CASE REPORT

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Autoimmune hepatitis with sclerosing cholangitis in a patient with thiopurine methyltransferase deficiency: case presentation

SORIN CLAUDIU MAN[1)], CRISTINA NICOLETA SCHNELL[1)], VALENTINA SAS[1)], ANCA DANA BUZOIANU[2)], DAN GHEBAN[3)]

[1)]*Department of Mother and Child, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2)]*Department of Functional Sciences, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[3)]*Department of Morphological Sciences, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

Abstract

The association between two autoimmune diseases is known in the literature as overlap syndrome. We present the case of an 18-year-old boy, diagnosed at the age of 13 with an overlap syndrome between type I autoimmune hepatitis and sclerosing cholangitis. The response to immunosuppressant therapy was hampered by azathioprine-induced toxicity causing severe pancytopenia, as a result of thiopurine methyltransferase enzyme genetic deficiency. Treatment was replaced by mycophenolate mofetil. Although the relapse rate was reduced, the disease progressed to cirrhosis. Specific features of this case were the overlap syndrome, young age of onset, especially for sclerosing cholangitis, azathioprine toxicity that influenced the prognosis and the treatment problems regarding the use and efficiency of alternative immunosuppressant agents in pediatric patients.

Keywords: autoimmune hepatitis, sclerosing cholangitis, thiopurine methyltransferase, mycophenolate mofetil.

Introduction

Autoimmune hepatitis (AIH) is a rare disorder in children [1] with a prevalence of two cases per 200 000 patients [2]. Serologically, it is defined by the presence of autoantibodies, high levels of liver transaminases and immunoglobulin G (IgG) and histologically by an interface hepatitis with unknown etiology [3]. Based on the type of autoantibodies identified, there were described two types of AIH in children: type I – characterized by the presence of smooth muscle antibodies (SMA) and/or antinuclear antibodies (ANA), and type II – characterized by the presence of liver kidney microsomal type 1 antibodies (anti-LKM1) or for liver cytosol type 1 antibodies (anti-LC1) [4]. Immunosuppressive treatment represents the standard therapy, aiming at preventing progression to advanced and terminal stage liver disease, and was shown to improve prognosis in most AIH cases and induce a symptom-free long-term survival [5]. The reduced response to immunosuppressive therapy can be due to an overlap syndrome with sclerosing cholangitis (SC) [6]. The long-term prognosis of SC cases is worse than for the AIH, due to the progression of bile duct lesions despite immunosuppressive therapy [7].

Here we present a case of a child who associated AIH/SC overlap syndrome and thiopurine methyltransferase (TPMT) deficiency, two particularities that influenced the outcome of this case. Both reduced the response to therapy and associated medical case management problems regarding the replacement of azathioprine treatment, with a negative outcome, as shown by the presence of cirrhosis at the second liver biopsy.

Case presentation

Patient DA, presented in our Service (Pediatrics Clinic III, Emergency Clinical Hospital for Children, Cluj-Napoca, Romania; May 26, 2011) at the age of 13 years for jaundice. At admission, the physical exam revealed good general condition, normal weight and height (51 kg, 1.68 cm), scleral and skin jaundice, and enlarged liver and spleen (4 cm, respectively 2 cm below the costal margin). Laboratory tests showed a high level of total and conjugated bilirubin levels, elevated liver transaminases, γ-glutamyltransferase (GGT) and alkaline phosphatase, with an alkaline phosphatase/aspartate aminotransferase ratio of 1/16. Abdominal ultrasound showed hepatosplenomegaly, with mild enlargement of the common biliary duct, with no signs of biliary obstruction. The tests for hepatitis A, B, C, Wilson's disease, hemochromatosis, and α1-antitrypsin deficiency were negative. Because of the high levels of IgG, we suspected AIH. Antinuclear antibodies, antimitochondrial antibodies, and anti-LKM1 antibodies were negative, but the anti-smooth muscle antibodies were positive (73 AU/mL, reference range <20 AU/mL). Based on these, we diagnosed AIH type I.

Following the initiation of oral prednisone therapy at 60 mg/daily, we observed a clinical and laboratory improvement – liver transaminases, bilirubin and IgG levels decreased, but the GGT increased (Figure 1). After four weeks of prednisone treatment, the bilirubin and IgG levels reached normal values, ultrasound showed a regression of the hepatosplenomegaly, but liver enzymes and GGT levels remained above the upper limit of the

normal ranges. The prednisone dose was slowly decreased during eight weeks, and azathioprine therapy (1 mg/kg/day) was initiated with further decrease in hepatic transaminases to normal levels after four weeks of therapy. Due to the maintenance of cholestasis, there was introduced the ursodeoxycholic acid. Liver biopsy and histopathological examination on paraffin-included pieces and stained with Hematoxylin–Eosin (HE) and green light trichrome [Goldner–Szekely (GS) technique] showed interface hepatitis with necroinflammatory activity grade I and Ishak fibrosis stage 5 (Figures 2a, 3a and 4a).

