CHRONIC HEPATITIS AT GROOTE SCHUUR HOSPITAL: 1978 – 1996.

A LITERATURE REVIEW OF THE SYNDROME, ITS CLINICAL SPECTRUM AND MANAGEMENT AT GROOTE SCHUUR HOSPITAL.

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A DISSERTATION SUBMITTED TO THE FACULTY OF MEDICINE, UNIVERSITY OF CAPE TOWN, IN FULLFILMENT OF REQUIREMENTS FOR PART III OF THE DEGREE OF MASTERS IN MEDICINE.

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PREFACE

Chronic hepatitis has multiple aetiologies which include viral hepatitis (hepatitis B, B+D and C), autoimmune hepatitis and drugs. In sub-Saharan Africa the major aetiological factor is chronic hepatitis B virus infection. In this region 10-15% of the population is chronically infected with the virus and 76% have serological evidence of past exposure to the hepatitis B virus. HDV infection has not been documented in Southern Africa but studies from Northern Africa show antibody positivity for HDV of 21-31% in patients with chronic HBV infection. Drug-induced hepatitis is also increasingly being recognised as a significant entity.

This study arose from the observation that there are a significant number of patients with autoimmune hepatitis on follow-up at the Groote Schuur Hospital liver clinic. This group includes patients who test negative for the standard autoimmune markers done at Groote Schuur Hospital but have liver histology that is typical of classical autoimmune hepatitis. They also show a clinical and biochemical response to steroid and azathioprine therapy that is indistinguishable from that seen in classical autoimmune hepatitis cases on similar treatment.

This study is retrospective and covers the period 1978 – 1996. The patients studied are those with chronic hepatitis as defined by the International Working Party in 1995, i.e. patients with necro-inflammatory disease of the liver lasting at least 6 months. This includes hepatitis B, B + D, C, autoimmune hepatitis and drug-induced liver disease. Several other liver diseases that may present with clinical and histological features of chronic hepatitis are specifically excluded. These include Wilson's disease, Primary biliary cirrhosis, Primary sclerosing cholangitis, alpha-1-antitrypsin deficiency, alcohol abuse and iron over load states.

DECLARATION

I declare that this dissertation is my own work. It is submitted for the Degree of Masters of Medicine. No part of it has been submitted before at another University. The information used in this thesis was obtained while I was employed at Groote Schuur Hospital, University of Cape Town.

Signed .

Henry N Hairwadzi.

30th APRIL, 1999.

DEDICATION

To my wife Kusile, children Chido and Manatsa, I would like thank you all for the patience and support.

My good friends Steven and vaZariro.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Professor P Hall for the guidance; support, encouragement and teaching which made this work a reality. Her tutoring of the intricacies of liver histopathology made the histological aspect of this work possible. While doing this work, I have learnt and acquired skills that certainly be will of great use in my future work. I would also like to thank Professors R Kirsch, J Seggie, R Hift and Doctor W Spearman for the encouragement, teaching, and the trust and belief that they have shown in me and the work that I am doing. Dr Isaacs at the Groote Schuur Hospital Medical Informatics Department was responsible for the statistical analysis of the data and guided me in planning the appropriate data collection and results recording.

I would also like to acknowledge the following people for all their help and assistance while I worked on this thesis:

. The Groote Schuur Hospital Medical Records department staff for tracing and retrieving as many of the old hospital notes as was practically possible.

. The University of Cape Town Department of Pathology staff for tracing and retrieving the liver histopathology sections dating back to the last 20 years.

. Mrs M Burger and Mrs F Pearce for all the secretarial and other assistance that they gave freely.

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ABBREVIATIONS

AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
AMA	Antimitochondrial antibodies
ANCA	Anti-neutrophil cytoplasmic antibodies
ANF	Antinuclear factor
ASGPR	Anti-asialoglycoprotein receptor antibodies
AST	Aspartate aminotransferase
CAH	Chronic active hepatitis
CLH	Chronic lobular hepatitis
CPH	Chronic persistent hepatitis
CRYP -	Cryptogenic chronic hepatitis
CRYP +	Cryptogenic steroid sensitive chronic hepatitis
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
HAI	Histological activity index
HBV	Hepatitis B virus
HBx	Hepatitis Bx protein
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HLA	Human leukocyte antigens
IFN α	Interferon alpha
INR	International normalised ratio
LKM-1, 2	Liver, kidney microsomal antibodies1, 2

NHL	Non-Hodgkin's lymphoma
NIH	National Institutes of Health
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction
PSC	Primary sclerosing cholangitis
RIBA	Radio-immunoblot assay
RNA	Ribonucleic acid
SLA	Soluble liver antigen
SMA	Smooth muscle antibodies

CHAPTER 1:

THE SYNDROME OF CHRONIC HEPATITIS

1:1 DEFINITION AND CLINICAL FEATURES

Chronic hepatitis is defined as a chronic inflammatory reaction in the liver continuing without improvement for at least 6 months (International Working Party 1995). The condition has several different causes with all age groups and races being affected. Viral Hepatitis B plus or minus D, hepatitis C, autoimmune hepatitis, cryptogenic and drug induced hepatitis are the main causes and their proportions vary geographically. Chronic hepatitis is frequently asymptomatic. Symptoms, when present, may be intermittent or constant and range from mild nonspecific complaints such as malaise, lack of energy and poor appetite through to jaundice, abdominal swelling, drowsiness, confusion, bleeding and bruising typical of severe liver disease. Physical signs may be minimal. Mild liver tenderness is often the only finding. In contrast some patients may present with jaundice, ascites and splenomegaly. Biochemical abnormalities are often picked up fortuitously. The most common abnormalities are increased plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity both usually to a similar degree (Williams and Hoofnagle 1988, Di Bisceglie and Hoofnagle 1996). The albumin and prothrombin international normalized ratio (INR) may be normal until hepatic decompensation occurs. Moderately raised serum globulin concentrations may indicate chronicity while high levels could suggest autoimmune hepatitis.

The histological features consist of varying degrees of necrosis, inflammation and fibrosis. Hepatocyte necrosis may be focal, confluent, bridging, interface or combinations of any of these patterns. The inflammatory cells are predominantly mononuclear in nature.

1:2 CHRONIC HEPATITIS: A HISTORICAL PERSPECTIVE

In 1950 Waldenstrom described young female patients who had chronic hepatitis, raised plasma globulins and a plasma cell infiltrate plus cirrhosis on histology. Mackay found that these patients demonstrated the LE phenomenon and proposed that the disease be termed *lupoid hepatitis* (Mackay et al. 1956). This led to the disease being inappropriately considered to be part of the systemic lupus erythematosus spectrum of disease. Confusion in terminology occurred in the 1960's as more studies on chronic hepatitis began to document its varied clinical, biochemical and histologic features. This led to new terms such as *plasma cell hepatitis, active juvenile cirrhosis* and *subacute hepatitis* being used to describe the different manifestations of chronic hepatitis.

The term *piecemeal necrosis* was introduced by Popper and co-workers to describe hepatocyte necrosis, the disappearance of parenchymal cells at the periphery of the lobule or along fibrous or inflammatory septa. Following their report piecemeal necrosis became synonymous with progression to chronic liver disease (Popper et al. 1960, Paronetto et al. 1962, Schiff L and Schiff ER 1993). It is now thought that other forms of cell death, in particular apoptosis, maybe of equal or even greater importance (Desmet et al. 1994). The histologic patterns of bridging necrosis and multilobular necrosis are associated with an increased propensity for progression to cirrhosis

(Baggenstoss et al. 1972, Schalm et al. 1977). Portal tract to central vein bridging is particularly aggressive compared to portal tract to portal tract bridging (Cooksley et al. 1986). The Mayo Clinic Group in 1972 identified bridging necrosis (confluent necrosis linking vascular structures) or multilobular necrosis as predictive for the development of cirrhosis in chronic hepatitis of more than 10 weeks duration (Soloway et al. 1972).

In 1968 De Groote et al. for the first time attempted to classify chronic hepatitis on the basis of histologic features and introduced the terms *chronic persistent hepatitis* (CPH) and *chronic aggressive hepatitis* (CAH) into the literature. CPH described predominantly portal chronic inflammation with preserved lobular architecture and little or no fibrosis. CAH described more severe disease with inflammation extending into the parenchyma with piecemeal necrosis, formation of intralobular septa and disturbed architecture but no lobular regeneration. The activity was graded as moderate or severe based on the degree of necrosis and inflammation. CPH was thought to have a better prognosis (De Groote et al. 1968). Popper introduced the term chronic lobular hepatitis (CLH) to describe the histological pattern of spotty parenchymal necrosis and inflammation with minimal or mild portal inflammation (Popper and Schaffner 1971). A comparison of the different histologic groups is shown in ***Table 1*** (Randall 1994).

TABLE: 1

Comparison of Histologic Groups (Randall 1994)

	Portal Inflammation	**Piecemeal Necrosis**	**Lobular Activity**	**Fibrosis**
Chronic Persistent Hepatitis	Present	None or slight	Variable	Nil
Chronic Active /Aggressive Hepatitis	Present	Mild to severe	Variable bridging to multilobular necrosis	Variable periportal bridging to cirrhosis
Chronic Lobular Hepatitis	Minimal or mild	Nil	Mild to moderate	Nil

1:3 MODERN VIEWS OF CHRONIC HEPATITIS

Over the years, documentation of the clinical and histologic evolution of chronic hepatitis, identification of new aetiologic agents and new diagnostic methods have resulted in a need to modify the original classification done by De Groote et al. in1968. The recognition that CAH, CPH and CLH are not distinct diseases by themselves, but part of a spectrum of histological activity which can occur in the same patient during the course of disease, e.g. chronic hepatitis B, argues for the discontinuation of these terms (Scheuer 1991). Scheuer proposed that a system that takes into account the aetiology of disease, various forms of necroinflammatory activity (CAH, CPH and CLH) and architectural changes (fibrosis) would be more comprehensive than the individual terms of CAH, CPH and CLH. The most recently proposed nomenclature emphasizes an aetiologic terminology and the discontinuation of the terms chronic active hepatitis, chronic persistent hepatitis (De Groote et al. 1968) and chronic lobular hepatitis (Popper and Schaffner 1971). These terms have

been the basis on which chronic hepatitis has been histologically classified (Desmet et al. 1994). By giving numerical scores to describe the intensity of necroinflammatory activity and fibrosis, the concepts of *grading* and *staging* of histology were also introduced (International Working Party 1995, Ishak et al. 1995). This gives a semi-quantitative and more reproducible assessment of the histological features of a particular liver biopsy.

1:3.1 AETIOLOGY

The causes of chronic hepatitis are viral hepatitis B, B plus D and C, autoimmune hepatitis and drug-induced liver injury (***Figure 1***). The diagnosis of chronic hepatitis should only be made after the proper exclusion of other causes of chronic liver disease, which have clinical and histological similarities to chronic hepatitis. The differential diagnosis includes genetic disorders with major extra-hepatic manifestations (e.g. Wilson's disease, haemochromatosis and alpha-1-antitrypsin deficiency), alcoholic hepatitis which has a different histological pattern, primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) where the major pathology is in the biliary system rather than the hepatic parenchyma. Nonalcoholic steatohepatitis and graft-versus-host disease in organ transplantation should also be considered (Desmet et al. 1994, International Working Party 1995).

Cryptogenic chronic hepatitis, unclassified as to viral or autoimmune is a chronic inflammatory disease of the liver for which no clear distinction between the two aetiologies can be made. Many of these patients present with already established cirrhosis. Laboratory tests for hepatotropic viruses are negative and should ideally

include the HCV-RNA PCR test. Autoantibodies found in autoimmune hepatitis are negative although a small minority of these patients may respond to a trial of steroids.

FIGURE: 1

Current Aetiological Nomenclature of Chronic Hepatitis
(International Working Party 1995)

WELL ESTABLISHED CAUSES

1. Viral hepatitis

- **Hepatitis B**
- **Hepatitis B and D**
- **Hepatitis C**

2. Drug- induced hepatitis

3. Autoimmune hepatitis

- **Classic (Type 1) autoimmune hepatitis**
- **Type 2 autoimmune hepatitis**
- **Type 3 autoimmune hepatitis**
- **Cryptogenic steroid responsive hepatitis**

4. Cryptogenic chronic hepatitis, unclassified as to viral or autoimmune

DIFFERENTIAL DIAGNOSIS

- **Resolving acute hepatitis**
- **Wilson's disease**
- **Alpha-1-antitrypsin deficiency**
- **Haemochromatosis**
- **Alcoholic hepatitis**
- **Nonalcoholic steatohepatitis**
- **PBC**
- **PSC**
- **Graft-versus-host disease**

1:3.2 PATHOPHYSIOLOGY

1:3.2(a) CHRONIC HBV INFECTION

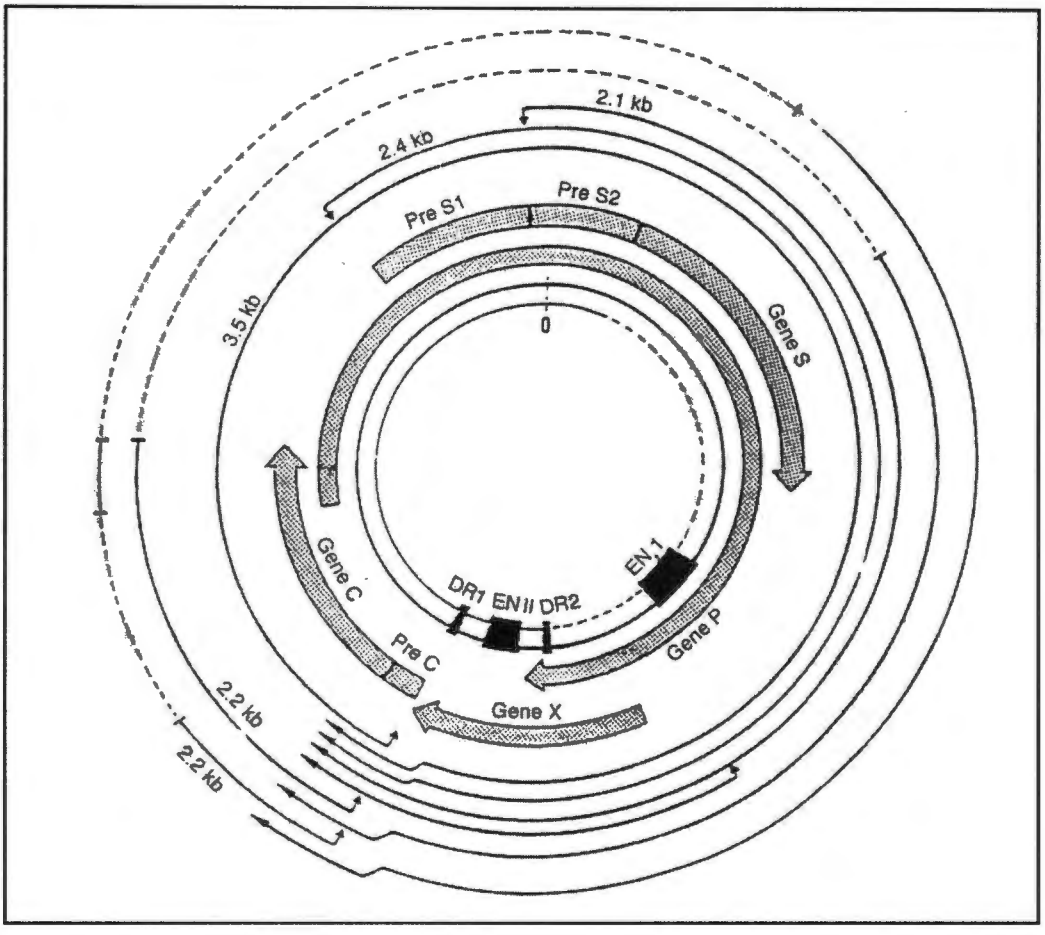
The HBV genome is a partially double-stranded circular DNA of about 3200 base pairs. It encodes four overlapping reading frames:

1. S for the surface or envelope gene.
2. C for the core gene.
3. X for the x gene.
4. P for the polymerase gene (***Figure 2***).

Following entry of the virus into the cell, the viral genome is delivered to the nucleus assisted by a nuclear localization signal in the viral core protein that is activated by protein phosphorylation. Viral genome replication and viral protein assembly follows and during these processes, small epitopes of HBV proteins (especially HB core antigen) are thought to initiate immune responses. The hepatitis B x protein (HBx) is a transactivator which is believed to stimulate cellular signal transduction and activation of regulatory genes to result in the expression of genes that induce or maintain inflammation. This effect is thought to be important in activating gene transcription and the development of hepatocellular carcinoma through the rearrangement of cellular and viral DNA. Some reports have shown that HBx alters the function of tumour suppressor gene p53 (Wang et al. 1994).

FIGURE: 2

Structure Of HBV Genome (Brechot 1996)



In HBV chronic hepatitis, the damage is not due to the virus itself. The hepatocyte necrosis is due to the host's immune response against the virus. The cellular responses are through HLA class I-restricted CD 8+ lymphocytes and HLA class II-restricted CD 4+ lymphocytes. The CD 8+ cells respond to HLA class I pathway processed HBcAg which is presented on the surface of the hepatocytes. This initiates a cytotoxic response mediated through cytokines; perforin release and Fas ligand mediated apoptosis. Nonhepatic antigen presenting cells can also process hepatitis B virions through the HLA class II pathway to stimulate CD 4+ T cells resulting in damage of

infected hepatocytes. The cytotoxic T cell response in those who clear the virus is vigorous, polyclonal and multispecific compared to those who develop chronic hepatitis (Chisari 1997). The differences in immune responses are due to the variable binding affinity of the class I binding groove for the immunodominant HBV peptides. In the infected neonate with an immature immune system, 95% become asymptomatic carriers while in children infected after the neonatal period but below 6 years, only 30% are carriers. In adults this figure drops further to only 3-5% (Lee 1997).

1:3.2(b) CHRONIC HBV and HDV INFECTION

The hepatitis delta virus (HDV) is a defective RNA virus, which can not be assigned to any of the known group of viruses. It resembles viroids and plant satellite viruses (Koshy et al. 1991). It requires the helper function of HBV (or other hepadna viruses) for its replication and expression. It is a spherical particle of 36nm in diameter, slightly smaller than HBV, with a circular 1700 nucleotide single-stranded RNA (minus strand). The outer envelope contains HBsAg but also has small, middle and large surface proteins. The genome has features of the rolling circle mode of replication common to genomes of the plant satellite viruses or viroids, with both genomic and antigenomic RNA being produced due to the ability for self-cleavage and self-ligation (Taylor et al. 1991). It replicates via RNA-directed RNA synthesis by transcription of genomic RNA to a complementary antigenomic RNA (plus strand). The antigenomic RNA strand in turn serves as a template for subsequent RNA synthesis. The genomic RNA is approximately 10 fold more abundant than the antigenomic RNA and remains primarily in the nucleus while the antigenomic RNA is within the cytoplasm where it is responsible for delta antigen production (Hess 1993).

The interaction between HBV and HDV replication is not clear, but it is known that HBsAg synthesis is sufficient to allow HDV production and that when an HBsAg carrier is superinfected with HDV, the synthesis of HBV is frequently shut down. This is due to an inhibitory effect of the HDV, however the activity of the chronic hepatitis is high due to the direct cytopathic effect of the HDV (Sureau et al. 1992). Most of the information on HDV comes from *in vitro* studies and animal models and cannot all be extrapolated to the human host. Cell injury is thought to be cytopathic through the molecular interactions of HBV and HDV. The development of hepatocellular carcinoma in these patients is rare and this may be due to the rapid course of disease to liver failure and cirrhosis such that there is no time for the evolution of primary liver cancer.

HDV infection can either be simultaneously with HBV (*co-infection*) or infection of a person already infected with HBV (*superinfection*). Chronicity following infection is varied with co-infection frequently resolving whereas superinfection tends to result in chronicity. Host factors may be important in the development of chronicity (Hess 1993). Superinfection can also transform asymptomatic or mild chronic hepatitis B into severe progressive disease and cirrhosis or alternatively can accelerate the course of chronic hepatitis B.

The transmission of HDV can be sexual, perinatal or percutaneous innoculation. HDV infection shows 2 epidemiological patterns. In Northern Africa, Southern Europe and the Middle East, HDV is endemic among those with hepatitis B infection and transmission is mostly by nonpercutaneous means. In the United States and Northern Europe meanwhile, HDV infection is confined to persons with frequent exposure to blood and blood products such as drug addicts and haemophiliacs (Dienstag and Isselbacher 1998). In Africa, there is very little epidemiological data on HDV. Studies

from Senegal and Kenya show 21% and 31% antibody positivity in chronic HBV carriers respectively (Cronberg et al. 1984, Greenfield et al. 1986). In Southern Africa, a study looking at Southern African black people with hepatocellular carcinoma and matched controls showed no cases of co-infection with HBV and HDV (Kew et al. 1984).

1:3.2(c) CHRONIC HCV INFECTION

HCV was recognized in the mid 1970s as the cause of post transfusion non-A, non-B hepatitis following the use of HB surface antigen assays to screen blood for transfusion. The genome was identified in 1989 and only after then did serological and polymerase chain reaction tests become available to test for the presence of this infection. The virus shows marked genetic heterogeneity with 6 major genotypes that differ by 31-34% at the nucleotide level and more than 50 subtypes that differ by 20-23% in nucleotide sequence being recognized (***Table 2***).

TABLE: 2

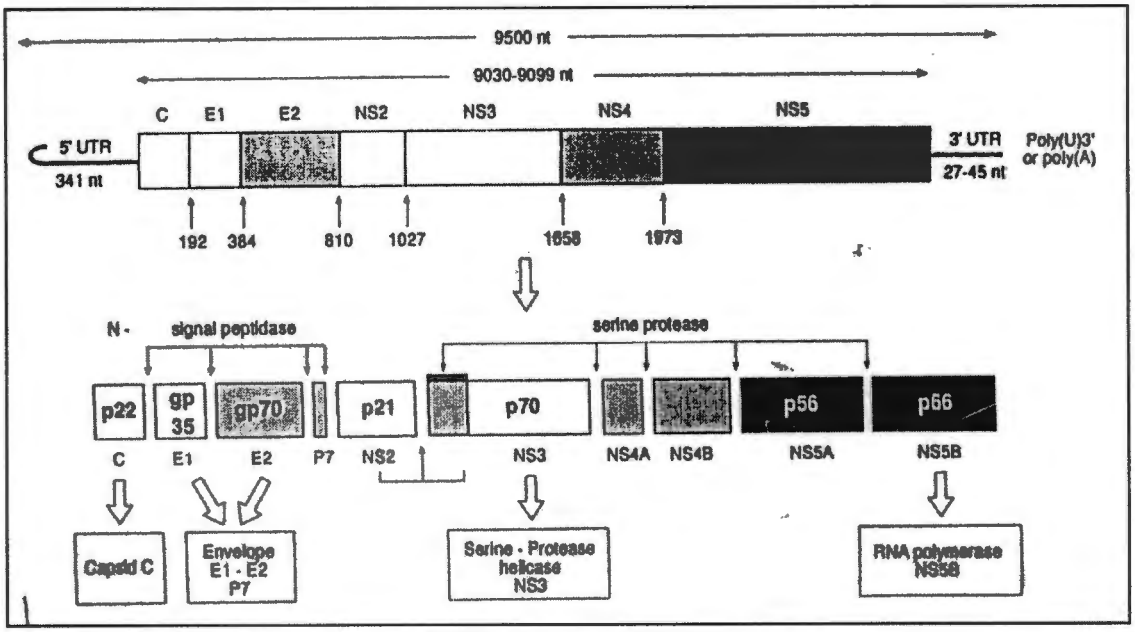
HCV genotypes and their distribution (Di Bisceglie 1998)

GENOTYPE	GEOGRAPHICAL PREDOMINANCE
1a	USA and developed western countries
1b	USA, Japan, Europe
2	Most developed countries, but not very common
3	Rising in prevalence among injection-drug users
4	Confined to the middle east and north Africa
5	South Africa
6	Asia

The genome is a single stranded RNA of approximately 10 000 nucleotides in length with a single open reading frame that codes for both structural and nonstructural proteins. There is homology of some regions of the HCV genome with similarly located regions of flavi-viruses genomes (yellow fever, dengue, Japanese B and pesti viruses) (Bisceglie 1998, Sharara et al. 1996). Two envelope proteins that coat the virus and a nucleocapsid core protein form the structural proteins. The E2/NS1 region has a hypervariable portion that mutates rapidly when the virus is under immunological surveillance. This occurs within the E2HV domain of this portion. The end result is the formation of quasi-species (nucleoside variability in viruses circulating within an individual) due to ongoing viral mutations (Ogata et al.1991). This ability to mutate and form quasi-species facilitates viral persistance and resistance to neutralisation. The nonstructural proteins are involved in viral replication with the NS3 gene producing a viral helicase, which is critical for viral replication. The 5' end contains an untranslated region of about 341 base pairs which functions as an interval ribosomal entry site. This region is highly conserved in the different HCV genomes and it makes an ideal target for diagnostic testing. The NS5A is known to be a serine phosphoprotein but its function is not clear presently. The NS5B has both RNA-dependent polymerase activity and terminal transferase activity (***Figure 3***).

FIGURE: 3

Structure Of HCV Genome (Brechot 1996)



Acute infections proceed to chronicity in 80%-85% of cases and recovery does not prevent re-infection on subsequent re-exposure (Dhumeaux et al. 1997, NIH consensus statement 1997). A direct cytopathic effect by HCV is not proven due largely to the absence of cell culture systems. The cellular damage appears to be due to an immunological attack as suggested by the presence of cryoglobulin, circulating immune complexes and autoantibodies. A role for excess hepatic iron in cell injury or resistance to interferon therapy is suggested in some studies (Olynyk et al. 1995, Hayashi et al. 1994). Studies from Italy have found an increased prevalence of HCV in patients with B cell Non-Hodgkin's Lymphoma (NHL); particularly low grade NHL with mixed cryoglobuliaemia (Pazzalo et al. 1994, Ferri et al. 1994). However similar studies, particularly in Germany, have failed to show such a connection (Ellenrieder et al. 1998).

The precise mechanisms of viral persistence and cellular injury are not well worked out. Viral persistence (unlike in HBV infection) is not dependent on integration in the

host genome but seems to be due to its ability to mutate and form quasi-species which can escape neutralisation by antibodies. To establish a persistent infection, a virus has to be able to establish a persistent non-cytopathic infection without disturbing vital cellular functions and secondly be able to evade the host's humoral and cellular immune response (Oldstone 1991). The lymphotrophic characteristics of the virus appear to allow the virus to alter immunological response possibly through the inhibition of interferon pathways, inhibition of complement pathways, interference with HLA expression, altered cytokine production and lymphocyte signal transduction (Booth and Thomas 1996, McChesney and Oldstone 1987, Gooding 1992). This allows the virus to persist in hepatocytes and T lymphocytes. The transmission of the virus by T lymphocytes has been well documented (Helling 1988).

1:3.2(d) CHRONIC DRUG-INDUCED HEPATITIS

The mechanisms underlying drug-induced chronic hepatitis are complex and poorly understood. This is due in part to a lack of an animal model since these reactions appear to be limited to susceptible humans and there is no relationship between the ingestion or amount of drug ingested and the occurrence or onset of liver disease. The cytochrome P450 system in humans handles ingested toxins and is thus located strategically in the liver and gastrointestinal tract, allowing it to digest and breakdown liposoluble xenobiotics (small foreign molecules) before they can reach the circulation (Robin et al. 1997). The products formed are stable or reactive metabolites that may covalently bind hepatic macromolecules to cause direct liver toxicity. The superposition of the cytochrome P450 system and immune system in human beings means that P450 generated reactive metabolites binding to proteins can modify the *self* of the individual and mislead the immune system. An immune attack against

hepatocytes occurs either against the self (autoimmune targets) or against the modified self (neoantigens). The cytochrome P450 system is responsible for oxidation and demethylation reactions, which generate hydroxyl groups. Through sulphation or glucuronidation reactions, a large water-soluble polar group is attached to the hydroxyl group. As a result of these reactions, cytochrome P450 activates drugs like hydrallazine and tienilic acid by forming adducts which generate a novel domain on the P450 protein. This is recognized by T-cells and induces an immune attack against cytochrome P450 in hepatocytes.

Drug induced chronic hepatitis may present with a spectrum of clinical, serologic and histologic features. At the one end of the histological spectrum, the pattern of liver injury resembles autoimmune hepatitis (e.g. methyldopa), at the other chronic viral hepatitis (e.g. isoniazid). However, most cases of drug-induced liver injury have features suggestive of both (***Table 3***).

TABLE: 3

Drugs Implicated in the Aetiology of Chronic Hepatitis
(Modified from Bass and Ockner 1996)

FEATURE	DRUG EXAMPLES	
Autoimmune-like	Methyldopa Nitrofurantoin Sulphonamides	Tienilic acid Phenytoin Diclofenac
Chronic viral Hepatitis-like	Amiodarone Isoniazid	Aspirin Etretinate

The number of drugs implicated is continually rising. Chronic liver damage appears to be both as a result of continued exposure to the agent and in some cases to a self-perpetuating process following the exposure. In the latter, removal of the agent may

not stop the progression of liver disease (Manns and Obermayer-Straub 1997). Methyldopa causes a wide spectrum of dysfunction and liver pathology ranging from asymptomatic enzyme elevations to acute and chronic hepatitis. It is well described as causing chronic hepatitis with autoimmune features (Maddrey1977). The liver disease is indistinguishable from other causes of chronic hepatitis. Liver injury tends to improve on stopping the drug or rarely may progress to cirrhosis or have a fatal outcome. The pathogenesis is not known. The major factors involved appear to be genetic predisposition, toxic metabolite generation and immune drug hypersensitivity. There is evidence for the formation of a quinone which binds and damages proteins. Suppressor T-cell function is inhibited resulting in up regulation of antibody production. The Coombs' test is positive with the formation of antinuclear and anti-smooth muscle antibodies (Kirtland et al. 1980).

1:3.2(e) AUTOIMMUNE HEPATITIS

Human leukocyte antigens have an antigen-binding groove and the polymorphisms studied as HLA types are largely related to specific aminoacid substitutions in and around the groove. In autoimmune liver disease, studies have identified specific aminoacid substitutions in the antigen-binding groove of human leukocyte antigen DR molecules that may clinically determine (1) the severity of disease and (2) disease susceptibility through their direct influence on antigen binding. The DR phenotype correlates with the age at disease onset, DR 8 appears to be associated with an increased frequency of disease. The anti-liver, kidney microsomal autoantibodies formed (anti-LKM) are directed against four linear epitopes on cytochrome P450 2D6. The adrenal and ovarian failure, which often accompanies this condition, may be due to autoantibodies to P450 cytochromes (P450 c 21, P450 scc and P450

c17) in these organs which destroy their normal function (Manns et al. 1991, Manns and Obermayer-Straub 1997). The autoantibodies associated with autoimmune hepatitis (AIH) are useful in defining patient groups with distinct clinical profiles and outcomes thereby assisting clinicians to improve diagnostic accuracy, standardise research and may form the basis for rational treatment protocols. Three major groups are recognised, Types 1, 2 and 3 which differ significantly in their autoantibody profiles, clinical progression and response to medical treatment (***Table 4***) (Czaja and Manns 1995). A cryptogenic group is also recognised.

