

Antibody titers and response to vaccination against hepatitis A and B in pediatric patients with portal hypertension

Mariana Nogueira de Paula Rosa, Gabriel Hessel, Adriana María Alves De Tommaso

Departamento de Pediatria / Faculdade de Ciências Médicas / UNICAMP. Ciudad de Campinas, Sao Paulo, Brazil.

Acta Gastroenterol Latinoam 2008;38:187-193

Summary

In Brazil, approximately 130 new cases of hepatitis A per 100,000 inhabitants occur annually and 15% of the population has been in contact with hepatitis B virus. Portal hypertension causes hypersplenism and reduces T cell production, which may lead to less effective response to hepatitis vaccination. The objective of the study was to evaluate the response to hepatitis A and B vaccination in patients with portal hypertension secondary to chronic liver disease or portal vein thrombosis. Twenty-three patients (2 to 18 years) with portal hypertension seen at the Pediatric Hepatology Service of Hospital das Clínicas, Universidade Estadual de Campinas, between 1994 and 2006 were studied. Hepatitis A and B serology was tested in all patients. Patients who had not been vaccinated before their visits received the vaccines during the study period. Patients who had been vaccinated before but had negative anti-HB antibodies received a booster dose, and their serology was repeated. Blood counts were performed in each patient to assess for immunosuppression. Eighteen patients received hepatitis A vaccine and all became positive for anti-HAV antibodies. All patients had received hepatitis B vaccine and 17 (73.9%) were anti-HBs positive at the time of the study. The other 6 received a booster dose and became anti-HBs positive afterward. The anti-HBs-positive and -negative patients did not differ significantly in age, leukocytes, lymphocytes, or duration between the vaccination and positive serology. In this study, hepatitis A vaccines elicited a 100% response and hepatitis B vaccine conferred protection and induced an anamnestic response in pediatric patients with portal hypertension.

Key words: portal hypertension, hepatitis A, hepatitis B, vaccine, children, adolescents.

Títulos de anticuerpos y respuesta a la vacunación contra hepatitis A y B en pacientes pediátricos con hipertensión portal

Resumen

Anualmente ocurren en Brasil aproximadamente 130 casos nuevos de hepatitis A, por 100.000 habitantes. Se cree que el 15% de la población haya estado en contacto con el virus de la hepatitis B. La hipertensión portal puede llevar al hiperesplenismo con reducción de células T, pudiendo disminuir la respuesta a la vacunación. El objetivo del presente estudio es evaluar la respuesta a la vacunación contra las hepatitis A y B, en la hipertensión portal secundaria a la hepatopatía crónica o trombosis de la vena porta. Se evaluaron 23 pacientes (2-18 años) con hipertensión portal atendidos en el servicio de Hepatología Pediátrica del Hospital de Clínicas de la Universidad Estadual de Campinas, desde 1994 hasta el 2006. Las serologías fueron realizadas en todos los pacientes. Los individuos no vacunados recibieron las vacunas durante el estudio. Los pacientes anti-HBs negativos recibieron refuerzo y la serología fue repetida. Valoraciones hematimétricas fueron realizadas para evaluar alteraciones recurrentes de la inmunosupresión secundaria a la enfermedad de base y al hiperesplenismo. Dieciocho pacientes recibieron vacuna contra la hepatitis A y presentaron respuesta. Todos los pacientes recibieron 3 dosis de la vacuna contra la hepatitis B, y diecisiete (73,9%) presentaron respuesta. Seis fueron sometidos a refuerzo y todos presentaron anti-HBs positivo. No hubo diferencias estadísticamente significativas entre los anti-HBs positivos y negativos con relación a edad, leucocitos, linfocitos, tiempo entre las vacunas y la realización de la serolo-

Correspondence: Mariana Nogueira de Paula Rosa
Address: Av. Doze de Outubro, 473, apto 34, Santo André-S/P,
Ciudad de Campinas, Sao Paulo, Brazil.
Tel.: (11) 44279561
E-mail: maridepaula@uol.com.br

gía. Se concluye que la vacuna contra la hepatitis A presentó 100% de respuesta en pacientes con hipertensión portal mientras que la vacuna contra la hepatitis B les proporciona protección y les induce respuesta anamnésica.

