Cystic Fibrosis related chronic liver disease

A study of its influence upon prognosis and possible mechanisms for this; with specific reference to pulmonary and systemic haemodynamics

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Abstract

A study of cystic fibrosis related chronic liver disease

To assess the influence of chronic liver disease on survival a time dependent multivariate Cox regression analysis was performed. The results confirmed that patients with evidence of chronic liver disease have a worse prognosis. The effect of liver disease upon survival was not related to hepatic decompensation, as only 2.2% of deaths were liver-related, suggesting an occult adverse effect.

To establish a non-invasive means for the diagnosis of chronic liver disease an ultrasound scoring system was developed, and validated for reproducibility by two independent radiologists. This scoring system correlated well with the capacity of the liver to metabolise lignocaine to monoethylglycine xylidide, with fasting serum bile acids and with the PGA index. While the measurement of the N-terminal propeptide of procollagen III revealed significant differences between subjects with and without liver disease, the results correlated less well with the ultrasound score. Abnormalities documented by quantitative hepatobiliary scintigraphy also correlated well with the ultrasound based scoring system.

Having established suitable criteria for diagnosing chronic liver disease in cystic fibrosis the systemic and pulmonary
circulations of patients with and without liver disease were evaluated non-invasively. The results confirm the presence of a hyperdynamic circulatory state in patients with chronic liver disease with significant elevations of cardiac output and left ventricular stroke work index and reductions of mean arterial pressure and systemic vascular resistance index. There were no differences in the peripheral circulatory status of the two cohorts.

Evaluation of the pulmonary circulation was made using shunt-perfusion scans and the 100% oxygen re-breathing technique. Significant pulmonary arterio-venous shunting, consistent with a diagnosis of the 'hepato-pulmonary' syndrome, was only detected in the cohort with liver disease (36% of those studied).

The results of studies presented within this thesis suggest that chronic liver disease can be confidently detected using ultrasound criteria, is important in the prognosis of patients with cystic fibrosis due to its covert adverse effect on survival, and may exert some of these effects through systemic and pulmonary circulatory changes.
Declaration

This thesis was completed while the author was a research fellow in the Department of Gastroenterology at Charing Cross Hospital, London.

All the studies presented were undertaken by the author, with appropriate assistance where necessary, with the exception of the routine laboratory tests, ultrasound scanning and some of the procollagen III propeptide assays.
Acknowledgements

My thanks to Dr David Westaby who first introduced me to the field of cystic fibrosis related chronic liver disease and who has been a constant source of advice and encouragement throughout.

The studies presented here would not have been possible without the co-operation of the cystic fibrosis patients who attend The Royal Brompton Hospital, London. They have proved to be a highly motivated and helpful group of subjects who remain charming and cheerful despite all the ramifications of their disease.

Access to these patients was possible due to the support of the physicians at The Royal Brompton Hospital. In particular my thanks to Drs Margaret Hodson, Duncan Geddes and Andrew Bush.

The work would not have been possible without help from a number of people in other departments at Charing Cross Hospital. Professor Abe Guz was instrumental in allowing me a 'free hand' in the Department of Medicine where many of the physiological studies were completed with the very able assistance of Ms Jo Samways, graduate scientific officer in the department. My thanks to her and also to Dr Alastair Innes who introduced me to supra-sternal cardiac output measurement and who has been a constant source of advice.

My thanks to Dr Jane Evanson in the Department of Radiology who helped develop the ultrasound scoring system and to Dr Joe Boultbee for free access to the ultrasound department, to Drs Reg
Jewkes and Kuldip Nijran from the Department of Nuclear Medicine for blinded analysis of the hepato-biliary and shunt-perfusion scans and to Mrs Julia Jones of the department of Biochemistry for her help with the procollagen III peptide assays.

No study would be complete without its statistical input. My thanks to Ms Karen Hayllar who performed the statistical analyses necessary to develop the prognostic model, to Dr Ken McRae who provided helpful comments and advice when more complex statistical approaches were required, and to Mr Tariq Rasheed of the Medical School computer department who helped me make the computer do what was needed.

Finally my thanks to my wife Siobhan, for being so supportive during the completion of these studies and more particularly while they were being written up, to Benjamin for ensuring that entertaining distractions were available at most times and to Oliver whose impending arrival provided the impetus to help me focus my thoughts on completing this work.
To Siobhan, Benjamin and Oliver
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Abbreviations

ALP    Alkaline phosphatase
ALT    Alanine aminotransferase
ANOVA  Analysis of variance
ANCOVA Analysis of co-variance
a-p    antero-posterior
APA    Apolipoprotein A1
AST    Aspartate aminotransferase
BSP    Brom-sulph-phthalein
C      Controls
cAMP   cyclic adenosine monophosphate
CaO2   Oxygen content of arterial blood
c-c    cranio-caudal
C02    End pulmonary capillary blood oxygen content
CFTR   Cystic fibrosis transmembrane regulator
cGMP   cyclic guanosine monophosphate
CI     Cardiac index
CO     Cardiac output
CO2    Carbon dioxide
CvO2   Oxygen content of mixed venous blood
CXR    Chest radiograph
D      Doppler
E      Electromagnetic catheter
E45    Isotope retention at 45 minutes
E60    Isotope retention at 60 minutes
ECG    Electrocardiogram
EHIDA  N,a-(2,6-diethylacetanilide)-iminodiacetic acid
exp    Exponential
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>FBF</td>
<td>Forearm blood flow</td>
</tr>
<tr>
<td>$F_E CO_2$</td>
<td>Mixed expired carbon dioxide concentration</td>
</tr>
<tr>
<td>$FEF_{25-75%}$</td>
<td>Forced expiratory flow between 25% and 75% volume</td>
</tr>
<tr>
<td>$F_E O_2$</td>
<td>Mixed expired oxygen concentration</td>
</tr>
<tr>
<td>$F_{ET}CO_2$</td>
<td>End tidal carbon dioxide concentration</td>
</tr>
<tr>
<td>$\gamma GT$</td>
<td>Gamma glutamyl transferase</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HbO$_2$</td>
<td>Oxyhaemoglobin</td>
</tr>
<tr>
<td>HEF</td>
<td>Hepatic extraction fraction</td>
</tr>
<tr>
<td>HLA</td>
<td>Human lymphocyte antigen</td>
</tr>
<tr>
<td>$^{125}I$</td>
<td>Radio-isotope of iodine</td>
</tr>
<tr>
<td>IDA</td>
<td>Imino diacetic acid</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>ILD</td>
<td>Intermediate liver disease</td>
</tr>
<tr>
<td>INR</td>
<td>International normalised ratio</td>
</tr>
<tr>
<td>KCO</td>
<td>Corrected carbon monoxide transfer factor</td>
</tr>
<tr>
<td>keV</td>
<td>Kilo electron volts</td>
</tr>
<tr>
<td>L</td>
<td>Number of counts (scintigraphy)</td>
</tr>
<tr>
<td>LD</td>
<td>Liver disease</td>
</tr>
<tr>
<td>1-1</td>
<td>Latero-lateral</td>
</tr>
<tr>
<td>LVSWI</td>
<td>Left ventricular stroke work index</td>
</tr>
<tr>
<td>MAA</td>
<td>Macro-aggregated albumin</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MBq</td>
<td>Mega Bequerel</td>
</tr>
<tr>
<td>MEG-X</td>
<td>Monoethylglycinexylidide</td>
</tr>
<tr>
<td>MHz</td>
<td>Mega Hertz</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MIGET</td>
<td>Multiple inert gas elimination technique</td>
</tr>
<tr>
<td>Mr</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>N2</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NLD</td>
<td>No liver disease</td>
</tr>
<tr>
<td>O2</td>
<td>Oxygen</td>
</tr>
<tr>
<td>p</td>
<td>Probability value</td>
</tr>
<tr>
<td>PaCO2</td>
<td>Arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PaO2</td>
<td>Arterial partial pressure of oxygen</td>
</tr>
<tr>
<td>PAS</td>
<td>Period acid Schiff</td>
</tr>
<tr>
<td>PAWP</td>
<td>Pulmonary artery wedge pressure</td>
</tr>
<tr>
<td>PBAR</td>
<td>Barometric pressure</td>
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<tr>
<td>PCO2</td>
<td>Partial pressure of carbon dioxide</td>
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<tr>
<td>PcO2</td>
<td>Partial pressure of oxygen in pulmonary end capillary blood</td>
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<td>PETCO2</td>
<td>End tidal partial pressure of carbon dioxide</td>
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<tr>
<td>PGA</td>
<td>Prothrombin, γ glutamyl transferase, apolipoprotein A1</td>
</tr>
<tr>
<td>PI</td>
<td>Predictive index</td>
</tr>
<tr>
<td>P-450 IIIA</td>
<td>Cytochrome P-450 IIIA</td>
</tr>
<tr>
<td>PIINP</td>
<td>Amino terminal propeptide of procollagen III</td>
</tr>
<tr>
<td>%PFEV₁</td>
<td>Percentage predicted forced expiratory volume in one second</td>
</tr>
<tr>
<td>%PFVC</td>
<td>Percentage predicted forced vital capacity</td>
</tr>
<tr>
<td>%PPEFR</td>
<td>Percentage predicted peak expiratory flow rate</td>
</tr>
<tr>
<td>PV</td>
<td>Peak velocity</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Q</td>
<td>Perfusion</td>
</tr>
<tr>
<td>Qs</td>
<td>Volume of shunted blood</td>
</tr>
<tr>
<td>Qt</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>r</td>
<td>Pearson rank correlation co-efficient</td>
</tr>
<tr>
<td>Rs</td>
<td>Spearman rank correlation co-efficient</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>SaO₂</td>
<td>Arterial oxygen saturation</td>
</tr>
<tr>
<td>SCLD</td>
<td>Signs of chronic liver disease</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SLD</td>
<td>Severe liver disease</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>T</td>
<td>Thermodilution</td>
</tr>
<tr>
<td>⁹⁹mTc</td>
<td>Radio-isotope of technetium</td>
</tr>
<tr>
<td>TE or t₁ᵩ₂</td>
<td>Excretion half-time</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TLCO</td>
<td>Transfer factor for carbon monoxide</td>
</tr>
<tr>
<td>Tmax</td>
<td>Time to maximal hepatic uptake</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour necrosis factor α</td>
</tr>
<tr>
<td>TU or t₁ᵢₚ</td>
<td>Uptake half-time</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper limit of normal (for assay)</td>
</tr>
<tr>
<td>V</td>
<td>Ventilation</td>
</tr>
<tr>
<td>VCO₂</td>
<td>Carbon dioxide production</td>
</tr>
<tr>
<td>Vₑ</td>
<td>Minute ventilation</td>
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<tr>
<td>Vₜ</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen uptake</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood count</td>
</tr>
</tbody>
</table>
The diagnosis of cystic fibrosis in patients included in this study

Cystic fibrosis was diagnosed on the basis of symptoms of malabsorption and pulmonary infection and confirmed with an elevated sweat sodium concentration (>70 mmol/l) (MacLusky & Levison, 1990).

Ethical approval for studies within this thesis

Ethical permission for the study was obtained from the Riverside Medical Ethics Committee and informed consent was obtained from each subject prior to the study. Where paediatric subjects were studied permission was obtained from both patient and guardian.
CHAPTER 1

INTRODUCTION

Cystic fibrosis and chronic liver disease
1.0. **Cystic fibrosis, an introduction**

Cystic fibrosis is the commonest autosomal recessive condition affecting white populations (Porteous & Dorin, 1990). It affects about 1:2,500 live births (Boat et al., 1989) and approximately 1:25 of the population are carriers of the mutation which is found on Chromosome 7 (Riordan et al., 1989). Heterozygotes have none of the clinical manifestations of the disease which affects exocrine gland secretions with changes in the sodium, chloride and calcium composition and results in changes in the mucus which is then capable of blocking secretory ducts. In homozygotes this results in bronchiectasis, pancreatic exocrine insufficiency and an elevation of sodium and chloride concentrations in the sweat. This last abnormality has been used as the most reliable marker of the disease (MacLusky & Levison, 1990), although the recent discovery of the affected gene and multitude of mutations associated with it have allowed genotyping to be introduced as diagnostic test where the results of the sweat test are equivocal (Porteous & Dorin, 1990).

Cystic fibrosis is usually diagnosed soon after birth with the development of the characteristic meconium ileus (intestinal obstruction), or later with recurrent chest infections or failure to thrive and steatorrhoea. Chest infections lead to bronchiectasis which accounts for the most disabling symptoms of the disease and for the majority of deaths which are due to respiratory failure or cor pulmonale (MacLusky & Levison, 1990). Recent advances in the management of the pulmonary complications of cystic fibrosis have resulted in increasing
longevity (British Paediatric Association Working Party on Cystic Fibrosis, 1988; MacLusky & Levison, 1990) and in increasing numbers of patients with other complications of cystic fibrosis such as hepatic disease and diabetes mellitus.

1.1. **Cystic fibrosis related chronic liver disease**

1.2. **Historical Review**

Liver cirrhosis has been a recognised manifestation of cystic fibrosis since its earliest descriptions. In the original report of cystic fibrosis Andersen described three case of biliary cirrhosis, several cases of mild portal fibrosis and many patients with fatty livers (Andersen, 1938).

The earliest descriptions of liver histopathology in cystic fibrosis are from Farber who described small bile ducts blocked by eosinophilic material resembling that found in the pancreatic ducts and acini of cystic fibrosis patients (Farber, 1944) and from Bodian who noted the patchy distribution of hepatic changes in cystic fibrosis and coined the term "focal biliary cirrhosis" (Bodian, 1952). Progression of this focal lesion to multi-focal disease and established cirrhosis has been termed "multilobular biliary cirrhosis" (di Sant'Agnese & Blanc, 1956). The evolution of the focal lesion to multilobular biliary cirrhosis was demonstrated by Oppenheimer and Esterly (Oppenheimer & Esterly, 1975a).
1.3. **Pathogenesis of liver disease**

1.3.1. Histology

Postmortem studies reveal the presence of the pathognomonic histological lesion of focal biliary cirrhosis in up to 72% of patients over 24 years old (Vawter & Shwachman, 1979).

Focal biliary fibrosis is characterised by oedema, chronic inflammatory cell infiltration, bile duct proliferation, and increased fibrosis in some portal tracts with the accumulation of amorphous PAS positive diastase-resistant material (Oppenheimer & Esterly, 1975a). These changes are consistent with the lesion of partial biliary obstruction.

In time focal lesions coalesce in some patients, progressing to a multilobular biliary cirrhosis characterised by irregular nodules larger than those seen in other forms of biliary and portal cirrhosis (Figure 1.1.) and with regenerative microscopic nodules, massive foci of necrosis, and bile duct proliferation adjacent to areas of preserved hepatic lobular architecture (di Sant'Agnese & Blanc, 1956; Oppenheimer & Esterly, 1975a). Bile plugged ducts may still be present, but bile stasis within lobules is uncommon even in advanced liver disease (Craig et al., 1957; Oppenheimer & Esterly, 1975a) (Figure 1.2.).

Obstructive plugging of the biliary ductules has been well documented in cystic fibrosis. Such changes are found at post mortem in 11% of cystic fibrosis patients at 3 months, 27% at one year, and 72% of those aged over 24 years (Vawter & Shwachman, 1979). Whether obstructive plugging of the bile
ducts is alone sufficient to explain the development of biliary cirrhosis in cystic fibrosis remains a matter of controversy. A recent light and electron microscopic study detected little evidence of cholestasis in liver biopsies obtained from patients with cystic fibrosis, with the most prominent histological feature being evidence of bile duct destruction and associated collagen deposition. The authors hypothesise that the damage may be mediated by a bile-related toxin (Lindblad et al., 1992).

Support for a bile-related toxin comes from studies of bile acids in cystic fibrosis. A change in the bile acid pool consequent upon malabsorption of bile acids (Colombo et al., 1984; O'Brien et al., 1993) causing excessive faecal loss of primary and secondary bile acids and taurine conjugates, results in an increase in the preponderance of hydrophobic glycine conjugates in the bile acid pool in cystic fibrosis (Setchell et al., 1985; Thompson, 1988). These hydrophobic bile acids are potentially hepatotoxic (Attili et al., 1986).

Subsequent exposure of the hepatocytes to lipophilic bile acids (chenodeoxycholic acid, deoxycholic acid and lithocholoic acid) exacerbated by bile acid reflux in the presence of a partially obstructed biliary system may result in hepatocyte injury and constitute part of the mechanism for the development of cystic fibrosis related liver disease.
Figure 1.1. Photograph showing the macroscopic appearances of the macronodular cirrhosis of cystic fibrosis
Figure 1.2. Typical microscopic appearances of the cirrhotic liver in cystic fibrosis

E - Eosinophilic material plugging the biliary ductules
B - Bile pigment
F - Fibrosis
1.3.2. The Cystic Fibrosis Transmembrane Regulator

Cystic fibrosis is characterised by a number of mutations in the cystic fibrosis transmembrane regulator gene (CFTR), which encodes a protein of 1480 amino acid residues that function as a chloride channel regulated by cyclic adenosine monophosphate (cAMP) (Anderson et al., 1991). Determining the relevance of the CFTR to the pathogenesis of the liver disease of cystic fibrosis has proved harder than in some of the other tissues affected in cystic fibrosis.

Primary bile is manufactured within the canaliculus, as a result of the active transport of solutes by the hepatocytes followed by the passive flow of water. Bile is modified by secretion and absorption in the intrahepatic bile ducts with some substances undergoing biliary-hepatic re-circulation. The larger ducts have two diametrically opposed rows of glands which produce both serous and mucous secretions (Psacharopoulos & Mowat, 1983) with sodium, potassium, chloride and bicarbonate accounting for most of bile osmolality.

Recent studies have suggested the presence of the CFTR at or near the apical plasma membrane of the biliary epithelium (Mulberg et al., 1993; Cohn et al., 1993). While most studies of the function of the CFTR have concentrated on its role as a cAMP dependent chloride channel (Welsh, 1990), it has also been shown to act as a cAMP bicarbonate (Smith & Welsh, 1992) and cAMP water (Hasegawa et al., 1992) transporter.
The role of the CFTR as a chloride transporter in bile duct cells is supported by evidence in the rat that secretin can increase cAMP levels (Lenzen et al., 1992), which can stimulate chloride channel opening (Fitz et al., 1993) and chloride/bicarbonate exchange (Strazzabosco et al., 1991). While extrapolation of these animal studies to the human bile duct cell should be made with caution, it may be hypothesised that secretin stimulated increases in intracellular cAMP open the CFTR-associated chloride channels and result in the efflux of chloride with concomitant movement of sodium and water and dilution of bile. In addition, the increased intraluminal chloride concentration may favour alkalinisation of bile through activation of chloride/bicarbonate exchange if this also occurs at the apical membrane (Strazzabosco et al., 1991). If this hypothesis applies to the human bile duct cell in cystic fibrosis then absence of the CFTR would lead to viscid bile which might cause obstruction of the intrahepatic ducts and may constitute part of the mechanism in the pathogenesis of the liver lesion of cystic fibrosis (Oppenheimer & Esterly, 1975a).

Alternatively there is evidence to support active bicarbonate secretion as an important process in the formation and flow of bile. If the defective CFTR fails to act as a bicarbonate transporter (Smith & Welsh, 1992) it provides an alternative mechanism for the production of viscid, low flow bile.

Support for this mechanism is derived from studies involving the administration of ursodeoxycholic acid. Unconjugated ursodeoxycholic acid has been shown to induce a bicarbonate
rich choleresis (Erlinger & Dumont, 1990) which may be the result of direct stimulation of hepatocyte bicarbonate transport (Erlinger & Dumont, 1990; Kitani, 1990). The improvement in both biliary excretion and the standard biochemical tests of liver function with ursodeoxycholic acid administration suggest that this mechanism may be important in the pathogenesis of cystic fibrosis liver disease (Colombo et al., 1992a).

1.3.3. Common Bile Duct Stenosis

A further mechanism proposed for the development of chronic liver disease in cystic fibrosis is stenosis of the intra-pancreatic portion of the common bile duct in association with the pancreatic disease of cystic fibrosis. Initial reports that this lesion was present in over 90% of cystic fibrosis patients with chronic liver disease (Gaskin et al., 1988) have not been confirmed by other investigators (Nagel et al., 1989; O'Brien et al., 1992b). Nevertheless isolated reports of secondary biliary cirrhosis caused by this lesion (Vitullo et al., 1978; Lambert et al., 1981; Bass et al., 1983), with improvement following surgical intervention (Gaskin et al., 1988), suggest that it is an aetiological factor in a minority of patients and that its presence should be excluded in all patients with cystic fibrosis related chronic liver disease.
1.4. Genetic aspects of cystic fibrosis related liver disease

The relationship of hepatic involvement in cystic fibrosis to current knowledge about specific cystic fibrosis gene mutations on chromosome 7 is unclear.

Studies evaluating the severity of liver disease and the prevalence of patients homozygous and heterozygous for the commonest gene deletion, ΔF508, do not agree about whether or not homozygotes for this deletion are at greater risk of developing liver disease (Johansen et al., 1991; Ferrari et al., 1991; De Arce et al., 1992). One study looking at three of the commoner gene mutations - ΔF508, G551D and R553X - found no correlation between the frequencies of each mutation and the presence or absence of liver disease (Duthie et al., 1992) as did another which looked at the frequencies of six defined CFTR gene mutations (Colombo et al., 1994a). A more recent report with a more detailed evaluation of currently identified mutations found no increase in the prevalence of any genotype in patients with liver disease. However, the prevalence of liver disease was low in the cohort of 798 patients studied (The Cystic Fibrosis Genotype-Phenotype consortium, 1993).

The report by Duthie et al, in agreement with others (Schuster et al., 1977; Scott-Jupp et al., 1991; De Arce et al., 1992), also noted some familial concordance in patients with liver disease suggesting the influence of genes outside the cystic fibrosis locus or an effect of environmental factors in the pathogenesis of the
disease. Further evidence for the influence of genes outside the cystic fibrosis locus is provided by a reported increase in the prevalence of liver disease in adolescent males (Scott-Jupp et al., 1991; Feigelson et al., 1993; Colombo et al., 1994a).

Liver damage may also be associated with an HLA-associated genetic factor controlling the lymphocyte-mediated immune response to liver antigens. There is an increased frequency of the HLA antigens A2, B7, DR2(DRw15) and DQw6 in those patients with liver disease which is probably due to an increase in the frequency of the HLA A2-B7-DR2-DQw6 haplotype (Duthie et al., 1990). The relative risk for liver disease increases from HLA A2 to DQw6, with 65.2% of patients with liver disease having DQw6 compared to 32.5% without, suggesting that the disease associated allele may lie near the DQB locus.

Lymphocytes play a key role in stimulating, via cytokines, the production of collagen and other macromolecules of the extracellular matrix during fibrogenesis (Friedman, 1990; Gressner & Bachem, 1990). Detection of a sub-population of lymphocytes, cytotoxic to hepatocytes and directed towards liver-specific lipoprotein gives further evidence for a role for immune mechanisms in the pathogenesis of liver disease (Mieli-Vergani et al., 1980).
1.5. **Epidemiology of cystic fibrosis related chronic liver disease**

Historical postmortem data shows that the incidence of liver disease rises steadily with age from 10.8% in infants less than three months old, to 15.3% in those 3 months to one year and 26.8% in those older than one (Salh et al., 1988).

A recent retrospective analysis of the case notes of 1100 children in central England revealed clinically overt liver disease in 4.2% of patients. The incidence rose from 0.3% in the 0-5 age group, to a peak of 8.7% in the 16-20 year old age group and in those over 20 years old it fell to 4.1%. There was a marked preponderance of affected males (5.4% of males compared with 2.6% of females), being most pronounced in the 11 to 15 year age group where three times as many boys as girls were affected (Scott-Jupp et al., 1991).

The correlation between liver function tests, which were abnormal in 12.9% of those in whom they had been measured, and clinical evidence of liver disease was poor. Normal serum liver enzyme activities were seen in 6 of 46 patients (13%) with hepatosplenomegaly, while 50 of 524 (10%) had abnormal hepatic enzyme activities in the absence of clinical evidence of liver disease (Scott-Jupp et al., 1991). Subsequent clinical reappraisal of a selected cohort of patients in the study revealed considerable under diagnosis of liver disease, but confirmed the male adolescent peak (Tanner, 1992).
The authors suggest that the reduced prevalence of liver disease in patients aged over 20 may be explained by selectively increased mortality in the liver disease group, but suggest that this is unlikely as liver related deaths due to such complications as hepatic failure and variceal haemorrhage were rare (7.8%). Their alternative explanation that this is a cohort effect, which will result in the peak age of liver disease steadily increasing, remains to be proven by a longitudinal study (Scott-Jupp et al., 1991).

1.6. **Clinical Presentation of chronic liver disease in cystic fibrosis**

Focal and multi-lobular biliary cirrhosis are the characteristic lesions of cystic fibrosis (di Sant'Agnese & Blanc, 1956) and tend to present late in the natural history of the disease. Other manifestations may rarely present in infancy.

Neonatal presentations of liver disease include deep cholestasis due to inspissated bile in the common bile duct which may have to be cleared by laparotomy and biliary lavage (Furuya et al., 1991) and presentation with haemorrhagic disease of the newborn due to vitamin K malabsorption (Torstenson et al., 1970). In malnourished infants fatty infiltration of the liver, massive hepatomegaly and hypoglycaemia, with carnitine deficiency as a possible exacerbating factor, has been reported (Treem & Stanley, 1989). Neonatal giant cell hepatitis has also been reported (Resti et al., 1990).
More commonly the features of biliary cirrhosis develop at any time from infancy to adult life. Clinically evident liver disease has been reported in only 20-30% of cystic fibrosis patients (Feigelson et al., 1975; Schuster et al., 1977; Nagel et al., 1989) with overt hepatic decompensation in only 2-5% (Psacharopoulos & Mowat, 1983). However, the prevalence of liver involvement is likely to increase as the care of the respiratory disease continues to improve and survival is extended (British Paediatric Association Working Party on Cystic Fibrosis, 1988; MacLusky & Levison, 1990). It is, therefore, likely to become a more significant management problem in cystic fibrosis.

A striking feature of cystic fibrosis related chronic liver disease is the paucity of clinical signs (Roy et al., 1982). Liver involvement is usually detected on the basis of abnormalities of the standard biochemical tests of liver function or hepatosplenomegaly in the patient under observation. Hepatomegaly has been shown to correlate well with histology and in particular fibrotic changes (Gaskin et al., 1988). Characteristically both lobes of the liver may be palpable when liver enlargement is first detected, but as the disease progresses the right lobe involutes while the left remains prominent (Vawter & Shwachman, 1979; Romano, 1981).

Evidence of decompensation in the form of jaundice, ascites or encephalopathy is uncommon, either as a presenting feature or as part of the natural history of the disease (Park & Grand, 1981). Similarly, variceal haemorrhage is a rare complication of cystic fibrosis but may occur in up to 30% of patients with
established focal or diffuse cirrhosis (a figure similar to cirrhotides of other aetiologies) (Navasa et al., 1987). However, variceal haemorrhage may be the presenting feature of underlying liver disease and may occur in the absence of any other signs of decompensation (Psachoropoulos et al., 1981; Penketh et al., 1987; Sinaasappel, 1989). Such early variceal bleeding may reflect the pre-sinusoidal component of portal vascular resistance which is well recognised in biliary cirrhosis and can occur in the pre-cirrhotic phase of the disease (Navasa et al., 1987).

The overall picture is, therefore, of a benign disease process which is similar in many respects to the other biliary cirrhotides, (primary biliary cirrhosis (Dickson et al., 1989) and primary sclerosing cholangitis (Farrant et al., 1991)), with a long history of evolution. However, the premature mortality caused by the pulmonary complications of cystic fibrosis may be a major factor reducing the incidence of decompensated cystic fibrosis liver disease (Scott-Jupp et al., 1991).

1.7. **Diagnosing liver disease in cystic fibrosis**

1.7.1. Standard laboratory biochemical tests

Standard biochemical tests of liver function have proved disappointing as predictors of underlying liver disease, and may even be normal in the presence of overt cirrhosis (di Sant'Agnese & Blanc, 1956; Stern et al., 1976). In addition, many series evaluating indirect measures to detect liver disease are
limited by lack of definitive parameters with which to compare them.

Previous reports have suggested that liver function tests become abnormal late in the disease (Feigelson et al., 1975; Park & Grand, 1981), but this contention has been challenged by a recent report which indicates that biochemical changes precede abnormalities on imaging in the majority of cases (Feigelson et al., 1993).

In a recent epidemiological survey 13% of those with clinical liver disease had normal biochemical tests of liver function while 13% with no clinical evidence of liver disease had abnormal blood tests (Scott-Jupp et al., 1991). In another series 30% of cystic fibrosis patients had transient moderate increases in circulating enzymes over a 38 year follow up period but did not go on to develop multilobular biliary cirrhosis (Feigelson et al., 1993). Histology for this sub-group of patients, when available, suggested focal biliary cirrhosis (Feigelson et al., 1993).

The pattern of blood tests is also variable with some patients with cirrhosis having entirely normal amino-transferase levels (Schwarz et al., 1978) and modest elevations frequently observed in the absence of liver disease (Kattwinkel et al., 1973; Feigelson et al., 1975). The reasons for this are unclear, but various explanations including chronic infection, hypoxaemia and drug ingestion have been proposed (Isenberg, 1982). Elevations of the liver iso-enzyme of alkaline phosphatase and gamma glutamyl transpeptidase (γGT) also have little specificity
(Feigelson et al., 1975; Scott-Jupp et al., 1991), although elevations of these enzymes to values four times above the normal level is almost always associated with some degree of biliary related disease.

The measurement of alkaline phosphatase is particularly problematic in cystic fibrosis where many patients have delayed puberty and the hepatic iso-enzyme may be elevated in the presence of a normal total serum alkaline phosphatase (Schwarz et al., 1978).

More refined analysis of alkaline phosphatase, by measurement of its high-molecular-mass iso-enzyme, has been proposed as a more sensitive test of hepato-biliary disease (Schoenau et al., 1989). However, while serum concentrations of this iso-enzyme correlated with other abnormalities of standard serologic liver function tests the authors provide no data on the patients clinical status with regard to hepatic disease, making informed evaluation of their data difficult.

The measurement of other parameters which are used to assess hepatic synthetic function are also unreliable in the early detection of the disease. The prothrombin time is usually normal in patients with liver disease, but when abnormal may be prolonged due to a due to a number of factors including reduced oral intake of vitamin K, reduced absorption of vitamin K and hepatic disease (Feigelson et al., 1970; Feigelson et al., 1975).

More detailed analysis of the clotting cascade has been undertaken with conflicting results. The measurement of Factor
VII levels in ninety one cystic fibrosis patients revealed low Factor VII levels, that failed to correct with vitamin K administration, in all six subjects with cirrhosis and twelve of twenty four subjects with other evidence of liver injury (Dominick & Sutor, 1981). In contrast a study of Factor II coagulant activity and immunoreactive prothrombin protein ratios in twenty four cystic fibrosis patients often found a low ratio that responded to vitamin K administration (Corrigan et al., 1981).

The serum albumin and bilirubin concentrations are further markers of hepatic dysfunction, but these tend to remain normal until late in the disease (Schwarz et al., 1978). While hypoalbuminaemia may be a marker of advanced liver disease (Feigelson et al., 1975; Green et al., 1960), it may also reflect the general nutritional status of the cystic fibrosis patient.

Given the disappointing results obtained with individual blood tests an alternative approach, using the immunoglobulin A (IgA) to transferrin ratio and the γGT concentration, has been proposed (Feigelson et al., 1975). When the ratio was high (>1.9) cirrhosis was indicated, but the γGT could be normal. Alternatively a low ratio (<1.0) was associated with a raised γGT suggesting cholestatic liver involvement. However, the ratio rose markedly in one patient shortly before death due to respiratory disease, because of an increase in IgA, suggesting that the applicability of the ratio is limited and while other investigators have suggested that serum IgA is related to liver involvement in cystic fibrosis.
there is no data to support this contention (Dietzsch et al., 1980a).

1.7.2. Bile acids

Bile acids have been evaluated as markers of liver disease in cystic fibrosis following an initial report that fasting serum bile acids were elevated in three of six patients, two of whom had evidence of cirrhosis (Goodchild et al., 1975).