Type 1 and **2** constitute 80% and 4% of the group respectively (Krawitt1996, Czaja 1996). **Type 1** is characterised by the presence of smooth muscle antibodies (SMA) and/or antinuclear antibodies (ANA) in serum. The presence of antibodies to polymerised F-actin being highly specific for the diagnosis. **Type 2** is characterised by the presence of anti-LKM-1 antibodies and this is mutually exclusive of SMA and/or ANA seropositivity. **Type 3** represents about 3% of the whole group. It is characterised by the presence of antisoluble liver antigen (SLA) which reacts against cytokeratins 8 and 18 within the cytoplasm of hepatocytes (see **section 1:3.4(e) page 32**). The trigger that sets in motion the autoimmune process is not clear but appears to be of viral origin in the setting of a susceptible individual with the appropriate genetic make-up. There is support for this from reports in which AIH developed after well-documented viral hepatitis (Lindberg et al. 1975, Vento et al. 1997) and also the demonstration of measles virus-specific nucleic acid sequences in peripheral blood lymphocytes of patients with AIH (Robertson et al. 1987).

TABLE: 4

Variants of Autoimmune Hepatitis
(Czaja and Manns 1995, Czaja 1995,Van Den Berg 1998)

FEATURES	TYPE 1 (80%)	TYPE 2 (4%)	TYPE 3 (3 %)
Antibody status *	Incidence (%)	Incidence (%)	Incidence (%)
Anti-nuclear	60	0	<5
Smooth muscle	70	0	30-40
Soluble liver antigen	0	0	100
Liver-kidney microsomal type 1	0	100	0
Anti-neutrophil cytoplasmic (ANCA)	75-90	?	?
Anti-asialoglycoprotein receptor (ASGPR)	80-85	?	?
Auto-antigen status	Unknown	Cytochrome P450 2D6	Cytokeratins 8 and 18
Genetic predisposition	HLA A1, B8, DR 3, 4	HLA B14, DR3, C4A-Q0	Unknown
Clinical profile	Adults ++ Two peaks of occurrence 10-20 years 45-70 years High globulin levels	2-14 years Paediatric Fulminant onset Lower globulin levels	Adults 30-50 years
Prognosis			
Cirrhosis rate	45%	82%	75%
Steroid response	70% and related to HLA haplotype	<70%	100%
HCV infection %	11%	44-86%	0%
Other immunological disease %	17%	34-40%	58%
Women	70%	89%	90%
Treatment**	Steroids alone or steroids with azathioprine	Same	Same

* — Van Den Berg 1998.
** — See also *Table 11 page 49*.

In Southern Europe, the prevalence of anti-HCV positivity is in the region of 80% and this is associated with AIH **type 2** in 40%-50% of cases in contrast with Northern Europe, which has a much lower positivity rate of anti-HCV and prevalence of AIH **type 2**. Unlike the typical profile of **type 2** AIH profile of females more than males, young age at onset, high LKM-1 antibody titres and a good response to immunosuppression, the anti-HCV associated **type 2** AIH has a different profile. This form of AIH **type 2** occurs in an older population, usually older than 40 years with more males than females being affected. The disease activity is much lower with a poor response to immunosuppression and lower LKM-1 antibody titres. Anti-GOR antibodies are often positive in this patient group (Michel et al. 1992).

Anti-GOR reflects HCV-induced autoimmunity. GOR47-1 is a cDNA clone that was isolated in the plasma of chimpanzees infected with HCV and was subsequently shown to encode a protein that is able to bind antibodies in patients with acute and chronic HCV infection. Its amino acid sequence (GRRGQKAKSNPNRPL) does not correspond to the reported sequences for HCV but to that of a single-copy gene of the host cellular genome (Mishiro et al. 1990). Anti-GOR is therefore not a proper autoantibody but is an anti-HCV core antibody that cross-reacts with a cellular self-antigen in the host. It is detectable earlier than anti-HCV, is strictly associated with replicating HCV infection and the titres correlate with antibodies to HCV-core antigen and the necroinflammatory disease activity on liver biopsy.

1:3.3 HISTOPATHOLOGY

Until recently, the use of the terms grading and staging in pathology has been restricted to the assessment of neoplasms with respect to the degree of tumour differentiation and extent of spread respectively. The use of these terms in chronic hepatitis aims at assessing, in a reproducible manner, the histologic features that have been shown in studies to impact on the prognosis, disease severity and progression of chronic hepatitis.

Grading is a measure of the severity of the necroinflammatory activity going on and as such will fluctuate with disease activity. Staging is an assessment of disease progression and hence is a measure of fibrosis and architectural alteration, which results from the necroinflammatory process. Staging therefore tends to progress or remain stationary. Staging and grading are clearly necessary for prognosis in ordinary practice and are vital in the study of chronic hepatitis and in evaluating trials of new therapies to allow the comparison of different patient groups and treatment regimens. Prognostic information, which influences patient management, can be obtained in a more reproducible manner (Ludwig 1995).

Any grading and staging system should be clinically relevant; reproducible with acceptable intra and inter-observer error; and, provide information of clinical and scientific use to the management of patients. The most widely quoted and used system is the **Knodell score** or **Histological Activity Index (HAI)** which scores four components of histological lesions. A numerical score gives a reproducible measure, which supplements written pathology reports (Knodell et al. 1981). The four components assessed by this system are shown in ***Table 5***, each with numerical scores as shown and a possible total score of 18. Better understanding of disease aetiologies

and prognostic markers has resulted in a number of valid criticisms of this system and the proposition of modifications.

TABLE: 5

Components for Histology Scoring
(Knodell et al. 1981)

Component	Range of scores
Periportal necrosis with or without bridging necrosis	0-10
Intralobular degeneration and focal necrosis	0-4
Portal inflammation	0-4
Fibrosis	0-4

The following problems have been identified with this scoring system.

1. The first three components represent grading of disease and should be separate from fibrosis, which is a staging assessment.
2. Piecemeal necrosis and bridging necrosis should be assessed separately since they are both grading components that are thought to have different prognostic implications (Soloway et al. 1972).
3. More rigorous definitions should be used to reduce variations in observer interpretation of histology.
4. On statistical analysis, cognizance should be taken of the fact that the scoring systems are discontinuous variables (Ishak et al. 1995). A proposed scoring system based on current understanding of disease pathology is as outlined in ***Tables 6(a)*** *and* ***6(b)***.

TABLE: 6(a)

Grading Of Inflammation (Ishak et al. 1995)

Grading Component	SCORE
Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	**0**
Mild (focal, few portal areas)	**1**
Mild/moderate (focal, most portal areas)	**2**
Moderate (continuous around<50% of portal tracts or septa)	**3**
Severe (continuous around >50% of tracts or septa)	**4**
Sub-total	
Confluent necrosis	
Absent	**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 (P-C) bridging	**4**
Zone 3 necrosis + multiple P-C bridging	**5**
Panacinar or multiacinar necrosis	**6**
Sub-total	
Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
Absent	**0**
One focus or less per 10x objective	**1**
Two to four foci per 10x objective	**2**
Five to ten foci per 10x objective	**3**
More than ten foci per 10x objective	**4**
Sub-total	
Portal inflammation	
None	**0**
Mild, some or all portal areas	**1**
Moderate, some or all portal areas	**2**
Moderate/marked, all portal areas	**3**
Marked, all portal areas	**4**
TOTAL SCORE	

TABLE: 6(b)

Staging Of Fibrosis (Ishak et al. 1995)

Change	Score
No fibrosis	**0**
Fibrous expansion of some portal tracts +/- short fibrous septa	**1**
Fibrous expansion of most portal tracts, +/- short fibrous septa	**2**
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	**3**
Fibrous expansion of portal areas with marked bridging P-P and portal to central (P-C)	**4**
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	**5**
Cirrhosis, probable or definite	**6**
SCORE	

Sample reporting options for chronic hepatitis would therefore read like:

. Chronic hepatitis C, mildly active with bridging fibrosis.

. Autoimmune hepatitis, minimally active with cirrhosis.

The question of which scoring system to use will arise many times. It seems appropriate to continue using Knodell's scoring system with appropriate modifications to take into account the need to separate grading from staging features and the importance of scoring histologic features known to be of prognostic value in patient management.

Interface hepatitis, portal inflammation, bridging necrosis and cirrhosis are illustrated in Appendix C.

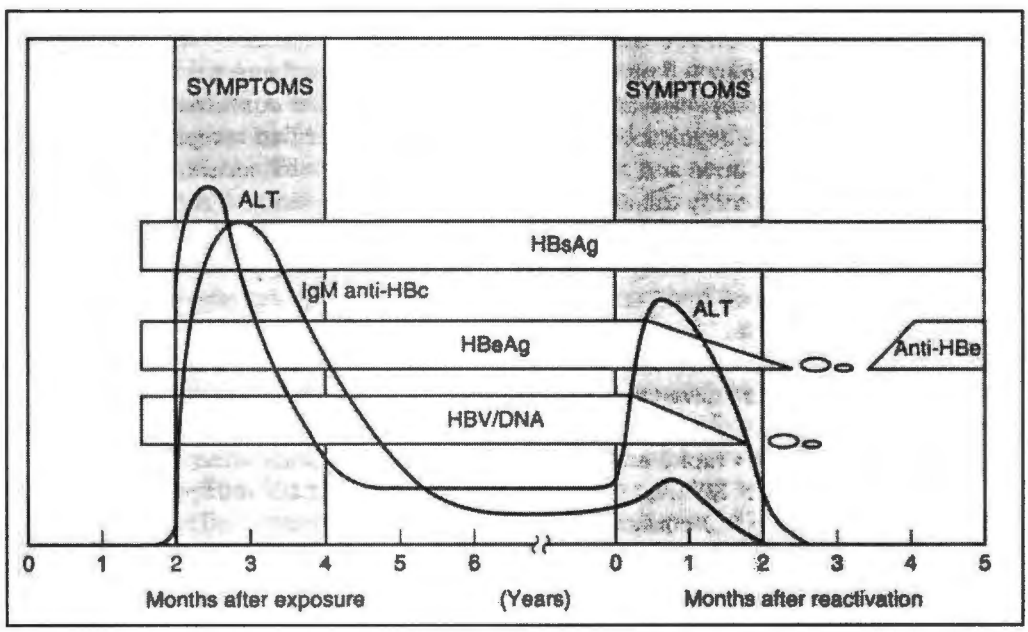
1:3.4 DIAGNOSIS

1:3.4(a) HEPATITIS B VIRUS INFECTION

In sub-Saharan Africa, 10-15% of the population is chronically infected with hepatitis B virus as compared to less than 1% in northern Europe and North America. The tests available for the serologic diagnosis of hepatitis B are HBsAg, HBeAg, HBcAg and HBV-DNA assays (***Figure 4***).

FIGURE: 4

Serological Profile of Hepatitis B Markers in Chronic Infection
(Decker 1993)



HBsAg is diagnostic of infection but gives no indication of the duration of the disease or the replication status of the virus. Infection without HBsAg in serum but positive HBV-DNA, is a rare finding, the significance is not clear (Brechot et al. 1985). HBeAg is a surrogate marker for active HBV replication and therefore the potential for viral infectivity. It may however be absent in infection with a mutant virus which cannot synthesize HBeAg. This is due to pre-core region mutations of the HBV

genome, which result in the formation of a stop codon (TGG to TAG) at the end of the pre-core region preventing HBeAg synthesis. Synthesis of HBcAg is intact since it is only the pre-core region that is deleted (***see Figure 2***). HBeAg is an immune telogen and its absence contributes to the development of more aggressive liver disease (Feitelson 1994, Liang et al. 1991, and Omata et al. 1991). HBcAg IgM appears in acute infection but may also be positive in chronic carriers where it is indicative of active replicative chronic HBV infection. Lower titres of HBcAg IgM are seen in chronic carriers. Anti HB core may be absent in children due to the relative immune deficiency which prevents them from making anti-HB core.

TABLE: 7

Comparison of Types of Hepatitis B Viral Infection
(Di Bisceglie and Hoofnagle 1996)

FEATURE	HEALTHY CARRIER	CHRONIC HEPATITIS B
Serum HBsAg	+	+
Serum HBeAg	–	±
Serum HBV-DNA	–	+
Serum aminotransferases	Normal	Elevated
Liver histopathology	Inactive	Active
Hepatic HBcAg	–	+
Risk of developing cirrhosis	Low	High
Risk of developing HCC	Low	High
Candidate for interferon therapy	No	Yes

HCC = Hepatocellular carcinoma
HBcAg = Hepatitis B core antigen

1:3.4(b) HEPATITIS B VIRUS AND HEPATITIS D VIRUS INFECTION

Infection with HDV can be demonstrated by showing an anti-HDV seroconversion through a rising titre or the de novo appearance of anti-HDV. HDV antigen can also be identified on liver tissue. HDV antigen is detectable only briefly in the serum after acute infection and its presence confirms ongoing HDV replication and potential infectivity. This assay is only available in research laboratories. Anti-HDV can take up to 30-40 days from the onset of symptoms before being detectable by ELISA techniques (Buti et al. 1986) and will also become undetectable the HBsAg is cleared, making retrospective diagnosis of HDV infections difficult.

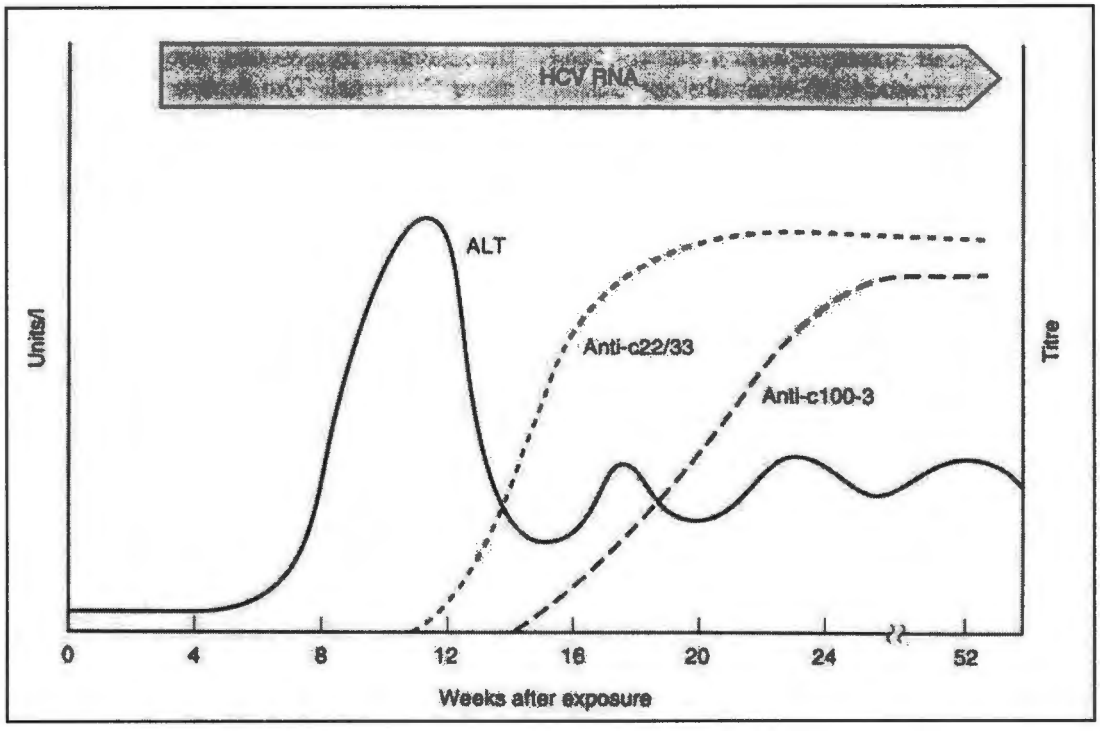
The presence of HBV core IgM antibodies, although not absolutely specific, are suggestive of co-infection when the anti-HDV antibody and HDV antigen are positive. In superinfection the HBV core antibodies will be IgG in nature together with a positive HDV antigen and anti-HDV antibody (Dienstag and Isselbacher 1998).

1:3.4(c) HEPATITIS C VIRUS INFECTION

At present, there are no diagnostic tests available that can test for HCV viral proteins. Diagnosis is based on the detection of the patient's antibody to HCV and testing directly for the virus RNA. The HCV antibody is not protective and does not confer any immunity. A liver biopsy is not required for diagnosis and aminotransferase levels by themselves are not helpful since only 40-60% of infected patients have raised enzymes and those with normal enzymes may have histological evidence of chronic hepatitis.

FIGURE: 5

Serological Course of Hepatitis C Infection
(Brown and Dusheiko 1993)

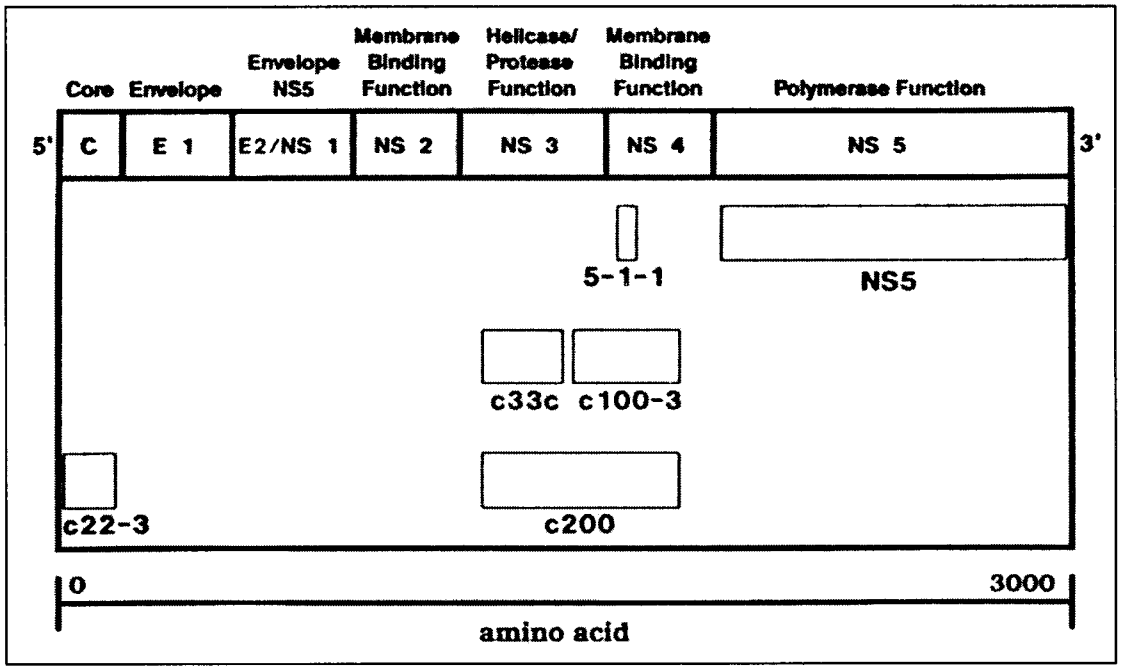


HCV ANTIBODY TESTS

The commercial kits currently available are reliable and have a sensitivity of almost 95%. The initial antibody test done is an enzyme immunosorbent assay (EIA) that uses four target HCV protein fragments in solution within the patient's serum. The fragments assayed are c22-3 of core region, c33c of NS3, c100-3 and 5-1-1 of NS4 region (***Figures 3 and 6***). False negative results with the EIA test can occur due to the use of one antigen only (as in c100-3 with the first generation assays), testing before seroconversion occurs and immunosuppression as with HIV or organ transplantation. Other problems include viral heterogeneity because current assays are based on the type 1a genotype, which is the most common in the USA. Hypergammaglobulinaemia and connective tissue diseases also interfere with the assays (Gross 1995 and 1998).

FIGURE: 6

Hepatitis C Virus Genome and Recombinant Proteins
(Medina and Schiff 1995)



If the EAI test is positive, the presence of antibody is confirmed with a radioimmunoblot assay (RIBA) which tests the patient's serum against the same four HCV protein fragments attached to a nitrocellulose strip to give a greater specificity. If the patient's serum reacts with two or more strips in the RIBA, the test is positive. More than 85% of patients with HCV viraemia show reactivity with two or more bands on second generation RIBA testing. A reaction with one strip is indeterminate. An indeterminate result may be seen in truly infected patients who have less common HCV genotypes (***Table 8***).

TABLE: 8

Interpretation of Serologic Tests for Hepatitis C Viral Infection
(Di Bisceglie and Hoofnagle 1996)

Anti-HCV EIA	ALT	HCV-RNA	RIBA	Interpretation
+	↑	+	+	Acute/chronic HCV infection
+	N	+	+	" Healthy carrier"
+	N	–	+	Recovered HCV infection
+	N	–	–	False positive

N = normal, ↑ = Elevated levels, + = Positive test, – = Negative test.

HCV-RNA TESTS

Qualitative and quantitative tests are available to assay for HCV-RNA.

Qualitative tests are designed to be very sensitive so as to pick up any virus present in the serum. The most sensitive test is the polymerase chain reaction (PCR) assay. This amplifies minute amounts of viral RNA under carefully controlled conditions to avoid false-positive and false-negative results. It can detect as few as 50-1000 genome-equivalents of HCV-RNA per ml of serum. Rapid separation of the serum from cellular blood components is important to maintain the integrity of the HCV-RNA genome and reduce inaccurate results. The variation in the HCV genome does not affect PCR testing because the PCR primers are based on the highly conserved 5' untranslated region of the genome.

Quantitative RNA tests measure the level of viral RNA in the blood. They are best suited for monitoring response to treatment and in making therapeutic decisions. These tests are less sensitive than PCR and should not be used for screening patients. The branched DNA (bDNA) signal amplification system has a reported sensitivity of

between 72 to 95% depending on the genotype. The assay can detect HCV-RNA down to a level of 1.6×10^5 genomes/ml. A low level of HCV-RNA, regardless of genotype, predicts those who in the long term are more likely to respond to interferon therapy (Lau 1993).

Immunostaining using polyclonal or monoclonal antibodies can detect HCV antigen (HCV Ag) within the liver. Only 60-70% of patients with a high level of HCV-RNA in serum are positive on immunostaining. The technique is difficult, has significant false positive results and is currently not available for routine clinical use (Krawczynski et al. 1992).

1:3.4(d) DRUG-INDUCED HEPATITIS

There are no specific tests available to confirm a diagnosis of drug-induced liver disease. Animal experiments cannot predict human idiosyncratic reactions and the clinical presentation may be with asymptomatic disease or fulminant liver failure. Each new drug is potentially hepatotoxic and a high index of suspicion is necessary to make a diagnosis (Kaplowitz 1986). Chronic drug-induced liver disease is considered to be present when biochemical evidence of liver injury has been present for more than 3 months. Biochemical evidence of liver injury is defined as an increase of more than twice the upper limit of the normal range of alanine aminotransferase (ALT) or conjugated bilirubin, or a combined increase in the aspartate aminotransferase (AST), alkaline phosphatase and total bilirubin. This is provided one of them is more than twice normal (Benichou 1990). There are no specific clinical features diagnostic of a drug aetiology for chronic hepatitis. Rash and fever with eosinophilia, although supportive of an acute drug reaction, occur rarely. Other features, which may occur, include renal failure, vasculitis and bone marrow suppression.

The temporal relationship is the first criterion used to establish an aetiological relationship of a drug to an illness. It is important to show that the patient was well before ingesting the drug, became ill while taking it and had a striking response after its withdrawal (Lee 1995). The latent period between ingestion and illness varies from several weeks for most forms of drug-induced hepatitis to more than 6 months in cases of chronic liver disease. Although variable, the latent period of many drugs is sufficiently reproducible to be of some diagnostic importance. Causality assessment is an integral part of the diagnostic process. The assessment attempts to quantify the likelihood of a particular disorder having been caused by a given drug. The drug history, temporal relationships between exposure, onset and resolution of liver abnormalities, clinical features and tests to exclude other causes of hepatobiliary disease are analysed to assess the likelihood of a drug being responsible.

The drug history should include ingested drugs and environmental toxins from present and past work environments or through recreational activities. Where more than one drug is being taken, it may be necessary to stop all drugs. The time to onset of the drug reaction ("challenge"), the course of the reaction after stopping the drug ("dechallenge") and the response to readministration of the suspected hepatotoxin ("rechallenge") can be scored and numerical cut-offs given. These can be used to define the reactions as suggestive, possible, dubious or incompatible with an adverse drug reaction (Benichou 1990).

Deliberate rechallenge to achieve a diagnosis is harzadous, has significant morbidity and mortality, and is not advised (Parrish 1990). From a practical and ethical viewpoint, in the individual case, rechallenge may be considered where further treatment with the implicated agent is absolutely necessary. Inadvertent rechallenge confirms the diagnosis but often with a fatal outcome (Seggie et al. 1979).

In vitro tests attempting to profile specific diagnostic tests for drug-induced liver disease have yielded conflicting results. No in vitro tests are specific and sensitive enough to predict an adverse drug reaction. The tests used presently include macrophage migration inhibition, lymphoblastic transformation and autoantibodies (LKM-2 antibody, which is anti-P450 2C9 and induced by tienilic acid and anti-liver microsomal antibody, which is anti-P450 IA2 induced by dihydrallazine). They all lack sensitivity and specificity and clinical use is limited. The individual susceptibility to have an idiosyncratic reaction is not necessarily immune mediated and rather depends on metabolic idiosyncrasy which consists of many factors impossible to measure/quantify in a single test. These include unusual metabolism of a drug, impairment of cellular antioxidant mechanisms or inhibition of enzymes that are already partially deficient from genetic defects (Farrell 1986).

Liver biopsy is not a prerequisite for the diagnosis of drug-induced hepatitis. There are histologic features that can be used to support such a diagnosis. These include microvesicular fatty change, zonal necrotic lesions disproportionate to the clinical severity, eosinophilic infiltration, bile duct destruction, granuloma formation and 'atypical cholestasis' such as cholestasis with hepatitis. Liver biopsy is also useful in diagnosing other causes of liver disease in patients who may be coincidentally using a potentially hepatotoxic drug. Use is also made of liver biopsy in cases where a drug in use for a serious medical condition with limited other choices is suspected of having an adverse effect (Farrell 1994).

1:3.4(e) AUTOIMMUNE HEPATITIS

Hypergammaglobulinaemia, serum autoantibodies and an unresolving inflammation of the liver characterize autoimmune hepatitis. Its clinical and histological features resemble those of other acute and chronic liver diseases. The immunological markers found in AIH can also be found in liver disease due to other causes. There is no single sensitive and specific diagnostic marker of AIH and so the diagnosis is therefore mainly one of exclusion. The diagnosis of autoimmune hepatitis implies the absence of the other diseases listed in ***Figure 1*** and can only be made from an assessment of a combination of clinical, biochemical, serological and histological features (Van Den Berg 1998).

Diagnosis may be difficult due to the many different autoantibodies associated with AIH, its co-existence and overlap with other diseases such as PBC and its varied clinical presentations. A panel of expects, The International Autoimmune Hepatitis Group, in 1992 made recommendations for diagnostic criteria for AIH based on a scoring system. This aims at achieving standard diagnostic and treatment approaches to AIH by clinicians (Johnson et al. 1993). Clinical, biochemical, immunological, virological and histological criteria are scored as well as the response to treatment. Points are given for particular features or deducted for conditions suggesting an alternative diagnosis. The scoring parameters are outlined in ***Tables 9(a)*** and ***9(b)***.

The autoantibodies used for diagnosis include anti-nuclear antibody (ANA), smooth muscle antibody (SMA), liver kidney microsomal antibody (LKM-1), soluble liver antigen (SLA), liver-pancreas antibody, asialoglycoprotein receptor (ASGP-A) and liver cytosol type 1. There is no single and specific diagnostic antibody for autoimmune hepatitis. ANA appear to be nonspecific manifestations of increased immunoreactivity associated with liver cell destruction (Sturgess 1992, Czaja 1995a).

In rheumatic diseases, patterns of ANA immunofluorescence characterize particular diseases and have diagnostic specificity. A diffuse pattern being associated with systemic lupus erythematosus (SLE), drug-induced SLE, rheumatoid arthritis, vasculitis and polymyositis. Mixed connective tissue diseases, Sjögren's syndrome and SLE have a speckled pattern. However no particular pattern of ANA indirect immunofluorescence positivity has been strongly associated with AIH (Czaja et al. 1997a). The patterns produced are variable, the most common being the homogeneous and speckled type. Younger patients have been shown tend to have speckled patterns at presentation but no prognostic value can be attached to individual patterns of immunofluorescence.

Smooth muscle antibodies are formed against actin and nonactin components (tubulin, vimentin, desmin and skeletin). When the reactivity is against actin cables in cultured fibroblasts, the specificity for AIH is high. The reactivity is against polymerized F-actin, which is closely associated with the hepatocyte membrane. Nonactin activity is common in viral infections and may give low positive SM titres.

Soluble liver antigen antibodies occur only in patients with AIH but are not mutually exclusive of other immunoserologic markers such as ANA and SMA. Their greatest value appears to be in assessing patients with cryptogenic chronic hepatitis in who up to 18% of cases can be reassigned to an autoimmune category (Manns et al. 1987, Czaja et al. 1993).

No separate criteria are needed for children but it should be noted that lower titres than those used in adults are considered significant. It is also important to note that overlap with PSC is a problem that needs exclusion with cholangiography.