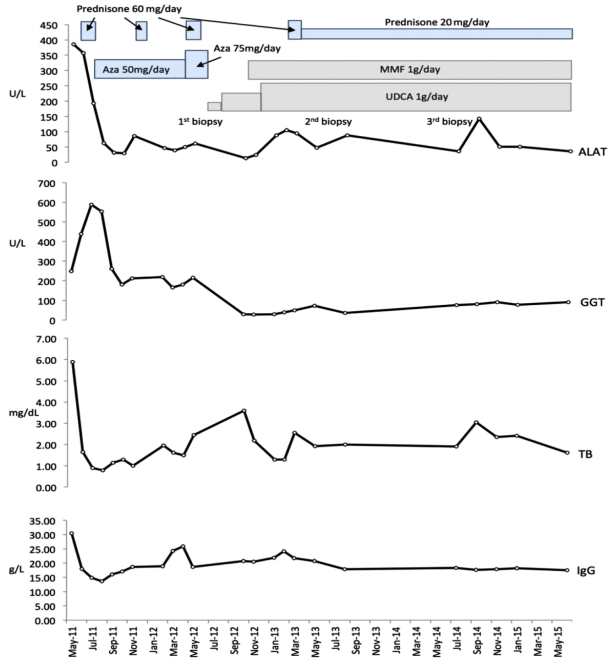

Figure 1 – ***Treatment and clinical course. Aza: Azathioprine; MMF: Mycophenolate mofetil; UDCA: Ursodeoxycholic acid; ALAT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; TB: Total bilirubin; IgG: Immunoglobulin G.***

During the first year after diagnosis, the patient presented two relapses at five and 10 months after onset and azathioprine dose was increased to 1.5 mg/kg/day. The consequence was severe pancytopenia with leukocytes 2600/μL, erythrocytes 1 890 000/μL, and platelets 69 000/μL. Thiopurine methyltransferase (TPMT) genotyping was performed and showed the presence of two mutations, p.Ala154Thr (TPMT*3B) and p.Tyr240Cys (TPMT*3C), associated with TPMT deficiency and azathioprine toxicity.

Following the genotyping, it was decided to stop azathioprine therapy, followed by another relapse with jaundice reappearance, increased levels of bilirubin, liver enzymes and cholestasis. Mycophenolate mofetil (MMF) was added at 1 g/day with clinical and laboratory improvement. Two months after the initiation of MMF treatment, liver enzymes increased again. This was interpreted as a relapse and the patient was put on high-dose prednisone. The liver ultrasound showed enlargement of the common biliary duct and collateral circulation in the hepatic hilum. Gastroduodenoscopy showed esophageal varices grade I and gastric mucosa congestion with multiple subepithelial hemorrhages (Lanza score 3). A proton-pump inhibitor was prescribed for four weeks and carvedilol 6.25 mg twice a day was given to reduce the portal pressure.

Because of persistently high levels of GGT and alkaline phosphatase and the presence of relapses in the first year after onset, we considered the possibility of an AIH/SC overlap syndrome. Magnetic resonance cholangiopancreatography (MRCP) showed a dilation of 8 mm of the diameter of the common hepatic duct, without obstructive lesions, and segmental dilations of intrahepatic bile ducts, with a maximum diameter of 4 mm. The MRCP also showed important collateral circulation in the spleen hilum, presence of a spleno-renal shunt and enlargement of the esophageal and perigastric veins. The second liver biopsy performed two years after the onset, showed signs of cirrhosis and SC (Figures 2b, 3b, 4b and 5).

The evaluation of liver lesions was completed by the performance of immunohistochemistry studies on the liver biopsy fragments included in paraffin, for the highlighting of the inflammatory cell reaction, and also of Ito cells. In this way, there were used the following antibodies: anti-CD3 (monoclonal mouse anti-human, clone F7.2.38, M7254, 1:25 dilution, Dako); anti-CD4 (monoclonal mouse anti-human CD4, clone MT310, M0716, 1:50 dilution, Dako); anti-CD8 (monoclonal mouse anti-human CD8, clone C8/144B, M7103, 1:100 dilution, Dako); anti-CD20 (monoclonal mouse anti-human, CD20cy, clone L26, M0755, 1:50 dilution, Dako); anti-CD68 (monoclonal mouse anti-human CD68, clone KP1, M0814, 1:100 dilution, Dako); anti-α-SMA (monoclonal mouse anti-human alpha-smooth muscle actin, clone 1A4, 1:100 dilution, Dako).

Our study allowed the highlighting of certain porto-biliary spaces with high dimensions, rich in collagen fibers and an abundant inflammatory infiltrate, made of numerous lymphocytes, plasmocytes and macrophages (Figure 6). Also, there were highlighted numerous porto-portal fibrous bridges containing collagen fibers with the tendency of fascicle organization and abundant inflammatory infiltrate. The immunohistochemical examinations showed the presence of CD3+ T lymphocytes in the porto-biliary inflammatory infiltrate, in high quantities, with a heterogeneous distribution (Figure 7). A high number of CD3+ T lymphocytes were also identified in the structure of liver lobes, present mainly in the Disse spaces, and also in the sinusoid capillaries (Figure 8a). CD4+ T lymphocytes were absent, both in Kiernan spaces and intralobularly (Figure 8b), while CD8+ T lymphocytes were numerous in the porto-biliary spaces (Figure 9) and rare in the liver parenchyma (Figure 10). In the portal inflammatory infiltrate, there were also identified CD20+ B lymphocytes, but in a smaller quantity compared to T lymphocytes (Figure 11).

The reaction of the macrophage system cells was quite intense in the porto-biliary space, where there was identified a number of CD68+ cells (Figure 12) as high as CD3+ T lymphocytes. In other words, we may appreciate that the inflammatory infiltrate in the porto-biliary space was mainly formed of T lymphocytes and macrophages. In the liver parenchyma, there was highlighted a high number of Kupffer cells of large sizes, which shows an intensification of their fagocyting activity (Figure 13).

