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# Liver Biopsy as a Useful Tool in the Management of Autoimmune Liver Diseases in Childhood

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## 1. Introduction

Autoimmune diseases of the liver and biliary tract are inflammatory diseases of unknown origin that progress spontaneously, in most cases, to severe fibrosis and cirrhosis (Maggiore et al, 2009). They are characterized by an inflammatory infiltrate in the liver tissue involving the lobule and the portal tract and, most frequently, by the presence of non-organ and liver-specific autoantibody reactivity (Mieli Vergani & Vergani, 2009). There are at least three principal disorders in humans, in which the liver damage is thought to be caused by an autoimmune mechanism: autoimmune hepatitis (AIH), where the target of the autoimmune attack is the hepatocyte, and two other conditions, autoimmune cholangitis (AIC) and primary biliary cirrhosis (PBC), where the target is the cholangiocyte. Between these three disorders exists a range of overlap of liver damage both at diagnosis and/or during the follow-up, recognized as "overlap syndrome" (Alvarez, 2006). Any autoimmune disorder may overlap with the other two. Autoimmune cholangitis may be limited, at diagnosis and during the follow-up, to the smallest intrahepatic (forth order) bile ducts in case of the so-called "small duct sclerosing cholangitis" (Chapman, 2002), or may be diffuse affecting also larger bile duct and giving the typical radiological or MRI imaging of "sclerosing cholangitis"). In children, primary biliary cirrhosis is exceptionally described, particularly before adolescence. Moreover, a rare and peculiar form of autoimmune hepatitis, typical of infancy exists, and is characterized by the unique association of Coombs positive autoimmune hemolytic anemia with the peculiar histopathological finding of diffuse giant cell transformation of the hepatocytes at a very unusual age even for infants (Maggiore et al, 2011).

Immunosuppressive treatment is usually efficacious in most cases of AIH, but it is less effective in autoimmune cholangitis, particularly in case of delayed diagnosis at the stage of diffuse sclerosing cholangitis (Maggiore et al, 2009). Immunosuppressive treatment is also the only life-saving treatment recognized in "infantile giant cell hepatitis associated with autoimmune hemolytic anemia" (Maggiore et al, 2011).

Liver biopsy is of paramount importance in the diagnostic work-up of autoimmune diseases of the liver and biliary tract as recognized by the major guidelines. Histological examination

of liver tissue allow to confirm the diagnosis through the presence of suggestive features; to evaluate the degree of biliary tract damage; to establish the severity of architectural injury and the eventual presence of cirrhosis and when necessary, to confirm the diagnosis of relapse in case of discontinuation of treatment.

## 2. Autoimmune hepatitis

Autoimmune hepatitis is a severe liver disease carrying a high mortality rate if untreated. All ages and genders are affected, with a peak of incidence in prepubertal girls, even if the disease has been diagnosed as early as age six months. The disease often presents acutely or can follow a chronic but fluctuating course, usually progressing to cirrhosis and liver failure, even though the rapidity of progression to end-stage liver diseases is highly variable (Lohse AW & Mieli-Vergani, 2011).

Waldenström first described this disease entity in 1950 in a young woman with a chronic inflammatory liver disease, rapidly evolving to cirrhosis, elevated gamma globulins and amenorrhea. The disease was defined as "lupoid hepatitis" because of the presence of antinuclear antibodies (ANA) and of lupus erythematosus cells. Further progress in the identification and characterization of the autoantibodies typically present in AIH patients, led to a consensus on the definition and classification of this disease.

Autoimmune hepatitis is a rare disease, occurring in all races and in all geographic areas, but with a wide range of prevalence in different populations. Studies in adults have reported, in Europe, a prevalence of 1: 10.000 (Boberg, 2002); however the insidious character of the disease in some patients suggests a considerable higher frequency. In childhood, the incidence of AIH is unknown, but it is apparently increasing in the last 20 years, representing today about 10% of all chronic liver diseases in patients referred to a tertiary pediatric liver centre, in Europe (Mieli Vergani & Vergani, 2008). Female gender is mostly involved, with an F/M ratio from 3:1 up to 9:1. Even though all ages are affected, AIH is mainly a pediatric disease since about 40% of diagnoses of AIH-1 and 80% of AIH-2 are made during childhood and adolescence (Alvarez, 2009).

### 2.2 Pathogenesis

The mechanisms leading to autoimmune attack of the hepatocytes are unknown, but several observations suggest that AIH is a multifactorial disease (Longhi et al, 2009). The histological pictures of interface hepatitis and immunohistochemical studies have identified, among the immune-reactive inflammatory cells, a large predominance of activated T lymphocytes positive for the CD4+ helper/inducer phenotype. The CD4+ T lymphocytes are believed to recognize one or more self antigens on the cell surface of the hepatocytes, thus triggering the autoimmune response.

#### 2.2.1 Autoantigen presentation

The first step in an immune reaction is the presentation of the antigen to naive CD4+ T lymphocytes. The antigenic peptide is carried by professional antigen-presenting cells (APC) on their membrane within the binding groove of class II human leukocyte antigen (HLA) molecules. These HLA class II molecules are encoded in close proximity to HLA I and HLA III genes, configuring a number of ancestral haplotypes due to strong linkage disequilibrium among the HLA *loci*. The HLA class II molecules expressed on the membrane of APC are able to hold only short peptides of 13-23 amino acid residues,

which are the final product of the internalization and partial digestion by the APC of extracellular proteins. The recognition of the complex "HLA II-exogenous peptide" is restricted by the specificity of the T-cell receptor and hampered by ligand to ligand co-stimulation. Consequently, HLA class II plays a central role in regulating CD4+ T helper activity. The functional site of HLA class II, the peptide binding groove, is hosted within the DRα and DRβ polypeptidic chain, which composes together the DR heterodimers. Alleles of the DRβ *locus* are highly polymorphous and present three hypervariable regions encoding amino acid motifs. The consequence of this polymorphism is that each individual carries different DR molecules with different binding properties and affinities. Consequently, the nature and the structure of HLA class II polypeptidic chains, and especially of the DR molecules, critically affect both the nature and the alignment of the antigenic peptide and the affinity and avidity of the linkage between the antigen major histocompatibility complex (MHC) II and the T-cell receptor. Thus, the suitability of certain autoantigens to trigger an immune response is genetically determined and depends strictly on the genotypic asset of HLA class II.

### 2.2.2 Genetic background

In the Caucasian population, the HLA A1-B8-DR3 haplotype is associated to AIH-1 as well as with several autoimmune disorders. The HLA DR3 and DR4 haplotypes were identified as independent risk factors for AIH-1, whereas HLA DR2 was accounted to have a protective role. With the availability of high-resolution HLA-typing methods, the molecular basis of this association became investigable and the principal susceptibility allele for AIH-1 was recognized in ***DRB1*0301*** (Czaja, 2000*)*. This allele not only confers an increased risk to develop AIH-1, but also influences some features of the disease. Patients bearing ***DRB1*0301*** in fact present at younger age, respond less favorably to corticosteroid treatment, carry a higher risk of relapse, and require liver transplantation more frequently for end-stage liver failure. Genetic predisposition to AIH-1 has, however, a relevant regional variation, and studies outside Europe and North America have found different susceptibility alleles at the DRB1 *locus*. In Japan, the prevalence of DR3 haplotype in the general population is very low and DR4 is the principal risk factor for AIH type. In an adult Brazilian mixed population with different percentages of whites, blacks, and Amerindians, a weak association with ***DRB1*1301*** (DR6) was found, while in Mexicans of mixed ancestry, the primary HLA association is with ***DRB1*0404*** as a part of the DR4 serologic subset.

Data concerning HLA typing in children with AIH-1 are scant. European children show the typical pattern for AIH-1 in Caucasoid patients, with a significant prevalence of DR3 (***DRB1*0301***) and DR52a (***DRB3*0101***) and with a low prevalence of DR4. In a large Argentinean series, DR6 (***DRB1*1301***) was the primary susceptibility allele, with a secondary association with ***DRB1*0301***, while HLA ***DRB1*1302***, which differs by only one amino acid, showed a weak protective role. A study concerning a mixed Brazilian population with a prevalence of patients older than 16 years reported comparable results: ***DRB1*1301*** was observed more frequently in children than in adults. A secondary association with ***DRB1*0301*** was recorded in all age groups, but ***DRB1*1301*** patients were significantly younger than the ***DRB*0301*** counterpart. More recently, in an array of 50 families from France and Quebec, once more, the ***DRB1*1301*** allele resulted as the primary genetic risk factor for AIH-1 in children.

Genetic background of AIH-2 has been poorly investigated to date because of the low prevalence of this form in adults. Two reports from Europe focused on the *DRB1*03* and *DQB1*02* alleles, whereas another study conducted in a German population reported an increased frequency of *DRB1*07*, *DRB4*01*, and *DQB1*06*. In a population from Brazil, composed for the large part by pediatric patients, a significant increase of *DRB1*07*, *DRB4*, and *DQB1*02* was observed when compared with healthy controls. The last two alleles were in strong linkage disequilibrium with *DRB1*07*. Speculating on this data in comparison with the patterns of susceptibility showed in adult patients, we can argue that *DRB1*1301* is a relevant risk factor peculiar to pediatric age and, interestingly, among adult patients it is associated with younger age.
The DR4 family of alleles seems not to be implicated in children, whereas in adults they have been described as a marker of a mild, late-onset disease. This might explain the peculiar epidemiology of AIH-1 in Japan, where HLA DR3 has a very low prevalence in the general population and pediatric cases of AIH are rare.

### 2.2.3 Autoantigens

The autoimmune response, independently of the trigger, develops against one or more autoantigens (Bogdanos, 2009). Recognition of these autoantigens might be the key factor in developing an etiologic-based therapy. Unfortunately, most of the antigens recognized by autoantibodies detected in AIH are either nonspecific or intracellular molecules and unlikely to be involved in breaking self-tolerance and provoking the emergence of liver-infiltrating immunocytes. The most likely candidate autoantigens seem to be the asialoglycoprotein receptor (ASGP-R) for AIH-1 and CYP2D6 for AIH-2. The ASGP-R is an organ-specific molecule located in the membrane and with a prevalent periportal expression. Both peripheral and infiltrating lymphocytes taken from adults and children with AIH show a proliferative response to purified human ASGP-R and can induce autologous B-lymphocytes to produce anti-ASGP-R autoantibodies *in vitro*. A lack of T suppressing function specific for ASGP-R has been also reported both in patients and in their healthy relatives. This defect seems to reside in a subpopulation of CD4+ T-cells and it is inherited as an autosomal, non HLA-linked trait and it is corrigible by immunosuppressive therapy. Unfortunately, auto-reactivity against ASGP-R is not AIH-specific and its pathogenetic role is far from being defined.
Seven isoforms of cytochrome P450 are highly expressed in human liver: two major isoforms, CYP2C and CYP3A that account for 50% of total CYP expression and five minor isoforms, including CYP2D6. All these isoforms have been identified as LKM targets in different types of liver diseases such as autoimmune, viral, and drug-induced liver diseases. The likely molecular target of AIH-2, CYP2D6, is an intracellular enzyme active in phase I detoxification of several drugs. The CYP2D6 was extensively studied to map the most frequent epitopes in LKM-1. Several short sequences were identified and each of them was labeled, *via* gene bank searches, as cross-reacting with viral proteins, or human proteins involved in other autoimmune disorders. The sequence 193-212 of CYP2D6 is recognized by 93% of the AIH-2 sera and 50% of the LKM-1/HCV-positive sera, and presents extensive cross-reaction with HCV and cytomegalovirus peptides. Furthermore, inhibition studies of the CYP2D6 enzymatic activity showed clearly that conformational epitopes exist and are functionally prevalent. By the effect of some cytokines, CYP2D6 can be expressed on hepatocyte surfaces, becoming a potential target for auto reactive T-cells. Several viruses

had been proposed as triggering factors in the pathogenesis of AIH such as measles virus, Epstein-Barr virus, hepatitis A virus, or herpes simplex virus. Molecular mimicry had been equally invoked between CYP2D6 and the IE 175 protein of herpes simplex virus, but presently none of these viruses is considered as a specific cause of AIH in genetically susceptible individuals.

