Case Report

Pitfalls in the Diagnosis of Autoimmune Hepatitis Associated with Liver and Kidney Microsomal Proteins

K. Paradis, F. Alvarez, E. Seidman, *J. M. Laberge, †R. Schreiber, ‡P. Brochu, and
A. Rasquin-Weber

Department of Pediatrics, Division of Gastroenterology, and ‡Department of Pathology, Hôpital Sainte-Justine, and
*Department of Surgery and †Department of Pediatrics, Division of Gastroenterology, Montreal Children's Hospital,
Montreal, Canada

Autoimmune hepatitis has been defined as chronic active hepatitis without biliary lesions, granulomas, siderosis, or copper deposits and with γ-globulins >1.5 times the upper limit of normal, elevated transaminases (usually), and no viral markers or other known causes of liver disease (1). Although the pathogenesis of autoimmune hepatitis remains unclear, further definition of associated antibodies detected in the sera of patients with this category of disease has allowed a more refined classification of this disorder. Antibodies recognizing actin filament epitopes [smooth muscle antibodies, (SMA)] (2,3), cytosolic proteins (4), and nuclear proteins (ANAs), as well as liver and kidney microsomal proteins [LKM, CYP2D6, or endoplasmic reticulum protein (5,6)], have been described and may have prognostic implications in terms of associated autoimmune phenomena and the course of the disease (7). Unfortunately, the hypergammaglobulinemia that is a common hallmark in these diseases may give false-positive results in various serological diagnostic tests.

Autoimmune hepatitis in the presence of circulating anti-LKM antibodies has also been described in patients having taken tielenic acid [LKM-2 (2)], as well as in patients with hepatitis δ (8). Anti–LKM-1–positive hepatitis is primarily seen in children and young adult women. Children with anti–LKM antibody–positive autoimmune hepatitis can present

with an asymptomatic finding of hepatomegaly on routine physical examination, acute hepatitis-like symptoms, or even fulminant hepatic failure, often at an earlier age than children present with other forms of autoimmune hepatitis (6,7,9,10). Once a diagnosis is made, treatment with immunosuppressive drugs can achieve normalization of liver function in the majority of cases, although some patients will progress to liver failure (9,10).

We report the case of a 4-year-old boy with anti-LKM–positive autoimmune hepatitis who required liver transplantation for end-stage liver failure. The clinical presentation—initially as an acute eosinophilic hepatitis compatible with a toxic reaction to antibiotics, then with findings compatible with hepatitis C infection, followed by giant-cell hepatitis—illustrates the difficulties encountered when managing patients with autoimmune hepatitis.

The importance of diagnostic tests using synthetic peptide fragments recognized by circulating antibodies in patients with anti-LKM–positive autoimmune hepatitis is also demonstrated by this case. Furthermore, the persistence of circulating antibodies to the same epitopes after liver transplantation and immunosuppression, without any evidence of recurrence of disease activity, raises doubts as to their role in the pathogenesis of autoimmune hepatitis.

CASE REPORT

The patient presented to his pediatrician at 2 years, 10 months, with fever. A diagnosis of bacterial pharyngitis was made, and an erythromycin–sulfamethoxazole preparation was prescribed for 10 days. Ten days after starting antibiotics, the patient

presented to another hospital with jaundice and dark urine. Liver enzymes and bilirubin were elevated (aspartate amino transferase (AST) 196 IU/L; alanine aminotransferase (ALT) 109 IU/L; conjugated bilirubin, 41 μM; total bilirubin, 76 μM), and the patient was transferred to Hôpital Sainte-Justine for further evaluation.

The patient was the second child of healthy non-consanguinous parents of French-Canadian descent. His height was over the 97th percentile and his weight was at the 50th centile; he had never been ill before except for benign viral illnesses. A maternal aunt had Crohn's disease, and three paternal aunts had been treated for thyroid diseases of an unspecified nature. There was no personal or family history of liver disease, recent travel, allergies, diabetes, blood transfusions, or known contact with persons with liver disease.

On admission, the patient was an active and thriving child with visible jaundice. There was mild pharyngitis and cervical lymphadenopathy; his liver was 3 cm below the right costal margin, which had slightly increased consistency. No splenomegaly, palmar erythema, finger clubbing, or spider angiomata suggestive of chronic liver disease was found.

