A RETROSPECTIVE ANALYSIS OF PATIENTS WITH AUTOIMMUNE HEPATIC DISEASES;

A STUDY FROM CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH).

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Declaration

I, Marc Ilan Ostrofsky, declare that this research project is my own work. It is being submitted for the degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature

Signed at Morningside, Sandton

Date 27/03/19

Presentation arising from MMED

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Abstract

Background: Overlap syndromes (OS) of the autoimmune liver diseases (AILD's) are characterized by the coexistence of features of autoimmune hepatitis (AIH) and features of cholestatic liver disease i.e. primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). There is a paucity of information with regards to characterizing these OS in the South African context.

Aim: Compare the clinical features and outcomes of autoimmune hepatitis primary biliary cirrhosis overlap syndrome (AIH/PBC OS), and autoimmune hepatitis primary sclerosing cholangitis overlap syndrome (AIH/PSC OS) with each other and also to the individual disease entities i.e. AIH, PBC and PSC.

Methods: This was a retrospective record review of patients at the liver clinic at CMJAH with the diagnosis of AIH, PBC, PSC, AIH/PBC OS and AIH/PSC OS during the period 01/01/1990 - 31/12/2016. The demographics, clinical characteristics, biochemical, histological and radiological results as well as treatment and outcomes were collected. A data collection tool was used. The overlap syndromes were compared with the individual diseases and with each other.

Results: There were 97 patients eligible for inclusion in the study. Forty four patients were diagnosed with AIH; 29 with PSC; four with PBC; 10 with AIH/PSC OS and 10 with AIH/PBC OS.

The majority of the cohort were Black i.e. 53 patients; 29 patients were White, 13 patients were Indian and two patients were Coloured. In the AIH/PSC OS group there were a higher proportion of Black patients (6/10). The racial profile of patients in the AIH/PBC OS group was different in that the majority were White (7/10).

The AIH/PSC OS group had the best outcome (50% remission). This was followed by the AIH group (32% remission) and the PSC group where the remission rate achieved was 14%. Importantly, there were no cases of remission in the AIH/PBC OS group.

Conclusions: Based on the results of the current study it can be surmised that ethnicity could influence the development of specific overlap syndromes [AIH/PBC OS (70% White) and AIH/PSC OS (60% Black)]. The liver enzyme profile alone cannot be used to differentiate between the OS. In our population AIH/PSC OS and AIH have better clinical outcomes and a lower complication rate compared both to AIH/PBC and PSC and Autoimmune hepatitis/ primary biliary cirrhosis overlap syndrome has a poorer prognosis than either AIH or PBC alone. Early recognition of the OS may assist in timely use of combination drugs which may improve outcomes
It was difficult to draw any meaningful conclusions from the comparison between PBC and the other diseases as there were only four patients with PBC.

Based on the literature together with the results of this retrospective study, it is important that definitive diagnostic criteria are developed in order to differentiate the autoimmune liver disease overlap syndromes from the separate disease entities. Further large volume prospective trials are warranted to better differentiate between the overlap syndromes and their single disease entities.

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Table of Contents

List of Figures

List of tables

Abbreviations/Nomenclature

AASLD	American Association for the Study of Liver Disease
AIH	Autoimmune Hepatitis
AIH/PBC OS	autoimmune hepatitis primary biliary cirrhosis overlap syndromes
AIH/PSC OS	autoimmune hepatitis primary sclerosing cholangitis overlap syndromes
AILDs	Autoimmune liver diseases
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AMA	Anti Mitochondrial Antibodies
ANA	Anti nuclear antibodies
ANOVA	Analysis of Variance
c-ANCA	Cytoplasmic Anti Neutrophil Cytoplasmic Antibodies
EASL	European Association for the study of Liver Diseases
ERCP	Endoscopic Retrograde Cholangiopancreatography
GGT	Gammaglutamyl Transferase
HCC	Hepatocellular Carcinoma
HLA	Human leukocyte antigen
IAIHG	International autoimmune hepatitis group
IBD	Inflammatory Bowel Disease
IgG	Immungolobulin G
IgM	Immunoglobulin M
INR	International Normalized Ratio
ITP	Immune Thrombocytopenic Purpura
LC1	liver cytosol type 1 antigen
LDL	low density lipoprotein
LKM1	anti/liver kidney microsomal antibody type 1

LKM3	liver/kidney microsomal antibody type 3
MELD	Model for End-stage Liver Disease
MMF	Mycophenilate Mofetil
MRCP	Magnetic Resonance Cholangiopancreatography
MTX	Methotrexate
NSDC	Non suppurative destructive cholangitis
OLT	Orthotopic Liver Transplant
OS	Overlap Syndromes
p-ANCA	Perinuclear Anti-Neutrophil Cytoplasmic Antibodies
PC	Paris Criteria
PBC	Primary Biliary Cirrhosis
PSC	Primary Sclerosing Cholangitis
PTT	Partial Thromboplpastin Time
RA	Rheumatoid Arthritis
RDC	Revised Diagnostic Criteria
SDC	Simplified Diagnositic Criteria
SLA/LP	anti-soluble liver antigen/liver pancreas antibodies
SLE	Systemic Lupus Erythematosis
SMA	Smooth muscle antibodies
SPSS	Statistical Packages for Social Sciences
STD	Standard Deviation
U/S	Ultrasonography
UC	Ulcerative Colitis
UDCA	Ursodeoxycholic acid
ULN	Upper Limit of Normal

CHAPTER 1:
INTRODUCTION
AND LITERATURE REVIEW

1.1 Introduction

There are three major hepatic autoimmune diseases; AIH, PBC and PSC. The co - occurrence of AIH and features of cholestatic liver disease has been termed OS. The term OS implies the presence of AIH and either PBC or PSC (1, 2). The major clinical relevance of these OS is their failure to respond in a consistent fashion to conventional corticosteroid therapy. The OS are clinical accounts rather than pathological descriptions and the overriding component of the disease determines its designation and choice of therapy (3).

A literature review of AIH/PBC OS and AIH/PSC OS in the Southern African context revealed a dearth of information. We therefore chose to review our cohort of patients to investigate whether their characteristics, treatment and outcomes are in line with the published literature.

1.2 Literature Review

1.2.1. Autoimmune Hepatitis

1.2.1.1 Definition

Autoimmune hepatitis is a non-resolving chronic liver disease that affects mainly women and is characterized by:

- Hypergammaglobulinaemia even in the absence of cirrhosis
- Circulating autoantibodies
- An association with human leukocyte antigens (HLA) DR3 or DR4
- Interface hepatitis on liver histology
- A favourable response to immunosuppression.
- Cirrhosis, liver failure and death if left untreated (4).

1.2.1.2 Epidemiology

In studies from Europe, the incidence is 0,9 to two per 100,000 per year, with a prevalence of 11 to 25 per 100,000. When looking at the prevalence in developing countries, the results of a prospective study show that AIH is not uncommon in India, constituting nearly 3.5% of all chronic liver disease patients. The prevalence of AIH among patients with chronic liver disease in North America is estimated to be from 11 - 23% (5). There is a female predominance (female to male ratio is 4:1). A bimodal age pattern at presentation has been reported. The first occurs during the childhood/teenage years and the second between the fourth and sixth decade of life (2, 6, 7)**.** Current literature reveals an increasing number of patients presenting at an older age. According to a meta - analysis that looked at AIH in the elderly, it was revealed that a significant proportion of the patients diagnosed with AIH are elderly with 1:4 being 60 years of age or older (8).

1.2.1.3 Clinical Manifestations

The spectrum of clinical manifestations is variable, ranging from an asymptomatic presentation to acute hepatitis, to fulminant liver failure. One third of patients present with one or more of the following non-specific symptoms: fatigue, general ill health, right upper quadrant pain, lethargy, malaise, anorexia, weight loss, nausea, pruritus, fluctuating jaundice and polyarthralgia involving the small joints without arthritis (2, 6, 9, 10).

Acute onset AIH occurs in approximately 25% of patients (11). This includes two different clinical entities: acute exacerbation of chronic AIH and genuine acute AIH without chronic histological changes (11).

Up to one third of patients are completely asymptomatic and the diagnosis is established during investigation for unexplained elevation of serum aminotransferases on routine testing (2, 6, 12). Approximately one third of patients have developed liver cirrhosis at the time of diagnosis suggesting chronic liver disease. This results in a poorer prognosis irrespective of the presence or absence of symptoms (2, 6, 13).
Physical findings range from completely normal, to features of chronic liver disease including palmar erythema, spider naevi, gynaecomastia, testicular atrophy and dupuytrens contractures. In more advanced stages, clinical features of portal hypertension can be

found including ascites, oesophageal varices, portal hypertensive gastropathy, cytopenias secondary to hypersplenism as well as hepatic encephalopathy (4).

1.2.1.4 Diagnosis

The diagnosis of AIH has been guided by a scoring system. This system was first published by a group of experts in the international autoimmune hepatitis group (IAIHG) in 1993. It was then revised in 1999 and called the revised diagnostic criteria (RDC) for AIH, and most recently a simplified scoring system i.e. simplified diagnostic criteria (SDC) has been proposed (14-16)**.** Refer to appendices A and B

Three subtypes of AIH have been proposed according to the pattern of autoantibodies detected:

- AIH - 1:
    - The most common type of AIH (accounts almost for 90% of AIH cases);
    - Characterized by the detection of antinuclear antibodies (ANA), anti-smooth muscle antibodies (SMA) or anti-soluble liver antigen/liver pancreas antibodies (SLA/LP);
    - Association with HLA DR3, DR4 and DR13;
    - Any age at onset;
    - Variable clinical and histopathological severity;
    - Good response to treatment but inconstant relapse rates after drug withdrawal and
    - May need long term maintenance therapy.
- AIH - 2:
    - Accounts for up to 10% of AIH cases;
    - Characterized by the detection of anti-liver-kidney microsomal antibody type 1 (LKM1), antibodies against liver cytosol type 1 antigen (LC1) and rarely anti liver kidney microsomal antibody type 3 (LKM3);
    - Association with HLA DR3 and DR7;
    - Onset usually in childhood and young adulthood;
    - Clinical and histopathological severity commonly acute and advanced;
    - Poor response to treatment and high relapse rates after drug withdrawal; need for long-term maintenance therapy very common.
- AIH - 3:
    - SLA/LP positive, often Ro52 antibody positive
    - Very similar to AIH - 1
    - Possibly more severe (15-20).

The validity of the sub classifications noted above is not conclusive as yet and requires further clarification (17).

Typical liver histology results include all of the following features:

- Interface hepatitis (hepatitis at the portal - parenchymal interface) with dense plasma cell - rich lymphoplasmocytic infiltrates,
- Hepatocellular rosette formation,
- Emperipolesis (active penetration by one cell into and through a larger cell) hepatocyte swelling and/or pycnotic necrosis.
  However it is important to note that there is no morphological feature that is pathognomonic for AIH (21, 22).

1.2.1.5 Associated Diseases

Autoimmune hepatitis is associated with a wide variety of extrahepatic autoimmune disorders. Up to 43 % of patients with AIH report a family history of another autoimmune disease in their first-degree relatives (2, 6, 10, 23, 24). The disorders include: Hashimoto thyroiditis - the strongest association, Grave's disease, vitiligo, alopecia, rheumatoid arthritis (RA), diabetes mellitus type 1, inflammatory bowel disease (IBD), psoriasis, systemic lupus erythematosus (SLE), Sjögren's syndrome, celiac disease, panniculitis, mononeuritis, urticaria pigmentosa, Sweet syndrome, idiopathic thrombocytopenic purpura (ITP), polymyositis, hemolytic anemia and uveitis.
A rare form of AIH occurs in 10 - 18 % of patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy. This condition is also known as autoimmune polyglandular syndrome type one. It is characterized by hypoparathyroidism, candidiasis, adrenal insufficiency, and gonadal failure.

1.2.1.6 Treatment

The primary objective in terms of treatment of AIH is to achieve biochemical and histological remission in order to prevent progression to chronic liver disease. Prednisone as initial therapy followed by the addition of azathioprine after two weeks is the standard first line treatment of AIH. The initial dose of prednisone is 0,5 - 1mg/kg/day. Azathioprine can be initiated whenever bilirubin levels are below six mg/dl (100 µmol/L) and ideally two weeks after the initiation of steroid treatment. The initial dosage should be 50 mg/day, and increased depending on toxicity and response up until a maintenance dose of 1 - 2 mg/kg is achieved.

According to the 2010 AASLD AIH guideline:

- Biochemical remission is defined as normalization of IgG, bilirubin and transaminases. Histological remission is defined as normal histology or minimal hepatitis (histology activity index less than four or equivalent)
- Incomplete response is defined when there is no progression in clinical, laboratory and histological findings within three years of treatment, despite good compliance.
- Treatment failure is defined as deterioration in the patient's clinical, biochemical and histological features despite good compliance, as well as the development of hepatic decompensation (19).

Immunosuppressive treatment should be continued for at least three years from the time of diagnosis. The immunosuppressive therapy can only be weaned two years after complete remission has been achieved. The current choices of second line immunosuppressive therapy include mycophenolate mofetil (MMF) and calcineurin inhibitors (cyclosporine or tacrolimus) (25).

1.2.1.7 Complications

The complications seen in AIH are the same as those that occur in any chronic, progressive liver disease. In the acute setting, fulminant liver failure and infectious complications predominate (4). The major concern is the development of liver cirrhosis in chronic liver disease. The complications associated with cirrhosis are portal hypertension and hepatocellular cell carcinoma (HCC). Hepatocellular cell carcinoma development does occur in AIH associated cirrhosis, but less frequently than in other chronic diseases (26). Ngu et al showed that patients with AIH have an increased risk of developing hepatic and extra hepatic malignancies (27). Extra hepatic malignancies were noted to occur in five % of patients in an unpredictable fashion with non - melanoma skin cancers being the most common. It is likely that this increased risk is due to the prolonged use of immunosuppressive agents (27, 28).

Drug related complications are also noteworthy and can occur in up to one quarter of patients. These occur frequently in patients who have used corticosteroids for a prolonged period; patients on azathioprine (related to toxicity) or drug intolerance (4).

1.2.2 Primary Sclerosing Cholangitis

1.2.2.1 Definition

Primary sclerosing cholangitis is a chronic progressive disorder defined as the presence of beading and stricture formation of the intra and/or extrahepatic bile ducts that cannot be ascribed to another cause, thus differentiating PSC from secondary sclerosing cholangitis (29).

1.2.2.2 Epidemiology

- Approximately 60 % of patients with PSC are male. The median age at diagnosis is 41 years. The incidence ranges from 0 - 1,3 cases per 100,000 persons per year, and the prevalence ranges from 0 - 16,2 cases per 100,000 persons (30). In the United States, approximately 29,000 patients have this disease (31). In a prospective study conducted in Cuba, which investigated the epidemiology, clinical characteristics and treatment outcomes of various AILD's, only 2,8 % of the cohort had PSC (32). In developing countries, the prevalence of PSC is poorly documented and probably underestimated. The likely reason for this is the lack of access to advanced health care as the diagnosis cannot be confirmed without advanced imaging in the form of magnetic resonance cholangiopancreatography/endoscopic retrograde cholangiopancreatography (MRCP/ERCP) (33, 34).

1.2.2.3 Clinical Manifestations

About 50% of patients are asymptomatic at the time of diagnosis. The symptoms, when present, that predominate are: abdominal pain (20%), pruritis (10%), jaundice (6%) and fatigue (6%.) The most frequent signs at diagnosis are hepatomegaly (44%) and splenomegaly 39%) (35, 36).

