AN INVESTIGATION OF THE DETECTION AND TREATMENT OF COLORECTAL LIVER METASTASES

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Abbreviations

APTT	activated partial thromboplastin time
APC	adenomatous polyposis coli
ALT	alanine transaminase
Alk Phos	alkaline phosphatase
AJCC	American Joint Committee on Cancer
ASA	American Society of Anaesthesiologists grading
AST	aspartate transaminase
ACPGBI	Association of Coloproctology of Great Britain and Ireland
AUC	area under the curve
CEA	carcinoembryonic antigen
CVP	central venous pressure
CRS	clinical risk score
CV	coefficient of variation
CD	compact disc
CT	computed tomography
CTAP	computed tomography arterial portography
CE-US	contrast enhanced ultrasound
CE-IOUS	contrast enhanced intraoperative ultrasound
DCC	deleted in colon cancer gene
DNA	deoxyribonucleic acid
DPI	doppler perfusion index
ECG	electrocardiogram

FAP	familial adenomatous polyposis
FUDR	5-fluorodeoxyuridine
18FDG	fluoro-2-deoxygenase
5-FU	5 fluorouracil
FiO_2	fraction of inspired oxygen
GGT	gamma-glutamyltransferase
HAI	hepatic arterial infusion
HPI	hepatic perfusion index
HNPCC	hereditary non polyposis colon cancer
HIFU	high intensity focused ultrasound
ILP	interstitial laser photocoagulation
ICCC	intraclass correlation coefficient
IOUS	intraoperative ultrasound
IHP	isolated hepatic perfusion
LUS	laparoscopic ultrasound
MRI	magnetic resonance imaging
MI	mechanical index
MCT	microwave coagulation therapy
MCE	myocardial contrast echocardiography
PMR	hepatic parenchymal to liver metastasis ratio
PEI	percutaneous ethanol injection
PVE	portal vein embolisation
PET	positron emission tomography
PT	prothrombin time

PIH	pulse inversion harmonic imaging
RF ablation	radiofrequency ablation
ROC	receiver operator characteristics curve
ROI	region of interest
RLV	residual liver volume
RNA	ribonucleic acid
SpO_2	saturation of oxygen via pulse oximetry
SIRT	selective internal radiation therapy
SD	standard deviation
TCT	thrombin time
TME	total mesorectal excision
TNM	tumour node metastases classification of cancer
US	unenhanced (fundamental) ultrasound
WCRF	world cancer research fund

Dedication

For Elizabeth Smillie and Isabella Moug.

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Declaration

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May 2006- Association of Surgeons of Great Britain and Ireland (Edinburgh)

Synchronous approach in the treatment of colorectal cancer with hepatic metastases does not adversely affect patient outcome. **SJ Moug**, E Leen, D Smith, WJ Angerson and PG Horgan.

May 2006- Association of Surgeons of Great Britain and Ireland (Edinburgh)

Prospective evaluation of components of the Clinical Risk Score in patients undergoing curative resection for colorectal hepatic metastases. **SJ Moug**, DC McMillan, E Leen, D Smith, PG Horgan.

November 2005 – Radiological Society of North American (Chicago)

Detection of Liver metastases with a second generation Ultrasound Contrast Agent, DMP 115 in Late Phase Imaging: Preliminary Report. **SJ Moug,** EL Leen, A Hunter, J Logue, PG Horgan.

May 2005 – Scottish Chapter of Coloproctology (Dundee)

Selective continuous vascular occlusion and fluid restriction in partial hepatectomy. **SJ Moug,** D Smith, E Leen, WJ Angerson and PG Horgan.

April 2005 – Association of Surgeons of Great Britain and Ireland (Glasgow)

Contrast-enhanced intraoperative ultrasound is an essential tool during partial hepatectomy for metastases. **SJ Moug**, E Leen, P Ceccotti, P Glen, T Albrecht, J Hohmann, A Oldenburg, JP, Ritz and PG Horgan.

July 2004 – International Congress of Radiology (Montreal)

Further findings on the use of contrast enhanced intra-operative ultrasound in the detection of colorectal liver metastases. **SJ Moug,** E Leen, P Ceccotti, T Albrecht and P Horgan.

January 2004 - Glasgow Gut Club (Southern General Hospital)

Preliminary Report on Contrast Enhanced Intra-Operative Ultrasound in the Detection of Liver Metastases: An Essential Investigation before Resection? **SJ Moug,** E Leen, P Ceccotti, T Albrecht and P Horgan.

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Poster Presentations

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Summary of Thesis

In the United Kingdom, colorectal cancer creates a significant health burden, with over 34 000 new cases diagnosed each year and over 16 000 deaths per year. Almost 50% of patients with colorectal cancer will develop liver metastases: up to 25% will have liver metastases at time of initial presentation with the remaining 25% developing liver metastases during the course of their disease. Death from hepatic metastases accounts for a large percentage of colorectal cancer mortalities and if left untreated the prognosis is poor, with median survival from 5 to 21 months with almost none alive at 5 years.

Surgical resection offers the only potential curative treatment for colorectal liver metastases with the five year survival rate varying in the literature from 25% to 51%. Hepatic surgery was associated with high morbidity and mortality and it is only since the 1990s that an evidence base has been published showing improved long term outcomes. Radiological imaging plays an essential role in the detection and characterisation of colorectal liver metastases. Accurate staging of the disease allows patient selection for hepatic surgery. Despite recent and significant technological advances in radiological imaging, up to 50% of patients that have undergone curative partial hepatectomy will develop hepatic recurrence in the first two years after surgery. Evidence from growth rate studies has shown that colorectal liver metastases are slow growing and that these recurrences were present at the time of initial staging. Therefore, the problem of occult liver metastases remains.

This thesis has assessed the potential clinical role of a new imaging modality in the detection of colorectal liver metastases: contrast enhanced ultrasound (CE-US). Initially a prospective trial using percutaneous CE-US with intravenous administration of an ultrasound contrast agent that has been used primarily in cardiac imaging was performed.

The results of this study found that CE-US enhanced late phase vascular imaging. This is an important finding as the persistence of a hypoechoic liver lesion in to the late phase of CE-US imaging is typical of a colorectal liver metastasis and an agent that optimises the late phase would allow improved characterisation of colorectal liver metastases. As a result, CE-US was then compared to percutaneous unenhanced ultrasound and found to have improved sensitivity and accuracy in the detection of colorectal liver metastases (sensitivity 100%, accuracy 90.8% versus 64.4% and 64.4% respectively). Furthermore, the optimal late phase imaging was achieved by the lowest dose of agent (0.4mL) that would allow repeated injections if incorporated into routine clinical practice. These findings support the growing evidence base for percutaneous CE-US and it is likely that CE-US will replace unenhanced ultrasound in routine clinical practice.

With promising results in the percutaneous detection of colorectal liver metastases, it was a logical step to perform CE-US intraoperatively. Unenhanced intraoperative ultrasound (IOUS) is widely regarded as the superior imaging modality for colorectal liver metastases and this thesis has documented the first prospective trial using CE-IOUS. CE-IOUS detected significantly more lesions than IOUS and preoperative CT/MRI as well as detecting smaller liver metastases than the other imaging modalities (mean lesion size 1.71cm versus 2.73cm for combined CT/MRI/IOUS). With smaller lesions being detected (the smallest was 0.4cm), alterations in staging and surgical management would be expected and this occurred in 35.1% and 29.8% of patients respectively. This prospective study contained 60 patients and inclusion of more patients with long term follow up will confirm if CE-IOUS is the superior imaging modality.

Previous researchers have developed techniques to detect the altered hepatic blood supply that occurs in the presence of colorectal liver metastases. This thesis developed a

novel and reproducible technique to measure hepatic perfusion using CE-US that found differences in perfusion between healthy controls and patients with overt colorectal liver metastases. To assess the ability of this novel technique to differentiate accurately between healthy controls and patients with colorectal liver metastases, ROC curves were drawn and the highest sensitivity and specificity achieved was only 85% and 58%, limiting the technique's clinical applicability as a screening tool for occult colorectal liver metastases.

Hepatic perfusion using CE-US was then used to assess changes in perfusion in patients undergoing curative partial hepatectomy with perioperative fluctuations occurring that were potentially reflecting the SIRS after surgical trauma. Perioperative perfusion changes were analysed for any relationship to hepatic recurrence with one parameter significantly higher in the recurrence group 3 months after the operation (peak intensity).

These two trials have shown that the measurement of hepatic perfusion in colorectal liver metastases can be reliably performed by percutaneous contrast enhanced ultrasound. However, this measurement of preoperative or postoperative hepatic perfusion does not accurately predict the likelihood of hepatic recurrence. Further work analysing the individual contributions of the hepatic blood supply to both healthy and diseased liver, alongside improved understanding of the physics of the microbubbles in the hepatic circulation may develop a clinically applicable technique for the detection of occult colorectal liver metastases.

With no widely accepted approach to performing partial hepatectomy this thesis analysed one small volume centre's novel approach to performing partial hepatectomy for colorectal liver metastases. The triphasic approach consists of: preoperative dehydration (using bowel preparation with no supplementary fluids); intraoperative fluid restriction (to allow CVP $<5cmH_20$) and continuous selective vascular occlusion (Half-Pringle).

Preoperative dehydration was shown to be well tolerated by patients as well as allowing straightforward maintenance of a low intraoperative CVP, with no intravenous nitroglycerins administered. The Half-Pringle renders the hemi liver under resection ischaemic whilst protecting the liver remnant and has been confirmed by Doppler studies. All three components minimised blood loss with a median intraoperative loss of 400mL (range 50-3000mL). As a result, postoperative morbidity and mortality were low with disease-free survival and long-term survival comparing favourably to the published literature from larger volume centres.

Particular attention was paid to the perioperative changes in renal and hepatic function with the triphasic approach since dysfunction in these organs is likely after undergoing partial hepatectomy. Using specific markers for renal function, in addition to routine biochemical blood markers, it was found that perioperative dehydration did not compromise renal function with no documented incidence of renal failure. No hepatic failure was documented and hepatic function blood tests displayed a distinct pattern which can be used as a guide to liver function postoperatively.

Colorectal cancer with hepatic metastases presents a unique opportunity to deal with the primary and secondary disease at the same laparotomy. It has been the choice of this surgical department to perform combined primary and secondary resections, with RF ablation as appropriate. A case matched study was performed that found no differences in blood loss, number of recurrences, disease free survival and overall survival between staged and synchronous procedures. There was increased morbidity and longer stay in hospital in the synchronous group, but these results are likely to be limitations of the study design rather than actual differences. Overall, the combination of major colonic

resections with synchronous bowel and /or RF ablation appears to be safe and does not compromise long term patient outcomes.

As a result of improved long term outcomes after hepatic resection for colorectal liver metastases, interest has increased in the development of prognostic scores to optimise patient treatment. This thesis assessed the potential clinical application of preoperative C-reactive protein as a predictor of outcome after partial hepatectomy. Although a significant predictor in other malignancies, C-reactive protein was not found to be an influence in this cohort. For comparison the clinical risk score (CRS) that was developed by one of the largest volume centres for hepatic resections in the world was applied in this study's cohort. The CRS was found to be predictive of poor outcome, thus validating it in another population and supporting its use in routine clinical practice.

This thesis has investigated the potential clinical role of contrast enhanced ultrasound in the detection of colorectal liver metastases. Improved percutaneous and intraoperative detection of metastases has led to optimised disease staging with significant alterations in surgical management. Partial hepatectomy using the triphasic approach has resulted in excellent short- and long-term patient outcomes and, with the inclusion of synchronous colonic and hepatic procedures, could extend the number of patients considered for surgical resection.

Section 1: Introduction.

The background of colorectal cancer and liver metastases

1.1 Natural history of colorectal cancer

1.1.1 Incidence of colorectal cancer

Incidence of colorectal cancer worldwide

Cancer of the colon and rectum is the fourth commonest cancer worldwide accounting for 9.7% of all new cancer cases. With an incidence of 500 000 new cases in 1975, 783 000 in 1990 and approximately 1 000 000 in the year 2000, colorectal cancer is a major cause of morbidity and mortality that continues to affect millions of the world's population. It is estimated that colorectal cancer results in 437 000 deaths each year (8.4% of all cancer deaths)[1;2].

Colorectal cancer affects men and women in almost equal proportion accounting for 9.4% of all cancer incidence in men (third most common malignancy in man) and 10.1% in women (second most common malignancy in women).

Colorectal cancer is not distributed evenly throughout the world. Indeed, it is more common in westernised countries (North America; Northern, Southern and Western Europe; Australasia and New Zealand) with an overall incidence in these countries of 12.6% and 14.1% in men and women respectively, which is significantly greater than 7.7% and 7.9% of all incident cases in men and women in the remaining areas of world. Of these Westernised countries, the United States has the highest incidence with 53.1 cases per 100 000[3].

Incidence of colorectal cancer in the United Kingdom

Colorectal cancer causes a significant health burden in the United Kingdom with over 34 000 new cases diagnosed each year: approximately 18 700 in men and 16 800 in women. In both sexes, it accounts for over 16 000 deaths a year in the United Kingdom[4].

In Scotland in 2000, colorectal cancer was the third commonest cancer in both sexes with over 3000 new cases diagnosed and 95% occurring in people over the age of 50. In 2002, 842 men and 713 women died from this disease[5]. The majority of colorectal cancers occur in the colon with incidence increasing with increasing age. Typically, the peak incidence of colorectal cancer occurs in the seventh decade, with over 80% of diagnosed patients being over the age of sixty. In comparison, less than 1% of cases are younger than 40 years at presentation[4].