The fasting serum conjugated cholic acid has been proposed as a marker of early liver involvement in cystic fibrosis with elevated levels in patients with hepatomegaly (Davidson et al., 1980). However, subsequent work on fasting serum cholic and chenodeoxycholic acid concentrations suggested a lack of correlation with liver histology obtained percutaneously (Strandvik & Samuelson, 1985).

The divergent results obtained in these and other studies of serum bile acids in cystic fibrosis highlight the complexity of bile salt metabolism, where associated pancreatic insufficiency and intestinal malabsorption may also influence the serum levels of bile acids in each individual patient (Colombo et al., 1984; O'Brien et al., 1993).

Detailed evaluation of urinary bile acids has also been undertaken following the observation that the urinary excretion of trihydroxy bile acids in cystic fibrotic patients was significantly higher and the monohydroxy bile acids lower than
in the control group (Arborgh et al., 1980). Correlating their findings with histology the investigators noted that those patients with liver disease had elevated urinary chenodeoxycholic acid, while liver disease patients, cystic fibrosis controls and normal controls excreted similar amounts of cholic acid. In addition, the cirrhotics had elevated chenodeoxycholic acid to 3β-hydroxy-5 chenoic acid ratios. Patients with normal biopsies or portal changes alone had ratios that were similar to the control subjects. The authors concluded that detection of elevated urinary chenodeoxycholic acid could be an early marker for cystic fibrosis liver disease (Arborgh et al., 1980). Difficulties in interpreting these data arise from the fact that the whole study group had normal galactose elimination, 24/25 had normal brom-sulph-phthalein (BSP) retention, there were no patients with clinical evidence of established liver disease and the histology specimens were obtained percutaneously (see below).

1.7.3. Quantitative liver function tests

Quantitative liver function tests have not been extensively evaluated in the detection of cystic fibrosis liver disease. BSP retention is reduced in advanced disease (di Sant'Agnese & Blanc, 1956; Feigelson et al., 1972; Feigelson et al., 1975). In addition, one report of BSP plasma clearance, using computerised BSP excretion curves, suggested that this technique can be used to detect early hepatic disease (Grand et al., 1978). It was,
however, evaluated on a relatively small cohort of patients (20), and has not been substantiated.

More recently the galactose elimination capacity, $^{14}$C-aminopyrine breath test and BSP clearance tests have been used in the sequential monitoring of the response of cystic fibrosis patients with liver disease to treatment with ursodeoxycholic acid, but have not been evaluated as a diagnostic tool for cystic fibrosis liver disease (Cotting et al., 1990).

1.7.4. Imaging techniques

1.7.4.1 Ultrasound

Real-time ultrasound scanning in cystic fibrosis allows the detection of hepatic parenchymal abnormalities and particularly the development of multi-lobular biliary cirrhosis (McHugo et al., 1987).

It also allows detailed evaluation of the splanchnic vasculature and in particular the portal vein, splenic size and whether or not portal-systemic collaterals are present suggesting the presence of portal hypertension (Vergesslich et al., 1989). It has been suggested that the portal vein diameter may be a useful marker for the early detection of portal hypertension in cystic fibrosis (Kumari-Subaiya et al., 1987). Patency of the portal and splenic veins, which may become thrombosed in the presence of cystic fibrosis related chronic pancreatitis, can also be determined.
Ultrasound is also valuable for assessing the biliary tree and, in the context of liver disease, for excluding dilatation of the common bile duct secondary to a biliary stricture in the intra-pancreatic portion of the duct (Lambert et al., 1981; Gaskin et al., 1988).

1.7.4.2 Computed tomography

Computed tomography has been shown to successfully detect both hepatic parenchymal abnormalities and evidence of elevated portal pressure, but in reality is felt to add little to the clinical data (Cunningham et al., 1980). It may be useful in evaluating the occasional patient in whom clinical and diagnostic doubt persists.

1.7.4.3 Endoscopic Retrograde Cholangiography

Cholangiography, most often using the endoscopic route, has also provided important diagnostic information (Nagel et al., 1989; O'Brien et al., 1992b), and in patients with common bile duct calculi and stenosis of the distal portion of the common bile duct can be employed for therapeutic intervention. Calibre irregularities of the intra-hepatic ducts, similar to those observed in patients with primary sclerosing cholangitis, appear to be specific for established chronic liver disease (Nagel et al., 1989; O'Brien et al., 1992b) (Figure.1.3.)
Figure 1.3. Endoscopic retrograde cholangiogram in a patient with cirrhosis and cystic fibrosis showing the typical changes of the intrahepatic bile tree.
1.7.4.4. Colloid liver scans

Radionuclide colloid (gold or technetium labelled) liver scans have been proposed as a useful adjunct in the diagnosis and monitoring of patients with cystic fibrosis liver disease (Feigelson et al., 1972). Scans reveal patchy uptake in the liver, an enlarged spleen and in the presence of marked liver damage increased isotope uptake by the vertebral column. In practice, the anatomical data obtained adds little to the clinical information available (Smith et al., 1977).

1.7.4.5. Hepatobiliary scintigraphy

Radionuclide imaging using iminodiacetic acid (IDA) derivatives labelled with 99m Technetium have been used to assess both the biliary tree and hepatobiliary function (Chervu et al., 1988) as IDA is taken up by the hepatocytes and then rapidly cleared in the bile.

A number of recent studies have utilised these derivatives as a means of assessing biliary function and have confirmed impaired drainage in patients with chronic liver disease (Gaskin et al., 1988; Nagel et al., 1989). Delay of excretion was documented in both intra and extra-hepatic sites, in keeping with delayed bile flow, rather than a single site of obstruction.

Semi-quantitative application of these techniques has shown delayed time to maximal uptake ($T_{\text{max}}$) and percentage excretion at 45 and 60 minutes in subjects with hepatic disease,
but while statistically significant differences in these parameters were documented, between those with and without liver disease, their applicability in facilitating the diagnosis of liver disease was not assessed (O'Brien et al., 1992b).

Further use of semi-quantitative IDA imaging has allowed objective documentation of the response of individual patients to treatment with ursodeoxycholic acid. In one study the half-time of hepatic washout and time to appearance in the intestine improved with ursodeoxycholic acid therapy (Colombo et al., 1992a), while in another the time to \( T_{\text{max}} \), and excretion at 45 and 60 minutes were not improved following treatment (O'Brien et al., 1992a). More detailed application of these techniques may allow more accurate monitoring of deterioration in liver disease or response to therapy.

IDA scanning has also been used to image the extrahepatic biliary tree. The results are contradictory with evidence of common bile duct stenosis reported in both patients with (Gaskin et al., 1988; O'Brien et al., 1992b) and without (O'Brien et al., 1992b) liver disease. Subsequent retrograde cholangiography in this latter study failed to demonstrate common bile duct strictures or dilatation in any of the patients with a dilated bile duct on IDA scanning. This discrepancy probably reflects biliary stasis or adherence of the tracer to abnormal mucous within the biliary tree and highlights the inaccuracy of this technique in diagnosing common bile duct stenosis.
1.7.5. Liver biopsy

Liver histology forms a cornerstone for the assessment of patients with most forms of liver disease. However, a sampling error as high as 66% has been reported in the diagnosis of cirrhosis by needle biopsy (Soloway et al., 1971) and the use of liver biopsies in the diagnosis of liver involvement in cystic fibrosis is controversial.

While some investigators have advocated the use of serial percutaneous liver biopsies for diagnosing and monitoring the progression of liver disease (Dietzsch et al., 1980a; Dietzsch et al., 1980b), it is accepted that the focal nature of the early lesion in cystic fibrosis associated chronic liver disease makes percutaneous liver biopsy an unreliable diagnostic tool (Schwarz et al., 1978; Psachoropoulos et al., 1981; Gaskin et al., 1988). In addition the risk of a pneumothorax in a patient with impaired pulmonary function is not, generally, deemed acceptable (Wilson-Sharp et al., 1984). It is possible that the use of ultrasound guided percutaneous biopsies would improve the diagnostic yield while minimising risks, but this remains to be prospectively evaluated.

In contrast the histology obtained from open or wedge liver biopsies taken at laparotomy is reliable for the diagnosis of liver disease (Tyson et al., 1968; Gaskin et al., 1988). One study has compared the histology obtained by open biopsy with that obtained percutaneously and confirms gross sampling error using the percutaneous approach (Gaskin et al., 1988). Consequently open liver biopsy has been advocated as the
method of choice for obtaining tissue (Schwarz et al., 1978), but the risks of the operative approach and of a general anaesthetic in patients with pulmonary disease appear to make this approach unacceptable.

1.8. Treatment

The treatment for cystic fibrosis related chronic liver disease falls into two areas. Firstly therapy directed at the complications of liver disease and secondly treatment for the liver disease itself.

1.8.1. Complications of liver disease

1.8.1.1. Specific Nutrients

In the presence of established cirrhosis the protein calorie requirements are frequently increased (Levine et al., 1994), so that attention to nutrition is important. Deficiencies of fat-soluble vitamins may occur, particularly in the cholestatic patient, so supplementation may be required. Caution should, however, be exercised with vitamin A replacement as complications related to hyper-vitaminosis have been documented even in the absence of overt over-dosage (Eid et al., 1990).
1.8.1.2. Portal hypertension

Variceal haemorrhage represents the most frequent serious complication of chronic liver disease and should be managed in the same way as it is in other forms of liver disease (Westaby, 1992).

Ascites is uncommon, and is usually accompanied by other evidence of decompensated liver disease such as jaundice and impaired synthetic function. This too should be treated in the conventional way (Moore et al., 1992).

Gross splenomegaly is an infrequent complication of portal hypertension (Psachoropoulos et al., 1981). Hypersplenism is a frequent incidental observation, but the low white cell and platelet counts are usually of no clinical significance and are not an indication for splenectomy. Left upper quadrant pain is the most troublesome symptom, but can usually be managed with simple analgesics. Splenectomy is reserved for those patients with intractable symptoms.

1.8.2 Treatment of cystic fibrosis related chronic liver disease

1.8.2.1. Ursodeoxycholic acid

There has been increasing interest in oral bile acid therapy for chronic liver diseases. Despite their essential function as detergents and key modulators of hepatic excretory function, bile acids are cytotoxic molecules and may cause cell death when
at high concentrations. Bile acid cytotoxicity is inversely related to the degree of hydrophilicity, which is in turn determined by their molecular structure (Attili et al., 1986). Hydrophilicity increases with the number of hydroxyl groups in the bile acid steroid nucleus and also whether or not it is conjugated, particularly with taurine. Ursodeoxycholic acid is very hydrophilic and non toxic due to the presence of an hydroxy group in the 7β position. In contrast, hydrophobicity and cytotoxicity is highest in the monohydroxy, dihydroxy, glycine-conjugated and free bile acids (Attili et al., 1986).

Ursodeoxycholic acid changes the relative concentrations of toxic and non-toxic bile acids in the bile acid pool. The mechanism for this is unclear but it may compete with endogenous bile acids for active ileal re-absorption resulting in reduced circulating concentrations of hepatotoxic bile acids (Marteau et al., 1990), or it may expand the overall bile acid pool size and diminish the relative concentrations of toxic bile acids (Beuers et al., 1992).

As well as protecting the hepatocyte from the toxicity of hydrophobic bile acids, ursodeoxycholic acid induces a bicarbonate rich choleresis (Renner et al., 1980), which should be of particular value in a cholestatic liver disease. However, this effect is mediated by unconjugated ursodeoxycholic acid which does not increase in concentration in duodenal bile when ursodeoxycholic acid is given to patients with cystic fibrosis (Roseau, 1990), suggesting that this effect may not occur in vivo. However, chole-hepatic recycling may result in the unconjugated
bile acid failing to appear in the final bile, so this mechanism cannot be discounted (Erlinger & Dumont, 1990).

A further mechanism for ursodeoxycholic acid efficacy may be via an immunomodulatory effect (Yoshikawa et al., 1992).

Beneficial effects of ursodeoxycholic acid administration have been reported in children (Colombo et al., 1990) and young adults (Cotting et al., 1990) with cystic fibrosis associated liver disease. The drug appears to be well tolerated and to have beneficial effects on standard and quantitative (BSP retention at 45 minutes and the aminopyrine breath test) liver function tests (Cotting et al., 1990). Dose response studies have shown that due to problems with ursodeoxycholic acid absorption encountered in cystic fibrosis 20 mg/Kg ursodeoxycholic acid must be administered to achieve similar biliary concentrations of ursodeoxycholic acid as those achieved with smaller doses in other chronic liver diseases (Colombo et al., 1992b).

Subsequent studies have confirmed the documented improvement in standard liver function tests reported by these early studies (Colombo et al., 1994b) but, while they are encouraging, long-term studies with data on clinically relevant events are still required before ursodeoxycholic acid can be accepted as an established form of treatment. Early data suggests that ursodeoxycholic acid therapy will not arrest disease progression in those patients who already have advanced chronic liver disease (Colombo et al., 1993), suggesting that intervention at the focal stage or even earlier in the disease
process will be necessary if ursodeoxycholic acid is to confer any benefit.

1.8.2.2. Somatic gene transfer

Following the identification of the cystic fibrosis gene attempts have been made to deliver a recombinant gene to the lung epithelium using adenovirus and liposome vectors.

Until recently attempts at delivering the recombinant gene to the liver have been focused on the hepatocyte (Wilson et al., 1992; Kay et al., 1992). However, the demonstration of the presence of the CFTR close to or in the apical membrane of the biliary epithelium (Cohn et al., 1993) has recently led to attempts at delivering liver-directed gene therapy to the biliary epithelium using recombinant adenoviruses (Yang et al., 1993). The adenoviruses were infused retrogradely into the biliary tract via the common bile duct using endoscopic retrograde cholangiography, and resulted in widespread expression of the CFTR gene throughout the biliary tract. This expression persisted for 21 days suggesting that this is a promising approach to the treatment of cystic fibrosis related chronic liver disease if the absence of the CFTR is the major pathogenetic defect related to the development of liver disease.
1.8.2.3. Transplantation

Orthotopic liver transplantation is being increasingly used to treat monogenetic liver-based metabolic disorders as the outcome following transplantation improves (Cohen et al., 1991). 1 year survival rates of over 85% are reported, with up to 78% surviving 5 years (Busuttil et al., 1991) and an annual attrition rate of 2-3%. The longest surviving recipient is reported to be well 20 years after transplantation (Salt et al., 1992).

Liver transplantation has been reported in about 35 cystic fibrosis patients, with 50% surviving with a very good quality of life (Starzl et al., 1989). However, progression of the lung disease may be anticipated after liver transplantation giving rise to further difficult management decisions.

For many patients who might be considered for liver transplantation the severity of lung disease precludes this approach. Combined heart-lung-liver transplantation has been reported, with limited success (Cox et al., 1987; Mieles et al., 1989; Madden et al., 1992; Feigelson et al., 1993). Success rates are unlikely to improve while small numbers of patients are being transplanted, and rates of triple organ transplantation are unlikely to increase while the shortage of donor organs exists.

Heart-lung or double-lung transplantation is now widely used as treatment for end-stage pulmonary disease in cystic fibrosis, with 50% 3 year survival reported (Madden et al., 1992).

There has been no systematic study assessing the influence of established liver disease upon survival. While most transplant
programmes have excluded patients with overt complications of liver disease, the occult nature of cystic fibrosis liver disease has resulted in patients with established focal or multi-lobular cirrhosis undergoing heart-lung transplantation. There is little evidence that the procedure causes hepatic decompensation in the short term, although this has been a complication in patients undergoing portal-systemic shunting (Tyson et al., 1968), and an uncomplicated post-operative course has been documented in some patients with a previous history of variceal bleeding (Williams et al., 1992). It, therefore, seems reasonable to offer heart-lung transplantation to all those patients with liver disease unless they have persistent jaundice, ascites or encephalopathy.
1.9. **Aim of Studies Presented here**

Liver disease in cystic fibrosis remains one of the less well studied manifestations of the disease. This has resulted from the apparent benign nature of the disease process and unavailability of an effective treatment for liver disease coupled with the fact that most of the significant morbidity and mortality in cystic fibrosis has been due to the pulmonary manifestations and their complications.

Following the report of Scott-Jupp *et al.* suggesting a possible adverse effect of liver disease on prognosis in cystic fibrosis (*Scott-Jupp et al.*, 1991) the initial aim of this thesis was to evaluate the prognostic significance, if any, of chronic liver disease in the cohort of cystic fibrosis patients attending The Brompton Hospital, London, UK.

The finding of a significant influence of liver disease on survival coupled with the anticipated increase in the prevalence of liver disease as longevity improves suggest that liver disease in cystic fibrosis may become central to the long-term management of people with cystic fibrosis. This prompts a number of questions some of which have been addressed in the further studies presented in this thesis:

(i) The need for an accurate, preferably non-invasive, test to accurately establish the diagnosis of liver disease is of paramount importance to allow the correct management of individuals with cystic fibrosis. This thesis evaluates a number of biochemical and imaging approaches to this problem.
(ii) Given that liver disease appears to indirectly influence survival in cystic fibrosis studies have been performed to attempt to elucidate a mechanism/mechanisms through which this effect might be mediated.
CHAPTER 2

PREDICTING SURVIVAL IN CYSTIC FIBROSIS

Development of a prognostic model and the influence of liver disease.
2.0. Introduction

Survival of patients with cystic fibrosis has improved considerably in recent years, predominantly as a result of improved respiratory care (British Paediatric Association Working Party on Cystic Fibrosis, 1988; MacLusky & Levison, 1990). Death however, continues to be due, in the majority, to respiratory failure associated with cor pulmonale and pulmonary hypertension (MacLusky & Levison, 1990). Transplantation (double-lung, heart-lung or occasionally heart-lung-liver) remains the cornerstone of treatment in the terminal phase of the disease (Madden et al., 1992) and results, which include enhanced survival and improvement in lung function, have been encouraging in both adults (Scott et al., 1988) and children (Whitehead et al., 1991b).

To optimise the timing and results of transplantation, particularly in the presence of a donor shortage, there is a need for an accurate prediction of patient survival. Current recommendations for transplantation include those patients whose quality of life is severely impaired and whose life expectancy is less than two years (Whitehead et al., 1991a). Current criteria suggested for recommending transplantation include percentage predicted forced expiratory volume in one second (%PFEV1) < 30% and significant carbon dioxide (CO2) retention (Kerem et al., 1992).

There have, however, been no assessments of the influence of liver disease upon survival despite the results of a large
retrospective study suggesting a possible covert influence of liver disease upon survival (Scott-Jupp et al., 1991).

The aim of the present study was, therefore, to document predictors of short and medium term survival in cystic fibrosis and to further evaluate the importance of liver disease in determining prognosis in cystic fibrosis.

The analysis was performed on a prospectively collected database of patients seen at The Royal Brompton Hospital, London. Following initial screening for variables significantly associated with survival by log rank analysis and univariate Cox regression analysis, significant variables were entered into a time dependent multivariate Cox regression analysis to select the subset of variables which best predict survival (Christensen et al., 1986).

These variables were combined to create a prognostic model which was then internally validated using split-sample testing (Christensen et al., 1986).

Data from a second cohort of patients also seen at The Royal Brompton Hospital after completion of the original study cohort were then used to externally validate the model.
2.1. Methods

2.1.1. Study Population

The study cohort consisted of 403 patients with cystic fibrosis seen at The Royal Brompton Hospital, London between 1969 and 1987, and followed up until death or 1989.

All patients underwent an initial assessment upon referral to the hospital and subsequent annual assessments. Evaluation of routine haematology and serum biochemistry, pulmonary function, nutritional and hepatic status were performed during periods of stability.

These data were collected prospectively on a computerised data base, with pulmonary function and weight expressed as a percentage of the expected value (Society of Actuaries (Chicago), 1959; Bates et al., 1971).

The year of entry to the study, considered to be the date of first full assessment at the Brompton Hospital, was included in all analyses to allow for the possible effect of changes in referral patterns and for the development of improved treatment regimes. In addition, as the diagnosis of cystic fibrosis had been made sometime prior to the initial assessment for the majority of the study population, the time from diagnosis to entry was calculated and entered into the analysis.

Sixteen patients were lost to follow-up but were included in the analysis until the date they were last known to be alive. 3 of
these patients returned to their homes abroad, 11 were transferred to other hospitals and no information is available for the other 2.

2.1.2. Statistical analysis

Variables were initially screened using two statistical tests:

(i) Log rank analysis was used to screen twenty static and continuous variables for correlation with survival. Median values were used as cut points for the continuous variables.

(ii) Univariate Cox regression analysis was performed separately on twelve variables as they changed with time. Those with a skewed distribution were transformed into a Gaussian distribution using a log transformation.

All significant variables identified by these initial screening tests were then entered in a stepwise manner into both forward and backward stepping, time dependent, multivariate Cox regression analysis to select the subset of variables which best predict survival (Christensen et al., 1986).

At each step only the significant variables were retained such that variables which did not add further information about survival were excluded.

Multivariate time dependent Cox regression analysis requires that all patients be represented by a complete set of data at the time of entry to the study. Thus as variables with missing data
were analysed and were excluded from the model, so more patients could be included in the final analysis. 30 patients had one or more variable in the final model missing and so the final analysis was performed on 373 patients. The significant variables were combined into a predictive index and used to produce estimates of survival.

2.1.3. Validation of the model

2.1.3.1 Internal Validation (within the study population).

The model was validated using split-sample testing (Christensen et al., 1986). The database of 373 patients was divided into two parts. The model was regenerated using the first 3/4 of the database and applied to the remaining 1/4. A predictive score was generated, at all time points, for each of the remaining patients using the model and, in combination with the underlying hazard function, used to calculate the probability of a particular patient dying within one year:

\[
\text{Probability of death} = 1 - \exp^{-\text{hazard} \times \text{time} \times \exp(\text{PI})}
\]

where
- \(\exp\) = exponential
- haz = underlying hazard function
- time = period of prediction (years)
- PI = predictive score for a particular set of observations

The sum of these probabilities represents the expected number of deaths within one year. Any death which occurred less than a
year after the observation was counted as an observed death. Only one set of observations per year per patient was used in the analysis.

The process was repeated by regenerating the model using the last 3/4 of the data base and applying it to the first 1/4.

The observed and expected totals were compared using the $\chi^2$ test.

2.3.1.2. External validation (using a cohort recruited between October 1988 and September 1993).

The predictive index (PI) was generated for each patient and the probability of dying within 1 year calculated using the same formula as above (Christensen et al., 1986).

The patients were then divided into three groups by PI, and the probabilities of death calculated for each group to give the expected number of deaths for that group. A comparison with the actual number of deaths was made using the $\chi^2$ test.

A further comparison of observed and expected deaths, as predicted by the model, was made by subdividing the observed deaths into PI ranges such that equal numbers of patients died in each of the three sub-divisions. The expected number of deaths for each of these ranges was calculated and comparison made using $\chi^2$. 
2.2. **Results**

2.2.1. Statistical analyses.

The patient characteristics are shown in Table 2.1 (pancreatic replacement being those subjects taking pancreatic enzyme supplements; malabsorption defined as frequent, loose, pale or offensive stools that were difficult to flush away). 373 patients were included in the final analysis of which 188 (50.4%) died. The median age was 20 years (range 11 - 63 years) with 169 males and 204 females.

54 (14.5%) had hepatomegaly at entry to the study and a further 74 (19.8%) developed hepatomegaly during follow-up. 24 (6.4%) had splenomegaly at entry and 89.7% were on pancreatic enzyme supplements.

The factors significant by either log rank analysis or univariate, time dependent, Cox regression analysis are shown in Table 2.1. Eleven variables were significant by Cox regression analysis.

Age, gender, the year of entry into the study and time from diagnosis to entry into the study were not significant after the final analysis.

Time dependent multivariate Cox regression analysis revealed hepatomegaly (as defined by palpable liver, confirmed by span >12 cm in the mid-clavicular line), height (m), percentage predicted forced vital capacity (%PFVC), percentage predicted forced expiratory volume in 1 second (%PFEV₁), white blood count (WBC) \( \times 10^9/l \), serum albumin (g/l), and serum alkaline
phosphatase (ALP) (iu/l), as factors significantly associated with survival.

Serum albumin and ALP were excluded from the final model. The former because its coefficient within the predictive model was only 0.0001 and the latter because for individual patients there was concern that bone related ALP might introduce considerable inaccuracy.

The remaining significant variables were combined to produce the final model (table 2.2.):

\[
PI = (0.99 \times \text{hepatomegaly}) - (3.41 \times \text{height}) - (0.038 \times \%\text{PFVC}) - \\
(0.0590 \times \%\text{PFEV}_1) + (0.090 \times \text{WBC})
\]

(presence of hepatomegaly =1; absence = 0)

2.2.2. Application of the model

Multivariate Cox regression analysis produces a model of two parts. The first part is related to the current medical status of the cohort and the second is a multiplying hazard function which is constant for all patients (the underlying hazard function) (Christensen et al., 1986). The hazard for this cohort of patients was constant as it changed over time (r=0.996), deviating only as the numbers of patients become small at 7 years (Figure 2.1.). As the aim of the analysis was to evaluate short and medium term predictors of survival, this line represented the underlying hazard function of the model well.
A linear hazard function implies a risk of death that is independent of time, and allows graphs relating the predictive index and the probability of surviving set periods of time to be generated (Figure 2.2.).

Using the model it is possible to calculate a predictive index (PI) for any patient with cystic fibrosis at any stage of their disease and to use it to obtain estimates of their predicted survival using the survival curves (Figure 2.2).

2.2.3. An example of model application:

A patient presents with

- Height = 1.54
- Hepatomegaly = 1 (present)
- %PFVC = 50
- %PFEV₁ = 35
- WBC = 20

The predictive index for this patient is obtained by multiplying each variable by its coefficient
\[ PI = -3.410 \times 1.54 + 0.990 \times 1 - 0.038 \times 50 - 0.059 \times 35 + 0.090 \times 20 = -6.4 \]

Using figure 2.2, it can be seen that this patient has a 75% estimated chance of surviving 6 months and a 56% chance of surviving 12 months.

2.2.4. Results of the validation

2.2.4.1. Internal Validation

The validation was performed twice. Once using the top 3/4 of the data, and once using the bottom 3/4.

In the first case the observed number of deaths was 65 and the expected number 60 (Chi squared = 0.41; p > 0.4 (ns)). In the second there were 33 observed and 33.65 expected deaths (Chi squared = 0.012; p > 0.8 (ns)).
2.2.4.2. External Validation

There were one hundred patients, fifty of each sex aged between 13 and 45 years old, recruited between October 1988 and September 1993. All had at least one year's follow-up, or died soon after referral. Comparison of observed and expected deaths using sub-division according to PI and sub-division to ensure that all groups contained the same number of observed deaths confirms the ability of the prognostic model to accurately predict 1 year survival in this cohort of cystic fibrosis patients (Tables 2.3. and 2.4.).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (n)</th>
<th>10-90% Range</th>
<th>Log Rank (p)</th>
<th>Univariate Cox Regression (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.17 (373)</td>
<td>16.0-27.0</td>
<td>p=0.421</td>
<td>N/A</td>
</tr>
<tr>
<td>% Pred PEFR</td>
<td>61 (364)</td>
<td>25.5-98.2</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>% Pred FVC</td>
<td>62 (373)</td>
<td>31.4-99.1</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>% Pred FEV₁</td>
<td>43 (373)</td>
<td>19.3-86.0</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.63 (373)</td>
<td>1.51-1.78</td>
<td>p&lt;0.0003</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>% Pred weight</td>
<td>82.0 (351)</td>
<td>71.1-100</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Alk Phos (iu/l)</td>
<td>225 (365)</td>
<td>130-571</td>
<td>p=0.897</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>38 (354)</td>
<td>32-46</td>
<td>p=0.0007</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Bilirubin (µmol/l)</td>
<td>7 (359)</td>
<td>3-16</td>
<td>p=0.968</td>
<td>0.1&lt; p&lt;0.2</td>
</tr>
<tr>
<td>WBC (x10⁹/l)</td>
<td>10.8 (373)</td>
<td>6.9-17.0</td>
<td>p=0.189</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.5 (364)</td>
<td>11.8-15.4</td>
<td>p=0.0006</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>3.5 (373)</td>
<td>1.0-7.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sex</td>
<td>male=169, female=204</td>
<td>p=0.556</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>no splenomegaly=349, splenomegaly=24</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>no hepatomegaly=319, hepatomegaly=54</td>
<td>p=0.010</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Malabsorption</td>
<td>no malabsorption=148, malabsorption=218</td>
<td>p=0.095</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>no diabetes=326, diabetes=47</td>
<td>p=0.005</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>no ascites=365, ascites=8</td>
<td>p=0.469</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Varices</td>
<td>no varices=367, varices=4</td>
<td>p=0.027</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Pancreatic replacement</td>
<td>no replacement=40, replacement=331</td>
<td>p=0.022</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

* These variables were recorded only at entry to the study.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>Coef/SE</th>
<th>p-value</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>-3.41</td>
<td>0.828</td>
<td>-4.12</td>
<td>p&lt;0.0001</td>
<td>0.033</td>
</tr>
<tr>
<td>Hepmeg (0,1)</td>
<td>0.99</td>
<td>0.154</td>
<td>6.48</td>
<td>p&lt;0.0001</td>
<td>2.69</td>
</tr>
<tr>
<td>% Pred FVC</td>
<td>-0.038</td>
<td>0.009</td>
<td>-3.27</td>
<td>p&lt;0.0001</td>
<td>0.963</td>
</tr>
<tr>
<td>% Pred FEV₁</td>
<td>-0.059</td>
<td>0.013</td>
<td>-4.46</td>
<td>p&lt;0.0001</td>
<td>0.943</td>
</tr>
<tr>
<td>WBC (x10⁹/1)</td>
<td>0.090</td>
<td>0.014</td>
<td>6.40</td>
<td>p&lt;0.0001</td>
<td>1.095</td>
</tr>
</tbody>
</table>

Table 2.2. Details of variables significant by multivariate Cox regression analysis and included in the final model
Figure 2.1. Graph of the cumulative underlying hazard plotted against time
Figure 2.2. Graph relating predictive index to survival probability (for example see text)
### Table 2.3. External validation sub-dividing patients by PI

<table>
<thead>
<tr>
<th>PI</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; -9.0</td>
<td>0</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>-9.0</td>
<td>13</td>
<td>6.42</td>
<td>6.7</td>
</tr>
<tr>
<td>-6.5</td>
<td>26</td>
<td>21.7</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Total $\chi^2 = 7.79; p>0.05$

### Table 2.4. External validation sub-dividing patients to ensure equal numbers of observed deaths within each sub-group

<table>
<thead>
<tr>
<th>PI</th>
<th>Observed</th>
<th>Expected</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; -5.4</td>
<td>13</td>
<td>6.6</td>
<td>6.2</td>
</tr>
<tr>
<td>-5.4</td>
<td>13</td>
<td>9.6</td>
<td>1.2</td>
</tr>
<tr>
<td>5.4</td>
<td>13</td>
<td>12.1</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Total $\chi^2 = 7.47; p>0.05$

Table 2.3. External validation sub-dividing patients by PI

Table 2.4. External validation sub-dividing patients to ensure equal numbers of observed deaths within each sub-group
2.3. Discussion

2.3.1. General

This analysis has enabled the construction of a model to predict short and medium term survival in patients with cystic fibrosis.

The model was validated within the study population and a cohort recruited after October 1988. Its application to other CF populations remains to be assessed, but its support by the results of the external validation, suggests wide applicability. This presumption is supported by reported similarities in the median survival for cystic fibrosis patients in the United Kingdom (British Paediatric Association Working Party on Cystic Fibrosis, 1988), United States of America (Corey et al., 1988), Canada (Corey et al., 1988) and Denmark (Niellsen & Schiotz, 1982).

The results of the external validation confirm the accuracy of the model in predicting the prognosis of cystic fibrosis patients who are particularly unwell. Sub-division by PI results in less accurate prognostic prediction for the intermediate category while sub-division to ensure equal numbers of observed deaths in the three groups results in successively less accurate prediction of survival in the intermediate and well sub-groups.

The purpose of this prognostic model is, however, to identify patients in need of transplantation and the results of the
validations showing accurate prediction of survival in the least well patients suggest that it will, in general, fulfil this aim.

