Autoimmune hepatitis in childhood

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AUTOIMMUNE HEPATITIS IN CHILDHOOD

GRADUATE THESIS

Zagreb, 2018.

This graduate thesis was made at the Department of Pediatrics, University Hospital Centre Zagreb, mentored by Prof. dr. sc. Jurica Vuković and was submitted for evaluation in the academic year 2017/2018.

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ABBREVIATIONS

A/G albumin : gamma
AASLD American Association for the Study of Liver Diseases
AIH autoimmune hepatitis
AMA antimitochondrial antibody
ANA antinuclear antibody
ANCA antineutrophil cytoplasm antibody
ASC autoimmune sclerosing cholangitis
CyA cyclosporine A
HLA human leukocyte antigen
IAIHG International Autoimmune Hepatitis Group
IgG immunoglobulin gamma
IgA immunoglobulin alpha
IL interleukin
INF interferon
LC1 liver cytosol type 1
LKM1 liver-kidney microsome type 1
MMF Mycophenolate mofetil
pANNA peripheral antinuclear neutrophil antibody
PBC primary biliary cholingitis
PSC primary Sclerosing cholingitis
SLA soluble liver antigen
SMA smooth muscle antibody
TGF-b transforming growth factor beta
Th T-helper (cells) Tregs regulatory T cells

CONTENTS

1. SUMMARY

Autoimmune hepatitis in childhood

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Autoimmune hepatitis (AIH) is an inflammatory process, where autoantibodies attack and damage liver cells. Like many autoimmune diseases, aetiology is unknown and it affects females more than males. It is characterised by both the presence of autoantibodies in serum and by high activity hepatic infiltrate patho-histology. There are two types of pediatric AIH identified; type I and type II. The types are differentiated on serological basis mainly: type I is positive for smooth muscle antibody (SMA) and/or antinuclear antibody (ANA); whereas, type II is positive for antibody to liver kidney microsome type 1 (ALKM1). Moreover, type II clinical presentation is more acute, usually at younger age as well as lower levels of IgA. In comparison to type I pediatric AIH, often present at older age/puberty. Nevertheless, the response to treatment and prognosis are comparable in both types. Genetic predisposition based on HLA typing has been found among different ethnicities. The treatment for both types is long- term immunosuppression classically with steroids and azathioprine; the treatment aims to normalise serology and liver histology – remission. Non-classic treatment is reserved for patients that are resistant to classic treatment; i.e. immunosuppression with budesonide, mycophenolate mofetil and cyclosporine. Relapses can occur; complete remission without the need of long-term immunosuppression is only achieved in 1 in 5 patients. Pediatric AIH has shown a significant association with other autoimmune diseases like autoimmune sclerosing cholangitis, inflammatory bowel disease, autoimmune thyroiditis, psoriasis and rheumatoid arthritis; some share clinical features and are collectively called overlap syndrome.

Keywords: Autoimmune hepatitis, immunosuppression, pediatric

2. SAŽETAK

Autoimunosni hepatitis u djece

Sadaf Khan

Autoimuni hepatitis (AIH) je upalni proces, gdje autoprotutijela napadaju i oštećuju jetrene stanice. Poput mnogih autoimunih bolesti, etiologija je nepoznata, a žene su više zahvaćene nego muškarci. Karakterizira ga prisutnost autoprotutijela u serumu i patohistologija visoke aktivnosti hepatičkog infiltrata. Postoje dvije vrste dječjeg AIH: tip I i tip II. Tipovi se uglavnom razlikuju serološki: tip I je pozitivan za glatko mišićna antitijela (SMA) i/ili antinuklearno antitijelo (ANA); dok je tip II pozitivan za protutijela na mikrosome jetre i bubrega tipa 1 (ALKM1). Štoviše, klinička prezentacija tipa II je akutnija, obično u mlađoj dobi, a IgA je nižih vrijednosti. U usporedbi s tipom I pedijatrijskog AIH, često prisutnog u starijoj dobi/pubertetu. Ipak, odgovor na liječenje i prognoza je usporediva u oba tipa. Genetska predispozicija na temelju HLA tipizacije pronađena je među različitim nacionalnostima. Liječenje oba tipa je dugotrajna imunosupresija, tipično - steroidima i azatioprinom; liječenje nastoji normalizirati serološki nalaz i histologiju jetre, odnosno postići remisiju. Netipičan tretman je rezerviran za bolesnike koji su otporni na tipičan tretman; tj. imunosupresija s budesonidom, mikofenolat mofetilom i ciklosporinom. Može doći do recidiva; potpuna remisija bez potrebe za dugotrajnom imunosupresijom postiže se samo u jednoj od 5 bolesnika. Dječji AIH je pokazao značajnu povezanost s drugim autoimunim bolestima kao što su autoimuni sklerotični kolangitis, upalna bolest crijeva, autoimuni tireoiditis, psorijaza i reumatoidni artritis; neki dijele kliničke osobine i zajednički se nazivaju sindrom preklapanja.

Ključne riječi: Autoimuni hepatitis, imunosupresija, pedijatrija

3. INTRODUCTION

Autoimmune hepatitis (AIH) is inflammatory disease of liver of unknown aetiology. Like many other autoimmune disease it affects mainly females. In pediatrics and young adults autoimmune hepatitis usually presents in acute form and often has a more aggressive course than a middle aged and elderly patients [1]. It can be divided into two types Type I and Type II, the classification is based on the presence of certain autoimmune antibodies, age at onset, severity and presence of certain immunoglobulins [1]. Table 1 enlists some of the main differences between type I and type II:

	Type I	**Type II**
Autoimmune antibodies	ANA +, SMA +	LKM1 +, LC1 +
Age of onset	Puberty	Younger even infancy
Severity	Less serious	More serious
Immunoglobulins	é IgG, 15% normal	é IgG, 25% normal, IgA deficiency common
Liver enzymes	é ASL/ALT	é ASL/ALT and bilirubin

Table 1: Type I v/s Type II autoimmune hepatitis in children. Derived from information obtained from Miele – Vergani et al 2009 [2,3]