#### 2.2.4 Autoimmune reaction as a defect of immune regulation

High titers of antibodies against different microbial antigens are present in patients with AIH; this non antigen-specific defect, also present in first-degree relatives, is correctable both *in vivo* and *in vitro* by pharmacologic doses of corticosteroids and is related to a generic impairment of "T-cell suppression". Children with this condition have low level of CD8-expressing T-cells, which segregate with the possession of HLA haplotype B8 DR3. Furthermore, patients with AIH have been reported to have a specific defect in a subpopulation of T lymphocytes controlling the immune response to liver-specific antigens expressed on the hepatocyte membrane. More recently, a CD4+ T-cell subset expressing the interleukin 2 (IL-2) receptor, known as CD25 regulatory T-cells, have been found to be reduced in number at diagnosis of AIH. This CD4+ T-cell subset regulates the proliferation of auto-reactive T-cells through the release of immunoregulatory cytokines such as IL-10.

### 2.3 Clinical features

Autoimmune hepatitis is heterogeneous in nature and it is usually classified in two types according to the pattern of the autoantibody panel detected at the time of diagnosis ( Odièvre, 1983). Type 1 AIH is characterized by the presence of anti-smooth muscle antibody and/or antinuclear antibody and AIH-2 by the presence of anti-liver-kidney microsomal antibody type 1 and/or anti-liver cytosol type 1 antibody. The ratio of incidence of the two types in Europe is 2:1, while it is 7:1 or greater in America and in Japan. In Europe, AIH-2 represents about 20% of new diagnoses of AIH, while in the USA AIH-2 represents only 5%. Differences between the two types consist in the epidemiologic distribution, genetic markers, and clinical presentation (table 1), which might underlie the different pathogenetic mechanism and include (Mieli Vergani & Vergani, 2009):

- AIH-1 affects children and adults, while AIH-2 is almost exclusively a childhood disease;
- Patients with AIH-2 are younger and have a higher tendency to present as an acute liver failure;
- Hypergammaglobulinemia, which is quite typical of AIH-1, is moderate and occasionally absent in AIH-2;
- AIH-2 progresses through "flares" of necrosis usually spontaneously regressing and this can explain why AIH-2 can be characterized by transitory phases of mild histological activity;
- AIH-2 is almost never associated to evidence of bile duct lesions, dissimilar to AIH- 1 where different degrees of bile duct lesion are common;
- Extrahepatic autoimmune disorders are reported in patients with both types of AIH and in first-degree relatives with a higher prevalence of autoimmune thyroid (Grave's and Hashimoto diseases) and skin (vitiligo and alopecia) disorders in AIH-2.

Table 1. Main clinical features of autoimmune hepatitis in children.

| | **AIH type 1** | **AIH type 2** | **Small ducts autoimmun sclerosing cholangitis** |
|---|---|---|---|
| **Age of onset** | infancy and early childhood | infancy and early childhood | all ages |
| **Symptoms at onset** | Symptomatic acute hepatitis | Symptomatic acute hepatitis | Often related to the associated inflammatory bowel disease |
| **Cirrhosis at onset** | not frequent | not frequent | not frequent |
| **Hypergammaglobulinemia** | possible | possible | possible |
| **Biliary lesions** | absent | absent | constant |
| **Autoantibodies** | LKM1, LC1 | LKM1, LC1 | ANA, SMA, p/cANCA, SLA |
| **Extrahepatic disorders except IBD** | Frequent | Frequent | possible |
| **Associated IBD** | Unusual | Unusual | frequent |
| **Response to immunosuppressive treatment** | usually good | usually good | possible |

Despite this heterogeneity, available data suggest a similar outcome and a similar response to treatment.

Beside these two main clinical-serological subtypes, in about 10% of patients AIH may present as a cryptogenic chronic hepatitis with the same demographic, biochemical, histological features and the same response to immunosuppressive therapy of both subtypes of AIH, but in absence of any recognizable autoantibody reactivity pattern. This entity recently described in adults as seronegative autoimmune hepatitis it has been also recognized in children. Recognition and treatment of SAIH are necessary to prevent progression to end-stage liver disease and liver biopsy in such patients play a pivotal role.

Overall, there are three clinical patterns of disease onset:

- The most frequent is indistinguishable from that of an acute viral hepatitis, with malaise, anorexia, nausea, vomiting, and abdominal pain followed by jaundice, dark urine, and pale stools. Some patients, particularly anti-LKM-1 positive, may develop acute liver failure with encephalopathy (Maggiore et al, 1990). Identifying autoimmune hepatitis as the etiology of acute liver failure is potentially important, because administering appropriate immunosuppressive therapy might avoid the need for liver transplantation. Even clinical and histological criteria of autoimmune acute liver failure have not been fully defined; liver histology may be particularly helpful in suggesting the diagnosis.
- About 30% of patients, with a higher frequency for AIH type 1, have an insidious onset with an illness characterized by progressive fatigue, anorexia, weight loss, or a relapsing course with jaundice eventually followed by a spontaneous partial recovery, sometimes even a complete normalization of liver function tests, even if acute exacerbation is usually experienced within a few months (Maggiore et al 1993). Patients may progress with a fluctuating course lasting for several months/years before diagnosis (Maggiore et al 1984). Firm hepatomegaly, splenomegaly and signs of liver function impairment are, however, frequent and cirrhosis or severe fibrosis is often present at diagnosis.
- Between 10-15% of patients may be completely asymptomatic when the underlying disease is accidentally discovered by the finding of hepatomegaly or by an increase of aminotransferase activity.
- Rarely, AIH may present with bleeding from esophageal varices as a complication of portal hypertension, or with symptoms related to an associated extrahepatic autoimmune disorder such as chronic diarrhea, weight loss, and goiter.

Autoimmune extrahepatic disorders are reported in about 30% of patients and include autoimmune thrombocytopenia, autoimmune hemolytic anemia, type 1 insulin-dependent, diabetes, autoimmune thyroiditis, vitiligo, cutaneous vasculitis, uveitis, glomerulonephritis, juvenile chronic arthritis, systemic lupus erythematosus, and Sjögren's disease. Celiac disease, in particular, may be associated with all types of autoimmune liver disorders with a particular high frequency (Caprai et al, 2008).

A form of AIH akin to AIH-2 affect some 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). APECED is an autosomal-recessive disorder caused by mutations in the autoimmune regulator (AIRE1) gene, and is characterized by a variety of autoimmune diseases accompanied by chronic muco-cutaneous candidiasis. These disorders include hypoparathyroidism and autoimmune adrenalitis. The AIRE1 gene is highly expressed in the thymus and the protein encoded by

the gene is involved in clonal deletion of self-reactive T-cells. AIRE1 monoallelic mutations have been reported also in a few children with severe AIH-2 and extrahepatic autoimmune disorders.

### 2.4 Laboratory findings

Besides the presence of specific autoantibodies that characterize the two types of AIH, abnormal laboratory findings exploring evidence of liver injury and/or abnormal liver function are frequent in AIH. Serum aminotransferase activity, which is the most sensitive test of ongoing liver injury, is increased in all untreated patients, sometimes markedly, up to or more than 50-times the upper normal limit. Gamma glutamyltransferase (GGT) activity commonly exploring evidence of bile duct injury, may be normal or slightly elevated. A significant increase of GGT should lead to suspect relevant bile duct damage as in the case of autoimmune cholangitis or overlap syndrome. Total serum gamma globulins are elevated in about 80% of patients, sometimes markedly; the increase concerns particularly immunoglobulin G class. This feature is typical of AIH-1, but may be absent in young patients with AIH-2 presenting with acute onset. Partial or complete serum immunoglobulin class A deficiency and genetically determined low levels of C4 can be found and are more common in AIH-2. Serum albumin may be normal in the early phases of the disease, but it may be reduced in case of cirrhosis with liver insufficiency. Reduction of protrombin activity reflects the severity of liver function impairment together with the level of total bilirubin, in case of acute presentation with severe liver dysfunction.

#### 2.4.1 Autoantibodies

Determination of traditional autoantibodies (ANA, SMA and LKM-1) is very helpful when diagnosing AIH (Krawitt, 2011). Their assessment is performed by indirect immunofluorescence (IF), a diagnostic technique, somewhat out of fashion because it is time-consuming and requires experienced technicians and laboratory physicians (Vergani et al, 2004). However, IF testing on murine tissue sections remains the gold standard for the detection ANA, SMA and LKM-1 reactivity. ANA fluorescence pattern, which in AIH is usually homogeneous, can be further characterized using Hep2 cells which are less specific when used as a screening. Both ANA and SMA are hallmarks of AIH-1, and they are usually present at high (≥ 1:100) titer and their presence is usually mutually exclusive.

Rat stomach is commonly used as substrate to detect SMA: uniform staining of the *muscolaris mucosae* as well as staining of the blood vessels walls (V) and of the parietal cell is characteristic. In rat kidney tissue, a faint staining of the mesangial area of glomeruli (G) and of the brush border of proximal renal tubular cells (T) is also present. The "VG" and "VGT" are the most frequent IF staining pattern detected in AIH. Smooth muscle antigen is directed against structural components of the cytoskeleton such as actin, desmin, and troponin. The SMA reactivity in AIH is mostly directed against filamentous (F) actin, present in the hepatocytes as a part of the cytoskeleton in close proximity to the plasma membrane. Anti-F-actin reactivity, which corresponds to the "VGT" IF pattern, is present in the majority of patients with AIH, especially children, even if its absence does not exclude the diagnosis of AIH (Maggiore et al, 1993). Antinuclear antibodies have various patterns of IF: homogeneous, the most common (60%), speckled (15-25%), and mixed homogeneous/speckled. Several nuclear antigens with a wide range of molecular weight were identified as a target of ANA reactivity. There is no evidence in ANA-positive AIH

patients of an association of a particular nuclear antigen with specific clinical manifestations of AIH or with treatment outcome. The IF ANA pattern is not counted on to have clinical importance and it could vary in the same patient during follow-up. The cut-off titer for the positivity of ANA is commonly indicated to be 1:40 in children. Our experience, however, suggests in clinical practice to raise the cut-off point to 1:100 to avoid over diagnosis due to low specificity of such autoantibody.