1.2.2.4 Diagnosis

The diagnosis of PSC is based on biochemical and radiological features including:

- Increased alkaline phosphatase (ALP) levels persisting for more than six months
- Cholangiographic evidence of bile duct strictures on imaging (MRCP/ERCP).
- Exclusion of causes of secondary sclerosing cholangitis (37).
- The presence of additional serological markers which are not diagnostic including: hypergammaglobulinaemia in 30%, increased serum immunoglobulin M (IgM) in 40 - 50%, perinuclear anti neutrophil cytoplasmic antibodies (p - ANCA) in 30 - 80%. (38) and HLA DRw52a in 0 - 100% (39).

  In terms of diagnosing PSC, a liver biopsy usually is not required, unless small-duct PSC is suspected or if there are concerns that a patient also has AIH. Histologic features of PSC are often nonspecific and prone to sampling variations, due to the heterogeneous involvement of the biliary tree. The classic description of concentric ductal fibrosis ("onion skinning") involving bile ducts within portal tract areas is rarely encountered in clinical practice (40).

There are several subtypes of PSC that have been defined. The classic sub type accounts for 90% of patients with PSC and involves the entire biliary tree (small and large ducts). Small duct disease accounts for approximately five % of patients with PSC and affects small intrahepatic ducts. Autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome accounts for the remaining five % of patients with PSC in the adult population (41).

1.2.2.5 Associated diseases

- Inflammatory Bowel Disease: The prevalence of IBD in patients with PSC approaches 90 %. Ulcerative colitis (UC), Crohn's disease , an indeterminate colitis of the colon account for 80%, 10% and 10%, respectively (42).
- Osteoporosis

1.2.2.6 Treatment

- Liver transplantation is the treatment of choice for patients with advanced liver disease due to PSC, and patients should generally be referred for liver transplantation once their Model for End - stage Liver Disease (MELD) score is ≥15 (43). Appendix D
- The guidelines for medical treatment of PSC are contentious regarding the use of UDCA.
    - The European Association for the study of Liver Diseases (EASL) endorses the use of moderate doses of UDCA (13 to 15mg/kg) (44).
    - The American Association for the Study of Liver Diseases (AASLD) and the American College of Gastroenterology do not support the use of UDCA (43).
- No other immunosuppressive agents have been proven to positively affect the disease progression.

1.2.2.7 Complications

- Gallbladder disease (cholelithiasis, cholangitis, cholangiocarcinoma, gallbladder carcinoma)
- Metabolic bone disease (osteoporosis)
- Fat soluble vitamin deficiencies
- HCC in patients with liver cirrhosis (45).
- Colon carcinoma. (The risk of colon carcinoma among patients with PSC and concomitant IBD is four times as high as the risk among patients with IBD alone and 10 times as high as the risk in the general population) (46).

1.2.3 Primary Biliary Cirrhosis

1.2.3.1 Definition

Primary biliary cirrhosis is a chronic inflammatory autoimmune disease characterized by a T cell mediated attack on the cholangiocytes of the small intralobular bile ducts of the liver. The sustained loss of intralobular bile ducts leads to cholestasis. In the absence of treatment, PBC generally progresses to cirrhosis and later liver failure over a period of 10 - 20 years (47).

1.2.3.2 Epidemiology

The incidence of PBC ranges from 0,7 - 49 per million per year and the prevalence from 6,7 - 402 per million (48). It is likely that substantive geographical differences exist both in terms of genetic susceptibility and environmental factors that potentially trigger the disease in genetically susceptible individuals. In a study evaluating the hypothesis that environmental toxins may trigger PBC in genetically susceptible individuals, it was found that patients living closer to toxic waste sites had a higher incidence of PBC (49).
The ratio of women to men is 10:1. Most patients are diagnosed between the ages of 30 and 65 years (47).

1.2.3.3 Clinical Manifestations

According to the literature, 50 - 60% of patients with PBC are asymptomatic at time of diagnosis**.** When symptomatic, fatigue and pruritus are the most common symptoms. In newly diagnosed patients, approximately one half complain of fatigue and one third pruritus (50). Patients with PBC may report right upper quadrant discomfort, which was noted by eight % of patients in one study (51).

12.3.4 Diagnosis

The diagnosis is based on the following three criteria:

- Elevation of serum ALP and gammaglutamyltranspeptidase (GGT) levels.
- Presence of anti - mitochondrial antibodies (AMA) with M2 (mitochondrial 2 antigen complex) specificity.
- Non suppurative destructive cholangitis (NSDC) on histology.

Any two of the above criteria are sufficient for the diagnosis considering the high specificity of the anti - M2 antibody and NSDC (44).

The effects of NSDC are predominantly seen in the interlobular and septal bile ducts. The term “florid duct lesion” is used to describe the inflammatory changes and necrosis seen around bile ducts. Lymphocytes and mononuclear cells form the bulk of the inflammatory infiltrate. These cells are adjacent to the basal membrane of the cholangiocytes undergoing necrosis. Occasionally epithelioid granulomas are identified in the early stage of disease (50).

There are three subtypes of PBC:

- The typical or classical subtype is represented by the slowly progressive decline of small bile ducts and parallel increase in liver fibrosis, leading to biliary cirrhosis over a period of 10 – 20 years (47).
- The second subtype involves overlapping features with AIH. It affects 10 - 20% of patients. These patients have a more severe clinical course with earlier development of liver fibrosis and liver failure (52).
- The third subtype is known as the premature ductopenic variant. It occurs in 5 - 10% of patients. It is characterized by a very rapid onset of ductopenia and severe icteric cholestasis, progressing very quickly towards cirrhosis in less than five years (53).

1.2.3.5 Associated Diseases

The following outlines the most common diseases associated with PBC:

- Approximately 40 - 65 % of patients have symptoms of Sjögren's syndrome, including keratoconjunctivitis and/or xerostomia (54).
- Thyroid disease occurs in 10 - 15 % of patients with PBC. The most common form is Hashimoto's thyroiditis (55).
- Approximately 5 - 15 % of patients with PBC have limited cutaneous scleroderma, which is frequently associated with anticentromere antibodies (56).
- RA develops in 5 - 10 % of patients with PBC (57).
- Hypercholesterolemia with increased low density lipoprotein (LDL) cholesterol is observed in about 20% of the patients (47).
- Patients with PBC are at increased risk for osteoporosis and osteopenia (58).

1.2.3.6 Treatment

Ursodeoxycholic acid is currently the mainstay of treatment in patients with PBC. The recommended dose is 13 - 15mg/kg/day. In both Europe and North America, the number of liver transplants for PBC is falling in parallel with the increased use of UDCA therapy (59).

The response to UDCA is assessed according to the Corpechot Criteria. Treatment is regarded as effective when serum ALP is less than three times ULN, AST less than two times ULN and serum bilirubin ≤17µmol/L after one year of UDCA treatment (60).

Patients who respond sub optimally to UDCA, or those who have concurrent features of AIH, interface hepatitis and increased bilirubin levels may warrant the use of adjuvant therapies. Currently, glucocorticoids (prednisone or budesonide) and methotrexate (MTX) could be considered for these patients.

A combination of glucocorticoids, particularly budesonide (six to nine mg/kg), and UDCA leads to both a better biochemical response and histological response in terms of inflammation and fibrosis in de novo PBC patients (61).

Methotrexate (MTX) improved biochemical test results and liver histological findings when it was added to UDCA in patients who had an incomplete response to UDCA (62).

Examples of some novel therapies being investigated are; peroxisome proliferator - activated receptor alpha agonists, farnesoid x receptor agonists, and biotherapies such as anti CD20 antibodies, glucagon - like protein - 1 receptor agonists, and oestrogen - alpha receptor agonists (47).

Liver transplantation has greatly decreased the mortality rate in patients with PBC. It is the only effective treatment for those with decompensated cirrhosis or liver failure (63).

It has been found that PBC recurs in about 20% of patients at five years (64).

1.2.3.7 Complications

- Hyperlipidaemia - High density lipoprotein (HDL) cholesterol is increased out of proportion to low density lipoprotein (LDL) cholesterol and therefore patients are not at increased risk of death from atherosclerosis (65).
- They may have decreased bile acid secretion resulting in increased risk of lipid malabsorption. However, despite this, clinically significant deficiencies of the fat soluble vitamins are rarely seen (66).
- Iron deficiency anaemia has been described in some patients with early stages of PBC. Some of these patients have unexpectedly severe portal hypertension, despite normal or nearly normal serum bilirubin and albumin concentrations and no evidence of cirrhosis on liver biopsy. The reason for this is thought to be due to nodular regenerative hyperplasia of the liver. These patients may have intermittent occult bleeding from congestive gastropathy or esophageal varices, rather than the massive upper gastrointestinal hemorrhage usually associated with portal hypertension (67).
- Osteoporosis is common and occurs in approximately one third of patients (68).
- Portal hypertension and liver failure secondary to liver cirrhosis occurs late in the course of PBC (69).

1.2.4 Autoimmune Hepatitis Overlap Syndromes

1.2.4.1 Definition

Autoimmune hepatitis overlap syndrome refers to the co - occurrence of AIH with features of a cholestatic liver disease. The term AIH/OS refers specifically to the coexistence of AIH and PBC or PSC.

There are five postulated explanations for AIH - PBC or AIH - PSC OS:

- The sequential presentation of two AILDs
- Concomitant occurrence of two distinct AILDs
- A clinico - pathologic midpoint in a continuum ranging from pure AIH to pure cholestatic AILDs
- OS are distinct entities on their own, with a variety of autoimmune manifestations presenting in a susceptible individual
- A primary AILD with one or more characteristics of another AILD.

The consensus among experts reviewing the evidence for OS favoured the fifth explanation (70).

The prevalence of AIH/OS is not well defined as there are no internationally recognized diagnostic criteria. The most recent IAIHG's critical review on OS concluded that current diagnostic criteria were arbitrary, lacked discriminate power and misused the RDC and SDC to diagnose AIH. They recommend that patients suspected as having AIH/ OS be classified on the basis of their primary disease as having AIH, PBC or PSC. The therapeutic modalities used should be based on the primary disease (70, 71).

1.2.5 Autoimmune Hepatitis/Primary biliary Cirrhosis Overlap Syndrome

1.2.5.1 Diagnosis

Several criteria have been proposed for the diagnosis of AIH/PBC OS; however none of these criteria have been independently validated.

The most widely used criteria come from two research groups: the so called ‘Paris Criteria’ (PC) proposed by Chazouillères et al in 1998 and the RDC and SDC for the diagnosis of AIH proposed by IAIHG (72).

The PC for AIH/PBC OS requires the presence of at least two of the three accepted criteria for diagnosis of PBC and AIH.

The diagnostic criteria for PBC are as follows:

- Serum ALP level twofold or greater the upper limit of normal (ULN) or serum GGT levels fivefold or greater the ULN;
- Positive test for AMA; and
- Liver biopsy specimen showing florid bile duct lesions.

The diagnostic criteria for AIH are as follows:

- Serum alanine aminotransferase (ALT) levels fivefold or greater the ULN;
- IgG levels twofold or greater the ULN or a positive test for SMA;
- Liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis (72, 73).

1.2.5.2 Epidemiology

In the two largest studies using the PC the prevalence of PBC/AIH OS among PBC patients ranged between 4,8 - 9,2%. In studies applying the revised IAHG scoring system to patients with PBC, the prevalence of PBC/AIH OS varied considerably between 2.1 - 19% (71).

In a retrospective study performed at the Mayo Clinic in 2002, whereby the revised IAHG scoring system was applied to 137 PBC patients, the prevalence of PBC/AIH OS was 19%. After eliminating scores for either female gender or the presence of other autoimmune diseases, the prevalence was reduced to four %. This observation highlights the fact that several criteria in the scoring system award positive scores for features that are common to both AIH and PBC and thus lack discriminative power (74).

According to a study done in Rotterdam in 2010, which looked at the effectiveness of the PC in diagnosing AIH/PBC OS, it was concluded that the sensitivity of the PC for the OS was 92% and the specificity was 97%. The sensitivity and specificity of the revised and simplified AIH scoring systems are considerably lower (73).

In the African context, a study was performed in Tunisia, whereby 36 patients with PBC were collected over a 15 year period (1995 - 2009). One third of the patients were diagnosed with AIH/PBC OS using the PC (75).

1.2.5.3 Clinical and Laboratory Characteristics

The median age of onset is generally consistent in the literature. It ranges between 50 and 55 and is similar to the median age of onset of both AIH and PBC (76, 77). However in the study by Huergue et al, the patients with AIH/PBC OS were significantly younger compared to PBC patients (median age 44 vs 59 years in PBC patients) (78). Common clinical findings are fatigue, jaundice and pruritis (72). Patients with AIH/PBC OS have a specific biochemical profile. Baseline serum transaminases and IgG levels in patients are similar to patients with AIH, and the ductal enzymes (ALP and GGT) are similar to patients with PBC (72, 73). Liver biopsies in patients may show features of one or both diseases. The histologic features are as follows:

- Interface or panacinar hepatitis with or without plasma cells;
- Non - destructive lymphocytic cholangitis;
- Destructive lymphocytic or granulomatous cholangitis;
- Ductopenia, portal and/or acinar granulomas;
- Biliary piece - meal necrosis (79).

In the study by Huergue et al, the biopsies showed interface hepatitis in 86% and lymphocytic cholangitis in 93% of patients with AIH/PBC OS (78).

1.2.5.4 Associated Autoimmune Conditions

Efe et al studied 71 patients with AIH/PBC OS in an attempt to document associated extra hepatic autoimmune diseases. The following conditions were noted:

- Thyroid diseases (18%)
- Sjögren syndrome (8%)
- Celiac disease (4%)
- Psoriasis (4%)
- RA (4%)
- Vitiligo (3%)
- SLE (3%)

A number of other autoimmune diseases occurred in single patients. These included: Autoimmune haemolytic anaemia; antiphospholipid syndrome; Multiple sclerosis; membranous glomerulonephritis; sarcoidosis; systemic sclerosis and temporal arteritis. The authors concluded that there is a significant correlation between AIH/PBC OS and the autoimmune diseases mentioned above (80).

1.2.5.5 Treatment and Outcomes

Despite the lack of randomized controlled trials, EASL guidelines, based on the results of small series, have recommended adding steroids either at the time of diagnosis of AIH/PBC OS or in the case of inadequate biochemical response to UDCA after a period of three months (4).

The results of a multicentre study comparing treatment with UDCA monotherapy vs. combination therapy of UDCA and immunosuppression (prednisone +/- azathioprine) in 88 patients with PBC/AIH OS are as follows.

- There was no response to therapy in the patients who received UDCA monotherapy (37%)
- The UDCA / immunosuppression combination was effective in most patients (73%) who were untreated or had not responded to UDCA alone.
- UDCA monotherapy and combination therapy had similar efficacy in patients with moderate interface hepatitis (80%). The efficacy was determined biochemically.
- UDCA monotherapy was relatively ineffective in patients with severe hepatitis.
- Patients with severe hepatic fibrosis did not respond well to combination therapy.
- Approximately half of the patients who failed to respond to initial immunosuppression did well on second line immunosuppressive agents including cyclosporine, tacrolimus, and MMF (81).

A retrospective study conducted by Chazouillères et al compared the treatment modalities (UDCA alone with a UDCA/ immunosuppression combination) and outcomes in 17 patients with AIH/PBC OS over a median of seven and a half years. The two groups had similar clinical presentations.