TABLE: 9(a) (Johnson et al. 1993)

Scoring system for diagnosis of autoimmune hepatitis: minimum required parameters

Parameters	Score
Gender	
Female	+2
Male	0
Serum biochemistry	
Ratio of elevation of serum alkaline phosphatase vs. aminotransferase	
>3.0	-2
<3.0	+2
Total serum globulin, gamma globulin or IgG	
Times upper normal limit	
>2.0	+3
1.5-2.0	+2
1.0-1.5	+1
<1.0	0
Autoantibodies (titres by immunofluorescence on rodent tissues)	
Adults	
ANA, SMA or LKM-1	
>1:80	+3
1:80	+2
1:40	+1
<1:40	0
Children	
ANA or LKM-1	
>1:20	+3
1:10 or 1:20	+2
<1:10	0
or SMA	
>1:20	+3
1:20	+2
<1:20	0
Anti-mitochondrial antibody	
Positive	-2
Negative	0
Viral markers	
IgM anti-HAV, HBsAg or IgM anti-HBc positive	-3
Anti-HCV positive by ELISA and/or RIBA	-2
Anti-HCV positive by PCR for HCV RNA	-3
Positive test indicating active infection with any other virus	-3
Seronegative for all of the above	+3
Other etiological factors	
History of recent hepatotoxic drug usage or parenteral exposure to blood products	
Yes	-2
No	+1
Alcohol (average consumption)	
Male <35 gm/day, female <25gm/day	+2
Male 35-40 gm/day, female 25-40 gm/day	0
Male 50-80 gm/day, female 40-60 gm/day	-2
Male >80 gm/day, female >60 gm/day	-1
Genetic factors	
Other autoimmune diseases in patient or first-degree relatives	+1

TABLE: 9(b) (Johnson et al. 1993)

Scoring System for Diagnosis of Autoimmune Hepatitis: Additional Parameters

Parameter	Score
Histology	
Chronic active hepatitis with piecemeal necrosis	
With lobular involvement and bridging necrosis	+3
Without lobular involvement and bridging necrosis	+2
Rosetting of liver cells	+1
Marked/predominantly plasma cell infiltrate	+1
Biliary changes	-1
Any other changes (e.g., granulomas, siderosis and copper deposits) suggestive of a different etiology	-3
Autoantibodies	
In patients who are seronegative for ANA, SMA, and LKM-1	
Any defined "liver autoantibody" (e.g., antibodies to SLA, ASGP-R, LSP, LC1, LP, HHPM and sulfatide)	+2
Positive	0
Negative	
Genetic factors	
HLA B8-DR3 haplotype, or DR4 allotype	+1
Response to therapy	
Complete response	+2
Partial response	0
Treatment failure	0
No response (in terms of disease activity)	-2
Relapse during or after treatment withdrawal after initial complete response	+3

Although invaluable as the basis for scientific studies on the diagnosis and the treatment of autoimmune hepatitis, the scoring system's usefulness in general practice is still debated. This is due to the fact that the system still has to be prospectively validated. Retrospective data from Czaja et al. in 1996 on 119 patients diagnosed with AIH by standard criteria and 131 patients with other chronic liver diseases gave favourable results. Ninety-seven patients (82%) diagnosed as having AIH by conventional criteria had a definite diagnosis by the scoring system while 22 patients (18%) had a probable diagnosis. Only 2 patients (2%) with other chronic liver disease had a score sufficient for a definite diagnosis of AIH (Czaja and Carpenter 1996a). However data from Newton et al. 1996, suggests that the proposed criteria may be too

stringent and hence could lead to withholding of effective treatment in some patients not fulfilling these criteria. The weaknesses noted to date include:

1. Some laboratory tests are not generally available e.g., HLA typing, LKM-1, SLA- and ASGPR autoantibodies.
2. Difficulty in diagnosing the overlap syndromes with other autoimmune disorders and liver infections e.g., PSC, PBC and HCV.
3. Features such as "relapse during or after treatment-induced remission" do not assist in deciding whether or not starting therapy is warranted in a new patient.
4. The scoring of the response to corticosteroid therapy can influence the final post treatment diagnosis assigned to the patient and hence the long term outcome.

In Czaja`s validation study of 1996, 22% of the study group (23 out of 107) had a probable diagnosis of AIH and these 43% had an upgrade of their diagnosis to definite AIH. In 18% of those with a definite AIH diagnosis pre-treatment, there was a downgrading to a probable diagnosis of AIH following corticosteroid therapy. This highlighted the over emphasis on steroid response. Clinical studies have shown clearly that this is not invariably the case. ***Table 10*** shows Czaja`s modified scoring system for AIH which is less complex, takes out the response to steroids score. The other objective is to ensure that the scoring system should be able to make a confident diagnosis of AIH prior to therapy and that this diagnosis be maintained in the post treatment period (Czaja 1998).

The association of AIH type 2 and HCV infection is well documented particularly in Southern Europe. These patients tend to be older patients, have previous histories of blood transfusions and men more than women are affected. They also have lower titres of anti-LKM-1 and serum aminotransferases levels compared to classical Type 2 AIH (Michel et al. 1992, Lunel et al. 1992).

Differentiation between the two depends heavily on serological characterization. There is significant overlap in ANA and SMA positivity with generally lower titres in patients with chronic HCV infection. The circulating immunoglobulins may occasionally be as high as those seen in AIH. The HCV- PCR is positive and the liver biopsy is helpful in the sense that HCV infection is suggested by the presence of portal tract lymphoid aggregates, mild microvesicular steatosis and severe bile duct damage or loss (Shapiro and Friedman 1996). A recent study by Czaja and Carpenter 1997, has shown that patients with AIH had more marked portal, interface and acinar hepatitis in various combinations associated with a plasma cell infiltrate compared to other causes of chronic hepatitis. They also had higher serum levels of gamma globulin and IgG, higher frequency of hypergammaglobulinaemia and cirrhosis.

TABLE: 10
(Czaja 1998)

MODIFIED SCORING SYSTEM FOR AUTOIMMUNE HEPATITIS

CATEGORY	FACTOR	SCORE	CATEGORY	FACTOR	SCORE
Sex	Female	+2	Alcohol	<25 g/d	0
				>40 g/d	-2
Alk Phos: AST ratio	≥3	-2	Immune disease	Patient or relative	+1
	<3	+2			
γ-Globulin or IgG levels above normal	>2.0	+3	Histological features	Interface and acinar hepatitis with bridging	+3
	1.5-2.0	+2		Interface hepatitis	+2
	1.0-1.5	+1		Rosettes	+1
	<1.0	0		Plasma cells	+1
				Biliary changes	-1
				Other features	-3
ANA, SMA, or anti-LKM 1 titres	>1:80	+3			
	1:80	+2			
	1:40	+1			
	<1:40	0			
AMA	Positive	-2	Blood transfusion or drugs	Yes	-2
				No	+1
Viral markers	HBsAg	-3	Pretreatment score		
	IgM anti-HAV	-3	Definite diagnosis		>15
	HCV RNA	-3	Probable diagnosis		10-15
	Other viruses	-3	Nondiagnostic		<10
	Anti-HCV	-2			
	All negative	+3			
HLA	DR3 or DR4	+1			

Abbreviations: Alk Phos: AST ratio, ratio of serum elevations above reference value of alkaline phosphatase and aspartate transaminase; IgG, serum immunoglobulin G levels; HBsAg, hepatitis B surface antigen; IgM anti-HAV, immunoglobulin M antibodies to hepatitis A virus.

1:4. MEDICAL THERAPY

The early diagnosis and recognition of chronic hepatitis is crucial for successful treatment and outcome. This is important considering that a significant number of patients are diagnosed only when they present with established cirrhosis and portal hypertension with some in this group having presented earlier but the diagnosis having been missed entirely.

The armamentarium available for the management of chronic hepatitis include:

(a) Early clinical recognition.

(b) Immunization.

(c) Immunosuppression with steroids and azathioprine.

(d) Interferon and second-generation nucleoside analogues.

(e) Liver transplantation.

The management of the complications associated with chronic hepatitis often ends up as the major function of the attending Physician. The major ones are portal hypertension with ascites, bleeding varices, hepatocellular carcinoma, osteoporosis and liver failure.

The early recognition of the syndrome is important with drug-induced hepatitis (for the immediate withdrawal of offending drug), viral hepatitis (to enable the early use of interferon before the onset of cirrhosis) and in AIH for early use of immunosuppression.

Immunisation to date has only been successful with HBV infection. Nationwide HBV vaccination in Taiwan using plasma-derived HBV vaccine in infants (at birth, one and six months), children and adults over a ten year period reduced the incidence of hepatocellular carcinoma (HCC) significantly. The average annual incidence of HCC

in children of 6-14 years was reduced from 0.70 per 100 000 children to 0.36 over this period (Chang et al. 1997). No vaccine is presently available for HCV due to the marked genetic heterogeneity shown by this virus.

1:4(a) HBV INFECTION

Interferon α-2b(IFN) therapy for chronic HBV and HCV infection is still evolving. Interferon α-2b induces the display of HLA class I molecules on the membranes of infected hepatocytes, promoting their lysis by CD 8+ cytotoxic T cells which can also inhibit viral protein synthesis directly. For HBV infection, IFN is the most effective treatment presently. It is able to achieve a 36% - 45% biochemical response rate, with only 10% loosing the HBsAg entirely. In untreated patients, only 5%-10% improve spontaneously and rarely do they loose their HBsAg. The patient groups who show the best response are those with:

(a) Well compensated disease.

(b) Detectable HBsAg and HBeAg in serum.

(c) Lower HBV-DNA levels at less than 200 pg/ml.

(d) Raised aminotransferase levels of more than 100 iu/ml (Response doubles if AST is more than twice normal).

(e) Short duration of disease of less than 1 year.

(f) Caucasian origin (Low response in Asians has been documented).

(g) No immunosuppression (HIV infection and transplants) (Brook et al. 1989).

(h) No HDV or HCV co-infection.

(i) Adult acquired disease and female sex.

Recent studies on the treatment of chronic hepatitis B infection in Asian patients with IFN have shown that the response rates are somewhat better than was initially reported in the early studies in Asian children (Lai et al. 1987 and 1991, Liaw et al. 1994). A recent randomised controlled study by Martin et al. 1998 compared the response rates in Asian-American adults with chronic HBV infection to a control group of Caucasian adults treated with the same antiviral regimen of prednisone withdrawal therapy initially followed by IFN α2b. The results showed that the initial response rates are identical with 62% of Asians and 60% of Caucasians clearing HBeAg and HBV DNA from the serum. At one year follow-up 67% of Caucasian responders and none of the Asian ones had cleared HBsAg from their serum. The loss of serum markers of active HBV replication was less sustained in the Asian responders as shown by the reappearance of serum HBeAg in 25% of Asians and 17% of Caucasians. This suggests that prolonged or combined therapy may be necessary in these patients to achieve better results.

The contra-indications to treatment with interferon include:

1. HIV infection associated with neutopaenia.
2. Severe liver disease with decompensation.
3. Serious medical illness such as renal failure, uncontrolled hypertension and heart disease.

A poor response is also associated with male sex, perinatally acquired disease, and the presence of anti-HBe, normal ALT and co-infection with HDV and HCV.

The recommended doses are 5Mu subcutaneously (sc) daily or 10Mu three times per week to complete a 16 week course. Pre-treatment a liver biopsy is needed to exclude other conditions that may cause a chronic hepatitis and to stage the disease. Base-line thyroid function tests and full blood count (FBC) are also necessary. The side effects

include depression, flu-like illness, hypothyroidism, neutropaenia and thrombocytopaenia. An axonal polneuropathy has been reported in the literature. Monitoring with FBC and serum aminotransferases should be done weekly for the first 4 weeks, two weekly for next 8 weeks and four weekly thereafter. The aminotransferases reach levels of 2-5 times above the starting baseline when the HBV-DNA and HBeAg are being cleared. If the side effects are intolerable, the IF dose should be reduced by half.

Nucleoside analogues are gaining increasing use in a bid to try and improve the response rates to treatment. They block reverse transcription of RNA and the most promise has been shown with the less toxic second generation group of lamuvidine and famciclovir. Lamuvidine alone for 4-12 weeks has been shown to clear HBV-DNA virtually by 100% but with very high relapse rates-only 19% achieving sustained remission (Dienstag et al. 1995). There is also a high mutation rate with longer-term treatment with at least 16% of the patients at one year having an associated recurrence in viraemia. The data from liver transplant patients suggests that nucleoside analogue treatment will need to be continued indefinitely (Honkoop et al. 1998). This has the attendant problems of cost and a high rate of mutations. Combination therapy with two different types of interferon or of two nucleoside analogues has been suggested but presently there is no data to support this as a better treatment.

The use of steroids for treating viral hepatitis reduces survival and fatal exacerbations can occur when the steroids are withdrawn. A few patients have been shown to clear their HBeAg and HBV-DNA during this withdrawal phase. Some workers have tried to take advantage of this phenomenon by combining steroid-withdrawal therapy and interferon alpha-2b with no real benefit being observed (Perrilo et al. 1990).

1:4(b) HCV INFECTION

In the treatment of HCV, interferon is the most widely accepted therapy although the relapse rates are significantly high. At the end of treatment 40-50% have normal aminotransferase levels with 30% showing a histopathological response. Only 15-20% will show a sustained response-that is, persistently normal serum aminotransferase concentrations and the absence of HCV RNA from serum at the end of treatment and sustained for at least six months (Brown 1998). Studies would suggest that prolonged courses of 18-24 months are needed to maximize the chances of having a sustained response to treatment (Poynard et al. 1995).

HCV has become the leading reason for liver transplantation in the USA. The natural history of untreated infection is cirrhosis after 21 years and the development of hepatocellular carcinoma at 29 years post infection. If infection occurs after the age of 40 years, cirrhosis develops in about 13 years in men and after 42 years in women who do not use alcohol. The sex difference is unexplained but alcohol is a significant co-factor in disease progression together with the viral load and the genotype (Poynard et al. 1997, Wiley et al. 1998).

Various indicators have been shown to predict good or poor response to interferon therapy. Poor response is predicted by:

1. Long duration of disease pretreatment (more than 10 years).
2. Associated HIV infection/immunosuppression of whatever cause.
3. Cholestasis.
4. Iron overload.
5. Presence of cirrhosis.
6. High level of viral multiplication (HCV RNA levels of >3million genomes/ml).
7. Genotype Ib.

8. High gammaglutamyl transferase levels.

The absence of the above predicts good response together with:

1. Lower body weight.
2. Genotypes 2 and 3 infections.
3. Mutations in the NS5 region.
4. Mild inflammation which is limited to the portal tracts.
5. Reducing HCV RNA levels after 12 weeks of therapy.
6. Prolonged treatment of 12-18 months (Kasahara et al. 1995, Poynard et al. 1995).

Doses of interferon higher than 3 million units three times per week do not improve the response rate and in non-responders, a second course is usually ineffective. Those who initially respond and then relapse may show a response on re-treatment. However caution should be observed in patients with established cirrhosis due to the risk of having a rebound of the activity of chronic hepatitis on stopping interferon (Macellin et al. 1991). A recent NIH conference on the management of chronic hepatitis C recommends treatment of those with compensated cirrhosis and other cirrhotics only under close supervision (NIH consensus statement Hepatology 1997). The different forms of interferon are not necessarily dose equivalent. Recombinant IFN alpha-2b and natural lymphoblastic IFN are of similar potency while recombinant IFN alpha-2a and consensus IFN need doses which are 2-5 times higher (Bacon et al. 1995, Tong et al. 1995).

The controversy of how to manage patients with normal or minimally elevated serum aminotransferase levels was recently addressed by a pilot randomised controlled study from Italy. This study showed that IFN alpha-2a treatment of HCV RNA carriers with

persistently normal transaminase levels for 6 months did not eradicate HCV RNA in these patients, instead it caused an ALT flare-up in 60% of them (Sagiovanni et al. 1998).

Alternatives to interferon have been considered for some time due to the poor response rates, cost and side effects that are associated with IFN. Combination treatment with ribavirin, n-acetylcysteine, iron-reducing treatments, ursodeoxycholic acid and nonsteroidal anti-inflammatory agents have all been looked at with variable outcomes. The best results have been obtained with ribavirin, which is a guanosine analogue with a broad spectrum of activity against RNA and DNA viruses. Its use for chronic HCV as single therapy was first reported in 1991.A 60% reduction in aminotransferase levels was achieved with some cases showing HCV RNA serum levels reduction. The relapse rate however was 100% on stopping treatment (Di Bisceglie et al. 1995, Richard et al. 1991). It is not clear how ribavirin enhances the effect of IF but this may be through cytokine modulation. A recent randomised, double-blind trial comparing interferon alone with interferon plus ribavirin in previously untreated patients showed that at 1 year post treatment, viral clearance rates were 42% for the combination therapy group and 20% for the interferon only group. This response rate was achieved mainly by those patients who had high viral titres of more than 3 Million genomes/ml (12/29 in the combination group vs 1/26 on interferon alone). Kinetic studies of HCV showing the high rates of viral turnover and rapid evolution of quasi-species (conferring possible drug resistance) suggest that like in HIV infection, combination therapy is likely to be the way forward. The development of inhibitors to the viral protease, helicase or RNA-dependent polymerase will enable such combinations to become a reality (Brown 1998, Reichard et al. 1998). McHutchison et al. 1998 have also recently shown similar results for

initial therapy of HCV. The response of genotype 1 to combination treatment was markedly improved to 28% compared to only 7% with IFN only therapy. In the treatment of relapsed HCV following IFN therapy, combination therapy with ribavirin gave better outcomes compared to IFN alone. The sustained virologic, biochemical and histologic response rates at 24 weeks follow-up were higher in the combination group at 49% compared to only 5% in the IFN group (Davis et al. 1998).

1:4(c) DRUG-INDUCED HEPATITIS

Diagnosis is the essential prerequisite for the treatment of a drug-induced chronic hepatitis. Prevention and early detection are important through appropriate dose adjustments for age and sex, assessing for pre-existing renal and liver disease, biochemical monitoring and a high index of suspicion.

Specific therapy centers around stopping the causative drug permanently. The use of antidotes is only appropriate in acute hepatic injury and is not applicable in chronic drug-induced hepatitis. Corticosteroids, although untested in controlled trials, have been used for patients with severe and protracted disease. Case series studies have shown limited benefit in chronic disease due to methyldopa, nitrofurantoin and dantrolene (Bernuau et al. 1986, Maddrey and Boitnott 1977). Case reports involving NSAIDS (diclofenac) and allopurinol have shown some benefit with histological improvement being shown after 3 months of treatment (Sallie et al. 1991). Most benefit appears to be more in cases of hepatocellular type injury that is not improving in 6 weeks to 3 months, rather than in cases with cholestatic disease.

Supportive therapy including rest, nutritional support, vitamin K and fresh frozen plasma should be used as required. Progressive deterioration to hepatic encephalopathy requires liver transplantation. The outcome is good (Mutimer and

Elias 1992) but associated medical conditions for which the drug was originally prescribed or psychological instability causing self-poisoning would preclude transplantation.

1:4(d) AUTOIMMUNE HEPATITIS

Immunosuppression is the mainstay of therapy in AIH. The drugs used are corticosteroids and azathioprine used alone or in combination. When the patient has no symptoms and the histology shows only mild inflammation, no treatment is needed as long as careful monitoring for possible disease progression is done biochemically and histologically. The initial treatment can either be monotherapy with steroids or a combination of steroids and azathioprine (***Table 11***). Maintenance treatment can be monotherapy with prednisone 5-15mg daily or azathioprine 100-200mg daily. Combination maintenance treatment is prednisone 5-10mg with azathioprine 50-150mg daily (Krawitt1996). Up to 80% of patients achieve remission with a biochemical response occurring within three months and remission after years of treatment. The majority requires long-term treatment and of those who relapse after stopping treatment, remission is much more difficult to achieve the second time round. The treatment failure group consists of patients with established cirrhosis, those with disease onset at a young age, HLA B8 and DR 3 phenotype and those who present with disease of long duration pre-treatment.

TABLE: 11

Treatment of Autoimmune Hepatitis
(Czaja 1995, Krawitt 1996 and Van Den Berg 1998).

TYPE OF THERAPY	MONOTHERAPY	COMBINATION THERAPY
Initial	Prednisone 20-30mg daily	Prednisone 10-20mg daily **and** Azathioprine 50-100mg daily
Maintenance	Prednisone 5-15mg daily **or** Azathioprine 100-200mg daily at ~ 2mg/kg body weight	Prednisone 5-10mg daily **and** Azathioprine 50-100mg daily
Investigational	Cyclosporin A* **or** Tacrolimus* **or** MMF*	Ursocholic acid and other immunosuppresants

*- Have been tried as first line therapy in steroid-resistant disease.
MMF= Mycophenolate mofetil.

Despite compliance, corticosteroid therapy has a failure rate of 9% and up to 13% have significant drug toxicity as to require early treatment withdrawal (Hegarty et al. 1983). In those who fail to achieve permanent remission, cirrhosis occurs in 40% within ten years (Davis et al. 1984). Cyclosporin A (CYA) at 5-6mg/kg /day has shown favourable outcomes, particularly in inducing remission in steroid resistant AIH. However it still needs to be tested against prednisone in controlled clinical trails. It is therefore not established therapy and its significant side effects should be considered when starting treatment, which is potentially indefinite. Relapse after stopping CYA occurs in all patients (Hyams 1987, Faulds 1993). Tacrolimus has not been fully evaluated but initial results show biochemical improvement after three months of therapy, but this is associated with a deterioration in the renal functions (Van Thiel 1992). Ursodeoxycholic acid has been used in the treatment of patients with chronic viral hepatitis and has been shown to reduce serum aminotransferases. The theoretical advantages of using ursodeoxycholic acid in AIH include:

1. The choleretic effect that it has in displacing potentially hepatotoxic hydrophobic bile acids from hepatocytes (Galle et al. 1990).
2. The prevention of ileal re-absorption of hydrophobic bile acids.
3. Reduction of direct toxic injury by protecting the hepatocyte membrane (Poo et al. 1992).
4. Its immunomodulating effects that can alter the expression of class I HLA antigens on hepatocyte membranes and reduce cytotoxic T cell attack (Calmus et al. 1990).
5. Its safety and low side effects profile (Ward et al. 1984).

The other possible new treatment options include mycophenolate mofetil and rapamycin. However the place of these therapies in the management of AIH still needs to be established in randomised controlled trails.

CHAPTER 2:

CHRONIC HEPATITIS AT GROOTE SCHUUR HOSPITAL: 1978-1996

2:1 INTRODUCTION

The spectrum of chronic hepatitis at Groote Schuur Hospital (GSH) was first described in 1980 by Poreh et al. who studied 54 patients who had been seen at GSH between 1964 and 1977 with clinical, biochemical and histological criteria for diagnosing chronic active hepatitis (CAH) as defined by the Fogarty International Conference on Standardization of Nomenclature, Diagnostic Criteria and Diagnostic Methodology of October 1974 (Leevy C et al. 1976). At this conference, CAH was defined as "a continuing inflammatory lesion of the liver with a potential to progress to more severe disease, including cirrhosis, to continue unchanged or to subside spontaneously with or without treatment."

Each patient had a liver biopsy, antinuclear factor (ANF), smooth muscle antibody (SM), anti-mitochondrial antibody (AMA), LE cell preparations, biochemical, haematological and HBV surface antigen assays. On assessment, the male to female ratio was almost 1 and the physical examination was unhelpful in diagnosing chronic active hepatitis. ANF was positive in 39%, SM antibodies in 40%, LE cells in 34%, HBsAg in 39% and all were anti-mitochondrial antibody negative. In those who were HBsAg positive, 38% were also SM antibody positive, 20% and 30% positive for

ANF and LE cells respectively. No comment was made on the histological features and survival of these patients, but the histological criteria for diagnosis included round-cell infiltration involving the portal tracts with moderate or severe piece-meal necrosis of liver cells extending outward into the parenchyma from the limiting plate as defined at the Fogarty International Conference.

Seggie et al. in 1979 described a subset of chronic hepatitis, that of methyldopa-induced liver disease. They described 12 patients with severe symptomatic methyldopa related liver disease who had presented between 1967-1977 at GSH. Methyldopa had been introduced for the treatment of hypertension in the early 1960s (Irvine et al. 1962) and soon thereafter there was a plethora of published literature on its potential for hepatotoxicity (Elkington et al. 1969, Maddrey and Boitnott 1977). In 75% of the patients described, illness occurred within 1-9 weeks of starting therapy (9 of 12 patients) and in the other 25%, the drug had been received for 13 months, 15 months and 7 years prior to experiencing any symptoms. A tender hepatomegaly and jaundice were frequent presentations and the histology ranged from mild fatty change, focal hepatocellular necrosis to massive hepatic necrosis. On withdrawal of the drug at presentation, 82% (9 of 11 patients) showed a rapid clinical improvement. Two patients died following a drug rechallenge by mistake resulting in a mortality of 16.66%. In three patients, jaundice recurred within two weeks of accidental drug re-exposure. The majority of the patients were female, 83.33% (10 of 12patients) giving a male to female ratio of 1:5.

The current study is a retrospective case file study looking at the various aetiologies of chronic hepatitis, clinical presentations, serologic and biochemical features. The treatments used and outcome in terms of prognosis, liver transplantation and death

will be discussed. The available liver biopsy slides were reviewed blindly, graded and staged according to current criteria (Desmet 1994, Ishak et al. 1995).

2:2 PATIENTS AND METHODS

2:2.1 STUDY POPULATION

The patients studied were seen at Groote Schuur Hospital between January 1978 and December 1996. These were primarily referrals from other hospitals or clinics and from private medical practitioners. The majority of the patients had been seen at the liver clinic at least once or had been assessed in the wards as referrals from other departments within GSH.

2:2.2 EPIDEMIOLOGICAL INVESTIGATION

A search of the computerized hospital records was done for the period January 1978-June 97 using the diagnostic codes chronic hepatitis, chronic active hepatitis, drug-induced hepatitis, lupoid hepatitis and viral hepatitis. The search obtained 2094 records. For inclusion into the study, the patients had to have had at least a 6 months history of illness and satisfy the definition of chronic hepatitis as proposed by the International Working Party in 1995 (Figure 1, International Working Party 1995). Alcohol or other alcohol-related diseases such as alcoholic pancreatitis were listed in 590 records. A further 1278 records were excluded following folder retrieval and analysis due to the following reasons:

REASONS FOR EXCLUSION FROM THE STUDY.

A. Missing or untraceable files and wrongly recorded folder numbers.

B. A diagnosis not fulfilling the current criteria for chronic hepatitis such as:

1. Acute or fulminant HAV and HBV infection.

2. Acute TB drugs hepatitis or acute poisoning with paracetamol, phenytoin and alcohol.

3. Felty's syndrome.

4. Congenital webs of the inferior vena cava and congenital hepatic fibrosis.

5. Haemochromatosis or secondary iron overload.

6. Primary biliary cirrhosis, primary sclerosing cholangitis and secondary biliary cirrhosis.

7. Metastatic deposits to the liver.

8. Budd-Chiari syndrome.

9. Sarcoidosis.

10. Ischaemic liver damage.

12. Septicaemic states.

This left 226 files available for further study. The records were searched for information on:

1. Full blood count, INR and liver tests results at the initial presentation to hospital.
2. AMA, SM antibodies, ANF, alpha feto protein and anti-LKM-1 antibodies.
3. Details of liver histopathology.
4. Other medical diagnosis.
5. Duration of follow-up recorded.
6. Therapy given or not given for a particular reason.
7. Complications and the cause of death if recorded.

2:3 RESULTS

2:3.1 DEMOGRAPHIC DATA AND CLINICAL FEATURES

Of the 226 patients available for analysis, **55%** (124 of 226 cases) were female and **45%** (102 of 226 cases) male giving a male: female ratio of **1:1** (***Table 12***).

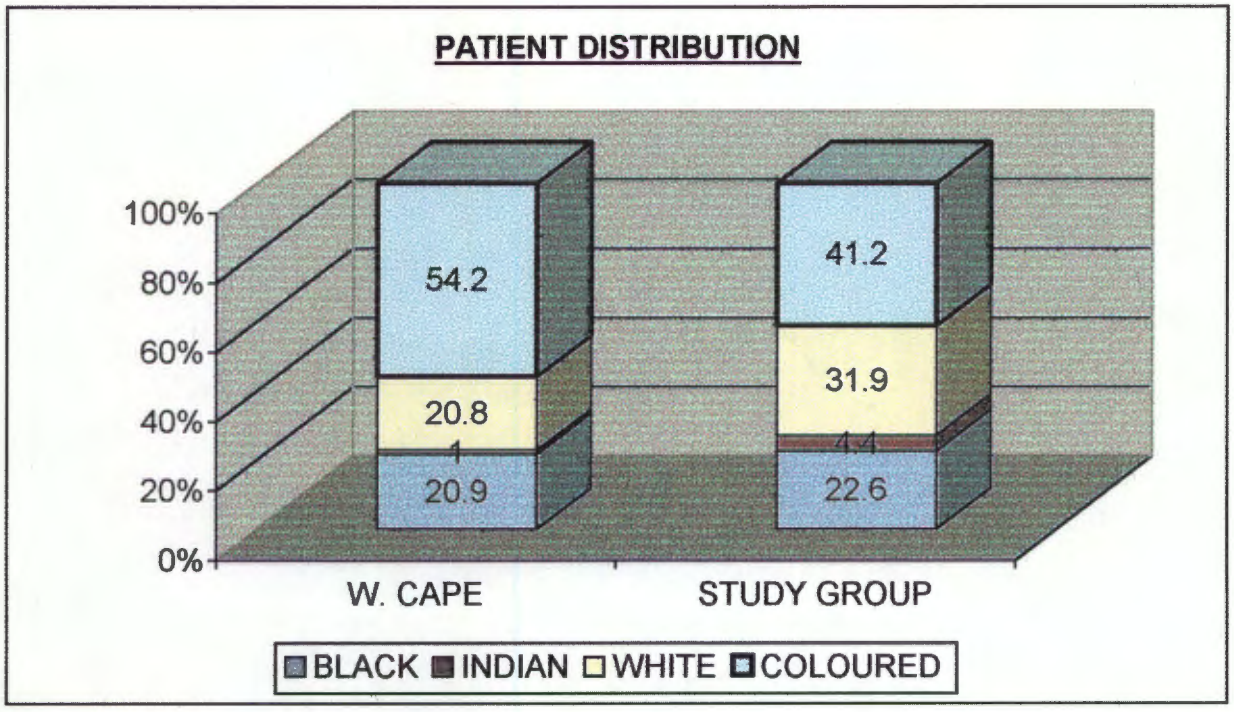
TABLE: 12

Gender and racial distribution

	BY RACIAL GROUP			WHOLE GROUP		
	MALE	FEMALE	% OF GROUP	MALE	FEMALE	% OF GROUP
COLOURED	34	59	**41.2%**	102	124	**Males 45%**
WHITE	37	35	**31.9%**			
BLACK	26	25	**22.6%**			**Females 55%**
INDIAN	5	5	**4.4%**			

Compared to the population demographics of the Western Cape from the 1996 population census of South Africa, the patient distribution by race is remarkably similar (***Figure 7***). In the census, the Coloured population was **54.2%**, Whites were **20.8%**, Blacks were **20.9%** and the Indians were only **1%** of the population.

FIGURE: 7



W. CAPE - Data of the Western Cape population obtained from the South African population census of 1996.

The ages at diagnosis of the study population had a uniform spread with a bimodal distribution curve on plotting. The age ranges were from as young as 3 years of age and the oldest patient was 78 years old. The mean age at diagnosis was 39.3 years with a median of 39 years (***Figures 8, 9***).