The use of the antibody against α-SMA allowed us to identify the myofibroblast cells involved in the synthesis of the collagen fibers. As it may be observed from our image (Figure 14), in the porto-biliary spaces there was highlighted an intense reaction to the anti-α-SMA antibody,

which denotes the presence of a very high number of myofibroblasts that participate in the synthesis of collagen fibers. The same antibody allowed the highlighting of Ito cells transformed into myofibroblast cells. It is well known that the stellate liver cells, also known as perisinusoidal dendritic cells or Ito cells, are localized in the Disse space, playing an essential part in the storage of vitamin A and some fat drops. In severe liver lesions (severe chronic hepatitis and liver cirrhosis), the liver stellate cells change, "become active", get contractile and chemotactic properties, multiply, synthesize and secrete collagen, participating in the formation of scar tissue. In our study, in the liver parenchyma, there were highlighted numerous perisinusoidal dendritic cells intensely positive to α-SMA (Figures 15 and 16).

The final diagnosis was an overlap syndrome between AIH and SC, which progressed to cirrhosis, and TPMT deficiency. The patient continued the treatment with MMF, low doses of prednisone (20 mg/day, every day) and ursodeoxycholic acid. The third liver biopsy performed two years after the onset, showed the same lesions as the second biopsy (Figures 2c, 3c and 4c). FibroScan examination showed F4 fibrosis.

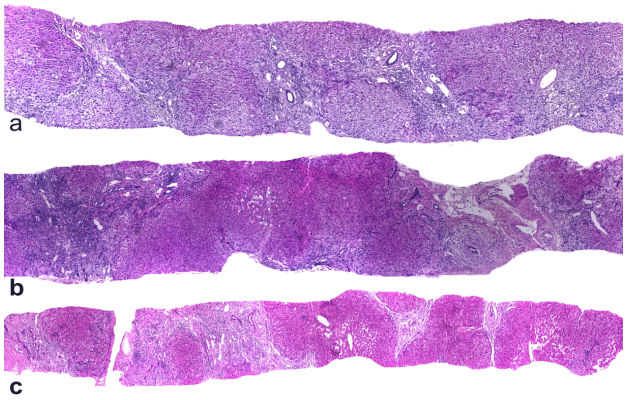

Figure 2 – ***HE staining (×100) showing moderate inflammation in 2012 (a), severe inflammation in 2013 (b) and reduced inflammation in 2014 (c). In all these biopsies, biliary tract hyperplasia is present in the portal tracts.***

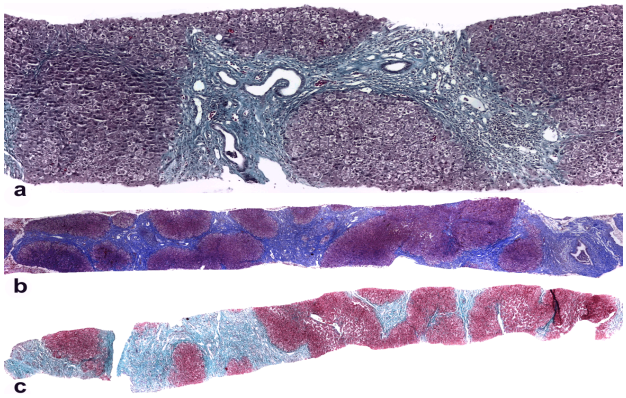

Figure 3 – ***Masson's trichrome staining for collagen showing a pre-cirrhotic state in 2012 (a) and established cirrhosis in 2013 (b) and 2014 (c).***

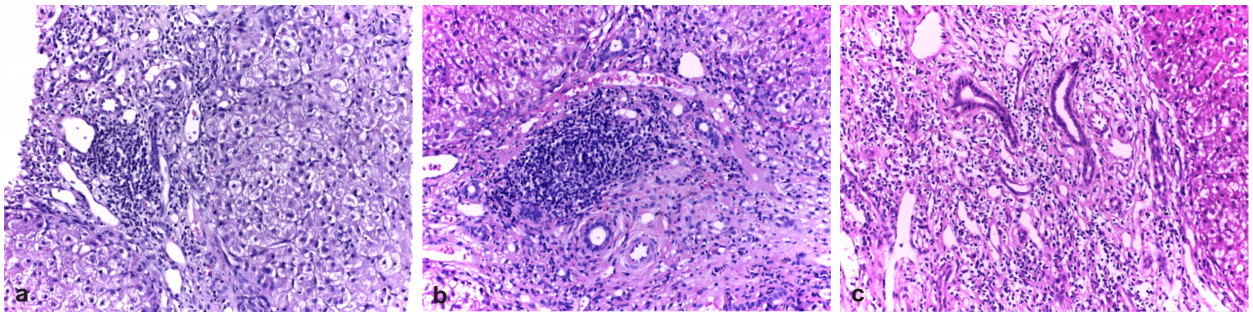

Figure 4 – ***Autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome – biopsies: (a) 2012; (b) 2013; (c) 2014. The 2012 and 2013 biopsies show abundant plasma cell infiltrate, interface hepatitis, lymphoid follicle formation and feathery degeneration of the hepatocytes suggesting autoimmune hepatitis. Reduced inflammation in the portal tract is present on the 2014 biopsy (c). All have also proliferated biliary tracts. HE staining, ×100.***