1.1.2 Aetiology of colorectal cancer

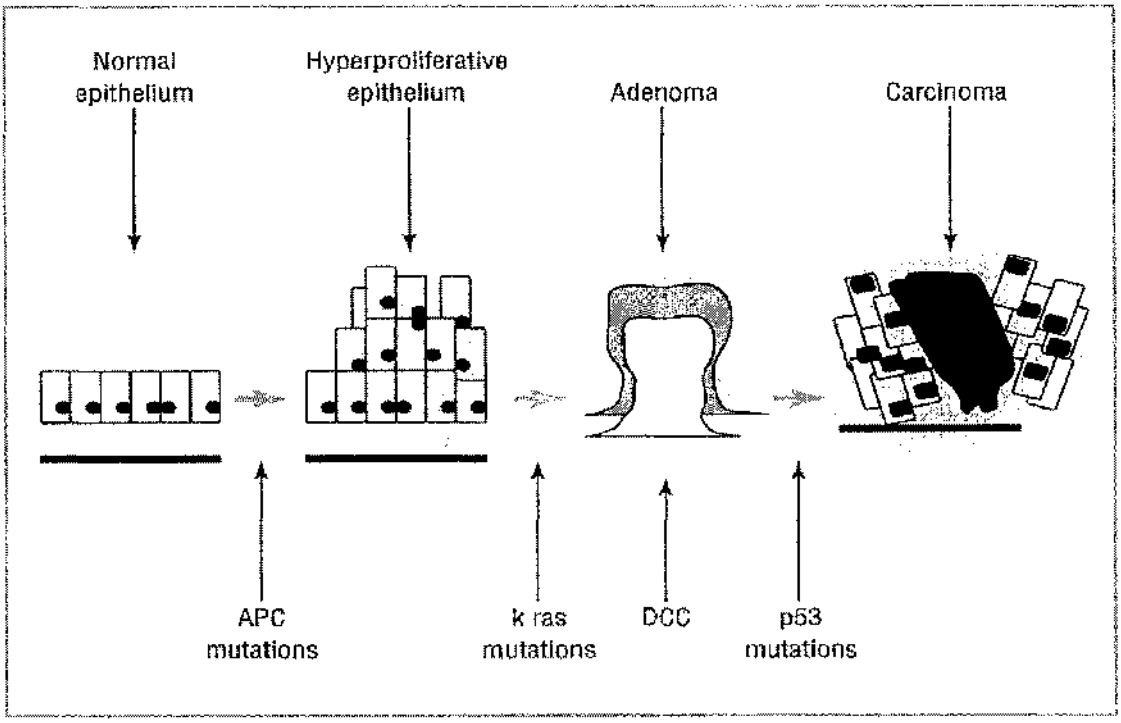
Colorectal cancers can be classified as: hereditary (isolated gene); familial (tendency within family, but unknown gene); colitic (arising from inflammatory bowel disease) and sporadic (no family history). It is widely accepted that the majority of colorectal cancers arise from pre-malignant adenomatous polyps, the so-called adenoma-carcinoma sequence[6-8]. This multi-step process starts with normal colonic mucosa, but from the accumulation of several mutations, ends with malignant adenocarcinoma [Figure 1]. Colorectal cancer is clonal in origin, but becomes heterogenous by the time patients become symptomatic. This process of clonal evolution driven by mutation has been confirmed by molecular studies identifying specific gene mutations: p53 and adenomatous polyposis coli (APC). p53 is a DNA binding protein transcriptional activator and can arrest the cell cycle in response to DNA damage and is termed the guardian of the genome. APC

is a tumour suppressor gene and is called the gatekeeper as it prevents the build up of molecules associated with cancer, such as catenins, which if left to accumulate, result in oncogene transcription.

Steps can be influenced by genetic mutations, environmental factors or a combination of both. With the majority of colorectal cancer cases being sporadic, environmental factors are thought to play a large role. However, it has been estimated that up to 30% of cases have a genetic component, a number that is likely to change as genetic research advances [9]. Currently, true hereditary cancer (families with strong histories and tumours developing at less than 50 years of age) accounts for less than 5% of colorectal cancer cases.

Figure 1: Proposed adenoma to carcinoma sequence in colorectal cancer.

Adenomatous polyposis coli (APC) gene mutations and hypermethylation occur early, followed by k ras mutations. Deleted in colon cancer (DCC) and p53 gene mutations occur later in the sequence, although the exact order may vary. Environmental influences affect normal epithelium and/or hyperproliferative epithelium[10].



Hereditary Colorectal Cancer

Most hereditary cancers can be attributed to two syndromes: Hereditary Non Polyposis Colon Cancer (HNPCC) or Familial Adenomatous Polyposis (FAP). The remaining causes are rarer: Peutz-Jeghers Syndrome; Juvenile polyposis; Turcot Syndrome and Cowden Disease.

HNPCC or Lynch Syndrome is the commoner of the two syndromes and manifests as multiple adenomatous polyps. Patients are diagnosed on average at 45 years with tumours being classically right sided and potentially synchronous or metachronous. The lifetime risk of developing malignant disease is high at 80% and tumours can change rapidly from benign to malignant. HNPCC results from germline mutations in DNA mismatch repair genes which normally remove misincorporated single or multiple nucleotide bases as a result of random errors during recombination or replications. There are several documented mismatch genes involved (MHS2, MLH1, PMS1 and PMS2) and mutations are particularly demonstrable in DNA with multiple microsatellites (microsatellite instability). As only 50% of patients will have mismatch gene mutation on genetic testing, diagnosis is made using the Amsterdam Criteria[11] or Bethesda Guidelines[12]. Patients will then undergo routine colonoscopy every 1-2 years alongside investigations to exclude other associated malignancies (ovarian, uterine, gastric, small bowel and renal).

FAP is also an autosomal dominant condition and is characterised by multiple (hundreds to thousands) adenomatous polyps in the large bowel with extra-colonic manifestations (congenital hypertrophy of retinal pigment epithelium, sebaceous cysts, desmoid tumours or osteomas). The majority of tumours are left sided and there is an associated risk for gastric and duodenal polyps, which have a high chance of becoming malignant. FAP also has associations with other malignant diseases: hepatoblastoma;

medulloblastoma; adrenal; pancreatic; biliary and thyroid. Polyps can appear as early as puberty to the second or third decades of life with approximately 100% being malignant by the age of 40. FAP has a clearer genotype than HNPCC with 90% of cases resulting from mutations of gene for adenomatous polyposis coli (APC) on chromosome 5. These patients will undergo yearly colonoscopy with upper gastrointestinal endoscopy every 3 years.

Familial colorectal cancer

In addition to these well recognized syndromes, clusters of colorectal cancer occur in families more often than could be explained by chance with immediate family members having an increased risk of developing the disease. Postulated reasons for this familial group include mild APC and mismatch repair gene mutations, as well as polymorphisms of genes involved in nutrient or carcinogen metabolism.

Colitic colorectal cancer – ulcerative colitis

Compared with the normal population, several studies have shown that patients with ulcerative colitis have a 2-8.2 relative risk of colorectal cancer, accounting for 2% of all colorectal cancers. One major factor influencing an individual's risk is duration of colitis: at 15 years disease duration the risk of malignancy is 5%, which increases to between 8 and 13% after 25 years of disease. The extent of disease is also important with the presence of pancolitis more likely to develop colorectal cancer. In addition, coexisting primary sclerosing cholangitis independently increases the relative risk of ulcerative colitis associated neoplasia by 3-15%. Finally, colorectal cancer resulting from ulcerative colitis may evolve from microscopic dysplasia with or without a mass lesion rather than from adenomas.

Colitic colorectal cancer – Crohn's disease

Crohn's disease was previously thought to carry a smaller risk of cancer development than ulcerative colitis. However, it is now thought that the risk is approximately the same as the risk for an equivalent extent and duration of ulcerative colitis[13]. A large study from Sweden found a relative risk of colorectal cancer for all patients with Crohn's disease of 2.5 (95% CI: 1.3 – 4.3)[14]. However, the relative risk potentially varies with the distribution of disease: ileal disease had no increased risk; ileocolonic disease had a relative risk of 3.2 (95% CI: 0.7 – 9.2) whilst colonic disease carried the greatest relative risk of 5.6 (05% CI: 2.1 - 12.2). These findings suggest that the development of Crohn's related colon cancer develops in the presence of chronic colonic inflammation. Indeed it has been noted that as well as a surprising number of cancers arising in fistulae and strictures, the majority of tumours in Crohn's disease arise in the proximal colon unlike sporadic and ulcerative colitis related colon cancer[15;16] . It is possible that Crohn's disease and ulcerative colitis share the same cancer development pathway (colitis-dysplasia-cancer pathway) as both can result in adenocarcinoma from flat, dysplastic mucosa rather than polypoid adenomas[17].

Environmental factors in colorectal cancer

Descriptive epidemiological studies have assessed colorectal cancer in worldwide populations and not only found that different populations have different incidences of colorectal cancer, but that these incidences change. Furthermore, within the same population, individuals with different lifestyles have different levels of colorectal cancer. Migrants provide further evidence of the environment's influence as they take on the colorectal cancer risk of their adopted country after only one generation of children.

The majority of research has been performed on potential dietary influences: animal fat and red meat; fruit and vegetables and fibre intake. The World Cancer Research Fund (WCRF) in 1997 reviewed the scientific and other expert literature linking foods, nutrition, food processing, dietary patterns and related factors with the risk of human cancers worldwide[18]. They stated that evidence for dietary protection against colorectal cancer was strongest for diets high in vegetables. There was not sufficient evidence that fruits decreased the risk of colorectal cancer with fibre and starches having only enough evidence for WCRF to conclude that a decreased risk may be possible. These findings were supported by the World Health Organisation[19]. They also set out to estimate the overall burden of disease resulting from suboptimal ingestion of fruit and vegetables and found that poor diets resulted in 2.635 million deaths a year worldwide. They found that by increasing daily intake to 600g/day, the burden of colorectal cancer could be reduced by 2%.

WCRF also assessed lifestyle influences on colorectal cancer risk[18]. They concluded that regular physical activity (to minimise obesity), avoidance of alcohol and not smoking all decreased the risk of developing colorectal cancer, findings that have been reported by a number of studies[20-24]. When added to positive dietary influences of a vegetable predominant diet that is low in meat, they documented that a potential decrease in colorectal cancer incidence of 66-75% could be achieved.

High socioeconomic deprivation has been shown to reduce survival in patients with colorectal cancer[25-27]. One such study assessed socioeconomic status in 21 905 patients with colorectal cancer in England and found significant differences in 5 year survival between the most and the least affluent group (40% and 32% respectively)[25]. Several potential explanations were proposed: delay in patient presentation; more advanced disease

at initial presentation or variations in treatment options. However, a study in 2002 analysed outcomes after pathologically confirmed curative resection in 2269 patients with colorectal cancer in varying socioeconomic groups. The authors found poorer overall and cancer specific survival rates in the deprived patients[28]. Furthermore, there were no significant differences between affluent and deprived groups in: mode of presentation; extent of disease; proportion of patients undergoing curative resection or postoperative mortality. The authors concluded that the tumour-host response, particularly the systemic inflammatory response may be involved.

The presence of an elevated systemic inflammatory response has been shown to predict reduced disease-free survival and long-term survival in several different types of malignant disease[29-34]. Colorectal cancer is one of these diseases, with an elevated preoperative C-reactive protein predicting reduced time to recurrence, overall survival and cancer specific survival in patients undergoing curative resection, independent of cancer stage[35-38] . The exact mechanism behind the increased C-reactive protein is unclear with reduced tumour T-lymphocyte infiltration (particularly CD4+ T-lymphocyte infiltration) being proposed[39]. One study compared socioeconomic deprivation and the systemic inflammatory response in 158 patients undergoing curative resection for colorectal cancer[36]. The authors found that both high deprivation and raised C-reactive protein predicted poorer overall survival, independent of tumour stage, after resection. However, only elevated C-reactive protein predicted poorer cancer specific survival, leading the authors to conclude that the presence of a systemic inflammatory response preoperatively has a greater impact on cancer specific survival than deprivation.

The influence of gender on the incidence of colon cancer has been researched with analysis of any potential protective effect of hormone replacement therapy. In 2002, the

Women's Health Initiative recruited 16 608 postmenopausal women with an intact uterus and randomised them to receive hormone replacement therapy (oestrogen and progesterone) or placebo[40]. The primary outcomes were the development of coronary heart disease and/ or invasive breast cancer, with the development of colorectal cancer, alongside other event categories, a secondary aim. After a mean follow up of 5.2years, the estimated hazard ratio for colorectal cancer was 0.63 (95% CI 0.43-0.92) with an absolute risk reduction per 10 000 patients of 6. This was the first randomised study and is supported by similar findings from two observation studies that have provided enough evidence for policy makers including the United States Preventive Service Task Force for chronic diseases acknowledging the protective effect of HRT on colorectal cancer development [41-43]. A potential mechanism may be the loss of expression of oestrogen receptor beta. This receptor is the predominant oestrogen receptor in colon tissue whose loss correlates with increasing Dukes stage and subsequently poorer outcome[44].

1.1.3 Staging and Prognosis of colorectal cancer

After pathological analysis of a biopsy specimen confirms a colorectal adenocarcinoma, each patient undergoes staging. Staging is the process of determining the local invasion of the primary tumour and the extent of metastatic disease. For colorectal cancer this involves preoperative visualisation of the entire lower gastrointestinal tract via colonoscopy and radiological imaging of the chest, abdomen and pelvis. Staging serves multiple purposes according to the American Joint Committee on Cancer (AJCC)[45]: planning of appropriate treatment (including consideration of surgical resection); indication of prognosis (by staging classifications) and evaluation of the results of treatment (i.e. neoadjuvant chemo/radiotherapy).

Cuthbert Dukes introduced his staging classification in 1932[46]. His simple, reproducible and clinically relevant classification, initially intended for the staging of rectal cancer, is still in widespread use for colorectal cancer today. Dukes divided his classification into 3 stages according to the extent of direct and lymphatic tumour spread: Dukes A (tumour confined to bowel wall), Dukes B (tumour extends beyond rectal wall, but no lymphatic involvement) and Dukes C (tumour has metastasised to regional lymph nodes). Dukes classification has undergone modification since 1932 with stages B and C subdivided into 1 and 2 and the introduction of distant metastases as stage D [Figure 2][47].

The Dukes classification is simple, but this simplicity is also its disadvantage, with no account taken of the depth of penetration of the primary tumour or the number of lymph nodes involved. The TNM staging was introduced in the 1950s and provides this missing information [Figure 3][48]. Subsequently, TNM has developed a prominent clinical role in colorectal cancer as well as other malignancies including: breast; gastroesophageal; ovarian; bladder and prostate[45]. TNM staging can be translated easily into the equivalent Dukes stage shown in Table 1.

Figure 2: The Modified Dukes Classification of colorectal cancer

Stage	Description
A	limited to mucosa
B1	extension into, but not through, the muscularis propria
B2	extension through the muscularis propria, no nodal involvement
C1	limited to bowel wall, metastases in the lymph nodes
C2	extension through bowel wall, metastases in the lymph nodes
D	distant metastases

Figure 3: International Union against cancer: TNM classification of Colorectal cancer

Tumour (T):

TX primary tumour cannot be assessed

T0 no evidence of primary tumour

Tis carcinoma in situ

T1 tumour invades submucosa

T2 tumour invades muscularis propria

T3 tumour invades muscularis propria into subserosa, or into non-peritonealised pericolic or perirectal tissues

T4 tumour invades other organs or structures and/or perforates visceral peritoneum

Nodes (N):

NX nodes cannot be assessed

N0 no regional lymph nodes metastasis

N1 metastasis in 1 to 3 regional lymph nodes

N2 metastasis in 4 or more regional lymph nodes

Metastases (M):

MX distant metastases cannot be assessed

M0 no distant metastases

M1 distant metastases

Table 1: Comparison of grades between Dukes's classification and TNM staging

Dukes	TNM Stage	T	N	M	5-year survival (%)
A	I	T1 or T2	N0	M0	90
B	II	T3 or T4	N0	M0	75
C	III	Any T	N1-N2	M0	35-60
D	IV	Any T	Any N	M1	<10

Table modified from International Union Against Cancer[48].

1.1.4 Treatment of Colorectal cancer

Surgery for colorectal cancer

Curative surgical resection of colorectal cancer is the current optimal treatment with recent domestic and international figures documenting declining rates of death[49-53]. In 1976, the Scottish Cancer Intelligence Unit documented a death rate of 33.6 per 100 000 of population in males and 26 in females. Figures in 2001 show improved rates with 24.4 per 100 000 in males and 14.8 in females, declines of 21% and 29% respectively. One study translated these declines into 5 year survivals of 40.1% (1974-1979) and 60.5% (1991-1994) after curative surgical resection[54].

The Association of Coloproctology of Great Britain and Ireland (ACPGBI) issued guidelines for the management of colorectal cancer that were updated in 2001[55].