The initial laboratory values are illustrated in Fig. 1. Acute and convalescent serologies for cytomegalovirus (CMV), hepatitis A virus, hepatitis B virus, and Epstein–Barr virus were negative. α-1-Anti-

FIG. 1. Laboratory values prior to liver transplantation. a: First hospital admission. b: Control blood work done by outside pediatrician. c: Second hospital admission. The serial fall in ALT and factor V levels parallels the increases in bilirubin levels and coagulation times.

trypsin and ceruloplasmin levels were normal. γ-Globulins were increased to 16.3 g/L (normal, 9–13), and there was no peripheral eosinophilia.

A percutaneous liver biopsy was performed; it revealed lobular disorganization with a moderate portal tract infiltrate and numerous eosinophils (Fig. 2). No piecemeal necrosis was seen, although the limiting plate was irregular due to the ballooning of adjacent hepatocytes. There were some areas of pseudoacinar formation as well as multinucleated hepatocytes. A lobular infiltrate composed of neutrophils, mononuclear cells, and eosinophils was also present, as were swollen Kupffer cells. Some minor interstitial fibrosis was seen on trichrome stain. Bacterial and viral cultures of the liver biopsy were negative. A tentative diagnosis of erythromycin toxicity was made; within days, the patient showed clinical and laboratory improvement of liver enzymes and bilirubin.

The patient was followed by his pediatrician over the following 3 months, during which his bilirubin levels normalized. Although AST levels remained six times the upper limit of normal and ALT eight times normal, the child was thriving, and no further laboratory evaluations were considered necessary by his physician.

Eight months following his initial hospitalization, the patient was transferred to us with reappearance of jaundice, vomiting, diarrhea, and edema of the lower extremities. Physical findings were similar to the ones on initial evaluation, and growth had been normal in the interim. Laboratory results (Fig. 1) revealed significant deterioration of liver enzymes, bilirubin, and coagulation parameters. Over the next few days, grade III encephalopathy developed with extreme agitation, and lactulose and neomycin were administered, resulting in some improvement.

Antibodies to rat liver microsomes were found in high titers by enzyme-linked immunosorbent assay (ELISA) (Fig. 3) (11). Retrospective analysis for LKM antibodies on stored serum from the previous hospitalization (the technique had not been available at that time) was also positive. This finding was then confirmed by Western blot, using rat liver microsomal proteins as antigens; a single 50-kD band was found (Fig. 4) (12). Dot-blot analysis, using synthetic peptides representing cytochrome CYP2D6-continuous (linear) antigenic sites (13), revealed that the main epitopes recognized were between amino acids 254 and 271 (Fig. 5). Antibodies to hepatitis C virus (HCV) were found to be present by second-generation ELISA (Abbott HCV EIA 2.0
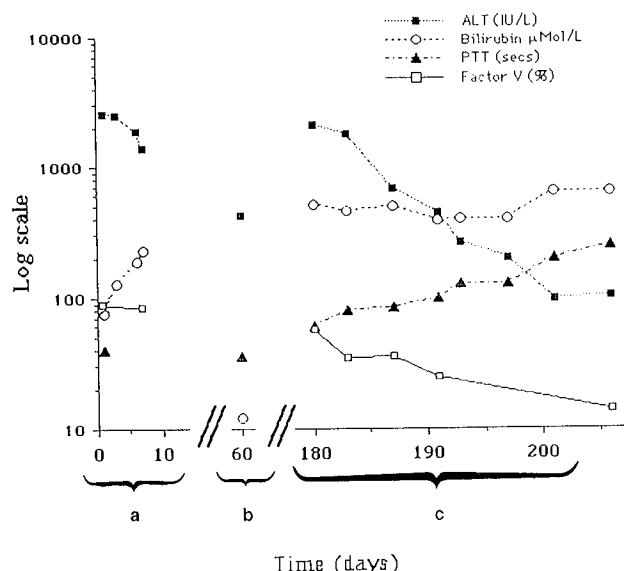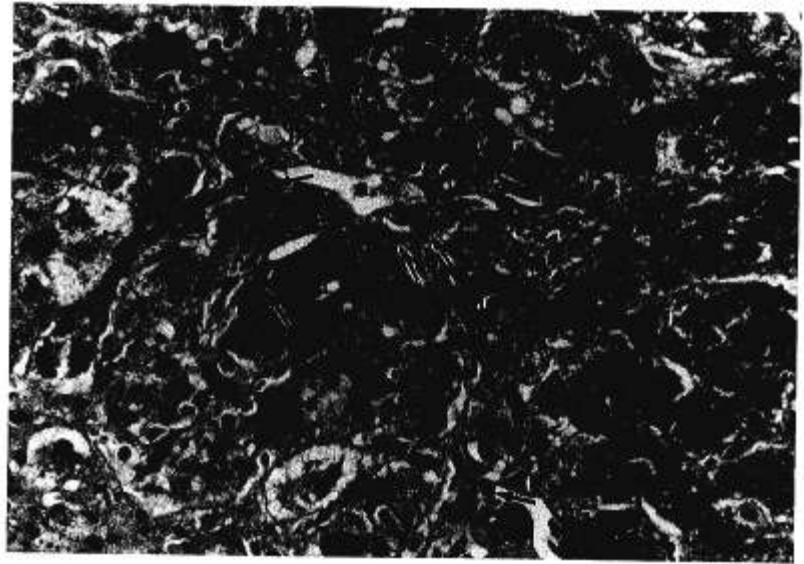
FIG. 2. Percutaneous liver biopsy on first admission. Portal tract shows an infiltrate composed of lymphocytes, plasmocytes, occasional neutrophils, and numerous eosinophils (arrows) (×400).