Palabras claves: hipertensión portal, hepatitis A, hepatitis B, vacuna, niños, adolescentes.

In Brazil, approximately 130 new cases of hepatitis A virus (HAV) infection per 100,000 inhabitants occur annually, and the country is considered a high-risk area for contracting the disease.¹ Vaccination is indicated for groups at high risk of infection, including individuals traveling from low endemicity areas to intermediate or high endemicity areas, homosexual men, intravenous drug users, individuals with a high occupational risk for infection, individuals with coagulation factor disorders, and patients with chronic liver disease.²

Patients with chronic liver disease have a higher mortality rate and a higher incidence of fulminant hepatitis when infected with HAV, and thus should receive vaccination.³⁻⁵ Hepatitis A vaccine is safe and well tolerated and has an extremely high immunogenicity with 98.2% to 100% of healthy individuals developing protective antibody levels after two doses of the vaccine within an interval of 6 months.^{4,6}

There are at least 350 million people infected with hepatitis B virus (HBV) worldwide and approximately 2 billion have come in contact with the virus. In Brazil, the Ministry of Health estimates that at least 15% of the population has been in contact with the virus and that 1% has chronic disease.⁷ High-risk areas in Brazil include the western part of the State of Paraná and some Amazon regions.⁸ About 1 million chronically infected people die each year from chronic liver disease through cirrhosis and hepatocellular carcinoma.⁹ Prevention of hepatitis B should be started at birth, with a series of three vaccine doses completed at 6 months of age. Vaccination against hepatitis B has been implemented since the 1980s, and the vaccine is one of the safest available.¹⁰

No response to hepatitis B vaccine is observed in 3% to 5% of healthy individuals, which may be associated with the frequency of some HLA class II alleles, such as HLA-DRB1*0301, DRB*0701 and DQA2 in recipients.^{11,12} A lower response to the vaccine has also been demonstrated in patients with chronic liver disease as well as in immunosuppressed patients.¹³⁻¹⁵

Portal hypertension is characterized by increased

blood pressure in the portal venous system at least 10mmHg higher than normal and is frequently associated with chronic liver disease. However, extrahepatic portal vein obstruction has been linked to portal hypertension in pediatric patients.^{16,17} Splenomegaly is the most prominent sign of portal hypertension, which is a result of hypersplenism due to fibrocongestive alterations of the spleen.^{16,18,19} Hypersplenism is characterized by the sequestration and destruction of red blood cells, leukocytes and platelets due to spleen enlargement, and hyperplasia of the respective precursors of the depleted cells due to blood cytopenia after splenectomy.²⁰ Because the immunological memory depends on T lymphocytes, the response to vaccination may be reduced in children with hypersplenism.

The objective of the present study was to evaluate antibody response to vaccination against hepatitis A and B in pediatric patients with portal hypertension secondary to portal vein thrombosis or chronic liver disease.

Patients and methods

Patients

Included in the study were patients who were treated at the Pediatric Hepatology Service of Hospital de Clínicas, UNICAMP (HC-UNICAMP) between 1994 and 2006. They must be younger than 18 years and have a diagnosis of portal hypertension established by ultrasonographic criteria (presence of splenomegaly, gallbladder wall thickness, lesser omental thickness at the venous ligament, lesser omental to aorta ratio, presence of splenorenal shunt)²¹⁻²⁴ and/or by presence of the esophageal varices.

Twenty-three patients ranging in age from 2 to 18 years (mean: 9.5 years, median: 9 years) with an ultrasonographic diagnosis of portal hypertension were included in the study. Fourteen (60.9%) of these patients were boys.

Sixteen (69.6%) of the 23 patients had a diagnosis of portal vein thrombosis and seven (30.4%) had chronic liver disease diagnosed by liver biopsy. The causes of chronic liver disease were congenital hepatic fibrosis in five patients and idiopathic in two. All patients were serologically negative for HIV, hepatitis C and hepatitis B (HBsAg and anti-HBc).

Exclusion criteria were the presence of positive serology for hepatitis B, hepatitis C or HIV, chronic

renal failure, diabetes mellitus, or diseases or medications resulting in immunosuppression. Patients with autoimmune hepatitis and Wilson's disease were also excluded from the study.