Colonic surgery should be considered in all patients and performed depending on the tumour/s sites: right hemicolectomy (lesions of the caecum, ascending colon and hepatic flexure); left hemicolectomy (lesions of the splenic flexure and descending colon) and sigmoid colectomy (sigmoid colon). Curative resection is based upon histological confirmation of complete excision of the tumour.

Total mesorectal excision (TME) is recommended for cancer in the lower two thirds of the rectum as part of an abdomino-perineal resection or low anterior resection. Local excision with curative intent should be restricted to T1 tumours, but it must be accepted that a small percentage of these will be found to be more advanced on pathological analysis and will require further surgery.

Laparoscopic and laparoscopic-assisted colonic surgery have shown promising initial results documenting improved morbidity and mortality when compared to open

colonic surgery [56;57]. There is limited research on laparoscopic rectal surgery which has commented on a potential reduction in local recurrence, but as with colonic surgery, long term results are required before any definitive decision can be made. Currently, the ACPGBI recommends that laparoscopic surgery be performed only by experienced operators as part of one of the national trials[55].

Chemoradiotherapy in colorectal cancer

In patients undergoing planned surgical resection, the surgeon must consider when and if chemotherapy and/ or radiotherapy will form part of their patient's treatment. These additional therapies can be given either preoperatively (neoadjuvant) or postoperatively (adjuvant).

In 1990, a Consensus Conference of the National Institutes of Health strongly recommended adjuvant treatment in patients with stage III colon cancer and stage II and III rectal cancer[58]. This postoperative chemotherapy is based around 5-fluorouracil (5-FU) which has a substantial evidence base with one meta-analysis concluding adjuvant chemotherapy may reduce the likelihood of death by 10–15% in patients with colorectal cancer [59;60].

More recent trials have focused on combining 5-FU with other chemotherapy agents (5-FU/leucovorin or 5-FU/levamisole) with comparative studies finding improved disease free survival and overall survival with the 5-FU/leucovorin combination [61-64]. As a result, this combination is used widely in clinical practice for stage III/ Dukes C colorectal cancers and more recent research is looking at further combinations including irinotecan and oxaliplatin.

The benefits of chemotherapy in Dukes C cancers raise the question of treating Dukes B colorectal cancers. However, the evidence is conflicting, leading several authors, including the ACPGBI, to recommend that clinicians decide on an individual basis, carefully balancing the side effects of chemotherapy against subsets of stage II that carry a poorer prognosis (serosal involvement, extramural vascular invasion, poorly differentiated histology)[65;66].

Rectal cancer differs from colonic cancer due to the high risk of local recurrence that may prove to be fatal. As a result, researchers have assessed the benefits of neoadjuvant radiotherapy to the primary rectal tumour in stage II and stage III cancer and found that patients show good tolerance as well as improved local control, disease free survival and overall survival[67;68]. It also appears that the combination of chemotherapy and radiotherapy may be better than radiotherapy alone as 5-FU is a radiosensitiser[69;70].

In the postoperative setting, chemoradiotherapy has also shown to be beneficial after resection of a rectal tumour resulting in 10–20% of patients having pathologically proven complete remissions[58;69;71;72]. However, preoperative chemoradiotherapy remains the preferred approach (especially in Europe) due to: greater efficacy of radiation therapy on well-oxygenated cells (as opposed to the relative ischaemia of remaining cancer cells in the postoperative pelvis); decreased spillage of viable tumour cells at the time of operation; down staging of the tumour and avoidance of radiating the anastomosis. One active area of research is the determination of the optimal chemotherapy agent for combined chemoradiotherapy, with new agents currently being assessed (capecitabine, uracil and tegafur)[66]. There is no evidence for radiotherapy in the management of colonic cancers.

Inoperable primary colorectal cancer

For a number of patients with colorectal cancer, surgical resection will not be an option. The main reasons for this include: advanced local disease that cannot be resected; high patient co-morbidity for a general anaesthetic or a combination of the two. There are several treatment options.

If the primary disease is rectal cancer that is locally advanced, the outlook is poor, with no evidence that surgical resection improves prognosis[73]. If the patient is fit (i.e. could undergo a general anaesthetic), then the ACPGBI recommend a course of either radiotherapy alone or in combination with chemotherapy to see if the tumour can be down staged, making resection a future possibility[65]. Chemoradiotherapy can also be administered to patients whose rectal cancer is unlikely to become resectable, but where a decrease in bulk could improve symptomatic control (pain in particular) leading to improved quality of life. A similar approach is proposed by the ACPGBI for the management of unresectable colonic cancer where a course of chemotherapy may allow down staging of the primary tumour leading to surgical resection.

Palliative options for both rectal and colonic cancers should be considered especially if the tumour is causing obstructive symptoms. These include: defunctioning colostomy or ileostomy; endorectal metallic stent insertion or transanal tumour ablation using laser or electrocoagulation [65]. There is some debate as to whether surgery should be performed to resect as much of the primary tumour as possible to prevent future complications including the aforementioned bowel obstruction and bleeding, however the literature consists of non-randomised small trials leaving many to conclude that the decision should be made on an individual case basis[74].

1.2 Natural history of colorectal liver metastases

1.2.1 Incidence of colorectal liver metastases

Colorectal cancer has an inherent tendency to spread in progressive stages: local, then lymphatic, followed by haematogenous and/or transcoelomic. As a result colorectal cancer can spread to many organs including: liver (>50%); lung (5%-50%); adrenal gland (14%); ovary (3%-8%); bone (5%); brain (5%) and mesentery (10%)[75]. The venous drainage of colon cancers is directed to the portal vein making it uncommon to have distant metastases without associated disease in the liver. In contrast, the venous drainage from rectal tumours can be directed into both the portal and systemic venous systems allowing haematogenously disseminated disease that can bypass the liver.

As a result, almost 50% of patients with colorectal cancer will develop liver metastases: up to 25% will have liver metastases at time of initial presentation with the remaining 25% developing liver metastases during the course of their disease[76-78]. Death from hepatic metastases accounts for a large percentage of colorectal cancer mortalities and if left untreated the prognosis is poor with median survival from 5 to 21 months with almost zero alive at 5 years[79-84].

1.2.1 The metastatic process

The metastatic spread of colorectal cancer is a complex process. Colorectal cancer has a clonal (single cellular progenitor) origin, but eventually the primary tumour becomes heterogenous with multiple subpopulations of cells with varying metastatic potential that then have to undergo a multitude of steps to metastasise: selection for detachment of primary tumour cells; invasion through the extracellular matrix; migration through blood

and/ or lymph channels; reattachment to a distant organ; growth and angiogenesis within the distant organ and further spread from distant organ to other distant organs[85-87]. Each stage involves a different set of proteins including: adhesion molecules; matrix degrading proteins; endothelial cell receptors and immunological recognition factors. With so many steps involved it is not unexpected that molecular research has not clarified the exact mechanisms driving the metastatic process.

Research has investigated why the liver is the first targeted organ for metastases. Two theories exist to explain this targeting: homing theory and cascade theory. The first believes that it is a property of the metastasising tumour cell to target the appropriate liver receptor. This homing theory proposes a non-random, highly organised multistep process, which will require years of research to clarify. However, if clarified may offer highly specific therapeutic solutions. In contrast, the cascade theory describes a random process. With the liver being the main site for venous drainage of the lower gastrointestinal tract, the millions of tumour cells that are released into the bloodstream filter through the liver and by a process of volume will eventually become embedded in the liver, leaving fewer cells to go elsewhere. If the cascade theory is true, then metastatic hepatic spread could be difficult to control therapeutically, with prophylactic liver chemotherapy potentially an option. It may well be that as molecular genetics progresses, that features of both theories are proved correct.

It is thought that lymphatic spread has a limited role in colorectal liver metastases primarily because there are no direct lymphatic channels connecting the colon or the rectum directly to the liver. However, it is possible that lymphatic spread could result from the pericolic to the para-aortic lymph nodes or from entering the blood system where the lymphatics drain directly into the superior vena cava via the thoracic duct. Therefore, in

patients with colorectal cancer it is uncommon to find metastatic lymph nodes along the hepatic artery (irrespective of liver involvement) without lymph node involvement at the primary tumour site.

1.3 Detection of colorectal liver metastases

1.3.1 Radiological imaging

Radiological imaging plays a fundamental role in the current management of colorectal liver metastases and has several functions: to make an accurate diagnosis of metastases and to differentiate from benign conditions; to clarify the number, size and position of metastases in assessment for potential hepatic resection and to detect changes in clinical state after treatment.

Preoperative radiological imaging – transabdominal ultrasound (US)

Ultrasound has been in medical use since the 1950s. It is based upon the pulse-echo principle, where pulses of high frequency sound waves are transmitted into the patient, reflected off tissue boundaries and returned to the machine as echoes [88].

Initially the instruments were cumbersome and the images were poor in quality. The development of real time imaging (or B-mode) and the linking of US to a computer (computed sonography) in the 1970s, dramatically improved these problems and took US forward to becoming part of routine assessment in many areas, including colorectal liver metastases.

US has many advantages over other available imaging modalities and can be applied directly to the skin or directly on to the organ being visualised allowing it to be used trans-abdominally, laparoscopically and intra-operatively. The machines are usually mobile, if not hand-held, allowing US to be used anywhere. In addition, US is not radioactive, easy to perform, well tolerated by patients and takes only a small amount of time to perform a full examination.

However, it is widely accepted that transabdominal ultrasound is less sensitive and specific in the visualisation of liver metastases when compared to other imaging modalities with the range of documented sensitivity from as low as 53% to as high as 90% [89-93]. This wide range is likely a reflection of varied reference standards used in each study and that US is operator dependent. As well as interference from body tissues (subcutaneous fat, muscle) reducing the acoustic signal, the detection and specificity of US decline as liver metastases become less than 2cm in size[93]. In one study, the sensitivity of ultrasound for detecting lesions less than 1cm was less than 20% [91].

Preoperative radiological imaging – computerised tomography (CT)

Along with transabdominal US, CT is considered a primary diagnostic tool for staging and has the added advantage over US of determining resectability of colorectal liver metastases. CT technology has advanced greatly and it is now widely accepted that all CT scans should be contrast-enhanced for superior sensitivity and specificity. In fact, un-enhanced CT is likely to produce a poorer image than un-enhanced ultrasound [94].

Carter and co-workers in 1996 compared unenhanced axial CT to pre-operative percutaneous US, intra-operative US and findings at laparotomy in 40 patients with resectable colorectal liver metastases [92]. They found that CT had a higher sensitivity (94%) than percutaneous US (77%). This finding is supported by other early studies that used various CT technologies with the sensitivities ranging from 38% to 88%[91;95-98].

More recent studies using enhanced CT have provided evidence of a higher sensitivity and specificity when compared to percutaneous US [91;98]. However, when all the studies comparing US with differing types of CT are combined, it becomes apparent that

with declining lesion size, the accuracy of CT declines accordingly. Therefore, further improvements in CT are still required to detect reliably sub-centimetre liver lesions.

Preoperative radiological imaging –magnetic resonance imaging (MRI)

MRI has undergone several recent technological developments, including the introduction of liver-specific contrast agents and breath-hold acquisition imaging.

Superparamagnetic iron oxides (SPIO) such as ferumoxides were initially described in 1987. Ferumoxides are dextran compounds that are predominately phagocytosed by macrophages in the liver and spleen. Normal liver parenchyma contains Kupffer cells which phagocytose ferumoxides, reducing the signal intensity. In contrast, liver metastases which do not contain any Kupffer cells remain unaffected and are displayed as areas of high intensity signal on MRI.

As the majority of radiology departments in the U.K. have both CT and MRI scanners available, there has been much research comparing CT with MRI. A recent study by Reimer et al [99] compared spiral CT (enhanced) with unenhanced MRI and ferumoxide enhanced MRI. Superior lesion detection was found with ferumoxide enhanced MRI and a combination of ferumoxide enhanced MRI and unenhanced MRI. These results are replicated in a similar study by Bluemeke et al [100] with both papers concluding that enhanced MRI was superior to enhanced CT in the detection of liver metastases. Furthermore, the combination of unenhanced and enhanced MRI was the superior technique in lesion characterisation. Although unenhanced MRI is generally accepted to be less sensitive than enhanced, it may have decreased the number of false positives that can result with enhanced MRI as there is documented difficulty in distinguishing small lesions from peripheral vessels that become more conspicuous after SPIO enhancement ;[93;99;101;102].

Another recent study by Ward et al (1999) examined 51 hepatic resection candidates. Patients underwent SPIO MRI and dual phase helical CT with the reference standard being intraoperative ultrasound, surgical palpation and histopathology. This study found statistically higher sensitivity with SPIO MRI when compared to CT (79.8% and 75.3% respectively) in the detection of all liver lesions.

Kinkel et al [103] performed meta-analysis of various non-invasive imaging modalities in the detection of liver metastases. Although the authors accepted that a limitation of their meta-analysis was poor documentation of exact imaging modalities performed in the studies reviewed, they found mean weighted sensitivities (specificity higher than 85%) of 55% for conventional ultrasound, 72% for CT (helical, non-helical, enhanced and unenhanced) and 76% for MRI (enhanced and unenhanced). Overall, research documents marginally improved detection of hepatic metastases with MRI, particularly when ferumoxide enhanced, when compared to CT [93;100;104].

There are disadvantages with MRI. Due to the nature of the scan, it cannot be used in patients with internal metal. Ferumoxides are not the only contrast agent available to MRI, but are the most expensive when compared to gadolinium. Ferumoxides also have some side-effects such as low back pain and a long infusion time.

Preoperative radiological imaging – positron emission tomography (PET)

Positron emission tomography (PET) is a relatively new imaging technique that evaluates cell metabolism using a glucose analogue, [^{18}F] fluoro-2-deoxygenase (^{18}FDG). This is quite different to CT and MRI in that it relies on changes in metabolic or physiological functions, rather than morphology, to detect disease. Therefore, it has the potential to demonstrate metastases before structural changes are detected.

Certain malignant cells including colorectal cancer have increased glucose utilisation which can be caused by increased glucose transport proteins and/or increased enzyme levels of hexokinase and phosphofructokinase that promote glycolysis [105;106]. Like glucose, ^{18}FDG is transported intracellularly then phosphorylated by hexokinase to FDG-6-phosphate. Unlike glucose, FDG-6 phosphate becomes trapped intracellularly as it is negatively charged with low membrane permeability [107]. After intravenous administration, ^{18}FDG accumulates in colorectal liver metastasis and performing a PET scan displays these tumours as areas of high glycolytic activity.

Currently the role of PET in assessing primary colorectal cancer is undetermined, however, the value of PET in the detection of recurrent colorectal cancer is well established [103;107-113]. The aforementioned meta-analysis by Kinkel et al compared non-invasive radiological imaging methods in the detection of hepatic metastases from gastrointestinal malignancies [103]. Their analysis included 111 data sets including approximately 3000 patients [Table I]. The mean weighted sensitivity was calculated for studies with specificity higher than 85%. The mean weighted sensitivity for detection of recurrent colorectal cancer with FDG-PET was 90% (MRI 76%, CT 72% and US 55%). Statistically, FDG-PET was significantly superior to ultrasound and CT. The authors concluded that FDG-PET is the most sensitive non-invasive imaging method for the detection of hepatic metastases for colorectal cancer.