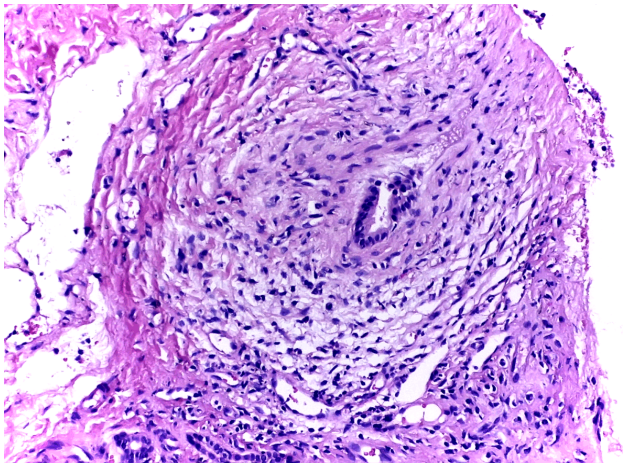

Figure 5 – ***"Onion skin" fibrosis characteristic for primary sclerosing cholangitis, seen in 2013 biopsy. HE staining, ×200.***

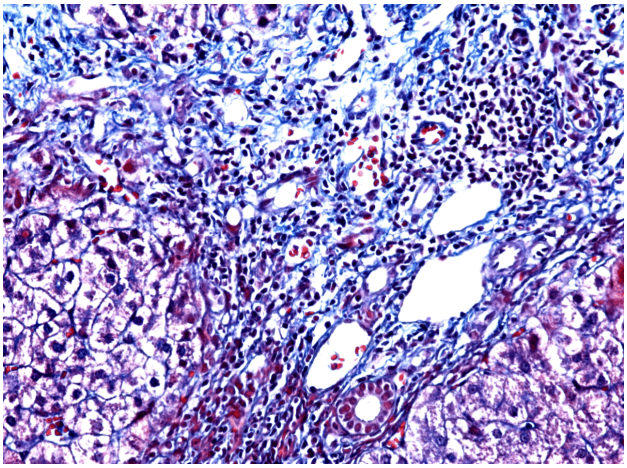

Figure 6 – ***Microscopic image of a porto-billiary space where we may observe the presence in high quantity of collagen fibers and an abundant inflammatory infiltrate. GS trichrome staining, ×200.***

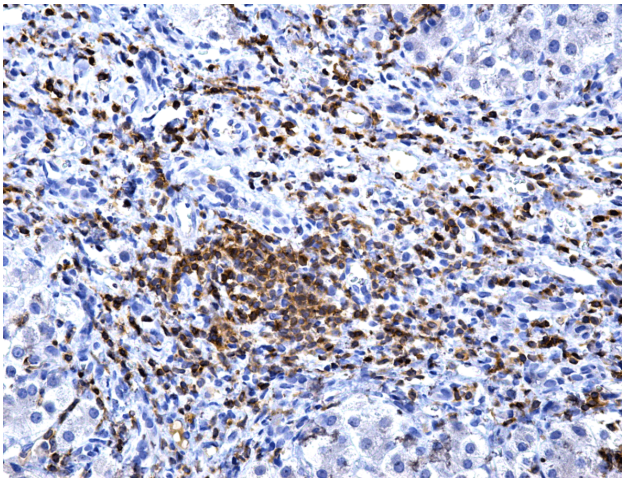

Figure 7 – ***Large size Kiernan space, intensely infiltrated with inflammatory cells and abundant CD3+ T lymphocytes. Anti-CD3 antibody immunomarking, ×200.***

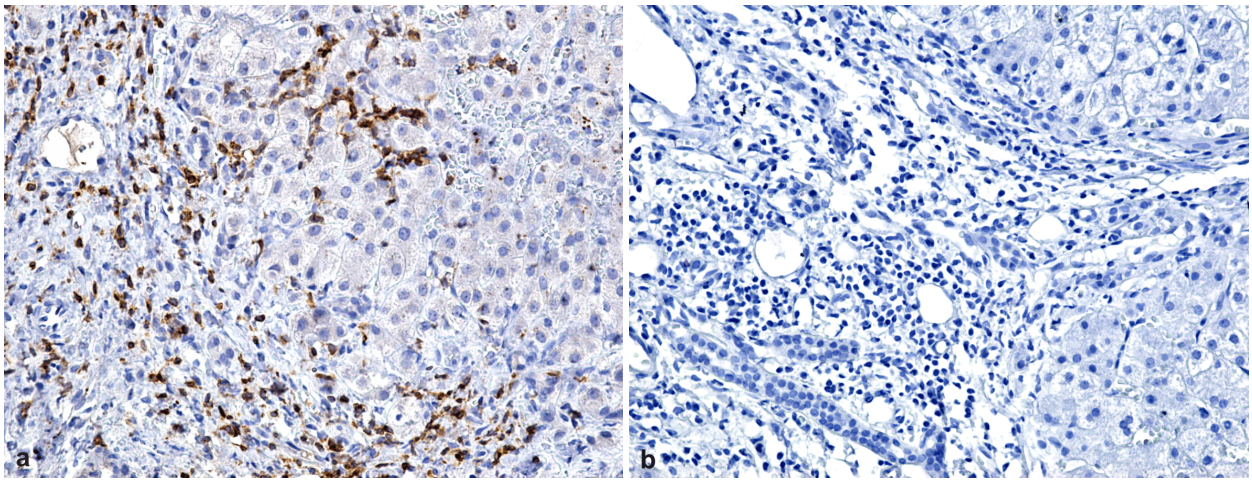

Figure 8 – ***(a) T-lymphocytes present in the structure of the liver lobe, both in the sinusoid capillaries and in the Disse space. Anti-CD3 antibody immunomarking, ×200; (b) Negative reaction of the inflammatory infiltrate cells of the porto-biliary space to the anti-CD4 antibody. Anti-CD4 antibody immunomarking, ×200.***