Methods

The study was approved by the Ethics Committee of Faculdade de Ciências Médicas, UNICAMP, and all patients participated in the study after the parents and guardians had signed the informed consent form.

All patients had serological tests for hepatitis A and B. Serology was performed by a microparticle enzyme immunoassay (MEIA) using the AXSIM[®] automatic system (Abbot, Wiesbaden, Germany). Serology for hepatitis A was qualitative, and hepatitis B antibody level was quantitatively determined using the AUSAB[®] kit. Samples presenting anti-HBs with a concentration of 10 mIU/mL or higher were considered to be reactive.

Patients not previously vaccinated received the vaccines during the study period. Previously vaccinated patients with a negative result for anti-HBs received a booster dose, and their serology was repeated after the booster.

The following hepatitis A vaccines were used: Havrix[®] (720U, SmithKline Beecham Biologicals) and Berna[®] laboratory vaccine (0.5mL). Two doses were administered at an interval of 6 months. The recombinant Euvax[®] vaccine was used for vaccination against hepatitis B (10µg HBsAg/0.5mL, LG Chem) and was given at 0,1 and 6 months.

Blood counts were performed at the same time as the serological tests to identify immunosuppression secondary to the underlying disease and hypersplenism. Leukopenia was classified as an absolute number of leukocytes less than 4000/mm³, and lymphocytopenia was defined as an absolute number of lymphocytes less than 1500/mm³.²⁵

For comparative analysis, patients were divided

into two groups: group A consisted of those with portal vein thrombosis and group B included those with chronic liver disease.

The results are presented as descriptive analysis including frequencies for categorical variables and measures of position and dispersion for continuous variables. Continuous or ordinal variables were compared between the two groups using the Mann-Whitney test with a level of significance set at 5%.²⁶ The SPSS for Windows program, version 7.5, was used for analysis of the data.

Results

Five of the 23 patients presented serology indicating natural immunity against HAV (mean age: 11 years and 9 months, median: 13 years); the other 18 patients had negative serology results and received the hepatitis A vaccine. Eleven patients received the Havrix[®] vaccine and 7 were vaccinated with the Berna[®] laboratory vaccine.

All patients received the primary vaccination series for hepatitis B, with 11 being vaccinated at the Immunobiology Sector of HC-UNICAMP (recombinant Euvax[®] vaccine), and 12 at community health centers in their towns. The first assessment after the vaccination showed that 17 (73.9%) patients became anti-HBs positive.

Blood counts were performed at the same time as the serological tests. Two patients had anemia, leukopenia, and thrombocytopenia resulting from hypersplenism; 11 had lymphocytopenia only.

Group A: Nine (56.3%) of the 16 patients with portal vein thrombosis were male. The age and hematological characteristics of the patients are shown in Table 1. Two (12.5%) patients presented natural immunity against hepatitis A. The other patients received the hepatitis A vaccine, and all became serologically positive. The duration between vaccination and the positive serology ranged from 2 months to 2 years and 8 months (mean: 10 months, median: 7 months).

Table 1. Age and hematological characteristics of patients with portal vein thrombosis (PVT) and chronic liver disease (CLD).

	Age	Hemoglobin (g/dL)	Leukocytes (mm ³)	Lymphocytes (mm ³)
PVT	9y3m (2-18y)	12.0 (9.4-13.9)	4793 (1730-10,120)	1458 (311-2390)
CLD	10y2m (6-14y)	11.9 (11.5-14.7)	5615 (4160-8720)	1491 (947-2177)

Values are the mean (range).

With respect to hepatitis B, 14 of the 16 patients (87.5%) presented positive serology for anti-HBs with titers ranging from 11 to 1000mIU/L (mean: 468.2, median: 349.5mIU/L). The duration between vaccination and positive serology ranged from 3 months to 6 years and 7 months (mean: 2 years and 7 months, median: 2 years and 10 months). The two anti-HBs-negative patients received a booster dose of the vaccine and showed a response after its administration.

Group B: Five (71.4%) of the seven patients with chronic liver disease were male. The age and hematological characteristics of the seven patients are shown in Table 1. Four (57.1%) patients with negative serology for hepatitis A received the vaccine at the Immunobiology Sector of HC-UNICAMP and all presented positive serology after the vaccination. The duration between vaccination and serology ranged from 9 months to 2 years and 3 months (mean: 1 year and 3 months, median: 1 year). The other three (42.9%) patients were naturally immune to hepatitis A.