In general, the published literature suggests that PET should be first line in recurrent colorectal disease as it would allow optimal preoperative identification and characterisation of extrahepatic disease, especially in the presence of an elevated carcinoembryonic antigen (CEA) [107;109;110;114-116].

The major disadvantage of PET is that it cannot determine the extent or depth of tumour involvement. As the clinical decision to operate is determined by the anatomical position of the liver metastases (which is information that PET cannot supply), PET has to be used in conjunction with CT or MRI to determine resectability.

There are limitations of the use of FDG since it is not tumour specific and any tissue with increased tissue metabolism will accumulate FDG and give a false positive result. Therefore, a positive result in patients with inflammatory bowel disease, diabetes or lung disease should be viewed with caution and a CT or MRI scan arranged to provide further information [117]. It is generally accepted that performing PET several months after any treatment as part of surveillance will reduce the likelihood of false positive results [106;118;119].

Another limitation is that although the majority of colorectal liver metastases do display increased uptake, there are some that do not, particularly the smaller liver metastases. Therefore, these metastases would remain occult to a FDG-PET scan [109;120].

Preoperative imaging modalities – hepatic perfusion measurement

The liver is unique in that it has a dual blood supply. Normal healthy hepatic parenchyma derives its main blood supply from the portal vein, with a smaller contribution from the hepatic artery. The presence of hepatic metastases alters the hepatic blood supply causing the hepatic artery to be the predominant supplier. Original evidence for this altered blood supply comes from both animal and human angiography studies with one of the earliest studies was by Breedis and Young in 1954[121]. From their previous unpublished work investigating hepatic circulation in rabbits, they found that although hepatic parenchyma stained when India ink was injected in to the portal vein, the hepatic tumours

did not [122]. However, the tumours did stain when the injection was into the hepatic artery, suggesting that the tumours were supplied mainly, perhaps even exclusively, by arterial blood.

In their following experiments using both animal tumour models and human autopsy specimens, they injected colloidal pigments (India ink, carmine, gelatine, Prussian blue, vinyl acetate) into the hepatic artery and portal vein then fixed the sample in formalin. After fixation the specimens were sliced, stained with haematoxylin to visualise the vessels that were then counted. The authors confirmed their hypothesis that liver metastases were predominately supplied by the hepatic artery irrespective of the primary tumour, with the hepatic artery contributing between 80% - 100% of the blood supply. Microscopically, the decreased portal vein contribution was thought to result from the neoplasm invading and subsequently occluding the portal branches.

A series of four papers from Ackerman et al from 1969 to 1974 continued the investigation in to the alteration in blood supply in colorectal liver metastases[123-126]. In their first paper the authors used isotope distribution (radioiodinated human serum albumin and yttrium-90 microspheres) to assess vascularity in animal models. They found that small tumours (less than 30mg) were fed by a combination of the hepatic artery and portal vein whilst large tumours (more than 30mg) received their main blood supply from the hepatic artery.

Their second paper used perfused silicone rubber to assess the microvasculature in Walker-256 carcinosarcoma implanted rats (tumour size 25-75μm). They injected silicone into 47 animals in various combinations: artery only; portal vein only or artery and portal vein together. After preparation, the sections were examined under the microscope. Of the nine animals that were perfused via the hepatic artery, the silicone formed a plexus of

irregular vessels at the periphery of the tumour that were seen to be adjacent to hepatic artery branches. In addition, some vessels of the portal vein were also filled. However, in the animals that were perfused by the portal vein (n=8), a typical branching pattern of treelike vessels was displayed that stopped short of the of the tumour plexus displaying a clear line of demarcation between liver and tumour. This not only confirmed that the hepatic artery solely supplied the tumour plexus, but suggested that one way hepatic artery to portal vein shunting may be present.

In the combined portal vein-hepatic artery perfusion group (n=14), two different colours of perfused silicone were used to determine the origin of the silicone. Again, the plexus of vessels at the tumour edge filled from the arterial route, whilst mixing of the colours was seen proximally to the tumour.

In the last group (n=16), the hepatic artery was ligated (in some animals the ligation was performed at the same time as tumour implantation, whilst in the remainder the artery was ligated 4 days after implantation) then the liver perfused via the portal vein followed by the aorta. Nine of the animals showed no tumour perfusion, reinforcing that the hepatic artery is the main tumour supplier. However, in the remaining 7 animals, the ring of tumour vessels was filled by the portal vein perfusion suggesting the portal vein can feed tumours after hepatic surgery ligation, a role that Nilsson and Zettergren have also documented, but not explained[127].

Ackerman's group explored this unexpected finding in their next paper where they ligated the hepatic artery or the portal vein in Walker-256 carcinosarcoma implanted rats, then injected radioactive tracers into the aorta or portal vein respectively. In conjunction with their previous findings, acute ligation of the hepatic artery resulted in a significantly decreased tumour/liver radioactivity ratio. This decrease was greater than the decrease

found with acute portal vein ligation, which is an expected finding due to the portal vein making a smaller contribution to the tumour as well as the known existence of unidirectional shunts from the arterial tree to the intrahepatic venous system. However, venous to arterial shunts have not been shown to exist and the authors proposed an acute coping mechanism for hepatic artery ligation as the tumor/liver ratio returned to levels seen in unligated animals four days later.

The authors found more complexities in their last paper, where the contribution of the hepatic artery and portal vein changed according to the size of the tumour. Using the same animal models, the authors classified the tumour into groups of small (1-2mm), moderate (3-7mm) and massive (7-33mm). Again, perfused silicone was used to assess tumour blood supply. Results found that small tumours had a variable blood supply that could be arterial, venous or both in origins. Moderate sized tumours had well developed arterial circulations that concur with their previous work in paper 2. Finally, massive tumours returned to a varied pattern. This paper reflects the varied results from many authors analysing the vascularity of cancers[128-131]. With different techniques and patient and animal populations, definite conclusions are difficult to draw, particularly as vascularity appears continually to change.

Researchers have taken advantage of this alteration in blood supply to clinically detect colorectal liver metastases. In 1983, Leveson and co-workers acknowledged the restriction of imaging modalities at that time to detect reliably small liver lesions and assessed in a prospective non-randomised trial, dynamic scintigraphy as a method of studying hepatic arterial and portal blood flow[132]. Fifty-nine patients with either colorectal or gastric carcinoma underwent dynamic flow scintigraphy and static isotope scans that were compared to values obtained from twenty healthy volunteers. To calculate the two

components of hepatic blood flow, a region of interest was drawn over the right kidney and the right lobe of liver from the stored dynamic scintigraphy images. This allowed time-activity curves to be generated with the peak of the kidney curve used to determine the division between the arterial and portal phases of the liver curve. The gradients of the two liver phases were calculated and the hepatic perfusion (HPI; arterial inflow gradient divided by total hepatic inflow) calculated for each patient/control. The HPI were then compared between controls, primary cancer only patients and patients with metastatic cancer with the diagnosis of metastases taken from laparotomy and static isotope scans.

The authors found that 96% of all patients with metastatic disease at laparotomy had abnormal perfusion indices (>0.42). The controls in contrast, all recorded normal HPI providing initial evidence that dynamic scintigraphy could provide improved detection of liver metastases. What is particularly interesting about this technique, which differs from the historical studies documenting altered blood supply, is that the region of interest was always placed over the right lobe of the liver, irrespective of the tumour position. Indeed, the HPI was recorded as abnormal in patients whose tumour/s were placed in the left lobe suggesting either the region of interest is detecting disseminated micrometastases in the right lobe or that the alteration in blood flow appears throughout the liver at the level of the microcirculation. The authors concluded that these were initial findings and further evidence was necessary to determine the clinical role of dynamic scintigraphy.

This group provided the further evidence in 1985 when 150 patients with gastrointestinal cancers underwent preoperative dynamic scintigraphy[133]. Again, regions of interest were drawn over the right kidney and right liver lobe. Gradients were calculated for arterial and venous inflow allowing the HPI to be generated for each patient. Twenty-three healthy volunteers were recruited which defined the upper limit of the normal range

of HPI to be 0.42. Similar detection rates were found in patients with overt liver metastases at laparotomy, where HPI was elevated in 94% of patients (n=47/50). In the remaining group with no apparent hepatic metastases, 50 had one year follow up. Eighteen had developed clinically detectable hepatic metastases all of which had elevated HPI prior to resection of their primary, resulting in a positive predictive value of 72% at one year and a negative predictive value of 98%.

As imaging technology progressed (primarily in computed tomography) the standard of reference for dynamic scintigraphy elevated. Several authors published trials that found HPI able to detect overt hepatic metastases, but of varying predictive value in the detection of occult metastases[134-136]. One of these studies concluded by saying that although the HPI is weak at detecting occult liver metastases, an abnormal HPI is associated with poor outcome in patients undergoing potentially curative resection for colorectal cancer and could be an indication for adjuvant therapy[134]. With further problems with reproducibility, HPI never became routine clinical practice.

The use of Doppler ultrasound in the measurement of hepatic perfusion to detect liver metastases was introduced in 1991 by Leen et al[137]. The authors hypothesised that duplex sonography would be a simpler and more reproducible technique as it has the capacity to measure hepatic blood flow directly. By measuring the cross sectional area and blood flow within the hepatic artery and portal vein, the Doppler Perfusion Index could be calculated (DPI = hepatic arterial flow / (hepatic arterial flow + portal venous flow).

The authors recruited 46 patients: 16 healthy controls; 11 patients with colorectal cancer but apparently disease free livers and 19 patients with overt colorectal liver metastases. The mean DPI results were: controls 0.13 ± 0.07; overt liver metastases 0.52 ± 0.13 and disease free patients 0.39 ± 0.17. With clear separation of the control and overt

liver metastases groups, the authors proposed that DPI may have the potential to detect metastases below the limits of conventional imaging techniques. The same group extended their recruitment and found similar promising results[138].

Further research followed up 68 patients that had DPI measured prior to undergoing potentially curative colorectal resection. 38 of these patients had abnormally elevated DPI (upper limit of normal 0.25 established from control group) with 21 developing liver metastases by one year follow up. Of 30 patients that had a normal DPI, all remained disease free at 1 year.

These results were extremely promising and to overcome potential criticism on operator variability a reproducibility study was performed[139]. Two independent blinded sonologists performed DPI on 20 patients and then a further 20 patients were recruited that underwent repeated measurements of DPI to determine intraobserver variability. The interobserver and intraobserver coefficient of variations were 20% and 16% respectively with agreement on DPI in 90% of cases. Leen et al then followed up patients for 5 years after resection and concluded that DPI can be used to identify patients at high risk of recurrence and that DPI should be incorporated in to widespread clinical practice.

To date, few investigators from other centres have managed to replicate successfully Leen et al's results[140-142]. It is has been suggested that the technique is too operator dependent and may require a period of specialist training. In addition, the principle of DPI may be flawed, as it measures directly the macroscopic blood supply which has been shown to vary in as many as 30% of normal healthy individuals[143]. Roumen et al published their findings on the application of DPI in 133 patients with colorectal cancer and followed them up for 4 years[141]. In addition to reliable DPI

measurements not being attainable in 29 patients, DPI using the previous normal range did not predict the presence of occult liver metastases.

Invasive imaging modalities – intraoperative ultrasound (IOUS)

IOUS was developed to combine the practicality of conventional ultrasound with the advantage of directly placing the probe over the liver. It was theorised that this direct placement would prevent the image degradation that often occurs as the sound beam traverses the layers of fat and muscle that comprise the body wall.

Machi et al. evaluated and compared the accuracy of IOUS to percutaneous ultrasound, conventional CT and surgical exploration[144]. Intra-operative ultrasound was the most sensitive modality in assessing colorectal liver metastases diagnosing 93.3% of metastases. Pre-operative ultrasound diagnosed 41.3%, CT 47.1% and surgical exploration 66.3%. Furthermore, intra-operative ultrasound imaged 22 liver metastases that none of the other modalities did. These 22 metastases were small in size (4 x 4mm to 15-18mm) proving that intra-operative ultrasound can visualise tumours less than 1 cm. One reviewer comments that intra-operative ultrasound may detect lesions as small as 2mm if they lie close to the liver surface [145]. Several studies have reported similar findings with resultant significant changes in surgical management. As a result, there is widespread acceptance of intra-operative ultrasound as the optimal modality for hepatic metastases imaging [146-151].

The main drawback with intra-operative ultrasound is the requirement for a laparotomy. The worst case scenario is when IOUS detects further lesions and /or unexpected locally advanced disease, leaving no surgical resection options. As a result, the patient has undergone an unnecessary laparotomy.

Invasive imaging modalities – laparoscopic ultrasound (LUS)

Laparoscopic ultrasound uses the improved accuracy of IOUS whilst preventing patients from an unnecessary laparotomy. Research has documented improved imaging of the liver with LUS compared to conventional US and CT, leading to altered patient management [152-154]. Rahusen et al found laparoscopic ultrasound prevented unnecessary laparotomy in 25% of patients when compared to pre-operative enhanced CT and percutaneous US[152]. However, in patients that have proceeded to laparotomy, IOUS detected a small number of previously missed metastases and it has been suggested that there may be a significant learning curve with laparoscopic ultrasound. Laparoscopic ultrasound is not a suitable procedure for all patients, especially the clinically obese, previous laparotomies and those patients with chronic pulmonary pathology.

Invasive imaging modalities – computed tomography arterial portography (CTAP)

CT arterial portography (CTAP) is an invasive technique that has a high sensitivity of 85-91% in the detection of colorectal liver metastases [90;96;155]. However, this technique has a high rate of false positives due to perfusion defects that may result in inappropriate conservative treatment [156]. In addition, CTAP has a decline in sensitivity to 61-79% for lesions less than 1cm. As a result of these limitations and because as an invasive test it could never be used for screening, CTAP has struggled to become part of routine practice [96;157-161].

Preoperative imaging modalities – recent advances

Contrast-enhanced ultrasound (CE-US) is a technique that dates back to 1968, but interest has increased recently due to the development of a new generation of contrast

agents [162]. These contrast agents consist of microbubbles that contain air or perfluorocarbon gas contained in an outer shell of denatured albumin, lipid or surfactant. These microbubbles are capable of modifying the acoustic properties of the liver, improving the ultrasound visualisation of colorectal liver metastases [94;163-165].

New imaging modes have been developed to maximise the effects of microbubbles: Pulse Inversion Harmonic (PIH) imaging was accepted as the superior technology for advanced contrast imaging at a recent meeting by leaders in the CE-US field [166]. PIH enhances signals from the microbubbles over those from the tissues, decreasing visual degradation from patients with poor body habitus and increasing image clarity and spatial resolution [167;168].

CE-US with PIH imaging has improved the detection of liver metastases when compared to percutaneous ultrasound imaging in B-mode [167;169-176]. This difference is conclusive with CE-US having a documented sensitivity of around 87% compared to 53-77% [91;175-177] with percutaneous ultrasound. CE-US has the capability of detecting sub-centimetre lesions that are a known restriction of conventional ultrasound [169;170;175;178]. Harvey et al. [179] stated that CE-US and PIH were capable of detecting lesions of around 3.14mm in size.

Del Frate *et al.* [170] documented a narrowing of the detection gap between transabdominal CE-US and ferumoxides-enhanced MRI. Although improved, CE-US still only detected 90% of liver metastases seen on MRI. When compared to contrast-enhanced dual phase CT, again although inferior, CE-US had narrowed the difference in sensitivity [169;179-181].