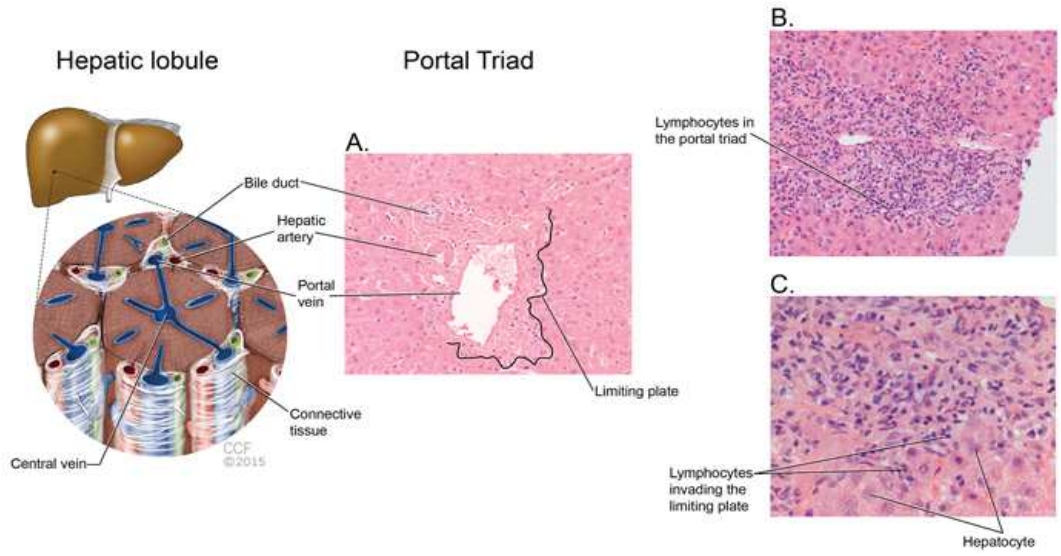


Figure 1: (A) Normal (B) Autoimmune hepatitis [34]

Autoimmune hepatitis is a progressive liver disease. Apart from serology and autoimmune antibodies, it is also characterised by liver histology (as shown in figure 1) by interface hepatitis especially in cases of unknown aetiology [1]. Autoimmune hepatitis responds to immunosuppressive treatment. Furthermore, autoimmune liver inflammation in children is due to these two liver disorders: a) classical AIH and b) AIH/sclerosing children cholangitis overlap syndrome (ASC)[2,3]. ASC has characteristic interface hepatitis along with bile duct damage.

4. ROLE OF GENETICS

Through our knowledge of other autoimmune disease, AIH is also believed to have link to genetics, predisposing some children to AIH as compared to others. Genetic study plays a crucial role in establishing such patterns. HLA typing reveals that population originating from northern Europe, and have type 1 AIH are also associated with the possession of the human leukocyte antigen (HLA) DRB1*03. Type 1 AIH is similar to AIH in adults [2,5]. Whereas, the adult patients who are HLA of DRB1*04 do not predispose to pediatric AIH, and can even wield a protective role against AIH of childhood.

HLA- DRB1*07 is associated with Type 2 AIH [6]. HLA typing differs across regions and ethnicities. For instance, in South America, possession of the HLA DRB1*1301 allele, predisposes to pediatric AIH type 1, as well as is associated with frequent infection with the endemic hepatitis A virus [6].

AASLD (American Association for the Study of Liver Diseases) shows that Pediatric AIH patients with either antiLKM1- or ANA/SMA-positive, have isolated partial deficiency of the HLA class III complement component C4, which is genetically determined.

Additionally, Type 2 AIH can present as a part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome; its an autosomal-recessive monogenic disorder, can comprise of liver disease is about 20% of cases. [7,8]

5. CLINCAL PRESENTATION

Clinically AIH can presents as three patterns of the disease presentation: [2]

1. *Viral presentation*: about 40% of AIH patients, the clinical picture is indistinguishable from that of an acute viral hepatitis; featuring nonspecific symptoms of malaise, nausea, vomiting, anorexia, and abdominal pain, followed by jaundice with dark urine, and pale stools. Some children, especially those who are anti-LKM1 positive (type 2), often develop acute hepatic failure with grade 2-4 hepatic encephalopathy in two to eight weeks from onset of symptoms.
2. *Insidious presentation*: about one third of patients have insidious onset, with presentation characterized by progressive fatigue, frequent jaundice, headache, anorexia, amenorrhea, and weight loss, the disease may not be diagnosed for several months and even years due to vague clinical picture.
3. *Portal hypertension presentation*: one tenth of the patients show no history of jaundice, such cases are diagnosed later when they present with complications of portal hypertension, such as splenomegaly, hematemesis from esophageal varices, bleeding diathesis, chronic diarrhea, and weight loss. [2]

Since there is quite some variability in pediatric AIH, the disease should be suspected and ruled out in all children presenting with symptoms and signs of liver pathology. The course of the disease may differ, with flares and spontaneous remissions, a pattern that can mislead and cause delayed diagnosis and treatment. Physical exam of most of these children will have clinical signs of an underlying chronic liver disease, like cutaneous stigmata (spider nevi, leukonychia, palmar erythema, striae), firm liver, and splenomegaly. The ultrasound of these patients often features nodular and heterogeneous liver parenchyma. [3]

In this thesis, serological data and patho-histological records of pediatric patients are reviewed that were referred to Rebro hospital Zagreb, Croatia; i.e.

immunology and histology essentially. In the last 34 years forty cases of pediatric AIH have been recorded in the pediatric department of University Hospital Centre Zagreb, Croatia. In this paper these forty cases are used to study the characteristic features and reoccurring pattern among these patients based on the following factors:

- Age at the onset of the disease
- Gender
- AIH Type I/II
- Autoimmune antibodies (ANA, ASMA, AGLM, LKM, ALA)
    - At the onset
    - During the course of the disease
- ESR
- Immunoglobulin A
- Immunoglobulin G
- Ratio A/G (albumin: calculated globulin)
- Liver biopsy (interface hepatitis/severity)
- Presence of another known autoimmune disease
- Presence/absence of inflammatory bowel disease (IBD)
- Treatment
    - Classical → steroids for induction and azathioprine for the rest
    - Non-classical

	TYPE I	TYPE II
Mean age at onset	11.2 years	4.0 years
Females %	55%	0%
Mode of presentation (%) Acute hepatitis	26%	0%
Acute liver failure	4%	0%
Insidious onset	17%	100%
Complication of chronic liver disease	35%	0%
Associated immune diseases (%)	45%	0%
Sclerosing cholangitis (%)	26%	0%
Inflammatory bowel disease (%)	25%	0%
ANA/SMA (AGLM) (%)	50%	
Anti-LKM1 (%)	0%	100%
Anti-SLA (%)	7.5%	
Increased IgG level (%)	61,5%	0%
Decreased Ratio A/G	10%	0%
Increased ESR	55%	Normal (0%)
Interface hepatitis (%)	66%	100%
Biliary features (%)	28%	0%