FIGURE: 8

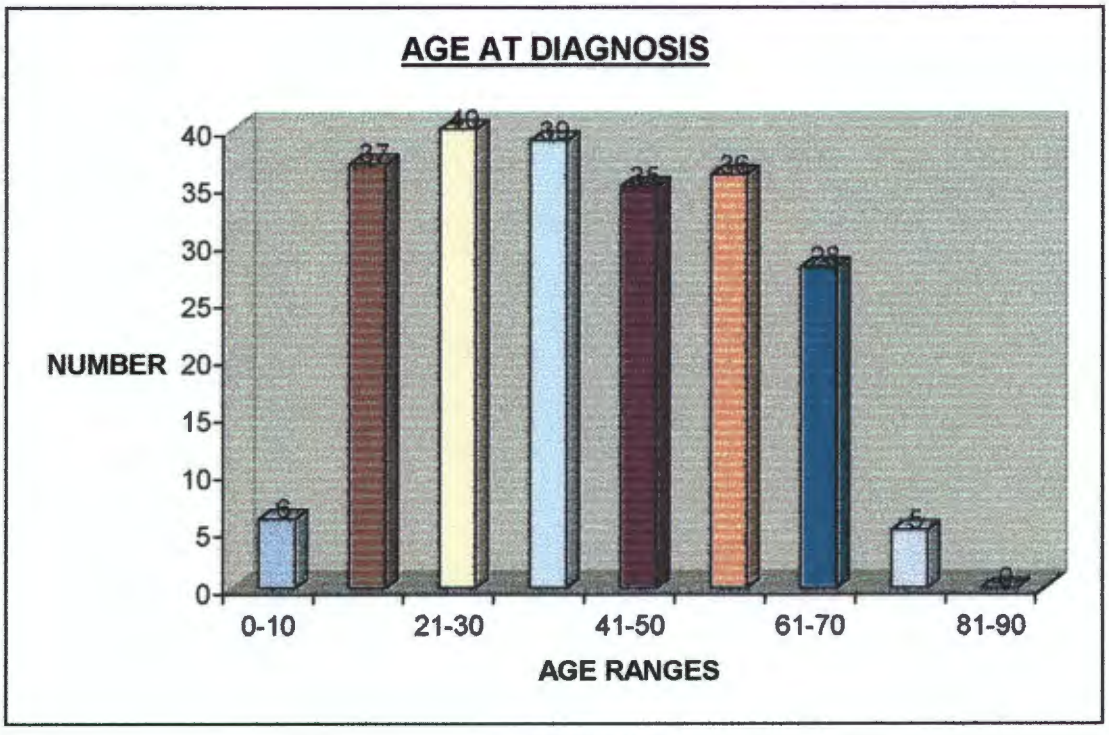
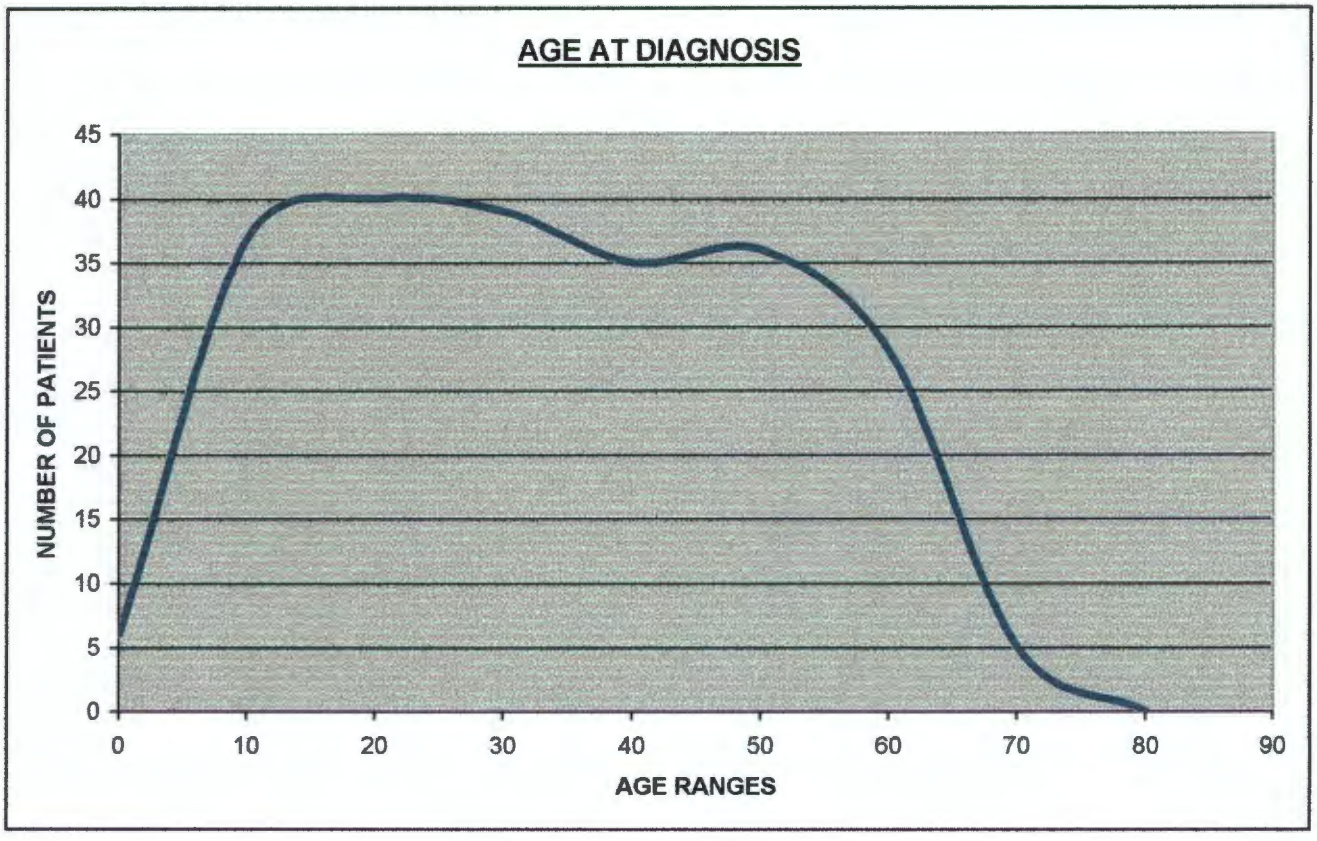


FIGURE: 9



The various clinical features recorded during the first clinical assessment were documented. These are summarized in ***Table 13***.

TABLE: 13

PRESENTING CLINICAL FEATURES

CLINICAL FEATURE	NUMBER OF PATIENTS	% OF TOTAL PATIENTS
Palpable liver.	111	49
Ascites.	89	39
Jaundice.	97	43
Abdominal pain.	79	35
Palpable spleen.	71	31
Loss of weight.	61	27
Malaise.	56	25
Spider naevi.	43	19
Haematemesis.	32	14
Nausea and/or vomiting.	36	16
Overt encephalopathy.	18	8
Foetor hepaticus.	19	8
Pruritis.	14	6
Amenorrhoea.	**17 of 124 women**	**14% of women**
Arthralgias.	10	4
SBP/Fever.	13	6
Testicular atrophy/Gynaecomastia.	**7 of 102 men**	**7% of men**
Abdominal striae.	5	2
Tattoos/traditional scrarifications.	6	3
Previous blood transfusions.	6	3
Acne.	4	2
Nose bleeds.	3	1
No problems.	2	1

SBP-Spontaneous bacterial peritonitis.

The most common mode of presentation was with one or more of the following: jaundice (**43%**), ascites (**39.4%**) with a "palpable liver" (**49%**). The precise size of the liver was not always recorded and so it is likely that in some instances the

"palpable liver" was the caudate lobe or the left lobe of liver rather than a truly enlarged liver (Di Bisceglie and Hoofnagle 1996). The common presentation was associated, to a lesser extent, with the presence of abdominal pain, a palpable spleen and significant loss of weight.

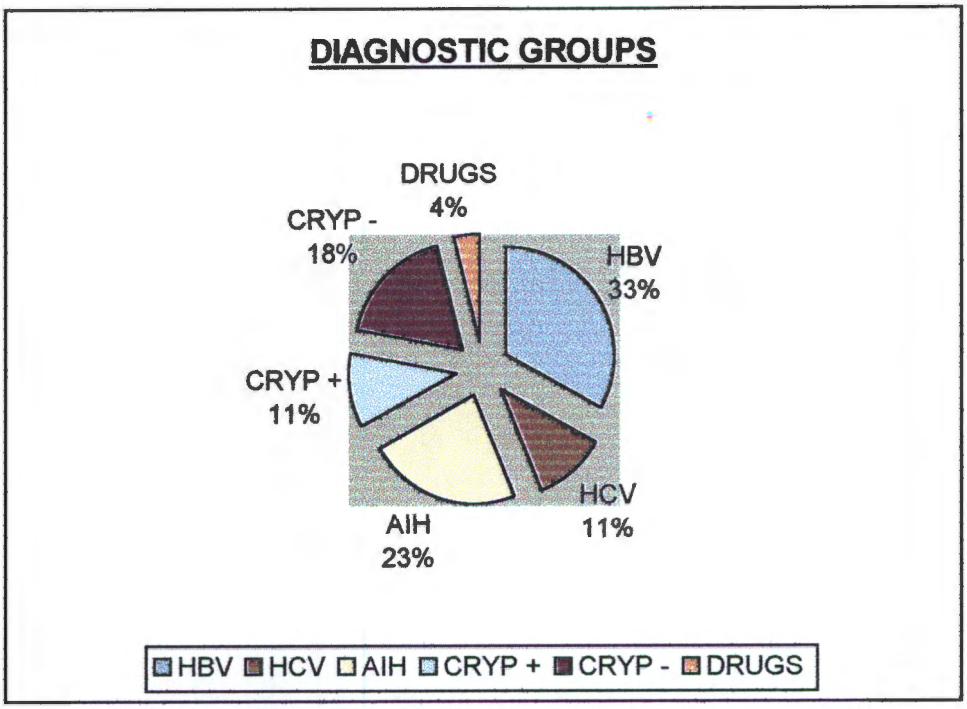
The diagnostic categories used are as outlined in ***Table 14*** and ***Figure 10***.

TABLE: 14.

DIAGNOSTIC CATEGORIES

DIAGNOSIS	NUMBER OF CASES	% OF TOTAL PATIENTS
1. HBV CHRONIC HEPATITIS	75	**33**
2. HCV CHRONIC HEPATITIS	25	**11**
3. AUTOIMMUNE HEPATITIS (Positive immune markers)	51	**23**
4. CRYPTOGENIC STEROID SENSITIVE CHRONIC HEPATITS (CRYP +) (Negative viral and immune markers)	26	**11**
5. CRYPTOGENIC CHRONIC HEPATITIS (CRYP –) (Negative viral and immune markers, no steroids given or no response to steroids)	41	**18**
6. DRUGS	8	**4**
Methyldopa	6	**3**
Carbamazipine	1	**0.4**
Chlorpromazine	1	**0.4**

FIGURE: 10



HBV – Hepatitis B virus chronic hepatitis, HCV – Hepatitis C chronic hepatitis, AIH – Autoimmune hepatitis, CRYP (+) - Cryptogenic steroid sensitive chronic hepatitis, CRYP (-) – Cryptogenic chronic hepatitis, DRUGS – Drug-induced chronic hepatitis.

2:3.2 LABORATORY INVESTIGATIONS

No statistical difference could be shown among the patients when the biochemistry and haematology data at presentation were analysed. The mean values of the haematology and chemistry are summarized in ***Table 15***. However there were differences in the biochemistry, haematology and ages at presentation when analysed by aetiology but these were not statistically significant. This is outlined in ***Table 16***.

TABLE: 15

Mean haematology and biochemistry values of the whole group

	HB	WCC	PLT	INR	TP	ALB	TB	ALP	AST	ALT	GGT
Mean value	12	6.9	179	1.5	75	33	68	265	177	176	167

HB- haemoglobin, **WCC**- white cell count, **PLT**- platelets, **INR**- international normalized ratio, **TP**- total protein, **ALB**- albumin, **TB**- total bilirubin, **ALP**- alkaline phosphatase, **AST**- aspartate aminotransferase, **ALT**- alanine aminotransferase, **GGT**- gamma-glutamyltranspeptidase.

TABLE: 16

Mean values by aetiology at presentation

DIAGNOSIS	INR	TB	TP	ALB	AST	ALT	ALP	AGE
1. HBV	1.4	40	72	32	157	171	206	35
2. HCV	1.4	72	77	37	120	149	194	51
3. AIH	1.6	96	82	32	224	186	266	38
4. CRYP +	1.6	85	73	32	300	264	360	32
5. CRYP -	1.4	67	71	34	124	150	348	45
6. DRUGS	1.6	73	81	36	85	90	280	52

From ***Table 16***, the sickest patients at admission when judged by the lowest albumin, highest AST/ALT/TB and ALP appeared to be from the autoimmune hepatitis and the cryptogenic steroid sensitive chronic hepatitis groups. The diagnostic groups with the less severe disease at presentation when assessed by the highest total protein and albumin, lowest AST/ALT/ALP and bilirubin were the HCV and drug-related hepatitis patients.

2:3.3 LIVER HISTOPATHOLOGY

Sections of liver were available for analysis in **211** of the **266** patients (i.e. **93.4%** of the patients). Slides could not be traced in **10** patients who had biopsies at other hospitals prior to referral to GSH. In **4** of the **16** patients with hepatocellular carcinoma, the diagnosis was made on the basis of a combination of ultrasound scan and/or CT scan abdomen, alpha feto-protein level and in **1** patient, a fine needle aspiration biopsy was done. All the slides were retrieved and reviewed blindly and without the knowledge of the clinical details by the author and Professor P Hall (PH), Department of Pathology University of Cape Town. The slides were scored for histological activity index (HAI) and stage of fibrosis using the Ishak system (Ishak et al. 1995, see ***Tables 6(a)*** and ***6(b) pages 22, 23***). A separate score for HAI using the Bedossa scoring system was also given with the intention of comparing two systems which assess the grade of disease (Bedossa et al. 1996, see ***Chapter 1 section 1:3.3 page 20***). The author did an initial score separately and then reviewed the slides with PH during which time a consensus score was agreed upon by both observers. Poor quality slides were restained or additional sections were made from the original biopsy blocks if these could be traced. These results are summarized in ***Figure 11*** and analyzed in ***Figure 12*** as the frequency of cirrhosis (i.e. presence of stage 5 or 6 score) in each diagnostic group and also as a breakdown of the cirrhotic group (the 120 patients with stage 5 or 6) by aetiology (***Figure 13***).

FIGURE: 11

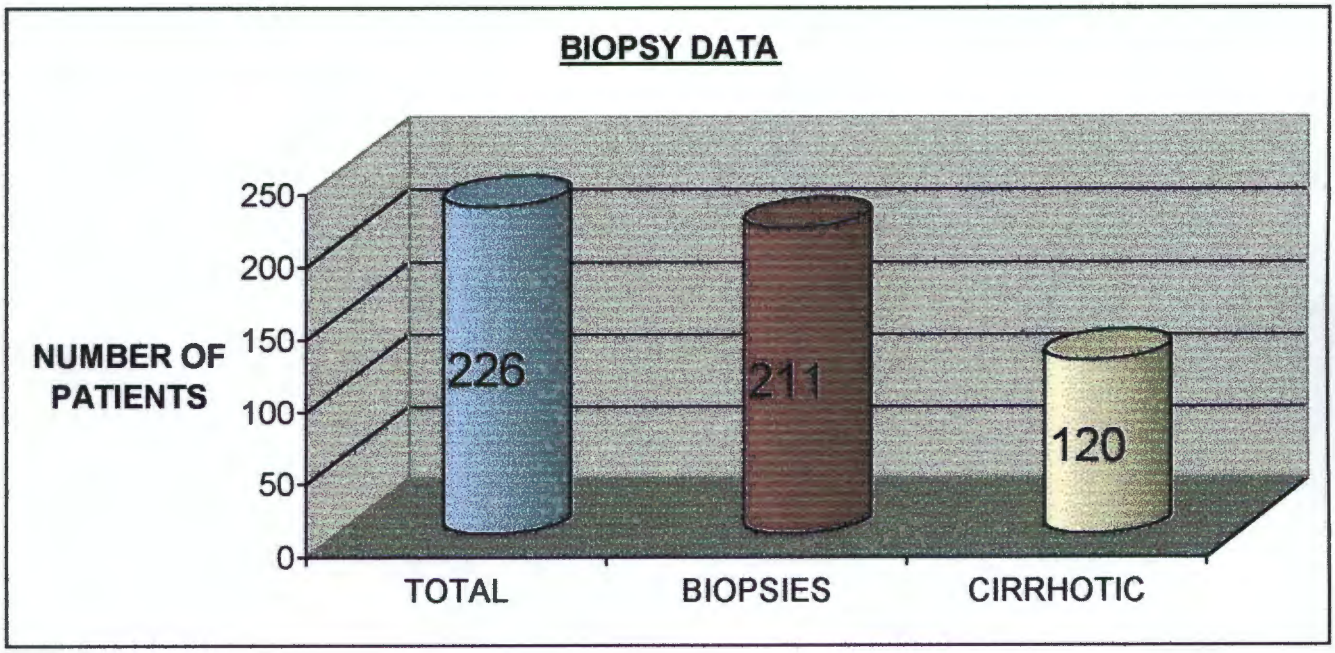


FIGURE: 12

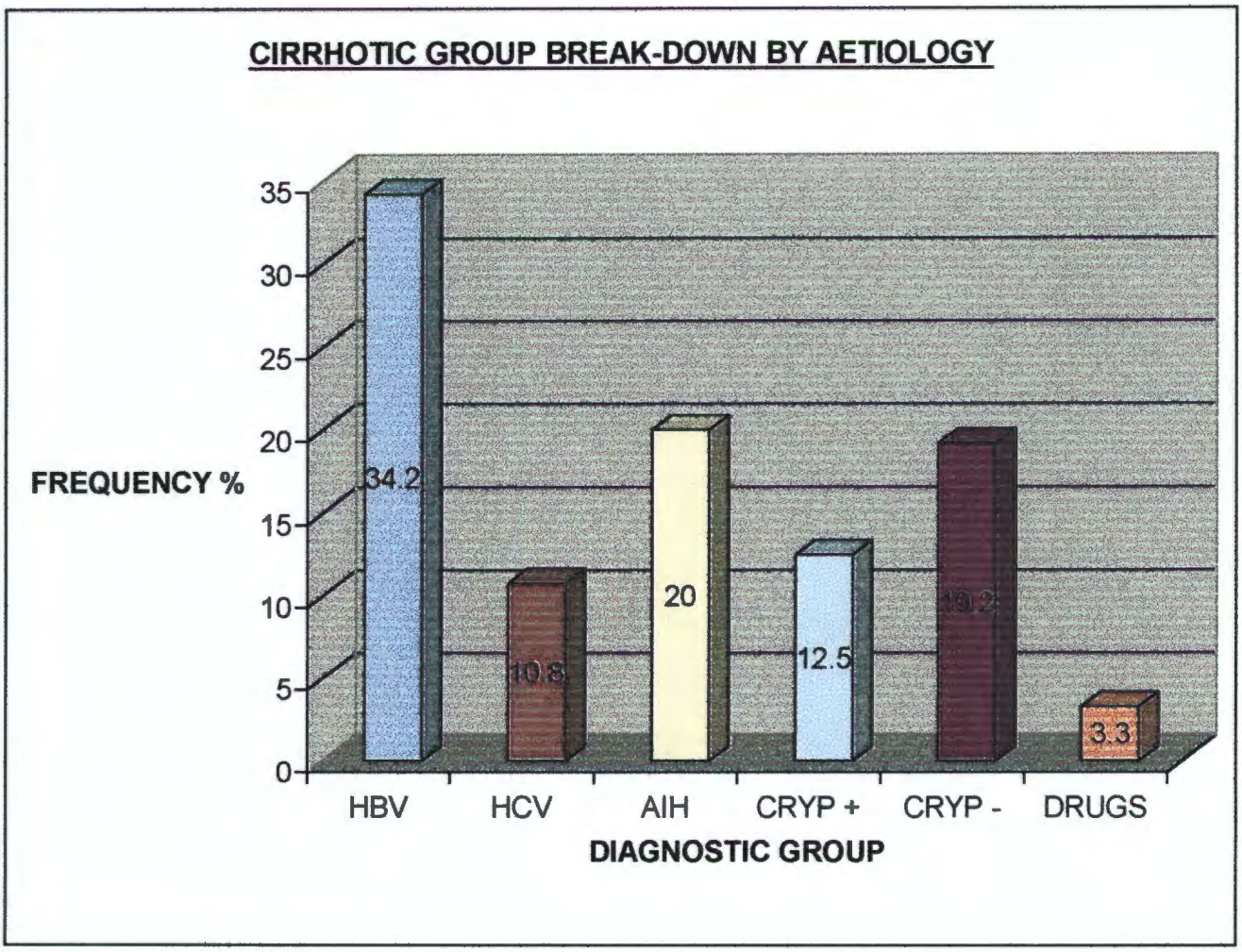
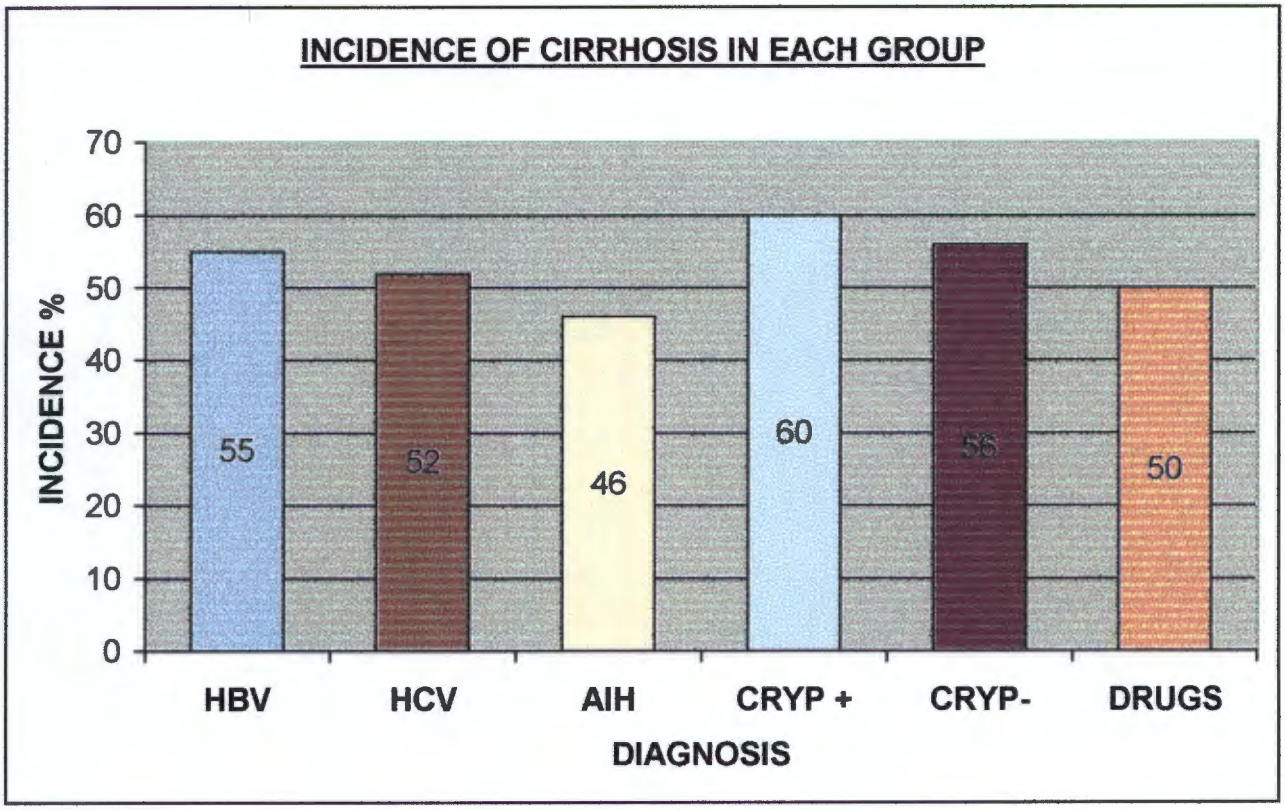


FIGURE: 13

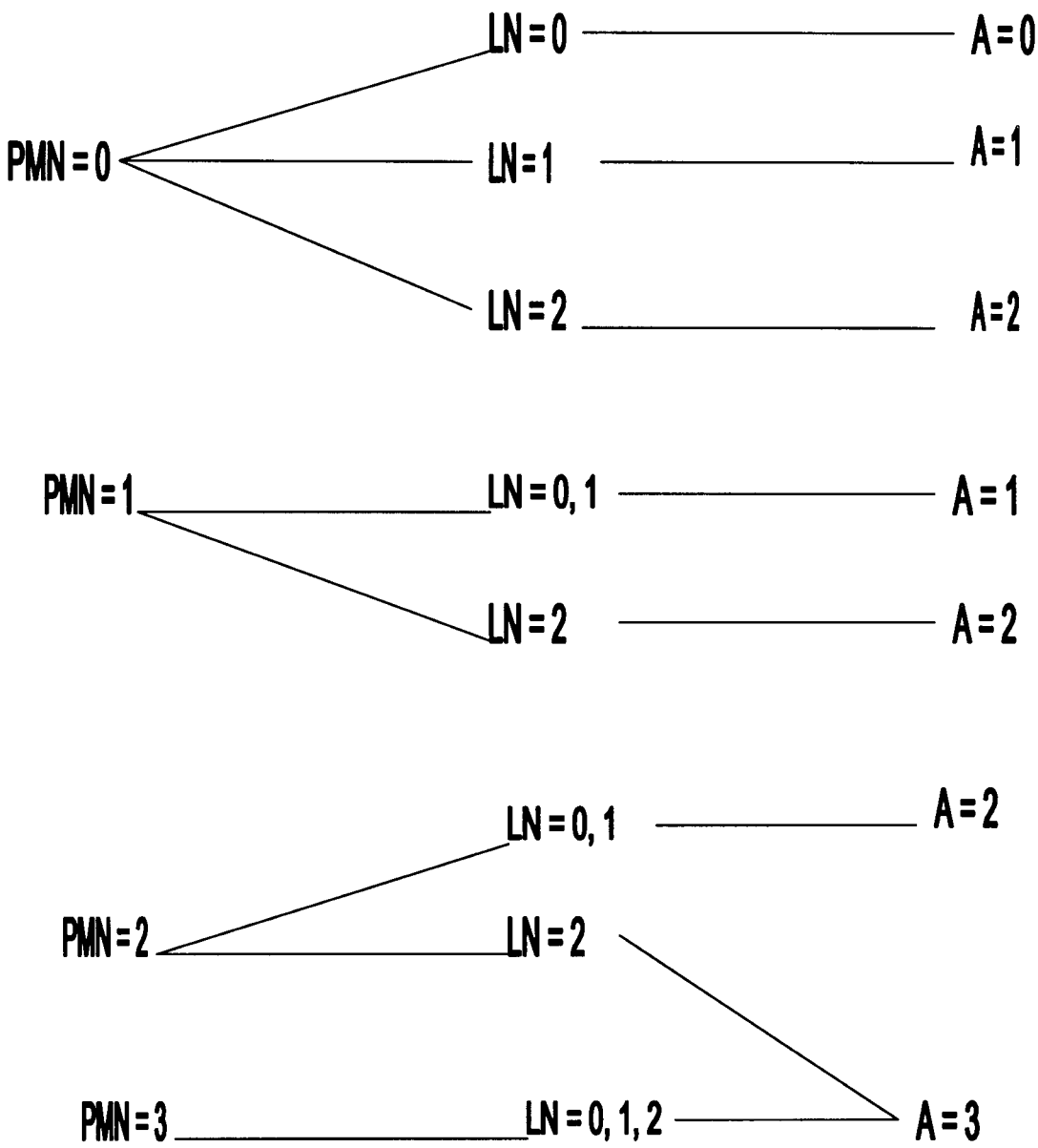


The Bedossa and Ishak scoring systems are compared in ***Figure 15***. The Ishak system has been previously discussed in ***Chapter 1 Section 1:3.3*** and summarized in ***Tables 6(a)*** and ***6(b)***. The Bedossa system was proposed in 1996 by the French METAVIR group for grading activity in chronic hepatitis C. A panel of 10 pathologists separately reviewed 363 chronic hepatitis C liver biopsies and a consensual algorithm on the grading of histological activity was established. In working out the algorithm, the consensus reached by the pathologists was that the intensity of piecemeal necrosis (PMN) was the major criterion that each one of them had used to decide on the severity of the grade. The lobular necrosis (LN) was not a major criterion but its intensity could be used to modulate the degree of activity. There was also a very

significant positive correlation between the severity of PMN and portal inflammation (p value < 0.00001). Portal inflammation itself was not thought to influence the assessment of the degree of activity and so is not scored in the Bedossa algorithm (***Figure 14***). This is of importance when looking at the correlation plot of Bedossa and Ishak grade scores in which cases with marked portal tract inflammation but less prominent PMN would have high Ishak scores but low Bedossa scores resulting in the rather wide scatter shown in ***Figure 15***. Compared to the Ishak scoring protocol, the proposed Bedossa scheme is simpler and faster to score mainly because it uses fewer criteria for scoring.

FIGURE: 14

BEDOSSA ALGORITHM FOR THE EVALUATION OF HISTOLOGICAL ACTIVITY (Bedossa et al. 1996)



PMN, piecemeal necrosis; 0, none; 1, mild; 2, moderate; 3, severe; LN, lobular necrosis; 0, no or mild; 1, moderate; 2, severe; A, histological activity; 0, none; 1, mild; 2, moderate; 3, severe.

Figure 15 shows the correlation that was shown between the Bedossa score and the Ishak grade score. The Ishak scoring was done initially and the Bedossa score done next at the same assessment. The r-value was 0.7, showing a very significant correlation.