In relation to lesion characterisation, several authors have documented that CE-US has improved lesion characterisation when compared to conventional US. Early results

suggest that again the gap may be narrowed between CE-US and CT and MRI [167;168;172;178;182-184].

Image fusion is another recent development that combines PET and CT scanning [185]. This combines anatomical with functional imaging and can be done by using specific hardware to generate hybrid images. Image fusion is in the early stages, but one paper shows interesting results. Cohade et al [186] analysed 45 patients with colorectal liver metastases. The authors compared detection with FDG-PET to FDG-PET and CT combined. They found an improvement in staging from 78% to 89% with image fusion. Further research will determine its clinical application.

Preoperative and intraoperative imaging modalities – limitations

There are two main areas of concern when discussing radiological imaging modalities. As technology has developed, studies have used varied forms of US, CT and MRI. Therefore, the reader must be careful to acknowledge that the terms US, CT and MRI can refer to completely different imaging techniques and interpret the results accordingly. In addition, discussion of the sensitivity of imaging techniques always involves a reference standard for comparison. Caution must be applied as the reference standards themselves are subject to limitations with none of them having 100% sensitivity and specificity.

It may seem that despite significant radiological advances just as many liver lesions are being missed as before. It is important to remember that the aforementioned reference standards have all progressed too, setting the standard higher. As the gap between the standards and imaging modalities is not diverging, we can assume that we are detecting smaller lesions at an earlier stage.

In summary, there are many imaging modalities currently available to detect colorectal liver metastases. Non-invasive imaging modalities have overlapping benefits with individual limitations. The invasive technique of IOUS is the current gold standard, but the challenge of detecting occult liver metastases and improving patient outcome remains.

1.3.2 Tumour markers

Carcinoembryonic antigen (CEA) is the most frequently used tumour marker in colorectal cancer. It was first described in 1965 by Gold and Freedman when they identified an antigen that was present in foetal colon and colonic adenocarcinoma, but absent in healthy adult colon[187;188]. As a result of only being present in embryonic and cancer tissue, the antigen was named carcinoembryonic antigen or CEA. Further work found that CEA was present in some healthy tissues, but that serum concentrations were greater in patients with colorectal cancer and that these concentrations increased with advancing disease[189;190].

An ideal or perfect tumour marker should: be highly specific for one disease process; appear early in disease process; change as disease progresses or declines; be cost effective and be a simple, non-invasive test to allow repeated testing of patient. In relation to the last two requirements, a relatively cheap serum test to measure CEA is available in most clinical laboratories, resulting in serial blood tests that most patients find convenient. However, CEA can be elevated by several disease processes, including smoking and benign diseases where excluding cancer is important, although if CEA is found to be markedly elevated then benign disease is unlikely [191-193].

This finding restricts the usage of CEA as a sole diagnostic tool and the guidelines from the Association of Coloproctology of Great Britain and Ireland do not currently include routine measurement of CEA. The role of CEA may lie in the surveillance of patients with resected colorectal cancer with evidence in the literature to suggest that raised serum levels postoperatively can be a good indicator of recurrent/ progressive disease, even in asymptomatic patients[194;195]. Furthermore, two prospective studies have concluded that elevated CEA was highly sensitive in diagnosing liver metastases, quoting sensitivities of 94% and 96%[195;196]. This role could be extended by monitoring the response of metastatic disease in patients undergoing chemotherapy, but data is lacking in this area[197]. Another potential role is the application of CEA as a prognostic indicator. The Clinical Risk Score has included CEA as one of their five variables to predict outcome after liver resection for metastatic colorectal cancer [65;198].

1.4 Treatment of colorectal liver metastases

1.4.1 Surgical resection

Long-term survival

Surgical resection of colorectal hepatic metastases is the only potentially curative option. The five year survival rate varies in the literature from 6% to 58%, however, the majority of studies document from 25% upwards. This contrasts sharply with untreated patients who have a median survival of 5 to 21 months with few alive at 5 years[79-84;199-201]. Hepatic surgery has evolved from the time when it was found to carry a high morbidity and mortality and it is only since the 1990s that an extensive evidence base has been published showing improved long term outcomes.

To document the long-term survival, a literature search of Medline, Embase and Pubmed was performed. The search included the keywords: colorectal liver metastases; surgical resection; partial hepatectomy; survival and patient outcomes. Over 400 papers were returned and the researcher decided to limit the search to include study populations of at least one hundred patients. If an author had published more than one paper on the same study population, but had analysed different long-term variables, then both papers were included. Otherwise, the paper with the greatest number of recruited patients was included. Table 2 displays the twenty-three studies in order of year published.

In one of the largest studies Scheele et al (1990) assessed 1209 patients with colorectal liver metastases over a 30 year period with the primary outcome being long term survival[200]. Patients were divided into three groups. Group 1 (n=921) were unresectable due to extensive liver metastases and/or extrahepatic spread found on imaging or laparotomy that resulted in 23 patients undergoing palliative hepatic debulking procedures

and 113 receiving hepatic arterial chemotherapy. Group 2 (n=62) patients had unresectable disease confined to the liver, but with current guidelines could undergo potentially curative hepatic resection. Of this group, only eight were treated with intra-arterial chemotherapy. The remaining 226 patients formed group 3 and underwent potentially curative resection, including 22 patients that underwent resection for other metastatic disease. Of these 226 patients, 183 had pathologically clear resection margins.

Group 1 patients had an extremely poor prognosis with only 7% alive at 4 years post diagnosis and none surviving 5 years. The median survival time was only 6.9 months. Group 2 patients with untreated resectable disease confined to the liver had improved median survival time of 14.2 months, but again none survived 5 years post diagnosis. These results contrast with group 3 that had an overall survival of 31% at 5 years, including those 22 patients with positive resection margins. If these 22 patients are excluded, the 5 year survival increased to 38% with 7 patients alive at 10 years (27%). The authors then looked at time to recurrence and found that disease-free survival in the curative resection group was promising with 29% of the patients disease-free at 5 years. In the 106 patients that had confirmed recurrences, liver recurrence accounted for the majority of cases (n=74), followed by lung (n=50) then primary tumour (n=16). The longevity of this study allowed the authors to assess changes in prognosis over time and although there was an increase in the number of curative resections being performed (2% in 1960s vs 9.5% in 1970s vs 20.5% in 1980s) there was no accompanying increase in 5 year survival (40% in 1960-1979 vs 39% in 1980-1987, $p>0.05$).

The authors concluded that hepatic resection is a safe and effective long term treatment for colorectal liver metastases with patients undergoing pathologically confirmed curative resections. If the resection margins are returned as positive the long term outcome

is shortened, but is still superior to untreated, but potentially resectable liver metastases.

Since 1990, there have been several large single centre studies confirming Scheele et al's results [198;202;203]. One such study by Kooby et al (2003) from the Memorial Sloan-Kettering Cancer Center prospectively assessed blood loss and its relationship to survival in 1351 patients undergoing curative hepatic resection for colorectal liver metastases [203]. The median survival was 42 months (95% CI 39-46 months) with one, three and five year survival rates of 88% (95% CI 86-90%), 56% (95% CI 53-59%) and 36% (95% CI 33-39%) respectively.

The majority of the evidence is provided by single centre studies with few multicentre trials in the literature. One large multicentre trial (Nordlinger et al 1996) analysed survival in 1568 patients from 85 institutions undergoing potentially curative resection for colorectal liver metastases[204]. No patient had evidence of extrahepatic disease and the median follow up was 19 months. After exclusion of 36 postoperative deaths, the 5 year overall survival was 28% with 5 year disease free survival at 15%. These results, although good, are not as good as figures from high volume specialist centres. It would be reasonable to conclude that hepatic resection should only be performed by specialist centres and indeed there is evidence to support this view[205]. However, many studies, including Nordlinger's multicentre study, have been performed over a long time frame[204]. With improvements in hepatic surgery having only occurred during the last 10-15 years, one would expect more recent studies to have improved long term outcomes. Indeed, this does seem to be the case as shown by improved 5 year survivals of 42%, 51%, 53% and 58% in studies where the study period is predominately from 1990 onwards[202;206-208].

Table 2: Long term survival in patients undergoing potentially curative resection for colorectal liver metastases.

							Survival in years (%)			
main author	**country**	**year published**	**study period**	**centre**	**patient no.**	**median survival (mnths)**	**1**	**3**	**4**	**5**
Child PW	Australia	2005	1990-1996	single	211	9-17		13-27		6-9
Bennett JJ	USA	2005	1990-2001	single	146	37		52		29
Jones OM	UK	2005	1986-2003	single	598				33-47	
Sasaki A	Japan	2005	1985-2003	single	103					43
Pawlik TM	USA, Italy, Switzerland	2005	1990-2004	multi	557	74	97	74		58
Schindl M	UK	2005	1988-2002	single	150	38	84	52		36
Nicoli N	Italy	2004	1975-2000	single	228					16
Abdalla EK	USA	2004	1992-2002	single	348			73		58
Stewart GD	UK	2004	1988-2001	single	137	23-59				25-40
Kooby DA	USA	2003	1986-2001	single	1351	42	88	56		36
Choti MA	USA	2002	1984-1999	single	226	46		57		40
Minagawa M	Japan	2000	1980-1997	single	235	3.1		51		38
Ambiru S	Japan	1999	1984-1998	single	174					20
Fong Y	USA	1999	1985-1998	single	1001	69	89	57		37
Iwatsuki S	USA	1999	1981-1996	single	305					32
D'Angelica M	USA	1997	1985-1991	single	456					21
Fong Y	USA	1997	1991-1993	single	133	36	84	55		
Nordlinger B	France	1996	1968-1990	multi	1568		88	44		28
Scheele J	Germany	1995	1960-1992	single	434					38
Gayowski TJ	USA	1994	1981-1991	single	204		91	43		32
Scheele J	Germany	1991	1960-1988	single	207					37
Hughes KS	USA, Germany, UK, Canada	1986	1948-1985	multi	899					33
Adson MA	USA	1984	1948-1982	single	141					25

Disease-free survival

Despite good long-term survival being achieved with hepatic resection, a large proportion of patients will recur. The documented range in the literature is from 15% to 54% at 5 years after surgical resection[200;204;207-210]. The majority of recurrences happen within the first two years after partial hepatectomy, with approximately 50% occurring in the liver.

This early recurrence was assessed by researchers in the late 1980s that performed growth rate studies[77;211]. Finlay et al took 15 patients that were undergoing bowel resection for their colorectal cancer. A total of 29 hepatic metastases were found: 11 at laparotomy (overt) and 18 with serial CT scanning in the postoperative period (occult). The estimate of mean tumour volume doubling time for the overt metastases was 155 +/- 34 days (+/- s.e.m.) compared with 86 +/- 12 days ($p<0.05$) for the occult metastases. In addition, the estimate of the mean age of the metastases at laparotomy was 3.7 +/- 0.9 years for the overt metastases and 2.3 +/- 0.4 years for the occult metastases. The authors concluded that colorectal liver metastases are slow growing. As a result, the high rate of hepatic recurrence in the first two years after surgery is more likely due to the growth of metastases that remained occult at the time of initial staging and surgery, rather than new metastases.

There is no widely regarded optimal treatment for this group of patients with systemic chemotherapy and local ablative therapies commonly selected. The role of repeat partial hepatectomy has received some analysis and appears to provide similar results to initial surgery with 5 year survival from 25% to 49% [212;213]. However, more research is required as these studies have only small patient numbers reflecting the small proportion of patients that would be suitable for repeat liver surgery.

Morbidity and mortality

Morbidity of hepatic resection has improved dramatically through the years with current postoperative rates lying between 8.2% and 47%[203;204;209;214-217]. Commonest hepatic related complications include: blood loss; hepatic dysfunction/failure; perihepatic sepsis; biliary (leaks, fistulae) and subphrenic collections. Cardiorespiratory complications account for the majority of non-hepatic complications.

It is acknowledged that mortality from hepatic resection should be low, with a mortality rate of less than 5% being acceptable [203;204;209;214-217]. Reasons for mortality vary, with hepatic failure, sepsis and cardiorespiratory complications accounting for the majority of cases.

Blood loss

Hepatic resection has been traditionally associated with a substantial risk of haemorrhage with some authors documenting losses of up to 10 litres in a single patient[218-220]. This risk is greater in patients with steatosis or cirrhosis of the liver and also increases with the volume of liver resected[221-226]. Table 3 displays documented blood losses from a variety of centres to highlight the varied ranges.

Several papers have documented that haemorrhage and subsequent blood transfusion during or after hepatic resection increases patient morbidity and mortality [203;227-229].

Table 3: Documented blood losses after hepatic resection in patients with colorectal liver metastases.

				blood loss (mL)				
main author	**study period**	**year published**	**cirrhosis**	**median**	**mean**	**range**	**transfusion rate**	**anaesthetic and surgical technique**
Tanaka K	n/a	2005	n/a	1000-2000	n/a	70-4030	480mL per patient	IO-Pringle (intermittent)
Otsubo T	1995-2000	2004	poss. 59/103	n/a	910-1177	n/a	32-42% patients transfused	±OO-IVC
Kooby DA	1986-2001	2003	0/1351	n/a	n/a	n/a	55% patients transfused	not clarified
Descottes B	1986-2001	2003	poss 16/87	n/a	n/a	n/a	1.5U/ patient	IO-Pringle, OO-IVC and OO-SVC
Jarnagin WR	1991-2001	2002	poss 84/1803	600	871	n/a	4 U/ patient	low CVP, IO-Pringle (intermittent) and OO-HV
Torzilli G	1994-1999	2001	135/329	690	853	61-4072	3.1% patients transfused	IO-Pringle (intermittent) OO-HV
Melendez JA	1991-1997	1998	poss 78/496	645	848	40-9000	2.6 U/ patient	low CVP plus IO-Pringle
Fong Y	1991-1993	1997	8/133	800	1200	50-8400	n/a	IO-Pringle
Gayowski TJ	1981-1991	1994	n/a	n/a	n/a	n/a	3.7 U/ patient	n/a
Cunningham JD	1991-1993	1994	poss 17/100	1000	n/a	450-1500	59% patients transfused	low CVP, IO-Pringle (intermittent), OO-HV
Hannoun L	1981-1991	1993	n/a	n/a	n/a	n/a	5.3 U/ patient	IO-Pringle ± OO-HV
Bismuth H	1979-1988	1989	0/51	n/a	n/a	n/a	4.5 U/ patient	OO-SVC+IVC; IO-Pringle (continuous)

Abbreviations to Table 3: *central venous pressure (CVP); outflow occlusion by clamping hepatic veins (OO-HV), outflow occlusion by clamping/controlling of inferior vena cava (OO-IVC); outflow occlusion by clamping of superior vena cava (OO-SVC); inflow occlusion by clamping portal triad/ Pringle's manoeuvre (IO-Pringle) and continuous v intermittent.*

Acutely, haemodynamic instability during the operation can occur. This can result in: delay or cessation of the operation; increased anaesthetic time; cardiorespiratory arrest or end organ damage due to poor tissue perfusion. Haemorrhage will decrease surgical field visibility increasing the technical difficulty of the operation and increasing the risk of damage to other structures, particularly the hepatic veins[230]. The administration of blood products to compensate for the blood loss carries a long list of potential side effects: allergic or anaphylactoid reactions; haemolysis; coagulopathy; increased postoperative infection rate; viral and bacterial infection transmission [231-234].