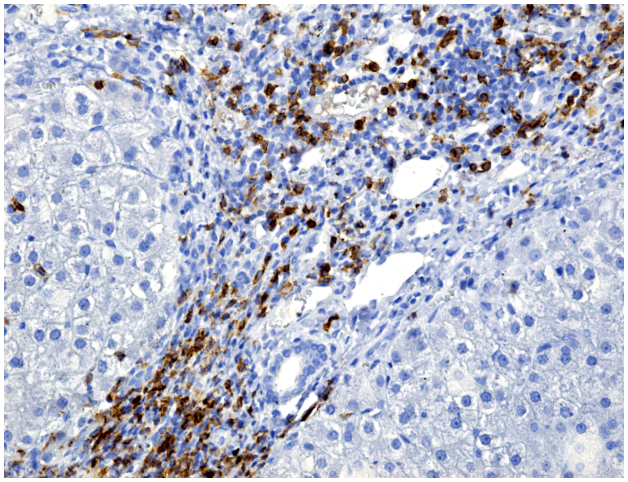

Figure 9 – ***CD8+ T lymphocytes present in large number in the portal space, and also in the porto-portal fibrous bridges. Anti-CD8 antibody immunomarking, ×200.***

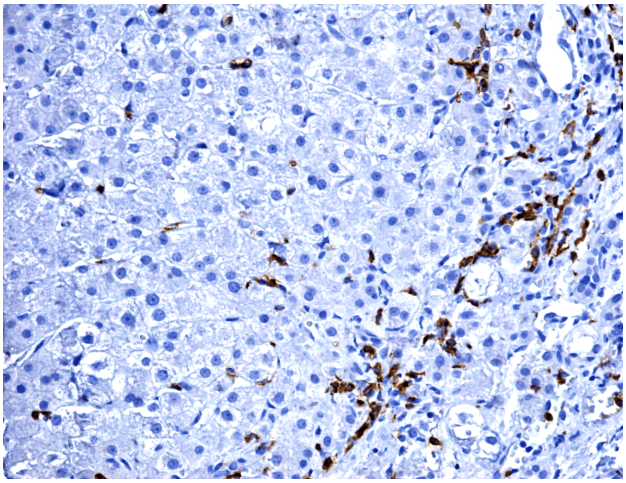

Figure 10 – ***Rare CD8+ T lymphocytes present in the liver parenchyma. Anti-CD8 antibody immunomarking, ×200.***

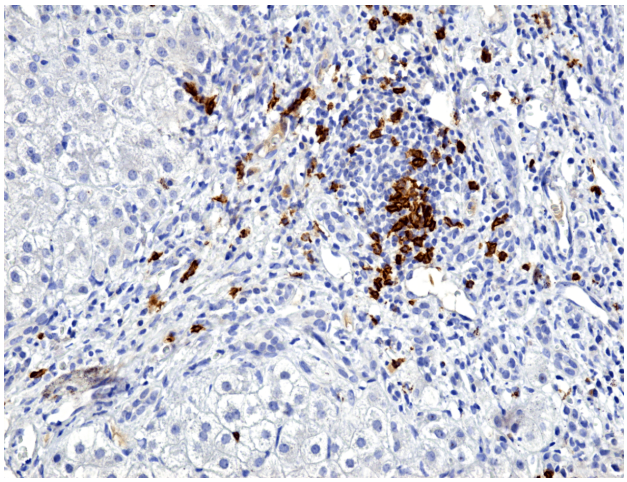

Figure 11 – *Kiernan space with a low number of CD20+ T lymphocytes. Anti-CD20 antibody immunomarking, ×200.*

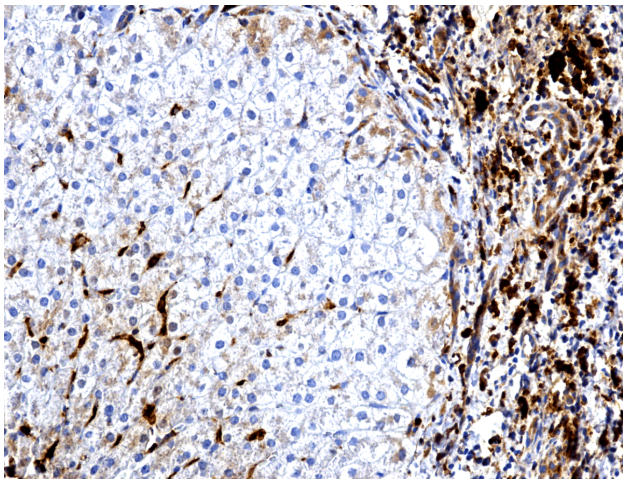

Figure 12 – *Intense reaction of macrophages in the porto-biliary space. Anti-CD68 antibody immunomarking, ×200.*