The characteristics of the cohort within this study were determined by hospital referral patterns and resulted in the analysis being of a predominantly adult population. This does not diminish its value, although its extrapolation to a paediatric population remains to be evaluated.

While there have been marked improvements in the management of patients with cystic fibrosis over the considerable time period of recruitment to this study, and also changes in the pattern of referral to the Brompton Hospital resulting more recently in a sicker patient population (predominantly those with complications of the disease that have proved difficult to manage or those in need of transplant assessment; the result of the development of specialist centres for the management of cystic fibrosis outside London), the failure of the year of entry to the study to reach statistical significance in the multivariate analysis suggests that improvements in patient management are accounted for by the variables included in the model. These changes do not, therefore, reduce the applicability of the prognostic model to patients diagnosed more recently. Furthermore, the prognosis of patients recruited more recently, whose prognosis is likely to reflect these changes in management and referral practice, appears to be well modelled by the predictive index as demonstrated by the results of the external validation.
Several previous reports have centred on the predictors of long-term survival and the factors associated with improved long-term outcome in cystic fibrosis (Corey, 1980; Wood, 1984; Phelan & Hey, 1984; Hudson & Phelan, 1987; Huang et al., 1987; British Paediatric Association Working Party on Cystic Fibrosis, 1988; Corey et al., 1988; Britton, 1989). Only one has attempted to address the issue of predictors of short term survival (Kerem et al., 1992).

The failure of both the age and sex of the patients to reach statistical significance within the model is at variance with some (British Paediatric Association Working Party on Cystic Fibrosis, 1988; Britton, 1989; Elborn et al., 1991; Kerem et al., 1992), but not all (Huang et al., 1987; Hudson & Phelan, 1987), previous reports. It has been suggested that the influence of both these factors on survival is related to the nutritional status of the patient (Corey et al., 1988). The inclusion of patient height, which may reflect nutritional status (see below), in this model may have resulted in the exclusion of both age and gender from the prognostic index. However, this inter-relationship between age, sex and nutrition has not been reported universally with the most recent report of predictors of prognosis suggesting an increased risk of death in young females with cystic fibrosis even when allowance is made for weight differences (Kerem et al., 1992).

While pulmonary function has not always correlated with survival (Huang et al., 1987), other reports have documented the
importance of deteriorating FEV1 and of CO2 retention (Wagener et al., 1980; Kerem et al., 1992).

Kerem et al. found that a %PFEV1 below 30% was the best predictor of survival, with other significant factors including a partial pressure of arterial oxygen below 55 mmHg, a partial pressure of arterial carbon dioxide above 50 mmHg. Within this model a reduced %PFEV1 and reduced %PFVC are positively correlated with shorter survival. Although a reduction of both variables reflects pulmonary outflow obstruction the inclusion of both by the statistical analysis suggests that each provides additional prognostic information.

Oxygen saturation and arterial blood gas analysis were not included in this analysis as these were not routinely performed before the mid 1970s.

Nutritional status, including short stature and reduced weight percentile, have also been documented as important predictors of prognosis (Huang et al., 1987; Corey et al., 1988), although there are no studies showing that nutritional intervention leads to an improved long-term survival (Levy et al., 1985). In the current analysis taller patients survived longer. The precise relationship of height to survival remains unclear, but failure of statural growth probably reflects a degree of malnutrition (Editorial, 1988) and may, therefore, be a longer term marker of disease severity.

The prognostic model also includes two factors that have not previously been reported as predictors of outcome:
The negative correlation of a high WBC with survival may reflect chronic on-going sepsis, presumably of pulmonary origin. The observation that WBC and pulmonary function are independent prediction variables suggests that mechanisms other than pulmonary disease alone may be involved. It may be speculated that inflammatory mediators secondary to sepsis, such as tumour necrosis factor α, may have a systemic adverse effect (Elborn et al., 1993).

Established chronic liver disease, as manifest by hepatomegaly, also proved to be an important negative correlate with survival. This previously largely unsuspected influence of chronic liver disease upon survival highlights two areas which may become increasingly important in the longer-term management of cystic fibrosis patients: firstly the accurate documentation of those cystic fibrosis patients with and without significant hepatic involvement (without the need to resort to a liver biopsy) and secondly the identification of a therapeutic agent effective for the treatment of cystic fibrosis related chronic liver disease.

Concerns that hepatomegaly might represent a poor predictor of chronic liver disease with inter-observer variation may have credence for individual patients (Tanner, 1992) but has proved of value both when compared with histological evidence of liver disease in cystic fibrosis (Gaskin et al., 1988) and within this statistical model (relative risk 2.69). Further support comes from two other cholestatic liver diseases: primary sclerosing cholangitis (Farrant et al., 1991) and primary biliary cirrhosis
(Roll et al., 1983) for which hepatomegaly has been shown to correlate significantly with reduced survival.

The importance of liver disease as a predictive factor is further supported by the finding that ALP was a significant variable.

2.3.2. Possible mechanisms for the influence of liver disease upon survival

In the present series very few deaths were directly attributable to overt complications of liver disease (2.2%). This observation is in keeping with a recent retrospective analysis of a cohort of 1100 cystic fibrosis patients in which only 7.8% of deaths in those patients with liver disease were directly related to the liver disease (Scott-Jupp et al., 1991) and suggests a covert influence of liver disease upon survival.

There are a number of potential mechanisms by which this effect could be mediated, including an adverse effect upon nutritional status (although liver disease and nutrition are independent variables within the prognostic model) (Sproul & Huang, 1964; Sokol & Stall, 1990); abnormalities of the immune system, both humoral (Feizi, 1968) and cell mediated (Webb et al., 1980), and changes in the pulmonary and systemic haemodynamics which occur in cirrhosis of other aetiologies and have resulted in hypoxaemia, a relative tissue oxygen debt and a detrimental effect on major organ systems (Bosch et al., 1983; Rodriguez-Roisin et al., 1988).
The documentation of the factors determining the adverse effect of liver disease upon survival would offer a potential for therapeutic intervention.

2.4. **Conclusions**

The importance of the accurate prediction of survival to facilitate assessment for lung transplantation is well recognised (Whitehead et al., 1991a; Kerem et al., 1992). This model represents a simple method, which should be applicable to other cystic fibrosis populations, for timing assessment for transplantation, and should be a valuable addition to the available criteria for judging the short and medium term management of cystic fibrosis patients.

The identification of chronic liver disease as an important factor reducing longevity in cystic fibrosis highlights a number of issues that have already been discussed. The simple, non-invasive diagnosis of chronic liver disease (given the inaccuracy of percutaneous liver biopsy) and some of the possible mechanisms for its covert effect upon survival are central to further work conducted as part of this thesis.

In addition, the identification of an effective treatment for cystic fibrosis related chronic liver disease would appear to be essential for the prospects of further improving longevity in cystic fibrosis.
CHAPTER 3

AN ULTRASOUND SCORING SYSTEM FOR THE DIAGNOSIS OF LIVER DISEASE IN CYSTIC FIBROSIS

and a detailed evaluation of the biliary tree.
3.0. Introduction

The prevalence of liver disease in cystic fibrosis has been estimated at up to 72% in post mortem studies (Vawter & Shwachman, 1979) and although clinical estimates of established chronic liver disease are lower at up to 25% of cases (Nagel et al., 1989) it is likely that improvements in the management of the pulmonary complications of CF and consequent increasing longevity (British Paediatric Association Working Party on Cystic Fibrosis, 1988; MacLusky & Levison, 1990) will result in more patients developing significant hepatic involvement.

The anticipated increase in prevalence coupled with the results of the multivariate Cox regression analysis presented in chapter 2 of this thesis suggest that the presence of liver disease may be important in the future management of patients with cystic fibrosis and highlights the need for a simple and accurate test for confirming the presence of established chronic liver disease.

Clinical examination and conventional biochemical tests of liver function are neither sensitive nor specific enough, in the individual patient, to confirm the diagnosis of established cystic fibrosis related chronic liver disease (Tanner, 1992).

In hepatological practice percutaneous liver biopsy is the standard for evaluating liver pathology (Van Ness & Diehl, 1989) as well as for guiding and monitoring treatment (Sherlock & Dooley, 1993b). However, liver biopsy has an associated morbidity (3%) and mortality (0.03%) (Garcia-Tsao & Boyer, 1993). This is further increased in cystic fibrosis because of the
risks of pneumothorax and the likely increase in mortality should any complication occur.

Percutaneous liver biopsy is subject to sampling error which may be as high as 66% (Soloway et al., 1971). In cystic fibrosis related chronic liver disease liver biopsy is particularly open to sampling error due to the patchy distribution of the early hepatic lesion (Bodian, 1952) and the macronodular pattern of the established cirrhosis (di Sant'Agnese & Blanc, 1956). Considerable discrepancy has been documented between liver biopsies taken percutaneously and wedge biopsies obtained laparoscopically in the same cystic fibrosis patients (Gaskin et al., 1988) with those obtained percutaneously being unrepresentative of the severity of the underlying liver lesion. This, coupled with the potential complications of the procedure, has made liver biopsy, in cystic fibrosis, unacceptable to many experts (Wilson-Sharp et al., 1984).

These major limitations have resulted in the evaluation of imaging techniques to detect evidence of liver disease. Reports of hepatic ultrasound (Wilson-Sharp et al., 1984; Graham et al., 1985; McHugo et al., 1987) and the measurement of the portal vein diameter (Kumari-Subaiya et al., 1987) in cystic fibrosis have supported the value of this non-invasive investigation.

In cystic fibrosis it might be reasonable to assume that the vast majority of chronic liver disease will be of biliary origin, and as such ultrasound does not need to be capable of providing a histological diagnosis, but rather to assess (and grade) the
degree of architectural disruption. Such assessment appears well within the capacity of modern ultrasound technology.

One small study (9 patients) has addressed the issue of an ultrasound diagnosis of liver disease in cystic fibrosis by comparing ultrasound findings with open or laparoscopic liver biopsies. This study showed that, in the few patients evaluated, ultrasound detected hepatic parenchymal abnormalities consistent with abnormal liver histology (Willi et al., 1980). In addition ultrasound abnormalities did not parallel histological change in the one patient who underwent a percutaneous liver biopsy (Willi et al., 1980).

The aim of this study was, therefore, to document the detailed clinical, biochemical and ultrasound characteristics of a cohort of cystic fibrosis patients in order to establish ultrasound criteria which could be applied universally for the diagnosis of cystic fibrosis related liver disease. Each of these parameters, if considered alone, would not be considered very specific or sensitive for the diagnosis of liver disease, but when combined represent the best 'guess'. This may provide problems for the individual case but overall provides a basis for statistical assessment.
3.1. Patients and Methods

3.1.1. Patients.

68 stable adult cystic fibrosis patients attending the out-patient clinics at The Royal Brompton Hospital were investigated.

The majority of patients were prospectively evaluated, although not all were randomly selected as some were known to have liver involvement prior to this study, and as such the cohort is not a truly representative cross-section of the cystic fibrosis patients attending The Royal Brompton Hospital.


3.1.2.1. Clinical Examination

Subjects were examined for evidence of the peripheral stigmata of chronic liver disease (SCLD) (Saraky, 1987), hepatomegaly (palpable liver with span >12 cm in the mid-clavicular line) and splenomegaly.

3.1.2.2. Haematological tests

Blood was taken for routine haematological and biochemical testing and was also screened for other aetiological factors of chronic liver disease in young adults/adolescents (Hepatitis B and C, autoimmune liver disease, α1-antitrypsin and Wilson's
Where these were detected the patient was excluded from the study.

3.1.2.3. Ultrasound scanning

The subjects underwent a detailed ultrasound scan following a 4 hour fast, using a realtime electronic curved array scanner (Ultramark 9-HDI, ATL, USA) and 3.5 MHz transducer, to assess the liver, spleen and portal circulation. Scanning was performed in the supine, right anterior oblique and left lateral positions by a single observer blinded to the available clinical and biochemical data.

3.1.2.3.1. Ultrasound scoring system

Previous studies have identified three ultrasound characteristics particular to liver disease in cystic fibrosis. These are parenchymal change similar to that observed in other forms of chronic liver disease (Wilson-Sharp et al., 1984), periportal fibrosis (increased periportal echoes) (Willi et al., 1980; Graham et al., 1985) and irregularity of the liver edge consistent with the macronodular pattern of cirrhosis in cystic fibrosis (McHugo et al., 1987).

Based on these criteria an initial cohort of 20 subjects was scanned to assess inter-subject variation in these features which were then used to develop an ultrasound based scoring system.
for the objective diagnosis of liver disease (Table 3.1.; Figures 3.1. to 3.5.).

A total score of 3 generated by the scoring system (Table 3.1.) was consistent with no liver abnormality (NLD group), while higher scores were indicative of a degree of hepatic abnormality up to a score of 9 which suggested established cirrhosis. All subjects with a score of four or more were allocated to the liver disease (LD) group.

The remaining 48 adult subjects were then evaluated prospectively, together with a cohort of 16 pre-pubertal subjects from the paediatric cystic fibrosis clinic who underwent hepatic ultrasound scanning to confirm the applicability of the scoring system to younger cystic fibrosis patients.

3.1.2.3.2. Validation of the scoring system

Inter-observer reproducibility of this scoring system was assessed by three independent radiologists, blinded to the patient's status, who used the scoring system to score hardcopy scans obtained from each of 52 ultrasound examinations.

3.1.2.3.3. Measurement of liver volume

The liver volume was estimated, following a small inspiratory breath, by measuring (in cm) the cranio-caudal (c-c) and antero-posterior (a-p) diameters of the liver in the mid-clavicular line
and by measuring the latero-lateral (l-l) diameter of the liver after rotating the ultrasound probe through 90°. The liver volume was calculated using the formula:

\[
\text{Volume (ml/Kg)} = \frac{(133.2 + 0.422(c-c \times a-p \times l-l)) \text{/patients weight (Kg)}}{Zoli \text{ et al., 1989}}
\]

3.1.2.3.4. Measurement of the splanchnic vessels

The diameter of the splanchnic vessels were measured following a small inspiratory breath. The portal vein at the porta hepatis, the splenic vein where it crosses anterior to the superior mesenteric artery and the superior mesenteric vein 1 cm proximal to its confluence with the splenic vein to form the portal vein. Doppler signal analysis was used to confirm patency of the splanchnic vessels and hepatic veins and to assess the direction of blood flow within the splanchnic vessels.

The porta hepatis, splenic hilum and gastric cardia were examined for the presence of portal-systemic collaterals and the longitudinal length of the spleen was measured.

3.1.2.3.5. The Biliary Tree

The biliary tree was evaluated to document intrahepatic and extrahepatic bile duct abnormalities. These included the diameter of the common bile duct and the status of the gallbladder, including whether or not it contained stones or
debris. When the gall-bladder was visualised it was classified as a micro-gallbladder when < 1.5-2.0 cm in width and < 3.0 cm in length (Kramer et al., 1983).

The pancreas was examined, when not obscured by bowel gas, for dilatation of the main pancreatic duct and increased echogenicity which suggests fibrosis.

3.1.3. Statistical analysis

Results for the adult subjects with a normal distribution are expressed as mean ± SEM, while those without a normal distribution and the results of the paediatric subjects are expressed as median (range).

Differences between the NLD and LD groups (as defined by the ultrasound scoring system) were calculated using the \( \chi^2 \) test for static variables and unpaired t tests for normally distributed and the Mann-Whitney U test for non-normally distributed continuous variables. A \( p \) value of <0.05 for a two-tailed test was considered statistically significant.

To assess the validity of the scoring system comparisons were made between the NLD and LD groups with respect to the results of their clinical examinations, haematological tests and ultrasound measurements. Correlation between the liver score and other measured variables was calculated using Spearman's Rank correlation co-efficient.
Inter-observer reproducibility of the ultrasound scoring system was calculated using Kendall's co-efficient of concordance 'W' which allows comparisons to be made between observations made by more than two observers.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic Parenchyma</td>
<td>Normal</td>
<td>Coarse</td>
<td>Irregular</td>
</tr>
<tr>
<td>Liver Edge</td>
<td>Smooth</td>
<td>-</td>
<td>Nodular</td>
</tr>
<tr>
<td>Periportal Fibrosis</td>
<td>None</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Table 3.1. The ultrasound scoring system
Figure 3.1. Hardcopy ultrasound scan of normal hepatic parenchyma and normal periportal echoes.
3.2. Results

3.2.1. Ultrasound scanning

Eighty four patients with cystic fibrosis underwent ultrasound assessment. Sixty eight were post-pubertal and sixteen pre-pubertal. These were sub-divided into NLD and LD groups on the basis of ultrasound criteria.

The post-pubertal group consisted of 35 NLD subjects (14 female and 21 male) with an average age of 24.5 ± 1.1 years and 33 LD subjects (12 female and 21 male) with an average age of 23.4 ± 0.9 years. These differences were not statistically significant.

The ultrasound results for the adult patients are shown in Table 3.2. 3 patients in the LD group had had a splenectomy: 1 for a childhood lymphoma, and 2 for symptoms related to splenomegaly. The splanchnic vessel diameters and blood count results for these three patients were excluded from further analysis.

Comparison of the ultrasound characteristics between the two groups shows no difference in the liver volume, but significant differences for the spleen length (NLD vs LD: 10 (8-12.5) vs. 16.8 (10.6-26) cm; p=0.0001) and the diameters of all the splanchnic vessels, and in particular the splenic vein (5.9 (3-12) vs. 11 (4.5-29.1) mm; p=0.0001).

Doppler assessment confirmed patency of hepatic veins in all patients, and patency of all splanchnic vessels although intra-abdominal gas limited visualisation of the splenic and superior
mesenteric veins in some subjects (numbers visualised given in Table 3.2). The direction of blood flow was hepatofugal in two of the patients in the LD group and a further patient in this group had cavernous transformation of the portal vein.

Portal-systemic collaterals were documented in seventeen of the LD group and none of the NLD group.

The pre-pubertal group consisted of nine NLD (3 females; age 10 (5-15)) and 7 [5 females; age 11 (5-15)] LD subjects. These differences were not statistically significant.

Ultrasound scanning identified changes identical to those of the post-pubertal subjects. The ultrasound characteristics of this cohort are shown in Table 3.3. The only significant difference between the two groups was in spleen size (9 (5.8-11) vs. 12.7 (8.6-17.4); p<0.02), with none of the other differences reaching statistical significance.

Following stratification into NLD and LD group on the basis of ultrasound scanning, a comparison of other group characteristics was possible:

3.2.2. Clinical findings

Examination of the adult patients for clinically detectable evidence of chronic liver disease: SCLD, hepatomegaly and splenomegaly, revealed no patients in the NLD group with any markers that are associated with liver disease. Seven of the LD group had palmar erythema (p=0.004 for LD vs NLD), and five of
the LD group had other SCLD (3 gynaecomastia, 1 spider naevi and 1 petechiae) (p<0.01 vs. NLD), although the subject with petechial haemorrhages was on long-term steroid therapy for allergic bronchopulmonary aspergillosis.

Seventeen of the LD group had hepato-splenomegaly, four had isolated hepatomegaly, and four LD patients had isolated splenomegaly. All these differences were significant when compared to the NLD group (p ≤ 0.05).

Comparison of the clinical findings in the pre-pubertal cohort revealed the expected differences in hepatomegaly +/- splenomegaly between the two groups (Table 3.3.). One subject in each group had palmar erythema and there were no other SCLD.

3.2.3. Haematological and Biochemical laboratory results

Comparison of measured haematological variables in the adults is given in Table 3.4. The LD subjects had significant elevations of the conventional biochemical tests of liver function, a significantly higher serum bilirubin (17 (5-71) vs 7 (4-44) µmol/l; p=0.0001) and significantly lower albumin (35 (25-44) vs. 39 (27-45) g/l; p<0.003) and platelet counts (120 (33-384) vs. 351 (215-834) x10⁹/l; p=0.0001). The international normalised ratio (INR), a test for coagulopathy, was not different between the two groups and was within the normal range (< 1.3) for the majority of the subjects.
3.2.4. Regression analyses between ultrasound score and other variables

Least-squares regression analysis confirmed significant correlations between measured variables and the ultrasound score (Table 3.5.). In particular increasing ultrasound score correlated with increasing splenic length ($R_s=0.86$, $p=0.0001$), increased splenic vein diameter ($R_s=0.74$, $p=0.0001$) and the presence of portal-systemic collaterals ($R_s=0.80$, $p=0.0001$). The majority of patients with varices (15/17; 88%) had marked ultrasonographic changes (score 8 or 9). There were weaker associations documented between ultrasound score and increasing portal vein diameter and superior mesenteric vein diameter (Table 3.5.).

Correlation with haematological/biochemical variables revealed three associations of note. Increasing ultrasound score correlated with reduced albumin ($R_s=-0.30$, $p=0.0008$), increased serum bilirubin ($R_s=0.69$, $p=0.0001$), and a reduced platelet count ($R_s=-0.60$, $p=0.0001$) (Table 3.5.). Further inspection of the data confirmed a predominance of the patients with a reduced serum albumin (<35 g/l) and elevated bilirubin (>17 μmol/l) in patients scoring 8 or 9 on ultrasound. All four subjects with prolonged INRs (>1.3) scored 8 or 9 with the ultrasound scoring system.

Furthermore, correlation of clinical data with ultrasound score revealed correlations between increasing score and the SCLD ($R_s=0.70$, $p=0.0001$), and with the number of subjects with hepato-splenomegaly ($R_s=0.76$, $p=0.0001$) (Table 3.5.). Ten of the
twelve subjects with SCLD (83%) and 13/17 (76%) with hepatosplenomegaly scored 8 or 9 when ultrasounded.

3.2.4. Ultrasound score reproducibility

The inter-observer scores for the hardcopy scans were strongly correlated confirming the reproducibility of the ultrasound scoring system ('W' = 0.92; p=0.00001).

3.2.5. The Biliary Tree

Evaluation of the biliary tree in the adult cohort revealed only one dilated common bile duct, and this in a patient who had had intrahepatic cholelithiasis treated by endoscopic sphincterotomy, basket retrieval of stones and who had had a pigtail prosthesis left in situ. There was no evidence of intra-hepatic bile duct dilatation.

The gall-bladder was visible in 31 (91%) of the NLD patients (one patient had had a cholecystectomy for symptomatic cholelithiasis), and 27 (82%) of the LD patients. 6 (17%) of the NLD group and 9 of the LD subjects (27%) had a micro-gall bladder. 5 (15%) of the NLD and 7 (21%) of the LD group had gall-stones identified by ultrasound scanning. None of these differences were statistically significant.

The pancreas was only visualised in 50 of the 68 adult subjects scanned. 24 of the 29 patients in the NLD group in whom the
pancreas was visualised had a pancreas of increased echogenicity, whereas all 21 visualised in the LD group were bright. No dilatation of the main pancreatic duct was documented.

In the pre-pubertal subjects there was no evidence of dilatation of the biliary tract. The gall bladder was visible in all nine of the NLD group, and only six of the LD group. 2 patients in each group (22% of the NLD and 29% of the LD group) had a micro gall-bladder.

The pancreas had increased echogenicity in all thirteen subjects in whom it was visualised, but was obscured by bowel gas in two of the NLD and one of the LD patients.
<table>
<thead>
<tr>
<th>GROUP</th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>35</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Volume (ml/Kg)</td>
<td>20.4 (11.7-33.6)</td>
<td>18.5 (7.6-44.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen length (cm)</td>
<td>10 (8-12.5)</td>
<td>16.8 (10.6-26)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Portal vein**

<table>
<thead>
<tr>
<th></th>
<th>NLD (100%)</th>
<th>LD (100%)*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number visualised</td>
<td>35</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>12.2 (5-15)</td>
<td>14.5 (8-26)</td>
<td>&lt;0.002</td>
</tr>
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</table>

**Splenic vein**

<table>
<thead>
<tr>
<th></th>
<th>NLD (86%)</th>
<th>LD (87%)*</th>
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</tr>
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<tbody>
<tr>
<td>Number visualised</td>
<td>30</td>
<td>26</td>
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</tr>
<tr>
<td>Diameter (mm)</td>
<td>5.9 (3-12)</td>
<td>11 (4.5-29.1)</td>
<td>0.0001</td>
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**Superior mesenteric vein**

<table>
<thead>
<tr>
<th></th>
<th>NLD (77%)</th>
<th>LD (63%)*</th>
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<tr>
<td>Number visualised</td>
<td>27</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>7 (3-12)</td>
<td>9 (6-18)</td>
<td>&lt;0.006</td>
</tr>
</tbody>
</table>

* Three cases, who had previously undergone splenectomy, deleted from the analysis of the LD group

Table 3.2. Ultrasound characteristics for the 68 adults (median (range))
<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Volume (ml/Kg)</td>
<td>24.9 (17.4-32.4)</td>
<td>18.8 (6.4-33.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen length (cm)</td>
<td>9 (5.8-11)</td>
<td>12.7 (8.6-17.4)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Portal vein diameter (mm)</td>
<td>10 (6-12)</td>
<td>10.3 (8-17.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Splenic vein diameter (mm)</td>
<td>4 (2.7-8)</td>
<td>6.3 (4.7-9)</td>
<td>NS</td>
</tr>
<tr>
<td>Superior mesenteric vein diameter (mm)</td>
<td>5 (3-8)</td>
<td>7 (5-9.7)</td>
<td>NS</td>
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Table 3.3. Ultrasound characteristics for the pre-pubertal subjects (median (range))
<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>35</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>241 (113-647)</td>
<td>1074 (217-3950)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(iu/l) (NR 90-250)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma glutamyl</td>
<td>14 (5-13.8)</td>
<td>169 (18-776)</td>
<td>0.0001</td>
</tr>
<tr>
<td>transferase (iu/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NR 0-32)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aspartate aminotransferase</td>
<td>28 (13-371)</td>
<td>72 (25-196)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(iu/l) (NR 10-50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>24 (5-89)</td>
<td>92.5 (16-289)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(iu/l) (NR 5-40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>7 (4-44)</td>
<td>17 (5-71)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(μmol/l) (NR 0-17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>39 (27-45)</td>
<td>35 (25-44)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>(NR 33-47)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Platelets (x10⁹/l)</td>
<td>351 (215-834)</td>
<td>120 (33-384)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(NR 150-400)</td>
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Table 3.4. Haematological and biochemical values for subjects with and without ultrasound evidence of liver disease (median (range))
<table>
<thead>
<tr>
<th>Variables</th>
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<th>p-value</th>
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</thead>
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<td><strong>Ultrasound</strong></td>
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<td></td>
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<tr>
<td>Splenic length</td>
<td>0.86</td>
<td>0.0001</td>
</tr>
<tr>
<td>Portal vein diameter</td>
<td>0.42</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Splenic vein diameter</td>
<td>0.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Superior mesenteric vein diameter</td>
<td>0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Portal-systemic collaterals</td>
<td>0.80</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Haematological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gamma glutamyl transferase</td>
<td>0.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.69</td>
<td>0.0001</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.30</td>
<td>0.0008</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.60</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signs of chronic liver disease</td>
<td>0.70</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hepato-splenomegaly</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 3.5. Correlations between clinical, biochemical and ultrasound parameters and the ultrasound scoring system.
### 3.3. Discussion

#### 3.3.1. The ultrasound scoring system

This study describes an ultrasound scoring system which allows the identification of patients with cystic fibrosis related chronic liver disease. It is based upon three characteristics which are particularly prominent in the liver disease of cystic fibrosis - the coarse liver parenchyma (Wilson-Sharp et al., 1984), nodularity of liver edge (McHugo et al., 1987) and increased periportal echoes (Willi et al., 1980; Graham et al., 1985).

The reproducibility of the scoring system has been independently validated by three blinded observers scoring hardcopy scans, which is more difficult than assessing realtime images (Joseph et al., 1991).

Support for an ultrasound based scoring system is provided by the reported sensitivity and specificity of ultrasound scanning in detecting hepatic fibrosis (Joseph et al., 1991) (although its limitations in detecting mild hepatic fibrosis are acknowledged), together with reports that abnormal hepatic ultrasonography represents strong evidence for significant hepatic pathology in chronic liver disease of other aetiologies (Bloom et al., 1992).

While no direct comparisons with previous studies can be made of scanning technique and assessment of hepatic parenchyma, detailed comparison of splanchnic vessel visualisation rates and vessel diameter measurements gives some comparison of the technical aspects and success of ultrasound scans in this series.
The visualisation rates of the splanchnic vessels were comparable with published series both in groups with (Wilson-Sharp et al., 1984; McHugo et al., 1987) and without (Bolondi et al., 1982) cystic fibrosis. In addition, comparison of the splanchnic vessel measurements with data from normal controls (Strohm et al., 1983; Zoli et al., 1985) reveals that the vessel diameters of the NLD subjects are very similar. Likewise the portal vein, splenic vein and superior mesenteric vein diameters of the LD group are similar to those reported for patients with chronic liver disease of other aetiologies (Zoli et al., 1985; Rector et al., 1986).

The optimal criteria to validate this scoring system would have been histological. However, as already discussed, percutaneous and open liver biopsy are unacceptable in cystic fibrosis due to the associated morbidity and mortality. In addition, as a result of sampling error, multiple percutaneous biopsies would have to be obtained. This approach would be unethical. We have, therefore, made an extensive and detailed comparison of the scoring system with potential markers of liver disease (markers of portal hypertension, clinical evaluation and conventional biochemical tests) as we believe this represents the best option available for validation.

Markers of portal hypertension including splenic size, splenic vein diameter (Doust & Pearce, 1976; Matsutani et al., 1991) and the presence of portal-systemic collaterals (Lebrec et al., 1980; Garcia-Tsao et al., 1985; Cales & Pascal, 1988) correlate strongly
with the scoring system as do the clinical, haematological and biochemical parameters.

The strong correlation of splenic vein diameter with ultrasound score is in keeping with reports that the splenic vein diameter is the best marker of portal hypertension (Doust & Pearce, 1976; Matsutani et al., 1991). Portal vein measurements, suggested by others to be an early marker of liver disease in cystic fibrosis (Kumari-Subaiya et al., 1987), correlated less well with ultrasound score. This observation is in keeping with reports that portal vein diameter correlates poorly with portal pressure in liver disease of other aetiologies (Bolondi et al., 1982; Subramanyam et al., 1983).

The positive correlation of the ultrasound score with the serum bilirubin and negative correlation with serum albumin, a marker of hepatic synthetic function which may reflect more advanced liver disease (Green et al., 1960; Feigelson et al., 1975), also suggest that increasing ultrasound score indicates more severe liver disease.

On this basis the scoring system identifies cystic fibrosis patients without liver disease (score 3) and with established cirrhosis (score 8-9). It also identifies a group of patients with definite but less advanced, possibly non-cirrhotic, liver disease (score 4-7).

Further analysis of the data according to these sub-groups would seem to confirm this distinction as the patients scoring 3 have normal biochemistry, those scoring 4-7 have normal hepatic
synthetic function but have conventional biochemical tests of hepatic function that mirror those of the more advanced group who scored 8-9. This last group have significant elevations of serum bilirubin and reductions of serum albumin (Table 3.6.). In addition there is a predominance of patients with varices, SCLD and hepato-splenomegaly in the patients scoring 8-9.
<table>
<thead>
<tr>
<th>Variable</th>
<th>3 (NLD)</th>
<th>4-7 (ILD)</th>
<th>8-9 (SLD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>35</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>a, b Alkaline phosphatase (iu/1)</td>
<td>241 (113-647)</td>
<td>1085.5 (217-2830)</td>
<td>1047 (255-3950)</td>
</tr>
<tr>
<td>a, b Gamma glutamyl transferase (iu/1)</td>
<td>14 (5-138)</td>
<td>168 (18-776)</td>
<td>199 (33-662)</td>
</tr>
<tr>
<td>c, d Aspartate aminotransferase (iu/1)</td>
<td>28 (13-371)</td>
<td>67.5 (42-135)</td>
<td>72 (25-196)</td>
</tr>
<tr>
<td>a, b Alanine aminotransferase (iu/1)</td>
<td>24 (5-89)</td>
<td>101 (24-220)</td>
<td>83 (16-289)</td>
</tr>
<tr>
<td>b, e Total bilirubin (umol/l)</td>
<td>7 (4-44)</td>
<td>10.5 (5-55)</td>
<td>23 (11-71)</td>
</tr>
<tr>
<td>b, e Albumin (g/l)</td>
<td>39 (27-45)</td>
<td>36.5 (30-43)</td>
<td>31 (25-44)</td>
</tr>
<tr>
<td>a, b, f Platelets (x10^9/l)</td>
<td>351 (215-834)</td>
<td>281 (107-384)</td>
<td>75 (33-383)</td>
</tr>
</tbody>
</table>

a Differences significant (p<0.001) for NLD vs ILD.
b Differences significant (p<0.001) for NLD vs SLD.
c Differences significant (p<0.05) for NLD vs ILD.
d Differences significant (p<0.01) for NLD vs SLD.
e Differences significant (p<0.01) for ILD vs SLD.
f Differences significant (p<0.001) for ILD vs SLD.