Diagnostic Kit). The results of subsequent analysis by RIBA assay and polymerase chain reaction (PCR) on serum were negative after transplantation (analysis generously carried out by Dr. S. V. Feinman, Toronto).

With confirmation of the diagnosis of autoimmune hepatitis, i.v. methylprednisolone was begun at a dose of 2 mg/kg/day. Despite this therapy, liver insufficiency progressed, and tense ascites and anasarca with severe deterioration of coagulation parameters developed (Fig. 1). Polyphagia and hyperglycemia developed prior to the initiation of steroid therapy and necessitated the administration of insulin. Screening for anti–islet cell antibodies was negative.

Twenty-seven days after this second admission, the patient underwent an orthotopic liver transplant with a reduced (segments 2, 3, and 4) ABO-compatible liver. Initial immunosuppression consisted of cyclosporine (trough levels, 300–450 ng/ml; Sandimmune), corticosteroids, and azathioprine, with rapid normalization of liver enzymes.

The resected liver was small and cirrhotic and weighed 322.8 g; the left lobe appeared more fibrotic than the right. Microscopic sections of the right lobe revealed marked ductular proliferation with occasional biliary thrombi in the lumen. Periportal hepatocytes contained microvesicular steatosis with some degree of pseudoacinar transformation. Centrolobular areas were very disorganized, with ballooned hepatocytes with macro- and microvesicular steatosis and numerous multinucleated giant cells (Fig. 6). In these areas, interstitial fibrosis and bridging fibrosis were prominent as was an in-

filtrate comprised primarily of lymphocytes and plasmocytes. Electron micrographs (kindly reviewed by Dr. M. J. Phillips, Toronto) did not reveal the characteristic cytoplasmic pleomorphic viral-like particles previously described (14). The left lobe was primarily composed of collapsed centrolobular zones with interstitial hemorrage and a
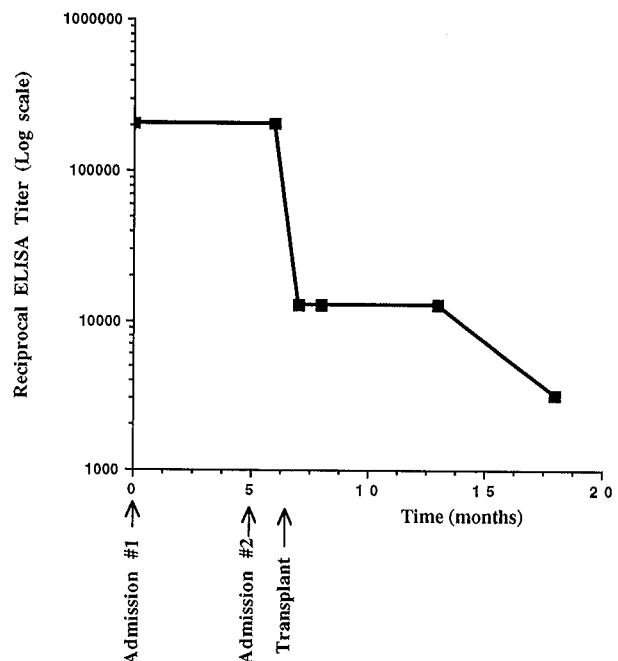