- The first group comprised 11 patients who were initially treated with UDCA alone. Three patients achieved biochemical response accompanied by stable or decreased fibrosis. The remaining eight patients were termed non - responders and seven of these patients received combination therapy subsequently. Six of the seven patients who received secondary combination therapy achieved a biochemical response with no further increase of their fibrosis.
- In the second group there were six patients; each of whom received combination therapy (UDCA and immunosuppression). Four patients (all non - cirrhotic) achieved a biochemical response along with non - progression of fibrosis (82).

- The progression of fibrosis was more marked in non - cirrhotic patients on UDCA monotherapy than in patients on the combination of UDCA and immunosuppressive drugs which led the authors to conclude that the combination of UDCA and immunosuppressive drugs appears to be the best therapeutic option for strictly defined PBC/AIH OS.

A study done at Mayo Clinic in 2007 looked at patients that had attended over a median of five and three quarter years of follow up appointments. It was found, that patients with AIH/PBC OS more frequently developed portal hypertension, features of decompensated disease and progressed to transplantation or death when compared to patients with AIH alone (77).

In a study conducted by Joshi et al, outcomes and response to treatment in patients with PBC and AIH/PBC OS were compared. A total of 331 patients with AMA positive PBC were included. 16 of the 331 patients had overlapping features of AIH. The biochemical and histological responses in the 16 patients with OS after two years of UDCA alone were similar to the cohort with isolated PBC (83).

In a meta - analysis of eight clinical trials that assessed the efficacy and safety of combination therapy (UDCA and budesonide) compared to UDCA monotherapy, it was found that the frequencies of death, need for liver transplantation and other adverse outcomes were similar in both groups. The combination therapy was significantly superior in improving ALP, ALT and other biochemical markers (84).

1.2.6 Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

This is a syndrome with overt cholangiography or histological findings typical of PSC, alongside robust histological and biochemical features of AIH concurrently or historically (85).

1.2.6.1 Diagnosis

The characteristic findings of PSC on ERCP/MRCP are focal strictures and dilatations of the biliary tree. In patients with features of AIH, together with a cholestatic picture on their liver function test and the above findings on ERCP/MRCP the diagnosis of AIH/PSC OS is warranted.

The histology on liver biopsy may show interface hepatitis with or without plasma cells, portal oedema or fibrosis, ductopenia, ductal tortuosity, ductular proliferation or rarely obliterative fibrous cholangitis (3).

1.2.6.2 Epidemiology

Large duct Primary Sclerosing Cholangitis/Autoimmune Hepatitis Overlap Syndrome

In a study assessing the prevalence of PSC/AIH OS in 114 patients with confirmed PSC, using the original IAIHG scoring system for AIH (1993), it was found that two % of the cohort were scored as definite and 33% as probable AIH. When applying the revised IAIHG scoring system for IAIHG (1999) to the same group of patients, it was found that two % still had definite AIH but there was a significant reduction in the group with probable AIH, i.e. nine %.

Similar results were found in a study of 211 patients with PSC using the above scoring systems for IAIHG. Two % of cases were found to have definite AIH and 19 % probable AIH using the original scoring system. The proportion of probable AIH was reduced to six% when the RDC was applied. In the largest PSC patient series (211 patients) evaluated according to the RDC for AIH, an overall of 7 - 14 % of patients were assessed as having features of AIH (71).

Small duct Primary Sclerosing Cholangitis/Autoimmune Hepatitis Overlap Syndrome

Patients who present with clinical features and a liver biopsy compatible with PSC but with a normal cholangiogram are classified as small duct PSC.

In a study performed in Sweden, which looked at 26 patients with AIH/PSC OS, seven of the 26 patients (27%) had small duct PSC. These seven patients fulfilled the revised IAIHG scoring system criteria for AIH. The diagnosis of small duct PSC in six patients was based only on MRCP. It is possible that the performance of ERCP may have revealed large duct PSC in some or all of these patients. This study concluded that small duct PSC is certainly prevalent in patients with AIH/PSC OS (86).

1.2.6.3 Clinical and Laboratory Characteristics

Autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome should be considered in patients who have clinical features and serological markers of AIH as well as:

- Pruritis
- UC
- Bile duct pathology on liver biopsy (such as portal oedema, cholestasis, and fibrous or obliterative cholangitis)
- Serologic features of cholestasis (ALP more than two times the upper limit of normal)
- No response to corticosteroid therapy
- An abnormal cholangiogram (1).

Overlap syndrome should also be suspected in patients diagnosed with PSC who have high levels of IgG, circulating ANA or SMA (titre > 1:40), and moderate to severe interface hepatitis on liver biopsy (87).

When Comparing seven of 41 PSC patients (17%) diagnosed with AIH/PSC OS and 34 classic PSC patients it was found that the OS patients were significantly younger (mean age 21.4 vs. 32.3) and had higher ALT (357 vs. 83.7 U/L,) and IgG levels (25.6 vs. 12.9mg/dL,) (88).

1.2.6.4 Associated Autoimmune Diseases

Inflammatory bowel disease occurs in approximately 90% of patients with underlying PSC (42). The frequency of IBD in AIH/PSC OS (90%) suggests that PSC is the primary disease. In AIH, IBD is relatively rare. The presence of AIH and UC in the same patient should raise the possibility of underlying PSC (88).

1.2.6.5 Treatment and Outcomes

Varying results have been reported in the treatment of AIH/PSC OS. In early studies, patients had both clinical and biochemical improvement following corticosteroid and azathioprine therapy. Ursodeoxycholic acid was occasionally added to the above regimens (71).

In 1998 Czaja evaluated 225 patients with AILDs. In his cohort there were 86 patients with AIH and nine patients with AIH/PSC OS.

- In the AIH group there were 25 patients on prednisone alone and 61 patients on a combination of prednisone and azathioprine.
  The majority in this sub - group i.e. 55 patients achieved remission.
- In the AIH/PSC OS group there were four patients on prednisone alone and five patients on a combination of prednisone and azathioprine.
  Only two patients achieved remission in the sub - group.

Patients with AIH/PSC OS had poorer outcomes (lower rates of remission; higher rates of liver failure and transplantation) when compared to those with classical AIH (89).

Van Buuren et al reported on nine patients with AIH/PSC OS. All nine patients received a combination of prednisone and azathioprine. The initial response was favourable in the entire cohort. Long - term remission however was obtained in only three patients; and three patients went on to require orthotopic liver transplant (OLT) (90).

In another study across six Swedish University Hospitals looking at patients with AIH/PSC OS, a good response to therapy (as judged by a decrease in aminotransferase levels) was concluded in 16/24 overlap cases treated with corticosteroids and azathioprine (tacrolimus in one). In seven patients in whom it was considered possible to assess the effect of UDCA therapy, remission or a good response on aminotransferase levels was obtained in two cases (86).

A prospective study compared seven patients with AIH/PSC OS to 34 patients with classical PSC. The AIH/PSC OS group were treated with prednisone (initial dose 0.5 mg/kg/day, tapered to 10 – 15 mg/day) and azathioprine (50 – 75 mg/day) plus UDCA (15 – 20 mg/kg/day) whereas the classical PSC group received UDCA alone. Serum AST decreased significantly in the AIH/PSC OS group but other liver biochemical tests were not significantly improved. No significant change in liver biochemical tests was observed in the classical PSC group. There were no deaths in the AIH/PSC OS group compared with nine in the classical group, suggesting improved survival in AIH/PSC OS (88).

There are very few large patient series describing long term outcomes in patients with AIH/PSC OS. A study at the King's College Hospital evaluated the long - term outcomes of patients with AIH/PSC OS overlap over a 35 year period. Two hundred and thirty eight patients with AIH, 10 patients with AIH/PBC and 16 patients with AIH/PSC OS were included in this study.

- In the AIH group all of the patients received combined immunosuppression (prednisone and azathioprine). Two hundred and twenty two of the patients (96 %) showed an initial complete response. Relapse occurred in 132 patients (55 %).
- Eight out of the ten patients (80 %) with AIH/PBC OS were started on standard immunosuppressive therapy and the remaining two patients (20%) were put onto UDCA monotherapy. Five patients (50%) showed an initial complete response. Relapse occured in 20% of the patients.
- In the AIH/PSC OS cohort, 14 patients (88%) were commenced on prednisone +/- azathioprine. One patient (6%) was put onto UDCA monotherapy and one patient (6%) was put onto a combination of UDCA and azathioprine. Fourteen patients (87%) showed an initial complete response. Relapse occurred in 10 of the patients (63%).

The outcomes were worse in the AIH/PSC OS group compared to the other cohorts. In the AIH/PSC OS group seven patients (44%) either demised or required liver transplantation; in the AIH group 57 patients (24%) had similar outcomes and two patients (20 %) in the AIH/ PBC OS group required transplantation or demised. Therefore, AIH/PSC OS patients had a decreased survival rate compared to patients with AIH and AIH/PBC OS on similar immunosuppressive therapy. This was despite most of them having had a good initial response (91).

In a study by Luth et al patients with AIH/PSC OS progressed despite an initial response. After a mean treatment period of 26 months 14/16 patients showed a good biochemical response. However despite this initial biochemical response, more than half of the cohort of patients went on to progress rapidly to cirrhosis (92).

The results of the above studies indicate that the prognosis of AIH/PSC OS patients may be better than in patients with classical PSC, but worse than in patients with AIH alone. Another important finding is that despite showing a good initial biochemical response to therapy, the AIH/PSC OS patients tend to progress to liver cirrhosis over time.
The EASL guidelines recommend that patients with AIH/PSC OS are treated with UDCA and immunosuppressive therapy, but emphasize that this is not evidence based (44). The AASLD guidelines on PSC also recommend the use of corticosteroids and other immunosuppressive agents in patients with AIH/PSC OS (93).

1.2.7 Alternative Immunosuppression for Autoimmune Hepatitis Overlap Syndromes

Mycophenolate mofetil has been used as second line therapy in AIH OS. There have been two reports on the viability of this therapeutic option (94, 95). Remission was based on the definition outlined by Manns et al. (Appendix E).
Wolf et al looked at 16 patients with AIH, AIH/PBC OS, or AIH/PSC OS. Their results were as follows:

- Five (31 %) ➔biochemical remission,
- Seven (44 %) ➔partial remission,
- Two (12.5 %) ➔incomplete responses,
- Two (12.5 %) ➔no response.

The decision to use MMF was based on azathioprine intolerance, non - response to prednisone and azathioprine, or physician preference (94).

Baven - Pronk et al determined the efficacy of MMF as a second line immunosuppressive agent in patients with AIH OS. The primary endpoint of this study was biochemical remission. The patients included were those who either did not respond to or were intolerant of azathioprine. Remission was induced in almost 60% of patients with AIH OS irrespective of whether they were intolerant or non - responsive to azathioprine. Based on the results obtained in this study, MMF is a good alternative immunosuppressive agent in AIH/OS (95).

1.2.8 Problem Statement

A literature review of AIH/PBC OS and AIH/PSC OS in the Southern African context revealed a dearth of information. It was therefore deemed pertinent to review a cohort of these patients in the South African context in order to investigate whether the characteristics, treatment and outcomes are in line with published literature.

The addition of a South African cohort to the literature that currently exists could contribute to the establishment of internationally accepted definitions of the autoimmune hepatitis overlap syndromes, in order to establish standardized diagnostic and management protocols.

Chapter 2: Methodology

2.1 Aims

1. To compare a cohort of patients with AIH/PBC OS and AIH/PSC OS at the liver clinic, at the CMJAH with respect to demography; clinical characteristics; treatment and outcomes.
2. To, retrospectively compare the clinical outcomes of overlap syndromes with those of AIH, PBC and PSC including biochemical characteristics, treatment modalities and responses, progression to cirrhosis and hepatic decompensation.

2.2 Study methods

2.2.1 Research design

This was a retrospective record review of all patients at the liver clinic at CMJAH with the following diagnoses: AIH, PBC, PSC, AIH/PBC OS and AIH/PSC OS. The file review included the period 01/01/1990 - 31/12/2016. The demography, clinical characteristics, biochemical, histological and radiological results were recorded on a data sheet. The treatment regimens and outcomes of patients with the above mentioned conditions were also recorded (Appendix F).

2.2.2 Location

This study was conducted at the liver clinic at the CMJAH which is situated in Johannesburg, South Africa. The CMJAH is one of the main teaching hospitals for the University of the Witwatersrand, faculty of health sciences. The hospital receives referrals from a number of hospitals in its catchment area i.e. Gauteng and neighbouring provinces. The liver clinic is run by consultant gastroenterologists, gastroenterology fellows as well as medical registrars rotating through medical gastroenterology. The Wednesday clinic is designated for patients with all liver diseases ranging from drug induced liver injuries to autoimmune liver conditions.

2.2.3 Study Participants

All patients at the liver clinic at CMJAH with the presumed diagnosis of AIH; PBC; PSC; AIH/PBC OS and AIH/PSC OS during the study period were included. At the liver clinic patients with auto immune liver disease are seen separately. The files of these patients were reviewed.

Exclusion Criteria:

- Patients under the age of 15 at the time of diagnosis
- Chronic liver disease caused by etiologies other than those mentioned above
- HIV infected patients

The records of patients who attend the liver clinic were collected. One hundred and twenty - three patients attended the clinic during this period. Ninety seven patients met the inclusion criteria for this study. Each patient was given a study number in order to maintain anonymity. The relevant data was captured using a data collection tool. Patients with HIV were excluded from this study as we felt that there were too many confounders in these patients regarding their liver pathology. The HIV itself, its treatment and possible concomitant opportunistic infections and the drugs used to treat them can all cause hepatic dysfunction. Therefore patients with AILD and concomitant HIV were excluded from the study. There were approximately 10 patients with HIV and AILD.

2.2.4 Data collection

Data was collected from the liver clinic database at CMJAH utilizing a data collection tool. The demographic data included patients' age; age at diagnosis of disease; gender and race. The diagnosis/ presumed diagnosis were also recorded.
(See Appendix F)

2.2.4.1 Biochemical Results and Serology

Biochemical and serological results were collected from medical records at the time of diagnosis and then subsequently at six months, one year, two years, three years, four years, five years, 10 years, 15years and 20 years after initiating treatment (where applicable).
See Appendix for details of biochemical results obtained
The following titre levels were accepted as a positive result:

- AMA >1:20
- ANA >1:40
- LKM - 1 $\geq$1:40
- SMA >1:80

2.2.4.2 Histology

Histological results of liver biopsies were documented where available. The findings were noted to be typical, atypical or inadequate.
Typical histology results conformed with the typical findings for the relevant conditions as highlighted in the literature review.
Atypical histology findings indicated that only some of the findings were typical.
In some patients the sample was inadequate and the result was termed inadequate.

2.2.4.3 Imaging

The imaging that was assessed consisted predominantly of abdominal ultrasonagraphy (U/S) as well as either MRCP or ERCP. Some CT scans were performed and the relevant results are included.

2.2.4.4 Concomitant Autoimmune disease

The presence of concomitant autoimmune diseases (autoimmune thyroid disease; sjogrens syndrome; coeliac disease; RA; psoriasis; SLE; vitiligo; IBD; ITP; auto immune haemolytic anaemia and diabetes mellitus type 1) was noted.

2.2.4.5 Outcomes

The outcomes of patients in the study were recorded as follows: remission; cirrhosis; portal hypertension; hepatic decompensation; liver transplant; death and lost to follow up.