Table 2: Clinical, Immunological, and Histological Features at Presentation of AIH referred to University Hospital Centre Zagreb patients [4]. Modified from Vergani et al 2009 [1]

Type1 AIH affects both children and adults and has characteristic autoimmune antibody against smooth muscle cells (SMA) plus/minus antinuclear antibodies (ANAs). Whereas, type 2 AIH is primarily a pediatrics disease; and is characterized by the presence of antibodies to liver-kidney microsome type 1 (anti-LKM1) plus/minus anti-liver cytosol type 1 (anti-LC1). [2,3] Both type 1 and type 2 AIH responds well to immunosuppressive treatment. If left untreated,

it generally progresses rapidly to cirrhosis and liver failure. Like most autoimmune diseases AIH has higher incidence of females as compare to males. The epidemiology of childhood AIH is unknown, but 2/3 of the AIH cases in children is type 1 AIH and usually presents around puberty, while type 2 AIH tends to present at a younger age some even during infancy. Laboratory work usually shows increased IgG at presentation in both types, although about 15% of children with AIH type 1 and 25% AIH type 2 may have normal levels [2]. Whereas, IgA deficiency is often seen in AIH type 2 [2]. Studies show that severity of disease is more or less similar in both types, but type2 AIH patients have higher levels of bilirubin and transaminases at presentation than those who are type 1 and present significantly more frequently with fulminant hepatic failure as reflected by bilirubin and transaminases values [2].

As far as patho-histology is concerned the severity of interface hepatitis at the time of diagnosis is similar in both type 1 and type 2, but cirrhosis on initial biopsy is more common to find in type 1 in comparison to type 2 AIH, indicating a more chronic course of disease in type 1. Furthermore, progression to cirrhosis while on treatment is more frequent in type 1 AIH. In both types of AIH, presence of antibodies to soluble liver antigen (SLA) suggests a more severe form along with increased tendency to relapse, about one in two patients of AIH are SLA positive, equal between the both types of AIH. [4] One fifth of al AIH of patients has other autoimmune disorders—like thyroiditis, vitiligo, type 1 diabetes, inflammatory bowel disease (IBD), and nephrotic syndrome and approximately 40% have a positive family history of autoimmune disease. [2]

6. DIAGNOSIS

Diagnosis of pediatric AIH is mainly based on serology, rheumatology and liver histology; i.e. a series of positive and negative criteria. [9,10] Liver biopsy is a requirement to establish the AIH diagnosis.

The second criterion of AIH diagnosis is elevated IgG levels and serum transaminases along with the presence of autoimmune antibodies ANA, SMA, or anti-LKM1. The International Autoimmune Hepatitis Group (IAIHG) introduced a

'negative criteria for the diagnosis of AIH that included hepatitis B or C viral infection or Wilson's disease and alcohol, among the positive criteria mentioned earlier [9,10]. The IAIHG positive and negative criteria have proven to be a useful scoring system for the diagnosis of AIH as well as for research purposes. It is a simple scoring system based on autoimmune antibodies, IgG, histology, and omission of viral hepatitis [11]. One of the cons of the IAIHG scoring system is that it has been produced for adult AIH and may not necessarily take into account the peculiarities of pediatric AIH.

6.1. Autoantibodies

As mentioned earlier autoantibodies detection is an important criterion for diagnosis and differentiating between types of AIH. These are detected using a technique called indirect immunofluorescence on a freshly prepared rodent substrate kidney, liver, and stomach tissue [48], to detect anti nuclei autoantibodies (ANA), smooth muscle autoantibodies (SMA), and anti-liver-kidney microsome type 1 (antiLKM1) autoantibodies. It must be noted that autoantibodies positivity is not diagnostic of AIH, as other liver diseases and viral hepatitis, Wilson's disease and non-alcoholic steatohepatitis [49] can also present with low titre autoantibodies. Hence, the titre value significant for AIH is also important. The immunofluorescence technique is clinician dependent and can be a cause of discrepancies in results along with the possibility of false positive results e.g. antiLKM1 can often be confused with AMA due to similar pattern. International Autoimmune Hepatitis Group (IAIHG) has issued guidelines in order to standardise the laboratory data, along with titre values to differentiate between adults and pediatric population [48]. , In healthy children autoantibody reactivity is uncommon, so that titres of 1/20 for ANA and SMA and 1/10 for anti-LKM1 are clinically significant. In comparison to adults autoantibody reactivity at 1/10 is common, 1/40 in adults is considered clinically relevant.

Other important autoantibodies are, anti-neutrophil cytoplasm antibody (ANCA), and soluble liver antigen (SLA) antibody; ANCA can be positive in autoimmune hepatitis. The autoantibody peripheral antinuclear neutrophil antibody (pANNA)

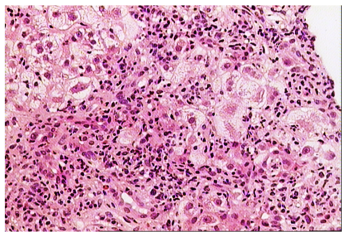
is found in AIH as well as other autoimmune diseases like inflammatory bowel disease (IBD) and sclerosing cholangitis; it is never indicative of type II AIH [50]. On the other hand anti-SLA autoantibody is also found in 50% of cases of both types AIH; the presence of anti-SLA autoantibody is indicative of a more severe form of AIH [50].

There are always some cases of AIH where the autoantibodies are undetectable. These sero-negative children are still treated with immunosuppression like seropositive types. [10, 51]

6.2. Histology

The typical histological picture of AIH liver includes:

- *Inflammatory infiltrate*: Presence of dense mononuclear and plasma cell infiltration of the portal areas, expanding into the liver lobule (Fig. 1A)
- *Interface hepatitis:* Peri-lobule destruction of the hepatocytes along with erosion of the limiting plate
- *Bridging collapse:* Connective tissue collapse due to hepatocyte death and spreading from the portal area into the lobule
- And hepatic regeneration with ‘‘*rosette”* formation



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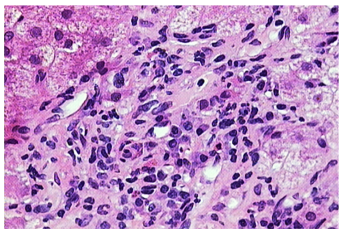