Table 3.6. Results of haematological and biochemical tests for the three groups as defined by the ultrasound scoring system (median (range))
3.3.2. The Biliary Tree

This study has allowed detailed evaluation of the biliary tree and gallbladders of patients with cystic fibrosis. The absence of demonstrable dilatation of the biliary tree (apart from the subject with a pig-tail stent in situ) on ultrasound confirms that there is no evidence in this study to support bile duct obstruction in the intrapancreatic portion of the duct as an important mechanism for the development of liver disease in the majority of cystic fibrosis patients (Gaskin et al., 1988; Nagel et al., 1989; O'Brien et al., 1992b).

The gallbladder visualisation rates (81%-91%) and the frequency of micro-gallbladder (17%-29%) are consistent with other reported series which document micro-gallbladder in up to 18% in clinical series and 30% at post mortem (Isenberg et al., 1976; L'Heureux et al., 1977).

Gallstones were detected by ultrasound in none of the paediatric subjects and in 15% of the NLD and 21% of the LD adult patients. This increase in the prevalence of gallstones with age in cystic fibrosis is well recognised (Warwick et al., 1976) and the prevalence rates are comparable with those published in the literature (Vawter & Shwachman, 1979).

3.4. Conclusions

The need for an early, accurate, diagnostic test for liver disease in cystic fibrosis is highlighted by the possibility of an occult
adverse effect of liver disease on survival, the potential for an increased prevalence of liver disease as survival improves and the availability of potential therapeutic agents such as Ursodeoxycholic acid (Cotting et al., 1990; Colombo et al., 1992b) and other anti-fibrotic agents such as colchicine (Burt, 1992) and the prolyl-4 hydroxylase inhibitors (Clement et al., 1991) which are as yet untried in cystic fibrosis liver disease. The ability to identify patients with earlier hepatic disease may be important as they may form a sub-group more likely to respond to therapy as compared with patients with advanced cirrhosis (Colombo et al., 1993).

Percutaneous liver biopsy is inaccurate and correlates poorly with open biopsy and ultrasound. Obtaining tissue for histology by either route is likely to be unacceptable.

The ultrasound scoring system reported here, which is supported by clinical, biochemical and ultrasound markers of liver disease, is probably useful for detecting and grading the severity of liver disease in cystic fibrosis. It may also allow the objective evaluation of response to therapy.

This study suggests that an ultrasound scan, preferably performed by a single experienced ultrasonographer using this scoring system, should be incorporated into the routine, regular assessments of all cystic fibrosis patients to allow early detection of hepatic disease and early institution of therapy.
Chapter 4

QUANTITATIVE HEPATOBILIARY SCINTIGRAPHY

for the diagnosis and assessment of disease severity in cystic fibrosis related chronic liver disease.
4.0. **Introduction**

Hepatobiliary scintigraphy represents an alternative means of imaging and evaluating liver disease (Heyman, 1994).

The present study evaluated this technique, which involves the use of Technetium labelled iminodiacetic acid derivatives to evaluate the integrity of the hepatobiliary system, for the detection of liver disease in cystic fibrosis and for the assessment of disease severity.

The handling of the isotope allows the measurement of both hepatic parenchymal and biliary excretory function. Measures of hepatic uptake may be a particularly useful marker of disease severity in patients with cystic fibrosis related chronic liver disease as the conventional parameters used to assess disease progression (hepatic decompensation and impairment of hepatic synthetic function) rarely occur until late in the disease process. Similarly, measures of hepatic excretion may be particularly useful in a disease which is believed to evolve from biliary obstruction.

Calculated measures of hepatic isotope uptake include the hepatic extraction fraction which is calculated using deconvolutional analysis and may be a useful estimate of hepatic reserve. In addition, other measures of hepatic isotope uptake (the half-time of hepatic uptake and time to maximal hepatic activity) and measures of biliary excretion (half-time of hepatic excretion and percentage retention of the isotope at particular time points) can be calculated from a non-linear least squares fit
to a background corrected liver time-activity curve. Furthermore, the anatomy of the biliary tree (both intra- and extra-hepatic) can be delineated.

For the purposes of this study a cohort of cystic fibrosis subjects who had already undergone hepato-biliary ultrasound scanning were evaluated. Normal ranges for the measured scintigraphic variables were established using ten normal healthy volunteers.
4.1. **Patients and Methods**

4.1.1. Patients and methods

Following an overnight fast 30 subjects underwent quantitative hepatobiliary scintigraphy (Brown et al., 1988; Howman-Giles et al., 1993). Of 20 subjects with cystic fibrosis, 13 had abnormal hepatobiliary ultrasounds (LD group) and 7 normal ultrasounds (NLD group), and there were 10 healthy Control subjects, with no clinical or laboratory evidence of hepato-biliary disease, who were recruited from the medical staff at Charing Cross Hospital.

150 MBq of technetium-99m labelled N,a-(2,6-diethylacetanilide)-iminodiacetic acid (EHIDA, Amersham Laboratories, Amersham, UK) was injected as an intravenous bolus. The dose was reduced for the paediatric subjects as appropriate for weight and age.

Serial analogue 64 x 64 matrix images were obtained every minute for 60 minutes by a wide field of view gamma camera (Scintronix, Aura Ltd., UK) fitted with a low energy general purpose collimator, that was interfaced to a dedicated computer system (Micas 5, Bartec). The spectrometer was set at a photon energy level of 140 KeV, with a 20% window.

The images were analysed by drawing regions of interest over the right lobe of the liver and the left ventricle of the heart. Care was taken to ensure exclusion of the major hepatic ducts, gallbladder and scatter from the heart from the liver region of interest, and to exclude the aorta and scatter from the liver from
the left ventricular one. A further area of interest was drawn over the spleen to give a measure of vascular background activity. The spleen was selected as background as it has a common arterial supply with the liver. Time-activity curves were generated from each of the areas of interest (Figure 4.1.). These time-activity curves were used by a single observer, who was blinded to the clinical status of each subject, to calculate measures of hepatic uptake and excretion.

4.1.2. Calculation of the hepatic extraction fraction

The heart curve was used as the input function and the liver curve as the output function for deconvolutional analysis (Gilbert et al., 1987; Howman-Giles et al., 1993).

The matrix algorithm of deconvolution (Valentinuzzi & Montaldo Volachec, 1975) was used to calculate the deconvolved liver curve. The resultant deconvolved liver curve is a hypothetical true liver response representing a direct bolus injection into the hepatic artery.

An exponential best-fit curve was matched to the deconvolving liver curve over the first 30 minutes of the study and extrapolated to time zero. The point where the curve crosses the y-axis is proportional to hepatocyte function and used to give a measure of the hepatic extraction fraction (HEF) by comparing the y-intercept of the fitted curve to the actual maximum value.
of the deconvolved liver curve.

\[ \text{HEF} = \left( \frac{\text{y-intercept}}{\text{y-max of liver response curve}} \right) \times 100 \% \]

4.1.3. Calculation of other measures of hepatic uptake and excretion

A further time-activity curve was generated by subtracting the background counts, measured over the spleen, from the liver time-activity curve. This background corrected liver curve was modelled by an uptake-excretion (bi-exponential) compartmental model:

\[ L(t) = k(e^{-0.693 \cdot t / TE} - e^{0.693 \cdot t / TU}) \]

where \( L \) is the number of counts at time \( t \), \( TE \) is the excretion half-time and \( TU \) is the uptake half-time (Brown et al., 1981).

The best fit was determined by a non-linear least squares technique (Bevington, 1969).

The background corrected liver curve was used to estimate the half-time of hepatic uptake (\( t_{UP} \)), the time to maximal hepatic activity (\( T_{max} \)), the half-time of hepatic excretion (\( t_{EX} \)) and to estimate the percentage radio-isotope retained at 45 and 60 minutes (\( E_{45} \) and \( E_{60} \) respectively).
4.1.4. Calculation of hepatic output efficiency

The output efficiency of the liver was calculated using a modification of a technique usually applied to renal studies (Britton & Brown, 1971).

The liver region of interest was modified to include both left and right lobes of the liver, but excluding scatter from other regions such as the major intrahepatic bile ducts. The integrated input (heart) curve was fitted to the first 5 minutes of the hepatic time-activity curve using a correction factor and the assumption that excretion from the liver over this time was zero. The counts from the actual hepatic time-activity curve were subtracted from this hypothetical fitted curve, which assumes that no isotope is excreted from the liver over the time course of the study, to produce a hypothetical curve of the cumulative amount of radio-isotope excreted by the liver.

The output efficiency was calculated from the average readings for the last 2 minutes of each curve and expressed as a percentage:

\[
\frac{\text{Mean t}_{58-60} \text{ excretion curve}}{\text{Mean t}_{58-60} \text{ integrated input curve}} \times 100 \%
\]

4.1.5. Assessment of the biliary tree

Subjective observations were also made, by a single blinded observer, of whether there was delayed excretion of the radio-isotope from the liver and whether this delay was intra- or
extra-hepatic. In addition the images were analysed for abnormalities of the common bile duct including dilatation, stricture and stenosis and for whether or not the gallbladder was visualised.

4.1.6. Statistical analysis

Differences between the three groups were analysed using $\chi^2$ for static variables and one-way analysis of variance (ANOVA) for continuous variables. When significant differences were detected between the three groups by ANOVA the results were analysed further using Fisher's $t$-based least significant difference.

Where comparisons were made between the 20 subjects with cystic fibrosis the Mann Whitney U test was used as the total numbers analysed were small.

A probability value <0.05 was considered statistically significant for a two-tailed test.
4.2. Results

4.2.1. Subjects

Of the 60 subjects undergoing EHIDA scanning, 13 had evidence of chronic liver disease detected on ultrasonography. This group was predominantly male (median age 47 years, range 17–75 years). There were no significant differences in these results compared to the normal control group (median age 50 years, range 20–65 years). These results were predominantly in the normal range suggesting that these differences were of no clinical relevance.

Figure 4.1. Regions of interest over the heart (pink), right lobe of liver (white) and spleen (green) and time-activity curves for each of these regions (as labelled) for quantitative EHIDA uptake and excretion analysis.
4.2. **Results**

4.2.1. Subjects

30 subjects underwent EHIDA scanning. 13 had evidence of cystic fibrosis related chronic liver disease detectable on ultrasound scanning (LD) (mean age 19.8 ± 2.2 years; 6 males: 7 females), 7 had completely normal ultrasounds (NLD) (mean age 19.3 ± 3.9 years; 3 males: 4 females) and 10 were normal Controls (mean age 27.4 ± 1.8; 5 males: 5 females). These differences in age and sex were not statistically significant.

4.2.2. Clinical, biochemical and ultrasound characteristics of the two cystic fibrosis groups.

The two groups had broadly similar characteristics to those already described in the ultrasound analysis.

There were significantly more subjects with hepatosplenomegaly (7/13 vs. 0/7; p<0.02) in the LD cohort (Table 4.1.). 3 of the LD group also had portal-systemic collaterals detectable on ultrasound against none in the NLD group, although this difference was not statistically significantly.

Routine haematological and biochemical laboratory tests revealed a significant elevation of AST, ALT and \( \gamma \)GT in the LD group (Table 4.1.). While statistically significant differences in the bilirubin and INR results were detected for the two groups, the results were predominantly in the normal range suggesting that these differences were of no clinical relevance.
The median ultrasound score for the LD cohort was 8 (range 4-9), with five subjects scoring 7 or less. The ultrasound characteristics confirmed splenomegaly in the LD patients and a significant difference in the splenic vein diameter (Table 4.2.).

None of the cystic fibrosis patients had abnormalities of the intra- or extra-hepatic bile ducts on ultrasonography. The gall-bladder was visualised in 5/6 (83%) of the NLD group (one of the NLD subjects had previously undergone cholecystectomy) and 9/13 (69%) of the LD group. Three subjects in the NLD and three in the LD group had micro gall-bladders (see above). These differences were not statistically significant.

4.2.3. Quantitative hepato-biliary scintigraphy

An example of an EHIDA scan in a cystic fibrosis subject without liver disease is shown in figure 4.2. There is rapid excretion of the isotope, whereas figure 4.3. shows a similar scan in a subject with liver disease and demonstrates marked retention of the EHIDA late in the time course of the study.

Overall there were no differences between the results for any parameter between the normal Controls and the NLD cystic fibrosis subjects. However, the LD subjects differed significantly from both the other groups for a number of uptake and excretion variables.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>NLD</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>a,bAST (iu/l)</td>
<td>24.8 ± 3.3</td>
<td>25.9 ± 2.5</td>
<td>83.2 ± 9.7</td>
</tr>
<tr>
<td>c,dALT (iu/l)</td>
<td>30.2 ± 9.5</td>
<td>49.7 ± 26.6</td>
<td>78.0 ± 13.6</td>
</tr>
<tr>
<td>b,eGT (iu/l)</td>
<td>6.8 ± 2.3</td>
<td>22.4 ± 9.5</td>
<td>204.2 ± 67.5</td>
</tr>
<tr>
<td>f,gBilirubin (µmol/l)</td>
<td>8.9 ± 1.7</td>
<td>7.7 ± 0.9</td>
<td>15.2 ± 2.2</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>-</td>
<td>38.9 ± 2.2</td>
<td>34.9 ± 1.1</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>fPlatelets (x10⁹/l)</td>
<td>250.7 ± 7.7</td>
<td>297.3 ± 51.9</td>
<td>193.4 ± 31.4</td>
</tr>
<tr>
<td>Clinical Finding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c,hHepato-splenomegaly</td>
<td>0/10</td>
<td>0/7</td>
<td>7/13</td>
</tr>
</tbody>
</table>

a p=0.004 NLD vs LD  
b p=0.0001 Controls vs LD  
c p<0.02 NLD vs LD  
d p<0.005 Controls vs LD  
e p<0.002 NLD vs LD  
f p<0.05 NLD vs LD  
g p<0.05 Controls vs LD  
h p<0.01 Controls vs LD

**Table 4.1. Clinical, haematological and biochemical variables for the subjects included in the EHIDA study**

(Statistical comparison by ANOVA and Fisher's t-based least significant difference; no significant differences between Controls and NLD subjects)
<table>
<thead>
<tr>
<th>Variable</th>
<th>NLD</th>
<th>LD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver volume (ml/Kg)</td>
<td>23.8 ± 2.4</td>
<td>19.4 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen (cm)</td>
<td>9.9 ± 0.7</td>
<td>15.5 ± 1.0</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Portal vein diameter (mm)</td>
<td>9.6 ± 1.1</td>
<td>12.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Splenic vein diameter (mm)</td>
<td>5.3 ± 0.6</td>
<td>9.0 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Superior mesenteric vein diameter (mm)</td>
<td>5.9 ± 0.9</td>
<td>8.1 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Varices</td>
<td>0/7</td>
<td>3/13</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4.2. Ultrasound characteristics of the cystic fibrosis subjects included in the EHIDA study
Figure 4.2. Hepato-biliary scintigram in an NLD subject

1.1 Appearance in the heart in the first minute
1.4 Rapid uptake into the liver by the end of the fourth minute
1.11 Rapid excretion, with radio-isotope in the small bowel at eleven minutes
1.27 Excretion almost complete by the twenty seventh minute
Figure 4.3. Hepato-biliary scintigram in an LD subject

2.1 Appearance in the heart and spleen after one minute
2.4 Uptake by the liver by the fourth minute
2.27 Marked intra-hepatic retention of the radio-isotope at twenty seven minutes with some excretion into the small bowel
2.60 Continued intra-hepatic retention at sixty minutes with more isotope excreted into the small bowel
4.2.3.1. Uptake (Table 4.3.)

Time to maximal uptake was significantly longer in the LD patients ($p<0.02$), although the delay in absolute terms was only a mean of 4 minutes with considerable overlap for the individual values of $T_{max}$ for the three groups (Figure 4.4.)

Deconvolutional analysis revealed no overall significant difference in HEF between the three groups. However $5/13$ (38%) of the patients with hepatic disease, all of whom scored 8 or 9 on ultrasound, had a reduced HEF ($<93.7\%$, or mean ± two standard deviations less than the normal controls; Figure 4.5.).

Uptake half-time was not different between the groups.

4.2.3.2. Excretion (Table 4.4.)

In the LD group there was a significant increase in retention of the radioisotope at both 45 and 60 minutes as well as a prolonged excretion half-time and reduced output efficiency ($p<0.0001$ for all comparisons).

Examination of the individual results shows an almost clear separation between the EHIDA retention at 45 minutes (Figure 4.6.), and 60 minutes (Figure 4.7.) and the hepatic output efficiency (Figure 4.8.) between NLD and LD cystic fibrosis groups.
### Table 4.3. Parameters measuring EHIDA uptake in the three groups (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>10</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>HEF (%)</td>
<td>98.9 ± 0.8</td>
<td>100.0 ± 0.0</td>
<td>93.7 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>*T\text{max} (min)</td>
<td>11.0 ± 0.8</td>
<td>10.1 ± 1.0</td>
<td>14.6 ± 1.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Uptake t\text{1/2} (min)</td>
<td>4.5 ± 0.4</td>
<td>3.9 ± 0.3</td>
<td>4.6 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Significant differences between LD and NLD (p<0.02) and LD and Controls (p<0.05)
Figure 4.4. Scattergram of time to maximal hepatic uptake (Tmax)
Figure 4.5. Scattergram of hepatic EHIDA extraction fraction (HEF)
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NLD</th>
<th>LD</th>
<th>p-value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>10</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>*E45 (%)</td>
<td>26.1 ± 4.3</td>
<td>18.4 ± 3.5</td>
<td>50.8 ± 3.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>*E60 (%)</td>
<td>15.4 ± 3.4</td>
<td>10.9 ± 2.9</td>
<td>39.0 ± 4.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>*Excretion</td>
<td>14.7 ± 2.0</td>
<td>11.9 ± 1.7</td>
<td>28.0 ± 2.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>t₁/₂ (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Output efficiency (%)</td>
<td>93.7 ± 1.5</td>
<td>93.6 ± 1.4</td>
<td>85.1 ± 1.1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Table 4.4. Parameters measuring EHIDA excretion in the three groups**

* Significant differences (p<0.001) between LD and both NLD and Control groups
Figure 4.6. Scattergram of EHIDA retention at 45 minutes
Figure 4.7. Scattergram of EHIDA retention at 60 minutes
Figure 4.8. Scattergram of hepatic output efficiency
4.2.3.3. Assessment of the biliary tree

Intrahepatic delay of EHIDA excretion was detected in none of the Control group, 1/7 of the N1D group and 7/13 of the LD group on subjective examination of the images. The differences between Control and LD groups was statistically significant (p<0.006), but all other inter-group comparisons were not.

Extrahepatic delay was detected in 2/10 of the Controls, 1/7 of the NLD group and 1/13 of the LD group. Delay in excretion was not associated with dilatation or tapering of the common bile duct, but did correlate with rapid filling of the gallbladder. These differences were not statistically significant. The single NLD subject had both intrahepatic and extrahepatic delay. The radioisotope cleared rapidly from all four subjects with extrahepatic delay following presumed relaxation of the sphincter of Oddi.

The gallbladder was visualised in 9/10 of the Controls, 4/6 of the NLD cohort and 5/13 of the LD group. The reduction in gallbladder visualisation rates in the LD group was statistically significant when compared with the Control group (p<0.02).

The two NLD subjects whose gall bladders were not visualised had micro-gallbladders on ultrasound scanning, as did one of those in whom it did fill with radio-isotope. Similarly, a further five of the LD cohort had gallbladders visualised on ultrasound. Only two of these had a micro-gallbladder which was also found in one of the LD subjects whose gallbladder was visualised using both techniques. Three of the LD group had no identifiable gallbladder either by EHIDA or ultrasound scanning.
4.3. **Discussion**

The results of EHIDA scanning have demonstrated delayed uptake and excretion of the radio-isotope in patients with the biliary cirrhosis of cystic fibrosis. In contrast those subjects who had normal hepato-biliary ultrasound scans extracted and excreted EHIDA at similar rates to the normal Controls. These results suggest that quantitative hepatobiliary scintigraphy may be useful for the identification of cystic fibrosis patients with significant hepatic involvement. The results also provide further support for the ultrasound based scoring system.

Iminodiacetic acid (IDA) compounds are transported bound to plasma proteins and in particular albumin. They enter the space of Disse through fenestrations in the endothelial cells and the IDA complex is extracted by the hepatocyte using a non-sodium dependent carrier mediated organic anion pathway (Gilbert *et al.*, 1987). In health this process is completed rapidly, but it is impaired in hepatocellular disease (Howman-Giles *et al.*, 1993).

The IDA molecule is transported to the canaliculus by an unknown mechanism and then excreted in the bile along with the hepatic bile. Intra- and extra-hepatic biliary disease results in impaired excretion (Brown *et al*., 1988).

The use of IDA derivatives in the quantitative assessment of hepatic and biliary function is dependent on their uptake and excretion via the liver. The loss of large amounts of the radio-isotope via the kidneys, for example, would invalidate quantitative measurements. More than 97% of administered
EHIDA is removed via the liver in animal studies (Wistow et al., 1977), supporting its use as an agent for the quantitative estimation of hepatobiliary function.

Hepatobiliary scintigraphy has previously been used in cystic fibrosis to evaluate the biliary tree (Gaskin et al., 1988; Nagel et al., 1989; Semith Dogan et al., 1994) and to assess the response to therapy of patients with chronic liver disease (Colombo et al., 1992a; O'Brien et al., 1992b). The study by Colombo et al. evaluated quantitative measures of biliary clearance only with respect to the half-time of hepatic washout and the time of appearance of isotope within the gut. They did not evaluate parameters of hepatic uptake. More recently a study detailing parameters representative of hepatic uptake and excretion has been reported (O'Brien et al., 1992b), with the baseline results similar to those obtained in the current study. However HEF and output efficiency were not calculated.

The HEF may accurately reflect hepatocyte function in adults (Brown et al., 1988; Doo et al., 1991) and children (Howman-Giles et al., 1993), falls with ischaemic liver injury (Tagge et al., 1987) and has also been used to serially monitor liver transplant function (Reichle et al., 1986). In this series 5/13 (38%) of the liver subjects, all of whom scored 8 or 9 on ultrasound, had an abnormally low HEF.

It is well recognised that patients with cystic fibrosis related liver disease rarely develop hepatic decompensation. Consequently conventional tests such as the bilirubin and INR
are rarely helpful in detecting advanced disease. Similarly there are no available criteria for evaluating liver disease in subjects who may require lung transplantation. It is possible that measurement of HEF will allow identification of a sub-group with more advanced parenchymal liver disease who are more likely to develop hepatic decompensation, particularly in relation to pulmonary transplantation.

While there are also significant differences in the time to maximal hepatic activity (Tmax) between the LD subjects and the other two sub-groups the mean delay of four minutes in the LD cohort is of doubtful clinical significance. The scattergram confirms marked overlap in this parameter for the three groups studied.

The differences in excretory parameters are similar to those previously documented (O'Brien et al., 1992b). However, the scattergrams of the excretory parameters do show good discrimination between cystic fibrosis subjects with and without liver disease. Measurement of these variables may supplement ultrasound for the diagnosis of liver disease particularly in subjects in whom doubt persists following baseline investigation.

Repeated documentation of these measures of hepato-biliary excretory function may also allow accurate monitoring of disease progression and the response to therapy.

The demonstration that the excretory delay is predominantly intrahepatic is in keeping with previous studies (Nagel et al., 1989; O'Brien et al., 1992b). In addition, the results of the
present study fail to provide evidence of common bile duct stenosis as a potential aetiological factor for chronic liver disease (Gaskin et al., 1988; Nagel et al., 1989; O'Brien et al., 1992b).

Extrahepatic delay was observed in all three groups. The delay appeared to be secondary to contraction of the sphincter of Oddi as evidenced by rapid filling of the gall bladder in all four cases and rapid clearance with appearance of the radio-isotope within the gut following presumed relaxation of the sphincter. Interestingly the NLD subject also had pooling of the radio-isotope in the intrahepatic biliary tree in a pattern similar to that previously described (Gaskin et al., 1988) and rapidly cleared it following relaxation of the sphincter. The detection of this pattern of abnormality in all three groups suggests that, contrary to previous reports (O'Brien et al., 1992b), biliary hypomotility and viscid mucous within the common bile duct may not be important mechanisms promoting the development of chronic liver disease.

The gallbladder abnormalities detected by biliary scintigraphy also show considerable inter-patient variation. The detection of a small gallbladder on ultrasound does not appear to imply that it is non functioning, and likewise the finding of a gallbladder with normal appearances on ultrasound does necessarily indicate a normally functioning organ as some of these were not demonstrated by biliary scintigraphy. However, it is recognised that a normal gall-bladder filled with bile following a prolonged fast may not be visualised using biliary scintigraphy (Larsen et al., 1982).
Other potential reasons for these variations in gallbladder visualisation are unclear but they may all may reflect different parts of the spectrum of the same disease process. At post mortem shrunken hypoplastic gallbladders with cystic duct obstruction due to plugging with mucous and sludge, mucosal hyperplasia, stenoses and even atresia have been demonstrated (Oppenheimer & Esterly, 1975a; Roy et al., 1982). These changes may result in non-visualisation of the gallbladder by both imaging techniques, or alternatively demonstration of a micro-gallbladder on ultrasound. Visualisation of an intact gallbladder by ultrasound but not by scintigraphy may reflect occlusion of the cystic duct by mucous at an early stage in the evolution of these changes or alternatively a gallbladder filled with bile following the pre-investigation fast.

4.4. Conclusions

Quantitative hepato-biliary scintigraphy may be of value in patients with cystic fibrosis where doubt about the hepatic status of the patient persists after more conventional tests and an ultrasound have been performed.

In addition the uptake parameters, and in particular the HEF, may prove of value in the assessment of disease severity pre-transplantation. Repeated measurement of both the uptake and the excretory parameters may allow the objective monitoring of disease progression or the response to therapeutic agents.

These potential applications require prospective evaluation.
CHAPTER 5

THE EVALUATION OF BIOCHEMICAL TESTS FOR THE PRESENCE AND SEVERITY OF LIVER DISEASE IN CYSTIC FIBROSIS.
5.0. Introduction

Individual biochemical tests of liver function (the transaminases, alkaline phosphatase and gamma glutamyl transferase) have proved disappointing markers for underlying liver disease in cystic fibrosis, and may be normal in the presence of overt cirrhosis (di Sant’Agnese & Blanc, 1956) (Stern et al., 1976).

In a recent epidemiological survey 13% of those with clinical liver disease (hepatomegaly ± splenomegaly) had normal conventional biochemical tests of liver function and conversely 13% with no clinical evidence of liver disease had abnormal blood tests (Scott-Jupp et al., 1991).

The purpose of the present study was to assess four alternative biochemical parameters as markers of liver disease in cystic fibrosis: the levels of monoethylglycinexylidide (MEGX-X) following the administration of lignocaine was used as an estimate of functioning hepatic cell mass (Oellerich et al., 1989); fasting serum bile acid concentrations were measured as a marker of biliary liver disease (Cravetto et al., 1985); the measurement of the concentration of the amino terminal propeptide of type III procollagen (PIIINP) was used as an estimate of ongoing liver fibrosis (Babbs et al., 1988); apolipoprotein A1 was measured and incorporated into an index for the detection of liver disease (the PGA index; P=prothrombin, G=gamma-glutamyl transpeptidase and A=serum apolipoprotein A1 concentration (Poynard et al., 1991)).
The MEG-X test is a quantitative liver function test (Reichen, 1993) and has been advocated as a useful test for the diagnosis of hepatic disease. It offers the potential of assessing disease severity (Huang et al., 1993) and may be of prognostic value (Sallie et al., 1991). MEG-X, the primary metabolite of lignocaine, was measured 15 minutes after a bolus injection of lignocaine using a fluorescence polarisation immunoassay (Oellerich et al., 1989).

Fasting serum bile acids have been advocated as a sensitive and specific test for liver disease (Cravetto et al., 1985), although they may not be sensitive enough to detect mild liver disease (Ferraris et al., 1987). Their concentration was measured using an enzyme linked immunosorbent assay.

Serum levels of PIIINP correlate with histological parameters in many forms of liver disease including primary biliary cirrhosis (Babbs et al., 1988; Mutimer et al., 1989) which suggests that serum concentrations may be of value in the assessment of the biliary cirrhosis of cystic fibrosis. PIIINP concentration was measured using a radio-immunoassay.

Hepatic fibrosis has also been estimated using a scoring index that has previously been validated in patients with alcoholic liver disease. The serum concentration of apolipoprotein A1, measured using rate nephelometry, gamma glutamyl transpeptidase and prothrombin time (expressed as the international normalised ratio (INR)) were assigned a score and the scores were combined (the PGA index). This index has been advocated as a sensitive test for the detection of liver fibrosis in
alcoholic liver disease (Poynard et al., 1991), and has been reported to correlate with disease severity, as judged by the Mayo score, in primary biliary cirrhosis (Teare et al., 1993).
5.1. **Patients and Methods**

5.1.1. Patients

Subjects were drawn from the pool of cystic fibrosis patients studied in chapter three. In addition twenty seven Control subjects with no clinical or laboratory evidence of hepatobiliary disease were recruited from the medical staff at Charing Cross Hospital.

All subjects undergoing hepatobiliary ultrasound evaluation provided fasting blood samples for the measurement of the standard biochemical tests of liver function (the transaminases, alkaline phosphatase, gamma-glutamyl transpeptidase) and for PIIINP, serum bile acid and apolipoprotein A1 measurement. Similar fasting samples were obtained from the Control subjects.

The MEG-X test was not performed on the normal healthy controls, although the safety of the test is well recognised.

5.1.2. Methods

All blood samples obtained for analysis were allowed to stand for 30 minutes and then centrifuged at 1,800 r.p.m. for 15 minutes to separate the serum. This was then decanted and stored at -20\(^\circ\)C until analysed.
5.1.3. Monoethylglycinexylidide measurement.

Lignocaine hydrochloride (2% aqueous solution) was given as a slow intravenous bolus (1 mg/Kg body weight) over 2 minutes. Blood samples were drawn before and 15 minutes after the completion of lignocaine administration (Oellerich et al., 1989).

The serum concentration of the lignocaine metabolite monoethylglycinexylidide (MEG-X) was determined, after thawing the stored serum samples, using a fluorescence polarisation immunoassay and an automated analyser (TDx analyser, Abbott Laboratories, Maidenhead, UK).

The TDx analyser was calibrated using standard samples obtained from the manufacturer to create a reference curve. The analyser automatically estimated the sample serum MEG-X concentration.

The MEG-X t0 values were subtracted from the t15 values to give the serum MEG-X concentration (ng/ml).

5.1.4. Total fasting serum bile acid assay

3α-hydroxy bile acid concentration was estimated using a commercially available kit (Enzabile, Nycomed Pharma, Oslo, Norway). The bile acids were reduced by 3α-hydroxysteroid dehydrogenase to produce 3α keto bile acids and NADH. Reaction of NADH and nitroblue tetrazolium salt, catalysed by diaphorase, resulted in the production of formazan which has a stable blue colour and a maximum spectrophotometric absorption at 540nm.
For each serum sample three microcuvettes were prepared. Two each containing 500μl of reconstituted sample reagent (Sample tubes), and one containing 500 μl of reconstituted blank reagent (Blank tube). 200μl of the serum was pipetted into each tube and mixed thoroughly. The time of addition was recorded and the mixture was incubated at room temperature (20-25°C) for 20 minutes. At the end of this period stop reagent was added to each of the micro-cuvettes and the tube mixed once more.

Absorbances of the sample and blank tubes were read in pairs at 540 nm (PU 8800 UV/VIS spectrophotometer, Phillips, Holland). The average of the absorbance recorded for the two sample tubes was used to estimate the serum bile acid concentration by reference to a standard curve constructed using standard samples obtained from the manufacturer (Enzabile standards, Nycomed Pharma, Oslo, Norway). The method shows a linear relation between absorbance at 540 nm and 3α-hydroxy bile acid concentration up to 200 μmol/l.