2.2.4.5.1 Remission

There are varying definitions of remission for the different disease entities. For AIH, biochemical remission, incomplete response and treatment failure were defined according to the AASLD 2010 AIH guideline (19).

With regards to PBC, the response to UDCA was assessed according to the Corpechot Criteria (60).

There are no well - defined criteria when it comes to assessing response to treatment in patients with PSC (45). We defined biochemical response as an improvement in the ductal enzymes.

2.2.4.5.2 Liver Cirrhosis

In this study, cirrhosis was defined based on histological as well as ultrasound findings (96).

2.2.4.5.3 Portal Hypertension

Patients were defined as having portal hypertension based on clinical, radiological and biochemical findings. Clinical findings include: jaundice and ascites. Radiological features on abdominal sonar include: cirrhosis, ascites and splenomegaly. Endoscopic features (where available) include portal gastropathy as well as varices. Biochemical features suggestive of portal hypertension include the presence of jaundice as well as thrombocytopenia. In this study platelet counts were not specifically recorded.

2.2.4.5.4 Hepatic Decompensation

In this study, a patient was regarded as having hepatic decompensation when he/she developed complications of portal hypertension, died as a result of the progression of liver disease or had a liver transplantation.

2.2.4.5.5 Liver Transplant

All patients who received a liver transplant secondary to hepatic decompensation were recorded as per medical records.

2.2.4.5.6 Death

All patients who demised as a result of hepatic decompensation were recorded as per medical records.

2.2.4.5.7 Lost to follow up

Patients were defined as being lost to follow up if it was noted in the medical records that they had not come back to the liver clinic six months or more after their last date, despite being given a follow up date.

2.2.4.5.8 Other

This group of patients included those with ongoing disease, those patients who are currently being worked up for a liver transplant as well as those patients who have been transferred to other centres for follow up.

2.2.5 Ethical Consideration

Permission to conduct the study has been obtained from the Human Research Ethics Committee of the University of Witwatersrand, Clearance Certificate number: M160307 (Appendix G)

2.2.6 Data Analysis

The findings are described and analyzed using descriptive and inferential statistics and percentages are rounded off to one decimal place.

Patients with AIH/PBC OS and AIH/PSC OS were compared to each other and to patients with single disease entities (AIH, PBC and PSC), with respect to demographic profiles, clinical characteristics, treatment and outcomes. The chi square test of proportions was used to compare the proportions of patients, in each of the five groups of presumed diagnoses.

To test whether biochemical results, at diagnosis, differ significantly across presumed diagnosis groups, analysis of variance (ANOVA) was carried out. If variables were normally distributed, the parametric test (ANOVA) was used; otherwise the non - parametric test (Kruskal Wallis) was used.

The Kolmogorov Smirnov test of Normality was carried out to determine whether each of the biochemical results was normally distributed. In this test, the Null hypothesis was that the variables were normally distributed and the alternative hypothesis was that it is not. If the p - value was less than 0.05, we rejected the hypothesis of normality. All the analyses were carried out using the SPSS (Statistical Packages for Social Sciences) version 13 and with the assistance of a statistician.

Chapter 3: Results

3.1 Introduction

A total of 97 files were reviewed. There were 26 (26,8%) males and 71 (73,2%) were females.

3.2 Demographic Data

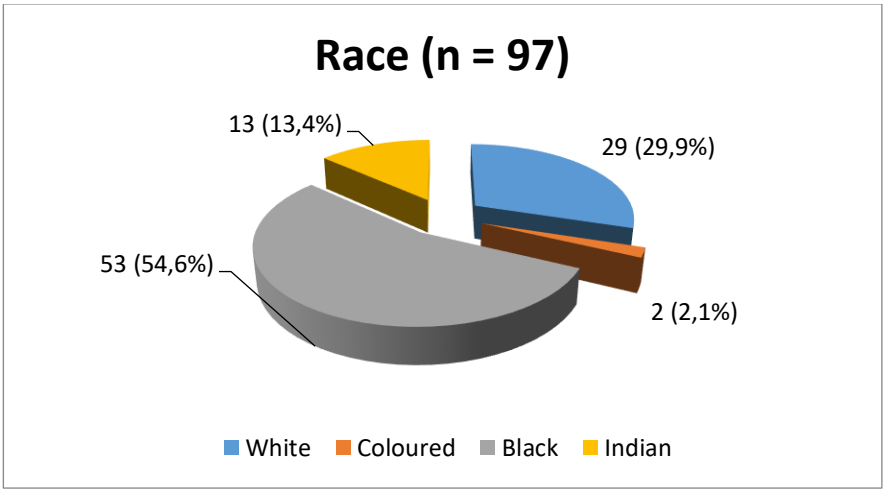


Figure 3.1: renumber the figures Frequency and distribution of the race groups of patients

The racial demographics were as follows: 29 patients (29,9%) were White, 53 patients (54,6%) were Black, 13 patients (13,4%) were Indian and two patients (2,1%) were Coloured.

3.3 Clinical Data

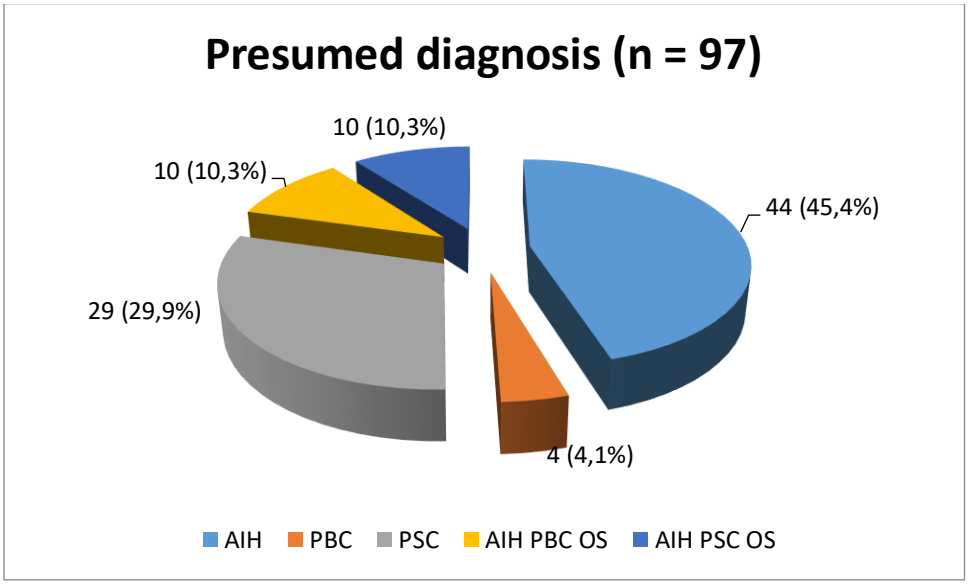


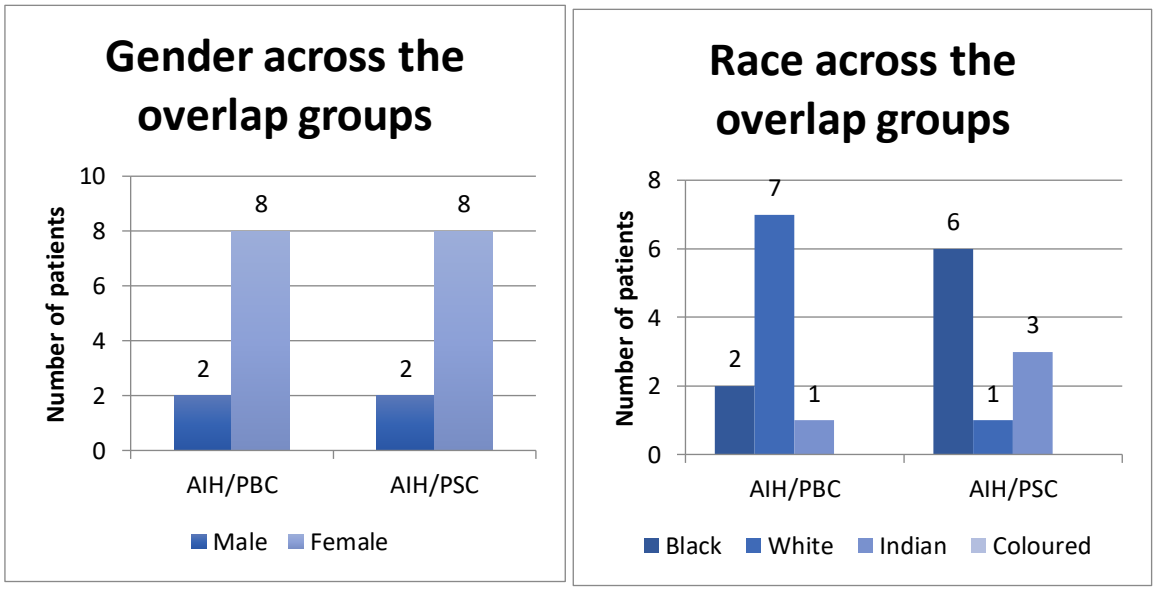
Figure 3.2 Presumed diagnoses

In the study, the majority of subjects i.e. 44 (45,4%) were diagnosed with AIH, four (4,1%) with PBC, 29 (29,9%) with PSC and 20 (20,2%) patients were diagnosed with overlap syndromes: 10 with AIH/PBC OS and 10 with AIH/PSC OS.

3.4.1 Summary: Comparison between the two overlap groups: Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis

The first objective of this study was to compare a cohort of patients with AIH/PBC OS and AIH/PSC OS at the liver clinic, at the CMJAH with respect to demographics, clinical characteristics, treatment and outcomes. In this section, the comparison between the two overlap groups is carried out.

3.4.1.1 Demography



Figures 3.3 and 3.4 Comparison of gender and race between Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes.

From figure 3.3 above, it can be noticed that the proportion of females: males as well as the actual number of patients in each gender were identical in both groups (AIH/PBC OS and AIH/PSC OS).

The racial profile differed between the two groups as seen in figure 3.4. There were a higher proportion of White patients in AIH/PBC OS group. This was in contrast to the AIH/PSC group where there were a higher proportion of Black patients. There were no coloured patients in the study.

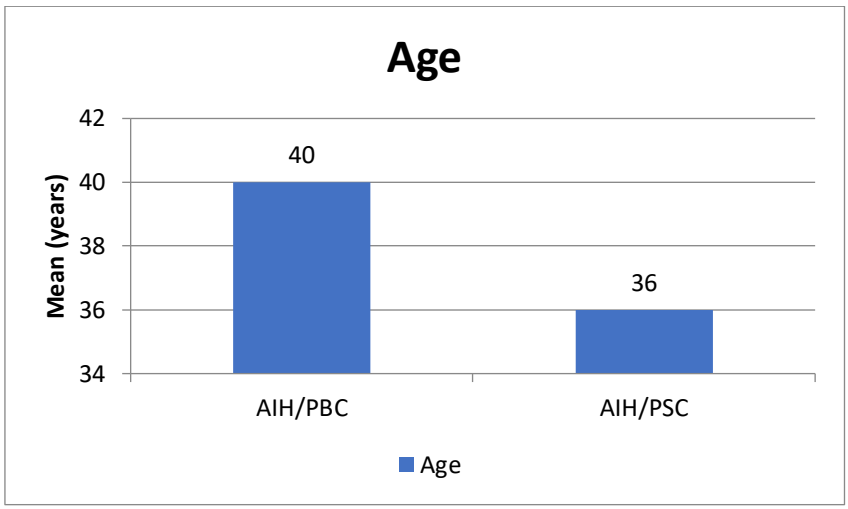


Figure 3.5: Comparison of the average age between the two Overlap Syndromes

There was no significant difference in the age at diagnosis of patients in both groups.

3.4.1.2 Biochemical Profiles

Table 3.1: Comparison of biochemical profiles between Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes

	AIH/PBC OS	AIH/PSC OS	Biochemical profiles (p - value)
Mean ± STD	333,9±236,9	267,5±151,7	
Median (Min – Max)	298 (78 - 752)	287 (0 - 445)	ALP (IU/L)(0,456)
Mean ± STD	589,6±543,6	245,9±216,4	
Median (Min – Max)	397 (73 - 1856)	147 (0 - 662)	GGT (IU/L) (0,079)
Mean ± STD	109,7±78,9	280,1±298,3	
Median (Min – Max)	89 (34 - 300)	165 (0 - 814)	ALT (IU/L) (0,097)
Mean ± STD	120,5±125,8	308,9±365,7	
Median (Min – Max)	69 (43 - 461)	124 (0 - 1125)	AST (IU/L) (0,140)
Mean ± STD	12,8±20,8	26,0±18,5	
Median (Min – Max)	0,0 (0,0 - 47,2)	33,3(0,0 - 45,1)	PPT (Seconds) (0,151)
Mean ± STD	1,0 ± 0,6	1,0 ± 0,6	
Median (Min – Max)	1,0 (0,0 – 1,6)	1,1 (0,0 – 1,7)	INR (0,754)
Mean ± STD	47,5 ± 34,6	96,2 ± 152,6	
Median (Min – Max)	38 (9 – 114)	36 (0 – 453)	Total Bilirubin (µmol/L) (0,338)
Mean ± STD	32,6 ± 32,4	76,8 ± 128,6	
Median (Min – Max)	21 (4 – 102)	23 (0 – 384)	Conjugated Bilirubin (µmol/L) (0,305)
Mean ± STD	8,6 ± 13,3	17,2± 22,5	
Median (Min – Max)	0,0 (0,0 - 39,2)	7,2 (0,0 - 67,2)	Serum IgG (g/L) (0,308)
Mean ± STD	1,3 ± 1,7	1,7 ± 2,3	
Median (Min – Max)	0,0 (0,0 – 4,1)	0,6 (0,0 – 6,6)	Serum IgM (g/L) (0,653)

STD stands for Standard Deviation; Min is the Minimum and Max, the Maximum.

Table 3.1 shows the comparison of biochemical profiles between the two overlap groups. To test whether the differences observed in table 3.1, between the two overlap groups, is statistically significant, the Mann Whitney U test, the nonparametric version of two independent samples, was carried out. The p - values in all the profiles are greater than 0.05. There was no significant difference in the values of biochemical profiles of patients, between AIH/PBC and AIH/PSC.

3.4.1.3 Autoimmune Serological Profiles

Table 3.2: Comparison of autoimmune serological profiles between Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes

Serological profile		
	AIH/PBC OS	AIH/PSC OS
	n(%)	n(%)
Total	10	10
AMA	**3(30,0)**	-
ANA	**5(50,0)**	**3(30,0)**
SMA	**1(10,0)**	**2(20,0)**
LKM - 1	-	-
P - ANCA	1(10,0)	-
C - ANCA	-	**3(30,0)**

Antinuclear antibody and SMA are the two profiles shared by both groups. There are 20,0% more patients with positive ANA results in AIH/PBC OS group than in AIH/PSC OS group. On the other hand, there were 10,0% more patients with positive SMA results in the AIH/PSC OS group than in the AIH/PBC OS group. Antimitochondrial antibody was positive in 30,0% of the AIH/PBC group and c-ANCA was positive in 30,0% of the AIH/PSC OS group.

Only one patient (10,0%) in each OS group had a concomitant disease. The single patient in the AIH/PBC OS group had Sjogrens syndrome and the single patient in the AIH/PSC OS group had IBD (UC).