Anti-LKM-1 serum reactivity defines AIH-2 (Maggiore et al, 1986). Anti-microsomal antibodies are a heterogeneous group, associated to a number of immune-mediated hepatic diseases such as drug-induced hepatitis, chronic viral hepatitis (HCV and HDV) and APECED. The distinctive IF pattern of LKM-1 on rodent liver and kidney sections is a diffuse cytoplasmic staining of microsome of hepatocytes and of the proximal renal tubular cells (P3 portion). A weak staining of the distal tubules is occasionally present and this can generate confusion with anti-mitochondrial antibody (AMA). Positivity for AMA in childhood patients should therefore be considered with caution since AMA-positive AIH or primary biliary cirrhosis are entities extremely rare in this age group. The LKM-1 target a 50 kDa antigen identified as cytochrome P450 2D6 (CYP2D6) (Gueguen et al, 1988) and inhibits *in vitro* but not *in vivo* CYP2D6 activity. Also, LKM-1 can be present in a small proportion (5-10%) of HCV-related hepatitis, even if the target epitopes are different. The role of LKM-1 antibody in the pathogenesis of liver cell injury is debated. The recent demonstration that CYP2D6 can be expressed on liver cell surface and the finding that immunization in mice with human CYP2D6 can induce liver damage suggests that LKM-1 might play a role in inducing liver cell damage in AIH (Muratori et al, 2000).

Anti-liver cell cytosol antibody 1 is an organ-specific autoantibody and its presence also characterizes AIH-2. It was identified by IF, immunodiffusion, and immunoblotting techniques and characteristically stains the cytoplasm of the rat hepatocytes in a homogeneous pattern, sparing the cellular layer around lobular central veins without staining of the proximal renal tubules (Martini et al, 1988 ). Liver cytosol-1 antibodies recognize a 58-62 kDa liver-specific antigen identified as formiminotransferase cyclodeaminase (Lapierre et al, 1999). Liver cytosol-1 can be present on its own as a sole autoantibody (Bridoux-Henno et al, 2004 ), can be found associated with LKM-1 reactivity in about 50% of AIH-2, and occasionally it has also been described associated with anti-SMA at low titer and in adults with HCV-related chronic hepatitis.

In 20-30% of adult patients lacking ANA, SMA, or LKM-1, the diagnosis of AIH can be suggested by the finding of other, less common autoantibodies such as anti-soluble liver antigen (SLA/LP) or anti-neutrophil cytoplasmic antibody (ANCA). None of these autoantibodies except SLA is specific for AIH since they are found either in several systemic autoimmune diseases, or in liver diseases of infectious etiology such as HCV infection. Anti-soluble liver antigen is a non specie-specific, non organ-specific antibody currently assessed by immuno-enzymatic or radio immunological assays. The target antigen is likely to be a 50 kDa 422-amino acid protein, identical to previous liver pancreas antigen. Soluble liver antigens are present in patients with AIH-1, AIH-2. Antibodies to SLA/LP are of a major diagnostic value for AIH-1, including overlap syndromes but are not found in association with anti-liver/kidney/microsome type 1 or antibodies to liver cytosol type 1. They are rarely present in other liver diseases such as hepatitis C and drug-induced hepatitis.

Antineutrophil cytoplasmic antibodies have a heterogeneous pattern of target antigens and are commonly distinguished as cytoplasmic (cANCA), perinuclear (pANCA) and atypical perinuclear (apANCA). They have been described in AIH-1 and in ASC patients with all types of pattern, while they are virtually absent in AIH-2.

Once diagnosis is made, autoantibody reactivity fluctuates during treatment, reducing in titer in case of remission, but also independently. Autoantibody status is not predictive of laboratory and histological features; moreover, high serum titers at presentation do not identify patients with more aggressive disease or different treatment outcomes. Finally, the disappearance of autoantibodies is not predictive of low risk of relapse during treatment or of sustained remission in case of stopping the treatment.

### 2.5 Histology

The histological hallmark of AIH is "interface hepatitis", defined as a dense, inflammatory infiltrate of mononuclear cells, lymphocytes (mainly T helper and natural killer), plasma cells and activated macrophages infiltrating the portal tract, invading the adjacent parenchyma and surrounding apoptotic hepatocytes with erosion of the limiting plate (Figure 1). Interface hepatitis may be present in other inflammatory liver diseases of different etiologies (viral, drug-induced, etc.) and this peculiar histological picture is to be properly integrated in clinical and biochemical setting.

Plasmacells are considered another significant histopathological hallmark of autoimmune disease; they may be diffusely evident in interface and lobular hepatitis areas, but in about one third of the cases plasmacells are patchy localized within liver parenchyma.

A considerable amount of eosinophilic granulocytes is often present within the portal infiltrate, especially in patients with co-existent celiac disease (Caprai et al, 2008). Most patients with autoimmune hepatitis have parenchymal complications of chronic inflammation such as severe fibrosis and about a third of them are already cirrhotic at presentation. In children, this proportion seems to be even higher. Only patients with very acute presentation may lack features of chronic hepatitis and fibrosis. Cirrhosis in autoimmune hepatitis is often macronodular, and may easily be overlooked by percutaneous liver biopsy. Anyway a confident histological diagnosis of cirrhosis should be proposed only in case of well-defined nodular fibrotic transformation, mainly in cases with severe features of activity, as massive necrosis with parenchymal collapse may lead to a nodular appearance of the liver. Laparoscopy, a minimally invasive technique with the currently available very small diameter endoscopes, is helpful in making a diagnosis of cirrhosis in autoimmune hepatitis, as cirrhosis maybe overlooked in up to 50% of cases without macroscopic assessment of the liver. Diagnosis of cirrhosis may influence the choice and dose of the immunosuppressive agents prescribed, has prognostic implications, and forms the basis for regular screening for complications of cirrhosis, such as esophageal varices bleeding.

Liver histology plays a major role in two particularly challenging diagnostic conditions: the AIH with very acute onset and the seronegative AIH. In case of very acute onset, the inflammatory liver injury, in contrast to classical autoimmune hepatitis, predominates in the centrilobular zone and it is often associated with reticular collapse. In such cases four features may suggest an autoimmune pathogenesis: the presence of massive/submassive hepatic necrosis (Figure 2), the presence of lymphoid follicles within portal tracts (Figure 3), a plasma cell-enriched inflammatory infiltrate and central perivenulitis. Ductular (intermediate) hepatocytes are considered a reactive/regenerative response to injury and more commonly present in case of massive/submassive necrosis serving as hepatic progenitor cells, and could be seen as cytokeratin 7 (CK7)-positive hepatocytes in immunohistochemistry. In case of seronegative autoimmune hepatitis the liver histology

represent an invaluable diagnostic tool showing features suggesting an autoimmune pathogenesis. Centrilobular changes are prominent due to the high frequency of acute icteric onset.

Bile duct inflammatory changes are not considered a typical lesion of autoimmune hepatitis. They can be found in limited proportion (about 25% of patients with AIH-1) in form of destructive or not destructive cholangitis and ductopenia. Patients with and without bile duct changes had similar laboratory findings but diagnostic scores for AIH are lower in case of bile duct damage. Patients with destructive cholangitis and/or ductopenia respond as well to therapy as patients with nondestructive cholangitis, and outcome is similar to those of patients without biliary changes.

### 2.6 Diagnosis

The diagnosis of AIH in children can be easy when all the typical hallmarks of the disease are present, such as the presence of another autoimmune disease in the same patient, hypergammaglobulinemia of IgG type with the demonstration of specific autoantibodies and a suggestive histology. However, in some patients the diagnosis be challenging, and in this case it is usually made by a combination of clinical, serologic, and histologic criteria and by the exclusion of other possible known causes of severe hepatic disease, such as chronic hepatotropic virus infections and Wilson's disease, by appropriate tests.

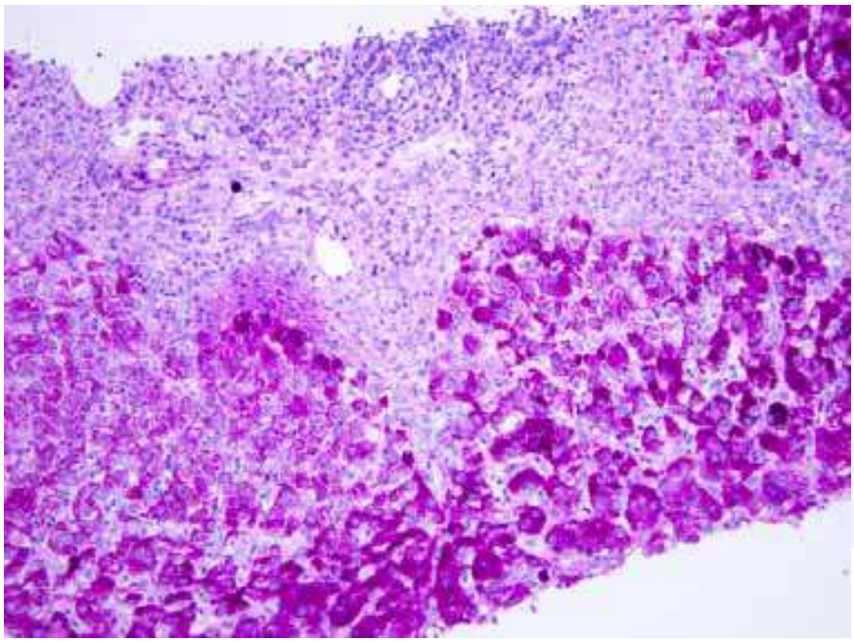

Fig. 1. Presence of dense portal and periportal inflammatory infiltrate with lymphocytes, histiocytes, neutrophilic and eosinophilic granulocytes and plasma cells with interface activity.

Even if histopathology features pathognomonic of autoimmune hepatitis are lacking, liver histology should be always performed at diagnosis if hemostasis allows it. The characteristic lesion of "interface hepatitis", characterized by a predominantly lymphoplasmacytic, necroinflammatory, and periportal infiltrate with or without lobular involvement should

suggest the diagnosis. Other suggestive features include the presence of portal-portal or central-portal bridging necrosis, formation of liver cell rosettes and the presence of nodular regeneration, even in the early stages, in the most severe cases. Storage of metals like iron, copper, or intracellular proteins have to be excluded by appropriate histochemical techniques.

Features of biliary damage are usually absent or limited. In case of absence of typical serum autoantibodies, it is mandatory to send a serum sample to a reference laboratory to investigate the presence of autoantibodies not routinely assessed such as ANCA or LC-1. If doubt persists, in case of severe cryptogenetic inflammatory disease, once Wilson's disease is excluded, it is advisable to attempt an immunosuppressive treatment for at least six weeks to evaluate the sensitivity of the disease to immunosuppressive therapy.