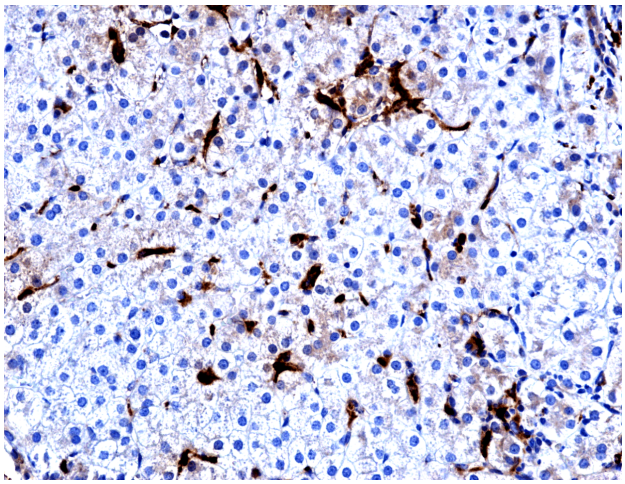

Figure 13 – *Liver parenchyma with numerous Kupffer cells, of large size, intensely positive to the anti-CD68 antibody. Anti-CD68 antibody immunomarking, ×200.*

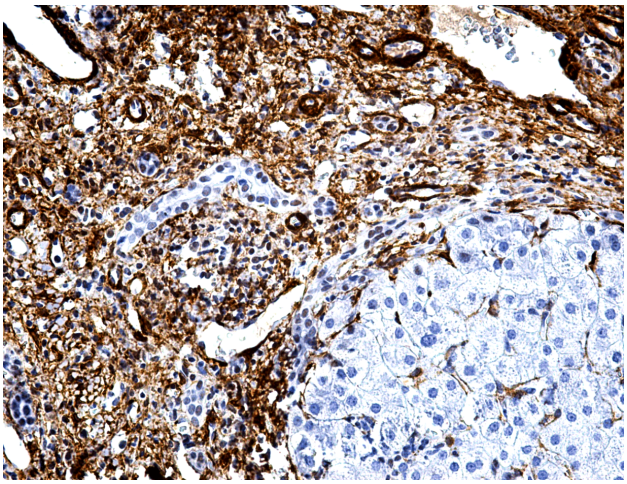

Figure 14 – *Porto-billiary space with numerous myo-fibroblasts, with an intense reaction to anti-α-SMA. Anti-α-SMA antibody immunomarking, ×200.*

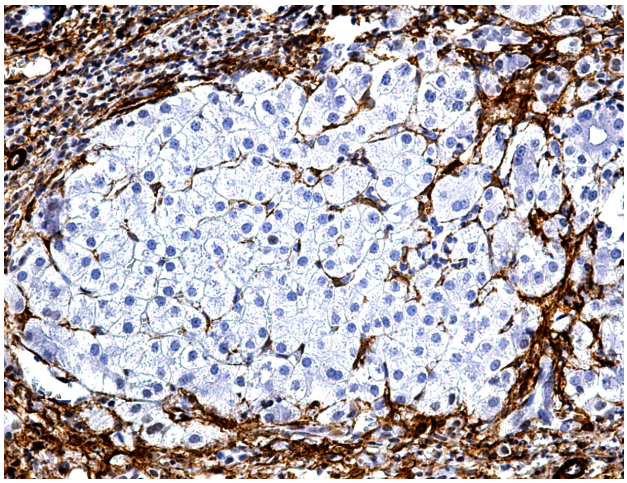

Figure 15 – *Image of cirrhotic nodule with dendritic cells positive to the anti-α-SMA antibody. Anti-α-SMA antibody immunomarking, ×200.*

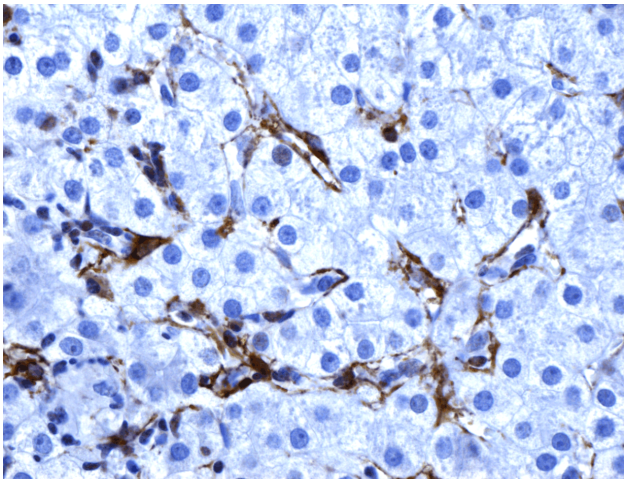

Figure 16 – *Activated perisinusoidal dendritic cells (Ito cells), transformed into myofibroblasts. Anti-α-SMA antibody immunomarking, ×400.*

Discussion

AIH and primary SC can be associated, in children and adults, with other autoimmune disorders like inflammatory bowel disease [7], autoimmune thyroiditis [7], insulin-dependent diabetes mellitus [7], systemic sclerosis-polymyositis [8], dermatomyositis and antiphospholipid syndrome [9, 10]. In children, as opposed to adults, AIH is frequently associated with SC. Previously, it was shown in a series of 55 children with evidence of liver diseases and autoimmunity that 27 (49%) had radiological features of cholangiopathy, a syndrome named by the authors autoimmune SC [7]. In adults, this association is present only in 2–10% of AIH cases [11, 12].

Although original [13], revised [14] or simplified [15] criteria for the diagnosis of AIH are currently used, none is recommended for SC diagnosis [16], due to the lack of lacking cholangiography findings among the diagnosis criteria. In clinical practice, the diagnosis of SC is made in patients with AIH criteria associated with cholangiographic or histological features characteristic for SC [16, 17]. Our patient had 8 points of simplified diagnosis criteria for AIH [15] and persistently high levels of GGT and alkaline phosphatase, histological features of SC and segmental dilatations of intrahepatic bile ducts.