3.4.1.4 Treatment Regimens

Table 3.3: Comparison of the treatment in Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes

Treatment		
	AIH/PBC OS	AIH/PSC OS
	n(%)	n(%)
Total	10	10
Prednisone	2(20,0)	2(20,0)
UDCA	1(10,0)	1(10,0)
Combination of UDCA and prednisone	2(20,0)	-
Azathioprine	-	3(30,0)
Combination of azathioprine and prednisone	4(40,0)	3(30,0)
Combination of azathioprine/UDCA/and prednisone	7(70,0)	5(50,0)
Liver transplant	1(10,0)	2(20,0)
MMF	-	-
MMF+Prednisone	-	-
Azathioprine+UDCA	-	1(10,0)
MMF+Prednisone+UDCA	-	1(10,0)

In the AIH/PBC OS group, figure 3.7 and tables 3.3 and 3.4, seven of the patients (70,0%) received a combination of azathioprine, prednisone and UDCA. Four of the seven patients were initially put onto a combination of azathioprine and prednisone for a period of time prior to the azathioprine/prednisone/UDCA regimen. Two patients were put onto an azathioprine/ prednisone combination for two years prior to changing to the azathioprine/prednisone/UDCA combination. Regarding the other two patients, one was put onto an azathioprine/prednisone for 11 years and the other 19 years prior to changing to azathioprine/prednisone/UDCA combination. Of the remaining three patients, two were given a regimen containing prednisone and UDCA and the last patient was initially put onto UDCA monotherapy and later received an OLT.

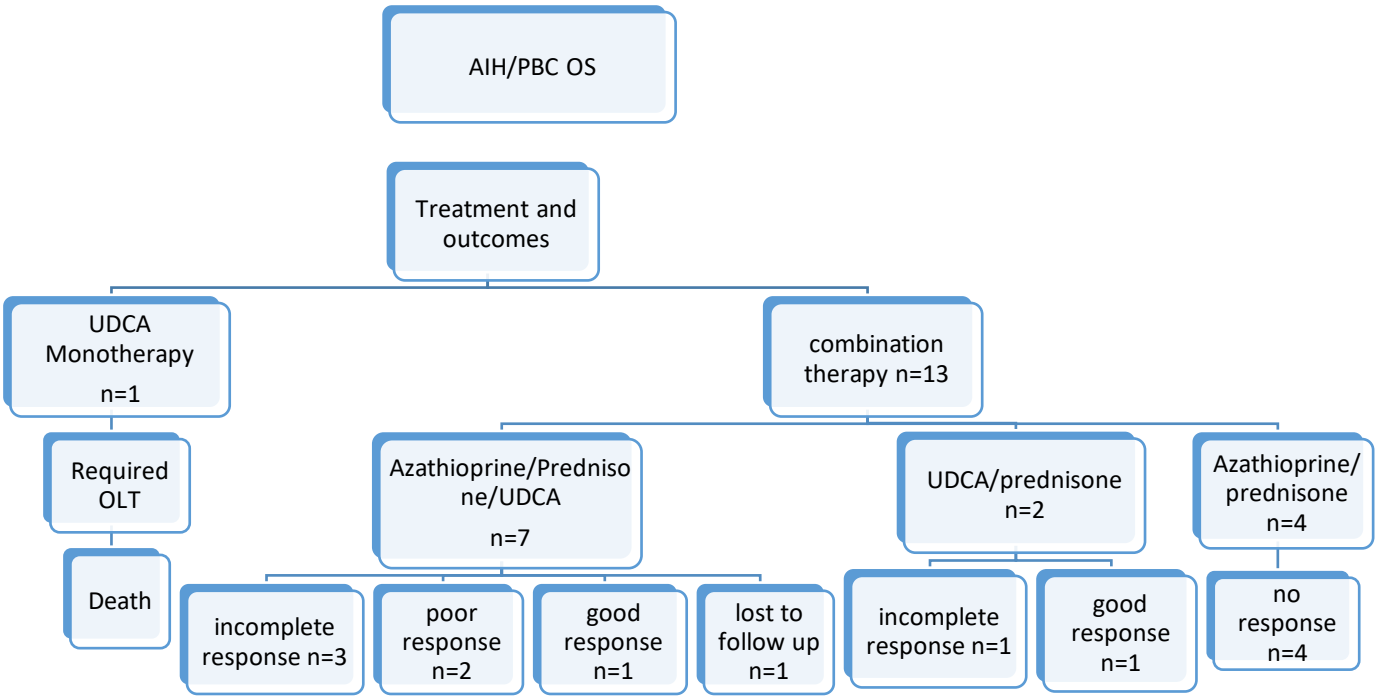


Figure 3.6 Treatment and Outcomes in the Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome group

In the AIH/PSC OS group, figure 7 and tables 3.3 and 3.4, five patients received a combination of azathioprine, prednisone and UDCA. One of the five patients was on the brink of acute liver failure after being on the above combination for one year and was put onto a salvage regimen with azathioprine, prednisone and MMF. This patient went into remission shortly after this. Another of the patient's who was originally on the azathioprine - prednisone and UDCA regimen, went into remission and was weaned to only requiring prednisone. The remaining three patients stayed on the azathioprine - prednisone and UDCA regimen. Two patients were put onto a combination of azathioprine and prednisone. Both of these patients went into remission. One patient was weaned successfully from this regimen to only requiring azathioprine. One patient was given UDCA monotherapy. There were two patients who received OLT's. It is important to note that one of the patients who received an OLT was initially diagnosed with AIH and subsequently developed AIH/PSC post liver transplant.

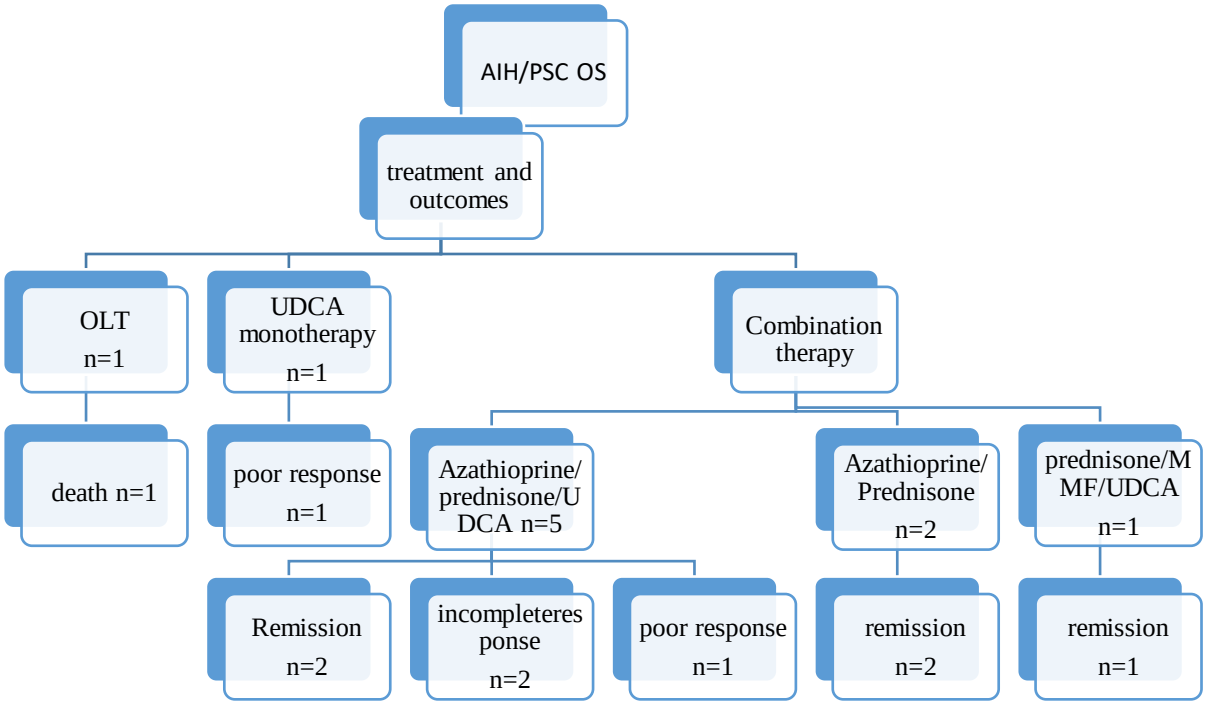


Figure 3.7 Treatment and outcomes in the Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome group

3.4.1.5 Outcomes

Table 3.4: Comparison of outcomes in Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes

Outcomes		
	AIH/PBC OS	AIH/PSC OS
	n(%)	n(%)
Total	10	10
Remission	-	**5(50,0)**
Liver cirrhosis	7(70,0)	4(40,0)
Portal hypertension	7(70,0)	4(40,0)
Hepatic decompensation	1(10,0)	2(20,0)
Liver transplant	1(10,0)	2(20,0)
Death	1(10,0)	1(10,0)
Lost to follow up	1(10,0)	-
Ongoing disease	4(40,0)	2(20,0)
Worked up for transplant	1(10,0)	1(10,0)
Transferred out	-	-

Seventy per cent of the AIH/PBC OS group developed cirrhosis and portal hypertension whereas only 40,0% of the AIH/PSC OS group developed these outcomes. Equal number of deaths occurred in both groups (10,0%).

Liver cirrhosis is present in AIH/PBC OS at diagnosis in 50,0% of the patients and in AIH/PSC at diagnosis in 40,0% of the patients. One patient in the AIH/PBC group developed cirrhosis after being on treatment for five years and another patient in the AIH/PBC group developed cirrhosis after being on treatment for 10 years.

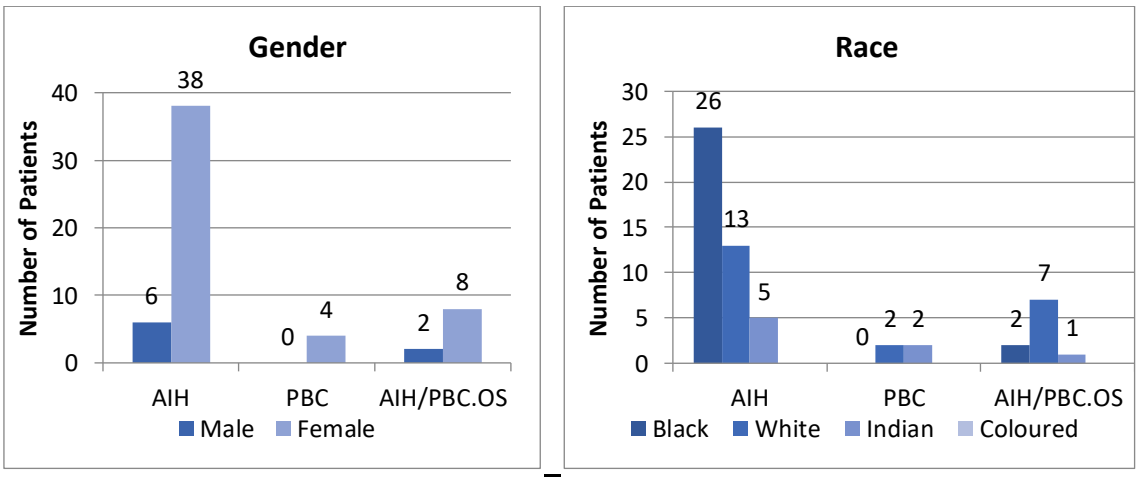
3.4.2 Comparing Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome with Autoimmune Hepatitis and Primary Biliary Cirrhosis

3.4.2.1 Demographic Profiles

Figures 3.9 and 3.10 below show the distributions of gender and race within the AIH, PBC and AIH/PBC OS groups.. In the AIH group, there were 44 patients of which 26 (59, 1%) were Black, five (11,4%) were Indian and 13 (29,5%) were White. Thirty Eight patients (86,4%) were female and six patients (13,6%) were male. The average age at diagnosis in the AIH group was 37 years of age. The average follow up period in the AIH group was five years.

In the PBC group, there were a total of four patients. Two patients were Indian and two were White. All four patients with PBC were female and the average age at diagnosis was 58 years of age. The average follow up period in the PBC group was seven and a half years.

In the AIH/PBC OS group there were a total of 10 patients, of which two were Black, one was Indian and seven were White. Eight patients were female and two patients were male. The average age at diagnosis of AIH/PBC OS was 40 years of age. The average follow up period in the AIH/PBC group was 6 and a half years.



Figures 3.8 and 3.9: Comparison of gender and race within Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome Groups

3.4.2.2 Biochemical Profiles

Table 3.5 Comparison of biochemical profiles in Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

	Presumed diagnosis			
	AIH	PBC	AIH/PBC OS	Biochemical profiles (p - value)
Mean ± STD	168,7±111,93	240,8±154,0	333,9±236,9	
Median (Min – Max)	131 (0 – 527)	213 (100 - 437)	298 (78 - 752)	ALP (IU/L) (0,083)
Mean ± STD	**139,6±148,6**	**172,3±99,5**	**589,6±543,6**	
Median (Min – Max)	**80 (0 - 686)**	**193 (41 - 263)**	**397 (73 - 1856)**	GGT (IU/L) **(0,001)**
Mean ± STD	305,6±422,2	42,5±20,1	109,7±78,9	
Median (Min – Max)	122 (0 - 1867)	42 (21 - 65)	89 (34 - 300)	ALT (IU/L) (0,180)
Mean ± STD	**382,0±555,2**	**38,5±37,0**	**120,5±125,8**	
Median (Min – Max)	**160 (0 - 2455)**	**33 (0 - 89)**	**69 (43 - 461)**	AST (IU/L) **(0,046)**
Mean ± STD	0,0±19,1	17,0±19,9	12,8±20,8	
Median (Min – Max)	0,0 (0,0 - 53,0)	14,8 (0,0-38,3)	0,0 (0,0 - 47,2)	PPT (Seconds) (0,912)
Mean ± STD	0,9 ± 0,7	0,9 ± 0,6	1,0 ± 0,6	
Median (Min – Max)	1,2 (0,0 – 2,6)	1,1 (0,0 – 1,3)	1,0 (0,0 – 1,6)	INR (0,839)
Mean ± STD	79,1±116,3	29,0 ± 39,4	47,5 ± 34,6	
Median (Min – Max)	41 (0 – 622)	11 (7 – 88)	38 (9 – 114)	Total Bilirubin (µmol/L)(0,350)
Mean ± STD	56,2 ± 90,1	22,3 ±37,2	32,6 ± 32,4	
Median (Min – Max)	22 (0 – 418)	5 (2 – 78)	21 (4 – 102)	Conjugated Bilirubin (µmol/L) (0,405)
Mean ± STD	17,2 ± 19,9	3,7 ± 7,4	8,6 ± 13,3	
Median (Min – Max)	14,4 (0,0 - 56,7)	0,0 (0,0 – 14,8)	0,0 (0,0 - 39,2)	Serum IgG (g/L) (0,212)
Mean ± STD	1,0 ± 1,4	0,9 ± 1,8	1,3 ± 1,7	
Median (Min – Max)	0,0 (0,0 – 4,8)	0,0 (0,0 – 3,6)	0,0 (0,0 – 4,1)	Serum IgM (g/L) (0,902)

Table 3.5 The ALP and GGT mean and median values are higher in the overlap group.

The p-values of the Kruskal Wallis tests in Table 3.5 above indicate that the null hypothesis is only rejected in GGT and AST, as their p-values are less than 0.05. There is a significant difference in the results of patients' blood tests across AIH, PBC and AIH/PBC OS, in GGT and AST only.