FIG. 3. Serial anti-LKM ELISA titers. High anti-LKM titers persisted posttransplantation despite normal liver enzymes and immunosuppression. Determination of the anti-LKM titer was performed retrospectively on frozen serum from the first admission (8).
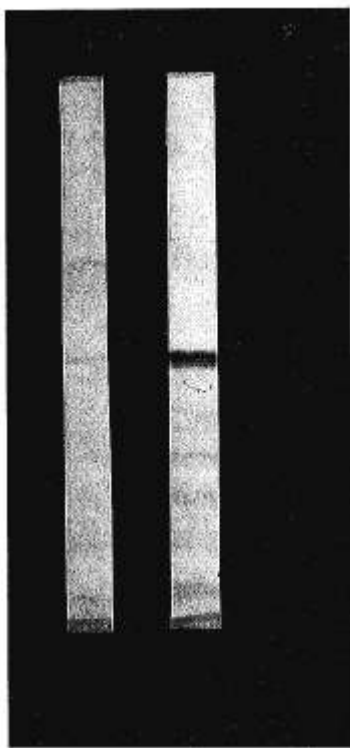
FIG. 4. Western blot confirmation of antimicrosomal antibodies. Nitrocellulose filters containing human microsomes previously separated by SDS–polyacrylamide gel electrophoresis were incubated with patient sera, washed, incubated with peroxidase-linked goat anti-human IgG antibodies, and developed with diaminobenzidine (9). **Left:** Control patient with anti-SMA autoimmune hepatitis. **Right:** Patient's serum shows recognition of a single 48-kD protein.

polymorphic infiltrate of neutrophils, lymphocytes, histiocytes, and plasmocytes. Around the central areas, some ductular proliferation was seen with a few residual ballooned hepatocytes suggestive of a massive hepatitis-like picture.

Insulin was required for the first postoperative week; boluses of corticosteroids were given during the first month for mild rejection. The patient has maintained normal glucose levels throughout the first year of follow-up. Since the second month posttransplant, liver enzymes remained within 1.5 times the upper limit of normal for the first 6 months and within normal values ever since, despite withdrawal of all steroid therapy, administration of azathioprine (1 mg/kg/day), and cyclosporine trough levels of 90–120 ng/ml. Control liver biopsies have been considered normal. Anti-LKM antibody titers have remained elevated with the same specificity as prior to transplantation (Fig. 3). Hepatitis C antibodies have been negative by second-generation ELISA since transplantation.

DISCUSSION

The present case illustrates many of the more common features of autoimmune hepatitis along with the pitfalls in making the diagnosis. Typically, children with antibodies recognizing CYP2D6 antigen present at a younger age, often with more advanced liver disease including cirrhosis or even fulminant hepatitis (9,10). In contrast to the present case, affected children are female in the majority of cases (6). Immunosuppression with corticosteroids with or without azathioprine, often for several years, may induce prolonged remission of the disease but does not preclude the need for liver transplantation (6).

The histological features associated with autoimmune hepatitis are protean and may lead to an incorrect diagnosis, as happened initially in this case. The first liver biopsy revealed an acute hepatitis-like picture with an abundance of eosinophils in the infiltrate. This histological picture can be found in acute viral hepatitis (15), parasitic infestation of the liver (16), drug-induced hepatitis including erythromycin-induced disease (17), pediatric sclerosing cholangitis (18), and various forms of autoimmune hepatitis (14,19).

The finding of giant cell hepatitis on the resected liver has also been described in patients with au-
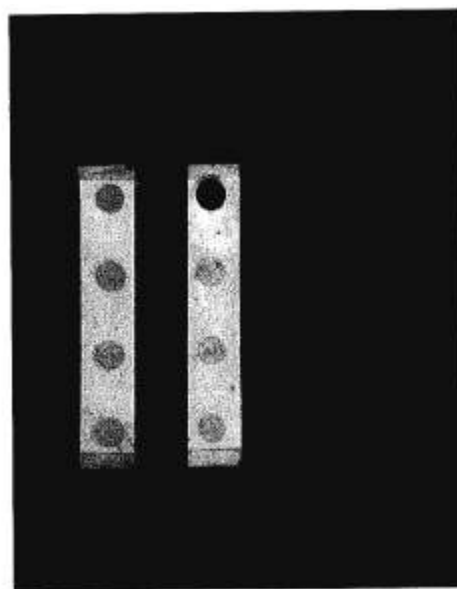

FIG. 5. Dot-blot using synthetic peptide fragments of cytochrome CYP2D6. Patient sera were incubated with nitrocellulose filters containing amino acid sequences 254–273, 321–351, 373–389, or 410–429 and developed as for Western blot (Fig. 4). Patient's serum recognized the usual epitope [254–273 (10)], while control serum recognized no epitope.