The histopathological and immunohistochemical changes of the liver present in our patient were intense and were characterized by the presence of an interface hepatitis, associated with an abundant inflammatory infiltrate of lymphocytes, plasmocytes and macrophages. According to some authors, these microscopic aspects suggest the involvement of an aggressive immune cellular response in the AIH etiopathogeny [18, 19]. In our study, the most numerous cell populations were represented by T lymphocytes and macrophages. Some of these cells were also identified in the liver parenchyma. Some studies showed that these cells surround the dead hepatocytes or the ones with dystrophic lesions [20].

In our case, the activation of perisinusoidal dendritic cells and myofibroblasts from the Disse space shows a tendency to accelerate the collagen production and the case evolution to cirrhosis. Other studies suggest that the activation of Kupffer cells and myofibroblasts leads to the increase of oxygen-reactive species, which determines the hepatocytes apoptosis, release of apoptotic bodies and activation of liver dendritic cells [21, 22].

We consider that autoimmune hepatitis may present various clinical and histopathological forms, related both to the immune response and to some genetic factors.

Compared to AIH alone, adults with AIH/SC overlap syndrome have been shown to have lower remission rates (22 *vs.* 64%), higher relapse rates (100% *vs.* 75%), treatment failure (33% *vs.* 10%), liver-related death or liver transplantation (33% *vs.* 8%), and develop cirrhosis more often (67% *vs.* 42%) [23]. In contrast, children with AIH/SC overlap syndrome appear to respond better than adults to immunosuppressive therapy, with response rates similar to the ones observed in children with AIH alone; however, the long-term prognosis remains worse compared to AIH alone due to progression of SC in approximately 50% of the cases [7].

According to guidelines [24, 25], AIH standard treatment is immunosuppression with prednisone/prednisolone in combination with azathioprine. The therapy of AIH/SC overlap syndrome in both children and adults is similar, but as opposed to AIH therapy, this recommendation this is based on low-quality evidence [26, 27]. The recommended dose for azathioprine is 50 mg/day or 1–2 mg/kg/day [24, 25]. In the first year after the onset of the disease, our patient received a dose of 1 mg/kg/day (50 mg/day) of azathioprine. Because of relapses, the dose was increased to 1.5 mg/kg/day, and this increase was associated with severe bone marrow suppression. Azathioprine is metabolized *via* two main pathways, involving two key enzymes: hypoxanthine-guanine phosphoribosyltransferase and TPMT. Genetic analysis in the case presented here showed the presence of two nonfunctional alleles (*3B/*3C) of the TPMT gene, representing a genotype with low TPMT enzyme activity [28] and consequently with increased risk of developing azathioprine toxicity. Despite this genotype, the patient did not present signs of toxicity at 50 mg/day (1 mg/kg/day); bone marrow suppression developed only at 75 mg/day azathioprine. The presence of these two nonfunctional alleles, which have rarely been reported in the literature – with a frequency of homozygotes with low activity alleles of about 0.3% [29], represents the peculiarity of the case presented here.

Alternative therapy for AIH includes multiple immunosuppressive agents but none, except budesonide [30], has been studied in controlled clinical trials [22]. In AIH cases non-responsive or intolerant to azathioprine therapy the next line of treatment is MMF, a prodrug of mycophenolic acid or calcineurin inhibitors [31]. In a retrospective study on 26 children with autoimmune liver disease (AIH and SC) resistant or intolerant to standard immunosuppression, 18 (69%) responded to MMF [27]. In the responders' group, only 2 (11%) had SC, compared with the non-responders group in which six out of eight (75%) had SC [32]. The response rate was not related to the indication of MMF: resistance to standard immunosuppression (response rate 70%) or intolerance to standard therapy (response rate 67%) [32].

There are many unanswered questions regarding AIH, such as "is azathioprine the most effective drug?" and "which is the best option in case of azathioprine resistance or intolerance?" [22].

Conclusions

Our patient developed a type I AIH, some clinical and laboratory aspects, such as significant conjugated hyperbilirubinemia and increased levels of GGT, suggested from the presentation the possibility of SC overlapping. The treatment response in this AIH/SC overlap case was less effective with two relapses during the first year after onset. The need of increased dose of azathioprine had a toxic effect on bone marrow due to genetic TPMT deficiency and lead to management problems concerning the safety and efficiency of alternative immunosuppressant therapy in pediatric patients. MMF treatment reduced the relapse rate and stopped the disease progress.

Conflict of interests

No conflict of interests to declare.

References

[1] Mieli-Vergani G, Heller S, Jara P, Vergani D, Chang MH, Fujisawa T, González-Peralta RP, Kelly D, Mohan N, Shah U, Murray KF. Autoimmune hepatitis. J Pediatr Gastroenterol Nutr, 2009, 49(2):158–164.

[2] Lohse AW, Mieli-Vergani G. Autoimmune hepatitis. J Hepatol, 2011, 55(1):171–182.

[3] Mieli-Vergani G, Vergani D. Autoimmune hepatitis. Nat Rev Gastroenterol Hepatol, 2011, 8(6):320–329.

[4] Krawitt EL. Discrimination of autoimmune hepatitis: autoantibody typing and beyond. J Gastroenterol, 2011, 46(Suppl 1): 39–41.

[5] Della Corte C, Mosca A, Vania A, Alterio A, Alisi A, Nobili V. Pediatric liver diseases: current challenges and future perspectives. Expert Rev Gastroenterol Hepatol, 2016, 10(2):255–265.

[6] Teufel A, Galle PR, Kanzler S. Update on autoimmune hepatitis. World J Gastroenterol, 2009, 15(9):1035–1041.