3.4.2.3 Autoimmune Serological Profiles

Table 3.6 Comparison of autoimmune serological profiles in Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

Serological profile	Presumed diagnosis		
	AIH	PBC	AIH/PBC OS
	n(%)	n(%)	n(%)
Total	43	4	10
AMA	**1(2,3)**	**4(100,0)**	**3(30,0)**
ANA	20(46,5)	2(50,0)	5(50,0)
SMA	5(11,6)	-	1(10,0)
LKM - 1	-	-	-
C - ANCA	3(7,0)	-	1(10,0)

3.4.2.4 Concomitant Diseases

Table 3.7 Comparison of concomitant diseases in Autoimmune Hepatitis, Primary Biliary Cirrhosis and their overlap groups

Presence of concomitant autoimmune diseases	Presumed diagnosis		
	AIH	PBC	AIH/PBC OS
	n(%)	n(%)	n(%)
Total	44	4	10
Autoimmune thyroid disease	4(9,1)	0	0
Sjogren's syndrome	1(2,3)	0	1(10,0)
Rheumatoid arthritis	1(2,3)	1(25,0)	0
Systemic Lupus Erythematosis	3(6,8)	0	0
Immune thrombocytopenic purpura	1(2,3)	0	0
Other concomitant autoimmune disease			
Crohn's	0	0	0
UC	0	0	0
Fibromyalgia	1(2,3)	0	0
Hypothyroid	3(6,8)	0	0
Grave’s disease	1(2,3)	0	0
Osteoporosis	0	0	0
pernicious anaemia	2(4,5)	0	0
SICCA	0	1(25,0)	0

3.4.2.5 Treatment

Table 3.8 Comparison of the treatment received by patients with Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

Treatment	Presumed diagnosis		
	AIH	PBC	AIH/PBC.OS
	n(%)	n(%)	n(%)
Total	41	3	10
Prednisone	3(7,3)	-	2(20,0)
UDCA	1(2,4)	3(100,0)	1(10,0)
Combination of UDCA and prednisone	-	-	2(20,0)
Azathioprine	9(22,0)	-	-
Combination of azathioprine and prednisone	34(82,9)	-	4(40,0)
Combination of azathioprine/UDCA/and prednisone	1(2,4)	-	7(70,0)
Liver transplant	6(14,6)	-	1(10,0)
Other treatments			
MMF	2(4,9)	-	-
MMF+prednisone	2(4,9)	-	-
azathioprine+UDCA	-	-	-

The treatment regimens of 41 patients in AIH group were documented. The majority (82,9%) received the combination of azathioprine and prednisone, 22,0% received only azathioprine as treatment, 14,6% received OLT. 9,7% received prednisone or UDCA. The three patients (100,0%) in PBC group received UDCA monotherapy. Some patients (9,8%), in AIH group, also received MMF or its combination with prednisone. In the overlap group (AIH/PBC), the majority (70,0%) of the patients received the combination of prednisone, UDCA and azathioprine as previously mentioned.

3.4.2.6 Outcomes

Table 3.9 Comparison of the outcomes in Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

Outcomes	Presumed diagnosis		
	AIH	PBC	AIH/PBC OS
	n(%)	n(%)	n(%)
Total	44	4	10
Remission	**14(31,8)**	**1(25,0)**	-
Liver cirrhosis	23(52,3)	3(75,0)	7(70,0)
Portal hypertension	21(47,7)	3(75,0)	7(70,0)
Hepatic decompensation	8(18,2)	-	1(10,0)
Liver transplant	6(13,6)	-	1(10,0)
Death	4(9,1)	-	1(10,0)
Lost to follow up	6(13,6)	-	1(10,0)
Other outcomes			
Ongoing disease	2(4,5)	-	4(40,0)
Worked up for transplant	5(11,4)	1(25,0)	1(10,0)
Transferred out	1(2,3)	-	-

Importantly, none of the AIH/PBC OS patients went into remission, compared to 31,8% in the AIH group.

3.4.2.7 Presence of Cirrhosis

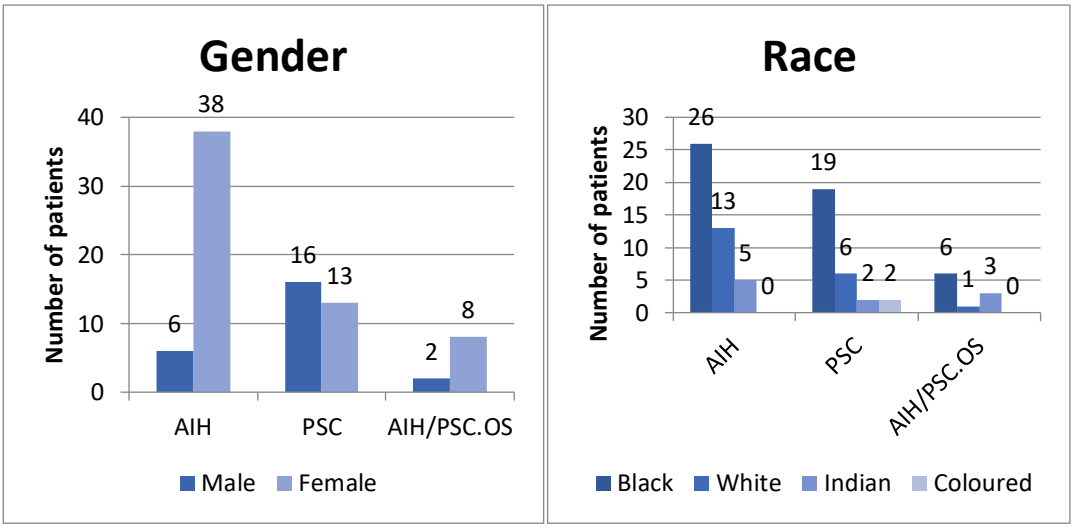
Table 3.10 Comparison of the presence of liver cirrhosis in Autoimmune Hepatitis, Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

Liver cirrhosis present	AIH	PBC	AIH/PBC OS
	n(%)	n(%)	n(%)
Total	44	4	10
At diagnosis	**20(45,5)**	**2(50,0)**	**5(50,0)**
One year after Rx	2(4,5)	-	-
Five years after Rx	1(2,3)	-	1(10,0)
Ten years after Rx	-	-	1(10,0)
Fifteen years after Rx	-	1(25,0)	-

At diagnosis, almost half of the patients were noted to have liver cirrhosis across all three groups.

3.4.3 Comparing Autoimmune Hepatitis/Primary Sclerosing Cholangitis with Autoimmune Hepatitis and Primary Sclerosing Cholangitis

3.4.3.1 Demographic Profiles



Figures 3.10 and 3.11: Comparison of gender and race within Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome groups

Figures3.10 and 3.11 above presents the frequencies of gender and race in the AIH, PSC and AIH/PSC OS groups respectively. It can be seen that in the AIH/PSC OS group there were a total of 10 patients, of which six (60,0%) were Black, three (30,0%) were Indian and one (10,0%) was White. Eight patients (80,0%) were female and two patients (20,0%) were male. The average age at diagnosis of AIH/PSC OS was 34 years of age, and the average follow up period was four and a half years.

In the PSC group, there were a total of 29 patients, of which 19 (65, 5%) were Black, two (6,9%) were Indian, two (6,9%) were Coloured and six (20,7%) were White. Thirteen patients (44,8%) were female and 16 patients (55,2%) were male. The average age at diagnosis was 36 years and the average follow up period in the PSC group was just under 5 years.

3.4.3.2 Biochemical profiles

Table 3.11: Comparison of biochemical profiles in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

	Presumed diagnosis			
	AIH	PSC	AIH/PSC OS	Biochemical profiles (p - value)
Mean ± STD	**168,70±111,93**	**516,0±534,9**	**267,5±151,7**	
Median (Min – Max)	**131 (0 – 527)**	**356 (0 - 2343)**	**287 (0 - 445)**	**ALP (IU/L) (0,002)**
Mean ± STD	**139,6±148,6**	**419,9±475,8**	**245,9±216,4**	
Median (Min – Max)	**80 (0 - 686)**	**225 (0 - 1930)**	**147 (0 - 662)**	**GGT (IU/L) (0,017)**
Mean ± STD	305,6±422,2	108,1±104,9	280,1±298,3	
Median (Min – Max)	122 (0 - 1867)	79 (0 - 455)	165 (0 - 814)	ALT (IU/L) (0,264)
Mean ± STD	382,0±555,2	111,5±87,0	308,9±365,7	
Median (Min – Max)	160 (0 - 2455)	108 (0 - 344)	124 (0 - 1125)	AST (IU/L) (0,111)
Mean ± STD	0,0±19,1	11,7±19,8	26,0±18,5	
Median (Min – Max)	0,0 (0,0 - 53,0)	0,0 (0,0 - 60,1)	33,3(0,0 - 45,1)	PPT (Seconds) (0,121)
Mean ± STD	0,9 ± 0,7	0,8 ± 0,8	1,0 ± 0,6	
Median (Min – Max)	1,2 (0,0 – 2,6)	0,9 (0,0 – 2,7)	1,1 (0,0 – 1,7)	INR (0,240)
Mean ± STD	79,1±116,3	175,3±495,6	96,2 ± 152,6	
Median (Min – Max)	41 (0 – 622)	47 (0 – 2700)	36 (0 – 453)	Total Bilirubin (µmol/L)(0,970)
Mean ± STD	56,2 ± 90,1	60,8 ±77,3	76,8 ± 128,6	
Median (Min – Max)	22 (0 – 418)	20 (0 – 241)	23 (0 – 384)	Conjugated Bilirubin (µmol/L) (0,934)
Mean ± STD	17,2 ± 19,9	6,1 ± 10,8	17,2± 22,5	
Median (Min – Max)	14,4 (0,0 - 56,7)	0,0 (0,0 – 37,9)	7,2 (0,0 - 67,2)	Serum IgG (g/L) (0,052)
Mean ± STD	1,0 ± 1,4	0,5 ± 1,0	1,7 ± 2,3	
Median (Min – Max)	0,0 (0,0 – 4,8)	0,0 (0,0 – 3,6)	0,6 (0,0 – 6,6)	Serum IgM (g/L) (0,216)

There is a significant difference in the blood results of patients in ALP and GGT.

3.4.3.3 Autoimmune Serological Profiles

Table 3.12: Comparison of autoimmune serological profiles in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

Serological profile		Presumed diagnosis		
		AIH	PSC	AIH/PSC OS
		n(%)	n(%)	n(%)
Total		43	29	10
AMA	% (Number +/Total)	1(2,4)	-	-
ANA	% (Number +/Total)	20(46,5)	3(10,3)	3(30,0)
SMA	% (Number +/Total)	5(11,6)	1(3,4)	2(20,0)
LKM-1	% (Number +/Total)	-	-	-
P-ANCA	% (Number +/Total)	-	1(3,4)	-
C-ANCA	**% (Number +/Total)**	**3(7,0)**	**1(3,4)**	**3(30,0)**

Tables 3.11 and 3.12 above show the results of comparison of biochemical results and serology tests, respectively, across AIH, PSC and their overlap (AIH/PSC OS). Table 3.12 c-ANCA is positive in 30,0% of the AIH/PSC group. This is significantly higher than in the AIH and PSC groups.

3.4.3.4 Concomitant Diseases

Table 3.13: Comparison of concomitant diseases in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

Presence of concomitant autoimmune diseases	Presumed diagnosis		
	AIH	PSC	AIH/PSC OS
	n(%)	n(%)	n(%)
Total	44	29	10
Autoimmune thyroid disease	4(9,1)	1(3,4)	-
Sjogren's syndrome	1(2,3)	-	-
Rheumatoid arthritis	1(2,3)	-	-
Systemic Lupus Erythematosis	3(6,8)	-	-
Inflammatory Bowel Disease	**-**	**20(69,0)**	**1(10,0)**
Immune thrombocytopenic purpura	1(2,3)	-	-
Other concomitant autoimmune disease			
Crohn's	-	2(6,9)	-
UC	-	18(62,1)	-
Fibromyalgia	1(2,3)	-	-
Hypothyroid	3(6,8)	-	-
Grave's disease	1(2,3)	1(3,4)	-
pernicious anaemia	2(4,5)	-	-

Table 3.13 above compares the concomitant diseases in AIH, PSC and AIH/PSC OS. These concomitant diseases are mostly encountered in AIH group. Only 69,0% of the PSC group were noted to have IBD. Twenty % of the IBD cases were Crohn's disease and 80,0% were UC. There was only one case of IBD (UC) noted in the AIH/PSC OS group.

3.4.3.5 Treatment

Table 3.14: Comparison of the treatment received by patients in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

Treatment	Presumed diagnosis		
	AIH	PSC	AIH/PSC OS
	n(%)	n(%)	n(%)
Total	41	27	10
Prednisone	3(7.3)	-	2(20,0)
UDCA	1(2.4)	10(37,0)	1(10,0)
Combination of UDCA and prednisone	-	2(7.4)	-
Azathioprine	9(22,0)	1(3.7)	3(30,0)
Combination of azathioprine and prednisone	34(82.9)	3(11.1)	4(40,0)
Combination of azathioprine/UDCA/and prednisone	1(2.4)	4(14.8)	5(50,0)
Liver transplant	6(14.6)	12(44.4)	2(20,0)
Other treatments			
MMF	2(4.9)	-	-
MMF+Prednisone	2(4.9)	-	-
Azathioprne+UDCA	-	3(11.1)	1(10,0)
MMF+Prednisone+UDCA	-	-	1(10,0)

The majority (82,9%) of the AIH group received a combination of azathioprine and prednisone. Of the 27 patients in PSC group, 10 received UDCA and 12 OLT's. Some patients (11,1%), in the PSC group, also received the combination of azathioprine with UDCA. In the overlap (AIH/PSC) group, half (5/10) of the patients received the combination of prednisone, UDCA and azathioprine as discussed previously.

3.4.3.6 Outcomes

Table 3.15: Comparison of the outcomes in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

Outcomes	Presumed diagnosis		
	AIH	PSC	AIH/PSC OS
	n(%)	n(%)	n(%)
Total	44	29	10
Remission	**14(31,8)**	**4(13,8)**	**5(50,0)**
Liver cirrhosis	23(52,3)	16(55,2)	4(40,0)
Portal hypertension	21(47,7)	15(51,7)	4(40,0)
Hepatic decompensation	8(18,2)	11(37,9)	2(20.0)
Liver transplant	**6(13,6)**	**12(41,4)**	**2(20,0)**
Death	4(9,1)	6(20,7)	1(10,0)
Lost to follow up	6(13,6)	2(6,9)	-
Other outcomes			
Ongoing disease	2(4,5)	7(24,1)	2(20,0)
Worked up for transplant	5(11,4)	4(13,8)	1(10,0)
Transferred out	1(2,3)	2(6,9)	-

Hepatic decompensation and OLT occurred more frequently in the PSC group (37, 9% and 41, 4% respectively) compared to the OS group (20,0% and 20,0% respectively) and the AIH group (18,2% and 13,6% respectively). The PSC group also had a higher percentage of deaths (20,7%) compared to the OS group (10,0%) and the AIH group (9,1%). The AIH/PSC OS group went into remission in 50,0% of cases compared to 31,8 % in the AIH group and only 13,8% of the PSC group.