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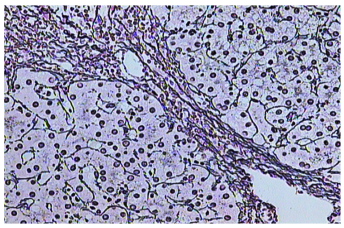

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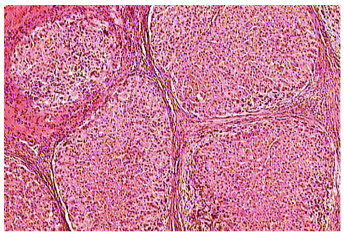

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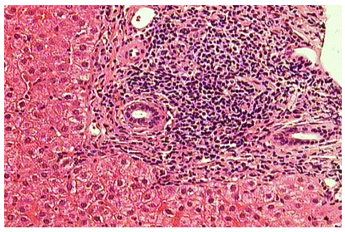

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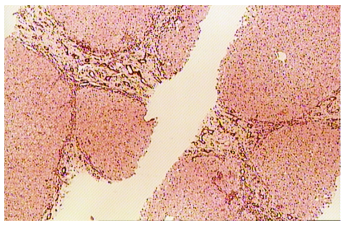

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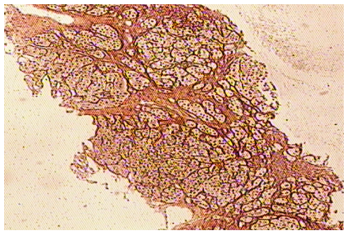

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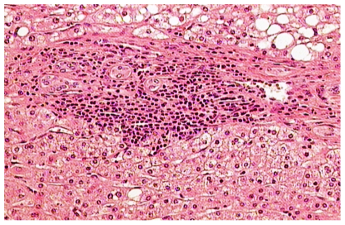

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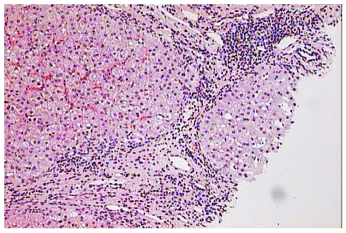

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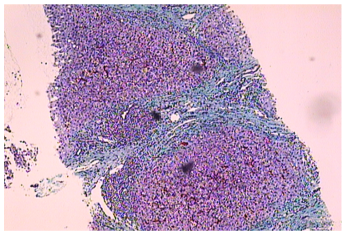

(J)

Figure 2: Autoimmune hepatitis patho-histology from University Hospital Centre Zagreb, Croatia histological records. (A) Autoimmune hepatitis 20x HE (B) AIH plasma cells HE 40x (C) Bridging necrosis Gomory (D) Cirrhosis HE (E) Mild fibrosis HE (F) moderate fibrosis HE 5x (G) Pericellular fibrosis Gomory (H) Piecemeal necrosis HE 20x (I) Portal septal bridging fibrosis HE (J) Severe fibrosis Mallory stain [66]

Figure 2 shows the typical features of hepatic patho-histology of AIH; it comprises mononuclear inflammation, high activity infiltrate, piecemeal necrosis, bridging necrosis, and fibrosis of varied severity depending on the stage of liver damage. The slides were obtained from the pathology department at University Hospital Centre Zagreb, Croatia.

6.3. HLA typing

See section 4, Role of genetics.

7. PATHOGENESIS

AIH is of unknown aetiology, it is believed that both genetic and environmental play part. Histologically, AIH is characterised by dense portal mononuclear cell infiltrate surrounding the parenchyma; the infiltrate consists of T and B-lymphocytes, macrophages, and plasma cells [58,59]. For the reason unknown, this strong inflammatory cellular reaction is initiated leading to liver damage. An auto-antigenic liver peptide is recognised by CD4 T lymphocytes. An HLA class II presents the auto-antigenic liver peptide to naïve CD4 T-helper cells (Th0) and it's then activated. The activated Th0 then differentiate into various cytokines triggering a cascade of immune reactions. Macrophages produce interleukin 12 (IL), IL-2 and interferon (IFN)-gamma; this increases HLA class I sensitivity and thus elevates CD8 T cell cytotoxic activity on hepatocytes as well as induction of HLA class II on liver cells. Elevated levels of IL-4 triggers differentiation of T-helper type 2 (Th2) cells from Th0 which mainly secretes IL-4, IL-10, and IL-13, this leads to autoantibody production by B lymphocytes. Furthermore, recent research shows that in the presence of transforming growth factor beta (TGF-b) and IL-6, a population of Th17 cells arise; Th17 cells have shown to play an important role in autoimmunity as well as inflammation. Regulatory T cells CD4 CD25 (T-regulatory cells), also a derivative of TGF-b is responsible for the recognising the auto-antigen. When there is a flaw in this regulatory system, autoimmunity persists. [59-62]

This regulatory mechanism has been an area of interest over that last three decades, in order to understand autoimmune pathogenic pathways and immune-regulations. Defective T regulatory cells have been seen in AIH. Recent studies have demonstrated that in AIH type 2 the main perpetrator is T-cells [59, 63]. There has been evidence that suggests that elevated levels of CD4 T cells and CD8 T cells producing IFN-gamma is linked with molecular hepatic damage. [63] In conclusion various studies and evidence indicates a multifactorial cellular immune attack.

8. TREATMENTS AND MANAGEMENT

The main aim of treatment is clinical remission, complete serological and histological resolution of inflammation and liver damage. The long-term goal is to sustain remission without the need for pharmacotherapy. AIH remission is defined as "*the resolution of symptoms; normalization of levels of serum aminotransferase, serum bilirubin, and g-globulin/IgG; and an improvement of liver histology either to normal or to only mild portal hepatitis*"[64]. Serological normalisation alone is not sufficient to deduce normal hepatic normalisation. The goals of the AIH can be stated as follows: [65]

- Initial aim is normalization of liver aminotransferase
- Normalization of the g-globulin and immunoglobulin G levels
- Histological normalization, reducing hepatic infiltrate
- Quaternary end point: resolution of fibrosis
- Final goal is to achieve sustained remission without the need for drug therapy and essentially preserving the hepatic function

8.1. Classic v/s non-classic

Classically AIH treated with steroids initially to induce remission; prednisone 20-30mg/day often combined with azathioprine. Azathioprine may cause dose-dependent hepatotoxicity in some rare severe AIH cases [1]. To avoid the side effects of azathioprine on decompensated liver, it can be commenced few weeks after the steroids [35]. Azathioprine in combination with prednisolone is

preferred as the side effects are fewer as compared to prednisolone alone i.e. 10% v/s 44%. [35] Furthermore protection to bone against long-term use of steroids is also suggested. Alternate steroids therapy is

Non-classical treatment options for AIH is considered to minimise adverse effects of the classical treatment or when there is tolerance observed with classical treatment. Remission and relapse is determined based on liver function test (AST and ALT) levels. One of the alternative therapy is Budesonide in addition with azathioprine can be considered for patients with comorbidities that can worsen with prednisone administration.