[7] Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, Mieli-Vergani G. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology, 2001, 33(3):544–553.

[8] Pamfil C, Zdrenghea MT, Mircea PA, Manzat Saplacan RM, Rednic N, Rednic S. Systemic sclerosis-polymyositis overlap syndrome associated with autoimmune hepatitis and cerebral vasculitis. J Gastrointestin Liver Dis, 2012, 21(3):317–320.

[9] Pamfil C, Candrea E, Berki E, Popov HI, Radu PI, Rednic S. Primary biliary cirrhosis – autoimmune hepatitis overlap syndrome associated with dermatomyositis, autoimmune thyroiditis and antiphospholipid syndrome. J Gastrointestin Liver Dis, 2015, 24(1):101–104.

[10] Stătescu L, Vâţă D, Ciobanu D, Gheucă Solovăstru L, Rotaru M. Histopathological aspects in autoimmune cutaneous manifestations associated with hepatitis C virus infection. Rom J Morphol Embryol, 2016, 57(3):937–941.

[11] Abdalian R, Dhar P, Jhaveri K, Haider M, Guindi M, Heathcote EJ. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: evaluating the role of routine magnetic resonance imaging. Hepatology, 2008, 47(3):949–957.

[12] Lewin M, Vilgrain V, Ozenne V, Lemoine M, Wendum D, Paradis V, Ziol M, Arrivé L, Beaugrand M, Poupon R, Valla D, Chazouillères O, Corpechot C. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: a prospective magnetic resonance imaging and histological study. Hepatology, 2009, 50(2):528–537.

[13] Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology, 1993, 18(4):998–1005.

[14] Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WGE, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston ALWF, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RNM, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Mieli-Vergani G, Nakanuma Y, Nishioka M, Penner E, Porta G, Portmann BC, Reed WD, Rodes J, Schalm SW, Scheuer PJ, Schrumpf E, Seki T, Toda G, Tsuji T, Tygstrup N, Vergani D, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol, 1999, 31(5):929–938.

[15] Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW; International Autoimmune Hepatitis Group. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology, 2008, 48(1): 169–176.

[16] Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E; International Autoimmune Hepatitis Group. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol, 2011, 54(2):374–385.

[17] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: autoimmune hepatitis. J Hepatol, 2015, 63(4):971–1004.

[18] Vergani D, Mieli-Vergani G. The impact of autoimmunity on hepatocytes. Semin Liver Dis, 2007, 27(2):140–151.

[19] Abe K, Takahashi A, Imaizumi H, Hayashi M, Okai K, Kanno Y, Watanabe H, Ohira H. Interleukin-21 plays a critical role in the pathogenesis and severity of type I autoimmune hepatitis. Springerplus, 2016, 5(1):777.

[20] Liberal R, Vergani D, Mieli-Vergani G. Update on autoimmune hepatitis. J Clin Transl Hepatol, 2015, 3(1):42–52.

[21] Czaja AJ. Review article: the prevention and reversal of hepatic fibrosis in autoimmune hepatitis. Aliment Pharmacol Ther, 2014, 39(4):385–406.

[22] Czaja AJ. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. World J Gastroenterol, 2014, 20(10): 2515–2532.

[23] Czaja AJ. Frequency and nature of the variant syndromes of autoimmune liver disease. Hepatology, 1998, 28(2):360–365.

[24] Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM; American Association for the Study of Liver Diseases. Diagnosis and management of autoimmune hepatitis. Hepatology, 2010, 51(6):2193–2213.

[25] Gleeson D, Heneghan MA; British Society of Gastroenterology. British Society of Gastroenterology (BSG) guidelines for management of autoimmune hepatitis. Gut, 2011, 60(12): 1611–1629.

[26] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. J Hepatol, 2009, 51(2):237–267.

[27] Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ; American Association for the Study of Liver Diseases. Diagnosis and management of primary sclerosing cholangitis. Hepatology, 2010, 51(2):660–678.

[28] Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, Stein CM, Carrillo M, Evans WE, Klein TE; Clinical Pharmacogenetics Implementation Consortium. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther, 2011, 89(3):387–391.

[29] Langley PG, Underhill J, Tredger JM, Norris S, McFarlane IG. Thiopurine methyltransferase phenotype and genotype in relation to azathioprine therapy in autoimmune hepatitis. J Hepatol, 2002, 37(4):441–447.

[30] Woynarowski M, Nemeth A, Baruch Y, Koletzko S, Melter M, Rodeck B, Strassburg CP, Pröls M, Woźniak M, Manns MP; European Autoimmune Hepatitis – Budesonide Study Group. Budesonide *versus* prednisone with azathioprine for the treatment of autoimmune hepatitis in children and adolescents. J Pediatr, 2013, 163(5):1347–1353.e1.

[31] Liberal R, Vergani D, Mieli-Vergani G. Paediatric autoimmune liver disease. Dig Dis, 2015, 33(Suppl 2):36–46.

[32] Aw MM, Dhawan A, Samyn M, Bargiota A, Mieli-Vergani G. Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. J Hepatol, 2009, 51(1):156–160.

Corresponding author

Sorin Claudiu Man, Associate Professor, MD, PhD, Department of Mother and Child, "Iuliu Haţieganu" University of Medicine and Pharmacy, 2–4 Câmpeni Street, 400217 Cluj-Napoca, Romania; Phone +40264–534 848, Fax +40264–590 492, e-mail: claudiu.man@umfcluj.ro

Received: March 22, 2016 *Accepted: May 4, 2017*