3.4.3.7 Presence of Liver Cirrhosis

Table 3.16: Comparison of the presence of liver cirrhosis in Autoimmune Hepatitis, Primary Sclerosing Cholangitis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

Liver cirrhosis present	AIH	PSC	AIH/PSC OS
	n(%)	n(%)	n(%)
Total	44	28	10
At diagnosis	**20(45,5)**	**12(42,9)**	**4(40,0)**
One year after Rx	2(4,5)	-	-
Four years after Rx	-	1(3,6)	-
Five years after Rx	1(2,3)	3(10,0)	-

The above table depicts the percentages of the presence of liver cirrhosis, at any time, in patients with AIH, PSC and AIH/PSC OS. At diagnosis, a similar percentage of patients were noted to have liver cirrhosis across all three groups. In the PSC group, cirrhosis was diagnosed again after being on treatment for four years in one (3,6%) patient and after being on treatment for five years in 3 (10,0%) patients. In the AIH/PSC OS group, no further patients were diagnosed with cirrhosis after being on treatment.

Table 3.17: Relationship between treatment and outcome in the Autoimmune Hepatitis group

Outcomes	Treatment								Total patients
	Pred	UDCA	Aza	Aza + pred	Aza+ UDCA+ Pred	Liver transplant	MMF	MMF+Pred	
Remission	1	0	5	12	1	0	0	1	14
Liver cirrhosis	2	1	4	17	0	6	1	1	22
Portal hypertension	2	1	3	15	0	6	1	1	20
Hepatic decompensation	0	0	1	6	0	3	0	0	7
Liver transplant	0	0	0	2	0	6	0	0	6
Death	0	0	0	3	0	1	0	0	3
Lost follow up	0	0	0	4	0	0	0	0	4
Ongoing disease	0	0	0	1	0	1	1	0	2
Total patients	3	1	9	34	1	6	2	2	41

KEY:

UDCA: Ursodeoxycholic acid

Pred: Prednisone

Aza: Azathioprine

MMF: Mycophenolate Mofetil

Table 3.17 indicates the cross tabling of treatment received by patients and their outcomes due to that treatment, in the AIH group. Of the three patients who received prednisone only, one went into remission, two developed liver cirrhosis and two developed portal hypertension. Of the 34 patients who received the combination of azathioprine and prednisone, 12 went into remission, 17 developed liver cirrhosis, 15 developed portal hypertension, six went on to develop hepatic decompensation, two received OLT, three died, four were lost to follow up, one with ongoing disease, five were worked up for OLT and one was transferred out. The total is above 34 as some patients received more than one treatment. Of the 14 patients with remission, the majority (12) received the combination of azathioprine and prednisone.

Table 3.18: Relationship between treatment and outcome in the Primary Sclerosing Cholangitis group

Outcomes	UDCA	UDCA+ Pred	Aza	Aza+Pred	Aza+UDCA +Pred	Liver transplant	Aza+UDCA	Total
Remission	2	0	0	0	1	1	1	4
Liver cirrhosis	5	1	0	1	2	10	2	16
Portal hypertension	5	1	0	1	1	10	2	15
Hepatic decompensation	3	0	0	0	1	9	2	11
Liver transplant	2	0	0	0	1	11	1	11
Death	3	1	0	1	0	3	2	6
Lost follow up	0	0	1	0	0	0	0	1
Ongoing disease	3	1	0	1	1	1	0	6
Total	10	2	1	2	4	11	3	25

KEY:

UDCA: Ursodeoxycholic acid

Pred: Prednisone

Aza: Azathioprine

Of the 16 patients with liver cirrhosis in PSC group, Table 3.18, most of them (10/16, 62,5%) received OLT as treatment. The same number of patients, as for liver cirrhosis, also received liver transplant among the 15 patients with portal hypertension.

Table 3.19: Relationship between treatment and outcome in the Primary Biliary Cirrhosis group

Outcomes	Treatment	
	UDCA	Total patients
Remission	1	1
Liver cirrhosis	2	2
Portal hypertension	2	2
Total of patients	3	3

KEY:

UDCA: Ursodeoxycholic acid

Of all the four patients in the PBC group, only three received treatment. They all received UDCA treatment. One patient went into remission after treatment, two developed liver cirrhosis and two developed portal hypertension.

Table 3.20: Relationship between treatment and outcome in Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

In the AIH/PBC OS							
Outcomes							Total patients
	Pred	UDCA	UDCA + pred	Aza+Pred	UDCA+Aza+Pred	Liver transplant	
Remission	0	0	0	0	0	0	0
Liver cirrhosis	0	1	2	2	4	1	7
Portal hypertension	0	1	2	2	4	1	7
Hepatic decompensation	0	1	0	0	0	0	1
Liver transplant	0	1	0	0	0	0	1
Death	0	1	0	0	0	0	1
Lost follow up	0	0	0	1	1	0	1
Ongoing disease	2	0	0	2	4	1	4
Total patients	2	1	2	4	7	1	10

KEY:

UDCA: Ursodeoxycholic acid

Pred: Prednisone

Aza: Azathioprine

Table 3.20 above depicts the relationship between treatment and outcome in the AIH/PBC OS group. Seven of the 10 patients developed both liver cirrhosis and portal hypertension. None of the patients went into remission in this group.

Table 3.21: Relationship between treatment and outcome in Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

In the AIH/PSC OS

Outcomes								Total patients
	Pred	UDCA	Aza	Aza + pred	Aza+UDCA +Pred	Liver transplant	MMF+UDCA+pred	
Remission	2	0	3	4	3	0	1	5
Liver cirrhosis	0	1	0	0	1	2	0	4
Portal hypertension	0	1	0	0	1	2	0	4
Hepatic decompensation	0	0	0	0	0	2	0	2
Liver transplant	0	0	0	0	0	2	0	2
Death	0	0	0	0	0	1	0	1
Ongoing disease	0	0	0	0	2	0	0	2
Total of patients	2	1	3	4	5	2	1	10

KEY:

UDCA: Ursodeoxycholicacid

Pred: Prednisone

Aza: Azathioprine

MMF: Mycophenolate Mofetil

Table 3.21 depicts the relationship between treatment and outcome in the AIH/PSC OS group. Four of the 10 patients developed both liver cirrhosis and portal hypertension. Five patients went into remission in this group.

Table 3.22: Histology Results

	AIH	PSC	PBC	AIH PSC OS	AIH PBC OS
	n(%)	n(%)	n(%)	n(%)	n(%)
Total number of patients who had Biopsies	34	17	2	6	8
Typical	22(64.7)	12(70.6)	2(100,0)	5(83.3)	6(75,0)
Atypical	3(8.8)	1(5.9)	-	-	1(12.5)
Inadequate	5(14.7)	-	-	-	1(12.5)
Unavailable result	1(2.9)	-	-	1(16.7)	-
Biopsies of transplanted liver	4(11.8)	4(23.5)	-	2(33.3)	-

It is important to note that in the AIH, PSC and AIH PSC OS groups some of the liver biopsies were taken from transplanted livers (11,8%, 23,5% and 33,3% respectively).

Chapter 4: Discussion

This retrospective study describes the demographics, clinical characteristics, diagnosis, treatment and outcomes in patients with the presumed diagnoses of AILDs and their overlap syndromes at the liver clinic at the CMJAH over a period of 26 years (1990-2016).

4.1 Epidemiology

4.1.1 Autoimmune Hepatitis

The cohort showed an overwhelming female predominance (6.3:1). This is consistent with the literature (4:1) (7). The average age at diagnosis of AIH was 37 years of age in our group. This is slightly younger than that reported in the literature (2, 6). Most of the data in the literature is based on White patients. One of the possible reasons for the younger age at diagnosis in our study could be the proportion of Black patients. The average age at diagnosis of AIH in our Black patients was 31.7 years, and in White patients was 42.6 years. There is very little published data on Black patients with AIH but a discrepancy in age at presentation has previously been noted. A study performed at a tertiary institute, in the USA, which assessed AIH in African Americans, found that the African American patients tended to be younger at presentation than Whites. (34.1 years vs. 41.5 years). It is important to note that this finding was not statistically significant (97).

A significant finding in our cohort of patients with AIH was that 45% had liver cirrhosis at the time of diagnosis. This is significantly higher than the figure quoted in the literature (6). A likely explanation for this finding would be that our patients are presenting later during the course of their disease.

4.1.2 Primary Biliary Cirrhosis

There were four patients in this group, all of whom were female. The demographic data in the literature, with regards to PBC, confirms a significant female to male predominance (10:1). The average age at diagnosis was 58 years. Most patients are diagnosed between the ages of 30 and 65 years (47).

There is limited data in Non - White patients. An American study evaluated the ethnic and gender proportions in 535 patients with PBC. The majority of their patients (86%) were White, eight % were Hispanic and four % were African American. The mean age and male - to - female ratio were similar across all ethnic groups (98). The small number of patients in this sub-group makes it difficult for us to comment on the ethnic differences.

4.1.3 Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome

In the AIH/PBC OS group there were a total of 10 patients. The majority were White (70%); two (20%) were Black and one (10%) was Indian. Ethnic differences have not been well documented but the disease maybe more common in Hispanics (99).

4.1.4 Primary Sclerosing Cholangitis

The average age at diagnosis in the current study was 36 years. This finding is consistent with published data i.e. even though all age groups are affected by PSC; the peak incidence is typically in the fourth decade of life. There was a male predominance in the study (55,2%), which is an expected finding. A review by Molodecky et al found that PSC is twice as likely to occur in males compared to females (30). In the current study the discrepancy was not as marked. A possible reason for this is the significant proportion of Black patients in our cohort (65%). An American study looked at the clinical and demographic characteristics in a multi ethnic population (African American; European American and Hispanic) with PSC. They found a predominance of males across all groups but this difference was lower in African Americans (100).

4.1.5 Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome

There were eight females and two males in this sub - group of the study. This proportion is different to the data from the study by Schramm and Lohse. They studied 71 adult patients with AIH/PSC OS and almost two thirds of their patients were male (101). This discrepancy could be due to the higher proportion of Black Africans in our study (60%) as discussed above (100) and/ or the small number of patients which lends itself to some skewing of the data.

4.2 Comparison of Autoimmune Hepatitis/Primary Biliary Cirrhosis and Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndromes

In our study, we had a total of 20 OS patients consisting of 10 in the AIH/PBC OS group and 10 in the AIH/PSC OS group.

4.2.1 Demography

The gender ratio and mean ages at diagnoses were similar in both groups. There was a significant racial difference in the two groups. Seventy % of the AIH/PBC group were White and 60% of the AIH/PSC group was Black. This may reflect a genetic predisposition in various racial groups. This has not been documented previously and warrants further investigation.

4.2.2 Biochemical Results

The difference between the two groups in terms of cholestatic (ALP and GGT) and hepatitic (ALT and AST) enzymes were not statistically significant. The higher GGT value in patients with AIH/PBC OS is to be expected as a high GGT is one of the Paris criteria (73). The mean serum IgG and IgM levels are higher in the AIH/PSC group, but this was not significant. It was very difficult to differentiate between the two OS based on the hepatic biochemical profile alone.

4.2.3 Autoimmune Serology

The autoimmune serology profiles differed amongst the two OS. Thirty % of the AIH/PBC cases had a positive AMA whereas none of the AIH/PSC case had a positive AMA. This is a significant finding and is in keeping with the literature as a positive AMA forms part of the PC for the diagnosis of AIH/PBC OS (72). The presence of ANA and SMA in both groups is consistent with previously documented diagnostic features of AIH overlap syndromes (99). Interestingly, c - ANCA was positive in 30% of the AIH/PSC group and negative throughout the AIH/PBC group. In a study which looked at the frequency of a positive c - ANCA in PSC compared to other chronic liver diseases (control group), it was concluded that c - ANCA is detected in a higher proportion of PSC patients (38.5%) compared to other chronic liver diseases (10.6%). The difference in detection rates was statistically significant (p - value<0.0001) (102).

4.2.4 Concomitant Diseases

The minimal presence of concomitant diseases (only 10%) across both groups of patients with OS is an unexpected finding. A study which looked at 71 AIH/PBC OS patients detected concomitant autoimmune diseases in almost half of their patients (44%) An improved understanding of the underlying genetic and immunologic mechanisms in these patients will provide additional information regarding the concept of 'mosaic of autoimmunity' and thus may provide some reasoning for this discrepancy in our group (80).

We only detected IBD in one patient with AIH/PSC. This is inconsistent with previously published findings. The prevalence of IBD in AIH/PSC OS should approach that of PSC, i.e. approximately 90% (42). The general consensus amongst experts is that autoimmune hepatic OS consists of a primary AILD with one or more characteristics of another AILD (71)

. Based on this, a possible explanation for low prevalence of IBD in the AIH/PSC OS group is that the majority of these patients primarily had AIH with characteristics of PSC.

4.2.5 Treatment and Outcomes

Multiple treatment regimens were utilized in both groups of patients. The AIH/PSC group had a better outcomes compared to the AIH/PBC group and a lower complication rate. An equal number of deaths occurred in both groups (10%). According to the literature, the definitive management of the overlap syndromes has not been established by randomized controlled trials. Therefore therapy is empiric and it should be individualized and guided by the severity of the cholestatic findings at presentation as well as by the patient's response (103, 104).

In terms of outcomes the available data is not clear. One retrospective study showed 10 patients with AIH/PSC OS had significantly better survival than 12 patients with AIH/PBC OS (90% vs. 50%) (105). In another retrospective study, survival was noted to be worse in the AIH/PSC OS group than in those with the AIH/PBC OS (91).

4.3 Comparison of Comparison of Autoimmune Hepatitis/Primary Biliary Cirrhosis Overlap Syndrome with Autoimmune Hepatitis and Primary Biliary Cirrhosis

The average age at diagnosis in the AIH group was 37 years, in the PBC group was 58 years and in the AIH/PBC OS group as 40 years. A similar finding was noted in a multicentre comparison of patients with AIH/PBC OS, AIH and PBC, where is was found that the AIH/PBC OS patients were younger at time of diagnosis compared to the PBC patients (Median age 44 years' vs. 59 years) (78).

4.3.1 Biochemical Results

The AIH/PBC OS group exhibited a distinct set of biochemical results in comparison to the AIH group and the PBC group:

- The AIH/PBC OS group had higher baseline ALT, AST and IgG levels than the PBC group
- The AIH/PBC OS group had higher baseline ALP, GGT and IgM levels than the AIH group

An interesting finding in this study was that ALP and GGT levels were higher in the OS group (GGT significantly so) than those in the PBC group. The laboratory findings elucidated above are in keeping with those described previously (78).

4.3.2 Autoimmune Serology

The OS group had a higher rate of AMA positivity than the AIH group and had a lower rate than the PBC group. All three groups had similar rates of ANA positivity.
It has been suggested in the literature that a difference in immuno reactivity to a distinct subset of AMA between patients with PBC and AIH/PBC may be important in differentiating between the two syndromes (106). This could provide an explanation for the higher percentage of AMA positivity in the PBC group compared to the AIH/PBC OS group. The findings described above need to be further elucidated and future serological studies may clarify these results.

Table 4.1 Comparison of specific autoimmune serology in three studies (107, 108).

		ANA	AMA	SMA
Korea	%(number/total)	100%(9/9)	67%(6/9)	11%(1/9)
China	%(number/total)	96% (140/146)	88%(129/146)	10%(14/146)
CMJAH	%(number/total)	50%(5/10)	30%(3/10)	10%(1/10)

The above table compares three studies that looked at the serum immunological profiles in patients with AIH/PBC OS. It can be seen that the percentage of SMA positivity was similar in all three studies. However a significantly higher percentage of patients in Korea and China were ANA and AMA positive compared to our cohort of patients. The reason for this disparity is not clear. An important consideration is that AMA is not a mandatory criterion when using the PC to diagnose AIH/PBC OS. Therefore the above findings may not signify clinical importance.