To help the diagnosis of autoimmune hepatitis, a panel of physicians and pathologists has published a descriptive set of criteria to classify patients as having either "definite" or "probable" autoimmune hepatitis. This scoring system has been used in a large number of studies and it has shown a good sensibility (89.9%) but a low specificity (60.8%), particularly in cases of immune-mediated biliary diseases like autoimmune cholangitis or primary biliary cirrhosis, which often score as a "probable" autoimmune hepatitis. In 1999 the score was reviewed; however, this scoring system still remains more adapted to adulthood than to childhood since some items, for example, concerning the use of the alkaline phosphatase/aminotransferase ratio due to the low discriminating role of this enzyme in childhood in exploring bile duct damage or alcohol consumption.

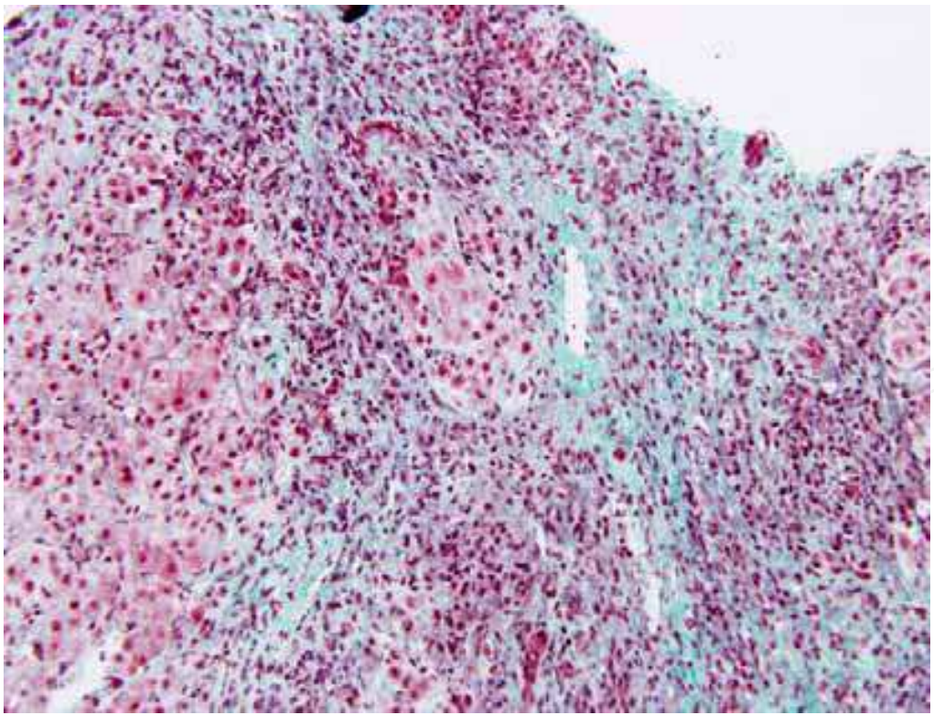

Fig. 2. Submassive necrosis with panlobular and portal inflammatory infiltrate with CD3 and CD20 positive lymphocyte plasmacells and polymorphonuclear eosinophils and neutrophils in a girl with AIH-1 with marked hypergammaglobulinemia and acute onset.

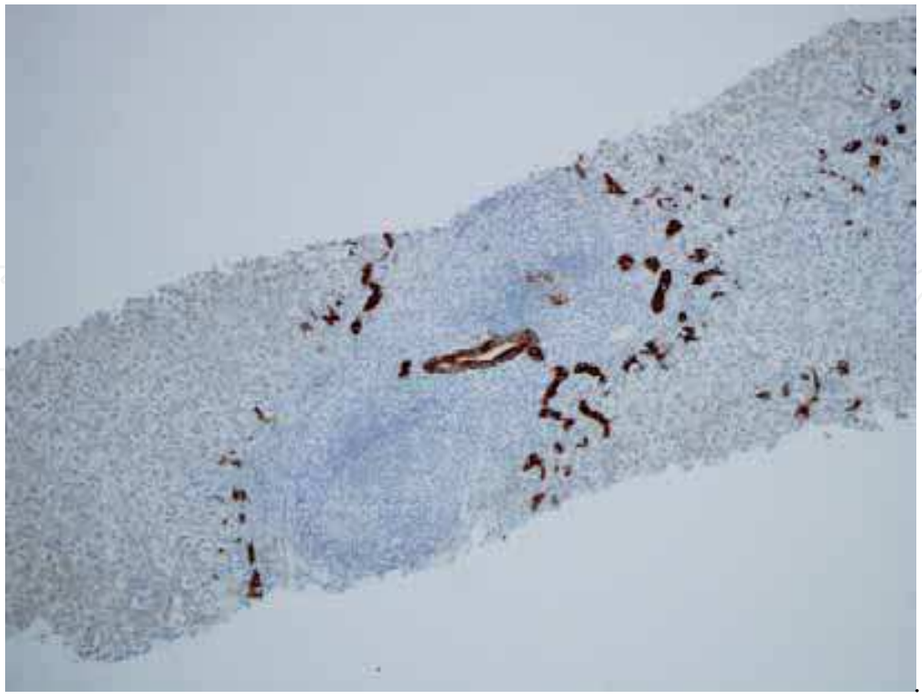

Fig. 3. Lymphocytic infiltrate shaped as lymphoid follicles surrounding two biliary structures with features of destructive cholangitis and marked ductular proliferation (CK7 immunostaining).

### 2.7 Management

Currently, the most effective therapy for AIH is immunosuppression. The degree of response to treatment depends on the severity of the disease at presentation. Standard treatment includes prednisolone as monotherapy or a combination of prednisone and azathioprine. Prednisone or prednisolone is used at a dose of 2 mg/kg/day (maximum daily dose of 60 mg in adolescents), and azathioprine is prescribed starting from 1 mg/kg/day up to a maximum 2.5 mg/kg/day. Combination therapy from the start of treatment is generally preferred because of the "steroid-sparring" effect of azathioprine that allows reducing the steroid dose more rapidly, avoiding the severe side effects related to their prolonged use at high doses (Maggiore et al, 1984).

#### 2.7.1 Efficacy of the treatment

The first goal of the treatment in AIH is to induce a complete remission of the clinical signs (jaundice, hepatomegaly, and/or splenomegaly) and of the biochemical "activity" of the disease (aminotransferases, gamma globulins). Treatment generally is associated with a measurable clinical and laboratory response within 6-10 weeks that is usually followed by restoration of liver function, when impaired at onset, as demonstrated by the normalization of protrombin activity (Maggiore et al, 1984). Once the response to treatment is established, prednisone doses are gradually decreased, being aware, however, that complete normalization of biochemical parameters takes a longer period of from 6-9 months. Different therapeutic schedules of treatment discontinuation exist; however, discontinuation of therapy should be tailored for individual patients in relation to their characteristics. The

shift to alternate-day use of corticosteroids is very suitable as soon as possible because it is associated with a lower incidence of side effects, particularly concerning growth, without increasing the risk of relapse (Maggiore et al, 1984). About 10% of cases, with severe liver function impairment at onset, however, deteriorate despite compliance to therapy. In these cases a "rescue" immunosuppressive therapy, with 2 mg/kg/day of steroids or 1 mg/kg day plus cyclosporine to a target blood level of 200 ± 50 ng/mL has been proven successful in 90% of patients in a mean period of 3 weeks (Cuarterolo et al, 2011). Eventually, the adjunction of cyclosporin as a third immunosuppressant agent or the substitution of azathioprine with mycophenolate mofetil should be assayed (Schramm C & Lohse AW, 2011). Treatment failure should lead to promptly discuss of the opportunity of an early liver transplantation.

### 2.7.2 Sustained response

Once complete remission is achieved, the goal of treatment becomes to maintain remission and to prevent relapses. The dose of prednisone is to be reduced further to the lowest dose compatible with a clinical and biochemical remission (strictly normal aminotransferases and gamma globulins levels). Small doses of prednisone on alternate-day schedule combined with azathioprine are in fact effective in maintaining remission. Once remission is achieved, a relapse can occur and in most cases it is related to inappropriate patient compliance to the prescribed treatment. To demonstrate histologic remission by a liver biopsy in a patient with long-standing, complete biochemical remission is a question of debate since histologic remission is not absolutely predictive of no risk of relapse. Hepatic fibrosis progresses only in a minority of patients who are compliant to treatment and who maintain a persistent remission. In some cases, fibrosis can even diminish during treatment.

### 2.7.3 Duration of treatment

No evidence-based data exist on the optimal duration of immunosuppressive treatment. Relapse is frequent if treatment is withdrawn within the first two years. Current experience suggests that sustained remission should be maintained for at least five years, then, in the case of treatment combining prednisone and azathioprine, prednisone should be stopped during the sixth year, maintaining a sustained remission on azathioprine monotherapy for at least another year. Azathioprine monotherapy, as in adults, reduces the likelihood of relapse and maintains sustained remission in most patients with AIH, independent of its serological type. Absence of serum autoantibodies is not predictive of an absence of relapse; however, a sharp increase of the titer of autoantibodies prompts caution in reducing the immunosuppression.

### 2.7.4 Side effects of treatment

Combination therapy is associated with side effects, mostly caused by prednisone that produces increased appetite, moderate weight increase, sometimes marked and a transitory reduction of height growth. Severe side effects are less frequent in specialized centers, but frequent in less experienced centers and include obesity, growth failure, severe cosmetic changes, cutaneous *striæ*, vertebral collapse, hyperglycemia, cataracts responsible for visual impairment, and psychosis. Azathioprine is rarely responsible for severe side effects such as cytopenia necessitating a dosage reduction. Teratogenicity and oncogenicity issues of azathioprine in humans have not been conclusively demonstrated. However, pregnancy

should be ruled out in adolescent girls before starting treatment since azathioprine therapy during pregnancy cannot be recommended.

#### 2.7.5 Alternative treatments

Failure to respond to conventional treatment or severe side effects of corticosteroids are clear indications for the use of cyclosporine A. Cyclosporine therapy is effective in inducing remission in patients with AIH at a median dose of 5 mg/kg/day to obtain serum cyclosporine initial concentration in the range of 200-250 ng/ml even if lower levels are often equally effective. Side effects of cyclosporine treatment are few, well tolerated, and disappear after reduction of the dose (Sciveres et al 2004). Once remission is achieved, treatment may be continued at lower doses or patients may be shifted to conventional treatment. Mycophenolate mofetil (MMF, 20 mg/kg twice a day) has been successfully employed in addition to steroids in patients who either did not tolerate azathioprine or did not respond to standard therapy, suggesting that it may represent another alternative strategy of treatment. Side effects of MMF include headache, diarrhea, dizziness, hair loss, and neutropenia.

Liver transplantation is the treatment option of choice in end-stage AIH or in patients with acute severe/fulminant onset who do not respond to rescue immunosuppression. The 5-year posttransplant survival for these AIH-patients is 86% and patient and graft survival, infectious and metabolic complications, and retransplantation rates did not differ between AIH and non-AIH patients (Martin et al 2011). The higher risk for late acute rejection and the greater degree of immunosuppression needed does not compromise outcomes of liver transplantation for AIH.

#### 2.7.6 Long-term prognosis

The long-term prognosis of children with AIH remains uncertain. A sustained remission can be maintained in most patients, without notable side effects, with low-dose immunosuppression. A limited number of patients maintain a sustained remission once the treatment is stopped. Some patients with cirrhosis, in the absence of an evident relapse of the disease, may develop progressive liver insufficiency and need liver transplantation.