FIGURE: 15

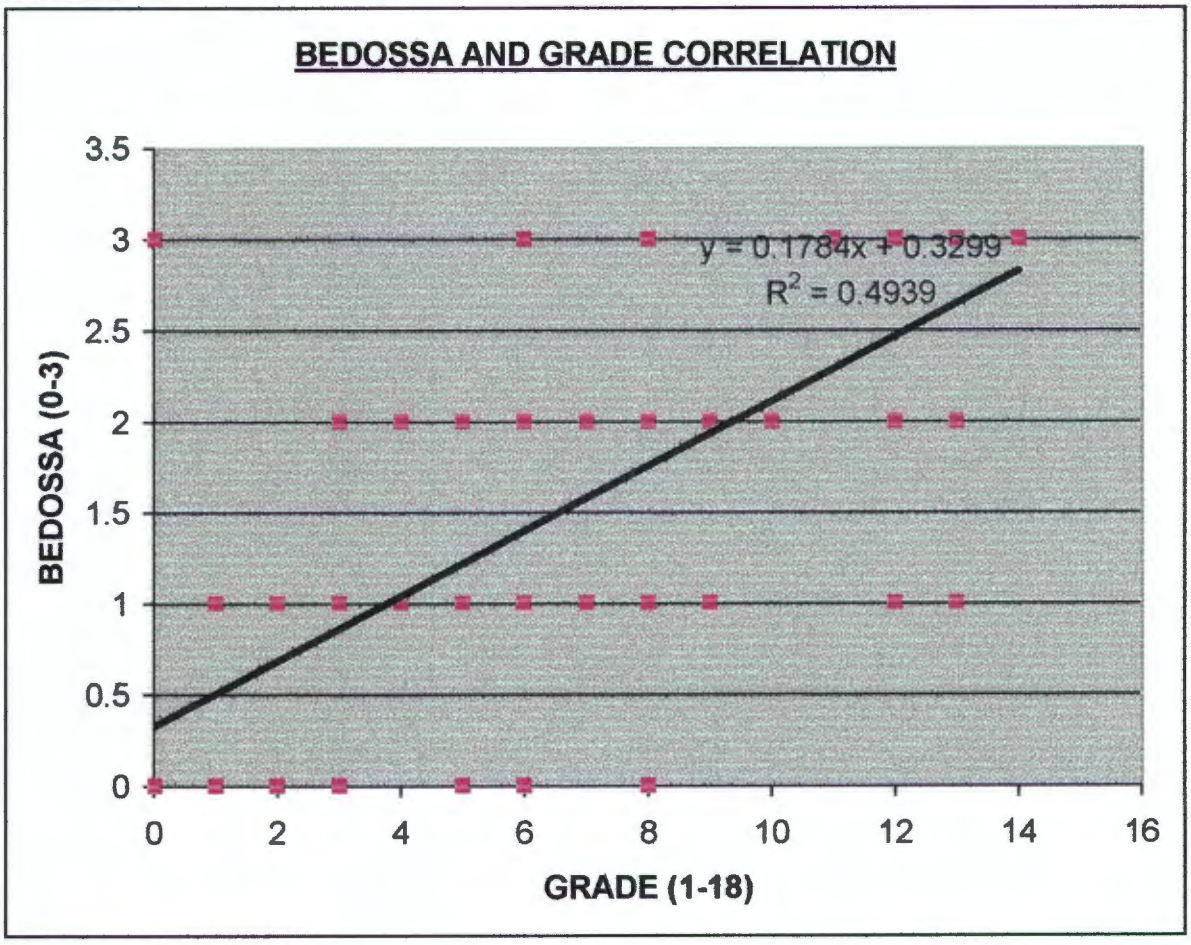
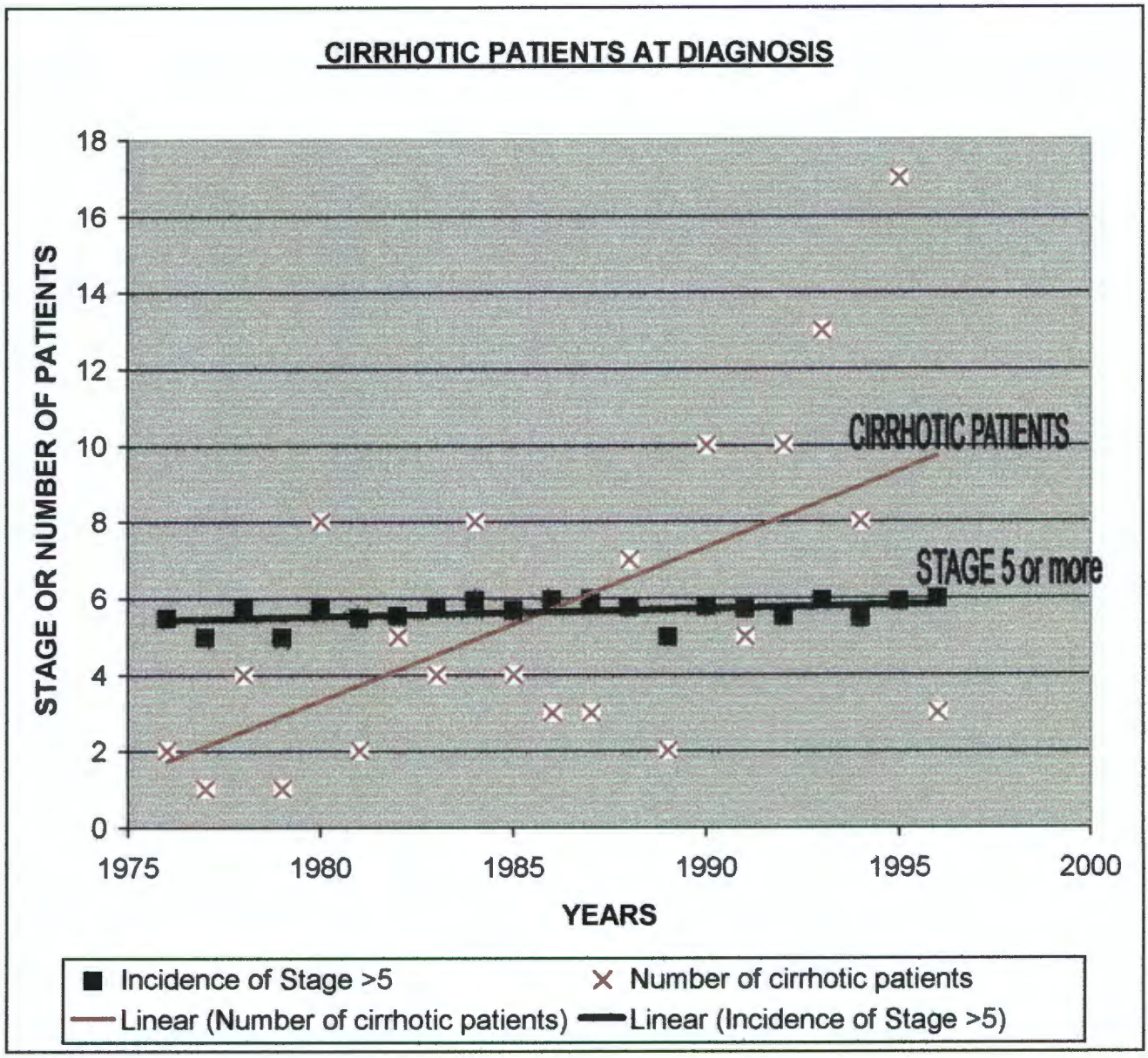


Figure 16 shows the changes in the stage of disease at diagnosis and the number of patients with cirrhosis during the entire period of the study. The patients with a stage of fibrosis ≥ 5 (i.e. with cirrhosis) are plotted together with their trend lines. The trend line of the stage of disease shows no significant change and specifically, there is no improvement in the severity of disease at the time of diagnosis. In fact the stage of disease plot shows a tendency to a worse stage of disease at diagnosis. There were

clearly more cirrhotic patients diagnosed with each year and this may be reflecting the increased rate of biopsy.

FIGURE: 16



The number and types of biopsies done in this study cohort are summarized in ***Table 17*** and ***Figure 17***. Most of the biopsies were done in the last 10 years of the study.

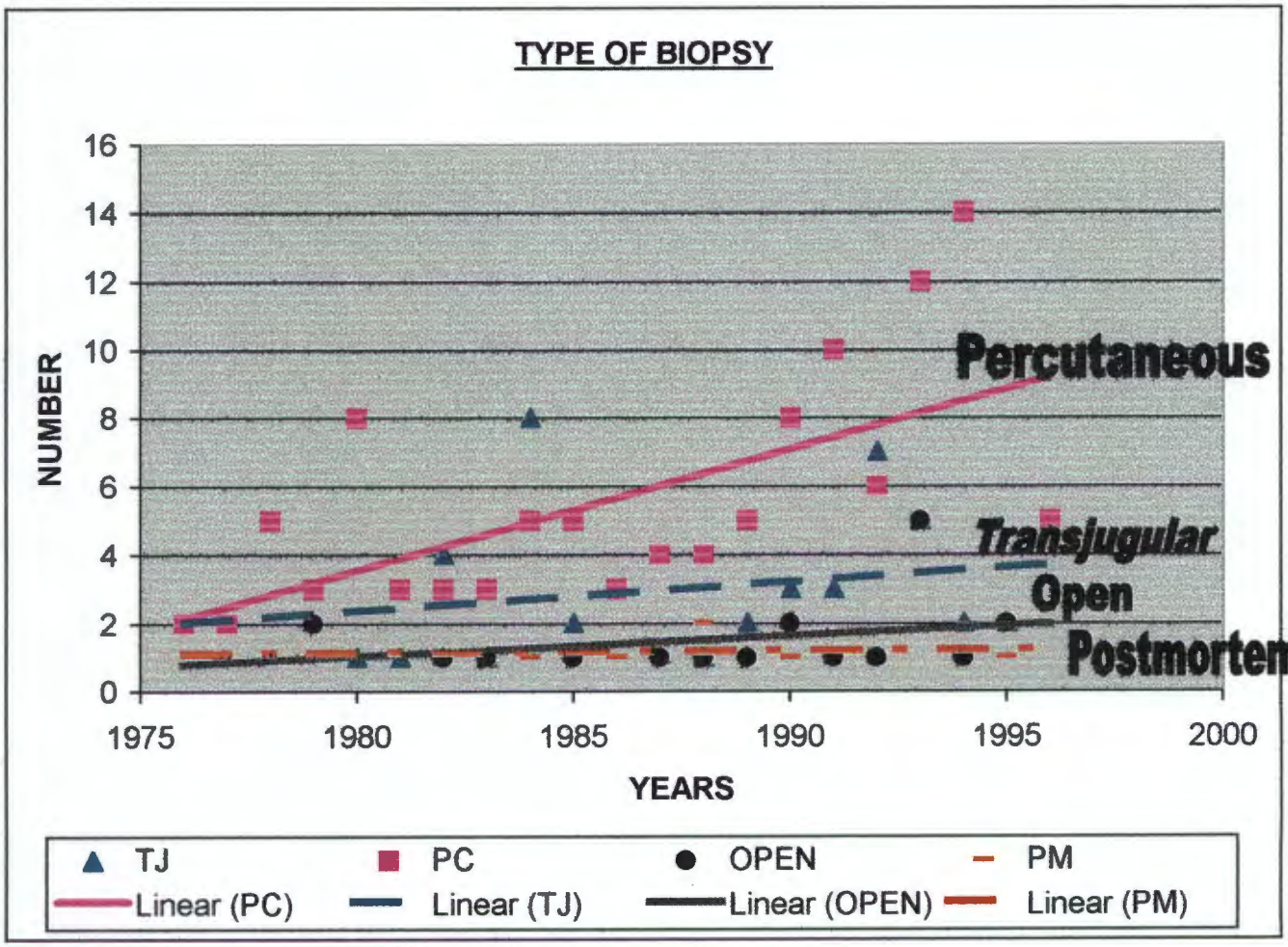
TABLE: 17

SUMMARY OF BIOPSIES DONE (1976-1996)

	TYPE OF BIOPSY				
PERIOD	**Transjugular**	**Percutaneous**	**Open**	**Explanted**	**Postmortem**
1976-1986	20	42	5	Nil	3
% of total	**29%**	**60%**	**7%**	**NIL**	**4.3%**
1987-1996	25	98	9	6	4
% of total	**18%**	**69%**	**6%**	**4.2%**	**2.8%**

The postmortem biopsies were done 35 to 120 hours after death and as a result, the grading components were not always possible to score due to the presence of tissue autolysis on the liver sections. The explanted liver histology appears from 1988 with the onset of the liver transplant programme at Groote Schuur Hospital. The explanted liver histology is included under the open biopsies in ***Figure 17***.

FIGURE: 17



TJ – Transjugular, **PC** – Percutaneous, **PM** – Postmortem.

The only complication recorded in this series of biopsies was of biliary peritonitis. This occurred in one patient and was managed conservatively. The patient made a full recovery. This would give a complication rate of 0.47% with no deaths being recorded. From ***Table 17*** and ***Figure 17***, most patients were biopsed percutaneously. During the first 10 years of the study, a third of the patients **(29%)** had transjugular biopsies and this dropped to **18%** in the last 10years of the study with more percutaneuos biopsies being done.

2:3.4 VIRAL CHRONIC HEPATITIS

2:3.4(a) HBV CHRONIC HEPATITIS

There were **75** patients with chronic hepatitis due to HBV infection. 51 were male (**68%**) and 24 female (**32%**) giving a male: female ratio of **2:1**. The ages at diagnosis showed a normal distribution curve and ranged from **3-67** years and the diagnosis was most frequent in the Coloured and Black populations (***Figures 18, 19***).

FIGURE: 18

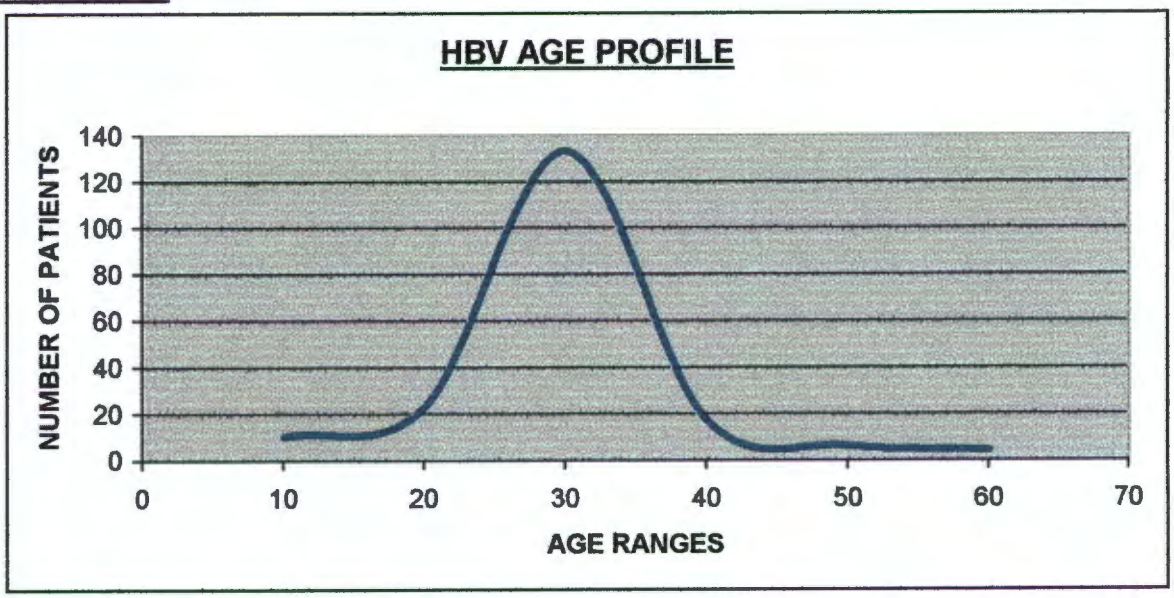
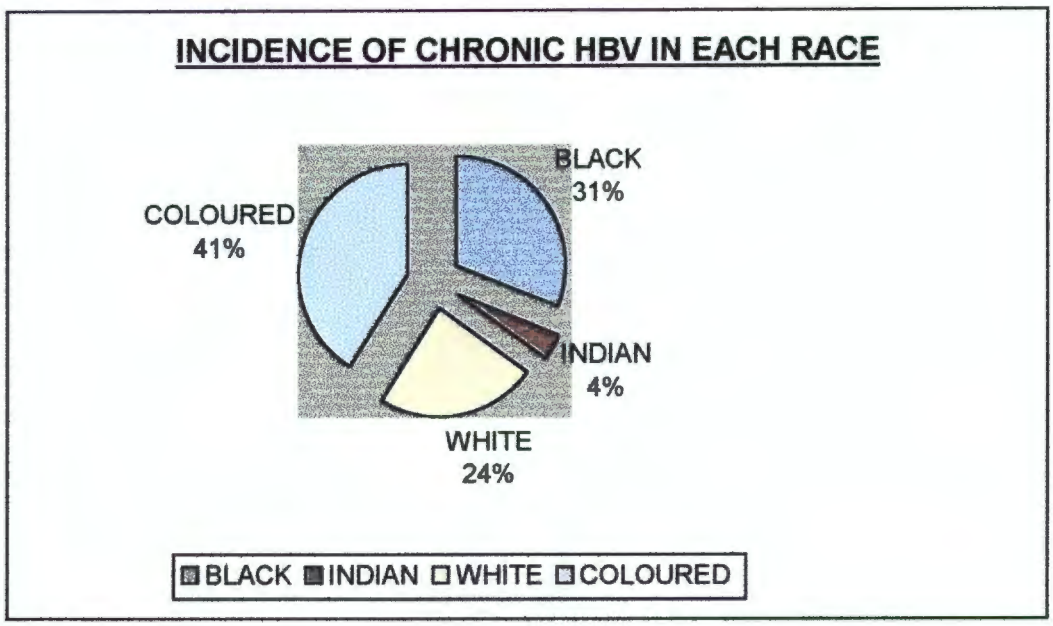


FIGURE: 19



At diagnosis, HBsAg and HBeAg were recorded as positive in **96%** (72 of 75 patients) and **43%** (32 of 75 patients) respectively. In 3 patients, only the HBeAg result was recorded in the folders. On immunohistochemistry studies of the biopsies, HBsAg was positive in **52.3%** of the HBV biopsies (34 of 65 biopsies) and the HBcore antigen was positive in **16.9%** (11 of 65 biopsies).

The amount of alcohol consumption was poorly recorded in most patients. An assessment of the weekly alcohol use was clearly recorded in only 10 patients. No accurate comment is possible in this group as regards their pattern and degree of alcohol use.

Interferon alpha (IFN) therapy was given in 6 patients of this group. The duration of therapy was 16 weeks. Four patients responded with loss of the HBeAg but one patient relapsed within a year. Four patients received corticosteroids, in two patients this was as part of steroid withdrawal therapy with IFN in 1989 and the other two patients received the treatment in 1981 as a trial of steroid therapy during episodes of raised transaminases. There were three patients who had lamuvidine. This was as part of a trial in two patients and in the third, it was given as monotherapy because IFN could not be used due to established cirrhosis with leukopaenia and thrombocytopaenia.

The follow-up documented in months up to December 1996 is summarized in ***Table18***.

TABLE: 18

Follow-up of the HBV group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	13	**17.3**
DEAD	23	**30.7**
LOST FOLLOW-UP	39	**52**
Less than 6months	20	26.7
6 to 12 months	9	12
More than 12 months	10	13.3

The majority of patients in this group were lost to follow-up (**52%**) and in most of the patients, this occurred within 6 months of being diagnosed (**26.7%**).

2:3.4(b) HCV CHRONIC HEPATITIS

25 patients had HCV infection with **14** males (**56%**) and **11** females (**44%**). The male: female ratio was **1.3: 1**. The disease was most frequent in the White and Coloured populations (**44%** and **32%** of the HCV group respectively), with age ranges of **14-73** years (***Figures 20, 21***). There were no cases from the Indian population and there was a tendency for the older age group to be affected, with the age distribution curve showing a significant skew to the right.

FIGURE: 20

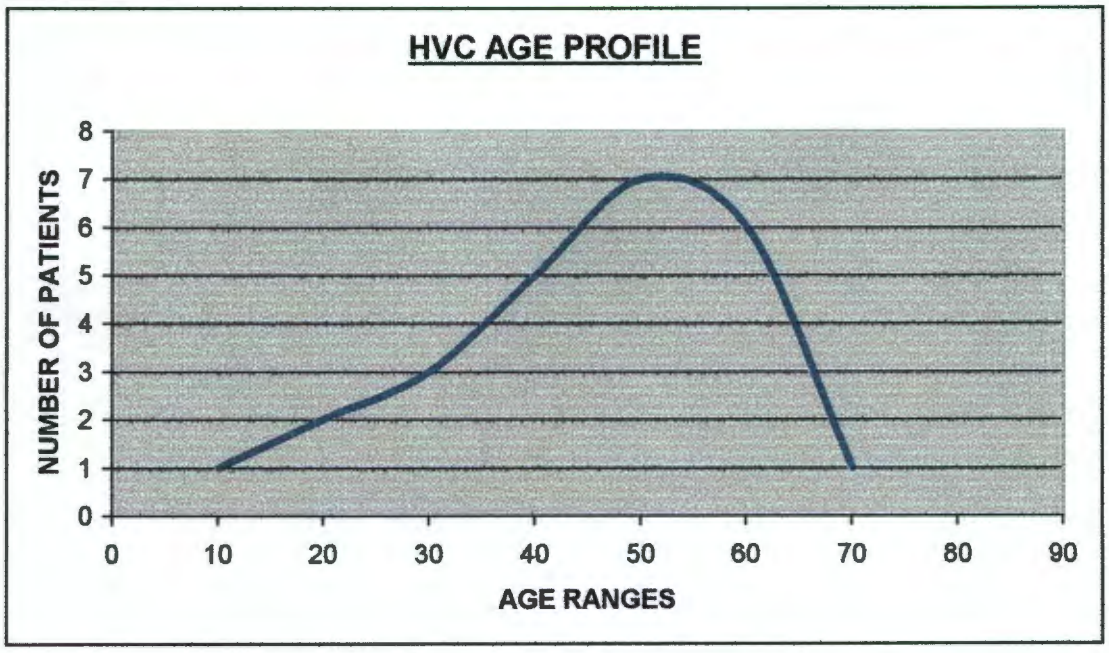
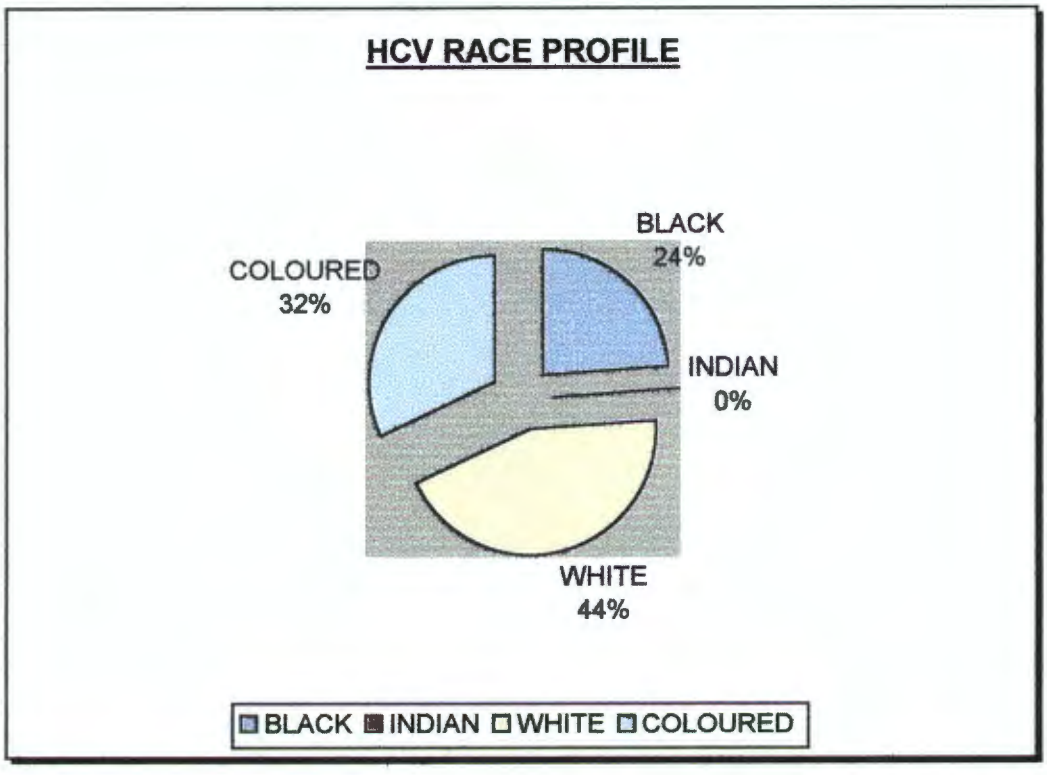
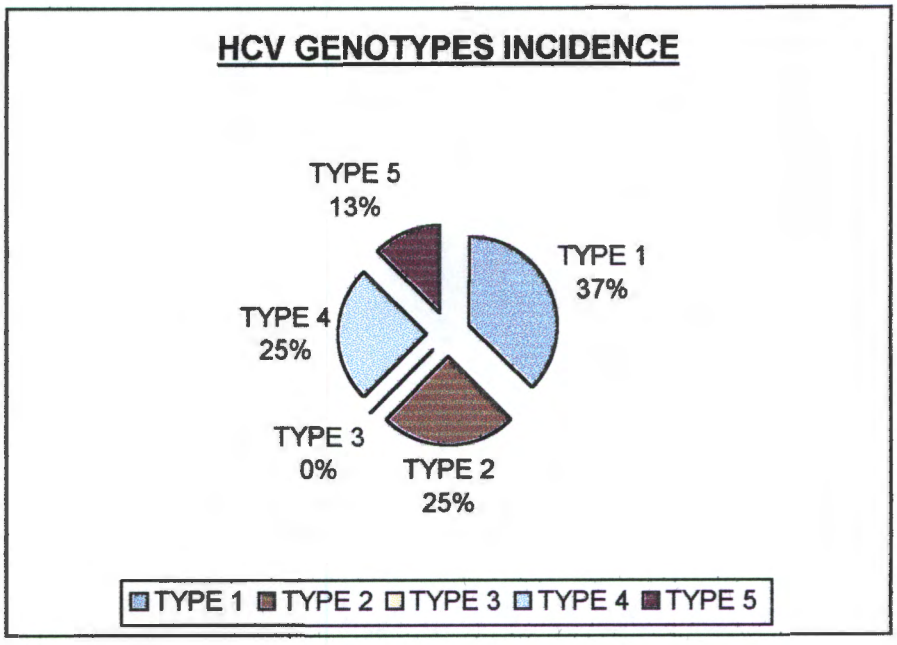


FIGURE: 21



The results of HCV PCR testing could be obtained for **16** of the **25** patients and the genotypes recorded are shown in ***Figure 22***.

FIGURE: 22



There were five patients who received IFN therapy. Treatment was given over a period of 24-52 weeks. In one patient the treatment was part of a trial but the follow-up is not available. In the remaining four patients, there was no response to the treatment. One patient had a liver transplant but died two years later from complications of colon resection surgery that was unrelated to the transplant. Steroids were used in three patients, in one patient this was for co-existing haemolytic anemia and in the other two patients steroids were given as a trial of therapy during 1982 and 1989 for severe hepatitis. The ANF was positive in two patients in titres of 1:10 and 1:2500. One patient was SM antibody positive with a titre of 1:60.

The history of alcohol consumption was again poorly recorded and an assessment of the intake could be made in only two patients and so this data is ignored.

The duration of follow-up until December 1996 is summarised in ***Table 19***.

TABLE: 19

Follow-up of the HCV group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	6	**24**
DEAD	4	**16**
LOST FOLLOW-UP	15	**60**
Less than 6 months	7	28
6 to 12 months	2	8
More than 12 months	6	24

2:3.5 DRUG-INDUCED CHRONIC HEPATITIS

This group was very small with only eight patients satisfying the criteria for drug-induced chronic hepatitis. There were **3** males and **5** females giving a male: female ratio of **1:2**. The age ranges at diagnosis were **21-68** years with a tendency for the older populations to be affected although the numbers are too small to really support this (***Figures 23, 24***).

FIGURE: 23

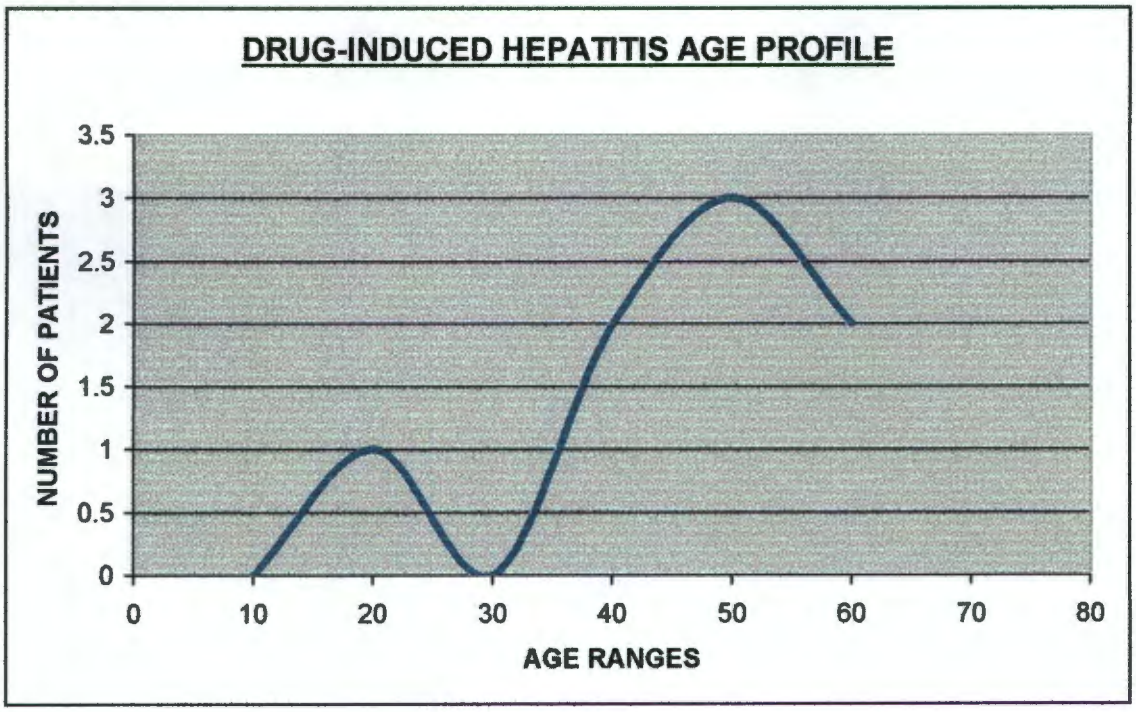
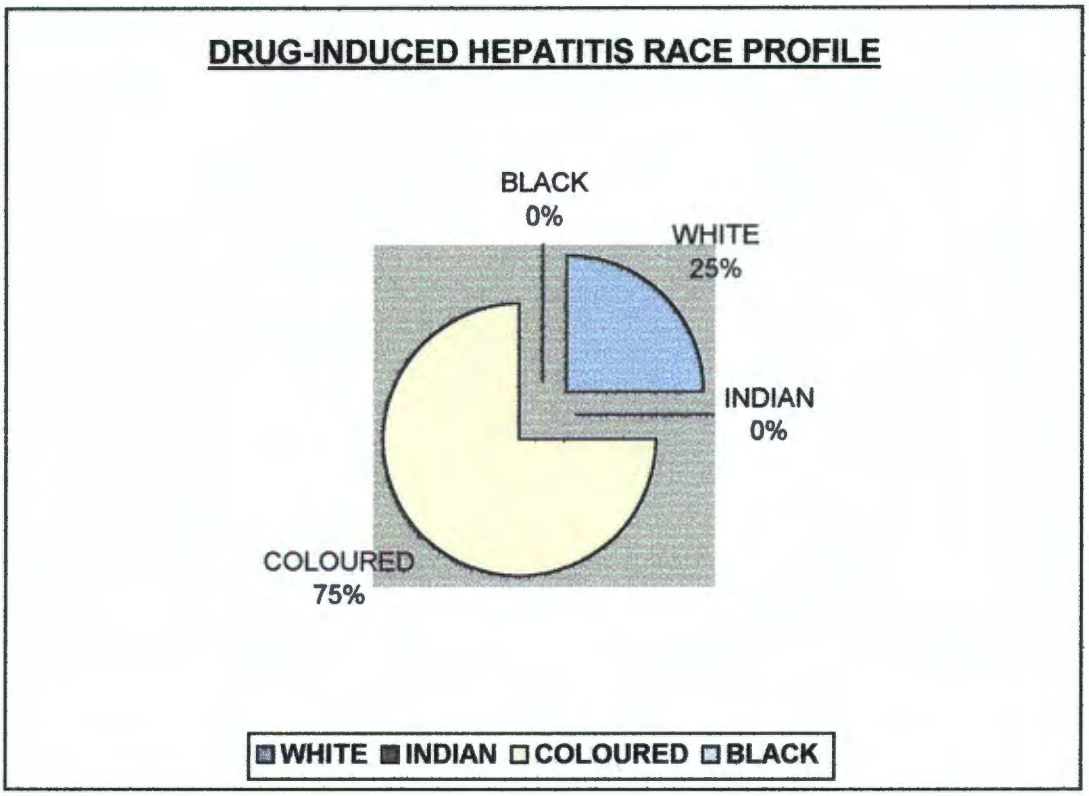


FIGURE: 24



Alpha-methlydopa was incriminated in six of the cases, carbamazepine and chlorpromazine in one case each. The alcohol intake was poorly recorded and the follow-up done is summarized in ***Table 20***.