4.3.3 Treatment and Outcomes

In our cohort of patients, 14.6% received OLT. This appears to be significantly higher than similar studies in the USA and Europe (4%) (4). A possible explanation for this is that our patients with AIH are presenting with advanced disease.

The AIH/PBC OS group had worse outcomes when compared to the AIH group. Of note, none of the OS group went into remission whereas 32% of the AIH group went into remission. The percentage of liver cirrhosis was higher in the OS group (70 vs. 52%). Autoimmune hepatitis/primary biliary cirrhosis overlap syndrome has previously been shown to have a worse outcome than PBC alone (72). It is very difficult for us to comment on this specifically as our PBC cohort was very small.

4.4 Comparing Autoimmune Hepatitis/Primary Sclerosing Cholangitis Overlap Syndrome with Autoimmune Hepatitis and Primary Sclerosing Cholangitis

4.4.1 Biochemical Results

When comparing the biochemical profile of the AIH/PSC OS group to the AIH group and the PSC group the following was found:

- The AIH/PSC OS group had higher baseline ALT, AST, IgG and IgM levels than the PSC group.
- The AIH/PSC OS group had higher baseline ALP, GGT and IgM levels than the AIH group.

The ALP and GGT values were significantly different amongst the three groups. A ratio of ALP to AST that is greater than three may be of value when trying to discriminate between AIH and AIH/PSC OS (89). When comparing PSC and AIH/PSC OS in adults, no differentiation could be made between the two regarding serum ALP levels (109).

4.4.2 Concomitant Diseases

The stand out result was the fact that only 69% of the PSC patients had IBD. The breakdown was 90% UC and 10% had Crohn's disease. The prevalence of IBD in our cohort of PSC patients is less than the 90 % quoted in the literature (42). As noted above there was only one patient in the AIH/PSC group with concomitant IBD. These results reflect the findings of Bowlus et al. They looked at PSC in a multi ethnic group of patients. The African American group was less frequently noted to have IBD in comparison to the European American group (60.7% vs. 71.6%). The lower frequency of IBD in African Americans was accounted for by a significantly lower frequency of UC (100).

4.4.3 Treatment and Outcomes

In keeping with the literature, the AIH/PSC OS group had better outcomes than the PSC group. The outcomes evaluated were clinical remission; requirement for OLT and death (83). The outcomes between the OS group and the AIH group did not differ very significantly. A higher percentage of OS patients went into remission compared to the AIH group (50% vs. 32%). Our results differ to those described in the literature. The concept of one primary AILD with features of a second disease may explain our findings. i.e. some of the patients in the AIH/PSC group may in fact have predominantly AIH with features of PSC and therefore the outcomes in these patients were more in keeping with those found in AIH.

The outcome of patients with adult AIH/PSC overlap appears to be worse than for patients with AIH. More patients experience treatment failure (33% vs. 10%) or unfavourable outcomes, such as OLT (33 vs. 8%) or the development of cirrhosis (89, 99).

Chapter 5: Limitations, Conclusions, Recommendations

5.1 Limitations

The cohort of patients consisted of a small sample size. The AIH/PBC OS and AIH/PSC OS groups consisted of 10 patients each and there were only four patients in the PBC group.

This was a retrospective file review and therefore not every file contained a full set of information. An example of this is that some liver biopsy results were not available.

There is a paucity of data in African countries with which we can compare our results.

There are no standardized diagnostic criteria for the overlap syndromes; therefore it may be difficult to accurately interpret some of the results and outcomes.

5.2 Conclusions

In this retrospective study, we described the demographics, clinical characteristics, diagnosis, treatment and outcomes in patients with the presumed diagnoses of AILDs and their overlap syndromes at the liver clinic at the CMJAH over a period of 26 years (1990 - 2016).

Based on the results of the current study it can be surmised that ethnicity could influence the development of specific overlap syndromes [AIH/PBC OS (70% White) and AIH/PSC OS (60% Black)]. The liver enzyme profile alone cannot be used to differentiate between the OS. In our population AIH/PSC OS and AIH have better clinical outcomes and a lower complication rate compared both to AIH/PBC and PSC and Autoimmune hepatitis/ primary biliary cirrhosis overlap syndrome has a poorer prognosis than either AIH or PBC alone. Early recognition of the OS may assist in timely use of combination drugs which may improve outcomes

5.3 Recommendations

Based on the literature together with the results of this retrospective study, it is important that definitive diagnostic criteria are developed in order to differentiate the autoimmune liver disease overlap syndromes from the separate disease entities. The reasons for this are that the overlap syndromes require different treatment regimens and have different outcomes to the separate disease entities. Further large volume prospective trials are warranted to better differentiate between the overlap syndromes and their single disease entities.

Another important aspect to consider is the genetic variation in these diseases. As noted, in our study there was a significant ethnic difference in the overlap groups, with a high prevalence of White patients in the AIH/PBC OS group and a high prevalence of Black patients in the AIH/PSC OS group. There was also a high prevalence of Black patients in the PSC group. Genetic studies in our patient population, with respect to HLA associations, may provide important clues as to which ethnic groups are at risk of certain disease entities.

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Appendix A:

Summary of the criteria for the diagnosis of Autoimmune Hepatitis (15)

Definite AIH	Probable AIH
Normal α-1AT phenotype	Partial α-1AT deficiency
Normal ceruloplasmin level	Non-diagnostic ceruloplasmin/copper levels
Normal iron and ferritin levels	Non-diagnostic iron and/or ferritin changes
No active hepatitis A,B,C infection	No active hepatitis A,B,C infection
Daily alcohol <25 g/day	Daily alcohol <50 g/day
No recent hepatotoxic drugs	No recent hepatotoxic drugs
Predominant AST/ALT abnormality	Predominant AST/ALT abnormality
γ-globulins or IgG level >1.5 times the upper normal limit	Hypergammaglobulinemia of any degree
ANA, SMA anti-LKM1 >1:80, in adults and >1:20 in children	ANA, SMA, anti-LKM1 >1:40 in adults
AMA negative	Other autoantibodies
Liver histology	Liver histology
Interface hepatitis moderate to severe	Interface hepatitis moderate to severe
No biliary lesions, granulomas or prominent changes suggestive of another disease	No biliary lesions, granulomas or prominent changes suggestive of another disease

Appendix B

Simplified diagnostic criteria of the International Autoimmune Hepatitis Group (16)

Feature/parameter	Discriminator	Score
ANA or SMA+	≥1:40	+1*
ANA or SMA+	≥1:80	+2*
or LKM+	≥1:40	+2*
or SLA/LP+	Any titer	+2*
IgG or γ-globulins level	>upper limit of normal >1.1x upper limit	+1 +2
Liver histology (evidence of hepatitis is a necessary condition)	Compatible with AIH Typical of AIH Atypical	+1 +2 0
Absence of viral hepatitis	No Yes	0 +2

Definite autoimmune hepatitis ≥7; Probable autoimmune hepatitis≥P6.

Addition of points achieved for all autoantibodies (maximum, two points).

Typical liver histology for autoimmune hepatitis = each of the following features had to be present namely, interface hepatitis, lymphocytic/lymphoplasmacytic infiltrates in portal tracts and extending into the lobule, emperipolesis (active penetration by one cell into and through a larger cell), and hepatic rosette formation.

Compatible liver histology for autoimmune hepatitis = chronic hepatitis with lymphocytic infiltration without all the features considered typical.

Atypical = showing signs of another diagnosis, like steatohepatitis.

Appendix C

Histology Activity Index in its original form, the Knodell score and modified form, the Ishak score (110)

Knodell Score	**Score**	**Ishak Grade**	**Score**
Periportal +/- bridging necrosis (piecemeal necrosis)		*Periportal or periseptal interface hepatitis (piecemeal necrosis)*	
None	0	None	0
Mild piecemeal necrosis	1	Mild (focal, few portal areas)	1
		Mild/moderate (focal, most portal areas)	2
Moderate piecemeal necrosis (involves less than 50% of circumference of most portal tracts)	3	Moderate (continuous around < 50% of tracts or septa)	3
Marked piecemeal necrosis (involves more than 50% of circumference of most portal tracts)	4	Severe (continuous around > 50% of tracts or septa)	4
		Confluent necrosis	
		None	0
		Focal confluent necrosis	1
		Zone 3 necrosis in some areas	2
		Zone 3 necrosis in most areas	3
		Zone 3 necrosis + occasional portal–central bridging	4
Moderate piecemeal necrosis plus bridging necrosis	5	Zone 3 necrosis + multiple portal–central bridging	5
Marked piecemeal necrosis plus bridging necrosis	6	Panacinar or multiacinar necrosis	6
Multilobular necrosis	10		
Intralobular degeneration and focal necrosis		*Focal (spotty) lytic necrosis, apoptosis and focal inflammation*	

None	0	None	0
Mild (acidophilic bodies, ballooning degeneration, and/or scattered foci of necrosis in <1/3 of lobules or nodules)	1	One focus or less per 10x objective	1
		Two to four foci per 10x objective	2
Moderate (involvement of 1/3–2/3 of lobules or nodules)	3	Five to ten foci per 10x objective	3
Marked (involvement of >2/3 of lobules or nodules)	4	More than 10 foci per 10x objective	4
Portal inflammation		*Portal inflammation*	
None	0	None	0
Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1	Mild, some or all portal areas	1
		Moderate, some or all portal areas	2
Moderate (increased inflammatory cells in 1/3–2/3 of portal tracts)	3	Moderate/marked, all portal areas	3
Marked (dense packing of inflammatory cells in >2/3 of portal areas	4	Marked, all portal areas	4
Fibrosis		*Ishak Stage*	
No fibrosis	0	No fibrosis	0
Fibrous portal expansion	1	Fibrous expansion of some portal areas, with or without short fibrous septa	1
		Fibrous expansion of most portal areas, with or without short fibrous septa	2
Bridging fibrosis	3	Fibrous expansion of most portal areas with occasional portal to portal bridging	3
		Fibrous expansion of portal areas with marked bridging	4

		(portal to portal as well as portal to central)	
Cirrhosis	4	Marked bridging (portal–portal and/or portal–central) with occasional nodules (incomplete cirrhosis)	5
		Cirrhosis, probable or definite	6

The original Knodell score was calculated as the sum or scores of periportal necrosis, intralobular injury, portal inflammation and fibrosis to yield a possible range of 0–22. As used in clinical trials, the first three categories are often totalled to give a necroinflammatory score (0–18), while the fibrosis score (0–4) is reported separately. The Ishak score separated the necroinflammatory components that are totalled to calculate the activity grade (0–18) from the stage (0–6), and varying degrees of confluent necrosis are listed in a separate necroinflammatory category.

Appendix D

Model of End Stage Liver Disease Score (111)

Serum bilirubin (mg/dL)
Serum creatinine (mg/dL)
INR

MELD=3.8 [Ln serum bilirubin (mg/dL)]+11.2 [Ln INR]+9.6 [Ln serum creatinine (mg/dL)]+6.4

*If a patient has had two or more hemodialysis treatments or 24 h of CVVHD in the week prior to the time of the scoring, creatinine will be set to 4 mg/dL

MELD score	**Mortality in 3 months (%)**
<9	1.9
10-19	6.0
20-29	19.6
30-39	52.6
>40	71.3

INR: International normalized ratio, MELD: Model for end-stage liver disease

Appendix E

Endpoints of Initial Immunosuppressive Therapy in patients with Autoimmune Hepatitis (19)

	2010 PG	2002 PG
Goals	1. Prevent progression and need for OLT 2. Minimize adverse events of immunosuppression	1. Reduce mortality and symptoms 2. Minimize adverse events of immunosuppression
Biochemical remission	Normalize: 1. ALT (<19 U/L women; <30 U/L men) 2. g-Globulin and IgG levels	Reduce: AST/ALT to 1.5–2 upper limit of normal
Histologic remission	Eliminate: 1. Interface hepatitis 2. Portal inflammatory infiltrates	1. Eliminate interface hepatitis 2. Confine inflammatory infiltrates to portal tracts
Fibrosis	Prevent: 1. Progression to cirrhosis 2. Progression of existing cirrhosis to decompensation or increasing Child-Turcotte-Pugh and Model for End-Stage Liver Disease scores	Reduce rate of: 1. Progression to cirrhosis 2. Worsening of existing cirrhosis
Immunosuppression	1. Use combinations of immunosuppressive drugs to minimize adverse events caused by any single drug 2. Use alternative therapies as needed to achieve remission	Minimize doses of standard immunosuppressive drug minimize serious adverse events

Appendix F

Data Collection Tool

Inclusion Criteria

1 presumed diagnosis:	
1.1 AIH	
1.2 PBC	
1.3 PSC	
1.4 AIH PBC OS	
1.5 AIH PSC OS	
2 Age >15	

Background Characteristics

Subject Number				
Age at diagnosis				
Race	White	Coloured	Black	Indian
Gender	Male		Female	
Date of Diagnosis (year)				
Follow up Period				

Biochemical Results

Biochemical Results reference range	Dx	6/12 after Rx	1 yr after Rx	2 yrs after Rx	3 yrs after Rx	4 yrs after Rx	5 yrs after Rx	10 yrs after Rx	15yrs after Rx	20 yrs After Rx
ALP (U/L) (42-98)										
GGT (U/L) (<40)										
ALT (U/L) (7-35)										
AST (U/L) (13-35)										
PTT (seconds)										
INR										
Total Bilirubin (µmol/L) (5-21)										
Conjugated Bilirubin (µmol/L) (0-3)										
Serum IgG (g/L)										
Serum IgM (g/L)										
Liver Cirrhosis present										

Serology Tests

AMA							
ANA							
SMA							
SLA							
LKM-1							
p-ANCA							
Other							

Histology:

	Yes
Liver Biopsy	

	Biopsy 1	Biopsy 2	Biopsy 3
Typical Histology			
Atypical Histology			
N/A (biopsy not done)			

Imaging Results

Abdominal U/S	Yes
Intra hepatic bile duct dilatation	
Extra hepatic bile duct dilation	
Cholelithiasis	
Features of cirrhosis	
Features of portal hypertension	
Additional Information	

MRCP/ERCP	Yes
multifocal strictures and segmental dilations of intrahepatic and/or extrahepatic bile ducts	

Presence of Concomitant Autoimmune Diseases

	Yes
Autoimmune thyroid disease	
Sjogren's syndrome	
Celiac disease	
Rheumatoid Arthritis	
Psoriasis	
Systemic Lupus Erythematosis	
Vitiligo	
Inflammatory Bowel Disease	
Immune thrombocytopenic purpura	
Autoimmune haemolytic Anaemia	
Type 1 Diabetes Mellitus	
Other	

Treatment	Yes	Date
Prednisone		
UDCA		
Combination of UDCA and Prednisone		
Azathioprine		
Combination of Azathioprine and prednisone		
Combination of azathioprine/UDCA/ and Prednisone		
Liver Transplant		
Other		

Outcomes	Yes
Remission	
Liver cirrhosis	
Portal hypertension	
Hepatic decompensation	
Liver transplant	
Death	
Lost to follow up	
Other	

Appendix G ethics clearance:

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr M Ostrofsky

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M160307

NAME: (Principal Investigator)	Dr M Ostrofsky
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine Liver Clinic Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A retrospective analysis of patients with auto-immune hepatic diseases, a study from Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)
DATE CONSIDERED:	01/04/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor A Mahomed
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	28/06/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **March** and will therefore be due in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

28/06/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES