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

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**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.*

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***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,*

*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]

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 se-

renal failure, diabetes mellitus, or diseases or medications resulting in immunosuppression. Patients with autoimunne 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/mm$^3$, and lymphocytopenia was defined as an absolute number of lymphocytes less than 1500/mm$^3$.[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, leucopenia, 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) |

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/mm$^3$ (mean: 1211.5/mm$^3$, median: 1247.5/mm$^3$). 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 atients 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 |

## 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 rate [31] have been reported. Keeffe 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 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/mm$^3$ in some patients, but there was no correlation between lymph-

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.

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