FIG. 6. Pathology of the resected liver. Section from the right lobe shows ballooned, often multinucleated, centrolobular hepatocytes and lobular disorganization (×160). **Inset:** several multinucleated giant cells are seen (×250).
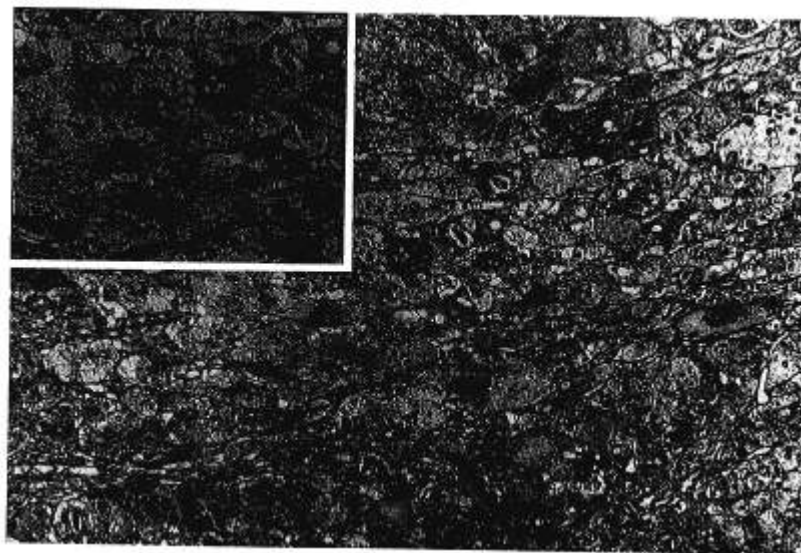
toimmune hepatitis and may represent a stage of the disease process (14). Giant cell hepatitis may also be seen in newborns with cholestasis of various etiologies, including Alagille syndrome (20), α-1-antitrypsin deficiency (21), Byler's progressive familial intrahepatic cholestasis (22), cholestasis associated with total parenteral nutrition (23,24), and idiopathic cholestasis and other disease states (25). Phillips et al. described a series of patients with syncytial giant cell formation associated with particles suggestive of a paramyxoviral infection on electron microscopy (14); they did not find the viral particles described in the present case.

The etiology of autoimmune hepatitis associated with anti–LKM-1 antibodies remains a subject of intensive research. Affected patients frequently have a positive family history for autoimmune disorders (6,7). An association with B8, DR3, and DR4 antigens of the major histocompatibilty complex has been found in adult patients with SMA and/or ANA autoimmune hepatitis (26), along with low levels of complement (27,28). Associated autoimmune phenomena such as vitiligo and diabetes are found in 27–50% of children with LKM-1 autoimmune hepatitis and/or their family members (6,7). The present patient had a positive family history of autoimmune disorders and a personal history of transient diabetes (preceding the trial with corticosteroid therapy), which reversed with liver transplantation and its associated immunotherapy.

The antigen recognized by patients with LKM-1 autoimmune hepatitis gives a typical fluorescence pattern on liver and proximal renal tubules of rat tissue (5). Antibodies were shown to recognize a

48-kD protein in human liver microsomes by Western blot (12). Disease activity was found to parallel anti–LKM-1 antibody titers as determined by ELISA (11). Studies by Yamamoto et al. at the molecular level allowed the antigen to be identified as CYP2D6, the only member of the cytochrome CYP2D subfamily identified in humans (13). Using synthetic peptide fragments, they found the main antigenic site in this molecule (as was the case for this patient) to be between amino acids 262 and 269 (13), as in our patient.

It is not unusual to find continued production of autoantibodies after liver transplantation for autoimmune hepatitis, as was found in our patient (29); this may represent an argument against the role of these antibodies in the pathogenesis of autoimmune hepatitis. The immunotherapy used to fight rejection probably suppresses the activity of the disease so that the autoimmune process has little clinical effect. Patients transplanted for other autoimmune liver diseases have been difficult to assess in terms of disease activity versus rejection of the liver graft as these two phenomena are usually closely related histologically (29).