Budesonide has a higher affinity for the glucocorticoid receptor as compared to prednisolone but about 90% of it is cleared through hepatic first pass metabolism. Study shows that remission is achieved with budesonide in 58-83% of cases along with improved tolerability [36, 37]. Another double blind randomised study consisted of 203 AIH patients that were given either budesonide plus azathioprine or prednisone plus azathioprine; the results showed remission at six months with budesonide in 60% whereas 40% with prednisone [38].
Although promising results were achieved with budesonide the 1st pass hepatic metabolism is a risk factor for corticosteroid induced side effects. This limits the use of budesonide in patients with severely damaged liver [39].

Mycophenolate mofetil (MMF) is a strong immunosuppressive drug, with side effects including diarrhoea and bone marrow suppression [40]. However a study has shown a decreased hepatic activity index along with normal transaminases achieved with three to nine months use of MMF; the patients used in the study were either intolerant or not effected by azathioprine [41]. Promising results of MMF were recorded in a pediatric study too [42]. The use of MMF is restricted to azathioprine tolerance/resistance. It is a teratogen thus it is not used in women planning to get pregnant. [35]

Calcineurin Inhibitors aka cyclosporine A (CyA) has shown positive results in inducing and maintaining remission, especially in pediatric population with AIH [43,44]. Another agent called tacrolimus is more potent than CyA and a studies has shown minimal side effects [45] with improved transaminases, IgG along with reduced inflammatory index [46]. A study done in Virginia on AIH patients treated with tacrolimus has observed increased remission rates. [47]. Tacrolimus seems to be much better choice of drug especially for AIH patients tolerant to classical therapy, but long-term data and follow up studies are required to be certain.

8.2. When to start

If left untreated autoimmune hepatitis has poor prognosis, while patients with adequate immunosuppressive therapy have good prognosis [12,13]. Autoimmune hepatitis is highly responsive to immunosuppression, and treatment should be started straightaway to avoid progression of liver fibrosis and failure. The treatment is targeted to decrease liver inflammation in order to reach remission, improve symptoms, prevent liver fibrosis, prolong survival and delay the need of liver transplant [20]. The response to therapy is dependent on disease severity at the time of presentation. Even though cirrhosis is present in 44 to 80% of children at diagnosis [21,22,23] mortality rate can be delayed and clinical stability is usually achieved, with well-regulated long-term therapy along with a good quality of life in pediatric AIH.

8.3. Duration

As each cases comes with individual characteristics and response to therapy, the optimal duration of immunosuppressive treatment for pediatric AIH is still a matter of research. Treatment withdrawal is dependent on liver histology, it is only recommended to stop the therapy if patho-histology shows little to no inflammation. This usually takes one to two years of normal liver function tests, normal immunoglobulin gamma levels and minimal to zero autoantibodies titres.

The relapses are more common just before puberty and within the first three years of diagnosis. Hence is recommended to avoid therapy withdrawal, just

before puberty and first three years of treatment. Treatment withdrawal can successfully be achieved in about one fifth of the patients with type 1 AIH but seldom in type 2 AIH [2]. Most of the patients require a long-term treatment and families may require counselling [11]. The AIH disease activity correlates with IgG levels, presence and titres of autoimmune antibodies and is important to monitor these values to maintain disease control and avoid flares [33].

9. PROGNOSIS

Overall prognosis of AIH in pediatric population is good with immunosuppressive therapy, even among patients that presented with cirrhosis. Survival rate is high with a very good quality of life on low dose immunosuppression therapy. 8.5% of patients from King's College Hospital Pediatric Liver Centre required transplant due to development of end-stage liver disease; the transplant was required 8-14 years post diagnosis [2]. The patients that present with acute liver failure are usually more resistant to treatment and initial therapeutic effect is more difficult to achieve. [27]

Table 3 Response to Treatment and Outcome in Children with Autoimmune Hepatitis type I and type II [2,25]

	TYPE I	TYPE II
Remission rate (%)	97	98
Median time to remission	6	9
Relapse rate (%)	42	46
Cessation of treatment (%)	19	0
Liver transplant rate (%)	6	13
Disease recurrence post-transplant (%)	0	0

Table 3:Data are from patients treated at the King's College Hospital Tertiary Pediatric Liver Centre [2,25]

10. REMISSION AND RELAPSE

Remission is achieved with clinical recovery as well as normal laboratory and histological data reports, including; normal reference range serum transaminase and IgG levels, zero or very low titre autoimmune antibodies, and histological resolution of inflammation. Although, patho-histological normalcy takes longer to achieve as compared to immunology and blood values [23, 24] and it may not correlate with the histological features. In 95% of cases it takes 4 years on average of treatment, to see histological improvement in portal inflammation and better fibrosis scores [23]. Remission without the treatment was achieved in 10% (four) of cases in patients referred to Zagreb teaching Hospital, Croatia. Two of the patients were females; both gave multiple births to healthy babies without relapse or any hepatic issues. [4]