5.1.5. N-terminal procollagen III propeptide measurement

Serum samples were analysed for PIIINP using a commercially available radioimmunoassay kit, (RIA-gnost PIIIP, Behringwerke AG, Marburg, Germany), in which anti-PIIINP antibodies coated on a test-tube combine with PIIINP in the sample.
20µl of standard or serum was pipetted into the bottom of the coated test tube and mixed with 400µl of buffer. The tubes were incubated in a horizontal shaker, at room temperature, for 2 hours and then washed twice with 1ml of wash buffer. The mixture within the tubes was discarded after each wash. After washing 400µl of $^{125}$I-labelled monoclonal antibody to PIIINP was pipetted into each tube and incubated for a further three hours in a horizontal shaker. The tubes were washed two further times with 1ml of wash buffer, with the contents of the tubes being decanted at each stage.

The amount of tracer specifically bound after washing was quantified by counting with a gamma scintillation counter (1261 Multigamma, Wallac UCB, Turku, Finland) over a two minute period. The content of unknown samples was estimated by reference to a standard curve constructed under identical conditions using standard samples provided by the manufacturer and the results expressed as serum PIIINP concentration (u/ml).

5.1.6. Apolipoprotein A1 and the PGA index

Apolipoprotein A-1 (APA) concentrations were measured by rate nephelometry using a commercially available kit (Beckman APA, Beckman Instruments Inc., Galway, Ireland) and a Beckman Array Protein System analyser (Beckman Instruments, High Wycombe, UK).
The analyser was calibrated using a calibrator, diluted 1:32, supplied by the manufacturer (APO Cal). 42 µl of the sample, also diluted 1:32, was injected into a cell and 42 µl of apolipoprotein A-1 antibody was added. The light scatter caused by the antigen-antibody complexes formed was detected by the analyser and converted to a peak rate signal which is a function of the sample APA concentration.

The gamma-glutamyl transferase concentration and INR were measured using standard laboratory techniques, and the three values were combined to produce the PGA index by scoring the results according to the table below and combining the scores to give a PGA value between 0 and 12 (Table 5.1.) (Poynard et al., 1991).

<table>
<thead>
<tr>
<th>Score</th>
<th>P (INR)</th>
<th>G (γGT)</th>
<th>A (apo A1)</th>
<th>mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1.25</td>
<td>&lt;20</td>
<td>≥200</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.25-1.41</td>
<td>20-49</td>
<td>175-199</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.42-1.65</td>
<td>50-99</td>
<td>150-174</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.66-1.99</td>
<td>100-199</td>
<td>125-149</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>≥2.00</td>
<td>≥200</td>
<td>&lt;125</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1. Scoring for the PGA index
5.1.7. Statistical analysis

The results were expressed as mean ± SEM.

Differences in the gender ratios of the groups studied were compared using $\chi^2$ and the results for all assays were compared between the two cystic fibrosis groups and Controls using ANOVA. When ANOVA was statistically significant (p<0.05), the results were further analysed by Fisher's t based least significant difference test. Where no control group was analysed (the MEG-X test) the results were compared using an unpaired t test.

Results were considered significant with a p-value of <0.05 for a two-tailed test.

Receiver operating characteristic curves were constructed, where appropriate, to estimate the optimal sensitivity and specificity of each test. Each curve was constructed by calculating the sensitivity (proportion of positives correctly identified) and specificity (proportion of negatives correctly identified) by the test at a particular value and plotting the sensitivity against (1-specificity) at each value (Poynard et al., 1991). The best cut-off point is that which maximises the sum of the sensitivity and specificity and corresponds to the point nearest the top left-hand corner of the curve (Altman, 1991).

Correlations between results for the four assays and the ultrasound score documented from chapter three were made using Pearson's simple least-squares regression analysis to
provide further analysis of the effectiveness of the ultrasound assessment.

5.2. Results

The diagnosis of cystic fibrosis related chronic liver disease and the sub-division into NLD, and LD was made on the basis of the ultrasound scoring system reported in chapter 3.

3.5.2.1. Conventional biochemical tests of liver function

The biochemical test results of the 68 adult subjects included in the ultrasound study have sensitivities and specificities as detailed in Table 5.2. when the results are analysed relative to the laboratory upper limit of normal (ULN). The greatest sensitivity was found for the alkaline phosphatase concentration which correctly identified 97% of the subjects with ultrasound evidence of liver disease, but was only 57% specific. The gamma-glutamyl transferase had the best combined sensitivity and specificity (85% and 89% respectively) corresponding to a positive predictive value of 88% and a negative predictive value of 86%.

When the data for these conventional tests of liver function were evaluated for their capacity to differentiate between the three ultrasound categories (NLD, ILD and SLD) none of them (except the platelet count) were able to adequately differentiate those patients with more severe hepatic involvement (Table 5.3.). The platelet counts are all within the normal range.
5.2.2. Monoethylglycinexylidide

Forty nine subjects underwent the MEG-X test. Serum MEG-X concentrations were measured before and fifteen minutes after the injection of lignocaine to give the corrected MEG-X concentration ($t_{15} - t_{0}$).

Thirty one subjects had liver disease (LD group) and nineteen no significant hepatic involvement (NLD). There was no significant difference in their ages (19.6 ± 1.1 vs 19.7 ± 2.2 years respectively) or gender distributions (18 males and 13 females vs 9 males and 9 females).

Corrected serum MEG-X concentrations for the LD group were significantly lower than those of the NLD group (38.5 ± 3.7 vs 67.0 ± 4.2 ng/ml; p<0.0001).

Eleven of the LD group fell into the intermediate (ILD) and twenty into the severe (SLD) sub-group as defined by the ultrasound scoring system. Sub division into NLD, ILD and SLD sub-groups revealed significantly different corrected MEG-X concentrations (67.0 ± 4.2; 52.0 ± 5.6; 31.1 ± 4.1 respectively; p<0.0001; (ANOVA)). Further analysis with Fisher's t-based least significant difference confirms statistically significant differences between all groups: NLD vs ILD, p<0.05; NLD vs SLD, p<0.001 and ILD vs LD, p<0.01. A scattergram of these data for the three sub-groups is shown in figure 5.1.
Construction of a receiver operating curve revealed the 'best' discrimination between the NLD and LD subjects at a corrected MEG-X concentration of ≤ 60 ng/ml. At this concentration the assay is 84% sensitive and 78% specific for an ultrasound based diagnosis of liver disease in cystic fibrosis (fig 5.2.). This corresponds to a positive predictive value of 87% and a negative predictive value of 74%.

Simple least squares regression analysis of corrected MEG-X concentration against the ultrasound score revealed a good correlation (r=0.71; p=0.0001).

5.2.3. Fasting serum bile acids

Ninety four subjects had their total 3α-hydroxy bile acid concentration measured. The Control group consisted of twenty seven volunteers (15 females and 12 males) with an average age of 26.9 ± 0.7 years. This group did not differ significantly from the thirty two NLD subjects (23.9 ± 1.4 years; 14 females and 18 males), but was significantly older than the thirty five LD subjects (average age 22.7 ± 0.9 years; p<0.005). The gender distribution of the LD subjects (14 females and 21 males) was well matched with the Controls. The NLD and LD groups were well matched with respect to age and sex.

There was no significant difference in the mean fasting bile acid concentration for the Controls (2.4 ± 0.5 µmol/l) and the NLD group (6.4 ± 0.7 µmol/l). Both groups differed significantly from
the LD group (mean concentration $36.1 \pm 5.3 \, \mu\text{mol/l}$) ($p<0.001$ for both comparisons).

Sub-division of the LD group into ILD and SLD sub-groups revealed a fasting bile acid concentration of $17.5 \pm 3.7 \, \mu\text{mol/l}$ for the 16 subjects in the ILD group and $51.9 \pm 7.7 \, \mu\text{mol/l}$ for the 19 subjects in the SLD group. Comparison between these groups by ANOVA revealed a statistically significant difference between the three sub-groups ($p=0.0001$), with significant differences ($p<0.001$) between the NLD and SLD and between the ILD and SLD groups when the data was analysed further. The differences in fasting bile acid concentration were not statistically different between NLD and ILD sub-groups. A scattergram of these data for the three sub-groups is shown in figure 5.3.

Construction of a receiver operating curve using $1 \, \mu\text{mol/l}$ cut-offs revealed that, at a cut-off of $8 \, \mu\text{mol/l}$, the serum fasting $3\alpha$-hydroxy bile acid concentration was 86% sensitive and 78% specific (positive predictive value 81%, negative predictive value 83%) (Fig 5.4.).

Simple regression analysis of fasting bile acid concentration against ultrasound score for the 67 cystic fibrosis subjects evaluated revealed a good correlation between increasing fasting serum bile acid concentration and ultrasound score ($r=0.72$, $p=0.0001$.)
5.2.4. N-terminal procollagen III propeptide

Due to the variation in serum PIIINP during childhood (Trivedi et al., 1989) data for subjects under 20 years of age was analysed separately.

The cohort of subjects 20 years or over consisted of 27 controls, 34 NLD subjects and 28 LD subjects. The groups were well matched for age (26.9 ± 0.7, 26.7 ± 1.1 and 25.3 ± 0.9 years respectively) and gender (12 males:15 females; 19 males:15 females, 17 males:11 females respectively).

Comparison of the fasting serum PIIINP concentrations between the three groups revealed significant differences for all comparisons. The Control group (mean concentration 0.48 ± 0.03 u/ml) differed significantly from the NLD (0.59 ± 0.02 u/ml; p<0.05) and the LD (0.81 ± 0.04 u/ml; p<0.001) groups. Similarly the NLD and LD groups had significantly different PIIINP concentrations (p<0.001) (Fig 5.5.).

Further analysis of the cystic fibrosis cohort incorporating the severity of the liver disease into the analysis revealed a significant difference in the NLD PIIINP concentrations with those of the ILD (0.79 ± 0.06 u/ml; p<0.01) and the SLD (0.82 ± 0.06 u/ml) groups (p<0.001 for both comparisons). There was, however, no significant difference between the ILD and SLD PIIINP concentrations (Fig 5.6.).

Construction of a receiver operating curve for the PIIINP concentration, using 0.1 u/ml cut-off points from 0.2 u/ml to 1.0 u/ml, revealed an optimum performance of the PIIINP assay in
detecting liver disease at 0.7 u/ml. The sensitivity at this concentration was 68% and the specificity 82% (Fig 5.7.). This corresponds to a positive predictive value of 76% and a negative predictive value of 76%. At 0.6 u/ml the sensitivity was 86% but the specificity fell to 59%.

Simple regression analysis of PIIINP concentration against the ultrasound score gave an r value of 0.42. This relationship was statistically significant (p=0.01).

Analysis of the data for the subjects aged between 15 and 20 and those aged under 15 revealed no significant difference in age, gender or PIIINP concentrations (Tables 5.4. and 5.5.).

5.2.5. Apolipoprotein A1 and the PGA index

80 cystic fibrosis patients (42 NLD and 38 LD) and 27 control subjects underwent measurement of APA and subsequent calculation of the PGA index. The groups were well matched for sex, but the controls were significantly older than the cystic fibrosis cohorts (Controls: 26.9 ± 0.7; NLD: 22.2 ± 1.2; LD: 21.8 ± 1.1 years; p<0.01 for comparisons between controls and both cystic fibrosis groups).

The mean APA concentration was not different between the three groups (150 ± 6, 170 ± 6, and 170 ± 8 mg/dl respectively).

The mean PGA index was not significantly different between the Controls and NLD cystic fibrosis group (2.9 ± 0.3 and 2.2 ± 0.2 respectively). Both groups had a significantly lower PGA index
scores than the LD cystic fibrosis group (5.1 ± 0.2; p<0.001 for both comparisons). A scattergram detailing the distribution of the individual PGA index scores is shown in Figure 5.8.

Sub-division of the LD group into ILD and SLD sub-groups revealed a PGA index score for the ILD group of 4.4 ± 0.3 which differed significantly from the NLD group (p<0.001), and from the SLD group (PGA index 5.7 ± 0.3; p<0.01). Similarly there were significant differences between NLD and SLD groups at the p<0.001 level.

Construction of a receiver operating curve revealed a sensitivity of 87% and specificity of 76% at a cut-off PGA index score of 4. This corresponds to a positive predictive value of 77% and a negative predictive value of 86% (Fig 5.9.)

Simple regression analysis of the PGA index against the ultrasound score revealed a significant correlation (r=0.72; p=0.0001) between the two variables.
<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+ve predictive value (%)</th>
<th>-ve predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (ULN 50 u/l)</td>
<td>68</td>
<td>97</td>
<td>95</td>
<td>77</td>
</tr>
<tr>
<td>ALT (ULN 40 u/l)</td>
<td>84</td>
<td>81</td>
<td>82</td>
<td>83</td>
</tr>
<tr>
<td>γGT (ULN 50 u/l)</td>
<td>85</td>
<td>89</td>
<td>88</td>
<td>86</td>
</tr>
<tr>
<td>ALP (ULN 250 u/l)</td>
<td>97</td>
<td>57</td>
<td>68</td>
<td>95</td>
</tr>
<tr>
<td>Bilirubin (ULN17 μmol/l)</td>
<td>42</td>
<td>91</td>
<td>82</td>
<td>63</td>
</tr>
</tbody>
</table>

Table 5.2. Table of sensitivity and specificity values for the conventional tests of liver function in the diagnosis of liver disease
<table>
<thead>
<tr>
<th>Test</th>
<th>NLD vs ILD</th>
<th>NLD vs SLD</th>
<th>ILD vs SLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
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<td>γGT</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Albumin</td>
<td>NS</td>
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</tr>
<tr>
<td>Platelets</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 5.3. Results of comparisons by Fisher's t based least significant difference showing lack of discrimination between ultrasound sub-groups by the conventional biochemical tests of liver function.
Figure 5.1. Scattergram and mean ± SEM of corrected MEG-X concentrations for the three ultrasound sub-groups
Figure 5.2. Receiver operating curve for the MEG-X assay
Figure 5.3. Scattergram of the fasting bile acid concentrations (and mean ± SEM) in control, NLD and LD groups
Figure 5.4. Receiver operating curve for fasting bile acid concentration
Fig 5.5. Scattergram and mean ± SEM of PIIINP concentrations
Figure 5.6. Scattergram and mean ± SEM of PIIINP concentrations for the ultrasound sub-groups
Figure 5.7. Receiver operating curve for the PIIINP concentration
<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Age</td>
<td>17.5 (16-19)</td>
<td>17.6 (16-19)</td>
</tr>
<tr>
<td>Sex</td>
<td>3 M: 3 F</td>
<td>6 M: 3 F</td>
</tr>
<tr>
<td>PIIINP (u/ml)</td>
<td>1.04 (0.81-1.2)</td>
<td>1.3 (0.48-1.9)</td>
</tr>
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</table>

Table 5.4. Age, gender and PIIINP (median (range)) data for the 15 to 20 year old cohort
<table>
<thead>
<tr>
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<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
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<td>7</td>
</tr>
<tr>
<td>Age</td>
<td>12 (5-15)</td>
</tr>
<tr>
<td>Sex</td>
<td>5 M: 2 F</td>
</tr>
<tr>
<td>PIIINP (u/ml)</td>
<td>1.05 (0.57-1.47)</td>
</tr>
</tbody>
</table>

Table 5.5. Age, gender and PIIINP (median (range)) data for the under 15 year old age group
Figure 5.8. Scattergram of the PGA index results for the three groups
5.3. Discussion

5.3.1. Monoethyglycine-glutamine

The measurements of plasma-free betaine-MG-X in this cohort have revealed an elevated plasma betaine metabolism in cirrhotic patient with an increase in fasting in addition to a significant serum concentration. These results suggest that the assay may be useful for the assessment of the severity of hepatic disease, especially in the absence of other biochemical assessments.

The results agree with previous reports and measurements in patients with cirrhotic liver disease. The increased levels of betaine-MG-X concentrations with liver disease and cirrhosis are indicative of decreased betaine-MG-X and could support for the need of assessing liver disease in cirrhotic patients.

However, the obesinolamia of glycine provides a number of advantages for this test. While only about 1% of glycine is secreted in the urine (Koizumi, J. et al., 1971), and MG-X formation is not affected by renal dysfunction.

Figure 5.9. Receiver operating curve for the PGA index
5.3. Discussion

5.3.1. Monoethylglycinexylidide

The measurement of the lignocaine metabolite MEG-X in this cohort has revealed reduced hepatic lignocaine metabolism in cystic fibrosis patients with chronic liver disease. In addition serum MEG-X concentrations were successively significantly lower in the ILD and SLD sub-groups suggesting that the assay may be useful for the assessment of the severity of hepatic disease (based upon ultrasound assessment).

The results of this study are in keeping with many previous reports of MEG-X measurement in patients with chronic liver disease. The close correlation of MEG-X concentration with histology in adults (Schiffman et al., 1994) and with a validated composite score in children (Gremse et al., 1990) provide support for this means of assessing liver disease in cystic fibrosis.

The advantage of the test, when compared with other quantitative hepatic function tests, is that it is simple, rapid and reliable and that the assay is easy to perform using a commercially available kit (Schiffman et al., 1994).

However, the pharmacokinetics of lignocaine provide a number of limitations of this test. While only about 3% of lignocaine is excreted intact in the urine (Keenaghan & Boyes, 1972), and MEG-X formation is not affected by renal dysfunction.
(Collinsworth et al., 1975), the conversion of lignocaine to MEG-X is dependent on oxidative N-deethylation by cytochrome P-450 IIIA (P-450 IIIA) (Bargetzi et al., 1989). This process can be influenced by the concomitant use of drugs that result in enzyme induction (phenobarbitone, dexamethasone, corticosteroids) (Guenerich, 1989) or substrate competition (Feely et al., 1982; Conrad et al., 1983).

In addition, there is a wide variation in P450 IIIA activity in the general population (Bargetzi et al., 1989; Watkins, 1990) that may have a genetic basis, and which probably accounts for the wide range of MEG-X concentrations obtained when the test is performed in normal controls (Thomson et al., 1987). Furthermore the presence of the P450 IIIA enzyme in the intestine (Kolars et al., 1992) might also contribute to lignocaine metabolism (Schiffman et al., 1994), although the high first pass hepatic metabolism would minimise this. This enzyme may, however, be important in patients with significant portal-systemic shunting (Schiffman et al., 1994).

Despite these caveats, in the present study the range of MEG-X concentrations obtained in the NLD group (27 - 107 ng/ml) corresponds well with other series (34 - 111 ng/ml) (Thomson et al., 1987) as does the mean concentration for both NLD and LD groups (Huang et al., 1993). In addition, the MEG-X value (60ng/ml in this study) which maximises the diagnostic sensitivity and specificity of the assay in detecting liver disease corresponds well with that previously reported (54 ng/ml) (Huang et al., 1993).
It should be noted that two of the LD subjects were on corticosteroids, which may in theory have resulted in enzyme induction and elevated concentrations of MEG-X. However, the serum concentrations of MEG-X for these two subjects were very much at the low end of the spectrum of values (26.2 and 34.4 ng/ml). None of the other patients were taking medications likely to affect lignocaine metabolism.

These results suggest that measuring the serum MEG-X concentration in no more sensitive or specific than some conventional biochemical tests of liver function in confirming an ultrasound based diagnosis of liver disease. However, its measurement in conjunction with ultrasound may give a measure of disease severity.

The MEG-X test may also predict the risk of developing the complications of cirrhosis (Schiffman et al., 1992) and may identify a cohort of patients more likely to die from their hepatic disease (Huang et al., 1993). It may also prove valuable in monitoring the response to therapy (Schiffman et al., 1994).

These hypotheses remain to be prospectively evaluated.

5.3.2. Fasting serum bile acids

The measurement of serum bile acids in this cohort of cystic fibrosis patients revealed significant elevations in subjects with LD and significant differences between the ILD and SLD subgroups as defined by the ultrasound scoring system. There was
also a highly significant correlation between increasing ultrasound score and the bile acid concentration.

The assay had an 86% sensitivity and 78% specificity for the detection of liver disease in cystic fibrosis when a cut-off of 8μmol/l was used. This cut off value is higher than the upper limit of normal for the assay quoted by the manufacturer of the Enzabile kit (6μmol/l), but is similar to the upper limit of the normal range quoted in other series (Rickers et al., 1982; Ferraris et al., 1987) and lower than the upper limit of the range of values obtained in the Control cohort in this study (9.76 μmol/l).

While these results were obtained in a predominantly adult cohort previous reports showing that adult and paediatric cystic fibrosis patients have similar fasting bile acid concentrations (Strandvik & Samuelson, 1985) suggest that they can be extrapolated to the cystic fibrosis population in general.

The sensitivity and specificity of fasting serum bile acids in the diagnosis of hepatic disease of aetiologies other than cystic fibrosis has been a matter of controversy. A 78% sensitivity and 94% specificity has been reported for bile acids, measured by radioimmunoassay, in detecting histologically proven liver disease (Ferraris et al., 1987). The poor sensitivity of the test was particularly marked in patients with histologically mild disease, in whom the serum aspartate aminotransferase was reported to be a more sensitive marker. In contrast Mannes et al. reported a 93% sensitivity of bile acids, measured by radioimmunoassay in detecting liver cirrhosis. This was
increased to 97% if the fasting and postprandial concentrations were used (Mannes et al., 1982). The sensitivity of the assay in this study is lower. This may be due to methodological differences, but may also be related to the fact that some of the cystic fibrosis patients may not have fasted.

It has been suggested that enzymatic methods, such as the one employed in this study, are less accurate in detecting the concentration of bile acids in the low or normal range (Kaplowitz, et al., 1973). The sensitivity of enzymatic methods is increased by coupling the assay to a fluorescein indicator (Hofmann, 1982) as in the present study, but it is this proposed inaccuracy at low concentrations that have led to other techniques for measuring bile acids, such as radioimmunoassay and gas liquid chromatography, being advocated (Kaplowitz et al., 1973; Hofmann, 1982; Ferraris et al, 1987).

In the present study the mean fasting bile acid concentrations for the controls and the mean concentration for the NLD group fall within the normal range suggested by the manufacturer of the assay. The differences between them (Control and NLD) can probably be explained by the possibility that some of the cystic fibrosis subjects were not in the fasting state. This is a well recognised limitation of this test (Hofmann, 1982).

Some investigators have suggested that the inaccuracy of the assay at low concentrations might be reduced by using post-prandial bile acid concentrations (Kaplowitz et al., 1973; Fausa, 1976). However, not all investigators have detected significant differences in pre- and post-prandial results (Pennington et al.,
1977; Linnet et al., 1983) and it may be that a 2 hour post-prandial concentration is less reliable as the post-prandial peak may be reached between 1 and 3 hours after the meal (Hofmann, 1982).

The factors contributing to fasting serum bile acid concentrations include intestinal absorption and hepatic uptake (Gilmore & Thompson, 1981). In patients with hepatic disease their concentration reflects hepatocyte loss and dysfunction, as well as portal-systemic and intra-hepatic shunting (Hofmann, 1982). Their concentration is inversely proportional to indocyanine green clearance and as such the functional hepatic blood flow (Islam et al., 1985).

In cystic fibrosis evaluation of fasting serum bile acid concentrations is further complicated by the evidence that ileal reabsorption of bile acids is abnormal (Fondacaro et al., 1982). More recently it has been suggested that terminal ileal malabsorption occurs predominantly in patients without chronic liver disease (O'Brien et al., 1993). These increased faecal losses of bile acids are compensated by increased hepatic bile acid synthesis and the resultant serum bile acid pool is of a similar size to that of normal controls (Setchell et al., 1985). However, the terminal ileal defect coupled with the high proportion of subjects with an abnormal gall bladder in cystic fibrosis (Isenberg et al., 1976) suggest that the post-prandial bile acid concentration is likely to be less accurate than the concentration estimated in the fasting state.
Previous reports evaluating the measurement of bile acids in patients with cystic fibrosis related chronic liver disease are hampered by the diagnostic criteria employed. An increased concentration has been reported in all those with palpable hepatomegaly and as a consequence the measurement of bile acids was advocated as a test for early liver involvement in cystic fibrosis (Davidson et al., 1980). However, more recent reports suggest that fasting serum bile acids correlate poorly with the degree of liver injury (Matsui et al., 1982; Roy et al., 1982). Subsequent work on serum cholic and chenodeoxycholic acid concentrations confirmed the lack of correlation with liver disease (Strandvik & Samuelson, 1985) but these evaluations were based upon clinical, biochemical and histological parameters which, as has already been discussed, all have major limitations in confirming a diagnosis of liver disease in cystic fibrosis.

The results obtained in this study suggest that the measurement of fasting bile acids correlates well with an ultrasound diagnosis of liver disease in cystic fibrosis. However, the fasting serum bile acid concentration did not distinguish between the NLD and ILD groups suggesting limited applicability of the assay in diagnosing early hepatic disease. In addition, the sensitivity of the test is little better than some of the conventional liver function tests and the specificity is somewhat worse.

Fasting bile acid concentration may be of prognostic significance in chronic liver disease of other aetiologies (Mannes et al., 1986). Patients with a serum bile acid concentration less than 20 μmol/l
may have a good prognosis, and those with a concentration over 50 μmol/l may have a poor prognosis. Whether these criteria have any applicability to prognosis in cystic fibrosis or to the assessment of the need for hepatic transplantation in patients undergoing lung transplant assessment requires prospective evaluation.

5.3.3. N-terminal procollagen III peptide

The results of the measurement of serum PIIINP concentration in this study revealed (in subjects 20 years old and over) a significant difference between Controls, NLD and LD groups. The PIIINP concentration did not, however, detect increasing liver disease severity as defined by the ultrasound score and was relatively poor at distinguishing between cystic fibrosis patients with and without liver disease on the basis of the sensitivity and specificity of the test as measured by the receiver operating curve.

The results for the small cohort of subjects under 20 years old confirm the inapplicability of this assay to patients who are still growing due to the contribution to PIIINP concentrations by interstitial procollagen turnover attributable to growth (Trivedi et al., 1989). The median values obtained in this study in the under 15s correspond well to those reported by the manufacturer, while the values in the 15 to 20 year olds are higher (manufacturers median 0.76 u/ml; range 0.29-1.95 u/ml).
The PIIINP concentration has been used as a means of monitoring veno-occlusive disease in children after bone marrow transplantation (Eltumi et al., 1993). To make it possible to make comparisons between subjects of differing age concentrations were standardised for age and sex by calculating a standard deviation score (Trivedi et al., 1989). While this approach may prove of value in comparing subjects of similar age with and without liver disease in cystic fibrosis, the detailed data necessary to make such standardised calculations are not currently available for the assay used in this study.

Procollagen III peptide is enzymatically cleaved from procollagen during the formation of collagen (Fessler & Fessler, 1978). Its serum concentration is also influenced by the breakdown of previously deposited procollagen III peptide and degradation by the liver (Alcorn & Chojkier, 1987; Bentsen et al., 1988). The relative contribution of these factors to serum PIIINP concentrations is unknown but a good correlation with the activity of hepatic prolyl hydroxylase, the enzyme related to the rate of collagen synthesis (Mann et al., 1979), in man (Torres-Salinas et al., 1986) and the level of hepatic fibrogenesis in a rat model of hepatic fibrosis has been demonstrated (Hayasaka et al., 1991).

Studies in patients with liver fibrosis reveal two types of collagen in association with liver disease (types I and III) (Gay et al., 1975), with evidence that the deposition of type III precedes that of type I (Wick et al., 1978). A significant correlation between PIIINP concentration and histologically
determined collagen content has been demonstrated, although it has been suggested that type I collagen may be a better marker of more advanced liver disease (Raedsch et al., 1982). PIIINP may, therefore, be a useful parameter for the assessment of less advanced liver disease in patients in whom a liver biopsy is not possible.

Studies of PIIINP in patients with the other biliary cirrhocides have yielded conflicting results. In primary biliary cirrhosis, PIIINP correlates with survival (Schuppan, 1991) and with the Mayo Prognostic index score (Teare et al., 1993). An increase in PIIINP concentration with worsening histological stage in primary biliary cirrhosis has also been demonstrated, although the results were not statistically significantly different (Teare et al., 1993).

In contrast, studies in patients with primary sclerosing cholangitis reveal a significant increase in PIIINP concentration when compared with control groups, which correlated with hepatic fibrosis and portal tract inflammation but not with the Mayo histological stage (Mehal et al., 1992). In addition, the PIIINP level did not correlate with survival (Mehal et al., 1992).

These results suggest that PIIINP measurements might have been of some value in the evaluation of hepatic involvement in cystic fibrosis. However, a major confounding factor is likely to be the documented elevation of PIIINP in about 20% of patients suffering from some forms of fibrotic lung disease (idiopathic pulmonary fibrosis and sarcoidosis) (Rohde et al., 1979; Low et al., 1992), and it is therefore not surprising that the results are
disappointing. Comparisons have not been made, however, with the amount of fibrosis on liver histology specimens.

It should also be noted that the assay used for this study detects both intact circulating PIIINP and low molecular weight fragments (such as Col 1, Mr 10,000) produced by fibrinolysis (Schuppan, 1991). Thus increased fibrinolytic activity might result in marked elevation of the PIIINP concentration measured by this assay. More sensitive assays are being developed which measure intact fragments of PIIINP alone and may allow more accurate assessment of hepatic fibrogenesis and fibrinolysis (by allowing comparison with less sensitive assays, the difference being due to the products of fibrinolysis) (Schuppan, 1991). The use of such assays may have improved the accuracy of the PIIINP level in detecting liver disease in this cohort.

In summary, estimation of the serum PIIINP concentration in cystic fibrosis is of limited applicability as a means of detecting hepatic involvement due to the probable confounding influence of the relatively young age of many of the affected cohort and the presence of active fibrotic lung disease in many of the patients.

It has been suggested that the measurement of other procollagen peptides such as the amino and carboxyl terminal propeptides of procollagen type IV and the laminin P1 fragment may be better markers of fibrotic liver disease in younger patients (Schuppan, 1991). It is not known whether the use of such assays would have yielded more information in this cohort.
One possible application for PIIINP measurement may be in the assessment of disease progression and the monitoring of the response to therapy (Schuppan, 1991). This remains to be evaluated.

5.3.4. Apolipoprotein A1 concentrations and the PGA index.

This study reveals a poor correlation between the apolipoprotein A1 concentration and the ultrasound evaluated hepatic status of the patients. The concentrations documented in all three groups are similar to those reported for controls in other series (males 171 ± 28, females 163 ± 33 mg/dl (Poynard et al., 1986)) and also correlate well with concentrations of APA in healthy controls in our laboratory (158.6 mg/dl).

Lipoprotein abnormalities in patients with liver disease are complex, but include a reduction in APA in patients with more advanced liver disease (Malmendier et al., 1983; Jahn et al., 1985). It has been suggested that serum APA has a higher predictive value for fibrosis than serum laminin and procollagen III propeptide (Poynard et al., 1989). However, of particular relevance to the present study is the elevation of APA in stage I and II primary biliary cirrhosis (Jahn et al., 1985) (according to the classification of Scheuer (Scheuer, 1973)), together with the fact that the intestine is also a significant source of the protein (Poynard et al., 1986).
If the progression of liver disease in cystic fibrosis follows a similar pattern to primary biliary cirrhosis it might be expected to follow a similar pattern of APA changes. APA might then help discriminate the early and late stages of the disease process. The results presented here suggest that this is not the case and it is probable that the serum APA concentration reflects the well maintained synthetic function usually found in patients with cystic fibrosis related chronic liver disease. While the contribution of disordered intestinal function to serum APA concentrations may be abnormally high in cystic fibrosis this has not been formally investigated.

The construction of the PGA index was based upon a study in patients with alcoholic liver disease in which APA, γGT and the prothrombin time were shown to be independent markers of the severity of liver disease (Poynard et al., 1986). Alcohol increases serum APA concentration early in the disease process, although the mechanism for this increase is unclear (Luoma et al., 1984; Poynard et al., 1986), suggesting that extrapolation of the index to populations of patients with other forms of liver disease may not be appropriate.