A search for the trigger initiating the process of autoimmune hepatitis has naturally included a possible viral etiology. The main epitope recognized by LKM-1 antibodies has been found to mimic proteins of herpes simplex virus and human T lymphotropic viruses I and II (30). Similarly, hepatitis A infection has been found to precede the development of autoimmune hepatitis (31). Hepatitis C viral infection has been associated with several autoimmune phenomena, and LKM-1 antibodies have

been found in adult patients with HCV (32). These findings were not confirmed in a study of pediatric patients with LKM autoimmune hepatitis; rather, it appears that false-positive results were found using the first- and second-generation ELISA test for detection of HCV antibodies, as in our patient (33). LKM antibodies of a different affinity have been described in patients with coinfection with hepatitis B and the δ agent (8). These issues require further studies to differentiate the true events from epiphenomena.

It is unlikely that erythromycin triggered the development of the disorder, as the drug induces a different P-450 enzyme, CYP3A (34). The two P-450 enzyme complexes, CYP2D6 and CYP3A, share <30% homology in terms of amino sequences. Furthermore, the main antigenic site sequence recognized by the present patient's serum (aa 254–271), which is the primary antigenic site recognized in the great majority of children with LKM-1 autoimmune hepatitis, is absent from any sequence of CYP3A.

The present case illustrates many of the common and unusual features of autoimmune hepatitis as well as the pitfalls in establishing the diagnosis. The eosinophilic infiltrate present in the initial liver biopsy can be seen in early biopsies of autoimmune hepatitis. Furthermore, the finding of giant cell hepatitis on the resected liver is also compatible with the diagnosis of autoimmune hepatitis, and no viral particles were seen. Finally, a false-positive hepatitis C result has been well-described in patients with anti–LKM-1 autoimmune hepatitis. The specific epitopes of the CYP2D6 recognized in the present case have not been seen in any other setting, to our knowledge, confirming the diagnosis. With the increased availability of such specific diagnostic tests to screen for the different forms of autoimmune hepatitis, therapy can be started at an earlier stage of the disease, in many cases avoiding or delaying the need for liver transplantation.

REFERENCES

1. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Study Group. Hepatology 1993;18:998–1002.
2. Homberg JC, Abuaf N, Helmy-Khalil S, et al. Drug-induced hepatitis associated with anticytoplasmic organelle autoantibodies. Hepatology 1985;5:722–7.
3. Toh BH. Smooth muscle autoantibodies and autoantigens. Clin Exp Immunol 1979;38:621–8.

4. Abuaf N, Johanet C, Chretien P, et al. Characterization of the liver cytosol antigen type 1 reacting with autoantibodies in chronic active hepatitis. Hepatology 1992;12:892–8.
5. Odièvre M, Maggiore G, Homberg JC, et al. Seroimmunologic classification of chronic hepatitis in 57 children. Hepatology 1983;3:407–9.
6. Codoner-Franch P, Bernard O, Maggiore G, Alagille D, Alvarez F. Clinical and immunological heterogeneity of anti-liver-kidney microsome antibody positive autoimmune hepatitis in children. J Pediatr Gastroenterol Nutr 1989;9:436–40.
7. Maggiore G, Bernard O, Homberg JC, et al. Liver-disease associated with anti-liver-kidney microsome antibody in children. J Pediatr 1986;108:399–401.
8. Crivelli O, Lavarini C, Chiaberge E, et al. Microsomal autoantibodies in chronic infection with the HBsAg-associated delta (δ) agent. Clin Exp Immunol 1983;54:232–8.
9. Maggiore G, Porta G, Bernard O, et al. Autoimmune hepatitis with initial presentation as acute hepatic failure in young children. J Pediatr 1989;116:280–2.
10. Porta G, Costa-Gayotto LC, Alvarez F. Anti-liver-kidney microsome antibody positive autoimmune hepatitis presenting as fulminant liver failure. J Pediatr Gastroenterol Nutr 1990;11:138–40.
11. Paradis K, Dib A, Homberg JC, Bernard O, Alagille D, Alvarez F. Detection of anti-endoplasmic reticulum antibody-positive autoimmune hepatitis in children, using an ELISA technique. J Pediatr Gastroenterol Nutr 1988;7:816–22.
12. Alvarez F, Bernard O, Homberg JC, Kreibich G. Anti-liver-kidney microsome antibody recognizes a 50,000 molecular weight protein of the endoplasmic reticulum. J Exp Med 1985;161:1231–6.
13. Yamamoto AM, Cresteil D, Boniface O, Clerc F, Alvarez F. Identification and analysis of cytochrome P450IID6 antigenic sites recognized by anti-liver-kidney microsome type-1 antibodies (LKM1). Eur J Immunol 1993;23:1105–11.
14. Phillips MJ, Blendis L, Poucell S, et al. Synctial giant-cell hepatitis. Sporadic hepatitis with distinctive pathological features, a severe clinical course, and paramyxoviral features. N Engl J Med 1991;324:455–60.
15. Simson IW, Gear JHS. Other viral and infectious diseases. In: MacSween RNM, Anthony PP, Scheuer PJ, eds. Pathology of the liver. 2nd ed. Edinburgh: Churchill Livingstone, 1987:224.
16. Dunn MA, Sodeman WA Jr. Liver diseases. In: Strickland GT, ed. Hunter's tropical medicine. 6th ed. Philadelphia: Saunders, 1984:27.
17. Zafrani ES, Ishak KG, Rudzki C. Cholestatic and hepatocellular injury associated with erythromycin esters. Report of nine cases. Dig Dis Sci 1979;24:385–96.
18. El-Shabrawi M, Wilkinson ML, Portmann B, et al. Primary sclerosing cholangitis in childhood. Gastroenterology 1987;92:1226–35.
19. Panush RS, Wilkinson LS, Fagin LS. Chronic active hepatitis associated with eosinophilia and Coomb's positive hemolytic anemia. Gastroenterology 1973;64:1015–9.
20. Alagille D, Odièvre M, Gautier M, Dommergues JP. Hepatic ductular hypoplasia associated with characteristics facies, vertebral malformations, retarded physical, mental and sexual development, and cardiac murmur. J Pediatr 1975;86:63–71.
21. Ghishan F, Greene H. Liver disease in children with PiZZ α1 antitrypsin deficiency. Hepatology 1988;8:307–10.
22. Reily C. Familial intrahepatic cholestatic syndromes. Semin Liver Dis 1987;7:119–33.
23. Merritt RJ. Cholestasis associated with total parenteral nutrition. J Pediatr Gastroenterol Nutr 1986;5:9–22.
24. Cohen C, Olsen MM. Pediatric total parenteral nutrition, liver histopathology. Arch Pathol Lab Med 1981;105:152–6.

25. Iau JY, Koukoulis G, Mieli-Vergani G, Portmann BC, Williams R. Synctial giant cell hepatitis—a specific entity? *J Hepatol* 1992;15:216–9.

26. Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson RJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocytes antigens DR4 and A1-B8-DR3 are independent risk factors. *Hepatology* 1991;13:701–6.

27. Vergani D, Wells L, Larcher VF, et al. Genetically determined low C4: a predisposing factor to autoimmune chronic active hepatitis. *Lancet* 1985;2:294–7.

28. Scully LJ, Toze C, Sengar DPS, Goldstein R. Early-onset autoimmune hepatitis is associated with a C4A gene deletion. *Gastroenterology* 1993;104:1478–84.

29. Balan V, Batts KP, Porayko MK, Krom RA, Weisner RH. Histological evidence for recurrence of primary biliary cirrhosis after liver transplantation. *Hepatology* 1993;18:1392–6.

30. Gueguen M, Boniface O, Bernard O, Clerc F, Cartwright T, Alvarez F. Identification of the main epitope on human cytochrome P450IID6 recognized by anti-liver-kidney microsome antibody. *J Autoimmunol* 1991;4:607–715.

31. Vento S, Garofaro T, DiPerri G, Dolci L, Concia E, Bassetti D. Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. *Lancet* 1991;337:1183–7.

32. Lenzi M, Ballardini G, Fusconi M, et al. Type 2 autoimmune hepatitis and hepatitis C virus infection. *Lancet* 1990;335:258–9.

33. Alvarez F, Martres P, Maggiore G, Bernard O, Dussaix E. False-positive result of hepatitis C enzyme-linked immunosorbent assay in children with autoimmune hepatitis. *J Pediatr* 1991;119:75–7.

34. Dalet C, Blanchard JM, Guzelian PS, Barwick J, Hartle H, Morel P. Cloning of a cDNA coding for P450 LM3c from rabbit liver microsomes and regulation of its expression. *Nucleic Acids Res* 1986;14:5999–6015.