TABLE: 20

Follow-up of the drug-induced hepatitis group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	0	**0**
DEAD	1	**12.5**
LOST FOLLOW-UP	7	**87.5**
Less than 6 months	2	25
6 to 12 months	1	12.5
More than 12 months	4	50

2:3.6 AUTOIMMUNE HEPATITIS

There were 51 patients with AIH. **15.7%** were male (8 of 51 patients) and **84.6%** were female (43 of 51 patients) giving a male: female ratio of **1:6**. The diagnosis was most frequent in the Coloured and White populations and rare in the Black and Indian communities (***Figures 25, 26***).

FIGURE: 25

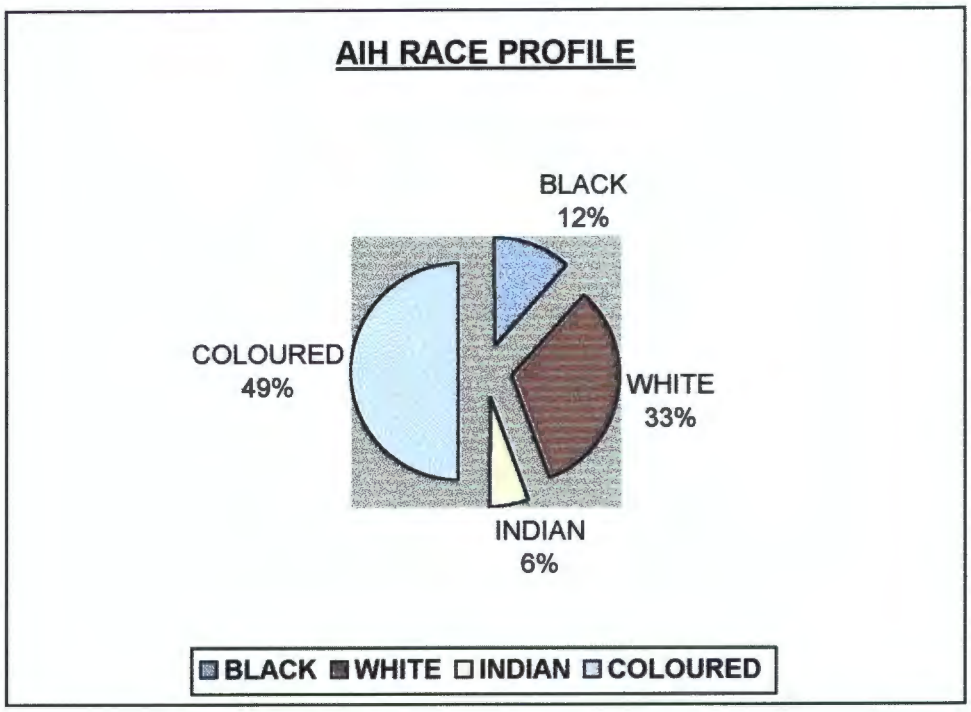
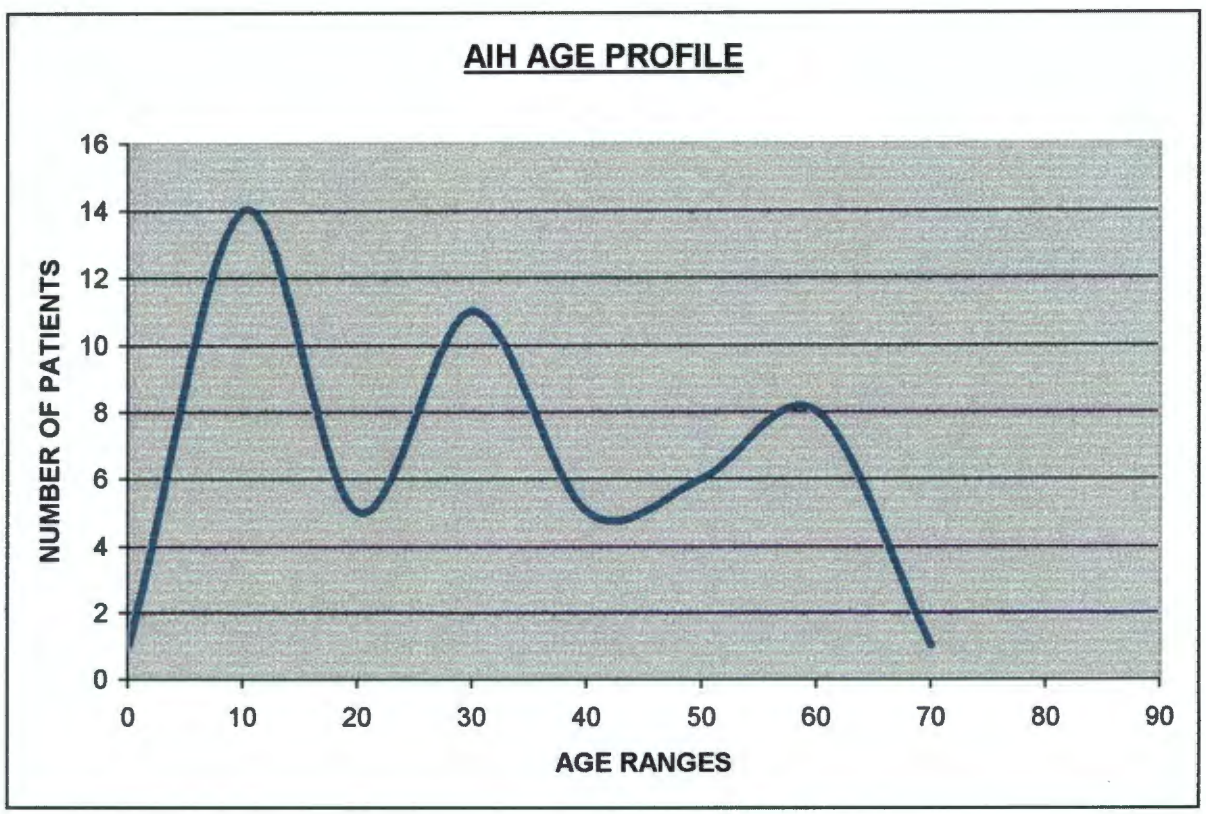


FIGURE: 26



The age ranges at diagnosis were **9-78** years. The ANF was positive in 36 patients (**71%**); SM in 27 patients (**53%**) and 13 patients (**25%**) had both ANF and SM positive. Only one patient (**2%**) was positive for the LKM-1 antibody (see ***Table 29***). The mainstay of treatment was steroids on their own in 29 patients (**56%**), steroids and azathioprine combination was used in 15 patients (**29%**), azathioprine alone in 1 patient (**2%**) and no treatment was given in 6 patients (**12%**). Only one patient received IFN therapy following a false positive HCV antibody test and she deteriorated on the treatment. The follow-up recorded is summarized in ***Table 21***.

TABLE: 21

Follow-up of the AIH group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	14	**27**
DEAD	14	**27**
LOST FOLLOW-UP	23	**44**
Less than 6 months	6	12
6 to 12 months	0	0
More than 12 months	17	33

The disease associations recorded included the following: HCV co-infection in two patients. In one of them the HCV results turned out to be a false positive. Genetic haemochromatosis was recorded in one patient who was a heterozygote for the C282Y mutation. PBC overlap syndrome was recorded in 2 patients but the histology was suggestive in one patient only. Inflammatory bowel disease was reported in 2 patients and discoid lupus erythematosus in one patient.

2:3.7(a) CRYPTOGENIC STEROID SENSITIVE CHRONIC HEPATITIS

There were 26 patients in this group with 6 males (**23%**) and 20 female (**77%**) giving a male: female ratio of **1:3**.The disease was most common in the White and Coloured populations, comprising 48**%** and **32% of** the group respectively. The age ranges at diagnosis were between **3 and 70** years (***Figures 27, 28***).

FIGURE: 27

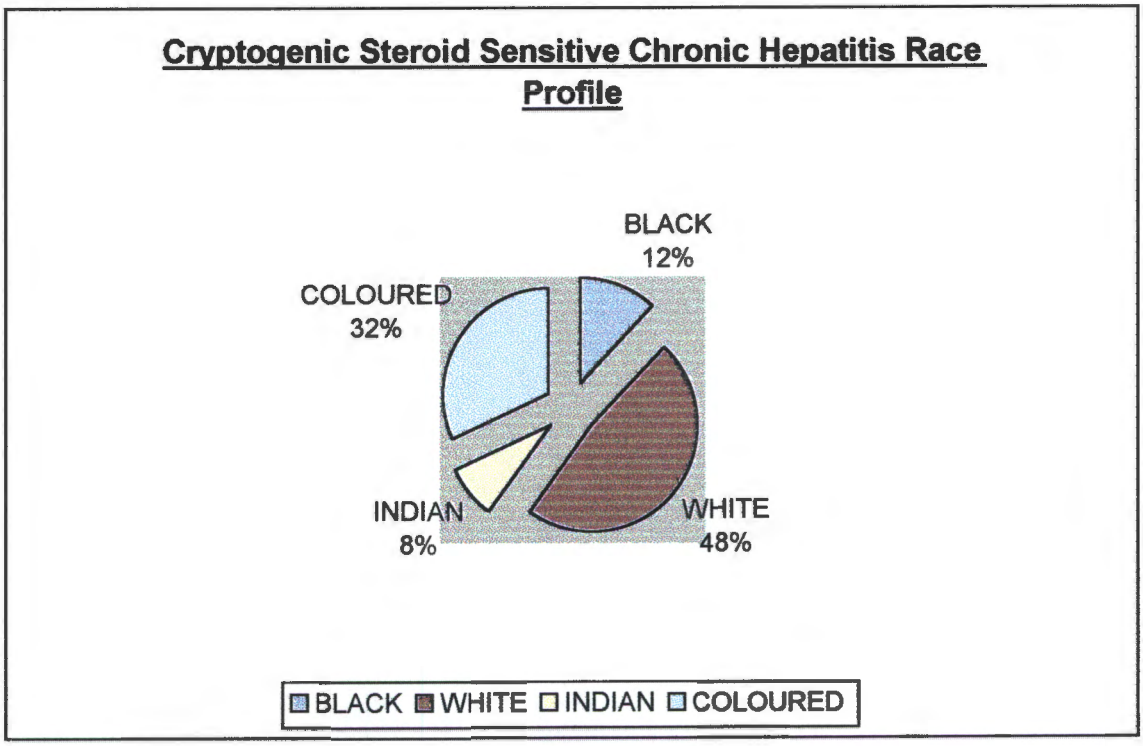
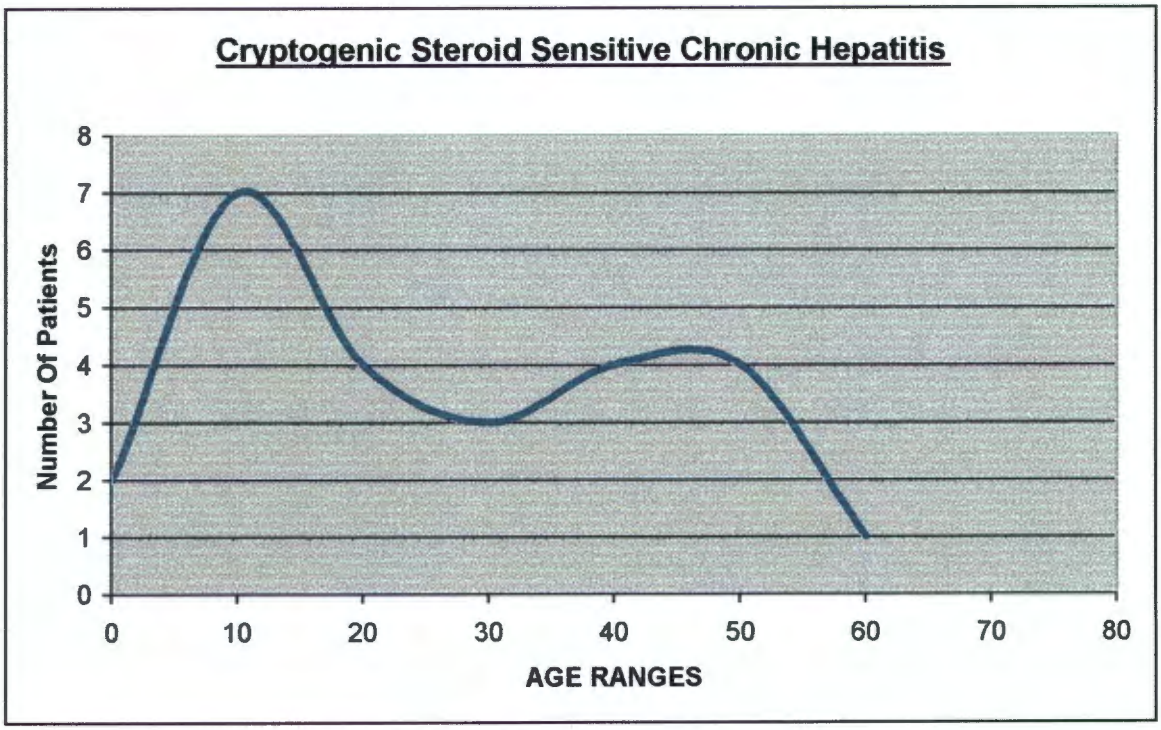


FIGURE: 28



All the patients were treated with steroids with or without azathioprine in combination. Most patients, **69%** (18 of 26 patients) were treated with steroids alone. In **31%** (8 of 26 patients) treatment was with steroids and azathioprine in combination and only one patient received IFN due to a false positive HCV antibody. One patient was transplanted and is still alive. The follow-up periods recorded are summarized in *Table 22*.

TABLE: 22

Follow-up of the cryptogenic steroid sensitive chronic hepatitis group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	13	**50**
DEAD	7	**27**
LOST FOLLOW-UP	7	**27**
Less than 6 months	2	8
6 to 12 months	1	4
More than 12 months	4	16

2:3.7(b) CRYPTOGENIC CHRONIC HEPATITIS

This group consists of those patients who were negative for viral markers (HBsAg, HBeAg, HBs antibody, HCV antibody and PCR, immunohistochemistry for HBsAg and HBcore Ag) and autoimmune markers (AMA, anti-LKM-1 antibody, ANF and SM antibody). They did not respond to a steroid course or were not given any steroids at all. There were **41** patients in this group with 21 males and 20 females giving a male: female ratio of **1:1**. The age ranges at diagnosis were from **10-76** years and the diagnosis was equally frequent in all the racial groups (***Figures 29, 30***).

FIGURE: 29

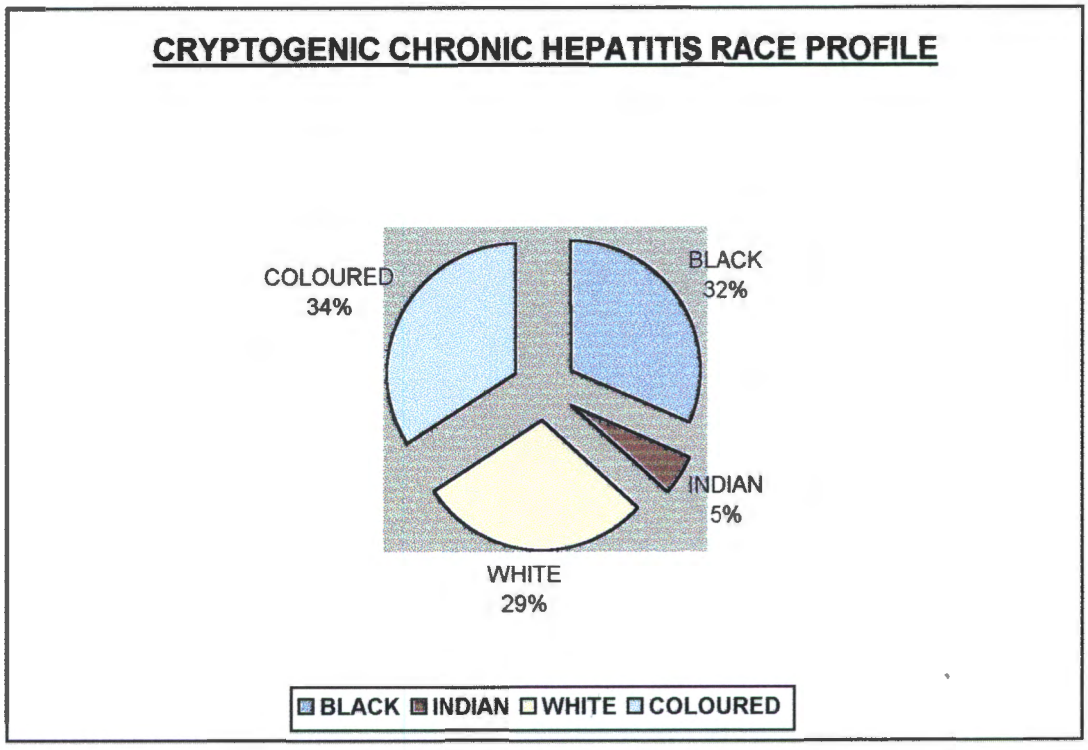
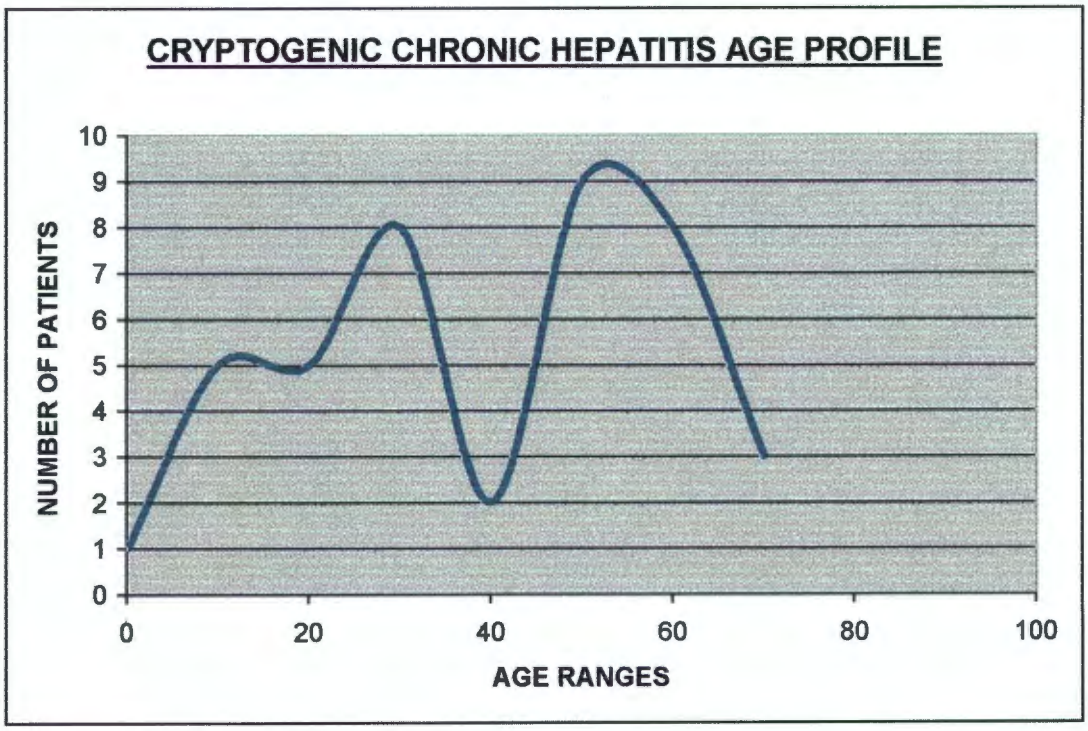


FIGURE: 30



Of the 41 patients, only three had a trial of steroid therapy. The lengths of the treatment courses were 3 to 7 months and one patient had both steroids and azathioprine over 7 months. None of the patients had a clinical response to the treatment given. Four patients had liver transplants done and two had documented HCC. The follow-up recorded in the patients is summarized in ***Table 23***.

TABLE: 23

Follow-up of the cryptogenic chronic hepatitis group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	3	7
DEAD	7	17
LOST FOLLOW-UP	31	76
Less than 6 months	18	44
6 to 12 months	7	17
More than 12 months	6	15

2:3.8 HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS

Hepatocellular carcinoma (HCC) was documented in **16** of the **226** patients in the study and this constitutes **7%** of the study group. There were **12** males and 4 females giving a male: female ratio of **3:1**. The tumour was common in the Black population (**50%** of the HCC patients) and was most frequent in the Black males who were **38%** of the HCC cases (6 of 16 patients). The age ranges at diagnosis were 18 to 63 years (***Figures 31, 32***).

FIGURE: 31

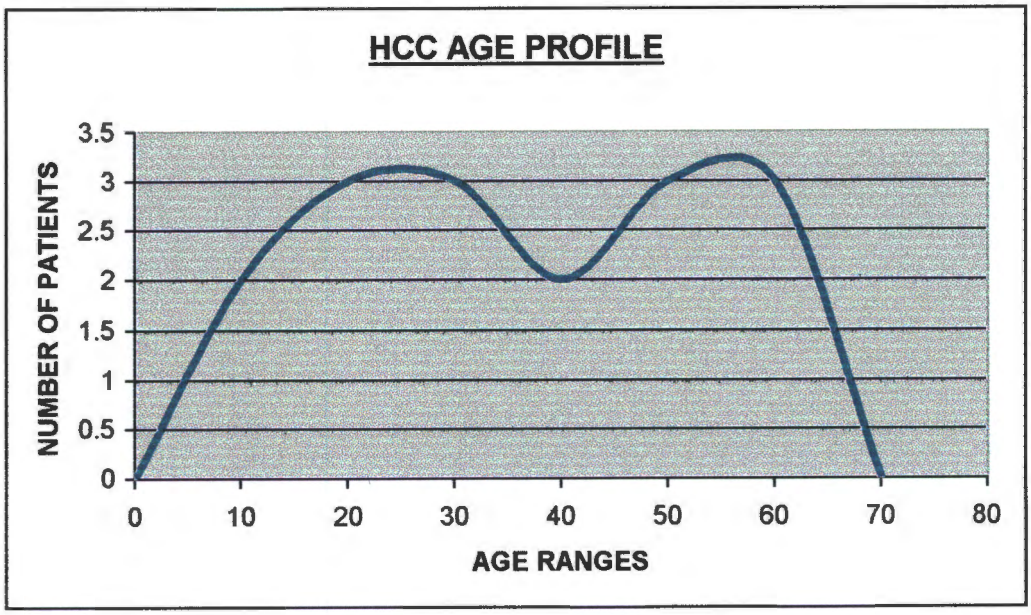
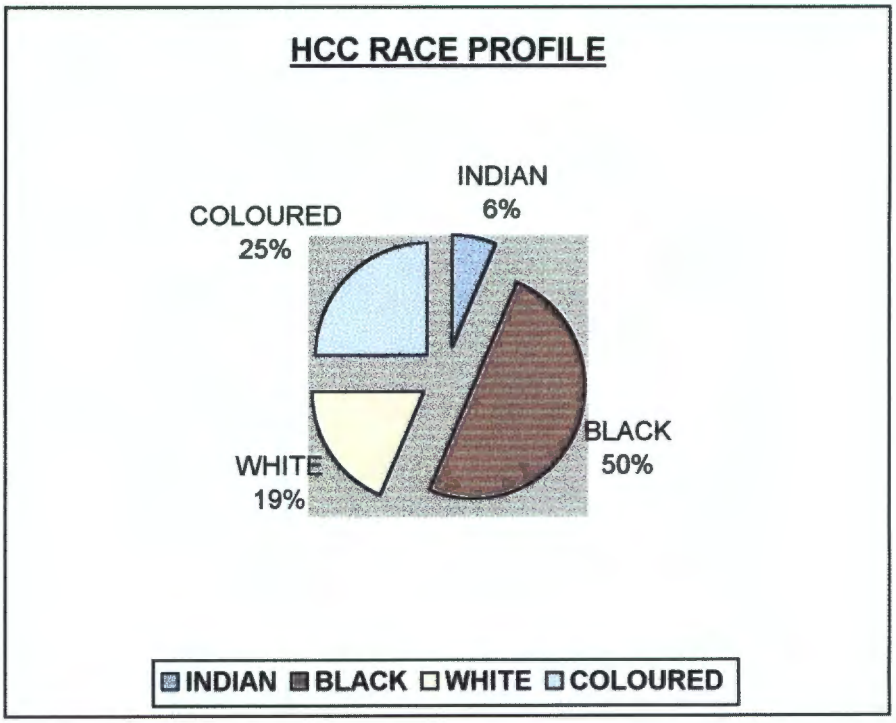
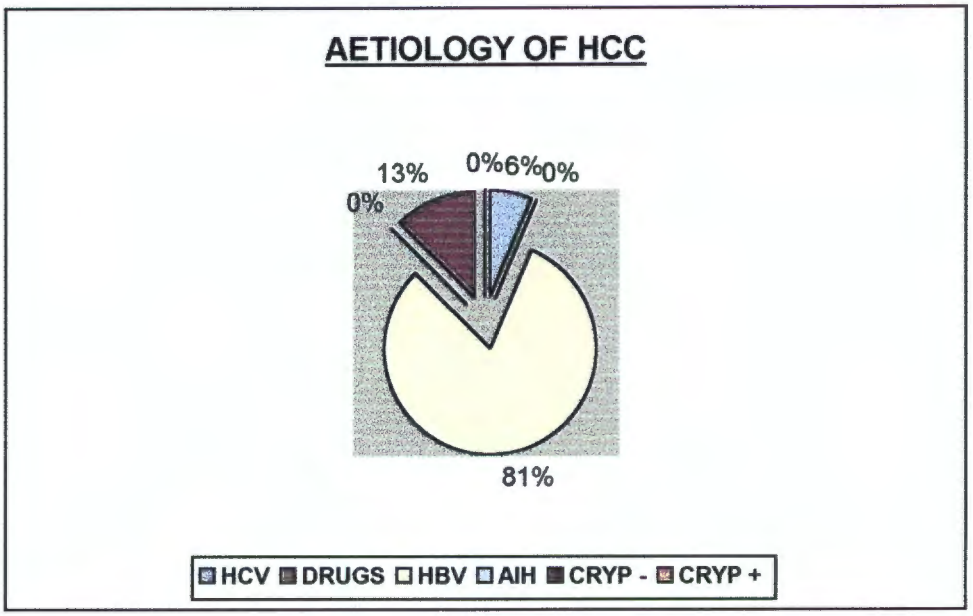


FIGURE: 32



Analysis by aetiology shows that HBV was the most frequent cause and there were no cases from the AIH and cryptogenic steroid responsive chronic hepatitis groups (***Figure 33***).

FIGURE: 33



HBV - 81%, HCV - 6%, AIH - 0%, Cryptogenic steroid sensitive chronic hepatitis - 0%, Drug-induced chronic hepatitis - 0%, Cryptogenic chronic hepatitis - 13%.

There were two patients who underwent liver resection, another two had chemotherapy and one patient had a liver transplant and the HCC was an unexpected finding in the explanted liver. The follow-up in these patients is summarized in ***Table 24 and the histopathology of HCC is illustrated in Appendix C***.

TABLE: 24

Follow-up of the HCC group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	1	**6**
DEAD	6	**38**
LOST FOLLOW-UP	9	**56**
Less than 6 months	6	38
6 to 12 months	0	0
More than 12 months	3	19

2:3.9 OTHER ASSOCIATIONS WITH HBV CHRONIC HEPATITIS

There were **11** patients with chronic HBV infection who had some other associated diseases that were not observed in the other diagnostic groups. Six patients (**55%** of the group) had haematological malignancies. These were non-Hodgkin's lymphoma in **4** patients, chronic myeloid leukemia and acute lymphoblastic leukemia in the other **2** patients. This was an interesting observation, which may reflect HBV infections that were acquired during the treatment of the malignancies since in all cases the malignancy preceded the diagnosis of HBV infection. There are case reports in the literature of a possible association between HCV and Non-Hodgkin's lymphoma but other workers (Pazzalo et al. 1994, Ferri et al. 1994, and Ellenrieder et al. 1998) have

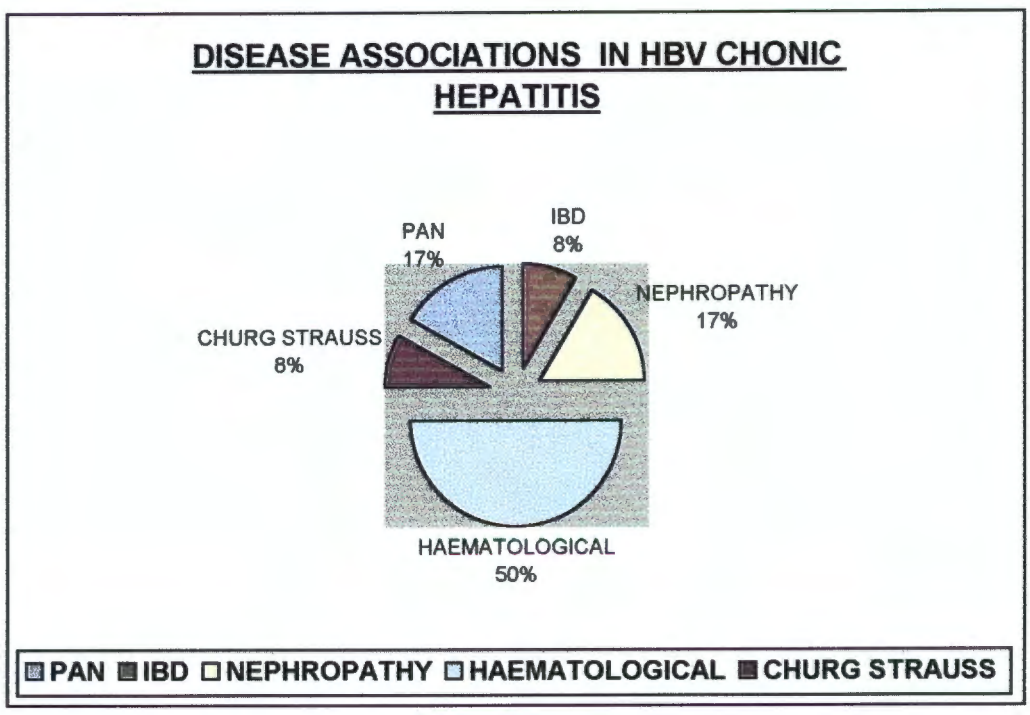
not substantiated this. The associations documented are summarized in ***Table 25*** and ***Figure 34***.

TABLE: 25

Disease associations with HBV chronic hepatitis

DISEASE	NUMBER
HAEMATOLOGICAL	**6**
Chronic myeloid leukemia	1
Acute lymphoblastic leukemia	1
Non-Hodgkin's lymphoma	4
POLYARTERITIS NODOSA	**1**
CHURG-STRAUSS SYNDROME	**1**
CROHNS' COLITIS	**1**
NEPHROTIC SYNDROME	**1**
PAN and Nephrotic syndrome	**1**

FIGURE: 34



PAN- Polyarteritis nodosa, **IBD**- Inflammatory bowel disease.

The follow-up record available in these patients is recorded in ***Table 26***.

TABLE: 26

Follow-up of the HBV disease associations group

	NUMBER OF PATIENTS	% OF TOTAL GROUP
KNOWN TO BE ALIVE	3	**27**
DEAD	2	**18**
LOST FOLLOW-UP	6	**55**
Less than 6 months	2	18
6 to 12 months	1	9
More than 12 months	3	27

2:4 DISCUSSION

The study population consists of patients with chronic hepatitis in the setting of strictly defined criteria (International Working Party 1995). As a result there are a number of patients who could not be included into the study due to the non-availability of hospital folders, wrong diagnostic codes or too short a period from the onset of illness (see ***section 2:2.2, epidemiological investigation***). There will therefore be cases that might have fulfilled the study criteria but are not included in this study cohort.