## 3. Autoimmune bile duct disorders

Autoimmune bile duct damage includes primary biliary cirrhosis in adults, autoimmune cholangitis and autoimmune overlap syndrome in adults and children. Since this chapter is devoted to autoimmune disorders in childhood PBC will not be examined.

### 3.1 Autoimmune cholangitis (AIC)

Autoimmune cholangitis is a chronic, immune-mediated, cholestatic disease of uncertain etiology, characterized by progressive inflammatory damage of intra and extrahepatic bile ducts resulting in obliterative fibrosis and destruction of small bile ducts and fibrosclerotic lesions of large bile ducts. Autoimmune cholangitis (AIC) may be distinguished in two main entities: the more traditional form, the so-called Primary Sclerosing Cholangitis (PSC), a chronic and diffuse inflammatory cholangiopathy potentially involving all orders of intra and extrahepatic bile ducts, characterized by radiographically visible biliary stricture and dilations of large and medium size bile ducts and a variant form with a damage at least initially limited to small bile ducts, the so- called small duct sclerosing cholangitis, (SDSC).

In this form, biliary imaging explored both endoscopic cholangiography or magnetic resonance is normal or present only minor abnormalities. PSC is more common and typical of adulthood, usually progresses to cirrhosis and liver failure and carries an increased risk of cholangiocarcinoma. SDSC is more typical of childhood, and diagnosis may be suspected only on clinical, biochemical, and histologic features showing evidence of biliary inflammatory injury.

### 3.1.2 Autoimmune (primary) sclerosing cholangitis (PSC)

Autoimmune sclerosing cholangitis is also defined as primary sclerosing cholangitis (PSC) to be distinguished by secondary forms where a wide range of insults may produce similar cholangiographic imaging pattern. Secondary sclerosing cholangitis (SSC) is thought to develop as a consequence of known injuries or secondary to pathological processes of the biliary tree. The most frequently described causes of SSC are longstanding biliary obstruction, surgical or blunt abdominal trauma to the bile duct, ischemic injury to the biliary tree, intra-arterial chemotherapy, portal biliopathy, eosinophilic and/or mast cell cholangitis, recurrent pyogenic cholangitis, primary immune deficiency, and AIDS-related cholangiopathy. PSC can usually be differentiated on the basis of patient's history and the strong association of PSC with inflammatory bowel disease (IBD).

PSC is a rare disease, the true incidence and prevalence of PSC in children is grossly underestimated: recent studies suggest a mean annual incidence rate of 0.23/100,000 compared to 0.77 cases per 100,000 person-year in adults. The incidence of PSC is similar in North American and European countries, but it continues to increase over time, this disease being diagnosed more frequently also in children and adolescents because of increased awareness of this condition and of the widespread use of noninvasive biliary imaging.

#### 3.1.2.1 Pathogenesis

The etiology and pathogenesis of PSC remain very poorly understood. As the disease is associated with autoantibodies and peculiar HLA haplotypes as well as being closely related to IBD, it would appear to be immune mediated. An autoimmune mediated destructive process is also suggested by lymphocytic infiltration into areas of portal damage. PSC is not however a classical autoimmune disease, as it occurs with a slight male predominance compared with the typical female predominance found in classical autoimmune liver diseases such as PBC and AIH. Moreover, PSC does not have the characteristic response to immunosuppressive treatment as seen in classical autoimmune diseases. Circumstantial evidence that PSC may be immune mediated comes from the independent association of PSC with a number of autoimmune diseases. Simultaneous or sequential occurrence of PSC and AIH has been described in both adult and pediatric populations. In general, PSC in children is characterized by more pronounced autoimmune features with a possible overlap with AIH. Serum autoantibodies and in particular, anti-neutrophil cytoplasmic antibodies (ANCA) are largely present in the serum of patients with PSC. They are however not specific for PSC and are also found in AIH. These ANCA may have atypical features distinct from perinuclear-staining anti-neutrophil cytoplasmic antibody (p-ANCA) and by cytoplasmic-staining anti-neutrophil cytoplasmic antibody (c-ANCA). The target antigen for these atypical ANCA is probably a neutrophil nuclear envelope protein. Some authors have suggested that the term p-ANNA is therefore more appropriate as the recognized antigen is not cytoplasmic but originating in the nuclear membrane. The importance of these autoantibodies in the

development of PSC is unknown, but current evidence suggests that they are unlikely to play a role in the pathogenesis of PSC. A high proportion of non-specific autoantibodies in addition to p-ANNA are found in patients with PSC, they are of unclear relevance and unhelpful in diagnosis. They include anti-nuclear antibodies anti-smooth muscle antibody, and anti-cardiolipin antibodies without however a demonstrated association with thrombotic disease.

Association studies have identified various HLA molecules and other immunoregulatory genes as determinants of disease susceptibility and progression in PSC. There is an increased frequency of *HLA B8* and *DR3* (*HLA DRB1*0301*) in PSC. *HLA B8* and *DR3* are in linkage disequilibrium and this haplotype is also associated with several organ specific autoimmune diseases including AIH-1, diabetes mellitus, myasthenia gravis and thyrotoxicosis.

There is a T cell predominant portal infiltrate in PSC although the relative proportions and importance of the CD4 and CD8 cells are not known. CD4 cells are seen more commonly in the portal tracts and CD8 cells predominate in areas of interface hepatitis, when present. The cell infiltrate may change as the disease progresses. These cells are functional and are likely to be involved in the pathogenesis of disease.

PSC is strongly linked to IBD but it also runs a course independent from the bowel disease since the disease can develop many years after colectomy. It has been suggested that T lymphocytes generated in the gut during active inflammation persist as long-lived memory cells and undergo enterohepatic circulation and can then trigger an inflammatory response in the liver when activated by an appropriate stimulus. The nature of the stimulus remains unclear; possibilities include hepatic expression of the original priming antigen or possibly mediation solely by the aberrant expression of gut specific adhesion molecules and chemokines. Bacterial antigens may act as molecular mimics in genetically susceptible individuals and cause an immune reaction responsible for initiating PSC. The bacteria are able to get through gut walls made permeable by colonic inflammation; chemokines and cytokines are then released from Kupffer cells in the liver attracting macrophages, monocytes, lymphocytes, activated neutrophils and fibroblasts to the portal tracts.

Finally, defects in the hepatobiliary transport system and in particular, in knockout mice for the *Mdr2* (*Abcb4*) gene, which corresponds to human *MDR3/ABCB4* gene defects, spontaneously develop sclerosing cholangitis with features similar to human PSC. A non-functional ABCB4 protein leads to the formation of a "toxic" bile with increased concentration of free, non-micellar bile acids which cause cholangiocyte injury, pericholangitis, periductal fibrosis and, eventually, sclerosing cholangitis. Studies in PSC patients, however, did not find ABCB4 mutations.

In summary, immune mechanisms play an important role in the pathogenesis of PSC, although it remains unclear whether it is a classical autoimmune disease. There are strong major histocompatibility complex genetic associations including HLA molecules. HLA haplotypes however do not account for all the genetic susceptibility in the development of PSC and there is uncertainty about the importance of genes outside this region. Bacterial antigens may act as molecular mimics in hosts who are genetically susceptible and therefore cause an immune reaction leading to PSC initiation. Lymphocytes may move from the inflamed gut in IBD *via* the enterohepatic circulation and cause inflammation of the liver when activated by a specific stimulus such as bacterially derived antigens.

#### 3.1.2.2 Clinical features

The mean age at diagnosis of PSC in the pediatric population is approximately 13 years with a slight (2:1) predominance in males, but there are an increased number of earlier diagnoses in the first decade of life (Feldstein , 2003). PSC is strictly associated with an inflammatory bowel disease (IBD) reaching more than 80% of cases, while, only 2 -7.5% of children or adolescents affected by IBD develop sclerosing cholangitis (Miloh 2009). This form of IBD predominantly has the features of an ulcerative colitis and in a minority of a Crohn's colitis. A distinctive IBD phenotype, consisting of a prevalence of diffuse colonic involvement (pancolitis), with however a less active disease and even asymptomatic in about 10% of cases; with eventual rectal sparing and backwash ileitis, but with an increased risk in the long term of colonic adenocarcinoma has been suggested (Loftus, 2005). The diagnosis of IBD may precede or closely follow the onset of PSC. A prior history of abdominal pain, diarrhea, and eventually blood in stools is frequently reported by patients or their parents. Two thirds of children who have PSC are symptomatic at the time of diagnosis and the most frequent initial symptoms are related to the IBD or to a generalized inflammatory process. The abdominal pain is fluctuant, whereas fatigue is progressive and usually followed by anorexia and weight loss. Signs or symptoms directly related to the liver disease are rare, particularly in younger patients, and include hepatomegaly, splenomegaly, jaundice, and pruritus. Frequently the disease is discovered because of an occasional finding of increased aminotransferase level without any sign or symptom. The association with other extrahepatic autoimmune disorders, such as autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, Sjögren's syndrome, and celiac disease, is less frequent than in AIH, but is reported in up to 25% of cases.

#### 3.1.2.3 Laboratory findings

Although there are no specific laboratory features specific of PSC, elevated serum GGT activity is almost always present in these children and reflects the presence of ongoing bile duct injury (Feldstein, 2003). Serum aminotransferase activity is generally elevated in most patients, but the increase of these enzymes is not specific. Serum alkaline phosphatase levels are not as helpful in childhood because of the wide range of normal values for this enzyme during pediatric age, resulting from the increase of bone formation related to growth. Total bilirubin concentration is usually within a normal range in most patients, and signs of hepatic failure are unusual at diagnosis. An increased direct bilirubin level usually characterizes more severe, long-lasting forms with evident macroscopic fibrosclerotic lesions of bile ducts and cirrhosis. On the other hand, jaundice may be transient, secondary to bacterial cholangitis, which can be recurrent and could even finally represent an indication for liver transplantation. Thrombocytopenia and diminished white blood cell count secondary to portal hypertension due to hypersplenism can found in children with splenomegaly. Elevated IgG levels are found in 70% of children with PSC, and circulating autoantibodies that usually characterize AIH-1, such as ANA and/or SMA, are detected in serum of more than 80% of patients. Antineutrophil cytoplasmic antibody, with staining mainly but not exclusively in the periphery of the nucleus, is usually found by indirect IF. Anti-LKM-1 is practically never detected in these patients.

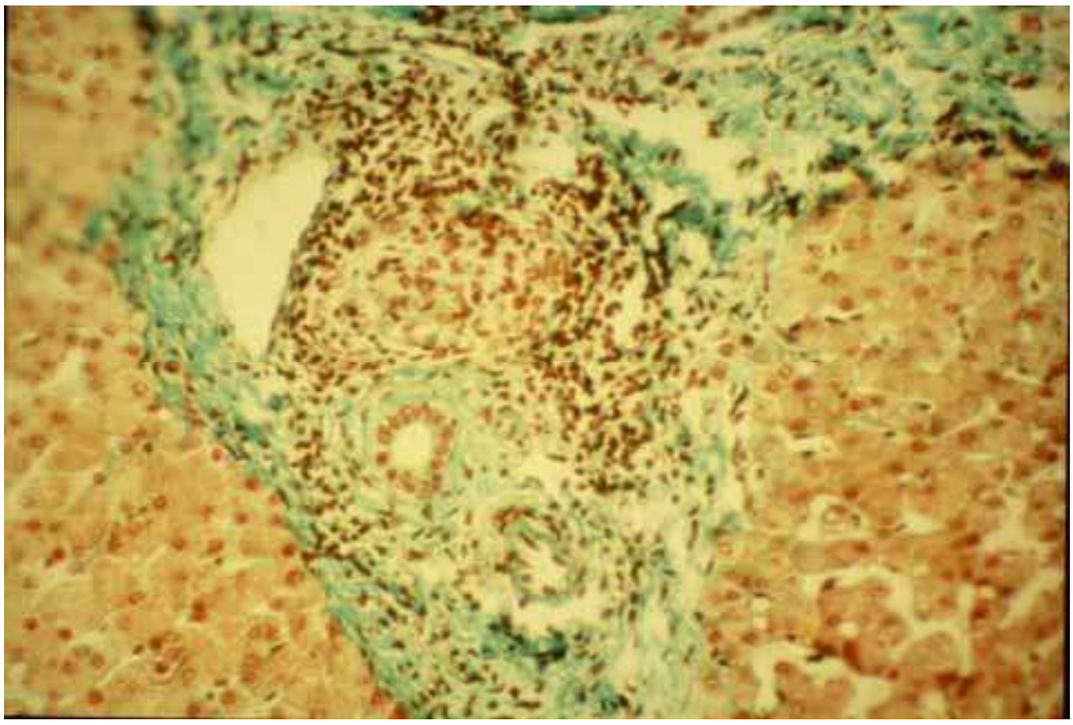

Fig. 4. Autoimmune cholangitis. An intrahepatic bile duct surrounded by an inflammatory infiltrate and ductal and periductal fibrosis.

#### 3.1.2.4 Histology

In patients with PSC, percutaneous liver biopsy documents the presence of an inflammatory and/or fibrotic cholangiopathy (Figure 4) with progressive damage, atrophy and ultimately loss of small size bile ducts (ductopenia) (Figure 5). Edema and fibrosis around the interlobular bile ducts progressing to concentric periductal fibrosis ('onion-skinning') ( Figure 6), narrowing , obliteration of the small bile ducts, leaving a bile duct scar may be considered the main histologic feature of PSC, although may be missed in the liver sample as it is a focal histological feature. However, often only lesions of acute cholangitis are present, such as infiltration and destruction of biliary epithelium (Figure 6 B) with the finding of lymphoid folliculi or even granuloma, usually encompassing a bile duct. Inflammatory infiltrate around the bile duct is polymorphous mainly with lymphocytes, histiocytes; however a significant number of eosinophils can be present around bile duct and in the colonic cryptae in case of associated IBD (figure 7).
Bridging fibrosis is frequent at diagnosis while typical lesions are present only in a limited number of patients with advanced fibrosis and more frequently findings are those of an aspecific cholangiopathy with ductular proliferation and loss of very small bile ducts. Septal fibrosis or cirrhosis is found in the initial liver biopsy of more than half of newly diagnosed children with PSC. An inflammatory infiltrate ranging from a mixture of lymphocytes and polymorphonuclear cells to a lymphoplasmacytic infiltrate with the features of interface hepatitis can be observed in about a third of patients. These features characterize the overlap syndrome. In advance disease, liver parenchyma show features of long standing cholestasis, i.e positive immunostaining to biliary-type citocheratin 7 and feathery degeneration of liver cells cytoplasm.

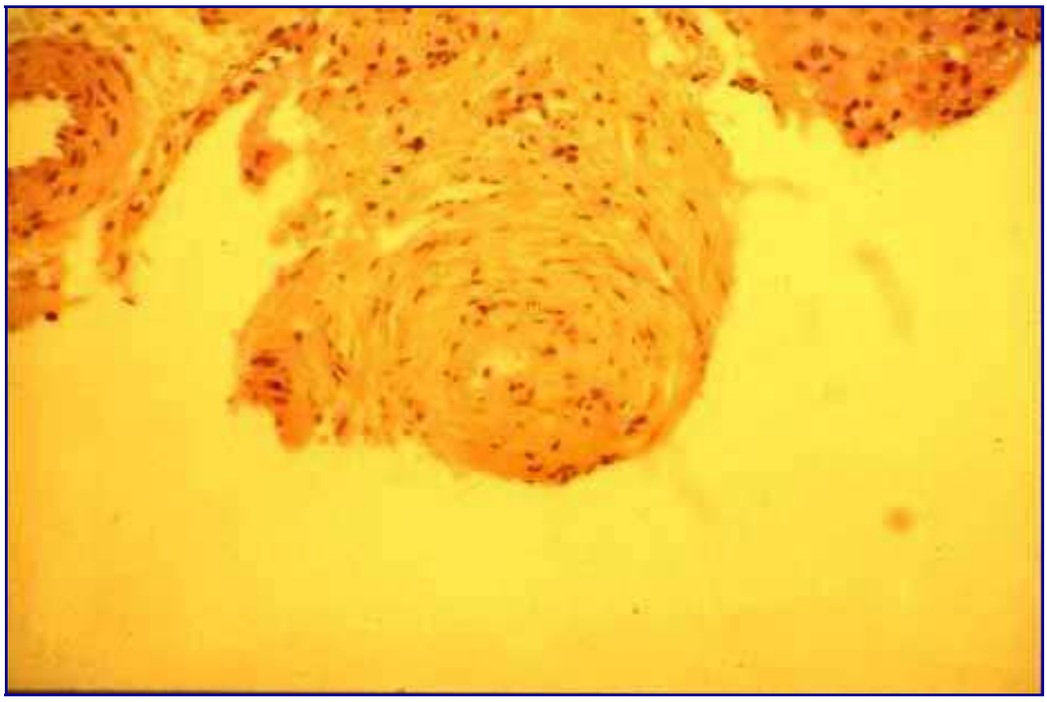

Fig. 5. Autoimmune cholangitis. A concentric fibrotic scar obliterating an intrahepatic bile duct.

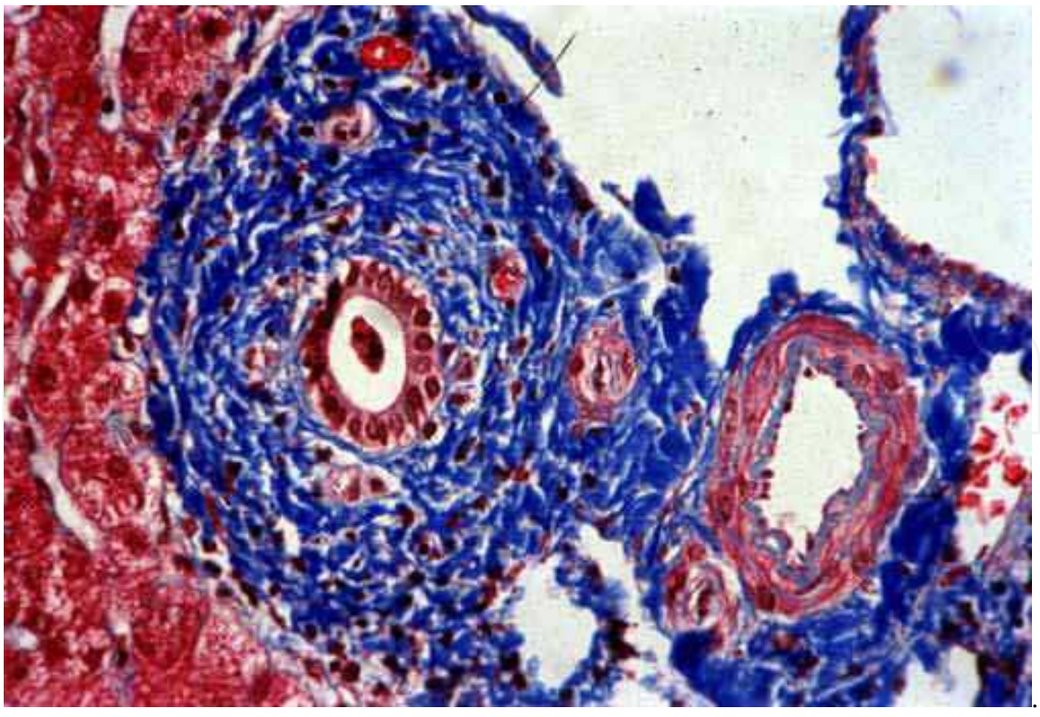

Fig. 6. Autoimmune cholangitis. An intrahepatic bile duct surrounded by concentric periductal fibrosis ('onion-skinning').

#### 3.1.2.5 Biliary imaging

Visualization of intra and extrahepatic bile ducts by direct opacification of the biliary tree or by magnetic resonance (MR) cholangiopancreatography is an essential part of the diagnostic workup. Until recently, endoscopic retrograde cholangiography or transhepatic, transcholecystic, percutaneous cholangiography were used to visualize the biliary tree.
Currently, MRI cholangiopancreatography provides an excellent quality of resolution for imaging intrahepatic and extrahepatic bile ducts in older children. Abnormal features observed include duct wall irregularities, strictures, irregular dilations, and beading resulting in the characteristic "bead-on-a-string" appearance (Figures 7 and 8). Lesions restricted to the intrahepatic branches are found in 30-40% of patients, whereas abnormalities limited exclusively to the extrahepatic ducts are rare. Cholelithiasis may be present, with the majority of patients being asymptomatic.

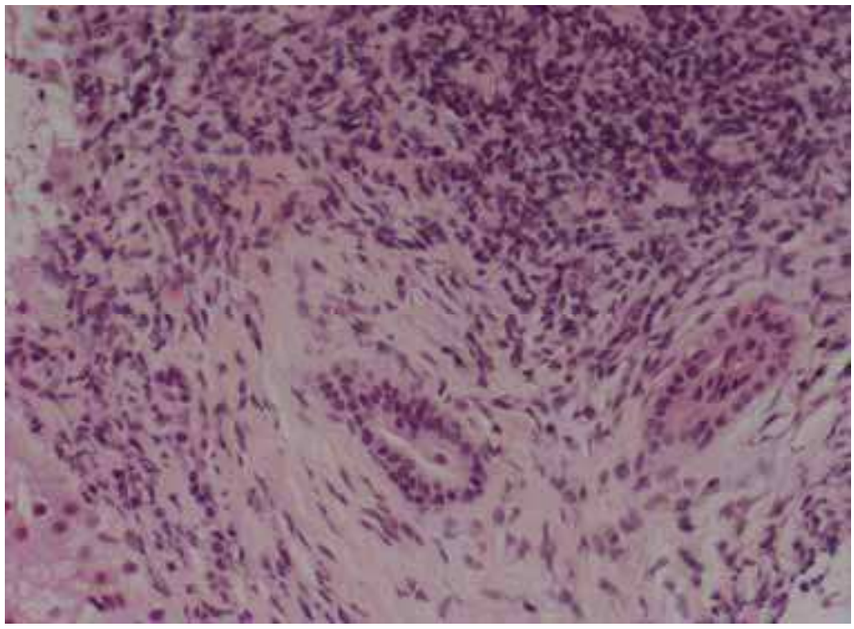

Fig. 7. Autoimmune cholangitis. Inflammatory portal fibrosis with destructive ductular lesions and biliary proliferation without ductopenia

#### 3.1.2.6 Diagnosis

Suspicion of PSC should arise when a patient presents with signs or symptoms of chronic liver disease, such as hepatomegaly, splenomegaly, stellar angiomas, palmar erythema, fatigue (more frequent in adolescents), anorexia, pruritus, or jaundice. The most common laboratory finding is an increase of the serum GGT activity. The possibility of PSC should be considered in any patient who has IBD and an increased liver enzyme serum activity, particularly if laboratory features suggesting cholestasis or symptoms or signs of chronic liver disease are present. Definitive diagnosis requires a liver biopsy and a biliary imaging. The diagnosis may be challenging in patients asymptomatic who do not have autoantibodies and hypergammaglobulinemia. In these patients, liver histology and the presence of an associated IBD may suggest the diagnosis. The differential diagnosis of PSC should include other causes of liver injury reported in patients who have IBD, including those such as drug-induced liver damage.

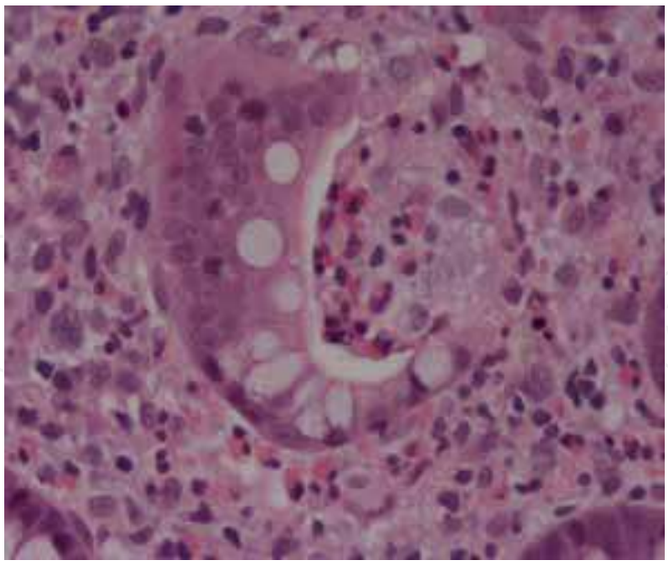

Fig. 8. Inflammatory bowel disease associated with Autoimmune cholangitis. Eosinophilic cryptitis

In children who have ulcerative colitis and who have abnormal liver laboratory tests, a liver biopsy and a biliary imaging are usually indicated. Moreover, distinction between PSC and AIH-1 is not always easy. Traditionally, AIH and PSC are regarded as separate disease entities, but recently it was hypothesized that both diseases are part of the same process.

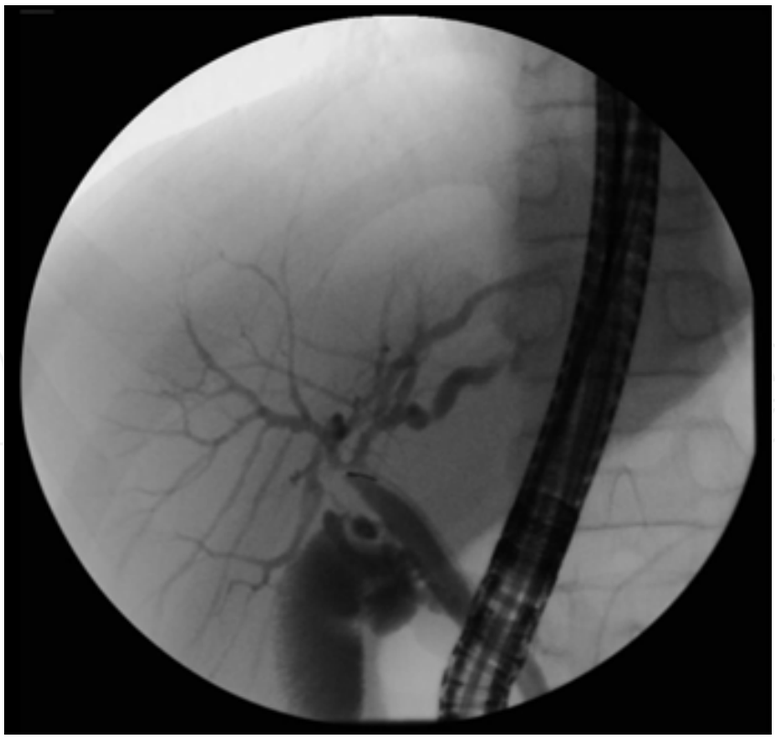

Fig. 9. Endoscopic cholangiopancreatography showing irregular intrahepatic bile ducts with stenosis and dilation prevalent in left liver sections.

This condition corresponding to the overlap syndrome, which would encompass both PSC and AIH, may respond satisfactorily to immunosuppressive therapy. In fact, approximately 50% of children with features of autoimmune liver disease present cholangiographic features typical of sclerosing cholangitis, though generally less advanced than those observed in adult patients with sclerosing cholangitis. All these patients present with the clinical features of typical AIH, many do not have typical histologic evidence of fibro-obliterative bile duct damage on liver biopsy, and virtually all have the autoantibody pattern of AIH-1

#### 3.1.2.7 Management and prognosis

Children with PSC have a significantly shorter survival than the expected for age-matched general population. Many children with PSC progress to end-stage liver disease with the consequent need for liver transplantation. Currently, no specific therapy is clearly standardized for PSC. Patients with PSC seem to have a more severe prognosis than those who have bile duct lesion limited to fourth order bile ducts (small duct sclerosing cholangitis, SDSC). Lesions of the large bile ducts and the consequent obstruction of bile flow contribute to the rapid progression of liver injury.

Treatment with ursodeoxycholic acid leads to improvement of biochemical tests in most patients, but does not seem to affect survival in children or adults. One third of newly diagnosed patients have biochemical signs of inflammation as well as interface hepatitis at liver biopsy. Immunosuppressive therapy is not effective in adults with PSC, even if there is increasing evidences of some benefit in adults and children with SDSC. However, in these cases corticosteroids and azathioprine are frequently administered. No impact on long term outcome has been reported with these drugs in small pediatric series or in larger adult series. This lack of improvement in outcome occurs despite a good control of the inflammatory process. Stabilization or even regression of the cholangiographic lesions has been however observed, however, in half of patients treated after a median follow-up of four years. Immunosuppressant drugs are indicated in the frequent occurrence of overlap syndrome and it should be considered that anti-inflammatory and immunosuppressive drugs administered to maintain remission of associated IBD might interfere with the natural history of PSC, particularly in children. It can be speculated that the duration of the inflammatory process and development of fibrosis in the bile ducts before diagnosis influence the outcome. The overall median survival rate without liver transplantation is approximately 12 years in children who have PSC. A younger age at onset and significant portal hypertension are clinical variables associated with shorter survival in children.

Surgical, endoscopic, or radiologic dilatation of bile duct strictures has been attempted in some patients who have PSC. Although transitory good results have been reported with this intervention, there seems to be no effect on long-term outcome. Any invasive procedure in these children can be complicated by bacterial cholangitis that would be difficult to treat, given the abnormal bile flow. Complications caused by chronic decrease in bile flow could be prevented with adequate nutritional support, including administration of liposoluble vitamins. Dominant strictures in PSC with significant cholestasis should be treated with balloon dilatation. Some patients also appear to benefit from short-term stenting. No randomized, prospective controlled trials have been performed to assess the efficacy of endoscopic treatment in PSC, and the application is presently performed based on individual assessment of each patient. Complications secondary to portal hypertension or

recurrent bacterial cholangitis can be considered possible indications for liver transplantation even if liver function is somewhat preserved

Liver transplantation is the only therapeutic option for a child who has PSC, cirrhosis, and signs of liver failure. However, retransplantation rates are higher for patients with PSC than other diagnoses with a 25% recurrence rate in transplanted liver. An increased risk of acute cellular rejection in PSC has been demonstrated in several series, it is not clear whether PSC patients are particularly at risk or whether the risk is related to autoimmune liver disease in general. Furthermore, there is an increased risk of acute rejection in patients with pre-transplant IBD as compared with patients without IBD and the risk of chronic rejection also seems to be higher in PSC patients with IBD.

Recently prolonged treatment with oral vancomycin has been shown may be beneficial in difficult-to-treat PSC associated with inflammatory bowel disease.

An increased incidence of colorectal neoplasia has been described in adult patients who have PSC-related IBD. This complication is the most frequent cause of death after liver transplantation in patients who have ASC. Cholangiocarcinoma may complicate PSC in adults, and is significantly more common in patients who have colorectal neoplasia.

### 3.1.3 Autoimmune overlap syndrome

Overlap syndrome in children and adolescents concerns only autoimmune hepatitis and autoimmune cholangitis features and includes patients within the spectrum of autoimmune liver diseases presenting with the characteristics of both PSC or SDSC and AIH. AIH and AIC are not homogeneous disorders and patients within each diagnosis may present with a range of clinical, biochemical, serological, and histological findings. However, the diagnosis of overlap syndrome should not be overlooked; certainly AIH should not be diagnosed in presence of definite bile duct pathology, but some coincidental biliary injury may be observed. The presence of some degree of biliary involvement in AIH should therefore not necessarily lead to a change in diagnosis but an adequate biliary imaging study should be considered in such patients. Several types of relationship between the autoimmune liver disorders in the same patient have been described: 1. sequential presentation of two disorders: 2. concomitant presence of two distinct disorders; autoimmune diseases are often associated with one another and it can be argued that an individual who has developed one autoimmune liver disease is predisposed to develop another one as well; 3. existence of a continuum of pathological changes between two disorders, without strict boundaries and with "overlaps" localized in the center; 4. "overlap syndromes" are distinct entities on their own, with a variety of autoimmune manifestations presenting in a susceptible individual; 5. the presence of one primary disorder that also has one or more characteristics of another: this contention has been supported by a majority. The diagnosis of AIH-AIC overlap syndrome could be established in a patient with inflammatory colangiopathy diffuse or limited to intrahepatic bile duct on the presence of: 1. significant biochemical disease activity (moderate to severe increase of aminotransferases, hypergammaglobulinemia); 2. ANA or ASMA antibodies present in a titer at least ≥1:40; 3. a liver histology with interface hepatitis, moderate to severe lobular necrosis, moderate to severe periportal or periseptal inflammation. The major guidelines suggest that patients with AIH-AIC overlap syndrome should be treated with immunosuppressive regimen associated with ursodeoxycholic acid (UDCA) even if this recommendation is not evidence-based. Ursodeoxycholic acid (UDCA) is widely used in autoimmune cholangitis due to its beneficial effects on serum liver tests,

histological features, prognostic surrogate markers, and development of colonic dysplasia associated with accompanying ulcerative colitis, although long-term efficacy of UDCA still remains unproven. UDCA at higher doses (> 20 mg/kg daily) may be superior to standard doses and has also been used in the treatment of AIH-PSC overlap syndrome. Use of UDCA in combination with immunosuppressive drugs in AIH-AIC overlap syndrome, and the long-term course has been considered favorable in the long term. The response to immunosuppressive therapy appears to be better in children with AIH-AIC overlap than in adults.

### 3.1.4 Autoimmune small duct sclerosing cholangitis (SDSC)

A minority of patients with sclerosing cholangitis of unknown etiology with similar cholestatic and histologic features as those with classic PSC has normal biliary imaging, and they have been referred to as SDSC (Chapman, 2002). Patients with SDSC: 1. Are generally younger than patients with PSC and presents with more active disease, with overlap features; 2. may however progress to PSC and to end-stage liver disease with the consequent need of liver transplantation; 3. may progress to end-stage liver disease even without evidence of development of large-duct disease; 4. may suffer from recurrence of SDSC in the allograft in case of liver transplant; 5. do not seem to develop cholangiocarcinoma; 6. generally seem to have a significantly better long-term prognosis as compared with patients with large-duct PSC.

### 3.1.5 IgG4-related sclerosing cholangitis (IgG4-SC)

Biliary stricture, mimicking bile duct carcinoma and primary sclerosing cholangitis are frequently seen in IgG4-related systemic diseases. IgG4-related sclerosing cholangitis is almost constantly associated with autoimmune sclerosing pancreatitis (AIP). This syndrome affects predominantly middle-aged and elderly patients, with male predominance. The patients present with symptoms referable to the involvement of one or more sites, usually in the form of mass lesions. also defined as type 1, most commonly presenting as painless obstructive jaundice with or without a pancreatic mass. Other common sites of involvement are the hepatobiliary tract, salivary gland, orbit, and lymph node, but practically any organ-site can be affected, such as retroperitoneum, aorta, mediastinum, soft tissue, skin, central nervous system, breast, kidney, prostate, upper aerodigestive tract, and lung. Some patients have low titers of autoantibodies such as antinuclear antibodies and rheumatoid factor. The natural history is characterized by the development of multiple sites of involvement with time, sometimes after many years. However, the disease can remain localized to one site in occasional patients. The patients usually have a good general condition, with no fever or constitutional symptoms. The disease often shows excellent response to steroid therapy with however relapse after steroid withdrawal. The main pathologic findings in various extranodal sites include lymphoplasmacytic infiltration, lymphoid follicle formation, sclerosis and obliterative phlebitis, accompanied by atrophy and loss of the specialized structures of the involved tissue (such as secretory acini in pancreas, salivary gland, or lacrimal gland). Immunostaining shows increased IgG4+ cells in the involved tissues (>50 per high-power field, with IgG4/IgG ratio >40%). The lymph nodes show an increase in IgG4+ plasma cells on immunostaining. The nature and pathogenesis of IgG4-related sclerosing disease are still elusive. Very few pediatric reports of AIP exist.

## 4. Giant cell hepatitis combined with autoimmune hemolytic anemia

Transformation of hepatocytes into giant multinucleated cells is mostly observed in the neonatal period, when it is considered a nonspecific reaction of the immature liver cell to various forms of aggression. In older children and adults, giant cell transformation is rare and has been described sporadically in cases of viral, toxic, autoimmune, and genetic disease, as well as in diseases of unknown origin. When present, it is considered to carry a severe prognosis, with a risk of recurrence after liver transplantation.

In 1981, four young children were reported associating a giant cell hepatitis and Coombs-positive hemolytic anemia of the immunoglobulin G-positive (IgG+) C type: three of them died of liver failure, but for the remaining child, immunosuppressive treatment with prednisone and azathioprine was beneficial. Because of the association with a positive Coombs test and a positive response to immunosuppressive treatment, it was postulated that this entity might be of autoimmune origin. Since this original description, a total of 18 children with giant cell hepatitis and Coombs-positive hemolytic anemia have been reported confirming the onset of this condition in early childhood after the neonatal period, the severity of the liver condition that can progress to liver failure, and in many instances, the response to immunosuppressive treatment, but with less favorable initial control than in conventional autoimmune hepatitis, which occurs later in childhood. In the studies reported, mortality or the need for liver transplantation were 39% of reported cases and were due to liver cell failure and/or bacterial infection as well as relapse after liver transplantation.

The diagnosis of giant cell hepatitis combined with autoimmune hemolytic anemia should be considered in any child between ages 1 month and 2 years presenting with autoimmune hemolytic anemia, acute liver disease of unknown cause, or both. Assay for serum aminotransferase activities should be part of the investigation in young children with autoimmune hemolytic anemia, both at the onset of the disease and during follow-up.

Unexplained elevated aminotransferase activity should lead to liver biopsy in a search for signs of giant cell hepatitis (Figure. 7). In such cases, any reduction of the dose of steroids initially given to treat hemolytic anemia should be gradual, because the liver condition may degenerate to liver cell failure if the dose is lowered too fast. Conversely, a direct Coombs test should be part of the search for acute hepatitis of unknown origin in early childhood. If the test is positive of the IgG+ C type, liver biopsy should be performed as soon as possible; if giant cell hepatitis is found, immunosuppressive therapy should be rapidly started.

Except in cases of acute refractory liver cell failure, which would require liver transplantation, immunosuppressive therapy with prednisone and azathioprine is the first-line treatment. This is similar to that used in the treatment of older children with the most common type of autoimmune hepatitis. If appropriately administered, this treatment may control the liver disease. Here again, extreme caution should be exercised when reducing steroid doses. Thus, the high initial dose should be maintained for as long as possible until serum aminotransferase activity has returned to normal, because early relapse may occur. Hepatitis relapses are difficult to manage: in some cases they may be controlled by increasing the steroid dosage or adding cyclosporine to the initial regimen. Sometimes, however, hepatitis is refractory to drug therapy and rescue treatment with other drugs or biological agent such as Rituximab can be attempted. Liver transplantation must be considered when signs of liver failure occur early or during a relapse and are refractory to immunosuppressive treatment even if a relapse of the initial liver disease can be observed.

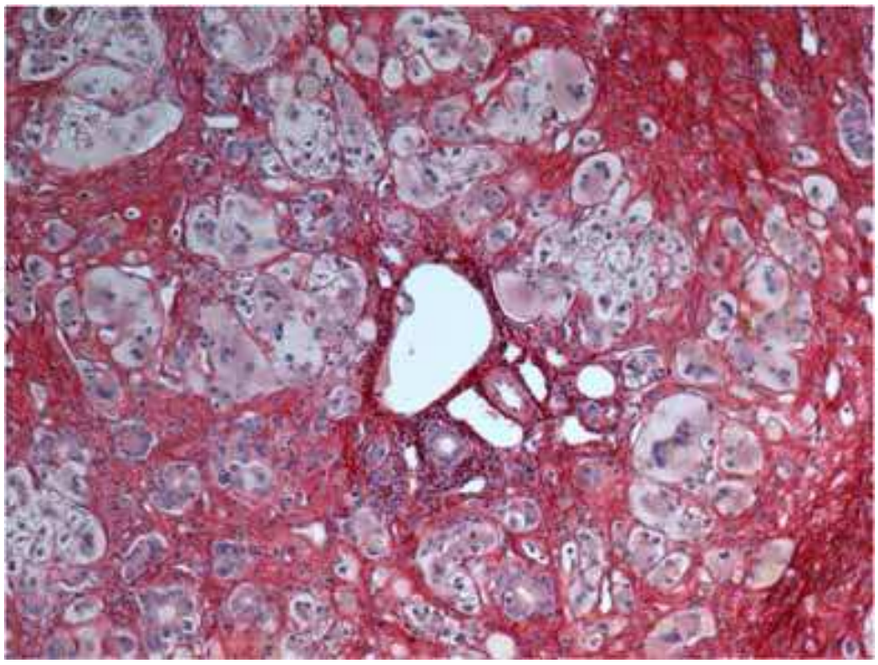

Fig. 10. Liver biopsy showing diffuse giant cell transformation in a child with autoimmune hemolytic anemia.

The evolution of anemia may be independent of the evolution of hepatitis: anemia can be extremely severe and require repeated erythrocyte transfusions and may be refractory to all kinds of drug therapy. In such cases, Rituximab should be considered. Before Rituximab became available, splenectomy was used with success. Once the disease has been under control for 5 years of management with immunosuppressive therapy, treatment can be stopped in most patients without relapse of the hepatitis.

## 5. Conclusions

In conclusion, in the pediatric population two predominant forms of autoimmune diseases of the liver, AIH and AIC, have been clearly identified, although the distinction between the two diagnoses is not always easy. The diagnosis of AIH must be considered in patients who have symptoms and signs of acute or chronic hepatitis, particularly when an extrahepatic autoimmune disorder is present. Hypergammaglobulinemia and circulating antibodies are of great help in supporting diagnosis of AIH. The fluctuating course of the disease can be responsible for alternating periods of remission and relapse. Therefore, a low-intensity inflammatory syndrome at onset does not preclude instituting immunosuppressive treatment. Rapid, complete, and sustained control of liver inflammation improves the short- and long-term outcome. Because it has been shown that liver fibrosis can regress in patients responding to treatment, an aggressive approach to confirm the diagnosis of AIH is justified.

The diagnosis of AIC must be evoked in patients who have IBD and clinical and laboratory features of cholestasis and/or bile duct injury. Liver biopsy and bile duct imaging are essential for the diagnosis and in distinguishing diffuse (PSC) from limited (SDSC) entities. Even if effective treatment has yet been described to control the progression of this disease,

patient with features overlapping with AIH may be efficaciously treated with conventional immunosuppressive treatment. Liver transplantation is the only definitive therapy, for patients with acute AIH unresponsive to rescue immunosuppressive treatment and for patients with end stage liver failure. Liver transplantation is also the treatment of choice for patient with sclerosing cholangitis and end stage liver disease. In both condition however the risk of relapse of the disease in the graft is not negligible.

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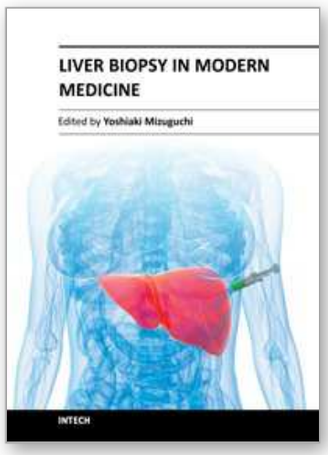


**Liver Biopsy in Modern Medicine**

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Liver biopsy, first performed by Paul Ehrlich in 1883, remains an important diagnostic procedure for the management of hepatobiliary disorders and the candidate/donated organ for transplantation. The book "Liver biopsy in Modern Medicine" comprises 21 chapters covering the various aspects of the biopsy procedure in detail and provides an up-to-date insightful coverage to the recent advances in the management of the various disorders with liver biospy. This book will keep up with cutting edge understanding of liver biopsy to many clinicians, physicians, scientists, pharmaceutics, engineers and other experts in a wide variety of different disciplines.

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