However, its application in chronic liver disease due to alcohol, hepatitis B and primary biliary cirrhosis has recently been reported (Teare et al., 1993). The PGA index in patients with primary biliary cirrhosis correlated well with the Mayo prognostic index score, a well validated measure of disease severity (Dickson et al., 1989).
The results for the PGA index obtained in this study confirm that it has some value in the discrimination between patients with and without liver disease in cystic fibrosis and that the PGA index increases as the ultrasound score of liver disease becomes more severe. However, the sensitivity and specificity of the index is not dissimilar from that of the standard biochemical tests of liver function in diagnosing liver disease. This probably reflects the similarity in NLD and LD APA concentrations, the fact that it is unusual for the INR to be elevated and the well recognised transient increases in γGT in cystic fibrosis patients without hepatic parenchymal involvement.

Overall the applicability of the PGA index is, therefore, likely to be of limited value.

5.4. Conclusions

This study has assessed four different biochemical measures of hepatic disease. Two are dependent on hepatic parenchymal function and two are markers of hepatic fibrosis. Their sensitivities and specificities for confirming an ultrasound based diagnosis of liver disease, which are no better than some of the conventional biochemical tests of liver function, are summarised in Table 5.6.

The most valuable information may be derived from the MEG-X assay, which has been shown to have a sensitivity and specificity similar to conventional biochemical tests of liver function while also allowing discrimination between patients
with mild and severe hepatic involvement as defined by the ultrasound scoring system. This discriminant capacity was not found for any other assay evaluated in this study. The use of the MEG-X test in conjunction with ultrasonography may prove useful in confirming hepatic disease and estimating disease severity in cystic fibrosis.

Of the other assays assessed both the fasting serum bile acid and PIIINP assays do not, in this study, distinguish between all subgroups and the PGA index is, as already discussed, of limited use given the pattern of disease in cystic fibrosis.

Both the fasting serum bile acid and MEG-X assays may be of value in assessing the severity of hepatic disease in patients pre transplantation and these two assays together with the PIIINP concentration may allow the response to therapy to be monitored.

It may be possible to combine some or all of these biochemical tests into a prognostic index allowing more sensitive and specific diagnosis of liver disease. However, their application, as evaluated in this study, will always be limited by the dependence on ultrasound criteria for the detection of hepatic disease.
### Table 5.6. Summary of sensitivity and specificity of the assays used to diagnose liver disease in cystic fibrosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+ve predictive value (%)</th>
<th>-ve predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEG-X</td>
<td>84</td>
<td>78</td>
<td>87</td>
<td>74</td>
</tr>
<tr>
<td>Bile acids</td>
<td>86</td>
<td>78</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td>PIIINP</td>
<td>68</td>
<td>82</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>PGA index</td>
<td>87</td>
<td>76</td>
<td>77</td>
<td>86</td>
</tr>
</tbody>
</table>

It is important to note that the +ve predictive value and -ve predictive value are crucial for understanding the accuracy of the tests. A high +ve predictive value indicates that a positive test result is likely to be accurate, while a high -ve predictive value indicates that a negative test result is likely to be accurate.
CHAPTER 6

SYSTEMIC HAEMODYNAMIC CHANGES IN PATIENTS WITH CYSTIC FIBROSIS

an adverse effect of underlying cystic fibrosis liver disease.
6.0. **Introduction**

There are well documented circulatory changes associated with chronic liver disease, although their mechanism remains obscure (Reichen, 1992). The circulation becomes hyperkinetic with an increased cardiac output associated with decreased peripheral vascular resistance and a tendency towards arterial hypotension (Kowalski & Abelmann, 1953).

Clinically there is evidence of increased peripheral blood flow (bounding pulse, capillary pulsations), and of increased cardiac output (tachycardia, active precordial impulse, ejection systolic murmurs) (Murray et al., 1958). In addition, there is a documented reduction in oxygen consumption in spite of the increase in oxygen transport (Moreau et al., 1988), thought to be related to physiological shunting of blood through the tissues.

It has been suggested that the hyperdynamic circulation, together with altered oxygen utilisation could be a factor in the pathogenesis of multiple organ failure in end-stage liver disease (Sherlock, 1990).

Following the demonstration that chronic liver disease was an independent risk factor for increased mortality in cystic fibrosis (as described in chapter 2 of this thesis), it was hypothesised that the presence of a hyperdynamic circulatory state in patients with cystic fibrosis related liver disease might be a contributory factor to accelerated mortality.
The aim of the present study was to test this hypothesis by evaluating the circulatory status of a group of patients with cystic fibrosis, with and without liver disease, for evidence of the hyperdynamic circulation.

Invasive measurement of circulatory parameters in cystic fibrosis patients was deemed unacceptable due to the small but appreciable risks involved - in particular the risk of pneumothorax from central vein cannulation and the likelihood that any complication associated with invasive evaluation was likely to be poorly tolerated. The study was designed, therefore, to use entirely non-invasive techniques.
6.1. **Patients and methods**

6.1.1. Patients

Fifty six stable cystic fibrosis patients attending the out-patient departments at The Royal Brompton Hospital were recruited for the study. Selection of patients was random in that all suitable candidates who attended the clinics were approached. Subjects known to have hepatic disease were selectively approached to ensure adequate recruitment to this cohort. Only subjects with very advanced pulmonary disease who were considered unlikely to be able to tolerate the exercise protocol were excluded.

Patients were categorised into the LD group on the basis of an abnormal hepato-biliary ultrasound scan using the criteria documented in chapter three.

The severity of liver disease was judged using the previously validated ultrasound scoring system (subjects were categorised into NLD, ILD and SLD groups on this basis (see Chapter 3)) and by using the Child-Pugh classification (a recognised system for grading severity of liver disease based upon five patient characteristics: encephalopathy, ascites, bilirubin, albumin and prothrombin time) (Pugh et al., 1973).

6.1.2. Control subjects

Mercury strain gauge plethysmography was performed on 12 healthy adult Control subjects, who had no clinical evidence of
pulmonary or hepato-biliary disease and who were members of the Departments of Gastroenterology or Respiratory Medicine at Charing Cross Hospital.

6.1.3. Methods

6.1.3.1. Clinical history and examination

Data on current clinical problems including whether or not the subject had been admitted to hospital during the previous year for complications related to cystic fibrosis (e.g. respiratory exacerbations), and current drug history (on the day of the exercise test) were obtained for all subjects.

Clinical examination placed an emphasis upon signs consistent with liver disease (hepatomegaly, splenomegaly or both) together with the peripheral stigmata of chronic liver disease (SCLD) (palmar erythema, spider naevi etc.) (Saraky, 1987) and any evidence of a hyperkinetic circulation (warm flushed extremities, bounding pulses etc.).

The subjects were weighed (Kg) and measured (m) using the same scales and height measure on the day of the test.

6.1.3.2. Sputum colonisation

The most recent sputum culture result was obtained for each subject.
6.1.3.3. Lung function tests

The subjects underwent baseline lung function tests (forced expiratory volume in 1 second (FEV$_1$) and forced vital capacity (FVC)) recorded on the day of haemodynamic assessment (Vitalograph spirometer, Vitalograph, Buckingham, UK). These were expressed as percentages of the predicted value for age and height (e.g. percentage predicted FEV$_1$ (%PFEV$_1$)) (Cotes, 1991).

Other lung function parameters (Total lung capacity (TLC), residual volume (RV) (Body plethysmograph, Fenyves & Gut, Basel, Switzerland), transfer factor (T$_L$CO) (Model B analyser, PK Morgan, UK) and transfer factor corrected for lung volumes (KCO)) were obtained from formal lung function tests performed as part of the patients' regular out-patient follow-up. These lung function tests were also expressed as percentage of predicted value (Cotes, 1991) and must have been recorded within six months of the study (without evidence of marked deterioration in the intervening period).

6.1.3.4. Chest radiograph and electrocardiogram

The most recent out-patient chest radiograph (CXR) and 12-lead electrocardiogram (ECG) were obtained for each subject to look for evidence of cor pulmonale (increased cardio-thoracic ratio, right axis deviation, right ventricular hypertrophy, peaked p waves).
6.1.3.5. Haemodynamic and ventilatory assessment

Subjects had the following variables recorded in the 4th minute of a period at rest and then at steady state in the 4th minute of 25 watt exercise seated on a bicycle ergometer (Lode, Holland) (Figure 6.1):

(i) Heart rate was continuously recorded with a 3 lead ECG (CASE, Marquette, USA).

(ii) Blood pressure was recorded using a semi-automated sphygmomanometer (Narco, Houston, USA) pre set to make recordings every 1½ minutes. Mean arterial pressure (MAP) was calculated using the formula MAP = diastolic blood pressure + 1/3 pulse pressure.

(iii) Respiratory airflow was measured using a heated pneumotachometer (Fleisch No.2) mounted on a mouthpiece. A nose clip was applied to prevent nasal ventilation. From this flow signal, an automatic system (Ergostar, Fenyves & Gut, Basel, Switzerland) computed averages every 30 seconds of respiratory rate (f), tidal volume (VT) and minute ventilation (VE). Oxygen uptake (VO2) and carbon dioxide production (VCO2) were calculated every 30 seconds from integrated measurements of airflow together with mixed expired carbon dioxide (FE CO2) (infrared analyser) and expired oxygen (FE O2) (paramagnetic analyser) measured in a flow-weighted sample of expired gas.

The system is able to derive volume from airflow over one breath and this function was used to calibrate the airflow: a known volume of air (1 litre) was passed, at physiological rates,
through the pneumotachometer and the amplifiers in the system were adjusted until the volume reported by the automated system equalled the volume used (± 3%). The gas analysers were calibrated before each study with air and two standard gas mixtures (100% N₂; 5% CO₂/12% O₂/balance N₂).

(iv) Arterial oxygen saturation was measured using a pulse-oximeter (Ohmeda Biox 3700, BOC Healthcare Ltd.). A vasodilator (Finalgon ointment, Boehringer Ingelheim) was applied to the left ear lobe and an ear probe attached. The pulse-oximeter was set to 'fast-response' mode to give a continuous reading of a 3 second moving average of the arterial oxygen saturation (SaO₂).

(v) End-tidal carbon dioxide, an estimate of arterial pCO₂, was measured from expired air sampled distal to the pneumotachometer through an infrared gas analyser (Beckman LB-2, Beckman Instruments). This system was calibrated with room air (0.03% CO₂) and a standard gas containing 5% CO₂/12% O₂/balance N₂. The ambient barometric pressure (PBAR) was used to convert FETCO₂ into PETCO₂.

(vi) Cardiac output (1 min⁻¹) was estimated by measuring aortic blood velocity using a 2MHz pulsed Doppler supra-sternal ultrasound probe (Pedof, Vingmed, Norway) interfaced to a Discrete Fourier Transform analyser (Doptek, Chichester) (Innes et al., 1987). By observing a spectral display the probe was angled and the depth of measurement adjusted at rest until the highest detectable peak ejection velocity was found. This setting was maintained for recordings made at rest and during exercise. The audiofrequency Doppler signals representing flow towards
the probe were recorded on an analogue tape-recorder (Store 4 DS, Racal Recorders Ltd.) for off-line analysis (see below).

A phonocardiogram was simultaneously recorded from the aortic area precisely to define end-ejection from the aortic second sound during Doppler signal analysis.

On a separate occasion, M-mode echocardiography was used to measure the diameter of the aortic root using the leading edge to leading edge technique; this measurement is required to calculate the cardiac output (see below).

Doppler signal analysis was performed off-line by replaying the taped audio-frequency signals through the spectrum analyser, and capturing the digital spectral data on a Research Machine 380Z microcomputer. The person performing the off-line analysis was blinded to the status of the cases (NLD or LD). An ensemble-averaged sonogram was obtained for 30-60 consecutive heart beats and the mean blood velocity for each 5 ms during ejection was calculated, then integrated for the entire ejection period and multiplied by the aortic root cross-sectional area (calculated from the M-mode aortic root diameter) to give an estimate of stroke volume (SV). This method has been validated against simultaneous thermodilution and invasive aortic blood velocity measurements (Innes et al., 1987).

Cardiac output was calculated from the product of heart rate and SV.

Cardiac index (CI) \( (1 \text{ min}^{-1} \text{ m}^{-2}) \) was calculated from the cardiac output and surface area of each subject which was estimated
from the height and weight using a nomogram (Du Bois & Du Bois, 1916).

6.1.3.6. Derived variables

Two other haemodynamic variables were derived from the available data:

The left ventricular stroke work index (LVSWI) (gm.min.m⁻²; normal range 29-39) according to the formula:

\[ \text{LVSWI} = \frac{\text{SV}}{\text{surface area}} \times (\text{MAP} - \text{PAWP}) \times 0.0136 \] (Haupt & Rackow, 1983), where PAWP is the pulmonary artery wedge pressure. In the absence of a direct measurement of PAWP this was assumed to be zero.

Systemic vascular resistance index (SVRI) (dynes.s.cm⁻⁵.m⁻²; normal value 2140 ± 240) according to the formula:

\[ \text{SVRI} = \frac{(\text{MAP} - \text{mean right atrial pressure}) \times 80/\text{CI}}{\text{CI}} \] (Fromm et al., 1987)

In the absence of a direct measurement of right atrial pressure, and in the knowledge that there was no clinical evidence of cor pulmonale, ECG or CXR evidence of right heart strain for any of the subjects included in the study, the right atrial pressure was assumed to be zero.
Figure 6.1. Experimental set-up for the haemodynamic study
6.1.3.7. Peripheral circulatory assessment.

Forearm blood flow (FBF) in mls/100mls tissue/min was measured using mercury-in-Silastic (Sonicaid, UK) strain gauge venous occlusion plethysmography (Hewlett & Van Zwaluwenberg, 1909-10; Whitney, 1953).

Measurements were made in a room kept at a constant temperature (20-21°C) and with the subject lying with the upper body at approximately 30° to the horizontal. The temperature and humidity of the room were recorded at the start of the study and the subjects' skin temperatures were recorded at the start and finish of the study (Ellab CTD 85, Ellab, Copenhagen; accurate to ± 0.1°C, manufacturers data).

The strain gauge was placed around the left forearm, at the point of its greatest circumference, and the forearm was supported on a piece of foam at about the level of the right atrium, such that the subject was comfortable and relaxed. An occlusion cuff was placed around the wrist and inflated to 200 mmHg to exclude the circulation of the hand. Two further cuffs were placed proximal to the elbow. The most distal of these was used for venous occlusion by intermittent inflation to 60 mmHg (Whitney, 1953). A large pressurised reservoir was used for rapid inflation of the venous cuff.

Changes in gauge resistance, consequent upon blood filling the capacitance vessels and stretching the gauge after venous occlusion, were recorded on a chart recorder (Mingograf 800, Siemens) with the paper speed set at 5mm/sec. and with the
gain adjusted to give an angle of about 45 degrees to the baseline of the recorded output of the strain gauge on venous occlusion.

To calculate the FBF the steepest straight line possible was drawn through the waveform produced by two beats at the same point in the cardiac cycle. This was either beats 1 and 2 or beats 2 and 3 (numbered from cuff inflation); beats 1 and 2 were only used if beat 1 occurred after full cuff inflation and the absence of cuff artefact had been demonstrated (Figure 6.2.) (Wilkins et al., 1938). Ten recordings of baseline flow were made over a 5 to 10 minute period, the angle of the slope was measured (to within ± 1 degree) and the results averaged. The product of the tangent of the angle of the slope and the paper speed (300mm/min) gives the change in limb circumference due to vascular filling following venous occlusion (divisions/min).

The strain gauges were calibrated off the limb on a former (Roberts et al., 1986). The gauge was stretched and, when the stretch increased the resistance sufficiently to return the recording needle to the baseline reading for the study, the length of the gauge corresponded to the forearm circumference. The length of the gauge was measured (mm).

It was then stretched further using a screw, whose pitch had been determined using a travelling microscope, to allow calculation of the number of divisions the record increased for a given stretch of the strain gauge (mm/division). The product of the calibration and change in limb circumference gives the gauge stretch (mm/min) and
FBF = 2 x Gauge stretch (mm/min) x 100 (ml/100ml tissue/min)
Limb circumference (mm)

The second cuff on the upper arm (arterial occlusion cuff) was used in the assessment of the hyperaemic response to ischaemia of the forearm (Strandness & Sumner, 1975). This cuff was inflated to 200 mmHg for 4 minutes following the base line measurements. Before deflating the arterial occlusion cuff at the end of the 4 minute period the venous occlusion cuff (distal upper arm cuff) was inflated to 60 mmHg to allow measurement of forearm blood flow immediately following cuff deflation ($t_0$). Further measurements of blood flow were made by inflating the venous occlusion cuff to 60 mmHg 30 and 60 seconds after deflating the arterial cuff and thereafter every minute for a further two minutes by which time the resting flow levels had been reached. Blood flow was calculated using the method described above.
Figure 6.2. Specimen plethysmography trace
6.1.3.8. Statistical analysis

6.1.3.8.1. Systemic haemodynamic data

The results are expressed as mean ± SEM.

Differences between groups were compared using $\chi^2$ for categorical data and unpaired t tests for continuous variables.

Discriminant function analysis was performed to define the subset of variables which best described the differences between the NLD and LD groups.

As the data for the Child-Pugh grades did not have a Gaussian distribution, the effect of deteriorating hepatic status on haemodynamic variables was evaluated using the Mann-Whitney U test.

Simple least-squares regression analysis (Pearson's correlation co-efficient) was used to assess correlations between deteriorating lung function (reduced FEV$_1$) and worsening liver disease as judged by the ultrasound grade (NLD, ILD and SLD) and haemodynamic variables for the two groups.

Forward stepping multivariate regression analysis of the resting data was used to assess whether or not the hepatic status (as judged by increasing ultrasound grade: NLD, ILD, SLD) and lung function status (as judged by worsening %PFEV$_1$) of the subjects were additive in their effect on the haemodynamic response at rest.
One-way analysis of co-variance (ANCOVA), where the baseline value of the variable was entered as a covariate, was used to compare the response to exercise between the two groups.

6.1.3.8.2. Plethysmography data

One-way ANOVA was used to compare the baseline plethysmography data.

For the purposes of analysing the hyperaemic response the data was analysed in two phases: the initial hyperaemic blood flow and then the recovery phase. Log transformation of the data was necessary due to the non-Gaussian distribution of blood flow immediately and 30 seconds after cuff deflation.

ANCOVA was used to compare the hyperaemic response (with the baseline value as the co-variate) and recovery phase (with the hyperaemic value as the co-variate).

Significant differences detected using ANOVA or ANCOVA were analysed further using Fisher's t-based least significant difference to detect which paired groups were significantly different.

p<0.05 for a two-tailed test was considered a statistically significant result for all comparisons.
6.2. **Results**

6.2.1. Patients

Fifty six patients were included: 28 with (LD) and 28 without (NLD) cystic fibrosis related liver disease.

The exercise test was stopped early for two patients, one from each group. The NLD subject developed marked bronchospasm and the LD subject profoundly desaturated from a resting SaO₂ of 83%.

Fourteen other studies were incomplete due to failure to obtain technically adequate Doppler signals (judged from the sonogram (Innes, 1987)) at rest and on exercise (11 studies: 5 NLD and 6 LD) or on exercise alone (5 studies: 2 NLD and 3 LD). Those studies where inadequate results were obtained both at rest and on exercise were excluded altogether (including ventilatory data) from further analysis, while those in which only the exercise data was inadequate were included in the evaluation of the resting data. Thus forty five resting (23 NLD; 22 LD) and forty paired rest and exercise (21 NLD; 19 LD) studies were available for final analysis.

Fifteen of the twenty two LD subjects included in the resting analysis were Child-Pugh grade A and seven were grade B (Pugh et al., 1973). There were no Child-Pugh grade C subjects. Two Child-Pugh grade A patients had technically inadequate Doppler recordings on exercise and one grade B subject failed to complete the four minute period of exercise.
Analysis of the data for the patients for whom resting haemodynamic data were available revealed that the two groups were well matched for age, sex, height, weight, body mass index and pulmonary function (Table 6.1.).

There were no significant differences, between the two groups, in the nature of pathogens isolated from the sputum (NLD:LD; non-mucoid *Pseudomonas aeruginosa* 13:8; mucoid *Pseudomonas aeruginosa* 10:13; *Staphylococcus aureus* 13:11), or in the number of subjects admitted for respiratory exacerbations (9:12), distal ileal obstruction syndrome (meconium ileus equivalent) (2:1) or variceal haemorrhage (0:2) during the year preceding the study. There were significantly more subjects with diabetes mellitus in the LD group (7:0; p<0.005).

The regular medications, recorded on the day of the study, being taken by the two groups differed in a number of ways (Tables 6.2. and 6.3.). There were significantly more LD subjects on nebulised Gentamicin, vitamin K and iron supplements, H2 antagonists and insulin injections.

The number of subjects who were clubbed was not statistically different between the two groups (13 NLD vs. 14 LD), but the number of subjects with peripheral manifestations of liver disease (Saraky, 1987) and/or hepato-splenomegaly was significantly greater in the LD group.

In the LD group there were twelve subjects with hepato-splenomegaly, two with hepatomegaly alone and two with splenomegaly alone. Ten (45%) of the LD group had one or more
of the cutaneous manifestations associated with chronic liver disease (Saraky, 1987): 7 (32%) had palmar erythema, 1 (5%) had spider naevi, 1 (5%) had petechiae and 2/11 (18%) of the males had gynaecomastia; one of the subjects had both palmar erythema and gynaecomastia. None of these features were detected in the NLD subjects.

None of the patients had any of the clinical features described as manifestations of the hyperkinetic circulation associated with cirrhosis (Murray et al., 1958), although both groups had an increased heart rate.

Analysis of the baseline characteristics of the patients following exclusion of the five subjects for whom exercise data was not available confirms the similarity of the two groups with respect to demographic data. The only differences between the two groups were for those factors detailed above which are directly related to the presence of hepatic disease.

Comparison of the haemoglobin concentration of the two groups revealed that the LD group had a significantly lower haemoglobin concentration (12.7 ± 0.3 vs. 14.3 ± 0.3 g/dl; p<0.001).

6.2.2. Resting haemodynamic results

Haemodynamic evaluation revealed that both groups were tachycardic and that the resting heart rates were not statistically significantly different. The LD group did, however, have a
significantly lower mean arterial pressure, elevated cardiac output and reduced systemic vascular resistance index (Table 6.4., Figures 6.3. and 6.4.).

The stroke volume and left ventricular stroke work index were also significantly higher in the LD group (Table 6.4.).

Resting ventilatory variables, in particular the oxygen consumption, but including the oxygen saturation, carbon dioxide production, respiratory rate and minute ventilation were not significantly different between the two groups (Table 6.5.).

6.2.3. Discriminant function analysis

Discriminant function analysis was performed to select the subset of variables that best describe the differences between the NLD and LD cohorts. Measured haemodynamic variables (excluding derived variables), ventilatory variables and haemoglobin were entered into the analysis. The results revealed that a combination of the MAP and CI produced the best model to allow discrimination between the two groups.

This model correctly identified 73.9% of the NLD cohort and 72.7% of the LD cohort. Thus 73.3% of the whole cohort was correctly categorised.
6.2.4. Exercise results

Evaluation of the haemodynamic and ventilatory data for the forty subjects for whom both resting and exercise data was available confirmed statistically significant differences in the cardiac output and systemic vascular resistance on exercise. The heart rate and mean arterial pressure were not significantly different (Table 6.6). Graphs showing the resting and exercise haemodynamic data are shown in figure 6.5.

Analysis of the paired data by ANCOVA revealed that the changes in haemodynamic variables on exercise were not statistically significant. Thus the increment in cardiac output caused by exercise was not influenced by the presence or absence of liver disease, suggesting that the peripheral muscular adaptation to exercise was similar for the two groups.

There were no differences in ventilatory parameters between the two groups on exercise. The oxygen saturation was similar for the two groups and their oxygen consumption and carbon dioxide production did not differ significantly (Table 6.7). Graphs showing resting and exercise ventilatory data are shown in figure 6.6.

6.2.5. Comparison of LD patients based on Child-Pugh grade

Comparison of the haemodynamic variables between Child-Pugh grade A and B subjects revealed a significantly higher cardiac
index (6.7 ± 0.6 vs 5.0 ± 0.3 l.min⁻¹.m⁻²; p<0.05) and lower SVRI (916.2 ± 93.8 vs 1240.4 ± 72.7 dyne.s.cm⁻⁵.m⁻²; p<0.05) in the Child-Pugh group B (those with more severe liver disease) subjects at rest. On exercise the Child-Pugh grade B subjects had a significantly higher CI (8.7 ± 0.7 vs 7.1 ± 0.3 l.min⁻¹.m⁻²; p<0.05).

6.2.6. Regression analyses

Least-squares regression was performed to assess whether deteriorating pulmonary function was associated with more severe haemodynamic changes. Correlating %PFEV₁ with haemodynamic variables (heart rate, MAP, CI, SVRI and oxygen saturation) revealed the following significant associations:

At rest %PFEV₁ correlated with the heart rate (r = -0.47; p<0.05), CI (r=- 0.45; p<0.05), SVRI (r = 0.42; p<0.05) (Figures 6.7. and 6.8.) and SaO₂ (r = 0.45; p<0.05) for the LD group. There was no significant correlation with the mean arterial pressure (Figure 6.8.).

In the NLD group %PFEV₁ correlated with heart rate (r = -0.44; p<0.05) and with SaO₂ (r = 0.48; p<0.05). There were no other correlations with haemodynamic variables at rest (Figures 6.9. and 6.10.).
6.2.7. Forward stepping multivariate regression analysis

For the purposes of this analysis severity of liver disease was categorised into the three gradings derived from the ultrasound scoring system: NLD (grade 1), ILD (grade 2) and SLD (grade 3). Haemodynamic variables at rest were correlated against this liver grade and %PFEV1 to evaluate any possible interaction between these two factors.

The results revealed a significant increase in correlation for CI when regression is performed against both ultrasound grade (USS grade) and %PFEV1. While the correlation coefficients for both heart rate and SVRI increased on adding the %PFEV1 to the statistical model these changes were not statistically significant (Table 6.8.).

6.2.8. Strain gauge plethysmography

Mercury-in-Silastic strain gauge plethysmography was performed on all 56 subjects entered into the haemodynamic study and 12 healthy adult Controls.

Only subjects with complete plethysmographic records, with recordings up to 180 seconds after the hyperaemic response, were included in the analysis. Consequently, ten of the cystic fibrosis subjects were excluded.

Four of the NLD group were excluded: one as she was unable to tolerate the four minutes that the upper cuff was inflated to
produce an ischaemic insult prior to assessment of the hyperaemic response and another because a leak from the rubber tubing resulted in loss of pressure in the cuff isolating the wrist from the forearm circulation. The remaining NLD subjects and the six LD subjects had to be excluded due a technical problem which resulted in a failure to record the hyperaemic response (the baseline of the record had drifted to the top of the recording paper and could not be re-centred resulting in loss of the immediate recording).

There was a small, but significant difference in the temperature of the room when the NLD and LD subjects were evaluated (20.3 ± 0.1 vs 20.7 ± 0.1 °C respectively; p<0.05). The room temperature for the Control subjects did not differ significantly (20.9 ± 0.3 °C) from either group. There were no differences in the humidity of the room at the time that subjects from the three groups were studied (Controls; NLD; LD: 55.3 ± 2.3 vs 59.6 ± 1.9 vs 60.9 ± 1.8 % respectively).

The cystic fibrosis subjects (both NLD and LD groups) had higher forearm skin temperatures than the normal Controls at the start (C; NLD; LD: 29.5 ± 0.4 vs 31.3 ± 0.2 vs 31.1 ± 0.3 °C respectively; p<0.01 for comparisons between Controls and the other two groups) and end (C ;NLD ;LD : 27.9 ± 0.4 vs 29.8 ± 0.3 vs 29.4 ± 0.3 °C respectively; p<0.01 for comparisons between Controls and the two other groups) of the study. There was a drop of about 1.5°C in skin temperature over the time-course of the study for all subjects.
Resting forearm blood flow was not statistically significantly different between Controls, NLD and LD sub-groups when analysed by ANOVA (2.5 ± 0.3 vs 3.5 ± 0.3 vs 3.5 ± 0.2 ml/100ml/min respectively).

The FBF response to ischaemia is documented in Table 6.9. and Figure 6.11. The log transformed data is shown in Figure 6.12.

ANCOVA of the log transformed data, with the baseline forearm blood flow used as the co-variate, revealed that there was no significant difference in the hyperaemic response for the three groups (p=0.448). Analysis of the recovery phase, with the hyperaemic blood flow entered as the co-variate, showed no difference at 30 or 120 seconds (p=0.051 and p=0.189 respectively). However, at 60 and 180 seconds the differences were significant (p=0.048 and p=0.033 respectively). Further analysis of the data using Fisher's t based least significant difference test revealed a difference in the recovery from hyperaemia in the Control group and the NLD cystic fibrosis subjects at 60 seconds and between Controls and both NLD and LD subjects at 180 seconds. There were no differences in recovery between the two cystic fibrosis groups at any stage.
<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>23</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>24.8 ± 1.5</td>
<td>22.2 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>15:8</td>
<td>11:11</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.6 ± 1.7</td>
<td>165.4 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>57.4 ± 1.9</td>
<td>53.9 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>19.9 ± 0.4</td>
<td>19.5 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>%PFEV₁</td>
<td>55.4 ± 4.8</td>
<td>49.6 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>%PFVC</td>
<td>78.8 ± 5.0</td>
<td>70.1 ± 5.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 6.1. Characteristics of the 45 patients included in the final analysis
(values expressed as mean ± SEM)

%PFEV₁ - percentage predicted forced expiratory volume in one second
%PFVC - percentage predicted forced vital capacity
<table>
<thead>
<tr>
<th>Pharmacological agent</th>
<th>NLD (n=23)</th>
<th>LD (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulised Colomycin</td>
<td>8</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Nebulised Gentamicin</td>
<td>1</td>
<td>8</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Nebulised Carbenicillin</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Nebulised Tobramycin</td>
<td>0</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Nebulised Ceftazidime</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>10</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Co-Amoxiclav</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>β2 agonists (neb/inh)</td>
<td>14</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>Salmeterol inhaler</td>
<td>2</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Ipratropium inhaler</td>
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<tr>
<td>Steroid inhaler</td>
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<td>NS</td>
</tr>
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<td>NS</td>
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<td>Aminophylline tablets</td>
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</tr>
<tr>
<td>Prednisolone tablets</td>
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<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Nebulised rhDNase</td>
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<td>NS</td>
</tr>
</tbody>
</table>

Table 6.2. Respiratory related drug therapy for the 45 subjects included in the final analysis
<table>
<thead>
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<th>Pharmacological agent</th>
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<th>LD (n=22)</th>
<th>p-value</th>
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<tr>
<td>Pancreatic enzymes</td>
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<td>21</td>
<td>NS</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>17</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin E</td>
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<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>4</td>
<td>12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>1</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>0</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Omeprazole</td>
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<td>NS</td>
</tr>
<tr>
<td>Insulin</td>
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<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glibenclamide</td>
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<td>NS</td>
</tr>
<tr>
<td>Hyoscine butylbromide</td>
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<td>NS</td>
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<tr>
<td>Metoclopramide</td>
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<td>NS</td>
</tr>
<tr>
<td>Quinine bisulphate</td>
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<td>NS</td>
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<tr>
<td>Spironolactone</td>
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<td>NS</td>
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<tr>
<td>Bendrofluazide</td>
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<td>1</td>
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</tr>
</tbody>
</table>

Table 6.3. Other pharmacological agents being taken by the 45 study subjects on the day of haemodynamic testing
### Table 6.4. Resting systemic haemodynamic results (mean ± SEM)

LVSWI - left ventricular stroke work index
Figure 6.3. Graphs showing mean (± SEM) heart rate and cardiac index for the two study groups

**p=0.0001
Figure 6.4. Graphs comparing mean arterial pressure and systemic vascular resistance index (mean ± SEM) for the two groups

*p=0.01; **p=0.0001

Figure 6.4. Graphs comparing mean arterial pressure and systemic vascular resistance index (mean ± SEM) for the two groups.
<table>
<thead>
<tr>
<th>Number (n)</th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td><strong>O₂ saturation (%)</strong></td>
<td>94.4 ± 0.6</td>
<td>93.8 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>O₂ Consumption</strong></td>
<td>311.7 ± 9.0</td>
<td>326.4 ± 12.0</td>
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</tr>
<tr>
<td>(ml.min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CO₂ Production</strong></td>
<td>280.0 ± 8.0</td>
<td>295.0 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>(ml.min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>End tidal CO₂ (mmHg)</strong></td>
<td>33.5 ± 0.6</td>
<td>33.8 ± 1.0</td>
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<tr>
<td><strong>Respiratory rate</strong></td>
<td>19.9 ± 1.2</td>
<td>22.1 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>(breaths.min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tidal volume (l)</strong></td>
<td>594.8 ± 25.4</td>
<td>577.0 ± 32.1</td>
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</tr>
<tr>
<td><strong>Minute ventilation</strong></td>
<td>11.3 ± 0.5</td>
<td>12.7 ± 0.7</td>
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</tr>
<tr>
<td>(l.min⁻¹)</td>
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<tr>
<td><strong>Respiratory quotient</strong></td>
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<td>0.89 ± 0.02</td>
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</tr>
<tr>
<td>(RQ)</td>
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<tr>
<td><strong>Ventilatory equivalent for O₂</strong></td>
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<td>37.6 ± 1.2</td>
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</tr>
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<td>uptake (VEO₂)</td>
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<td></td>
</tr>
<tr>
<td><strong>Ventilatory equivalent for CO₂</strong></td>
<td>40.5 ± 1.3</td>
<td>41.6 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>production (VECO₂)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.5. Resting ventilatory results
(mean ± SEM)
Table 6.6. Measured haemodynamic variable results after 4 minutes of 25W exercise (mean ± SEM)
Figure 6.5. Graphs showing changes in selected haemodynamic parameters from rest to exercise
<table>
<thead>
<tr>
<th></th>
<th>NLD</th>
<th>LD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>21</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>$O_2$ saturation (%)</td>
<td>94.4 ± 0.4</td>
<td>94.4 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>$O_2$ Consumption (ml.min$^{-1}$)</td>
<td>916.7 ± 37.0</td>
<td>910.0 ± 29.0</td>
<td>NS</td>
</tr>
<tr>
<td>$CO_2$ Production (ml.min$^{-1}$)</td>
<td>865.7 ± 27.0</td>
<td>875.3 ± 27.0</td>
<td>NS</td>
</tr>
<tr>
<td>End tidal $CO_2$ (mmHg)</td>
<td>35.3 ± 0.5</td>
<td>36.3 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Respiratory rate (breaths.min$^{-1}$)</td>
<td>28.1 ± 1.9</td>
<td>31.4 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Tidal volume (l)</td>
<td>1059.5 ± 65.2</td>
<td>993.7 ± 78.4</td>
<td>NS</td>
</tr>
<tr>
<td>Minute ventilation (l.min$^{-1}$)</td>
<td>27.4 ± 0.9</td>
<td>29.0 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Respiratory quotient (RQ)</td>
<td>0.95 ± 0.03</td>
<td>0.95 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Ventilatory equivalent for $O_2$ uptake ($V_{E}O_2$)</td>
<td>30.5 ± 1.0</td>
<td>31.7 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Ventilatory equivalent for $CO_2$ production ($V_{E}CO_2$)</td>
<td>32.0 ± 0.6</td>
<td>33.1 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 6.7. Ventilatory variable results for the two groups following 4 minutes of 25W exercise (mean ± SEM)
Figure 6.6. Graphs showing changes in selected ventilatory parameters from rest to exercise
Figure 6.7. Correlations of heart rate and cardiac index with %PFEV \(_1\) for the LD group
Figure 6.8. Correlations of mean arterial pressure and systemic vascular resistance index with %PFEV<sub>1</sub> for the LD group
Figure 6.9. Correlations of heart rate and cardiac index with %PFEV_1 for the NLD group

$\text{Heart rate (b/min)}$

$\text{Cardiac index (L/min/m}^2\text{)}$

$r = -0.44$

$p < 0.05$

$r = -0.13$

$p = 0.6$
Figure 6.10. Correlations of mean arterial pressure and systemic vascular resistance index with %PFEV₁ for the NLD group
<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Step 1</th>
<th>r</th>
<th>p-value</th>
<th>Step 2</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>%PFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.40</td>
<td>&lt;0.02</td>
<td>USS Grp</td>
<td>0.46</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>USS Grp</td>
<td>0.59</td>
<td>0.0001</td>
<td>%PFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.66</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>SVRI</td>
<td>USS Grp</td>
<td>0.56</td>
<td>0.0002</td>
<td>%PFEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.60</td>
<td>&lt;0.0003</td>
</tr>
</tbody>
</table>

Table 6.8. Results of the forward stepping multivariate analysis for each of the haemodynamic variables.

r - correlation co-efficient
MAP - mean arterial pressure
CI - cardiac index
SVRI - systemic vascular resistance index
USS - ultrasound
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NLD</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>12</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Time (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest (Pre)</td>
<td>2.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Hyperaemia (0)</td>
<td>23.9 ± 1.9</td>
<td>28.4 ± 2.3</td>
<td>32.5 ± 2.6</td>
</tr>
<tr>
<td>+30</td>
<td>8.5 ± 1.6</td>
<td>15.8 ± 2.4</td>
<td>11.3 ± 1.0</td>
</tr>
<tr>
<td>+60</td>
<td>4.4 ± 0.8</td>
<td>8.1 ± 1.2</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>+120</td>
<td>2.7 ± 0.4</td>
<td>4.7 ± 0.6</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>+180</td>
<td>2.2 ± 0.3</td>
<td>4.0 ± 0.6</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

Table 6.9. The mean (± SEM) resting forearm blood flow (ml.100ml⁻¹.min⁻¹) for the three study groups and their hyperaemic response and recovery phase following 4 minutes ischaemia
Figure 6.11. Forearm blood flow for the three study groups at rest and in response to 4 minutes ischaemia.
Figure 6.12. $\log_{10}$ forearm blood flow for the three groups at rest and following 4 minutes ischaemia
6.3. Discussion

6.3.1. Haemodynamics

The results of this haemodynamic study are in keeping with the documented hyperdynamic circulation associated with other forms of liver disease (Kowalski & Abelmann, 1953; Murray et al., 1958; Martini et al., 1972) in the finding of an elevated cardiac output with a reduced systemic vascular resistance index in subjects with cystic fibrosis associated chronic liver disease.

In addition, the circulatory changes significantly correlated with the severity of liver disease as judged by their Child-Pugh grade. This is in keeping with previous reports of a progression of the hyperdynamic circulatory state as the cirrhosis progresses (Braillon et al., 1986a), and with an increase in the degree of hepatic dysfunction as measured by Child's classification (Braillon et al., 1986a).

To avoid the risk of complications related to the invasive assessment of cardio-pulmonary status in patients with cystic fibrosis this study has used a number of non-invasive techniques to measure cardio-pulmonary variables.

The pulsed Doppler system (D) used in the present studies to measure cardiac output has been validated in man against indicator thermodilution (T), cardiac output measurements and also electromagnetic catheter (E) measurements of aortic blood velocity;
\[ D_{PV} = 1.12E_{PV} - 1.97 \text{ cm.s}^{-1}; r = 0.91 \] (where PV is the peak velocity)

and

\[ D_{SV} = 0.86T_{SV} + 5.6 \text{ mls}; r = 0.90; D_{CO} = 0.89T_{CO} + 0.36 \text{ l.min}^{-1}; r = 0.92 \] (Innes et al., 1987; Innes et al., 1988).

Due to anatomical variability technically acceptable results could not be obtained for some subjects, but the failure rate was not unusually high in this patient group (Innes et al., 1988) (the technique requires a free path of ultrasound from sternal notch to ascending aorta; in some subjects this path is obstructed by lung tissue, especially at peak inspiration, resulting in loss of signal. In patients with airflow obstruction hyperinflation of the lungs may increase this problem).

The other potential methodological problem in this study is the use of a pulse oximeter to estimate arterial oxygen saturation. Data supporting the use of a pulse oximeter for these measurements was derived from twenty five cystic fibrosis subjects, included in the assessment of pulmonary arteriovenous shunting reported in this thesis (chapter seven), who had simultaneous measurement of the oxygen saturation of arterial blood (OSM 2 Hemoximeter, Radiometer A/S, Copenhagen) and oxygen saturations measured by pulse oximeter (Ohmeda Biox 3700). The mean difference between measured and estimated oxygen saturations was 2.6% (95% confidence interval: 1-4.2%), suggesting that resting measurements of SaO2 by pulse oximeter are reproducible and accurately reflect the true SaO2.
These conclusions cannot be extrapolated to the exercise data as the accuracy of pulse oximeters is not as good when the SaO2 falls below 90% (Orenstein et al., 1993). However, the demonstration that paired oximeter measurements accurately detect changes in SaO2 from rest to exercise (Ries et al., 1985), coupled with the assumption that any methodological inaccuracy will apply equally to both cohorts, suggests that any methodological limitations will not influence the final results in this study.

Concerns that the SaO2 measured by pulse oximetry may have been influenced by hyperbilirubinaemia in the LD cohort (Chaudhary & Burki, 1978) do not arise as any elevations of serum bilirubin were minor.

The documented haemodynamic changes in the LD cohort included in this study would not have been suspected on clinical examination of the subjects, who in addition to having few of the recognised peripheral manifestations of liver disease (a finding consistent with the clinical picture of primary biliary cirrhosis (Wright, 1987)), had no evidence of the flushed warm extremities (7 of the LD subjects did have palmar erythema), bounding pulses and systolic ejection murmurs described with the hyperkinetic circulation associated with cirrhosis (Murray et al., 1958).

It is interesting to note, however, that the skin temperatures of both cystic fibrosis groups (measured as part of the plethysmography protocol) were significantly higher than those of the normal Control subjects, suggesting that there may have
been some cutaneous vasodilatation in both of the cystic fibrosis
groups. This may be related to the elevated basal metabolic rate
which is a feature of cystic fibrosis (Vaisman et al., 1987;
Buchdahl et al., 1988).

While the LD subjects had evidence of a hyperdynamic
circulation on exercise the statistical analysis suggests that the
response of the two groups to exercise is not significantly
different. While it may have been possible to obtain further
information by performing a maximal exercise test, the design of
this study was to compare cardiovascular variables in the two
groups under matched degrees of metabolic stress; the matched
oxygen consumptions show that we achieved this aim. Whether
or not the observed cardiovascular difference would have been
reflected in different maximal oxygen uptakes is outwith the
scope of the present study.

The response of all subjects to exercise suggests that the patients
studied here have a similar capacity to respond to the increased
metabolic demands of exercise, albeit with increased
haemodynamic demands consequent upon the resting
hyperdynamic circulation in the LD group. These results concur
with previous studies showing a normal response of cirrhotics to
exercise (Abelmann et al., 1955), although other reports have
suggested that subjects with alcoholic liver disease, and no
detectable cardio-pulmonary abnormality, have an impaired
capacity to respond adequately to physical stress (Kelbaek et al.,
1987). It may be that this latter study identified a group with
sub-clinical cardiac disease, which may not be surprising in the light of the cardio-toxicity of alcohol.

The documented haemodynamic changes were not due to differences in the severity of pulmonary disease, or to differences in lung pathogens. Although there were significantly more diabetics in the LD group this is unlikely to have influenced the haemodynamic changes and while there were some documented differences in the medical therapy of the two groups none of the drugs taken by significantly more of the LD group are likely to account for the haemodynamic differences.

The similarities in oxygen consumption between the two groups indicate that the increased cardiac output was not due to an increased metabolic rate, either at rest or on exercise, in the LD group. The elevated oxygen consumption in both cystic fibrosis groups relative to normal subjects is consistent with the reported increase in resting energy expenditure in cystic fibrosis (Vaisman et al., 1987; Buchdahl et al., 1988).

While there were significant differences in the haemoglobin concentrations between the two groups the mild degree of anaemia observed in the LD group, which may, in part, be explained by plasma volume expansion which occurs in chronic liver disease (Murray et al., 1958) (although we have not measured plasma volume), is unlikely to be of clinical significance. This contention is supported by two lines of evidence: firstly the results of the discriminant function analysis which included the haemoglobin as a variable and defined the MAP and CI as the factors producing the best discriminant
model, and secondly by the comparison of the product of haemoglobin and CI which reveals statistically significant differences both at rest (NLD vs LD: 52.5 vs 70.0 g Hb/min output; p<0.002) and on exercise (78.6 vs 94.2 g Hb/min output; p<0.02) suggesting that the changes in haemodynamic status in the LD group are independent of the differences in haemoglobin concentration.

6.3.2. Plethysmography

Mercury-in-Strain gauge plethysmography allows non-invasive assessment of peripheral blood flow. The results of the baseline measurement of forearm blood flow (FBF) in this study show that there are no differences between NLD and LD cystic fibrosis subjects and healthy Controls. The difference in room temperature (0.4 °C) between the two cystic fibrosis groups is unlikely to be of any clinical significance.

FBF measurement using strain gauge plethysmography has been validated by comparing it with blood flow measurement using an electromagnetic flow cuff around the brachial artery;

\[ \text{FBF}_{\text{plethysmography}} = 1.21 \times \text{FBF}_{\text{electromagnetic}} + 0.87, \ r=0.87 \]

(Longhurst et al., 1974).

The higher estimate obtained by plethysmography may be due to flow through the skin missed by the arterial flow cuff. Comparison of values within individuals gave a correlation coefficient of 0.96.
The mean FBFs of all three groups fell within the normal range which has been estimated at between 2 and 4 ml/100ml/min (Elia & Kurpad, 1993). Further examination of the published literature shows that the Control subjects' FBF values are lower than previously documented in normal subjects by many investigators (Whitney, 1953; Lunzer et al., 1975; Jiron et al., 1988) but higher than those reported by others (Okumura et al., 1990), while the cystic fibrosis subjects with liver disease have FBFs similar to those reported by some investigators (Whitney, 1953; Okumura et al., 1990), but lower than those reported by others in studies of cirrhotic patients (Lunzer et al., 1975; Jiron et al., 1988). It is likely that a part of these differences between studies can be attributed to methodological differences including the ambient temperature of the room in which the studies were performed (20-21°C in this series), and the fact that, in this study, the circulation to the hand was arrested whereas other investigators have not measured blood flow in the isolated forearm (Lunzer et al., 1975; Jiron et al., 1988).

The similarities in FBF of the NLD and LD groups is in keeping with observations made in other studies of well compensated cirrhotics (Lunzer et al., 1973; Lunzer et al., 1975; Jiron et al., 1988) where the FBF did not differ from normal controls. It is only in patients with more advanced liver disease that increased peripheral blood flow has been demonstrated (Abramson & Lichtman, 1937; Martini & Hagemann, 1956).

While there was no statistically significant difference in the FBF in the three groups, the cystic fibrosis subjects had an increased
skin temperature and the room was significantly warmer for the LD when compared to the NLD cystic fibrosis patients, albeit by a mean of only 0.4 °C. This small but significant difference in room temperature during the study is unlikely to have affected the forearm blood flow as FBF does not change in normal subjects in response to heating (Weinberg, 1989).

The increase in skin temperature has previously been documented in cirrhotics with a significantly increased FBF (Okumura et al., 1990) and has been proposed as a marker of increased blood flow (Kontos et al., 1964). The cystic fibrosis cohort also had FBFs of approximately 1ml/100ml/min greater than the normal Controls, and while this difference was not statistically significant it may reflect the reduced numbers of Controls studied. Previous studies have suggested an increase in skin blood flow in cirrhotics (Abramson & Lichtman, 1937; Martini & Hagemann, 1956), whereas skeletal muscle blood flow, which is the major component of forearm blood flow, was not increased (Lunzer et al., 1973). It is, therefore, possible that the cystic fibrosis subjects were cutaneously vasodilated, although this component of peripheral blood flow was not formally evaluated in this study.

Why cystic fibrosis subjects without liver disease should have evidence of cutaneous vasodilatation is unclear, but it may be hypothesised that this is a consequence of their pulmonary disease (see below) or may be related to their elevated basal metabolic rate.
Further information on cutaneous vasodilatation might have been obtained by using experimental techniques that assess capillary blood flow. Plethysmography can be used to estimate finger blood flow, which is mainly a measure of capillary blood flow through the skin, and has been reported to be increased in cirrhotics (Martini, 1955).

Another technique available for assessing cutaneous blood flow is laser Doppler flowmetry (Oberg et al., 1984). This allows estimation of the total amount of blood moving in the area beneath the Doppler probe to a depth of about 1.5mm and includes arterio-venous anastamotic flow. However, the technique is difficult to reproduce and does not allow absolute measurements of blood flow to be made. Consequently it is better applied to changes in blood flow within an individual.

An alternative method is television capillaroscopy which allows examination of the skin capillaries and in particular those of the nailfold (Intaglietta et al., 1979). By measuring capillary velocity and capillary diameter it is possible to calculate capillary volume flow, and by estimating capillary density derive the skin capillary blood flow. The technique has been used to show marked capillary dilatation in the nail beds of patients with chronic liver disease (Pirovino et al., 1988).

Whether this latter technique would have provide more information is not known.

The plethysmography results add further information to the results of the systemic haemodynamic study. The lack of
correlation between peripheral arterial flow and systemic haemodynamic changes in the two cystic fibrosis groups suggests that other vascular beds are more important than the peripheral vascular bed in determining overall haemodynamics. This is in agreement with studies using the rat model of portal hypertension which suggest that it is the splanchnic vasculature and in particular the portal-systemic collaterals which are the major determinants of cardiac function (Braillon et al., 1986b). Further support for this hypothesis in cystic fibrosis liver disease comes from studies documenting increased portal blood flow in subjects with cystic fibrosis related chronic liver disease (Vergesslich et al., 1989).

This study has also attempted to assess the hyperaemic response of all three groups to ischaemia. While there have been no previous studies assessing the response of the peripheral vasculature of subjects with chronic liver disease to vasodilatory stimuli, an impaired vasoconstrictor response to tilting from the horizontal to the vertical, to the Valsalva manoeuvre, in response to mental exercise (Lunzer et al., 1973; Lunzer et al., 1975) and to cold stimulation (Okumura et al., 1990) has been documented.

Analysis of the results of this study suggests that there is no difference in the hyperaemic response or indeed in the initial recovery phase following hyperaemia. These results confirm the capacity of the peripheral vasculature to vasodilate in response to ischaemia and provide further support for the concept that
the peripheral circulation is not significantly dilated in the presence of well compensated liver disease.

6.3.3. Possible underlying mechanisms for the documented haemodynamic changes

The mechanisms for the circulatory changes associated with liver disease have not been evaluated in cystic fibrosis related chronic liver disease. In addition to factors reported in subjects with liver disease of other aetiologies the pathogenesis is likely to be complicated by additional factors such as chronic lung sepsis.

6.3.3.1. Mechanisms specific to liver disease

Hepatic dysfunction is associated with most, but not all (the syndrome has been described in patients with portal vein thrombosis), described cases of the hyperdynamic circulation (Lebrec et al., 1983), there is evidence that the syndrome progresses as the cirrhosis progresses (Braillon et al., 1986a), and that it correlates with the degree of hepatic dysfunction as measured by Child's classification (Braillon et al., 1986a), the functioning hepatic cell mass as measured by indocyanine green clearance (Ito et al., 1988) and liver histology (Dicarlo et al., 1979). These data correspond to the observation in this study of more severe haemodynamic changes in association with increasing Child-Pugh grade and with increasing ultrasound
score. This suggests a positive correlation between the degree of haemodynamic derangement and the severity of liver disease.

Various mechanisms have been proposed for the development of the hyperdynamic circulation which has been reported in up to 85% of cirrhotic patients (Martinez et al., 1992). These include an excess of circulating vasodilatory substances (Benoit et al., 1984) and an insensitivity to endogenous vasoconstrictor mechanisms (Reichen, 1992).

The mechanism for increased vasodilator concentrations in cirrhosis is contentious, but includes intra- and extra-hepatic portal-systemic shunting and the formation of vasodilator substances by the diseased liver (Sherlock, 1990).

Many substances have been evaluated as potential vasodilators. These include:

- Nitric oxide
- Glucagon
- Calcitonin gene-related peptide
- Eicosanoids
- Adenosine
- Bile salts
- Platelet-activating factor
- Gamma-aminobutyric acid

The most recent addition to this list of potential vasodilators is nitric oxide, originally known as the endothelium-derived relaxing factor (Palmer et al., 1987). Its role in the pathogenesis
of the hyperdynamic circulation associated with liver disease has been proposed in response to circulating endotoxins and cytokines (Vallance & Moncada, 1991). These are found in increased concentrations in the circulation of cirrhotics, probably as a result of portal-caval shunting, even in the absence of clinically overt infection (Ohnishi et al., 1987). Alternatively release of nitric oxide can be induced by shear stress (Palmer et al., 1987) and this may be an important mechanism for increased release in patients with chronic liver disease.

Support for a role for nitric oxide in the pathogenesis of the hyperdynamic circulation has been derived from studies showing an improvement in circulatory changes in a rat model of portal hypertension following the administration of an inhibitor of nitric oxide synthetase (Pizcueta et al., 1992) and an increase in urinary cyclic guanosine monophosphate (cGMP), which dilates blood vessels following activation by nitric oxide, in the urine of cirrhotics (Miyase et al., 1990).

Nitric oxide is unlikely to be the sole mediator of the haemodynamic vasodilatation as nitric oxide inhibitors do not completely reverse the circulatory changes (Pizcueta et al., 1992). Consequently other potential vasodilators must be considered.

Of the other mediators listed glucagon is the most likely to be important as it is responsible for about 30% of the increase in splanchnic blood flow in the rat model (Benoit et al., 1986). It has been suggested that glucagon may be an important mediator...
in the early stages of the development of portal hypertension (Lee et al., 1988).

There is evidence supporting a role for all of the other vasodilators listed in the pathogenesis of the hyperdynamic circulation. This evidence has either been contradicted or is currently insufficient to attribute a major role to them in the pathogenesis of the syndrome (Reichen, 1992).

An alternative explanation for the hyperdynamic circulation is a blunted response to endogenous vasoconstrictors. However, this is not a uniform observation as the response to some vasoconstrictors, such as serotonin, is actually increased in portal hypertension (Cummings et al., 1986). Vasoconstrictors which have been implicated in the pathogenesis of the hyperdynamic circulation include:

**A. Increased production/blunted response**

- β-adrenergic agents
- Angiotensin II
- Vasopressin
- Endothelin?

**B. Increased sensitivity**

- Serotonin
- Endothelin?
The blunted response to vasoconstrictors may simply reflect the presence of increased concentrations of vasodilator substances. There is evidence that nitric oxide can impair the response of splanchnic arteries to α-adrenergic agents (Sieber & Groszmann, 1992) and glucagon the response of the rat circulation to norepinephrine (Pizcueta et al., 1990).

6.3.3.2. Mechanisms related to cystic fibrosis

The theories behind the pathogenesis of the hyperdynamic circulation in liver disease are complicated by several other potential contributory factors that are related to pulmonary and intestinal dysfunction in cystic fibrosis.

6.3.3.2.1. The lung as a metabolic organ

The lung functions as an organ of first pass, and is responsible for the metabolism of a number of substances in the systemic circulation (Junod, 1975). These include glucagon (Geddes, 1979), which as discussed above may be an important mediator in the pathogenesis of the hyperdynamic circulatory state. Prostaglandins E₁, E₂ and F₂α are also affected (Ferreira & Vane, 1967).

The effect of pulmonary disease on the concentrations of these substances is unclear, but it may be speculated that impaired metabolism in the diseased lung results in an increase in systemic concentrations. Impaired metabolism by the lung may be exacerbated by intra-pulmonary arteriovenous shunting.
In addition the lung synthesises a number of vasoconstrictor substances including angiotensin II (Ng & Vane, 1968). The effect of disease on their concentration remains unknown.

6.3.3.2.2. Chronic sepsis

Chronic pulmonary sepsis in cystic fibrosis is characterised by colonisation of the airways by *Pseudomonas aeruginosa*, an elevation of circulating immunoglobulins (Wheeler *et al.*, 1984) and a leukocytosis (Suter *et al.*, 1989).

Bacterial cell wall lipopolysaccharides may stimulate the production of cytokines by monocytes and macrophages (Smith *et al.*, 1988). Tumour necrosis factor α (TNFα) is among the cytokines produced (Suter *et al.*, 1989; Norman *et al.*, 1991) and has been shown to circulate in increased concentrations in cystic fibrosis patients without overt evidence of lung sepsis (Norman *et al.*, 1991).

TNFα may be responsible, at least in part, for the changes in resting energy expenditure and body composition found in cystic fibrosis (Elborn *et al.*, 1993). Some of its effects may be mediated through an increase in serum glucagon (Van der Poll & Sauerwein, 1993), which may in turn exacerbate the hyperdynamic circulatory state in patients with liver disease.

An increase in cytokines related to chronic lung sepsis may be responsible for the proposed cutaneous vasodilatation
(suggested by the increased skin temperature in the plethysmography study) in the NLD group.

6.3.3.2.3. Intestinal permeability and orocaecal transit time

The permeability of the small intestine is increased and the orocaecal transit time prolonged in cystic fibrosis (Escobar et al., 1992). It may be speculated that these changes will result in an increased prevalence of bacteremia or endotoxaemia. This may contribute to the circulatory changes via mechanisms discussed above.

6.4. Relevance of these circulatory changes to a possible covert effect of cystic fibrosis related liver disease upon survival

The circulatory changes in patients with advanced chronic liver disease of other aetiologies is associated with reduced oxygen consumption despite the increase in oxygen transport (Moreau et al., 1988).

It has been proposed that the discrepancy between oxygen delivery and consumption is due to microcirculatory disturbance causing a failure to deliver oxygen to the tissue beds where it is required (Rappaport, 1979), or in an abnormal limitation of oxygen extraction (Moreau et al., 1988). Limitation of tissue oxygen extraction is suggested by a low oxygen extraction ratio
and elevated mixed venous oxygen concentration (Witte & Witte, 1983) and may be caused by arterio-venous shunting (Robin, 1980). Unless the individual can respond by increasing oxygen transport (by increasing blood flow) tissue hypoxia will result (Moreau et al., 1988), and may contribute to multi organ failure in patients with advanced liver disease (Sherlock, 1990). There is good evidence to show that the maintenance of oxygen consumption through increases in systemic oxygen delivery (mediated by increases in cardiac output) does not continue as the hyperdynamic circulatory state worsens (Ito et al., 1988). This occurs with deteriorating liver function and worsening of Child-Pugh grade (Moreau et al., 1988).

In the present study we have documented a hyperdynamic circulatory state in the LD cohort with normal levels (for patients with cystic fibrosis) of tissue oxygen consumption and carbon dioxide production. These findings are typical of the well compensated cirrhotic patient (Braillon et al., 1986a). However, we have also shown a correlation between deteriorating pulmonary function and worsening of the hyperdynamic circulation and between deteriorating pulmonary function and a falling SaO2. In addition, forward stepping multivariate analysis suggests that worsening liver disease and deteriorating pulmonary function have an additive effect on the severity of haemodynamic changes in the LD cohort.

It may be hypothesised that as the liver disease and/or lung disease progress, so the circulation will become more hyperkinetic and result in increased left ventricular work. If this
process is associated with a falling SaO2 it is possible that subclinical myocardial ischaemia may occur and result in myocardial damage and sub-endocardial fibrosis. Myocardial fibrosis, of unknown aetiology, has been documented at post mortem in patients with cystic fibrosis (McGiven, 1962; Oppenheimer & Esterly, 1975b).

Ultimately the cardiac output may not be able to increase sufficiently to maintain oxygen delivery. These factors may contribute to the accelerated cardio-pulmonary mortality suggested by studies showing increased non-liver related mortality in the cohort of cystic fibrosis patients with chronic liver disease.

6.5. **Conclusions**

The results presented here suggest that patients with cystic fibrosis associated liver disease have a hyperdynamic circulation which may be more severe in the presence of both advanced pulmonary and liver disease.

When these factors are combined using forward stepping multiple regression analysis there is evidence for an interaction between the two factors causing an exacerbation of the haemodynamic derangement.

This interaction, while not clinically apparent, may result in increased left ventricular work in the face of deteriorating
pulmonary function in the cystic fibrosis patient with liver disease.

It is possible, therefore, to speculate that the hyperdynamic circulation may in the presence of progressive pulmonary disease, combine to accelerate mortality from cardio-pulmonary disease. It is not possible, however, to take the findings of a study made on individual patients at a single time point in the disease process and extrapolate these findings to conclude that these patients always retain a sufficient cardiac reserve to cope with increasing haemodynamic derangement as their liver disease progresses or a sufficient cardiac reserve to respond to the increased venous return and oxygen demands of exercise. To evaluate this further would require sequential haemodynamic studies as the liver disease progressed, and it might not prove ultimately possible to evaluate the patients at the crucial time when their capacity to exercise is severely impaired by both advancing pulmonary and hepatic disease.
CHAPTER 7

PULMONARY ARTERIO-VENOUS SHUNTING IN PATIENTS WITH CYSTIC FIBROSIS

evidence for the 'hepato-pulmonary' syndrome
7.0. Introduction

A number of changes in the pulmonary circulation have been well documented in patients with cirrhosis.

The more severe pulmonary vascular changes of chronic liver disease may be manifest by cyanosis, finger clubbing and a hyperkinetic circulation. They may complain of shortness of breath exacerbated by standing up (orthodeoxia). Although most have abnormal liver function and peripheral stigmata of liver disease the pulmonary abnormalities may antedate severe hepatic failure (Bank et al., 1983). These changes are described in patients who have no underlying intrinsic respiratory disease and have both anatomical and physiological explanations.

Intrapulmonary arteriovenous anastamoses were first described in patients with cirrhosis by Rydell and Hoffbauer in 1956 (Rydell & Hoffbauer, 1956). Berthelot et al. related pulmonary vascular changes to documented hypoxaemia in cirrhotic patients using post-mortem specimens injected with micro-opaque gelatin to highlight the pulmonary vascular tree (Berthelot et al., 1966) and demonstrated marked dilatation of the fine peripheral branches of the pulmonary artery (at the precapillary and capillary level). Spider naevi were apparent on the pleura of half of their patients, but true arteriovenous communications were found in only one such patient. Subsequent post-mortem studies have confirmed these findings (Stanley et al., 1977; Davis et al., 1978).
The physiological basis for them has been the subject of much debate with proposed mechanisms including changes in the affinity of oxyhaemoglobin, intrapulmonary and portopulmonary shunts, alveolar diffusion limitation for oxygen and ventilation-perfusion (V/Q) inequalities (Agusti et al., 1990). The clinical consequences of this complex series of interactions have been called the hepato-pulmonary syndrome (Sherlock & Dooley, 1993a).

We hypothesised that, if patients with cystic fibrosis related chronic liver disease had the hepato-pulmonary syndrome, it might be a contributory factor to the covert effect of liver disease upon survival.

The purpose of the present study was, therefore, to evaluate subjects with cystic fibrosis related chronic liver disease for evidence of the hepato-pulmonary syndrome. The results were compared with a matched group of cystic fibrosis patients without liver disease as the interpretation of the results was complicated by the presence of underlying pulmonary disease, possible V/Q mismatch (Dantzker, 1987) and the development of broncho-pulmonary shunts in the brochiectatic lungs of patients with cystic fibrosis (Mack et al., 1965; Moss et al., 1968), although this latter manifestation is rarely, if ever, associated with systemic arterial desaturation (Liebow et al., 1949).

Lung function was measured as detailed in chapter six and oximetry was used to estimate postural and exercise induced changes in the oxygen saturation. The size of pulmonary arterio-venous shunts was measured.
Intrapulmonary arterio-venous shunting and V/Q mismatch were assessed using radionuclide 'shunt-perfusion' scans and the 100% oxygen rebreathing technique. The combination of these techniques should allow the evaluation of both anatomical and functional (physiological) shunting.
7.1. Patients and Methods

7.1.1. Patients

The study cohort included all 56 subjects who were entered into the haemodynamic study. 28 subjects were randomly selected from this cohort (14 NLD and 14 LD) for the shunt estimation studies.

Patients were categorised into the LD group on the basis of their hepatic ultrasound score as detailed in chapter three.

7.1.2. Methods

The following methods were used to evaluate the subjects:

7.1.2.1. Lung function

Pulmonary function variables were recorded as documented previously and expressed as percentage of predicted values for age and height (Cotes, 1991).