2:4.1 DEMOGRAPHIC DATA AND CLINICAL FEATURES

The recorded clinical features would suggest a late presentation by these patients (***Table 13***). The presence of jaundice, splenomegaly, loss of weight and ascites are in keeping with advanced liver disease (Di Bisceglie and Hoofnagle 1996). These features were very prominent in these patients. A palpable liver was a frequent recording although in most of the cases the precise liver size was not recorded. It is likely that in some cases this merely reflected the presence of a palpable left lobe of liver or a caudate lobe in a cirrhotic liver rather than a definitely enlarged liver. A proper assessment of the incidence of clinical problems such as amenorrhoea, acne, striae and pruritis in this population is not possible due to the poor recording of these findings at clinical visits. The absence of proper recording of the history of alcohol use means that the study is unable to assess the impact that alcohol would have made on the progression of chronic hepatitis to cirrhosis and hepatocellular carcinoma. Alcohol intake has been shown to accelerate the progression to cirrhosis in HCV (Wiley et al. 1998) and to increase the risk to developing HCC in chronic HBV infection (Mohamed et al. 1992).

The bimodal distribution of the age at diagnosis of the whole group shows two small peaks, the first in the third to fourth decades and the second the sixth to seventh decades (***Figures 8, 9***). The first peak would relate to the predominance of AIH in the early years (***Figure 26***) and the late peak is explained by the higher incidence of chronic viral hepatitis in these decades (***Figures 18, 20***).

The predominance of HBV chronic hepatitis is not surprising, as this is a common infection in Southern Africa with a reported 76% prevalence of positive HBV makers in South Africa (Kiire 1990, Kew 1992). The cryptogenic chronic hepatitis group i.e. (negative viral and autoimmune markers, no steroids given or no response to steroids) was significant, comprising 18% of the study group. This group was largely made up of patients who did not receive any steroids. Only **3** of the **41** patients received steroid therapy over periods of three to seven months, with one patients receiving both steroids and azathioprine without any clinical or biochemical response. The size of this group can be partly explained by the lack of HVC testing in most of the patients, as this may be a significant cause of cirrhosis in this setting (Jeffers et al. 1992). Only **39%** (**16** of the **41** patients) were tested for the HCV antibody with only 2 of them having both antibody and PCR testing. The low use of a trial of steroids also raises the possibility that some of the patients may indeed have been steroid responsive. There is also the possibility of there having been a drug aetiology that was missed in the history, as the use of a drug was not consistently recorded. Lastly the possibility of an unknown viral cause is a possibility worth considering.

The biochemistry and haematology profiles were not helpful in separating the patient groups by aetiology as most presented with advanced disease on the whole as shown by the high incidence of established cirrhosis at diagnosis (***Figure 13***). The biochemical abnormalities showed the pattern of the ALT being somewhat higher

than the AST (***Table 16***) except in the AIH and cryptogenic steroid responsive group who seemed to have slightly worse parameters (Williams and Hoofnagle 1996). The follow-up periods achieved differed markedly in the different diagnostic groups. This is summarized in ***Table 27***.

TABLE: 27

Follow-up of the whole study group

	NUMBER OF PATIENTS	% OF WHOLE GROUP
KNOWN TO BE ALIVE	48	**21**
DEAD	56	**25**
LOST FOLLOW-UP	122	**54**
Less than 6 months	56	25
6 to 12 months	19	8
More than 12 months	47	21

When compared to the follow-up in each diagnostic group, the best follow-up was in those patients in whom treatment was available and in whom regular monitoring was done i.e. the AIH, cryptogenic steroid responsive and the liver transplant patients. The loss of follow-up was highest in the HBV and HCV patients where disease modifying treatments (IFN and lamuvidine) were not available until after 1991. From ***Table 27***, more than half of the group (**54%**) was lost to follow-up at GSH and this occurred within 6 to 12 months of diagnosis. However some of the loss in follow-up can be attributed to the discharge of patients back to the referring hospitals since the Liver Clinic at GSH serves areas other than the Western Cape region.

2:4.2 HISTOPATHOLOGY

The high incidence of cirrhosis at the first clinical presentation is a major indicator of the late presentation that occurred. When the presenting stage scores of **≥5** for biopsies (see ***Table 6(a) page 22***) in each year are plotted over the 21 year period, it is interesting to note that the stage score at diagnosis and initial presentation did not improve but showed a slight worsening from **5.5** to **6** (***Figure 16***). The number of cirrhotic patients i.e. Ishak stage score of **≥5**, showed a steady increase over the period. This may be a reflection of an increased biopsy rate as shown in ***Table 17*** and therefore an increased rate of diagnosis of those patients who were cirrhotic. These changes show that there was no improvement in the time from illness to diagnosis over the study period i.e. the patients on average still are referred late with advanced disease and invariably with established liver fibrosis. The correlation that was shown between the Bedossa and Ishak grade scores shows that the simpler method does give the same information as the more complex and rarely used system. The correlation obtained could also be due to the fact that both scores were done at the same histological assessment resulting in a tendency for the initial Ishak score to influence the scores given for the Bedossa assessment. Also the fact the author was taught liver histopathology assessment by the reviewing pathologist would have increased the tendency for agreement in the scoring process. On average the disagreement in the scores was by one or two points during the combined review of the slides. The points of disagreement would then be reviewed and a consensus score agreed upon. In spite of the good correlation between the two systems, there is a fairly wide scatter in values i.e. a grade score of 14 would correlate with a Bedossa score of 1 or 2. This is explained by the absence of a portal inflammation score in the Bedossa system. It means therefore that those sections which had prominent portal inflammation and less

marked piecemeal necrosis had higher scores on the Ishak system but this would not reflected in the Bedossa score which does not score portal inflammation.

The liver biopsy practice did change during the study period. In the first **10** years of the study, **30%** of biopsies were by the transjugular route but this reduced to less than **20%**, this in spite of more transjugular biopsies per year being done. This is explained in part by:

(a) The availability of a safer and easier to use Menghini needle (Hepafix needle) resulting in more percutaneous biopsies being done.

(b) The use of ultrasound scan guided percutaneous plugged biopsies by the Radiology department in the presence of coagulation disorders. The biopsy tract is plugged with 2ml of gelatin before the Tru-Cut biopsy needle is withdrawn. Using this procedure, patients who previously would have undergone transjugular biopsies can be biopsed in a safe manner and a shorter operating time.

The postmortem biopsies were not very helpful due to tissue autolysis. This is due to the fact that all the postmortem tissues were obtained during the routine postmortem study, which in all the cases was after 35 hours.

2:4.3 HBV CHRONIC HEPATITIS, DISEASES ASSOCIATED WITH HBV, HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS

HBV chronic hepatitis and hepatocellular carcinomas were most prevalent in the male gender i.e. **81%** of the HCC were associated with HBV infection and the male: female ratio in the HCC group was **3:1** (***Figure 33***). This association has been shown in China and the Far East particularly (Lai C et al. 1987, Lok 1988) and studies from Southern Africa also show a male preponderance (Kew 1988). The explanations for this include: the higher carrier rates of HBV in males, the greater use of alcohol and

smoking in males (Lam 1982, Lai C 1987), greater exposure to chemical carcinogens in the work place, differences in the rates of metabolism of chemical carcinogens such as aflatoxins between the sexes (Linsell 1984) and a possible permissive role that the male hormonal milieu may have on HCC carcinogenesis (Kew 1996). Alcohol could also act as a promoter or a co-carcinogen and by inducing the hepatic microsomal P450-dependent biotransformation system, could produce reactive metabolites from ingested chemicals resulting in the formation of carcinogens (Lieber 1982). It is also interesting to note that although the cryptogenic steroid sensitive hepatitis and AIH groups had high incidences of cirrhosis at presentation, **60%** and **46%** respectively, none of them developed HCC during the follow-up period. This is very significant taking into account the fact that they had the longest periods of follow-up. The impact of alcohol unfortunately cannot be assessed in this study and a prospective study would be more appropriate to obtain such information.

The age distribution curve of the HBV chronic hepatitis group does not take into account the young patients who are diagnosed and treated at the Red Cross Children's Hospital in the Western Cape. It would be expected that if these were included, there might be a small peak at around 10 to 20 years. The racial preponderance in the Coloured population most likely reflects the population demographics of the Western Cape where 54.2% of the population is Coloured (South African census 1996, ***Figure 7***).

Only 6 patients in the study received IFN during the follow-up period. A sustained response was obtained in 3 patients and one patient responded but relapsed within one year. The other three patients did not respond at all. The current use of IFN therapy in chronic HBV can not be assessed by this study since regular use of interferon began after 1992, initially as part of drug trials. A study specifically designed to assess the

efficacy of IFN therapy in HBV chronic hepatitis in South Africa needs to be undertaken.

HBV immunohistochemistry on the liver biopsy was valuable in one patient to achieve a diagnosis. In this patient, HBV infection was only proven on the liver histology studies of the explanted liver following liver transplantation for cryptogenic cirrhosis. The diagnosis before liver transplantation was cryptogenic chronic hepatitis. The serological makers for HBV infection had been negative in the serum and there had been no liver biopsy pre-transplantation due to the presence of a small and nodular liver on ultrasound scanning, deranged clotting and ascites. However in this series of biopsies, the HBsAg was positive in **52.3%** of liver biopsies and the HBcore antigen in only **16.9%**. The question does arise therefore of how many of the crytogenic chronic hepatitis group may fall into this category in spite of having negative serological markers for known viral infections.

Chronic HBV infection was associated with other diseases and these were mostly haematological (55%) but also polyarteritis nodosa (PAN), churg-strauss syndrome, crohn's colitis and the nephrotic syndrome (NS). The associations with the NS and PAN are well documented (Lai KN 1987, Guillevin et al. 1992). The nephrotic syndrome occurs in less than 1% of adults and is associated with active viral replication. The histological lesion in the kidney is that of membranous or membrano-proliferative glomerulonephritis. These patients tend to have a good response to INF therapy. None of the patients in this group received IFN therapy. The pathogenesis of polyarteritis nodosa is not clear and it tends to be associated with the Churg-Strauss syndrome. It is characterized by the inflammation of medium-sized arteries and presents with severe hypertension, renal failure, and asthma with ischaemic events to various organs. These may present as stroke, ischaemia of the gut, a mononueritis

multiplex and acalculous cholecystitis. In these patients the diagnosis of PAN was made on the basis of renal biopsy, muscle and nerve biopsy, peripheral neuropathy and the histology of resected ischaemic bowel. Haematological malignancies are not reported in the literature as associations of chronic HBV infection although non-Hodgkin's lymphoma has been associated with chronic HCV infection (Ferri 1994, Ellenrieder 1998). The association of HBV infection with chronic myeloid leukemia (1 of 6 patients), acute lymphoblastic leukemia (1 of 6 patients) and non-Hodgkin's lymphoma (4 of 6 patients) in this group is not clear. The association could be entirely coincidental as the number involved is small. The other plausible explanation is that the HBV infection was picked up during treatment for the haematological malignancy. This could occur through blood products or re-use of blood lancets used for finger-prick haemoglobin meters and glucometers. An outbreak of acute HBV infections did occur in the Haematology Department at GSH previously, which was traced to the re-use of blood lancets (unpublished observation). The presence of Crohn's colitis (1 patient) in this group is likely coincidental considering the significant incidence if inflammatory bowel disease in the Western Cape (Wright et al 1986).

2:4.4 HCV CHRONIC HEPATITIS

This form of chronic hepatitis was most prevalent in the White and Coloured races and it is interesting to note that of the White population, 4 patients (**16%** of the HCV group or **44%** of the White patients with HCV infection i.e. 4/9 white patients) were Italian immigrants living in the Western Cape. The presence of immigrants from Europe must have influenced the HCV genotype incidence (***Figure 22***) which shows that type 1 genotype was most frequent at **37%**, types 2 and 4 at **25%**, type 5 with a

low presence of **12%** and no type 3 present in the study cohort. The corresponding figures quoted for blood donors in the Western Cape in 1996 show a predominance of type 5 at **28%**, type 1 at **20%**, type 3 at **12%**, type 2 at **8%** and there was no type 4 detected. The genotype could not be determined in **12%** of the blood donors (Voigt et al. 1996). An earlier study in 1995 using serum samples from around South Africa had also shown that type 5 was most prevalent, occurring in **40%** of the group, type 1 in **33%**; and types 2, 3, and 4 occurring in **13.8**, **7.7** and **2.3%** respectively. In **3.8%** of these patients the genotype could not be determined (Smuts and Kannemeyer 1995). There was a history of a previous blood transfusion in 6 patients and 3 had tattoos suggesting the possible sources of infection in those patients. The population group was older with a mean age of 51 years making this the diagnostic group with the oldest patients (***Table 16***).

None of the patients with HCV chronic hepatitis who received IFN therapy responded. This is summarized in ***Table 28***.

TABLE: 28

IFN treatment in the HCV patients

Genotype	IFN dose per week	Year of treatment	Duration	Outcome
1	3Mu three times	1992	16 weeks	No response, transplanted
2	2.5Mu three times	1993	24 weeks	No response
2	3Mu three times	1992	24 weeks	No response and IFN stopped due to side-effects
5	3Mu three times	1995	52 weeks	No response

Mu - million units, **IFN** - interferon.

The response of genotype 1 to interferon is known to be poor. Genotypes 2 and 3 have a better response to INF therapy. In the patients under discussion, the two patients

with genotype 2 received treatment for standard recommended duration. One patient had 2.5 million units due to the presence of clinical depression. According to the current recommendations, their therapy would have been stopped once they had shown no biochemical response within three months of treatment (National Institutes of Health consensus statement 1997). The use of a longer duration of treatment has been shown to improve the sustained remission rate in those who respond to IFN but not the response rate (National Institutes of Health 1997, Alberti et al. 1996, and Par et al. 1998). The patient with the genotype 5 infection would according to the current knowledge and practice, not be expected to respond with prolonged treatment and the treatment would have been stopped after three months as well. The two patients who were ANF positive did not receive any specific therapy. The patient who was SM antibody positive had an autoimmune haemolytic anaemia for which a splenectomy was done in 1989. This patient was also treated with steroids for the haemolytic anemia.

2:4.5 DRUG-INDUCED CHRONIC HEPATITIS

This diagnostic group was the smallest and so it is difficult to make any definite deductions from the data of these patients. The major factor relating to why this group was disproportionately small is the poor recording of the drug histories of the majority of the patients. It is plausible that some of the cryptogenic chronic hepatitis group patients could be cases of drug-induced hepatitis. There were also cases that had possible drug aetiologies but were not biopsed because of clinical improvement and so could not be included in the study. The older hypertensive patients who reacted to methyldopa explain the bimodal distribution of the group and the other peak is due to the younger patients who reacted to other drugs (***Figure 23***).

2:4.6 AUTOIMMUNE HEPATITIS

A diagnosis of autoimmune hepatitis (AIH) was made in 23% of the study patients. The diagnosis was most frequent in the female gender with a male: female ratio of **1:6** as described elsewhere (Czaja 1984). By definition, these patients would be mostly **type 1** AIH because they were diagnosed on the basis of either a positive ANF, SM antibody or the presence of both antibodies (see ***Table 4 page 18***). Only one patient had a positive LKM-1 antibody that is specific for **type 2** AIH. From ***table 4***, the only way to separate **type 2** AIH from **type 3** AIH immunologically would be by testing for the soluble liver antigen in those patients who were only positive for the SM antibody and negative for ANF. Although **type 3** AIH can have a positive ANF, the incidence is extremely low at a rate of less than 5%. There were 14 patients who were only SM antibody positive and could potentially have **type 3** AIH. The patients who were both ANF and SM antibody positive and those who were only ANF positive are **type 1** AIH by definition. The immunological profiles of these patients and their probable AIH subtype are summarized in ***Table 29***.

TABLE: 29

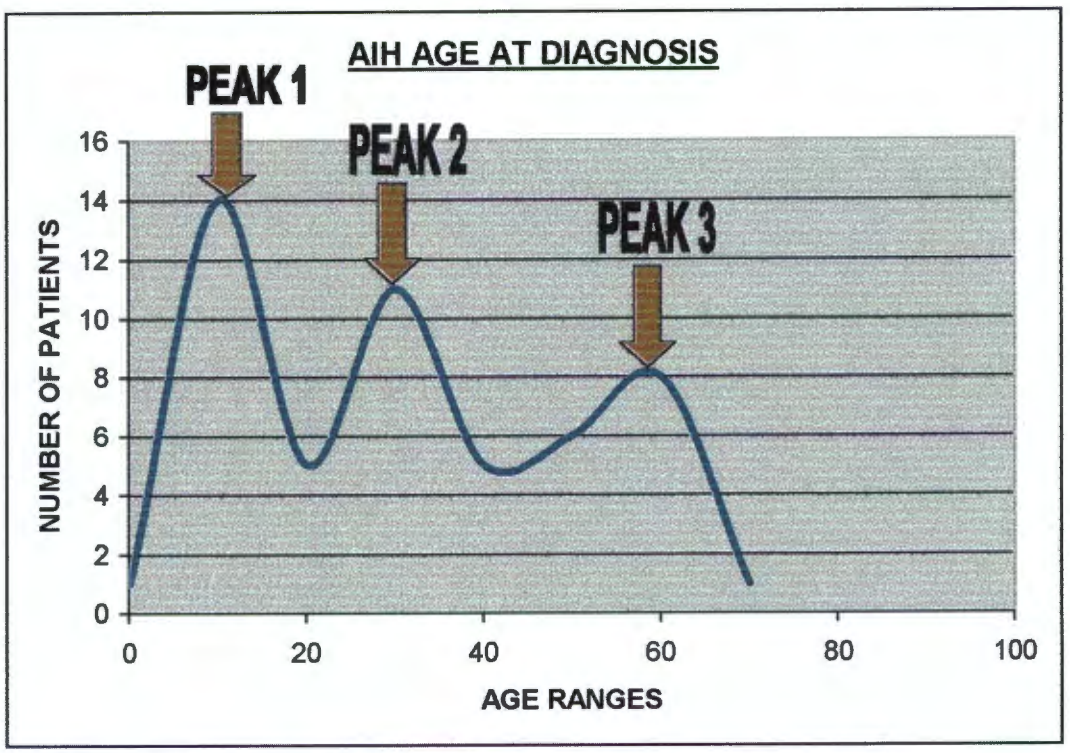
Immunology profiles of the AIH group

	POTEINTIAL AIH SUBTYPE			
ANTIBODY TEST	**TYPE 1**	**TYPE 2**	**TYPE 3**	**% OF GROUP**
LKM-1		1		**2**
SM + ANF	13			**25.5**
ANF ONLY	23			**45.1**
SM ONLY			14	**27.5**
Soluble Liver Antigen	**NOT AVAILABLE**			

From ***Table 29***, the diagnostic label of AIH **subtype 1** could be assigned to 36 of the 51 patients (**70.6%**) i.e. those ANF, SM and ANF positive; AIH **subtype 2** to only 1patient (**2%**) i.e. anti-LKM-1 positive. The SM only positive patients are potentially AIH **subtype 3**. They were **27.5%** of the patient group (14 of 51 patients). Further differentiation would have been possible if soluble liver antigen antibody studies were done.

The possibility that this group of patients may contain some patients who fit the diagnostic criteria for **type 3** AIH is also suggested by the presence of three peaks on the age at diagnosis profile plot (***Figure 26***). The three peaks are analyzed further in ***Figure 35***.

FIGURE: 35



PEAK 1 – 5 to 20years. **PEAK 2** – 25 to 40 years. **PEAK 3** – 50 to 70 years.

The first and third peaks would fit in with the age incidences of **type 1** AIH while since these are the two classically described peaks. The second peak would be

explained by the presence of **type 3** AIH in this group of patients. **Type 3** AIH has an incidence peak around the third and fifth decades.

The Coloured community of the Western Cape has a high incidence of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. The associated high incidence of AIH in this population would therefore not be a surprise. A study emanating from this work is planned to investigate the HLA status and other features of the autoimmune hepatitis and cryptogenic steroid responsive groups as this would also help to classify the patients into autoimmune subtypes. As a group the AIH patients had the best follow-up and of those who were subsequently lost, this happened after more than 12 months (see ***Table 21***). This was clearly due to the availability of treatment and the required follow-up needed to adjust steroid and azathioprine doses.

In most of the patients (**56%** or 29 of the 51 cases) disease control was achieved with steroids alone but it is significant to note that **29%** (15 of the 51 cases) of the patients required combination treatment with azathioprine to achieve biochemical control of the disease. There was one patient who received IFN therapy in 1992 and had a marked clinical deterioration over 12 weeks of treatment. INF was stopped and prednisone was started with a good clinical response. The repeat HVC studies done 2 years later in 1994 were negative. Only one patient has a record of a persistently positive HCV antibody and PCR with a positive ANF. She had no IFN given but was treated with steroids and azathioprine successfully. There was one patient who had genetic haemochromatosis and was heterozygote for the C282Y mutation. She was treated successfully with regular venesections, steroids and azathioprine combination. In the two patients who had the diagnostic label of AIH with PBC overlap, 1 had a suggestive histology with paucity of bile ducts and a positive AMA of 1: 40 titre. The

other patient's histopathology was not suggestive although there was marked cholestasis.

2:4.7 CRYPTOGENIC STEROID RESPONSIVE CHRONIC HEPATITIS

This is a group of particular interest due to its many similarities to the autoantibody positive AIH in spite of the absence of serological markers. The points of significance were:

1. The similar racial incidence profile.
2. The similar female gender preponderance although this was half of that observed in the AIH i.e. **1:3** and **1:6** respectively.
3. A similar clinical response to corticosteroids as well as the use of azathioprine. There was a need for azathioprine in 31% of this group and 29% in the AIH group.
4. The age distribution plot showed a profile similar to that of classic **type 1** AIH with two incidence peaks at 5 - 20 years and 40 - 60 years (***Figure 28***).

This diagnostic group raises a number of questions about the diagnosis of autoimmune hepatitis in the Western Cape. The possibility that there are other immune markers that are present and can help to tease apart this group further arises since the mixed ancestry population in the Western Cape consists of a heterogeneous gene pool. This is expressed in the high incidence of rheumatoid arthritis and systemic lupus erythematosus in this population group. The presence of such a genetic predisposition can be better assessed through HLA studies in the patients with autoimmune liver disease.

The size of this group might have been reduced if all of them had had HCV studies and a more thorough history search made for a possible drug aetiology for the cryptogenic cirrhosis.

2:4.8 CRYPTOGENIC CHRONIC HEPATITIS

This patient group was very different from the AIH and cryptogenic steroid responsive groups. The incidence in all the major racial groups was virtually the same and the sexes were equally affected. These patients also showed a tendency to developing hepatocelluar carcinoma while no HCC occurred in the AIH and cryptogenic steroid responsive groups. They also comprised 31% of the patients with liver transplants (4 of 13 patients) which was the same as the latter two groups combined (see ***Figure 37, Chapter 3***).

Clearly the issues for debate on these patients again are:

1. How many could have been a drug aetiology that was missed? This is impossible to exclude because the drug history is poorly recorded if at all.
2. How many could have been a missed viral aetiology due to HCV or HBV infections. Only 16 of the 41 patients (**39%**) had an HCV antibody test done and in only 2 of these was a PCR test done as well.

As a result of this study a number of observations and questions arise. It is clear that the AIH group at GSH is very heterogeneous and requires further study. A follow-up study of this group is planned to address the issues of:

1. The subtypes of AIH.
2. Further immunological and viral characterization of the cryptogenic steroid sensitive and the cryptogenic chronic hepatitis groups.

CHAPTER 3:

MANAGEMENT OF CHRONIC HEPATITIS AT GROOTE SCHUUR HOSPITAL

3:1 MEDICAL THERAPY

The complications of chronic liver disease include cirrhosis with portal hypertension in association with ascites and oesophageal varices, infections (particularly spontaneous bacterial peritonitis), osteoporosis and complications of the treatments used to treat or control the primary liver disease. The treatment related complications include steroid-induced diabetes mellitus, osteoporosis and infections.

The available treatments that would qualify as "disease modifying therapies" at the moment are:

1. Steroids and azathioprine in the treatment of autoimmune hepatitis. The use of immunosuppression allows for the control of inflammation and retards the tendency to progress to cirrhosis.
2. Interferon alfa alone or in combination with ribavirin. The use of combination therapy has now been shown to improve the success rate in the treatment of chronic hepatitis C infection (Davis et al. 1998, Reichard et al. 1998, McHutchinson et al. 1998). Studies have also shown that responders to interferon therapy have a lower incidence of developing hepatocellular carcinoma (Nishiguchi et al. 1995, Harper and Dienstag 1996, Kasahara et al. 1998).
3. Lamivudine in the treatment of HBV infection with or without a liver transplant.
4. Liver transplantation for end stage liver failure.

Chronic viral hepatitis therapy

The early management of chronic viral hepatitis at Groote Schuur Hospital was primarily the management of the complications of chronic liver disease. The availability of interferon has meant the improved referral of the HBV and HCV cases from the Blood Transfusion Service and medical practitioners in the areas serviced by Groote Schuur Hospital. However this study is not designed to assess how this has affected the management of chronic viral hepatitis because the period of study does not adequately cover the period over which interferon has been available at Groote Schuur Hospital. A study that should be inclusive of every patient who received interferon would need to be undertaken.

The success rate in the patients who received IFN in this cohort was very low. In the HBV cases, only 6 of the 75 patients (**8%**) in the study group received IFN. Of these only 2 responded with clearance of their HBeAg. In 1 patient the follow-up is not available as the patient was discharged back to the referring hospital (see ***pages 72*** and ***95***). In the 5 patients in whom follow-up is available, 4 were alive at the end of 1996 and 1 patient had died from liver failure 60 months after IFN therapy to which there had been no response. Of the 4 patients alive, 2 of them were the patients who had responded to IFN with the loss of HBeAg. They were both lost to follow-up at 36 and 12 months respectively after the completion of their IFN treatment courses.

The remaining 2 patients were non-responders to IFN therapy. The one patient has complications of established liver cirrhosis with intra-hepatic stones, portal hypertension and intractable ascites. This patient has recurrent ward admissions for spontaneous bacterial peritonitis, cholangitis and tense ascites that requires regular drainage. The last patient initially responded to IFN therapy with the loss of HBeAg but relapsed at one year of follow-up. During this period she also developed

hypothyroidism as a complication of IFN treatment. Presently she has established cirrhosis with a low normal albumin of 35g/l and a mildly deranged clotting profile. In the HCV patient group, 5 of 25 patients (**20%**) received IFN treatment and none of them responded (see ***pages 75*** and ***98***). One patient received the IFN as part of a drug trial and the follow-up details are not available. In the remaining four patients, one patient had a liver transplant but died 24 months post liver transplantation from septicaemia following colon resection surgery for perforated colon diverticuli. One patient was lost to follow-up and the other 2 patients are alive with established liver cirrhosis.

Lamivudine use in non-drug trial patients was done in 2 patients of this study cohort. In one patient lamivudine was used due the presence of cirrhosis with low platelet and leukocyte counts. This patient was alive at the end of the study and this was 2 years after starting the lamivudine treatment. The second patient was treated with lamivudine after acquiring acute HBV infection 10 months post liver transplantation from a household contact. The other patients, who are in the majority, received symptomatic therapy for the associated complications of chronic liver disease. The emphasis in these patients was on the management of ascites, infections and the eradication of oesophageal varices.

Autoimmune liver disease therapy

The only therapy that made an impact on the progression of disease was immunosuppression in the treatment of AIH or steroid sensitive chronic hepatitis. As a result it is not surprising that the follow-up rate of the other chronic hepatitis cases was poor. As discussed in Chapter 1, the availability of reasonably effective therapy

meant that the AIH and steroid responsive patients required more regular follow-up to monitor dosages and the response to treatment.

All the autoimmune marker positive patients received prednisone. Azathioprine was added if there was no biochemical response or if the steroid doses could not be tapered down without deterioration in the liver tests. The autoimmune markers that are done at Groote Schuur Hospital in the investigation of possible autoimmune liver disease are ANF, SMA, AMA and anti-LKM1 and 2 antibodies. The patients, who had inactive "burnt-out disease" i.e. established cirrhosis with normal liver tests, were not treated with immunosuppressive therapy. Only **11.8%** (6 out of 51 patients) of the cases with AIH who fell into this category and these patients were treated for any complications of chronic liver disease that they had at presentation.

3:2 LIVER TRANSPLANTATION

There were 13 patients in this study cohort who underwent liver transplantation. Eight were male and 5 female. Although the numbers are small, there were no Indian patients in this group, Blacks comprised **23.1%** (3 of 13 patients), Coloureds comprised **30.8%** (4 of 13 patients) and Whites were **46.1%** (6 of 13 patients) of the whole group. Most of the patients were young or in the 3rd to 5th decade (***Figure 36***). The youngest was 13 years and the oldest was 56 years old. The aetiology of the end-stage liver disease leading to liver transplantation is summarised in ***Figure 37***.