All patients in this group had been vaccinated against hepatitis B and three of them (42.9%) were anti-HBs positive. The antibody titers ranged from 411 to 1000 mIU/L (mean: 638, median: 503mIU/L). The duration between vaccination and serology ranged

from 3 months to 3 years and 2 months (mean: 1 year and 7 months, median: 2 years and 2 months). The four anti-HBs-negative patients had lymphocytopenia, with a lymphocyte count ranging from 1012 to 1339/mm³ (mean: 1211.5/mm³, median: 1247.5/mm³). None of the anti-HBs-positive patients presented lymphocytopenia. The duration between vaccination and serology ranged from 3 months to 2 years and 4 months (mean: 1 year and 5 months, median: 1 year and 8 months) in patients with negative anti-HBs, and from 4 months to 3 years and 2 months (mean: 1 year and 10 months, median: 2 years and 2 months) in the anti-HBs-positive patients.

The four anti-HBs-negative patients (57.1%) received a booster dose of the hepatitis B vaccine as soon as negative results were known and all became anti-HBs positive after the booster. All four patients had a diagnosis of congenital liver fibrosis. The mean time between vaccination and serology was 1 year and 5 months.

Statistical analysis for comparison of anti-HBs-positive and anti-HBs-negative patients revealed no significant difference in age, leukocyte counts, lymphocyte counts, or duration between vaccination and serology in either group A or group B (Tables 2 and 3).

Table 2. Comparison between anti-HBs-positive and anti-HBs-negative patients in the group with portal vein thrombosis (N=26).

	Anti-HBs positive	Anti-HBs negative	p*
Age	8y9m/7y	12y5m/12y5m	0.333
Leukocytes (mm3)	4863/4150	4300/4300	0.700
Lymphocytes (mm3)	1520/1650	1016/1016	0.333
Time	2y6m/2y7m	3y3m/3y3m	0.500

Values are reported as mean/median. Time= time interval between vaccination and serology for hepatitis B. *Mann-Whitney.

Table 3. Comparison between anti-HBs-positive and anti-HBs-negative patients in the group with chronic liver disease (N=9).

	Anti-HBs positive	Anti-HBs negative	p*
Age	9y4m/9y	10y9m/10y	0.400
Leukocytes (/mm3)	5390/4600	5785/5130	0.857
Lymphocytes (/mm3)	1822/1651	1160/1215	0.057
Time	1y10m/2y2m	1y5m/1y8m	0.629

Values are reported as mean/median. Time = time interval between vaccination and serology for hepatitis B. *Mann-Whitney test.

Discussion

Five (21.7%) of the 23 patients with a median age of 13 years presented natural immunity against hepatitis A. A low frequency of natural immunity has also been reported by Ferreira et al.²⁷ who found a 24% prevalence of anti-HAV IgG antibody in 59 children with chronic liver disease ranging in age from 12 months to 16 years (mean of 7 years). These percentages are considered low by us and by Ferreira et al., mainly because these children belonged to families of low socioeconomic status.

The response rate to hepatitis A vaccination was 100% in the 18 patients vaccinated in our study. Ferreira et al.²⁸ have reported, a seroconversion rate of 76% after the first vaccine dose and of 97% after the second dose using quantitative serology tests in 34 children with chronic liver disease (17 with biliary atresia, 6 with cryptogenic cirrhosis, 7 with cirrhosis due to autoimmune hepatitis, and 4 with other causes of cirrhosis).

In the present study, we used qualitative serological tests for hepatitis A, which are indicated for the detection of the disease rather than a vaccine response. Because all the vaccinated patients were anti-HAV positive, we inferred that the vaccine elicited an immunological response. Most studies evaluating the immunogenicity of hepatitis A vaccine in patients with chronic liver disease have been conducted on adult transplant patients and the results are conflicting. Both low response rates, (6/23 - 23% and 0/8 - nonresponse)^{29,30} and a 97% response rate³¹ have been reported. Keffe et al.³² evaluated the response to hepatitis A vaccine in adults with chronic liver disease and found a response rate of 94.3% in patients infected with hepatitis C and of 97.7% in patients infected with hepatitis B virus.

In our study, 73.9% (17/23) of the patients were anti-HBs positive at the time of the study. The mean time between vaccination and serology was 2 years and 4 months (median of 2 years and 3 months).

Previous studies have demonstrated lower response rates to hepatitis B vaccine in patients with chronic liver disease than in healthy subjects. Aziz et al.³³ observed a 72% response in adult patients who received a vaccination series consisting of 10 double shots after they failed to respond to the primary dose. Factors related to the low response rate in cirrhotic patients include peripheral blood lymphocytopenia secondary to hypersplenism, changes in the composition of T lymphocyte subpopulations, and

inappropriate interactions among antigens, T cells, T-cell receptors, and MHC molecules. In addition, advanced age, disease severity and malnutrition also play an important role in the immune response of these patients.^{13,34,35} In one study, Ferrante et al.³⁶ evaluated the leukocyte subpopulation in six children with portal hypertension and observed reduction in the absolute and relative number of CD3+ and CD4+ T cells and cytotoxic T cells (CD8+). McGovern et al.³⁷ observed low levels of CD4+ cells in 65% of 60 adult patients with liver cirrhosis, which was associated with splenomegaly, leukopenia, thrombocytopenia and anemia. In addition, they associated reduced CD4+ cell counts with esophageal varices and manifestations of portal hypertension. The authors suggested that the low CD4+ counts may be attributed to an overall sequestration of blood cell lines because of portal hypertension. Tokushige et al.³⁸ found, in 24 Japanese children with portal hypertension, reduced numbers of T helper 2 (Th2) cells in both peripheral blood and splenic cells, but their Th1 cell counts were similar to that of healthy subjects. Generally, Th1 cells are involved in cellular immunity and Th2 cells play a role in humoral immunity.

After immunization, anti-HBs concentration declines over time. A previous study found that 15% to 50% of vaccinated infants and children had undetectable levels of antibodies 9 to 15 years after the primary vaccination.³⁹ The presence of immunological memory can be demonstrated by a rapid increase in antibody levels after a booster dose including individuals with undetectable levels.⁴⁰

In our study, two patients with portal vein thrombosis and four patients with chronic liver disease were anti-HBs negative at the time of the study. Since anti-HBs was measured years after vaccination in some patients, these individuals cannot be regarded as nonresponders to the initial vaccination. All 6 patients presented protective titers after receiving a booster dose, demonstrating an anamnestic response.

To evaluate the effect of hypersplenism in the patients, blood counts were performed and showed no correlation between the presence of lymphocytopenia and anti-HBs positivity or negativity. Thus, the presence of hypersplenism did not seem to be related to a decline in anti-HBs titers. The absolute number of lymphocytes was less than 1500/mm³ in some patients, but there was no correlation between lymphocytopenia and anti-HBs negativity in either group of patients in which this correlation was analyzed.

The two groups studied here had conditions that make patients vulnerable to leukopenia, lymphocytopenia, and changes in CD4+ cell counts, all of which may contribute to reduced response to the vaccine. All patients vaccinated against hepatitis A were anti-HAV positive after vaccination, thus demonstrating the effective immunogenicity of the two vaccines we used. Regarding the hepatitis B vaccine, most patients were vaccinated at community health centers in their towns, which made it difficult to assess the efficacy of the vaccine used. An anamnestic response was observed in patients who had received a booster dose. In the case of eventual contact with the virus, memory B lymphocytes would be stimulated to combat infection.

In conclusion, the hepatitis A vaccines elicited a 100% response and the hepatitis B vaccine was able to confer protection and induce an anamnestic response in pediatric patients with portal hypertension.

References

1. Brasil. Ministério da Saúde. Tópicos de saúde - Hepatites [on line] 2006 [Accessed January 10, 2006] Available at: <http://portal.saude.gov.br/portal/saude/visualizar-texto.cfm?idtexto=22248>.
2. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 1999;48:26-27.
3. Akriviadis EA, Redeker AG. Fulminant hepatitis A in intravenous drug users with chronic liver disease. *Ann Int Med* 1989;110:838-839.
4. Keffe EB. Is hepatitis A more severe in patients with chronic hepatitis B and other liver diseases? *Am J Gastroenterol* 1995;90:201-205.
5. Vento S, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, Ferraro T, Concia E. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998;338:286-290.
6. Clemens R, Safary A, Hepburn A, Roche C, Stanbury WJ, André FE. A clinical experience with an inactivated hepatitis A vaccine. *J Infect Dis* 1995;171:S44-S49.
7. Ferreira CT, Targa C, Silveira TR. Hepatites virais: aspectos da epidemiologia e da prevenção. *Revista Brasileira de Epidemiologia* 2004;7:473-487.
8. Foccacia R, Conceição OJG, Sette Junior H, Sabino E, Basit L, Nitrini DR, Lomar, AV; Lorencio, R; Souza, FV; Kiffer, CRV; Santos, EB; Gonzales, MP; Sáez Alquezár, A; Riscal, JR; Fisher, D. Estimated prevalence of viral hepatitis in the general population of the municipality of São Paulo, measures by a serological survey of a stratified, randomized and residence based population. *Braz J Infect Dis* 1998; 2:269-284.
9. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997;337:1733-1745.
10. Kline MW, Shearer WT. Active and passive immunization in the prevention of infectious diseases. In: Stiehm ER, ed. *Immunologic disorders in infants and children*. 4a ed. Philadelphia: Saunders Company; 1996:916-938.
11. Alper CA, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, Dienstag JL, Awdeh Z, Yunis EJ. Genetic prediction of nonresponse to hepatitis B vaccine. *N Engl J Med* 1989;321:708-712.
12. Craven DE, Awdeh ZL, Kunches LM, Yunes EJ, Dienstag JL, Werner BG, Polk F, Snyderman DR, Platt R, Crumpacker CS, Grady GF, Alper CA. Nonresponsiveness to hepatitis B vaccine in health care workers. Results of revaccination and genetic typings. *Ann Intern Med* 1986;105:356-360.
13. Idilman R, Colantoni A, De Maria N, Ustun C, Sam R, Ingin TS, Akan H, Koc H, Thiel DHV. Impaired antibody response rates after high dose short interval hepatitis B vaccination of immunosuppressed individuals. *Hepato Gastroenterol* 2003;50:217-221.
14. Karasu Z, Ozacar T, Akarca U, Ersoz G, Erensoy S, Gunzar F, Kobat A, Tokat Y, Batur, Y. HBV vaccination in liver transplant patients: not an effective strategy in the prophylaxis of HBV recurrence. *J Viral Hepat* 2005;12:212-215.
15. Lo CM, Liu CL, Chan SC, Lau GK, Fan ST. Failure of hepatitis B vaccination in patients receiving lamivudine prophylaxis after liver transplantation for chronic hepatitis B. *J Hepatol* 2005;43:283-287.
16. Henderson, JM. Portal Hypertension. In: Corson J, Williamson R, eds. *Surgery* [on line] London: Mosby; 2000 October [Accessed September 12, 2006]. Available at: <http://www.elsevier-international.com/e-books/pdf/8.pdf>
17. Pinto RB, Silveira TR. Trombose de veia porta em crianças e adolescentes: série de 14 casos. *AMRIGS, Porto Alegre* 2002;46:45-72.
18. Martinelli ALC. Hipertensão portal. *Medicina (Ribeirão Preto)* 2004;37:253-261.
19. Schettino GCM, Fagundes EDT, Roquete MLV, Ferreira AR, Penna FJ. Portal vein thrombosis in children and adolescents. *J Pediatr (Rio J)* 2006;82:171-178.
20. Jacob HS. Erythrocyte disorders – anemias related to hyperactivity of the reticuloendothelial system. In: Williams WJ, Beutler E, Erslev AJ, Rundles RW, eds. *Hematology*. New York: McGraw-Hill; 1972.p.511-520.
21. MacGahan JP, Phillips HE, Cox KL. Sonography of the normal pediatric gallbladder and biliary tract. *Radiology* 1982;144:873-875.
22. Patriquin H, Tessier G, Grignon A, Boivert J. Lesser omental thickness in normal children: baseline for detection of portal hypertension. *AJR* 1985;145:693-696.
23. Irigoyen N. A importância da medida ecográfica do pequeno epíplon no diagnóstico da hipertensão portal em pediatria. *Rev Imagem* 1991;13:145-148.
24. West MS, Garra BS, Horii SC, Hayes WS, Cooper C, Silverman PM, Zeman RK. Gallbladder varices: imaging findings in patients with portal hypertension. *Radiology* 1991;179:179-182.
25. Hoffbrand AV, Pettit JE, Moss PAH. *Essential Haematology*, 4a ed. Massachusetts: Blackwell Science; 2003.p.331.

26. Conover WJ. The use of ranks. Two independent samples. In: Conover WJ. *Practical Nonparametric Statistics*, 1a ed. New York: John Wiley & Sons Inc; 1971.p.223-236.
27. Ferreira CT, Taniguchi AN, Vieira SM, Pereira-Lima J, da Silveira TR. Prevalência do anticorpo da hepatite A em hepatopatia crônica. *J Pediatr (Rio J)* 2002;78:503-508.
28. Ferreira CT, Silveira TR, Vieira SM, Taniguchi A, Pereira-Lima J. Immunogenicity and safety of hepatitis A vaccine in children with chronic liver disease. *J Ped Gastroenterol Nutr* 2003;37:258-261.
29. Arslan M, Wiesner RH, Poterucha JJ, Zein NN. Safety and efficacy of hepatitis A vaccination in liver transplantation recipients. *Transplantation* 2001;72:272-276.
30. Dumot JA, Barnes DS, Younossi Z, Gordon SM, Avery RK, Domen RE, Henderson JM, Carey WD. Immunogenicity of hepatitis A vaccine in decompensated liver disease. *Am J Gastroenterol* 1999;94:1601-1604.
31. Stark K, Günther M, Neuhaus R, Reinke P, Schröder K, Linnig S, Bienzle U. Immunogenicity and safety of hepatitis A vaccine in liver and renal transplant patients. *J Infect Dis* 1999;180:2014-2017.
32. Keefe EB, Iwarson S, McMahon BJ, Lindsay KL, Koff RS, Manns M, Baumgarten R, Wiese M, Fourneau M, Safary A, Clemens R, Krause DS. Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease. *Hepatology* 1998;27:881-886.
33. Aziz A, Aziz S, Li DS, Murphy L, Leone N, Kennedy M, Dhillon S, Van Thiel DH. Efficacy of repeated high-dose hepatitis B vaccine in patients with chronic liver disease. *J Viral Hepat* 2006;13:217-221.
34. De Maria N, Idilman R, Colantoni A, Van Thiel DH. Increased effective immunogenicity to high-dose and short-interval hepatitis B virus vaccination in individuals with chronic hepatitis without cirrhosis. *J Viral Hepat* 2001;8:372-376.
35. Wiedmann M, Liebert UG, Oesen U, Porst H, Wiese M, Schroeder S, Halm U, Mossner J, Berr F. Decreased immunogenicity of recombinant hepatitis B vaccine in chronic hepatitis C. *Hepatology* 2000;31:230-234.
36. Ferrante A, Davidson GP, Beard LJ, Goh DHB. Alterations in function and subpopulations of peripheral blood mononuclear leukocytes in children with portal hypertension. *Int Arch Allergy Appl Immunol* 1989;88:348-352.
37. McGovern B, Golan Y, Lopez M, Pratt D, Lawton A, Moore G, Epstein M, Knox TA. The impact of cirrhosis on CD4+ cell counts in HIV-seronegative patients. *Clin Infect Dis* 2007;44:431-437.
38. Tokushige K, Yamauchi K, Komatsu T, Takasaki K, Hayashi N. Predominant T helper 1 cells in patients with idiopathic portal hypertension. *J Gastroenterol Hepatol* 2000;15:1312-1317.
39. Williams IT, Goldstein ST, Tufa J, Tauillii S, Margolis HS, Mahoney FJ. Long term antibody response to hepatitis B vaccination beginning at birth and to subsequent booster vaccination. *Pediatr Infect Dis J* 2003;22:157-163.
40. Boxall EH, Sira JA, El-Shunkri N, Kelly DA. Long-term persistence of immunity to Hepatitis B after vaccination during infancy in a country where endemicity is low. *J Infect Dis* 2004;90:1264-1269.