7.1.2.2. Arterial oxygen saturation

To assess whether or not patients with cystic fibrosis liver disease were more likely to desaturate on exercise or to have a postural fall in oxygen saturation (orthodeoxia) arterial oxygen saturation was measured in two situations:
(a) pre and post exercise following the same protocol as detailed in chapter six.

(b) supine and after standing upright for 60 seconds.

For the purposes of the exercise induced desaturation only the 21 NLD and 19 LD subjects who had paired data available from the exercise study were included. For the postural desaturation study all subjects entered into the haemodynamic study were eligible for inclusion in the analysis.

Arterial oxygen saturation was measured using a pulse-oximeter with an ear probe attached (Ohmeda Biox 3700, BOC Healthcare Ltd.) following the application of a vasodilator (Finalgon ointment, Boehringer Ingelheim) to the left ear lobe. The pulse-oximeter was set to 'fast-response' mode to give a continuous reading of a 3 second moving average of the arterial oxygen saturation (SaO₂).

7.1.2.3. Shunt estimation

Two methods were used to evaluate the presence of pulmonary arterio-venous shunting:

(a) 13 patients underwent radionuclide 'shunt-perfusion' scanning with technetium-99m labelled macroaggregated albumin (⁹⁹ᵐTc-MAA) and lung, brain and kidney imaging with a gamma-scintillation camera (Krowka & Cortese, 1989).
The technique is dependent upon the size of the $^{99m}$Tc-MAA particles (20 to 60 µm) which should not pass through the normal pulmonary capillaries which are less than 15 µm in diameter. The presence of true arterio-venous anastamoses or a dilated capillary bed will, in theory, allow passage of $^{99m}$Tc-MAA particles. The shunt can be estimated on the basis that the combined renal and cerebral circulations account for 32% of the cardiac output in the resting state (Wade & Bishop, 1962).

100 MBq of $^{99m}$Tc-MAA (Amersham Laboratories, Amersham, UK) were injected into an antecubital vein with the patient sitting upright. Ten minutes after the injection the subject was placed in the supine position, and the amount of radioactivity was measured within posterior lung images using a wide field of view gamma camera (Scintronix, Aura Ltd., UK) fitted with a general purpose, low energy collimator. Immediately afterwards, the radioactivity was measured within the brain (anterior view of the skull) and kidneys (posterior view of the abdomen).

Measurements were made by drawing regions of interest over the lungs, brain and kidneys and estimating the amount of radio-activity in each of these areas using a dedicated micro computer (Micas V, Bartec). In some subjects radioactivity had already reached the bladder at the time of abdominal scanning, so a further region of interest was drawn over the bladder.

A further region of interest was drawn between the kidneys for the estimation of background counts to allow measurements to be corrected for scatter from the lungs. The kidney and bladder counts were corrected by subtraction of background activity.
The number of counts in a region of interest was corrected for area by dividing by the measured area of the organ and for the acquisition time by dividing by the number of seconds over which the images were obtained. The corrected counts were expressed as counts/pixel/second.

The amount of shunted radio-labelled albumin was estimated from:

\[
\frac{(\text{Corrected kidney & bladder} + \text{corrected brain counts}) \times 100/32}{\left\{(\text{Corrected kidney & bladder} + \text{brain}) \times 100/32\right\} + \text{Corrected lung counts}}
\]

This ratio was expressed as a percentage.

(b) 27 patients underwent shunt estimation using the 100% oxygen rebreathing method.

Subjects underwent baseline arterial blood gas analysis (ABL 300, Radiometer A/S, Copenhagen), sampled from the radial artery using a heparinised 5 ml. glass syringe and 23 gauge needle, to ensure that there was no carbon dioxide retention. The seated subject then breathed 100% oxygen, from a Douglas bag, via a mouthpiece and two way valve with a nose clip applied to ensure that no nasal ventilation occurred. The subject was encouraged to keep their lips tightly sealed around the mouthpiece.

Arterial oxygen saturation was measured using a pulse-oximeter (Ohmeda Biox 3700), with ear probe attached, following the application of Finalgon ointment to the ear lobe.
To ensure that an adequate end-point was reached for all subjects the end expiratory nitrogen concentration was measured using a mass spectrometer (MGA 200, Centronic, UK) that had already been calibrated using room air and a standard gas mixture (5% CO₂, 12% O₂, balance N₂).

The oxygen saturation, end expiratory nitrogen and end-tidal carbon dioxide concentration were recorded on a multi-channel recorder (TA400, Gould Electronics, USA). The end-point, (complete nitrogen washout), was defined as less than 1% nitrogen detectable in the expired gas.

Once the end-point had been reached a further arterial sample was obtained from the other radial artery whilst ensuring that the subject continued to breathe 100% oxygen and maintain an airtight seal around the mouthpiece. The sample was immediately placed on ice and analysed in the blood gas analyser.

Correction of the result obtained was made to allow for the non-linear relationship between the actual PaO₂ and the measured value obtained from the blood gas analyser for partial pressures greater than 100mm Hg. The correction factor was obtained by tonometering 10 mls. of heparinised venous blood, from a healthy non-smoking adult, with 100% oxygen for 20 minutes (Tonometer 237, Instrumentation Lab, Italy), and measuring the PaO₂ of the tonometered blood using the blood gas analyser. The expected value is given by the formula:
Expected $\text{PaO}_2 = \text{P}_{\text{BAR}} - 47 \times 100$ where $\text{P}_{\text{BAR}}$ is the barometric pressure.

The corrected $\text{PaO}_2 = (\text{Expected PaO}_2 / \text{Tonometered PaO}_2) \times \text{Measured PaO}_2$

Right to left shunting was calculated from the $\text{PaO}_2$ value obtained and the haemoglobin (Hb; g/dl) concentration and $\text{SaO}_2$ (%) measured on the same sample (OSM 2 Hemoximeter, Radiometer A/S, Copenhagen) by using the classical equation to give the volume of the shunt as a percentage of cardiac output (Bergrren, 1942; Chilvers et al., 1990).

$$\frac{Q_s}{Q_r} = \frac{(\text{CcO}_2 - \text{CaO}_2)}{(\text{CcO}_2 - \text{CvO}_2)} \times 100$$

where

$Q_s$ is the volume of the shunt
$Q_r$ is the cardiac output
$\text{CaO}_2$ is the oxygen content of arterial blood (ml/100ml)
$\text{CvO}_2$ is the oxygen content of mixed venous blood (ml/100ml)
$\text{CcO}_2$ is the end pulmonary capillary blood oxygen content (ml/100ml)

These values are calculated from the following formulae:

$\text{CaO}_2 = (\text{Hb} \times 1.34) + (\text{PaO}_2 \times 0.003)$

$\text{CcO}_2 = (\text{Hb} \times 1.34) + (\text{PcO}_2 \times 0.003)$ where $\text{PcO}_2$ is the partial pressure of oxygen in pulmonary end capillary blood and is given by the formula $\text{PcO}_2 = \text{P}_{\text{BAR}} - 47 - \text{PaCO}_2$ (the arterial partial pressure of carbon dioxide).
These calculations assume a physical solubility of oxygen in blood of 0.003 ml/100ml/mm Hg and a blood oxygen content equal to dissolved O₂ and HbO₂.

\( CVO_2 \) was not measured but was assumed to be 5ml/100ml below the \( CaO_2 \) (\( CVO_2 = CaO_2 - 5 \)).

7.1.2.4. Statistical analysis

Results were expressed as mean ± SEM and where the sample size was small as median (range).

Results for the two groups were compared using \( \chi^2 \) for categorical data and Mann-Whitney U (for the 99mTc-MAA shunt-perfusion scans) and unpaired t tests (for the remaining comparisons) for continuous data. A probability value <0.05 was considered a significant result for a two-tailed test.

7.2. Results

7.2.1. Pulmonary function tests

There were no significant differences in the following lung function variables: FEV₁, FVC, forced expiratory flow between 25 and 75% volume (FEF25-75%), total lung capacity (TLC), residual volume (RV), transfer factor (TLCO) or its value corrected for lung volume (KCO) (Table 7.1.).
7.2.2. Oximetry

7.2.2.1. Exercise

Paired readings for pre and post exercise oxygen saturations were available for 40 subjects (21 NLD; 19 LD). There were no age or sex differences between the two groups (see chapter 6).

There were no significant differences in SaO2 when these were compared, and the mean change in oxygen saturation with exercise was not different for the two groups (-0.3 ± 0.2 vs. -0.5 ±0.2 %; p=NS). The maximum fall in oxygen saturation recorded with exercise was 3% for both groups (Table 7.2.).

7.2.2.2. Posture

Data on postural changes in oxygen saturation was available for fifty subjects (26 NLD; 24 LD). It was not recorded in the other six subjects who underwent haemodynamic evaluation. The groups were well matched for age (NLD vs. LD: 24.8 ± 1.3 vs. 22.6 ± 1.3 years) and sex (9 female; 17 male vs 11 female; 13 male).

There were no significant differences in lying or standing oxygen saturations between the two groups and there was no difference in the mean change in saturation on moving from lying to standing (0.8 ± 0.3 vs. 0.0 ± 0.4 %; p=NS). The maximum desaturation recorded on standing was 4% in the NLD group and 5% in the LD group (Table 7.2.).
7.2.3. Shunt estimations

The oximetry data for the twenty eight subjects included in the shunt analysis is documented in tables 7.3. (NLD group) and 7.4. (LD group).

7.2.3.1. $^{99m}$Tc MAA 'Shunt-perfusion' scans

13 randomly selected subjects (5 NLD; 8 LD) underwent $^{99m}$Tc MAA 'shunt-perfusion' scans. The groups were well matched for age (20.2 ± 1.5 vs 20.3 ± 1.8 years) and sex (2 female:3 male vs 4 female:4 male). All the scans demonstrated lung perfusion abnormalities consistent with parenchymal lung disease.

Four (44%) of the LD group had significant shunts. In three subjects these were greater than 10% and in one larger than 6% (Tables 7.5. and 7.6.; Figure 7.1.). However, the median shunt fraction was not statistically significantly different for the NLD and LD groups (4% (range 0.6-5%) and 6.9% (range 3.1-13.3%)) respectively.

7.2.3.2. 100% oxygen re-breathing shunt estimation

27 subjects were included in the oxygen re-breathing study (13 NLD and 14 LD). The groups were well matched for age (25.1 ± 1.9 vs 21.9 ± 1.4 years) and sex (4 female:9 male vs 6 female: 8 male). Twelve of the subjects who underwent $^{99m}$Tc MAA shunt-
perfusion scans were included. The thirteenth subject declined to participate in the study due to a needle phobia.

Arterial blood gas analysis revealed mild hypoxaemia in both groups, but the partial pressures of oxygen did not differ significantly between the two groups (Tables 7.5. and 7.6.). The other baseline characteristics of the two groups were normal and were well matched.

Shunt estimations documented no statistical difference in the size of shunt in both groups (4.8 ± 0.6 vs. 5.3 ± 1.1 %). However, two subjects in the LD group had shunts >10% (Tables 7.5. and 7.6.; Figure 7.2.). One of these had had a similar level of shunting demonstrated by 99mTc MAA shunt-perfusion scan. The other two subjects who had had a shunt >10% documented by 99mTc MAA shunt-perfusion scan did not have an elevated shunt fraction on measurement by the oxygen re-breathing technique.
Table 7.1. Pulmonary function test details for the whole of the cohort included in the haemodynamic study (mean + SEM)

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<tr>
<td>%PFEV₁</td>
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<td>%PFEF25%-75%</td>
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<td>%PRV</td>
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<td>%PT₁CO</td>
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<tr>
<td>%PKCO</td>
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%PFVC - percentage predicted forced vital capacity
%PFEV₁ - percentage predicted forced expiratory volume in one second
%PFEF25%-75% - percentage predicted forced expiratory flow between 25 and 75% of vital capacity
%PTLC - percentage predicted total lung capacity
%PRV - percentage predicted residual volume
%PT₁CO - Percentage predicted transfer factor for carbon monoxide
%PKCO - percentage predicted transfer factor corrected for lung volume
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<td>SaO₂ rest (%)</td>
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<td>SaO₂ lying (%)</td>
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<td>SaO₂ standing (%)</td>
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Table 7.2. Oxygen saturation data from rest to exercise and from lying to standing for the whole evaluable cohort (mean ± SEM)
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Table 7.3. Oxygen saturations pre and post exercise and from lying to standing for the NLD cohort who underwent shunt estimation.
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| 274 |

Table 7.4. Oxygen saturations pre and post exercise and from lying to standing for the LD cohort who underwent shunt estimation.
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Table 7.5. Arterial blood gas and shunt data for the NLD cohort

(subject number corresponds to number in table 7.3.)
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Table 7.6. Arterial blood gas and shunt data for the LD cohort.

(subject number corresponds to number in table 7.4.)

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Figure 7.1. Scattergram of $^{99m}$Tc MAA shunt-perfusion shunt estimations
Figure 7.2. Scattergram of 100% oxygen rebreathing shunt estimations
7.3. Discussion

7.3.1. General

The hepato-pulmonary syndrome is characterised by a number of abnormalities which combine to cause hypoxaemia, cyanosis and clubbing. The first is a reduced affinity of haemoglobin for oxygen due to an elevated concentration of 2,3-diphosphoglycerate (Agusti, 1985), although this is not thought to be a major contributory factor. The second is arterio-venous shunting through anatomical arterio-venous communications (Rodriguez-Roisin et al., 1992) and pre-capillary dilatation of the pulmonary vessels (Berthelot et al., 1966). These dilated pulmonary vessels are preferentially located at the lung bases and may explain the fall in arterial PaO\textsubscript{2} sometimes seen in patients with cirrhosis when rising from the supine to upright position (orthodeoxia) (Agusti, 1985). The third is the so called 'diffusion-perfusion' defect in which the combination of a dilated vascular bed in association with a high cardiac output results in impaired access of oxygen to the midstream of the capillary blood (Agusti et al., 1989). The reduced T\textsubscript{i}CO frequently seen in these patients (Agusti, 1985) and the tendency for the arterial PaO\textsubscript{2} to fall during exercise when the increased cardiac output will result in even less time for the haemoglobin to be oxygenated (Krowka & Cortese, 1989) might be related to this mechanism. The fourth is the presence of ventilation-perfusion (V/Q) inequality which may result from a disproportionate increase in capillary perfusion (due to abnormal vessel dilatation) to some lung units and a relative failure of hypoxic
pulmonary vasoconstriction to redress the balance (Agustí et al., 1990). Exercise might also result in a significant fall in PaO₂ if this were the mechanism.

V/Q mismatch is also the predominant mechanism by which patients with parenchymal lung disease become hypoxic (Dantzker, 1987).

While all of these mechanisms may be contributory, it is generally believed that the hepato-pulmonary syndrome is more functional than anatomical with a combination of a dilated pulmonary vascular bed, impaired hypoxic pulmonary vasoconstriction and oxygen diffusion limitation being the predominant features (Rodríguez-Roisin et al., 1992). This hypothesis is supported by the reversibility of the syndrome following orthotopic liver transplantation (Eriksson et al., 1988; Stoller et al., 1990).

Not all cases with a dilated intrapulmonary vascular bed are hypoxaemic, suggesting that subclinical pulmonary vasodilatation can occur before the development of a PaO₂ <70 mmHg (Krowka & Cortese, 1990). Anatomical shunting may be important in the more severe cases, but is relatively uncommon (Rodríguez-Roisin et al., 1992).

The results of the present study suggest that there is no limitation of pulmonary diffusion in cystic fibrosis patients as both groups had normal carbon monoxide transfer when this was corrected for their increased lung volumes (KCO). However, a normal diffusing capacity for carbon monoxide does not imply
a normal diffusing capacity for oxygen as they have different diffusive capacities.

None of the subjects demonstrated a significant fall in oxygen saturation upon exercising or upon standing suggesting that neither of these manifestations of the hepato-pulmonary syndrome were problems for the cohort evaluated in this study.

The results of the shunt estimations would also suggest that large anatomical and physiological pulmonary shunts are not a significant problem in this cohort. However, 5/14 (36%) patients in the LD group did have larger shunts than would have been expected on the basis of their underlying pulmonary disease.

A small amount of blood is normally shunted through the bronchial and thebesian system and amounts to less than 3% of the cardiac output (Dantzker, 1989). However, in a study of patients with underlying pulmonary disease, (lobar collapse, pleural effusion or pneumothorax), using the 100% oxygen rebreathing technique it has been shown that as much as 6.9% of the cardiac output may reach unventilated lung segments (Bergrren, 1942). The shunt fraction in normal subjects in this study was less than 0.6% (Bergrren, 1942).

These results concur with the shunt fractions obtained in the majority of subjects within this study, with shunts of less than 8.5% in all the NLD subjects and in 10/14 (71%) of the LD group.

However, four of the LD subjects had shunt fractions that might be clinically important (>10%), with a fifth subject in the LD group having a $^{99m}$Tc MAA shunt of 7.6%. The largest
documented shunt (15.7%) was in the only LD subject with spider naevi. Patients with spider naevi and liver disease tend to have the most severe pulmonary vascular changes (Rodriguez-Roisin et al., 1987) and it has been suggested that spider naevi are one of the most useful markers of altered pulmonary blood vessels in these patients.

Interestingly spider naevi were an uncommon finding in the cohort of cystic fibrosis patients included in the ultrasound study presented in chapter three, suggesting that true anatomical shunts may not have a high prevalence in patients with cystic fibrosis related chronic liver disease.

Various methods have been proposed for quantifying the shunt fraction including the $^{99m}$Tc MAA shunt-perfusion (Krowka & Cortese, 1989) and 100% oxygen rebreathing (Bergren, 1942) methods used in the present study. These techniques primarily detect anatomical shunts although both will give abnormal results in the presence of a dilated pulmonary vascular bed (physiological shunt). The former will be abnormal if the vascular bed is sufficiently dilated to allow the passage of the $^{99m}$Tc-MAA particles which are 20-60 μm in diameter and should normally be trapped in the pulmonary vascular bed (diameter up to 15 μm) and the latter if the vascular bed is sufficiently dilated and the cardiac output high enough to ensure rapid transit of red blood cells past the alveoli causing reduced haemoglobin oxygenation as a result of the so called 'diffusion-perfusion' defect (Krowka & Cortese, 1990).
100% oxygen rebreathing will also be abnormal in the presence of significant V/Q mismatch. Neither technique will allow the differentiation of the various mechanisms thought to be involved in arterial hypoxaemia in cirrhotic patients, although it has been suggested that the 100% oxygen rebreathing method or a combination of tests may help.

If (following oxygen rebreathing in subjects without underlying lung disease) the PaO₂ remains essentially unchanged, then an anatomical problem is likely to be the predominant mechanism, if there is a moderate increase in PaO₂ (>300 mmHg) a true arterio-venous shunt cannot be excluded, and if there is a substantial increase (>500 mmHg) then V/Q mismatch is likely to be the predominant problem (Davis et al., 1978; Krowka & Cortese, 1989).

In this study the two LD subjects with shunts >10% increased their PaO₂ to above 300 mmHg making a true anatomical shunt unlikely and suggesting that V/Q mismatch is the likely mechanism for shunting. A similar mechanism is suggested for all the other NLD and LD subjects who underwent oxygen rebreathing shunt estimation as they had a substantial increase in their PaO₂ to greater than 500 mmHg (Tables 7.5. and 7.6.).

However, comparison of the shunt fractions obtained for the twelve subjects who were studied using both techniques may provide additional information. It has been suggested that up to 6% of the injected ⁹⁹mTc-MAA particles may pass into the systemic circulation in a normal person (Wolfe et al., 1977), suggesting that four of the LD subjects had elevated shunt
fractions by this technique (3 were >10%). However, only one of the subjects had a shunt of similar size measured by the oxygen re-breathing technique suggesting that this subject may have had a true anatomical shunt that did not correct at all with 100% oxygen rebreathing.

The reduction of shunt, on oxygen rebreathing, documented in the other three subjects, coupled with the increase in PaO2 to above 500 mmHg suggest that these three subjects had a functional problem similar to the 'diffusion-perfusion' defect or had V/Q mismatch which was largely corrected by the administration of 100% oxygen. The other LD subject with a large oxygen rebreathing shunt did not have a 99mTc MAA scan, but it may be hypothesised that some or all of the shunt had an anatomical basis due to the presence of cutaneous spider naevi.

These results are in agreement with previous studies which have suggested a good correlation between the two methods of shunt estimation when they are used to measure anatomical shunts (Whyte et al., 1992), and others which have shown a large discrepancy when they are used to evaluate some patients with chronic liver disease (Wolfe et al., 1977). The study in patients with chronic liver disease suggested that the oxygen rebreathing technique underestimated shunting in patients with a dilated intrapulmonary vascular tree because the increased driving pressure of oxygen resulted in improved oxygenation of haemoglobin carried by red blood cells in the middle of the blood stream with a consequent attenuation of any 'diffusion-perfusion' problems and reduction in the measured shunt. In
contrast the radionuclide passed through the dilated pulmonary vessels and gave a true reflection of shunt size. This would appear to be the case in the subjects studied here.

On this basis, therefore, the patients with LD in this study appear to exhibit many of the features of the hepato-pulmonary syndrome. Particularly elevated shunt fractions are probably due to a combination of the 'diffusion-perfusion' defect, V/Q mismatch and true anatomical shunts. It is likely that in the other patients inappropriate perfusion of diseased or unventilated lung segments resulted in V/Q mismatch and oxygen rebreathing shunts of larger size than would be expected in the normal population.

The techniques used in this study are well recognised for the evaluation of arterio-venous shunting. There are, however, other techniques for investigating pulmonary abnormalities which may have provided additional information. These include pulmonary angiography which may reveal a 'spongy' distal arterial tree in the lung bases corresponding to the basilar infiltrates sometimes seen on chest radiographs (Oh et al., 1983) and contrast-enhanced two-dimensional echocardiography (Krowka & Cortese, 1989). The latter technique identifies echoes (microbubbles of air or indocyanine green) in the left atrium within three to six beats of their visualisation in the right heart when a shunt is present (Krowka & Cortese, 1989). The microbubbles (up to 90μm) are usually trapped in the pulmonary vascular tree. The technique does not, however, allow the differentiation of functional and anatomical shunts.
(Rodriguez-Roisin et al., 1992), and will not allow the detection of V/Q mismatch. Its only advantage over the methods used in this study is its ability to differentiate intrapulmonary and intracardiac shunting (when the bubbles will appear immediately) (Krowka & Cortese, 1990).

An alternative approach is the use of the multiple-inert gas elimination technique (MIGET) (Evans & Wagner, 1977). This technique allows the estimation of the functional distribution of V/Q ratios within the lung on the basis of the pattern of elimination of dissolved infused inert gases of different solubility. On the basis of studies using this technique it has been proposed that V/Q mismatching (perfusion of underventilated lung segments by dilated pulmonary vessels) and intrapulmonary shunting are the principal causes of a reduced PaO2 in cirrhosis (Rodriguez-Roisin et al., 1987; Melot et al., 1989; Edell et al., 1989; Castaing & Manier, 1989; Hedenstierna et al., 1991). Whether or not the use of this technique would have been more sensitive or yielded different results in this study involving patients with significant parenchymal lung disease is not known.

7.3.2. Potential mechanisms and therapeutic implications

This study has detected shunting in 36% of the subjects in the LD group, while there was no evidence of significant shunts in the NLD cohort. Four of the LD group had a shunt fraction >10% and
one a shunt fraction of 7.6% using the shunt-perfusion technique. In at least three of these patients this is likely to be the result of intrapulmonary shunting and V/Q mismatch due to a dilated pulmonary vascular tree.

The mechanisms for the vascular abnormalities in the lung in cirrhotic patients are likely to be similar to those proposed for the hyperdynamic systemic circulatory disturbance. A putative pulmonary vasodilator originating from the mesenteric circulation which fails to be metabolised or cleared by the diseased liver has been suggested (Krowka & Cortese, 1989). Potential pulmonary vasodilators including prostacyclin (Zipser et al., 1979), atrial natriuretic factor (Gines et al., 1988) and platelet activating factor (Caramelo et al., 1987) have been detected and may be responsible for attenuating hypoxic pulmonary vasoconstriction. Alternatively an absent or impaired vasoconstrictor may be responsible (Krowka & Cortese, 1989). In addition, there is likely to be a contribution from the hyperkinetic systemic circulation documented in these patients.

In cystic fibrosis perfusion of underventilated or diseased lung segments in association with anomalous vascular formations associated with bronchiectatic lung segments may have an additional effect (Mack et al., 1965; Moss et al., 1968).

The implications for treatment in the context of cystic fibrosis are unclear. While drug therapy has not been shown to consistently benefit patients with the 'hepato-pulmonary' syndrome (Krowka & Cortese, 1987; Cadranel et al., 1992), liver transplantation has been shown to reverse it (Eriksson et al.,
1988; Stoller et al., 1990). It is possible, therefore, that patients with cystic fibrosis related liver disease and severe hypoxemia thought to be due to pulmonary shunting could be considered for isolated hepatic transplantation or triple organ transplantation to attempt to reverse the functional changes.

7.4. Conclusions

This study has identified features of the hepato-pulmonary syndrome in 36% of patients with cystic fibrosis associated chronic liver disease. Documentation of larger shunts in 4/14 (29%) of LD subjects is similar to the prevalence of the hepato-pulmonary syndrome reported in other series (Rodman et al., 1960)

When present the hepato-pulmonary syndrome may result in an inappropriately low SaO2. It may be speculated that the combination of an increased cardiac output and arterio-venous pulmonary shunting in a patient with liver disease might accelerate the deaths of patients (perhaps by causing subclinical myocardial ischaemia) with progressive lung and liver disease. Sequential monitoring of shunting and systemic circulatory changes would be required to test this hypothesis further.
Chapter 8

CONCLUSIONS

and suggestions for further work
8.0. **Summary**

As the median age of survival continues to increase with improvements in the respiratory care of patients with cystic fibrosis it is likely that there will be an increase in the prevalence of cystic fibrosis related chronic liver disease.

The work presented within this thesis suggests that liver disease is an important factor influencing survival. However, very few patients died as a direct consequence of chronic liver disease (<3%), thus implicating indirect effects of the liver disease upon survival.

The prognostic value of chronic liver disease highlights the need for the early diagnosis of the chronic biliary liver disease of cystic fibrosis and also of identifying effective therapeutic agents.

Considerable sampling error has seriously restricted the reliance of accurate diagnosis upon histology. Ultrasonography seems, in experienced hands, to be an ideal method for evaluating hepatobiliary involvement. Having confirmed the characteristic ultrasonographic features of cystic fibrosis liver disease as coarseness of the hepatic parenchyma, periportal fibrosis and marked irregularity of the margin of the liver, we have been able to construct an ultrasound based scoring system which allows earlier identification of patients with hepatic involvement. The simplicity of the scoring system should allow experienced ultrasonographers in other centres to confidently assess the severity of liver disease in cystic fibrosis.
As an alternative to histology the scoring system was validated against a number of biochemical tests measuring functioning hepatic cell mass and hepatic fibrosis. In general, these tests correlated well with the ultrasound scoring system.

Quantitative hepato-biliary scintigraphy also appeared to provide objective data regarding the presence or absence of liver disease. In particular a normal ultrasound scan and a normal EHIDA scan correlate closely.

The biochemical tests evaluated for the detection of liver disease proved disappointing with no greater sensitivity or specificity than some of the conventional tests of liver function in the identification of patients with an ultrasound diagnosis of liver disease.

Of the tests evaluated the measurement of the primary metabolite of lignocaine, MEG-X, appeared to be the most promising both with the regard to the diagnosis of hepatic disease and assessment of disease severity in that it allowed discrimination between the NLD, ILD and SLD groups defined by the ultrasound scoring system as well as correlating well with the ultrasound score. While the other biochemical parameters measured proved disappointing in the assessment of disease severity, they may all, together with quantitative hepato-biliary scintigraphy, have a role in monitoring the response of patients to therapeutic intervention for hepatic disease.

This thesis has also assessed one of the potential mechanisms by which liver disease might influence survival. It was
hypothesised that this adverse influence might be attributable, in part, to the systemic and pulmonary circulatory changes documented in chronic liver disease of other aetiologies.

Two potentially important inter-relating factors have been observed. Firstly patients with cystic fibrosis related chronic liver disease have a hyperdynamic circulation similar to that observed in other forms of chronic liver disease. Secondly some patients with liver disease have significant pulmonary arterio-venous shunts. These changes were documented in patients who were stable and not in an advanced disease state at the time of the study and who may not, therefore, fully express the extent of the abnormalities in question.

It may be speculated that these changes interact to accelerate mortality in the cohort of cystic fibrosis patients with liver disease. The exact mechanism for this effect on survival may relate to the increased left ventricular work, associated with the increased cardiac output and tachycardia, causing increased myocardial oxygen demands and reduced diastolic myocardial perfusion time. This may result, particularly in the context of a falling oxygen saturation consequent upon progressive pulmonary disease and pulmonary arterio-venous shunting, in myocardial ischaemia, myocardial dysfunction and myocardial damage. Such sub-endocardial fibrosis has been documented at post mortem in cystic fibrosis.

In addition, a falling oxygen saturation in patients with pulmonary arterio-venous shunting may accelerate the development of pulmonary hypertension and cor pulmonale. It
is possible, therefore, that it is the patients who do develop pulmonary arterio-venous shunting who are most vulnerable to the premature decline.

These hypotheses remain a matter for speculation. To test them further would require sequential studies of individual patients over time and it may not ultimately prove possible to study the patients at the crucial time when they begin to decline.

8.1. Conclusions

This thesis highlights the importance of chronic liver disease in the long-term management of cystic fibrosis despite the perceived slow progression of the hepatic lesion.

The need for simple and reliable, non-invasive tests to confirm the presence of liver disease has been addressed with the conclusion that a hepato-biliary ultrasound in conjunction with the measurement of MEG-X may represent the best approach.

It is suggested that part of the mechanism for the increase in mortality associated with hepatic involvement is related to the systemic and pulmonary circulatory changes documented in patients with chronic liver disease.

8.2. Suggestions for further work

A number of questions are posed by the work presented in this thesis:
1. The prognostic index presented in this thesis must now be prospectively evaluated to confirm its applicability, not only in the Brompton Hospital patients, but also in other cohorts of patients with cystic fibrosis.

Such an assessment in patients undergoing transplantation would also give further guidance as to the appropriate time to initiate transplant assessment in other patients.

2. There are currently no criteria by which to judge the severity of liver disease in patients undergoing transplantation. Current criteria allowing estimation of disease severity are confined to the documentation of episodes of decompensation (jaundice, ascites, encephalopathy) in the absence of a precipitating insult such as a respiratory exacerbation.

Use of quantitative hepato-biliary scintigraphy, in particular the hepatic extraction fraction, the MEG-X test and possibly total fasting serum bile acids, may give an estimate of how well preserved hepatic function is. Prospective evaluation of these tests in patients undergoing transplantation will provide data on whether or not they are useful in predicting patients who will decompensate following surgery or alternatively those who will benefit from triple-organ transplantation.

3. Further work elucidating the covert effect of liver disease upon survival may present further therapeutic options.

(a) Sequential evaluation of systemic and pulmonary haemodynamics may give further insight into whether this mechanism is important.
(b) Measurement of potential mediators such as glucagon, and the inflammatory cytokines IL-1, IL-6 and TNFα may provide further insight and produce new avenues for therapy.

(c) Evaluation of other proposed mechanisms, such as the immune function of cystic fibrosis patients with and without chronic liver disease may be instructive.

4. If liver disease has an important negative influence on longevity in cystic fibrosis, and if as many experts anticipate the prevalence of liver disease increases as a consequence of improved survival, so the identification of an effective therapeutic agent for treating liver disease becomes even more important.

Ursodeoxycholic acid has already been identified as a potential therapeutic agent, but while improvements in conventional biochemical tests and the half-time of hepatic excretion of IDA agents have been documented there is no data to show an improvement in other parameters of hepatic function. A double blind placebo controlled trial addressing this issue is urgently needed.

In addition, in the light of the fact that ursodeoxycholic acid is an extremely well tolerated medication, its use for prophylaxis against the development of liver disease in a high risk population, such as those patients who present in infancy with meconium ileus, must be considered.

Furthermore, the role of other therapeutic agents, such as the prolyl-4-hydroxylase inhibitors, should be addressed.
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