FIGURE: 36

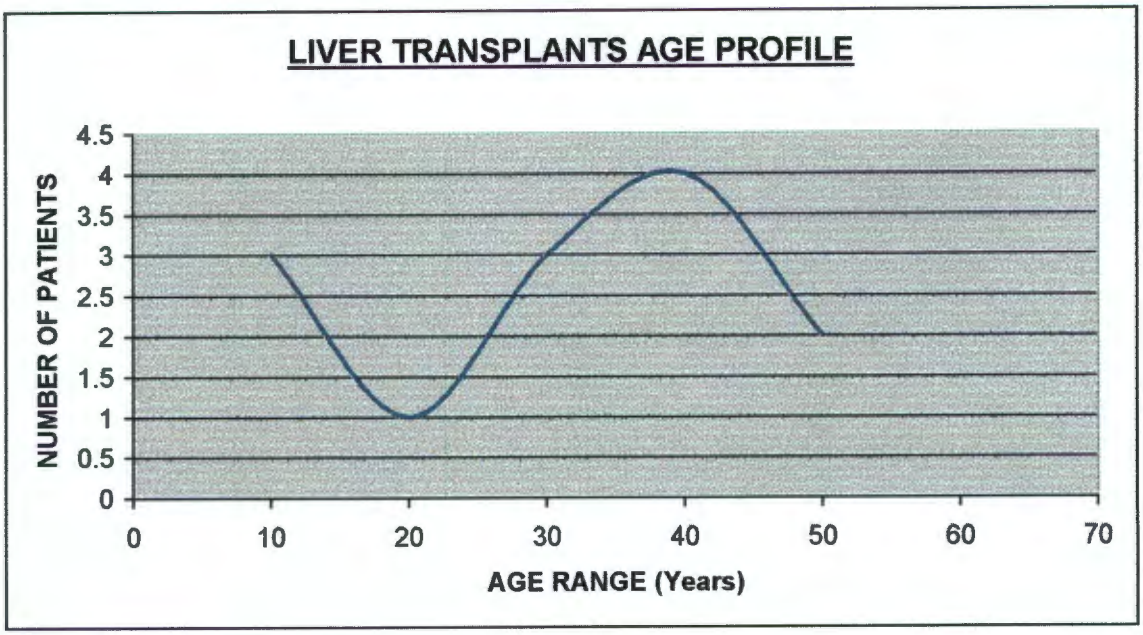
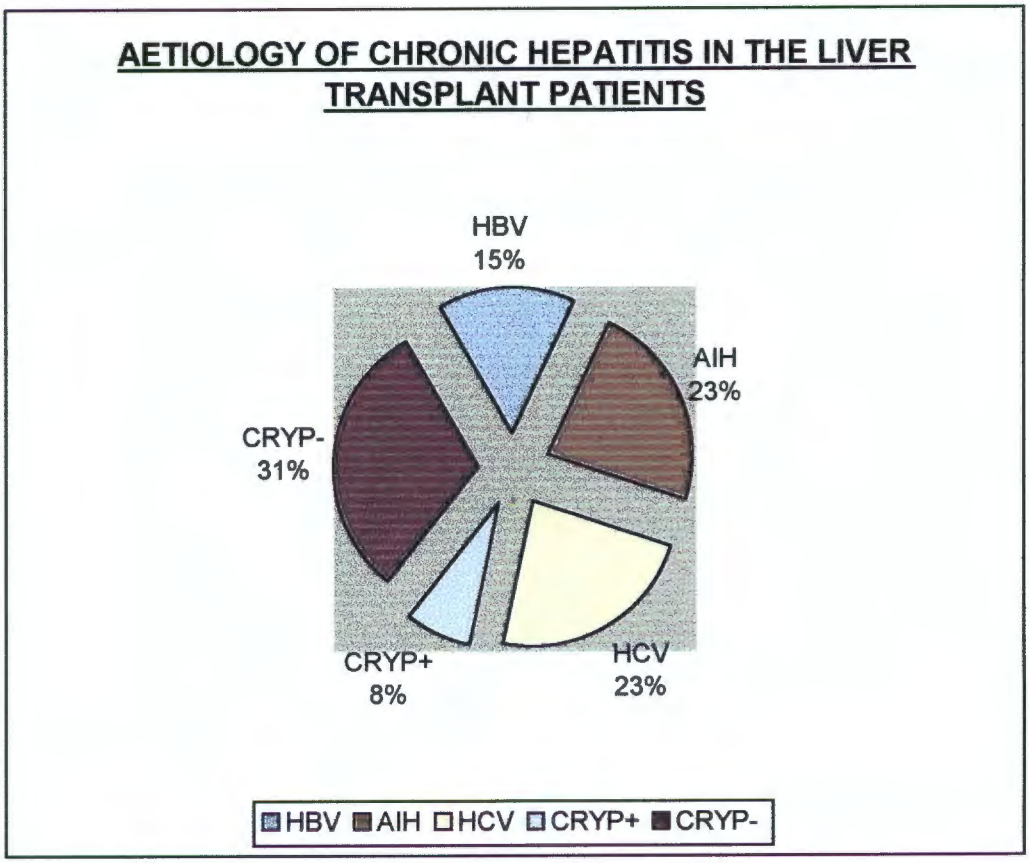


FIGURE: 37



CRYP -, Cryptogenic chronic hepatitis, CRYP +, Cryptogenic steroid sensitive chronic hepatitis, AIH -Autoimmune hepatitis, HBV - Hepatitis B virus hepatitis, HCV - Hepatitis C virus hepatitis.

In terms of numbers, the aetiological groups of the transplant patients were: HBV - 2 patients, HCV - 3 patients, AIH - 3 patients, Cryptogenic chronic hepatitis - 4 patients and Cryptogenic steroid sensitive chronic hepatitis - 1 patient. There were no cases with drug-induced hepatitis in the transplant group. One of the cryptogenic chronic hepatitis patients developed acute HBV infection 1 year after transplantation but has done well on lamivudine treatment.

Table 30 summarises the total adult liver transplants done during the study period. In the study patients, there were 5 deaths, one was in the immediate post-surgical period and the other deaths were 7 to 36 months post liver transplantation (***Table 31***).

TABLE: 30

Adult liver transplants done 1988-1996

YEAR	TRANSPLANTS DONE	STUDY GROUP (Chronic hepatitis)
1988	1	1
1989	2	
1990	5	3
1991	4	
1992	6	
1993	6	4
1994	7	1
1995	7	4
1996	6	
TOTAL	**44**	**13**

TABLE: 31

Causes of death in the liver transplant cases

	DIAGNOSIS	CAUSE OF DEATH	FOLLOW-UP
1	HCV	Intra-abdominal bleed, adult respiratory distress syndrome	1 Week
2	Cryptogenic chronic hepatitis	Disseminated CMV infection, chronic rejection	7 months
3	HCV	Perforated colon, septicaemia	24 months
4	AIH	Acute HBV infection	36 months
5	AIH	Chronic rejection, disseminated CMV and aspergillosis infections	36 months

3:3 DISCUSSION

3:3.1 Transplantation in chronic viral liver disease

Liver transplantation for chronic liver disease became available at Groote Schuur Hospital in October 1988. The first 10 patients were reported in 1992 and among them were 2 cases of HBV chronic hepatitis who are included in this study cohort (Robson et al. 1992). Since then 47 liver transplants have been done in adults inclusive of the 44 during the study period. The outcome following liver transplantation for chronic liver disease has improved in the last decade and the management of these patients is still evolving.

Transplantation in HBV chronic hepatitis results in a high incidence of re-infection and progression to chronic hepatitis with cirrhosis often occurring within 2 years (Lake et al. 1991). This may be associated with a rare histological variant called fibrosing cholestatic hepatitis that is rapidly progressive and has a fatal outcome. This is typified by the presence of periportal and perisinusoidal fibrosis, ballooned hepatocytes with cell loss, pronounced cholestasis and mild inflammatory activity (Davies et al. 1991). The European Hepatitis Project (EUROHEP) evaluated the results of liver transplantation in hepatitis B surface antigen positive patients in European centres between 1977 and 1990 (Samuel et al. 1993). This review showed that a lower recurrence risk was independently predicted by:

1. The long-term administration of anti-HBs immunoglobulin for at least six months or more. Prophylaxis for 2 months or less had the same outcome as not giving any immunoglobulin.
2. The absence of HBV DNA in serum before transplantation.
3. The absence of HBeAg in serum before transplantation.

A lower risk of recurrence was also associated with HDV superinfection and acute fulminant liver disease. The association with HDV superinfection may be due to the ability of HDV to switch-off HBV replication (Sureau et al. 1992).

The treatment strategies for patients with hepatitis B infection undergoing liver transplantation should include:

(a) Patient selection to exclude those with a high level of viral replication before transplantation. The patients to be considered are those with acute fulminant hepatitis B or D, chronic HDV infection, or chronic HBV infection without detectable HBeAg or HBV DNA.

(b) Modification of immunosuppression to reduce the risk of re-activation. Steroids have a deleterious effect and this is likely to be due to the presence of a steroid responsive promoter region in the HBV genome. The activation of the promoter region results in increased viral replication (Lam et al. 1980, Tur-Kaspa et al. 1986). For these reasons, the clinical practice is for a rapid reduction in the dose of steroids in these patients post liver transplantation.

(c) Passive immunoprophylaxis with hepatitis B immunoglobulin has now been shown to reduce the rate of recurrent hepatitis B infection significantly. In the EUROHEP study, the rate of recurrent HBV infection after 3 years was only 30% in patients on long-term prophylactic HBsIg treatment (for at least six months) compared with 67% in those given no prophylaxis (Samuel et al. 1993). However the cost is prohibitive and the availability is limited. In 1997 figures, HBsIg therapy in the United States adds $10 000 - $50 000 to the first year's charges for a liver transplant and $5000 - $20 000 to each subsequent year (Terrault and Wright 1997). Such costs are clearly not affordable in South Africa, which makes the transplantation of these patients economically unaffordable.

(d) Antiviral treatments pre-transplantation and post-transplantation to prevent graft re-infection and avert the recurrence of HBV infection. Interferon-α has the theoretical risk of enhancing HLA expression on epithelial cells of the bile ducts and lead to allograft rejection. The magnitude of this problem is controversial and the risk of acute rejection cannot be quantified (Wright T 1994,Terrualt 1996, Terrualt 1997).

The use of nucleoside analogues has had good results in both transplant and non-transplant cases. In liver transplant patients, lamivudine given as 100mg daily for 4 weeks before the transplant showed undetectable HBV DNA concentrations or borderline positive results by bDNA assay (Perrillo et al. 1996).

The HBV cases transplanted at Groote Schuur hospital have done well. Two cases were transplanted and another patient acquired acute HBV infection post-transplantation. One case was known to be HBV infected pre-transplantation. This patient was done in April 1990 and had a negative HBeAg, positive HBsAg and HBeAb pre-transplantation. HBV DNA levels were not done because the assay was not available at Groote Schuur Hospital at the time. Graft re-infection occurred in September 1990 and the patient became HBeAg positive. IFN therapy was started but this was stopped after the patient developed severe depression and became suicidal. The patient is alive and has good graft function. The other patient had negative serological markers for HBV infection pre-transplant. However on immunohistochemistry staining of the explanted liver, the HBsAg was positive. This patient is alive and also has good graft function. The patient who had post-transplant acute HBV was transplanted for cryptogenic chronic hepatitis in November 1995 and acquired HBV infection from a household contact in September 1990. Lamivudine

was given and over the years she has done very well. The current results show that she is now negative on testing for HBV DNA, HBsAg, and HBeAg.

In the three patients who were transplanted for HCV infection, 2 have since died and 1 is still alive. Patient number **1** in ***Table 31*** died a week post transplantation after developing an adult respiratory distress syndrome 2 days post-surgery and intra-abdominal bleeding 6 days later. Patient number **3** in ***Table 31*** died 2 years following liver transplantation due to complications of colon resection surgery after diverticuli perforation. The patient who is alive was transplanted in 1993. He did not receive IFN at any stage and is still HCV PCR positive but with good synthetic liver function.

One of the transplant patients had an incidental hepatocellular carcinoma (HCC) found on the histology of the explanted liver. This patient is still alive and there has been no evidence of tumour recurrence. The management of HCC in this series of patients has been discussed earlier (see **section 2:3.8** ***page 84***).

3:3.2 Prevention of chronic hepatitis

The management of chronic hepatitis today should involve preventative measures, the treatment of complications of chronic liver disease and the use of "disease modifying therapies" where appropriate. The prevention of chronic hepatitis is possible in drug-induced liver disease and chronic viral hepatitis, as these causes are amenable to interventions that can prevent disease.

In chronic viral hepatitis, education, improvement of public health, follow-up of cases and their close contacts, screening of appropriate target groups and vaccination are necessary to impact on the incidence of disease in the population. Education can be effected through the print and electronic media, school education programmes and via institutions such as the Blood Transfusion Services which have frequent and regular contact with communities. The Western Province Blood Transfusion Service

(WPBTS) provides such a service. The WPBTS also initiates the follow-up and assessment of clients who test positive for hepatitis B or C serological markers. Such patients are referred to the liver clinic at Groote Schuur hospital for assessment and initiation of contact follow-up and education. These clinic visits are important because they provide an opportunity to assess the individual patient for interferon therapy and also to effect the follow up of close contacts of the patient, who may benefit from vaccination. If already infected with HBV, the contacts would also require a clinical assessment for possible interferon use.

The provision of appropriate sanitation, clean drinking water and uncontaminated food are important to modify risk factors for acquiring viral hepatitis. The gross under-notification of cases of hepatitis impacts negatively on the ability of the health system to follow-up contacts of cases and keep reliable data on viral hepatitis. Accurate data is important for identifying trends and for planning health interventions by Government. In South Africa, it is estimated that due to under-reporting, the national incidence of hepatitis B is at least 7 times higher than that calculated from notification data (Abdool Karim 1991).

Screening is the routine investigation of an apparently healthy population to detect an unsuspected condition for which treatment would be beneficial. Therefore to justify the exercise, the detection test should be specific and sensitive enough to pick up disease and the treatment good enough to justify the screening procedure. If used successfully, screening will prevent infection or transmission and identify those cases that require vaccination for hepatitis B. Screening has already been used for blood donations to prevent the transmission of HBV, HCV and other transmittable diseases with remarkable success. The screening of pregnant women is important in the strategy to control hepatitis B virus infection because the use of hepatitis B

immunoglobulin and immunisation of infants is highly effective in preventing vertical transmission of hepatitis B virus. Universal vaccination against hepatitis B virus infection of newborn babies and infants under the age of 1 year was started in South Africa in April 1995. The vaccine in use is a plasma derived flash heated vaccine (Hepaccine-B). This is given at birth, 1 and 6 months of age to coincide with the other clinic visits for immunisations. The vaccine is given intramuscularly in the deltoid muscle at a dose of 10g per dose in children and 20g per dose in adults. Administration in the buttock has been shown to be less effective and should not be used (Buchel et al. 1994). A booster injection is recommended for every 5 years to maintain protective levels of anti-HBV immunoglobulin. A recent study has shown the benefit of universal vaccination in Taiwan where the average annual incidence of hepatocellular carcinoma is showing dramatic reductions in children following the introduction of a vaccination programme for HBV infection (Chang et al. 1997). The other population groups who should be targeted for vaccination include:

1. Recipients of blood products on a regular basis. Those with clotting disorders who receive factor concentrates and those with hypo-immune disorders that require regular immunoglobulin infusions.
2. People at occupational risk of acquiring HBV infection. This includes health care workers and students, staff at mental institutions who are at risk to assaults, bites and needle stick injuries.
3. Patients on haemodialysis and those in mental institutions and special schools.
4. Sexual and household contacts of HBV carriers. Vaccination should be combined with education about the spread of HBV and how to minimise the risk of transmission.

5. Those at risk of sexual transmission i.e. commercial sex workers and homosexual and bisexual men (Practices advisory committee 1991).
6. Non-immune travellers who are going to stay in an endemic area for more than six months. Vaccination should ideally begin 6 months before travel to allow for the completion of the vaccination series.

In drug-induced hepatitis, the only effective preventive measure available is the education of the health workers and their patients. The severity and incidence of this problem can only be reduced through safe prescription practises and the immediate cessation of any drug that a patient who presents with jaundice and raised liver enzymes may be using. Clearly a causality assessment has to be done by the attending doctor but any potential hepatotoxin is best stopped until a cause for the liver dysfunction can be established (see ***section 1:3.4(d) pages 30 - 32***).

CHAPTER 4:

REVIEW AND CONCLUSIONS

This study has raised a number of interesting questions that need further investigation and has also exposed the limitations of analysing data that has not been uniformly collected and recorded. These points can be discussed under the following subheadings:

1. The quality of the available data.
2. Treatments used and the use of liver biopsy in managing chronic hepatitis.
3. The subtypes of AIH and the need to further evaluate the nature of the diagnostic categories of chronic hepatitis and cryptogenic steroid sensitive chronic hepatitis.

4:1 QUALITY OF DATA AVAILABLE

The data available was not consistent in its detail and accuracy. The best detail was available from the liver clinic records where the emphasis was more on the management of the chronic liver disease. Even so, details of factors like alcohol consumption were universally poorly recorded and a comment on the impact of alcohol in chronic hepatitis or the development of HCC can not be made from this study. It was clear that the liver clinic itself needs an easily accessible computerised database and that for uniformity and accuracy, a standard clinic form for recording essential details would be ideal. A good database would allow for:

1. Retrospective studies to monitor disease prevalence and admission trends.
2. Easier auditing of patient management at the clinic to see where changes may be necessary to improve patient care.

3. The tracing of patients in the event of new therapies or new diagnostic procedures becoming available.
4. Linking of patient details to pathology records and possibly the storage of blood and tissue samples for future study and reference.

This is important when considering the need currently to further evaluate the patients who fall into the diagnostic categories of cryptogenic chronic hepatitis.

4:2 TREATMENTS AVAILABLE AND THE USE OF LIVER BIOPSY

The limited therapies available in the past impacted negatively on the ability to sustain long follow-up periods in most patients other than the AIH cases. The availability of liver transplantation in 1988 and interferon in the 1990s improved the treatment modalities available to patients at Groote Schuur Hospital and this has improved the outcome in patients with chronic liver failure. Transplantation in patients with HBV related liver failure is unfortunately not economically viable in the South African setting despite this being the biggest cause of chronic liver disease in sub-Saharan Africa. The major reason is the cost of HBs-immunoglobulin, which is necessary for the survival of the graft (see ***section 3:3.1***).

The use of liver biopsy is increasing and from the analysis of the biopsies done in this patient cohort, all modes of liver biopsy are in increasing use. This suggests that every attempt is being made to obtain liver tissue in the assessment of these patients at the liver clinic. Of interest to the author was the fact that the use of transjugular liver biopsies has continued to increase (***Table 17, Figure 17 on pages 69, 70***). This is so because the early use of this procedure was by the surgical hepato-biliary team at Groote Schuur Hospital in their studies of bleeding oesophageal varices in portal hypertension. The continued use of the transjugular liver biopsy method presently is a reflection of other transjugular liver biopsy procedures being done which are not

related to any study protocol. This is most likely due to the continued late presentation of patients at Groote Schuur Hospital with established cirrhosis and therefore difficult to biopsy livers.

4:3 PATIENT GROUPS AND AIH SUBTYPES

The patients sub-groups documented in this study have shown the presence of a group of patients who therapeutically behave like AIH but do not have the markers of autoimmunity on testing i.e. the cryptogenic steroid sensitive chronic hepatitis group. This group warrants further study to characterise their immunological status and clinical response to immunosuppression treatment long term as compared to classical AIH. While the liver clinic at Groote Schuur Hospital has been good in separating the steroid sensitive patients and treating them in the same way as the AIH patients, these patients require further characterisation in every respect. It is possible that the Coloured population of the Western Cape in particular has an immune phenotype that makes them susceptible to autoimmune hepatitis. From ***Table 12, page 55***, the female Coloured population comprises **47.6%** of the study population women (59 of 124 women) and **26%** of the whole group (59 of 226 patients). While the rest of the racial groups had equal numbers of both sexes affected in the Coloured population the male: female ratio was **1: 1.7**. All these clues tend to suggest that this population may have a unique genetic pool resulting in the high incidence of a group of patients who have chronic liver disease indistinguishable from AIH but have negative autoimmune markers on standard testing. This will be the subject of a study arising from this work. The accompanying group of cryptogenic chronic hepatitis also needs to have their virological and immune status further looked at to rule out the possibility of missed chronic hepatitis C infection or drug aetiologies.

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APPENDIX A DATA CAPTURE FORMS

Name:
DOB:
Ethnic Group: White
Mixed
Black
Asian
Diagnosis: !._____
2._____

Hospital No_____
Sex: _____
Phone: _____
Address:_____
_____
_____

Clinical Data:

	Yes/No	Duration		Yes/No	Duration
Nausea and vomiting			Hepatomegaly		
Abdominal pain			Splenomegaly		
Jaundice			Acne		
Ascites			Striae		
Pruritis			Haematemesis		
Amenorrhoea			Spider naevi		
Loss of weight			Arthralgias		

Drug History:

	Dose	Duration
Methyldopa		
NSAID's		
Phenytoin		
TB drugs		
Homeopathic		
Other		

APPENDIX A DATA CAPTURE FORMS

Histology:

Liver biopsies done: 1._____
2._____
3._____

Histological diagnosis:_____

Scoring of Histological Inflammatory Activity and Staging/Degree of fibrosis:

* **Inflammatory Activity Grade**

0	10
1	11
2	12
3	13
4	14
5	15
6	16
7	17
8	18
9	

* **Stage / Degree of Fibrosis**

1
2
3
4

APPENDIX A DATA CAPTURE FORMS

Treatment

	Steroids	Azathioprine	Interferon	Transplantation
Date started				
stopped				
Dose				
Duration of treatment				
Date of biopsy				

	Diagnosis	Remission	Relapses	Transplantation	Death and cause
Date					

Treatment Complications:

1. Infections
2. Osteoporosis
3. Peptic Ulcer disease
4. Diabetes Mellitus
5. Other

NAME Race

Sex Folder Number

Biopsy Number

Age

Alcohol Use

VIRAL MARKERS

HCV antibody HBsAg HBcAg
HVC pcr HBeAb

AUTOIMMUNE MARKERS

ANF

Anti-SM antibody

Anti-LKM-1 antibody

Anti-mitochondrial antibody

HISTOLOGY REPORT

Iron

TREATMENT

Interferon

Steroids

Transplantation

Survival

POSTMORTEM

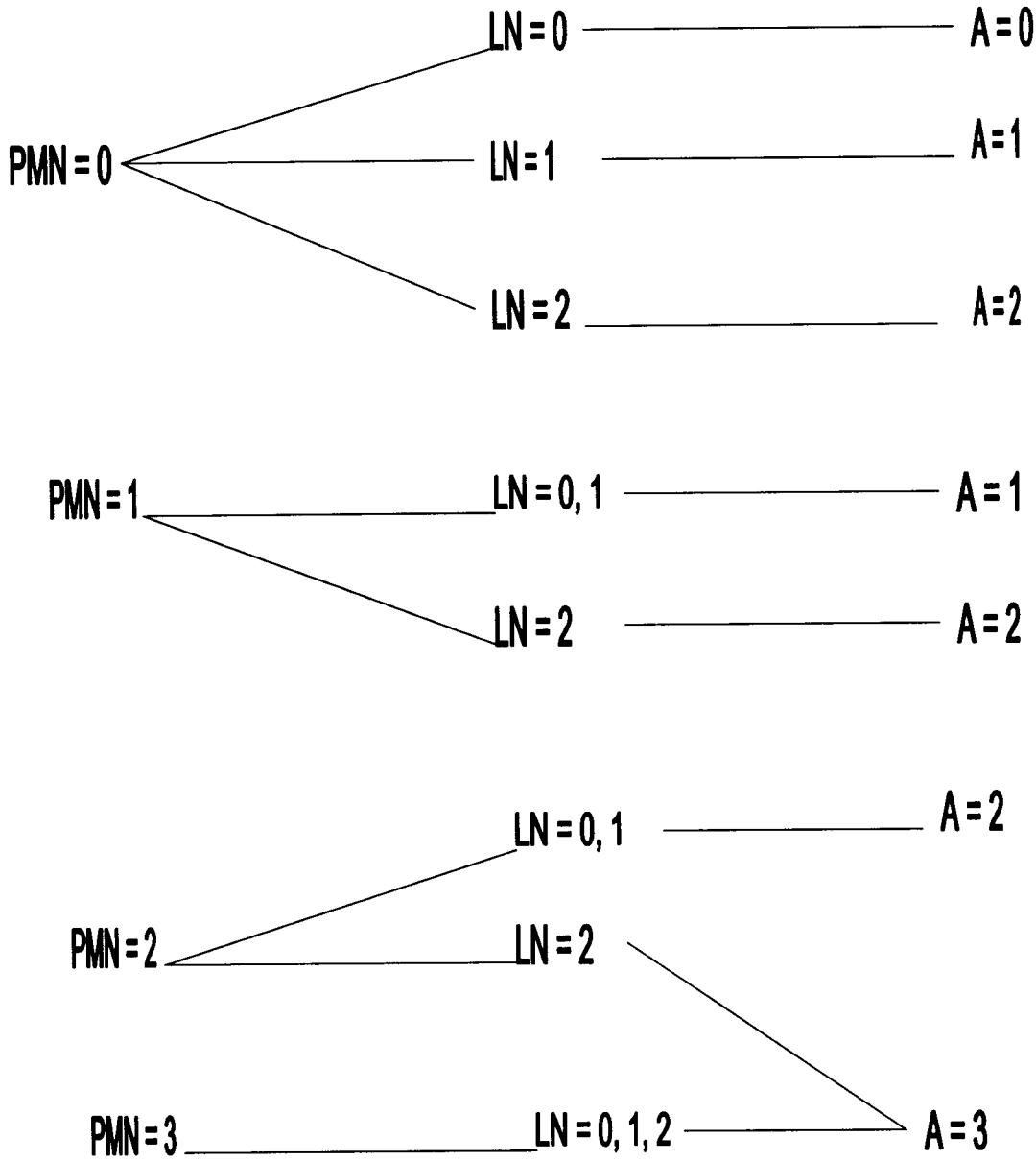
Grading of inflammation (Ishak et al 1995)

Grading Component	SCORE
Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around<50% of portal tracts or septa)	3
Severe (continuous around >50% of tracts or septa)	4
Sub-total	
Confluent necrosis	
Absent	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 (P-C) bridging	4
Zone 3 necrosis + multiple P-C bridging	5
Panacinar or multiacinar necrosis	6
Sub-total	
Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
Absent	0
One focus or less per 10x objective	1
Two to four foci per 10x objective	2
Five to ten foci per 10x objective	3
More than ten foci per 10x objective	4
Sub-total	
Portal inflammation	
None	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Sub-total	
TOTAL SCORE	

Staging of fibrosis

Change	**Score**
No fibrosis	0
Fibrous expansion of some portal tracts +/- short fibrous septa	1
Fibrous expansion of most portal tracts, +/- short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging P-P and portal to central (P-C)	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6
TOTAL SCORE	

BEDOSSA ALGORITHM FOR THE EVALUATION OF HISTOLOGICAL ACTIVITY (Bedossa et al. 1996)



PMN, piecemeal necrosis; 0, none; 1, mild; 2, moderate; 3, severe; LN, lobular necrosis; 0, no or mild; 1, moderate; 2, severe; A, histological activity; 0, none; 1, mild; 2, moderate; 3, severe.

APPENDIX C HISTOPATHOLOGY ILLUSTRATIONS

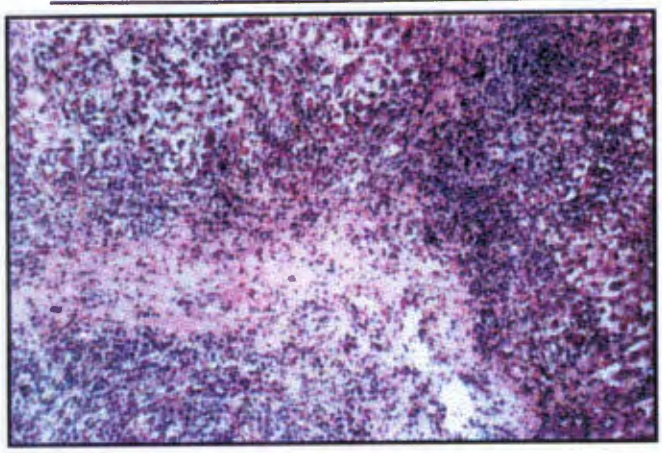


Figure 1

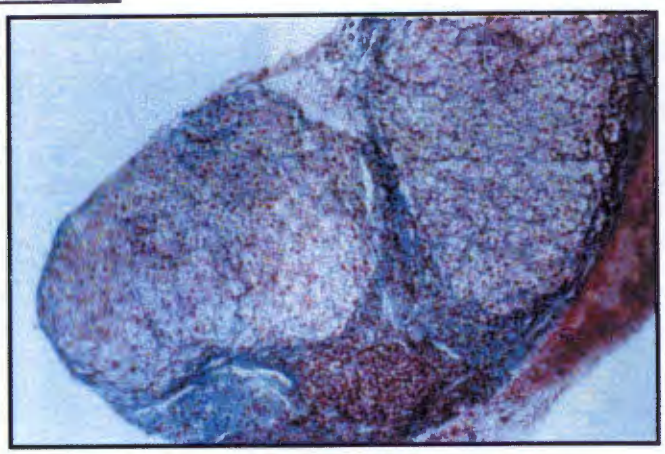


Figure 2

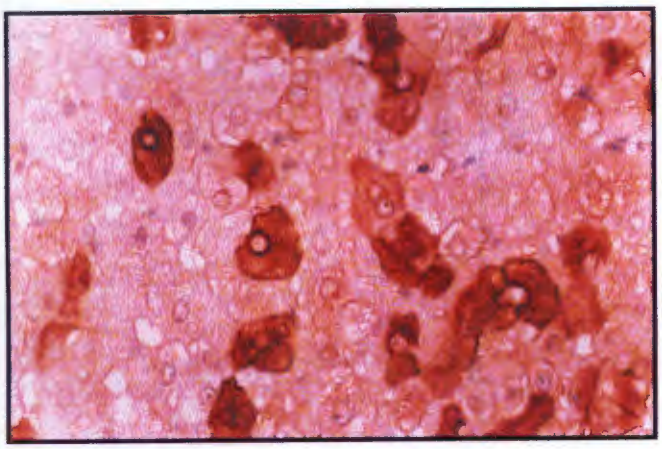


Figure 3

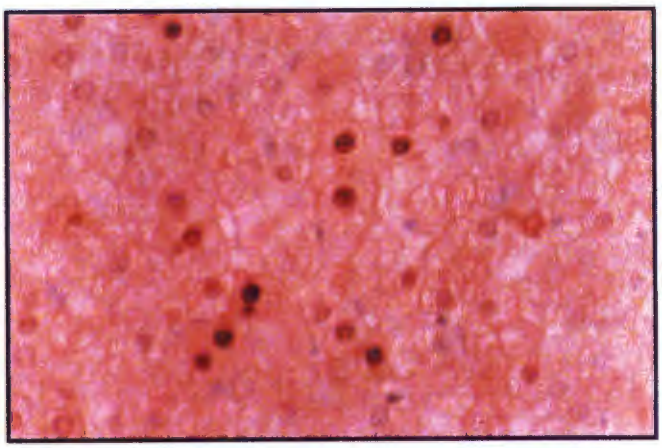


Figure 4

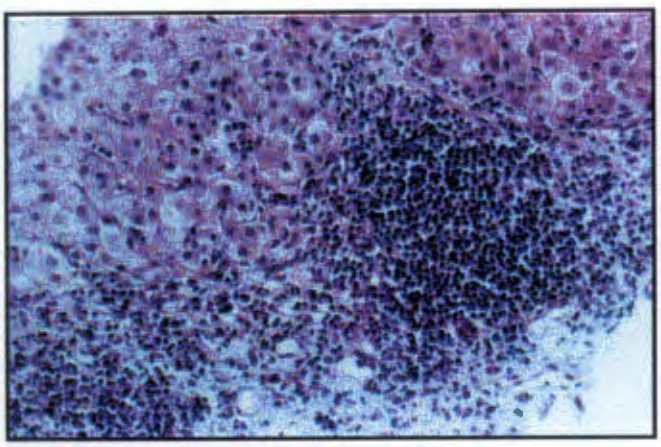


Figure 5

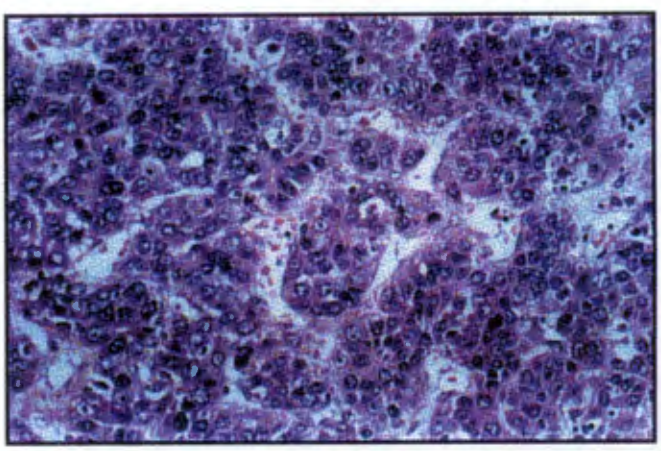


Figure 6