanine aminotransferase and antibody to hepatitis B core antigen on the risk of hepatitis C virus transmission. *Europ. J. clin. Microbiol. infect. Dis.*, 12: 668-672, 1993.
21. KAHN, R.A.; JOHNSON, G.; AACH, R.D. et al. - The distribution of serum alanine aminotransferase levels in a blood donor population. *Amer. J. Epidem.*, 115: 929-940, 1982.
22. KATKOV, W.N.; FRIEDMAN, L.S.; CODY, H. et al. - Elevated serum alanine aminotransferase levels in blood donors: the contribution of hepatitis C virus. *Ann. intern. Med.*, 115: 882-884, 1991.
23. KRAWCZYNSKI, K. - Hepatitis E: clinical course and prevention. In: AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES. Postgraduate course - viral hepatitis A to F: an update. Chicago, AASLD, 1994. p. 83-91.
24. KUO, G.; CHOO, Q-L; ALTER, H.J. et al. - An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*, 244: 362-364, 1989.
25. LARREY, D.; RUEFF, B.; PESSAYRE, D. et al. - Cross hepatotoxicity between tricyclic antidepressants. *Gut*, 27: 726-727, 1986.
26. LEARY, T.P.; MUERHOFF, A.S.; SIMONS, J.N. et al. - Consensus oligonucleotides primers for the detection of GB virus C in human cryptogenic hepatitis. *J. virol. Meth.*, 59: 119-121, 1996.
27. LINNEN, J.; WAGES Jr., J.; ZHANG-KECK, Z.-Y. et al. - Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science*, 271: 505-508, 1996.
28. LOVE, E.; PENDRY, K. & HUNT, L. - Analysis of pre-and post-donation haematological values in plateletpheresis donors. *Vox Sang. (Basel)*, 65: 209-211, 1993.
29. MASUKO, K.; MITSUI, T.; IWANO, K. et al. - Infection with hepatitis GB virus C in patients on maintenance hemodialysis. *New Engl. J. Med.*, 334: 1485-1490, 1996.

30. MENGHINI, G. - One-second needle biopsy of the liver. *Gastroenterology*, 35: 190, 1958.
31. MOATTER, T.; TARIQ, S.A.; FISHER-HOCH, S. et al - Hepatitis G virus in Karachi, Pakistan. *Lancet*, 348: 1318-1319, 1996.
32. MOAVEN, L.D.; HYLAND, C.A.; YOUNG, I.F. et al. - Prevalence of hepatitis G virus in Queensland blood donors. *Med. J. Aust.*, 165: 369-371, 1996.
33. NATIONAL INSTITUTE OF HEALTH - Consensus development panel on infectious disease testing for blood transfusions: infectious disease testing for blood transfusions. *J. Amer. med. Ass.*, 274: 1374-1379, 1995.
34. PALMER, M. & SCHAFFNER, F. - Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology*, 99: 1408-1413, 1990.
35. POPPER, H. & SCHAFFNER, F. - The vocabulary of chronic hepatitis. *New Engl. J. Med.*, 284: 1154-1156, 1971.
36. RAVAGGI, A.; PRIMI, D. & CARIANI, E. - Direct PCR amplification of HCV RNA from human serum. *PCR Meth. Applic.*, 1: 291-292, 1992.
37. SHERMAN, K.E. & DODD, R.Y. - Alanine aminotransferase levels among volunteer blood donors: geographic variation and risk factors. *J. infect. Dis.*, 145: 383-386, 1982.
38. SIMONS, J.N.; LEARY, T.P. & DAWSON, G.J. et al. - Isolation of novel virus-like sequences associated with human hepatitis. *Nature Med.*, 1: 564-569, 1995.
39. SIMONS, J.N.; PILOT-MATIAS, T.N.; LEARY, T.P. et al. - Identification of two flavivirus-like genomes in the G B hepatitis agent. *Proc. nat. Acad. Sci. (Wash.)*, 92: 3401- 3405, 1995.
40. VANDERBORGHT, B.O.; REIS, A.M.; ROUZERE, C.D. et al. - Prevalence of anti-hepatitis C virus in the blood donor population of Rio de Janeiro. *Vox Sang. (Basel)*, 65: 122-125, 1993.

Received: 09 January 1998

Accepted: 19 June 1998