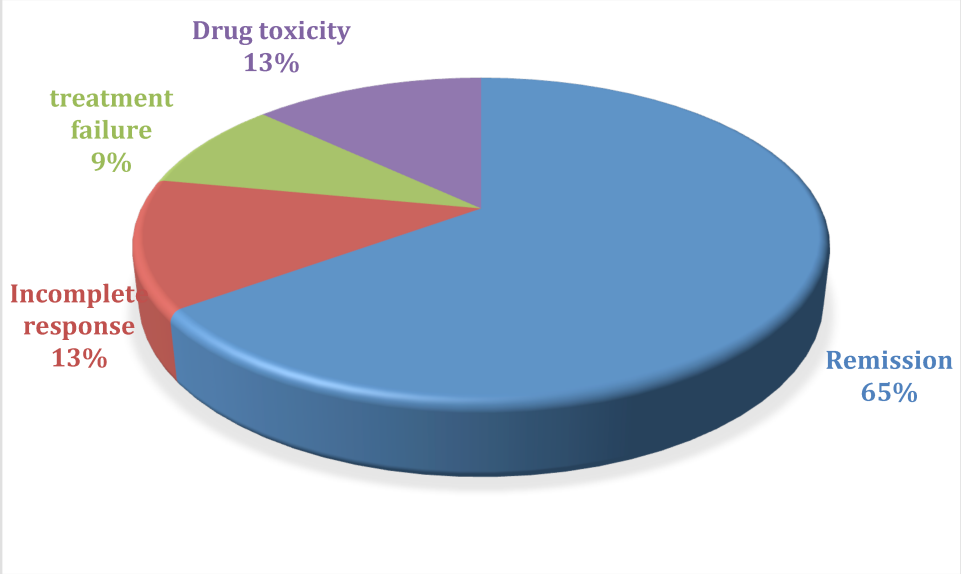

Figure 3: Outcomes of corticosteroid treatment of AIH; Drug toxicity, disease progression treatment failure, and incomplete response are the possible outcomes of AIH treatment, and relapse is the most common problem after drug withdrawal. Modified from: Czaja AJ. Autoimmune hepatitis: treatment. [52]

Relapse is defined as elevated serum aminotransferase levels following remission. Relapse during treatment is not uncommon, approximately 40% of patients experience relapse [25] that is dealt by a temporary increase in the steroid dosage. Compliance among adolescent is a one of the major factor

resulting in relapse [26]. In severe cases, the risk of relapse is greater if steroids schedule is an alternate-day therapy, the aim of alternate-day steroid schedule is to minimise its side effect on child's growth. However, it has seen that small daily doses, have better effect in maintaining disease control. It minimises the need for high-dose steroid dosage during relapses and thus fewer/less severe side effects i.e. abating negative affect on final height [25,27]. Classical treatment of AIH consists of prednisolone (also known as prednisone) 2 mg/kg/d maximum 60 mg/ d, this is gradually decreased over a period of 4 to 8 weeks, according to corresponding decline of serum transaminase levels, bringing it to a maintenance dose of 2.5 to 5 mg/d [28,30].

Usually an 80% decrease of the serum aminotransferase levels is achieved in the first eight weeks, but lowering it to normal range may take months [28,31]. Weekly liver function test must be carried out in first 6-8 weeks of therapy to adjust steroid dosage and thus minimising steroid side effects in children. Azathioprine may be added in the presence of severe steroid side effects, or as an adjuvant drug if the serum transaminase levels fail to decrease with steroid treatment alone. The starting dose is 0.5 mg/kg/d, which in the absence of signs of toxicity is increased up to a maximum of 2.0 to 2.5 mg/kg/d until the desired serum transaminases values are achieved. In some cases a combination of steroids and azathioprine is prescribed from the start, but caution is recommended because azathioprine can cause hepatotoxicity, especially in patients with severe jaundice. A preliminary report suggests that measurement of the azathioprine metabolites 6-thioguanine and 6-methylmercaptopurine is useful in identifying drug toxicity and compliance issues. Additionally, the metabolite 6-thioguanine is considered therapeutic for inflammatory bowel disease [32].

11. TRANSPLANT

Liver transplant is indicate in end-stage AIH/liver disease as the last resource when the conventional treatment methods are not effective often due to long term hepatic damage prior the start of the immune suppression therapy. These

patients usually present with fulminant liver failure along with encephalopathy [1]. Two patients of Rebro KBC Zagreb hospital patients were indicated for liver transplant, which makes it 5% of the data collected. Whereas another AIH pediatric study done at Kings Hospital London by Mieli-Vergani recorded that 10-20% children diagnosed with AIH require liver transplant, with 20% recurrence rate with transplanted liver [1]. Insufficient data is available to calculate recurrence rate with liver transplant patients from Rebro KBC Zagreb hospital patients. As the recurrence is possible even after liver hence the state of immunosuppression must be maintained using steroids, higher than non-transplant AIH patient. Immunosuppression is also recommended to avoid graft versus host disease. [27]

12. DISCUSSION

The limitations and AIH associations with other autoimmune diseases are as follows:

12.1. Limitations

There are some limitations that must be considered when reviewing the research data as some patients with primary biliary cirrhosis (PBC) may present features of autoimmune hepatitis [14,15] another differential diagnosis is primary sclerosing cholangitis (PSC) [16]. As found in the pediatric patients database from Rebro hospital Zagreb these cases, termed as overlap syndromes, are subject to debate and further research and classification [17]. The treatment via immunosuppressive therapy in AIH patients also has divided opinions, but majority of experts agree with the need to use immunosuppression given the high activity inflammatory hepatitis infiltrate [18]. The distinction between overlap syndrome and primary AIH and PBC or PSC associated AIH is done by the positive and negative criteria [19] of diagnosis [see diagnosis section].

Secondly, most research and data available about AIH is based on adult AIH and thus some discrepancies may exist in areas like clinical features, aetiology, response to treatment and prognosis. Reason being pediatric AIH is not as common as in adult population.

12.2. In relation to other autoimmune diseases

AIH in pediatric population is sometimes associated with other autoimmune disease; the correlation and pattern of such associations vary from case to case. Some of the common association of AIH is with autoimmune sclerosing cholangitis (ASC), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), inflammatory bowel diseases (IBD); ulcerative colitis/crohn's disease and rheumatoid arthritis (RA).

ASC presents with typical presentation of autoimmune disease i.e. elevated IgG increased titres of autoantibodies especially ANA, SMA along with interface hepatitis [53]. About 5-10% of PSC and PBC patients have serology-indicating AIH and vice versa and some patients shares clinical pictures too. A Mayo Clinic study comprised of 225 AIH patients showed a significant association between AIH and other autoimmune diseases [54]. This study showed that 18% of the patients with overlapping features; i.e. 7% with AIH-PBC, 6% with AIH-PSC, and 11% with AIH-autoimmune cholangitis (AIC). [55, 56, 57]

13. CONCLUSION

Since the follow up data from the University Hospital Centre Zagreb patients is out of this thesis scope. The detailed information about their duration of treatment, remission and relapse is not reviewed. In order to obtain a whole picture of treatment outcome of AIH, a more detailed and long-term review is needed. Nevertheless, it is safe to conclude that a long-term management is required for pediatric AIH, which requires regular serological and patho-histological (if serology indicates hepatic pathology) monitoring to ensure remission and/or detect relapse so that appropriate therapy/intervention can be adjusted accordingly.

New drugs have been studied for the achievement and maintenance of remission with minimal or no side effects. Hopefully, future studies will allow us to pin point the trigger of pathogenesis of AIH so that a more targeted therapy can be devised.

14. ACKNOWLEDGMENT

I would like to thank my mentor, Prof. Dr. Sc. Jurica Vuković for the academic support and guidance he provided me throughout the thesis writing process. In addition to Dr. Vuković I would like to thank the rest of the thesis committee Prof. Dr. Sc. Duška Tješić-Drinković and Prof. Dr. Sc. Marija Jelušić; as well as Dr. Marijana Ćorić from the Pathology department for her assistant with the patho-histology of AIH. I would also like to extend special thanks to Faraz Khan for proof reading my graduate thesis and my colleague Ana Stručić for translating the summary to Croatian language.

Furthermore, I want to thank my family, Jindaree and friends for their support during my medical studies and the writing of this thesis.

15. REFERENCES

1. Vergani D, Mieli-Vergani G. Autoimmune hepatitis. Textbook of Hepatology: From Basic Science to Clinical Practice. 3rd ed. Oxford, UK: Blackwell Publishing; 2007: 1089–1101
2. Gregorio GV, Portmann B, Reid F, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 1997;25(3):541–547
3. Martini E, Abuaf N, Cavalli F, Durand V, Johanet C, Homberg JC. Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. Hepatology 1988;8(6):1662–1666
4. KBC Rebro Hospital patients' data, Zagreb, Croatia 1984-2018.
5. Donaldson PT. Genetics in autoimmune hepatitis. Semin Liver Dis 2002;22(4):353–364
6. Ma Y, Bogdanos DP, Hussain MJ, et al. Polyclonal T-cell responses to cytochrome P450IID6 are associated with disease activity in autoimmune hepatitis type 2. Gastroenterology 2006;130(3):868–882
7. Liston A, Lesage S, Gray DH, Boyd RL, Goodnow CC. Genetic lesions in T-cell tolerance and thresholds for autoimmunity. Immunol Rev 2005;204:87–101
8. Simmonds MJ, Gough SC. Genetic insights into disease mechanisms of autoimmunity. Br Med Bull 2004;71:93–113
9. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology 1993;18(4):998 - 1005.
10. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31(5): 929–938
11. Hennes EM, Zeniya M, Czaja AJ, et al; International Autoimmune Hepatitis Group. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48(1): 169–176
12. Krawitt EL. Autoimmune hepatitis. N Engl J Med 2006;354:54–66.
13. Kanzler S, Lohr H, Galle PR, Meyer zum Büschenfelde KH, Lohse AW. Long- term management and prognosis of autoimmune hepatitis (AIH): a single center experience. Z Gastroenterol 2001;39 (339–41):344–348.

14. Lohse AW, Meyer zum Büschenfelde KH, Franz B, Kanzler S, Gerken G, Dienes HP. Characterisation of the 'overlap syndrome' of primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) in genetically susceptible individuals. Hepatology 1999;29:1078–1084.
15. Chazouilleres O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology 1998;28:296–301.
16. Lüth S, Herkel J, Kanzler S, Frenzel C, Galle PR, Dienes HP, et al. Serologic markers compared with liver biopsy for monitoring disease activity in autoimmune hepatitis. J Clin Gastroenterol 2008;42:926–930.
17. Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E, Overlap syndromes: The International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol 2011;54: 374–385.
18. Neuhauser M, Bjornsson E, Treeprasertsuk S, Enders F, Silveira M, Talwalkar J, et al. Autoimmune hepatitis-PBC overlap syndrome: a simplified scoring system may assist in the diagnosis. Am J Gastroenterol 2010;105:345–353.
19. Qiu D, Wang Q, Wang H, Xie Q, Zang G, Jiang H, et al. Validation of the simplified criteria for diagnosis of autoimmune hepatitis in Chinese patients. J Hepatol 2011;54:340–347.
20. Alvarez F. Autoimmune hepatitis and primary sclerosing cholangitis. Clin Liver Dis 2006;10(1):89–107
21. Costa M, Rodr´ıguez-Sa´nchez JL, Czaja AJ, Gelp´ı C. Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP(Ser)Sec complex recognized by autoanti- bodies from patients with type-1 autoimmune hepatitis. Clin Exp Immunol 2000;121(2):364–374
22. Saadah OI, Smith AL, Hardikar W. Long-term outcome of autoimmune hepatitis in children. J Gastroenterol Hepatol 2001;16(11):1297–1302
23. Ferreira AR, Roquete ML, Toppa NH, de Castro LP, Fagundes ED, Penna FJ.

Effect of treatment of hepatic histopathology in children and adolescents with autoimmune hepatitis. J Pediatr Gastroenterol Nutr 2008;46(1):65–70

24. Al-Chalabi T, Heneghan MA. Remission in autoimmune hepatitis: what is it, and can it ever be achieved? Am J Gastroenterol 2007;102(5):1013–1015
25. Mieli-Vergani G[1], Vergani D. Autoimmune hepatitis in children: what is different from adult AIH? . 2009 Aug;29(3):297-306
26. Kerkar N, Annunziato RA, Foley L, et al. Prospective analysis of nonadherence in autoimmune hepatitis: a common problem. J Pediatr Gastroenterol Nutr 2006;43(5): 629–634
27. Czaja AJ, Bianchi FB, Carpenter HA, et al. Treatment challenges and investigational opportunities in autoimmune hepatitis. Hepatology 2005;41(1):207–215
28. Mieli-Vergani G, Vergani D. Autoimmune hepatitis in children. Clin Liver Dis 2002;6(3):623–634
29. Czaja AJ, Freese D; KAmerican Association for the Study of Liver Disease. Diagnosis and treatment of autoimmune hepatitis. Hepatology 2002;36(2):479–497
30. Czaja AJ, Menon KV, Carpenter HA. Sustained remission after corticosteroid therapy for type 1 autoimmune hepatitis: a retrospective analysis. Hepatology 2002;35(4): 890–897
31. Krawitt EL. Autoimmune hepatitis. N Engl J Med 2006; 354(1):54–66
32. Rumbo C, Emerick KM, Emre S, Shneider BL. Azathio- prine metabolite measurements in the treatment of auto- immune hepatitis in pediatric patients: a preliminary report. J Pediatr Gastroenterol Nutr 2002;35(3):391–398
33. Gregorio GV, McFarlane B, Bracken P, Vergani D, Mieli- Vergani G. Organ and non-organ specific autoantibody titres and IgG levels as markers of disease activity: a longitudinal study in childhood autoimmune liver disease. Autoimmunity 2002;35(8):515–519
34. http://www.clevelandclinicmeded.com/medicalpubs/diseasemanageme

nt/hepatology/chronic-autoimmune-hepatitis/ - Google image

35. Treatment of Autoimmune Liver Disease Palak J. Trivedi, BSc (Hons), MBBS, MRCP, Gideon Hirschfield, MB BChir, FRCP, PhD Ther Adv Chronic Dis. 2013;4(3):119-141.
36. Danielsson, A. and Prytz, H. (1994) Oral budesonide for treatment of autoimmune chronic active hepatitis. *Aliment Pharmacol Ther* 8: 585–590.
37. Czaja, A. and Lindor, K. (2000) Failure of budesonide in a pilot study of treatment-dependent AIH. *Gastroenterology* 119: 1312–1316.
38. Manns, M., Woynarowski, M., Kreisel, W., Lurie, Y., Rust, C., Zuckerman, E. *et al.* (2010b) Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. *Gastroenterology* 139: 1198–1206.
39. Mederacke, I., Helfritz, F., Puls, F., Ringe, K., Klempnauer, J., Manns, M. *et al.* (2012) Budd-Chiari syndrome after treatment with budesonide in a cirrhotic patient with autoimmune hepatitis. *Ann Hepatol* 11: 143–144.
40. Hlivko, J., Shiffman, M., Stravitz, R., Luketic, V., Sanyal, A., Fuchs, M. *et al.* (2008) A single center review of the use of mycophenolate mofetil in the treatment of AIH. *Clin Gastroenterol Hepatol* 1036–1040.
41. Richardson, P., James, P. and Ryder, S. (2000) Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. *J Hepatol* 33: 371–375.
42. Aw, M., Dhawan, A., Samyn, M., Bargiota, A. and Mieli-Vergani, G. (2009) Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. *J Hepatol* 51: 156–160.
43. Alvarez, F., Ciocca, M., Canero-Velasco, C., Ramonet, M., de Davila, M., Cuarterolo, M. *et al.* (1999) Short-term cyclosporine induces a remission of autoimmune hepatitis in children. *J Hepatol* 30: 222–227.
44. Cuarterolo, M., Ciocca, M., Velasco, C., Ramonet, M., González, T., López, S. *et al.* (2006) Follow-up of children with autoimmune hepatitis treated with cyclosporine. *J Pediatr Gastroenterol Nutr* 43: 635–639.
45. Aqel, B., Machicao, V., Rosser, B., Satyanarayana, R., Harnois, D. and Dickson, R. (2004) Efficacy of tacrolimus in the treatment of steroid refractory autoimmune hepatitis. *J Clin Gastroenterol* 2004; 38: 805–809.

46. Larsen, F., Vainer, B., Eefsen, M., Bjerring, P. and Hansen, B. (2007) Low-dose tacrolimus ameliorates liver inflammation and fibrosis in steroid refractory autoimmune hepatitis. *World J Gastroenterol* 13: 3232–3236.
47. Tannous, M., Cheng, J., Muniyappa, K., Farooq, I., Bharara, A., Kappus, M. *et al.* (2011) Use of tacrolimus in the treatment of autoimmune hepatitis: a single centre experience. *Aliment Pharmacol Ther* 34: 405–407.
48. Vergani D, Alvarez F, Bianchi FB, et al; International Autoimmune Hepatitis Group. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol 2004;41(4):677–683
49. Cotler SJ, Kanji K, Keshavarzian A, Jensen DM, Jakate S. Prevalence and significance of autoantibodies in patients with non-alcoholic steatohepatitis. J Clin Gastroenterol 2004; 38(9):801–804
50. Ma Y, Okamoto M, Thomas MG, et al. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. Hepatology 2002; 35(3):658–664
51. Gassert DJ, Garcia H, Tanaka K, Reinus JF. Corticosteroidresponsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. Dig Dis Sci 2007;52(9):2433–2437
52. Czaja AJ. Autoimmune hepatitis: treatment. (figure 19-1, page 135) In: McNally PR, ed. GI/Liver Secrets Plus. Philadelphia: Mosby Elsevier; 2010
53. Gregorio GV, Portmann B, Karani J, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology 2001;33(3): 544–553
54. Czaja AJ. Frequency and nature of the variant syndromes of autoimmune liver disease. Hepatology 1998;28:360–365
55. Boberg KM, Fausa O, Haaland T, et al. Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of 114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. Hepatology 1996;23:1369–1376 75.
56. Poupon R, Chazouilleres O, Corpechot C, Chretien Y. Development of autoimmune hepatitis in patients with typical primary biliary cirrhosis. Hepatology 2006;44:85–90 76.

57. Czaja AJ. The variant forms of autoimmune hepatitis. Ann Intern Med 1996;125:588–598
58. Senaldi G, Portmann B, Mowat AP, Mieli-Vergani G, Vergani D. Immunohistochemical features of the portal tract mononuclear cell infiltrate in chronic aggressive hepatitis. Arch Dis Child 1992;67(12):1447–1453
59. Longhi MS, Mitry RR, Samyn M, et al. Vigorous activation of monocytes in juvenile autoimmune liver disease escapes the control of regulatory T-cells. Hepatology 2009; February 18 (Epub ahead of print)
60. Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli- Vergani G, Vergani D. Impairment of CD4(þ)CD25(þ) regulatory T-cells in autoimmune liver disease. J Hepatol 2004;41(1):31–37
61. Longhi MS, Ma Y, Mitry RR, et al. Effect of CD4þ CD25þ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. J Autoimmun 2005; 25(1):63–71
62. Longhi MS, Hussain MJ, Mitry RR, et al. Functional study of CD4þ CD25þ regulatory T cells in health and autoimmune hepatitis. J Immunol 2006;176(7):4484–4491
63. Longhi MS, Hussain MJ, Bogdanos DP, et al. Cytochrome P450IID6-specific CD8 T cell immune responses mirror disease activity in autoimmune hepatitis type 2. Hepatology 2007;46(2):472–484
64. Czaja AJ, Freese DK. Diagnosis and treatment of auto- immune hepatitis. Hepatology 2002;36:479–497
65. Hiromi Ishibashi, M.D., Atsumasa Komori, M.D., Shinji Shimoda, M.D., and M. Eric Gershwin, M.D. Guidelines for Therapy of Autoimmune Liver Disease. Volume 27, number 2 2007 215-226
66. Pathology department at Rebro Hospital Zagreb, Croatia. April 2018.

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