The main long term complication from haemorrhage is reduced disease free survival for several types of malignant disease[203;229;235-241]. Yamamoto et al assessed survival in 250 patients undergoing complete resection of hepatocellular carcinoma[235]. Recurrence occurred in 55 out of 74 patients (74.3%) who had received a blood transfusion compared to 89 out of 178 patients (50%) that did not ($p=0.0001$). In addition, perioperative blood transfusion was found to be a significant predictor for accelerated recurrence on multivariate analysis ($p=0.003$).

In relation to colorectal liver metastases, the largest study assessing the influence of blood transfusion on long term survival after resection is by Kooby et al[203] who analysed more than 1300 patients undergoing hepatic resection. Approximately half of these patients received a blood transfusion and it was found that these transfused patients were more likely to die in the first 60 days following resection. The authors concluded that the major effect of transfusion is on perioperative outcome, as although there was a perceptible influence on long term survival, other factors were more dominant in predicting survival than transfusion administration.

Documented blood losses and techniques developed to minimise loss

Discussion of the blood losses documented with hepatic resection must be interpreted in the context of the various surgical and anaesthetic techniques that have evolved to minimise haemorrhage (Table 3). There are three main phases when bleeding can occur during hepatic resection: dissection; parenchymal transection and revascularisation. Subsequently, a multitude of anaesthetic and surgical techniques have been developed that target these 3 phases.

Anaesthetic technique

After the induction of anaesthesia, it was a traditional approach to administer intravenous fluid to expand the intravascular volume in anticipation of significant blood loss. This additional volume would increase the central venous pressure (CVP), distending the central veins and compounding any bleeding from damaged or undetected vessels. Maintenance of a low intraoperative CVP during extrahepatic dissection and parenchymal transection was introduced and analysed for any effect it may have on intraoperative blood loss[226]. Cunningham et al assessed blood loss and transfusion requirements in 100 consecutive patients undergoing partial hepatectomy with a low CVP. They found blood losses ranging from 450mL (segmentectomy) to 1500mL (extended left hepatectomy) with 59% of patients receiving a blood transfusion. The authors concluded that low intraoperative CVP resulted in an acceptable and low blood loss.

The same research group published further findings on outcomes with low CVP in 1998 where the median blood loss in 496 partial hepatectomies performed under low CVP was 645mL and only 33% of patients had a perioperative blood transfusion[242]. Jones et

al[243] performed a randomised controlled study on one hundred patients undergoing hepatic resection: group 1 underwent partial hepatectomy with a CVP greater than or equal to 5cmH_2O whilst group 2 underwent partial hepatectomy with a CVP less than 5cmH_2O. Median intraoperative blood loss was significantly lower in the low CVP group (200mL, range 0-1000), compared to the high CVP group (1000mL, range 0-8000; $p<0.001$). Transfusion rate was also significantly lower in the low CVP group (5% v 48%, $p=0.008$). After further statistical analysis the authors concluded that these results were not influenced by other variables: the presence or absence of portal occlusion; the resection type; the length of operation; the number of lesions resected; the method of liver dissection or the presence or absence of cirrhosis.

Low CVP can be achieved by several routes with the two most commonly used being administration of intravenous nitroglycerins and/or intraoperative intravenous fluid restriction. Intraoperative haemodynamic instability has been reported as a consequence of maintaining a low CVP and has to be carefully balanced against the risk of haemorrhage. Intraoperative hypotension can lead to further complications including renal dysfunction and/ or failure but the exact incidence is unknown with estimates around 13%[230]. The largest study that has analysed renal dysfunction in low CVP conditions, found only 3% of 496 patients developed renal dysfunction after partial hepatectomy, suggesting that low CVP anaesthesia does not appear to compromise renal function[242].

Surgical techniques

In 1908, J. H. Pringle demonstrated that hepatic inflow vascular occlusion could reduce hepatic bleeding[244]. In 1952, control of hepatic inflow was combined with hepatic

outflow occlusion to allow the first major anatomical liver resection to be performed[245]. Since then, the lower morbidity and mortality associated with reductions in blood loss have led many authors to experiment with different vascular occlusion techniques. Currently, there are many techniques available for the hepatobiliary surgeon: hepatic inflow; hepatic inflow and outflow; total or selective occlusion and intermittent or continuous[246]. Each technique varies in level of technical difficulty, prevention of haemorrhage, intraoperative patient tolerance and hepatic tolerance, with the risk of hepatic failure being foremost[246]. Indeed, with the documented incidence of hepatic dysfunction and/ or failure being as high as 18%, some surgeons have disregarded hepatic occlusion techniques altogether [247,248].

To understand vascular occlusion techniques, the anatomy of the liver must be described. At the hilum of the liver, the dual blood supply (portal vein and hepatic artery) divide into right and left branches that supply the right and left lobes respectively. This is the classical description, but there are anatomical variations with aberrant hepatic arteries commonly arising from the left gastric (aberrant left) and superior mesenteric arteries (aberrant right). Venous drainage is provided by three large hepatic veins (right, middle and left) that lie posterior to the liver and open into the inferior vena cava just inferior to the diaphragm. Again, in 20% of cases there can be variations in the anatomy with the presence of: a significant right inferior hepatic vein; communications between the larger veins and/or smaller veins and small veins draining the posterior right lobe directly into the inferior vena cava. All of these anatomical variations have a significant impact on the effectiveness of the chosen approach to hepatic vascular occlusion.

Hepatic pedicle clamping is commonly referred to as Pringle's Manoeuvre and interrupts the arterial and portal venous inflow to the liver. The occlusion requires minimal

dissection and the pedicle can be occluded with a vascular clamp or encircled with a tape. One of the problems with Pringle's manoeuvre is that there is no attempt to prevent venous bleeding (termed back bleeding). This bleeding can be significant and result in the morbidity described previously. Another significant problem is the risk of hepatic failure. Although the exact duration of clamping that can be safely tolerated by the liver is unknown, sixty minutes is a widely quoted figure[249]. This led to research assessing the value of performing Pringle's manoeuvre intermittently (e.g. 15 minutes clamping followed by 5 minutes unclamped, repeat as required). Although there appears to be little difference between the two approaches in healthy liver (similar blood losses and hepatic dysfunction), intermittent clamping may be beneficial in cirrhotic livers[250]. Perhaps more importantly, intermittent clamping has led to hepatic preconditioning. Clavien et al found that the protective effects of intermittent clamping appeared to arise from the initial period of ischaemia (ten minutes) followed by reperfusion[251]. The authors concluded that this initial clamp-unclamp sequence could be manipulated to maximise liver function during the remainder of the hepatic occlusion.

Selective arterial occlusion interrupts the arterial and venous inflow to the region of liver to be resected, avoiding ischaemia to the liver remnant. This technique can be performed at two levels: lobe (right or left hepatic artery, for hemi-hepatectomy) or liver segment (segmental artery and vein, for hepatic segmentectomy). The haemodynamic trauma to the patient is minimised as well as the risk of hepatic dysfunction, making this technique attractive. However, this has to be balanced with the need for significant hilar dissection accompanied by potential bleeding from the raw surface of the non-occluded liver remnant.

Outflow occlusion is normally performed in conjunction with inflow occlusion. Again there are several options. The conventional technique for hepatic outflow occlusion involves clamping of the infra- and supra-hepatic inferior vena cava. The main advantage is the minimising of back bleeding. However, complete mobilisation of the liver is required as well as dissection of the inferior vena cava from the retroperitoneum. Furthermore, the patient has to be carefully monitored, as they are put under significant haemodynamic strain: decreased blood pressure; decreased pulmonary artery pressure; tachycardia; increased systemic vascular resistance and decreased cardiac output. These potential complications have led to other techniques being preferred leaving inferior vena caval clamping to be used when others cannot.

Another type of outflow occlusion is extraparenchymal hepatic vein clamping. This approach exposes and controls the three main hepatic veins without significant haemodynamic insult due to the preservation of caval flow. This technique can also be used selectively, by occluding only the appropriate hepatic vein. However, there are disadvantages: significant dissection and mobilisation of the liver is required; collateral circulation is not controlled and this approach cannot be used if tumour involves the cavo-hepatic junction.

Each of these hepatic occlusion techniques has a role to play depending on the patient status, tumour location, associated liver disease and experience of the anaesthetist and hepatic surgeon. The hepatic surgeon should understand the benefits and potential complications associated with each and apply them on an individual basis.

Liver dysfunction and failure after partial hepatectomy

Liver dysfunction or failure after partial hepatectomy has been documented to occur within the range 3-18% with the cirrhotic liver at greater risk[215;247;248;252;253]. The main reasons for liver dysfunction are the use of hepatic vascular occlusion techniques and resection of a large tumour volume leaving inadequate liver remnant function.

It has been traditionally thought, although not proven, that up to two thirds of the liver can be safely resected, with the risk of hepatic dysfunction or failure being low. Schindl et al (2005) addressed this question by performing preoperative estimation of residual liver volume by CTAP in 104 patients undergoing hepatic resection (colorectal metastases n=92, cirrhotic patients n=12) [248]. The authors categorised postoperative hepatic dysfunction as mild, moderate or severe with a total of 77 patients developing a degree of hepatic impairment: mild in 42 patients (40.4%); moderate in 22 (21.2%) and severe in 13 (12.5%). The patients who developed severe hepatic dysfunction had a significantly smaller %RLV (residual liver volume) compared to those with mild hepatic dysfunction (24.5% vs 42.9%, p=0.005) with further analysis determining a critical minimum %RLV of 26.6% must be achieved. If less than this threshold, then serious hepatic dysfunction is likely to occur.

These results are supported by two other papers which found a residual liver volume of ≤25% significantly increased the likelihood of postoperative hepatic dysfunction[254;255]. These authors concluded that volumetric analysis should become part or a patient's preoperative assessment for hepatic resection.

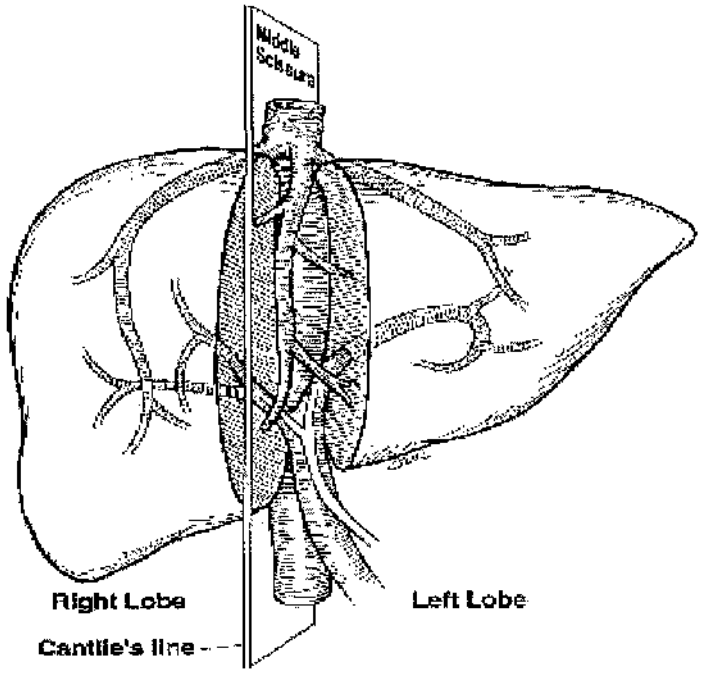
The rationale for hepatic surgery

The first recorded right lobectomy was performed in 1911 by Wendel, who based his approach on Cantlie's description as well as using hilar ligation[256]. At that time, the concept of hepatic segmental anatomy was not accepted and it took until the 1950s with the work of Healey (1953) and then Couinaud (1954, 1957, 1981) to achieve acceptance of segmental anatomy[257-261].

These anatomists divided the liver into functional lobes and segments according to the arterial blood supply, portal venous blood supply, biliary drainage and hepatic venous drainage. A plane passing through the bed of the gallbladder and the notch of the inferior vena cava divides the liver into right and left lobes and is commonly referred to as Cantlie's line [Figure 4] [262;263].

One of the most widely accepted definitions of liver segments are those by Couinaud[258]. Couinaud based his system on the fissures (or scissurae) of the three hepatic veins that divide the liver into four sections. This led to 8 segments being described (Figure 5). Most of the other well known definitions are similar to Couinaud's system, but there are some differences with much debate on how to classify which lobe the quadrate and caudate functionally belong. The quadrate is regarded as belonging functionally to the left lobe. The caudate also belongs to the left lobe and is classified as segment I. Some of the other nomenclatures are clinically useful, including Healey and Schroy's subdivision of segment IV: IVa (left superomedial segment) and IVb (left inferomedial segment)[257]. In addition, Bismuth (who also based his system on the three fissures) described a transverse fissure passing through the right and left portal branches, allowing simple diagrammatic explanation of the eight segments. (seen in Figure 5)[264].

Figure 4: Division of liver into left and right hepatic lobes by Cantlie's Line.

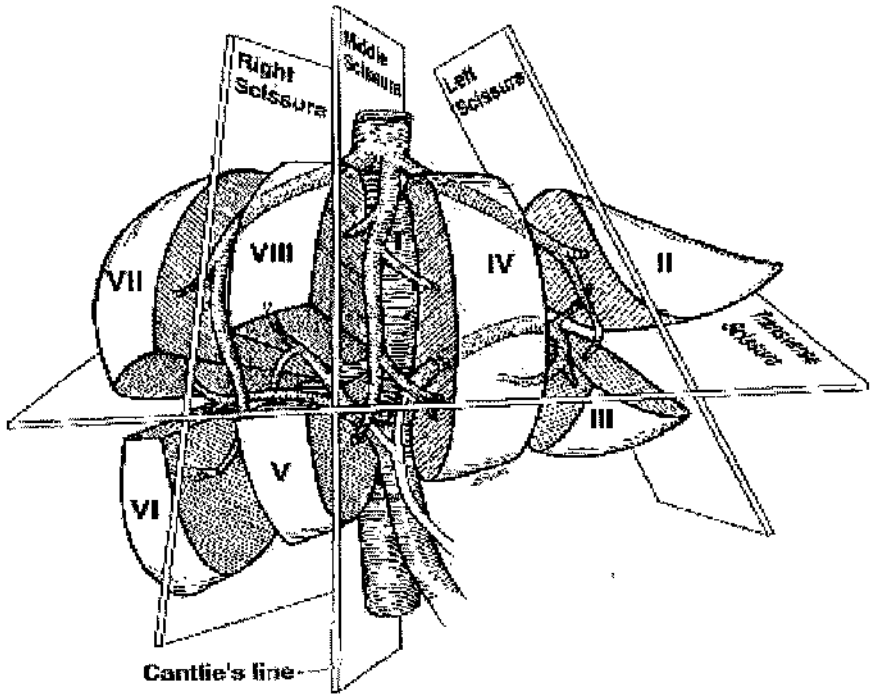


This delineation of the liver into planes forms the basis for anatomical liver resection as these planes are relatively devoid of major blood vessels and bile ducts.

Table 4 shows the current definitions of hepatic surgery used by one of the major hepatobiliary units[265]. Many definitions of hepatic resection are currently in widespread use, all based on Couinaud's work. As a result, attention should be paid to authors' individual nomenclature in the methodology section of their published work.

At the same time the anatomists were documenting their findings, Jean Louis Lortat-Jacob and HG Robert performed an anatomical hepatic resection. The operation was performed in Paris in 1952 on a patient that had a solitary liver lesion that turned out to be a colorectal liver metastasis. Lortat-Jacob and Robert's work caught the attention of surgeons and became a catalyst for hepatic resection being widely performed[245;266]. The majority of these resections were major anatomical resections which were thought to ensure a clear resection margin, conferring the best long term outcome for the patient with metastatic colorectal disease. More recently, segment-oriented surgery has become increasingly popular with various approaches available[267-269]. The popularity is due to maximal preservation of the functional liver parenchyma, reducing the risk of hepatic failure which is particularly attractive in patients with cirrhosis. Oncologically, the patient appears not to be compromised with several studies documenting similar long term survival to patients that have undergone traditional lobectomies[202;270;271]. Subsequently tri-, bi- and monosegmentectomies are now accepted approaches for small volume or bilobar disease. The picture is not as clear for subsegmentectomies, with some authors documenting reduced disease free and overall survival[272].

Figure 5: Couinaud's anatomical segments of the liver



I caudate lobe

II left posterolateral segment

III left anterolateral segment

IV left medial segment

V right anteroinferior segment

VI right posteroinferior segment

VII right posterosuperior segment

VIII right anterosuperior segment

Table 4: Extent of hepatic resection definitions.

Type of resection	**Couinaud segments**
Left lobectomy	II, III, IV ± I
Right lobectomy	V, VI, VII, VIII ± I
Extended left lobectomy	II, III, IV, V, VIII ± I
Extended right lobectomy	IV, V, VI, VII, VIII ± I
Trisegmentectomy	any three segments (adjacent or non-adjacent)
Bisegmentectomy	any two segments (adjacent or non-adjacent)
Monosegmentectomy	one segment
Wedge resection	less than one segment

Adapted from nomenclature in Weber et al (2000) from the Memorial Sloan-Kettering Cancer Center, New York, US[265].

Synchronous resections

The 20-30% of patients having liver metastases at the time of initial presentation presents an opportunity to perform combined hepatic and colonic resection. This combined or synchronous approach would allow the patient only one hospital stay which as well as being beneficial to the patient, would be cost effective.

Earlier studies explored patient outcomes after synchronous resections when compared to staged or interval resections and found significantly increased morbidity and mortality in patients undergoing synchronous resection[82;273-276]. As a result, these studies favoured staged resections due to lower postoperative complications with the additional benefit of the interval allowing detection of supposed 'occult' liver metastases.

However, the majority of these studies were non-randomised, limited by small patient numbers and in addition, were performed before or partly during the time frame of significant improvements in patient outcomes after hepatic resection. Also, comparisons are difficult as the definition of synchronous resection has varied. For some authors, this means hepatic resection at the time of laparotomy for the colonic primary. For others, synchronous refers to resection of the hepatic metastases up to 3 months after the primary bowel surgery.

Two recent studies have been published that overcome some of the limitations of the earlier studies[277;278]. Chua et al (2004) retrospectively analysed 96 patients that presented with colorectal cancer and liver metastases[277]. These patients underwent either synchronous (n=64) or staged bowel and hepatic resections (n=32) within the same surgical unit. For analysis of outcomes in the staged group, variables from both the primary and secondary surgery were added together and compared with the synchronous resections

group. The results found trends for lower volume liver resections (p=0.09) with an increased blood transfusion rate after synchronous resections (mean 326mL vs 185mL, p=0.08). Postoperative complication rates were similar between groups (53% synchronous vs 41% staged, p=0.25) with no operative mortality. The synchronous group experienced a significantly shorter hospital stay (mean 11 vs 22 days; p=0.001). In relation to long term outcomes, no significant differences between groups (synchronous vs staged) in disease free survival or overall survival were found (median 13 vs 13 months, p=0.53; median 27 vs 34 months, p=0.52). These authors concluded that synchronous resections are safe, effective and should be the procedure of choice for selected patients in experienced centres.

Martin and colleagues from the Memorial Sloan-Kettering Cancer Centre performed a similar analysis from a prospective database over a 17 year period [278]. One hundred and thirty four patients (group I) underwent synchronous resections, whilst 106 patients underwent staged resections (group II). Again, the extent of hepatic disease was less in the synchronous resections group (fewer number of tumours, p=0.001; smaller tumours, p=0.009) resulting in significantly smaller hepatic resections being performed (major resections 34% group I vs 72% group II, p=0.001). In addition, right hemicolectomy was performed in 40% of synchronous resections compared to only 14% in staged resections. Although the blood loss was greater in group II (median 550mL group I vs 1100mL staged, p<0.001), there was no difference in transfusion rates between groups (31% group I vs 38% group II). Regarding postoperative complications, the synchronous resections had significantly less (49% vs 67%, p<0.003) than group II, which on further analysis appeared to be a direct result of a second laparotomy in the staged group. This reduced complication rate also contributed to the significantly shorter hospital stay (median

10 vs 18 days, p=0.001). Perioperative mortality was exactly the same in both groups (n=3). Unfortunately this study did not analyse long term outcomes.

These two studies provide promising evidence that synchronous bowel and hepatic resections are safe without compromising long term outcome. With the introduction of local ablative therapies treating liver metastases at the same laparotomy for hepatic resection the debate for optimal timing of treatment in patients with synchronous colorectal cancer and liver metastases continues.

Patient selection and prognostic indicators after hepatic resection

With the publication of many centres' work, hepatic resection has finally become the widely regarded optimal treatment for colorectal liver metastases. Exact guidelines for selecting patients for hepatic resection currently do not exist and remain an issue for discussion.

Patient selection for hepatic resection has been divided into three groups: medical fitness for anaesthesia; the absence of disseminated disease and lastly, tumours that are confined to the liver so that adequate liver parenchyma is preserved. Several studies have set up uni- and multivariant analyses to determine prognostic factors for patient selection (Table 3).

In relation to overall survival, the burden of disease is prognostic. Nodal status of primary tumour and number and size of hepatic tumours have been found to be important outcome markers in many studies. In addition, the presence of extrahepatic disease has been shown to be an independent predictor of survival in three of the largest studies, resulting in subsequent studies excluding such patients from analysis[204].

Bilobar disease has previously been an exclusion criterion for undergoing hepatic resection. However, approximately half the studies shown in Table 5 have not found metastases on both lobes to be indicative of patient outcome. This disparity between studies can be explained in that some cases bilobar disease reflects a high burden of disease (i.e. multiple widespread hepatic metastases) whilst in others only a small burden of disease is present (i.e. two small metastases, one either lobe). It is a similar picture for extent of hepatic resection. Assuming enough healthy parenchyma is left behind, the extent of resection does not always reflect the burden of disease. Indeed, the decision for the extent of resection can be based on surgeons' preference rather than disease burden (i.e. performing a right hepatectomy rather than a monosegmentectomy for a tumour in segment 6). As a result, many studies have not found extent of resection to be an independent prognostic factor.

Achieving adequate resection of the hepatic tumour is an independent prognostic factor. An area of debate is what constitutes an adequate margin. The majority of studies state that the best survival outcome occurs when the resection margin is greater than 1 cm[204;209;279]. However, Fong et al divided their patient population into resection margins >1cm and those <1cm and found no difference in survival between the two groups[280]. The general consensus is that any R0 margin is acceptable, but ideally a margin of 1cm should be achieved.

CEA may have a role in determining prognosis after hepatic resection, with two large studies finding that an elevated CEA was an independent determinant of survival after hepatic resection[198;204]. However, other studies have not found as convincing evidence for

CEA, which may be a reflection of the different thresholds for determining what constitutes an elevated CEA.

Table 5: Summary of analyses of prognostic determinants of disease free survival and overall survival after resection of colorectal liver metastases.

				Prognostic factors													
				patient			primary cancer			liver metastases				liver resection			
Authors	Country	year	OS / DFS	nos	age	sex	side	node positive	disease free interval	nos	size	bilobar	CEA	extrahepatic disease	RO margin	extent of resection	post op chemo
Fong	USA	1999	OS	1001	-	-		+UM	+UM <12/12	+UM	+UM >5cm	+U	+UM	+UM	+UM	+U	
Sirichindakul	Thailand	2004	OS	96	-	-	-	+UM	-	-		-	-	+UM			-
Iwatsuki	USA	1999	OS	305	-	-	-	+U	+UM >30/12	+UM ≥3	+UM >8cm	+UM		+U	+U <1cm	+U	-
			DFS		-	+U female	-	+U	+UM >30/12	+UM ≥3	+UM >8cm	+UM		+U	+U <1cm	+U	-
Ambiru	Japan	1999	OS	168	-	-	-	+UM	+U	+UM ≥3	-	-	-	+U	+UM ≤0.5cm	-	+UM
D'Angelica	USA	1997	OS	96	-	-	-	+U	-	+U ≥4	-	-	-	-	-	+U	
			DFS		-	+UM female	-	-	+UM <12/12	-	-	-	-	-	+U	-	
Fong	USA	1997	OS	456	-	-	-	+U	+UM	+UM	+UM >5cm	+U	+U	+UM	+UM	-	-

				patient			primary cancer			liver metastases				liver resection			
Authors	Country	year	OS / DFS	nos	age	sex	site	node positive	disease free interval	nos	size	lillobar	CEA	extrahepatic disease	RO margin	extent of resection	post op chemo
Nordlinger*	France	1996	OS	1568	+UM		-	+UM	+UM <12/12	+UM	+UM >5cm	-	+UM	excluded	+UM <1cm	+U	-
Gayowski	USA	1994	OS	204	+U ≥60yrs	-	-	+U	+U ≤24/12	+UM	-	+U		+U	+UM ≤1cm	-	
			DFS		-	+U female	-	+U	+U ≤24/12	+UM	-	+U		+U	+UM ≤1cm	+U	
Scheele	Germany	1991	OS	266	-	-	-	+U	+U ≤24/12	+U satellite	-	-		-	+U	+U	
			DFS		-	-	-	+U	+U ≤24/12	-	-	-		-	-	+U	
Adson	USA	1984	OS	141	-	+UM men		+UM	-	-	-			+UM		-	

* the only multicentre study in this table

OS = overall survival; DFS = disease free survival (highlighted in grey)

+ = prognostic factor; - = not prognostic factor; blank = not studied.

U = univariant analysis; M = multivariant analysis.

Prognostic indicators for disease free survival are similar to overall survival, reflecting the importance of burden of disease. There has been much research into the role of chemotherapy after hepatic resection and this will be discussed in detail in the next section. It should be noted that of the studies discussed here, only one has found chemotherapy to be an independent prognostic indicator of survival[216].

Several authors have tried to collate the prognostic factors to develop a prognostic score, clarifying patient selection for undergoing hepatic resection. In one of these, Fong et al retrospectively reviewed 1001 patients that were undergoing hepatic resection for metastatic colorectal cancer[198]. Actuarial survival was 37% at 5 years with positive resection margin, extrahepatic disease, >1 tumour, elevated CEA, tumor size greater than 5cm, node positive primary and disease free interval <12 months all to be independent predictors of survival. The authors then selected five clinical criteria and assigned one point to each to produce the Clinical Risk Score (CRS) [Table 6]. Patients with a CRS of 0, 1, or 2 have a highly favourable outcome with a 5 year survival of 40-60%, whilst groups 3-5 have a poorer prognosis with patients scoring 5 having a 5 year survival of only 14%. The authors concluded that patients with scores 0-2 should be considered for aggressive treatment with patients scoring 3-4 having a more guarded prognosis, and resection should be planned in the context of adjuvant therapies. Finally patients with a score of 5 have very poor outcomes, and resection without additional effective adjuvant therapy or outside of adjuvant trials is highly questionable. This clinical risk score has recently been validated by another centre and further research will determine its widespread applicability[206].

Table 6: Clinical Risk Score for Tumour Recurrence.

	Survival (%)					
CRS	**1 year**	**2 year**	**3 year**	**4 year**	**5 year**	**Median (mnths)**
0	93	79	72	60	60	74
1	91	76	66	54	44	51
2	89	73	60	51	40	47
3	86	67	42	25	20	33
4	70	45	38	29	25	20
5	71	45	27	14	14	22

Each risk factor is one point: node positive primary; disease free interval <12 months; >1 tumour; tumor size >5cm and CEA >200ng/mL

From Fong et al, Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer [198].

Section 1.4.2 Chemotherapy

The role of chemotherapy in primary colorectal cancer is clearly defined. In contrast, the indication for chemotherapy in unresectable and post resected colorectal liver metastases remains unclear. Chemotherapy can be administered systemically or regionally with the latter dividing into hepatic arterial infusion (HAI) or isolated hepatic perfusion (IHP).

Systemic chemotherapy

5-Fluorouracil (5-FU) was one of the first chemotherapeutic agents and is still the backbone of chemotherapy for patients with metastatic colorectal cancer. Used alone, researchers have found that 15-20% of patients undergo tumour regression, but with no evidence of improved survival[281]. When used in conjunction with other agents (biomodulation) prolonged survival has been found when compared to patients receiving the best supportive care[282]. The most commonly prescribed regime (5-FU with folinic acid) has been documented to result in a median overall survival of 14.8 months and the introduction of new agents (irinotecan, mitomycin, oxaliplatin, cisplatin, topoisomerase I inhibitor) has provided a new area of research with delayed disease progression and improved long term survival[283-286 287-290].

The clinical role and application of systemic chemotherapy is continually changing and large multicentred trials are required to determine the exact combination and optimal administration of both old and new agents[291].

Hepatic artery infusion chemotherapy

Hepatic artery infusion (HAI) chemotherapy is based on two principles: liver metastases deriving the majority of their blood supply from the hepatic artery and certain drugs having a high first pass hepatic extraction[292]. These result in high doses of chemotherapeutic drugs to the tumour yet sparing the normal hepatic parenchyma and reducing systemic toxicity, both of which are major disadvantages to systemic treatment.

Several randomised studies have documented improved response rates (reductions in liver tumour size, longer time for tumour progression and patient compatibility/ quality of life) but no survival advantage for patients with unresectable disease undergoing HAI with 5-fluorodeoxyuridine (FUDR; commonly used because of its solubility properties and ability to be concentrated in a small volume) compared to systemic 5FU[293-295]. Kerr et al in 2003 performed a multicentred, prospective randomised trial comparing HAI to intravenous chemotherapy using fluorouracil and folinic acid[284]. The authors found a median overall survival of 14·7 months for the HAI group and 14·8 months for the intravenous group (hazard ratio 1·04 [95% CI 0·80–1·33], log-rank test p=0·79) with no significant difference in disease progression survival. In addition, there were documented problems with catheter insertion (resulting in 37% of the patients allocated to receive HAI not undergoing HAI) and catheter failure (29% of patients having failure of the catheter during their treatment course).

An area that requires more analysis is the use of HAI in combination with systemic chemotherapy in patients that have undergone hepatic resection. The Sloan Kettering Group in 1999 found improved long term survival with adjuvant HAI (FUDR) when it was

combined with systemic chemotherapy (5FU) compared to control group that received only systemic chemotherapy[296].

Isolated hepatic perfusion chemotherapy

Isolated hepatic perfusion (IHP) is able to administer high doses of chemotherapeutic agents to the liver by isolating the liver from the systemic circulation[297]. Outflow catheters are inserted into the inferior vena cava and portal vein which are connected into a bypass circuit that drains into the axillary vein[298].

Conclusions about the disease-free and long-term survival with IHP in patients with colorectal liver metastases are hard to draw due to few comparative studies with other approaches and varying methodology, especially in the type of agent administered. The largest series analysed only 51 patients and found increased median survival when IHP was combined with HAI compared to IHP alone (27 months versus 16 months respectively)[299]. Interestingly, in a smaller study from the same group, partial response was attained in patients undergoing IHP that had previously not responded to both systemic chemotherapy and HAI, an area that needs further evaluation[300].

Section 1.4.3 Radiotherapy

Traditionally, radiotherapy has had a limited role in the treatment of colorectal liver metastases with a recent multicentre trial supporting this statement[301]. Radiotherapy is limited in that it cannot treat extrahepatic disease and any dose greater than 30Gy will have a significant risk of developing radiation hepatitis[302;303].

Selective internal radiation therapy (SIRT) is a new modality for patients with unresectable disease that takes advantage of the hepatic tumours being predominately supplied by the hepatic artery. Treatment consists of delivery of radioactive microspheres (usually 90Yttrium) into the hepatic artery supplying the tumour, allowing selective tumour uptake that minimises inadvertent perfusion of other non-diseased organs. Initial results show good patient compliance and long term results are awaited, especially when used in conjunction with postoperative chemotherapy[304-307].

Section 1.4.4 Local ablative therapies

There are many local ablative treatments available for the treatment of surgical unresectable colorectal liver metastases. There is limited evidence for inclusion into clinical practice for the majority, including: hepatic cryosurgery; percutaneous ethanol injection; embolisation; focal hyperthermia and high intensity focused ultrasound. More recent evidence has focused on the clinical application of laser photocoagulation, microwave coagulation and predominately, radiofrequency ablation[308].

Interstitial laser photocoagulation (ILP)

Lasers produce a coherent, monochromatic and highly collimated beam that results in tissue vaporisation, necrosis and coagulation when placed inside a solid tumour[309]. In an early study, Amin et al[310] performed ILP on 26 patients with liver metastases, of which 21 were colorectal in origin. Each patient had between 1-8 sessions until follow up CT confirmed complete necrosis of the liver tumour. The authors concluded that ILP had a good safety profile and potential for improving survival with 2 year survival at 70%. One

of the largest studies assessed long term outcome in 393 patients with colorectal liver metastases that were not suitable for surgical resection (recurrence after previous surgery; bilobar disease; irresectable disease or refused surgery)[311]. After undergoing ILP the mean survival was 41.8 months with 5 year survival at 30%. Although this group were highly selected, compared to the natural progression of untreated metastatic colorectal disease, ILP appears to offer a survival advantage[312].

Microwave coagulation therapy (MCT)

Microwave coagulation therapy produces electromagnetic radiation that is delivered into the tumours by a needle, resulting in rapid agitation of the water molecules. This agitation causes frictional heating that can achieve temperatures of 60-100°C[313]. There are only a few documented clinical trials assessing MCT. In one of these trials, Beppu et al (1998) performed percutaneous MCT in 40 patients with colorectal liver metastases and found recurrence in 14/ 40 with 5 year survival of 33%[314]. These results are promising, but require further trials to confirm.

Radiofrequency ablation (RF ablation)

An alternating high frequency current is transmitted from the tip of an electrode in to the tissue surrounding the electrode. Ions in the tissue try to follow the current and become agitated, producing frictional heating that results in coagulation necrosis. RF ablation produces temperatures greater than 100°C destroying tissue microvasculature and can be performed percutaneously, laparoscopically or at laparotomy.

A multicentre study group analysed the complications after performing ultrasound guided percutaneous RF ablation in 1,139 patients in 11 institutions. The overall complication rate was 2.4% with a mortality rate of 0.09%. This study and others have concluded that the morbidity and mortality of RF ablation is acceptable[315-317]. Although the long term survival of patients with colorectal liver metastases that have undergone RF ablation is unknown, results to date compare favourably to surgical resection. In by far the largest study to date of RF ablation and colorectal liver metastases, Solbiati et al[318] found 1, 2 and 3 year survival rates of 93%, 69% and 46% respectively in 117 patients. As more research is published, the need for a randomised trial may become apparent.

Section 2: Hypotheses and Aims of thesis.

The hypotheses and aims of this thesis are:

1) Contrast enhanced ultrasound has a clinical role in the detection and characterisation of colorectal liver metastases that challenges current available imaging modalities.

This study aimed to determine the clinical role by performing four separate prospective trials. The first trial aimed to document the percutaneous detection and characterisation of colorectal liver metastases using a second generation ultrasound contrast agent. The second trial aimed to investigate the detection of colorectal liver metastases with the first documented use of intraoperative contrast enhanced ultrasound. The next trial aimed to develop an original technique using percutaneous contrast enhanced ultrasound to measure altered hepatic perfusion that could reliably detect colorectal liver metastases. The final trial assessed changes in hepatic perfusion using contrast enhanced ultrasound in patients undergoing curative partial hepatectomy for colorectal liver metastases.

2) The short and long term outcomes in patients undergoing partial hepatectomy for colorectal liver metastases using a novel triphasic approach is superior to the published literature.

One centre's prospective hepatic database on patients undergoing partial hepatectomy for colorectal liver metastases using a novel triphasic approach is analysed with emphasis on the perioperative changes in renal and hepatic function in addition to

blood loss, tumour recurrence and overall survival. Outcomes in relation to synchronous bowel and/or hepatic procedures are investigated and the application of prognostic scores in this study's population determined.

Section 3 – Investigative chapters

Chapter 1: The novel application of a second generation ultrasound contrast agent in the percutaneous detection and characterisation of colorectal liver metastases.

Chapter 2: A prospective trial determining a new clinical application of contrast enhanced ultrasound in the intraoperative detection of colorectal liver metastases.

Chapter 3: The development of a novel technique for the functional imaging of hepatic perfusion in colorectal liver metastases using contrast enhanced ultrasound.

Chapter 4: Perioperative changes in hepatic perfusion in patients undergoing curative partial hepatectomy for colorectal liver metastases using contrast enhanced ultrasound.

Chapter 5: Short and long term outcomes in patients with colorectal liver metastases undergoing partial hepatectomy with a novel triphasic approach.

Chapter 6: Conclusions.

Section 3

Chapter 1: The novel application of a second generation ultrasound contrast agent in the percutaneous detection and characterisation of colorectal liver metastases.

3.1.1 Introduction and aims

Contrast enhanced ultrasound (CE-US) is an evolving imaging modality. The introduction of a new generation of contrast agents, termed microbubbles, and improved imaging technology (pulse inversion harmonic imaging) allows diagnosis of focal liver lesions based on lesion vascularity. Three vascular phases can be identified (arterial, portal and late/sinusoidal) that allow pattern recognition based on the echogenicity of the lesion through the phases. Typically, a colorectal liver metastasis appears as a hypoechoic lesion during the arterial phase, accompanied by a halo of contrast defining its outer border. The metastasis then remains hypoechoic throughout the portal and late phases of scanning.

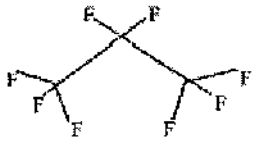
Several studies have compared CE-US with unenhanced US and found improvements in liver lesion detection and characterisation [94;167-169;175;180;181;319-321]. This difference appears to be significant with CE-US having a documented sensitivity of approximately 87% compared to 53-77% with percutaneous ultrasound [91;175-177]. In addition, CE-US appears to have the capability of detecting sub-centimetre lesions that are a known limitation of conventional ultrasound [169;170;175;178;179]. Further work is required to confirm these promising results and to understand the interaction between ultrasound contrast agents, ultrasound beam and hepatic microcirculation.

Definity® (DMP 115; Bristol-Myers Squibb Medical Imaging, Billerica, MA, USA) is a second generation ultrasound contrast agent comprising octafluoropropane gas encapsulated in an outer perflutren lipid shell (Figure 6). Each mL contains a maximum of 1.2×10^{10} perflutren lipid microspheres with a mean diameter of 1.1μm – 3.3μm. Definity® is a transpulmonary blood pool agent that has a good safety profile with the only

contraindications in patients with known hypersensitivity to octafluoropropane or in patients with cardiac shunts.

Definity® is licensed for use in cardiac imaging, but not for hepatic imaging. As a consequence, there is little published work on the application of Definity® in the detection of colorectal liver metastases. The primary aim of this study was to assess the effect of three different intravenous doses of ultrasound contrast agent Definity® on the three phase vascular enhancement of colorectal liver metastases, with particular attention paid to late phase enhancement. The secondary aim was to compare the detection rate of Definity® enhanced ultrasound to unenhanced ultrasound in the detection of colorectal liver metastases.

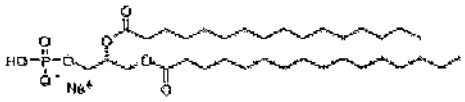
Figure 6a: Structural formula of Octafluoropropane.[322]



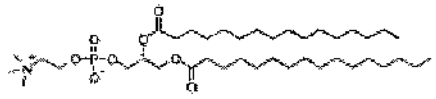
Octafluoropropane is chemically characterised as 1, 1, 1, 2, 2, 3, 3, 3-octafluoropropane with a molecular weight of 188, empirical formula of C_3F_8.

Figure 6b: The three components of the Perflutren lipid microspheres: DPPA; DPPC and MPEG5000 DPPE.[322]

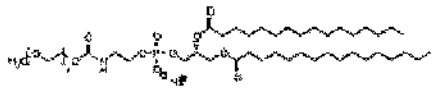
DPPA



DPPC



$MPEG_{5000}$ DPPE



3.1.2 Patients and Methods

Contrast uptake by colorectal liver metastasis

Ten consecutive patients (mean age 64.7 years, SD 8.1, range 56-79 years) undergoing percutaneous ultrasound as part of their pre-treatment assessment of their colorectal liver metastases were recruited from one centre. Ethical approval was obtained from the local research and ethics committee and each patient gave their informed consent.

Unenhanced ultrasound (US) was performed first using a HDI 5000 scanner (Philips, Bothell, Washington, USA) with a percutaneous C5-2Hz probe to target the required image: one liver metastasis surrounded with a region of healthy liver parenchyma. The patient was instructed to maintain stationery with regular breathing for the duration of each scan to minimise movement of the required image.

The scanner setting was then changed to pulse inversion harmonic imaging (PIH) with the ultrasound gain, focal zone and output power (Mechanical Index (MI): 0.04-0.08) all standardised. PIH is a sequence of two transmitted ultrasound pulses instead of one. The first pulse is an in-phase pulse and the second a mirror image of the first. For any linear target, the response to the second pulse is an inverted copy of the response to the first pulse. These responses are then summated and all linear echoes cancelled. Microbubbles, however, are non-linear and oscillate asymmetrically resulting in different responses to the two pulses. As a result the fundamental components of the echo are cancelled (i.e. the background noise from liver tissue) whilst the non-linear components (i.e. harmonics) are added, giving twice the level of a single pulse.

A bolus injection of 0.4mL of Definity® was administered via a peripheral venous cannula followed by a 3mL saline flush and the required image was maintained throughout a four minute scan (Figure 7). After this time period, the remaining contrast (Definity® has a half life of > 5 minutes post injection) was burst by a high power ultrasound beam (MI: 1.00-1.20) and a five minute interval started before the next contrast injection. Following the interval, the patient then underwent the same protocol for two further scans performed after injections of 0.6mL and 0.8mL of Definity® respectively.

Data analysis

Each four minute scan was recorded onto a video loop and saved to CD to allow data analysis by computer quantification software (Q-Lab®, Quantification software, Philips, Bothell, USA). In each patient and for each dose, a region of interest (ROI) was drawn over the liver metastasis with another duplicate ROI drawn over liver parenchyma allowing signal intensity against time curves to be drawn (Figure 8). The liver parenchyma to liver metastasis contrast ratio (parenchymal signal intensity divided by liver metastasis signal intensity; PMR) was calculated for each dose at selected time points during the four minute scans: 0s; 60s; 90s; 120s; 150s; 180s; 210s and 240s.

Statistical analysis was performed to compare any differences between the three intravenous doses of Definity® using the Mann Whitney test with significance taken at the 5% level (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA).

Detection of colorectal liver metastases using percutaneous contrast enhanced ultrasound

A further twenty three consecutive patients (mean age 67.2 years, SD 8.1, range 53-78 years) undergoing percutaneous ultrasound as part of their pre-treatment assessment of their colorectal liver metastases were recruited from the same centre. Ethical approval was obtained from the local research and ethics committee and each patient gave their informed consent. Each patient underwent CT and/or MRI scanning that was used as the standard of reference for comparison. Unenhanced ultrasound (US) was performed using a HDI 5000 scanner with a percutaneous C5-2Hz probe. The scanner setting was then changed to PIH with the ultrasound gain, focal zone and output power (MI: 0.04-0.08) all standardised. A bolus injection of 1.0mL of Definity was intravenously injected. Both ultrasounds were performed systematically in axial, sagittal and oblique sweeps to ensure complete liver coverage. For each ultrasound the number of metastases detected were recorded and compared to the standard of reference.

CT and MRI scanning

In all patients, CT and/ or MRI were performed as part of the patient's routine assessment to stage their disease. Two phase CT examination was performed using two Multidetector CT scanners (Siemens, Sensation 4 and Sensation 16, Erlangen, Germany) with 2-4mm slice thickness/ 16 x 1.5mm collimation and enhanced with 150mL of Omniscan 300 (Amersham, UK) injected at 3-5mL/second. Scanning was performed at 25-30 seconds for the arterial phases and 55-60 seconds for the portal venous phase following the start of the bolus contrast injection in a peripheral vein.

MRI was performed using two 1.5T MRI scanners (Philips, Gyroscan, Eindoven, Netherlands; Siemens Vision, Erlangen, Germany) with body and phase coils. Two different liver specific MRI contrast agents, MultiHance (Bracco Spa, Milan, Italy; dose 1mL/kg body weight) and Resovist (Schering AG, Berlin, Germany; 8 micromols Fe/kg body weight) were used. Pre contrast axial T2 and T1 weighted 2D scans with and without fat saturation were first performed. MultiHance enhanced axial 3D scans were carried out at 17 seconds, 45 seconds, 120 seconds and 60 minutes following a bolus peripheral venous injection (at 2mL/second followed by 20mL flush at 2mL/second), whilst Resovist enhanced axial T2 weighted scan was performed 10 minutes after a bolus injection.

Data analysis

Statistical analysis was performed using non-parametric testing (Mann Whitney and Wilcoxon Sign Rank Tests) with significance taken at the 5% level (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA). Changes in surgical management following CE-US were recorded (e.g. abandoned planned resection; more extensive resection; limited resection or combined resection with RF ablation). Sensitivity, accuracy and positive predictive value were calculated for unenhanced and enhanced ultrasound.

Figure 7: Percutaneous contrast enhanced ultrasound (portal phase) showing two hypoechoic lesions consistent with colorectal liver metastases (white arrows).

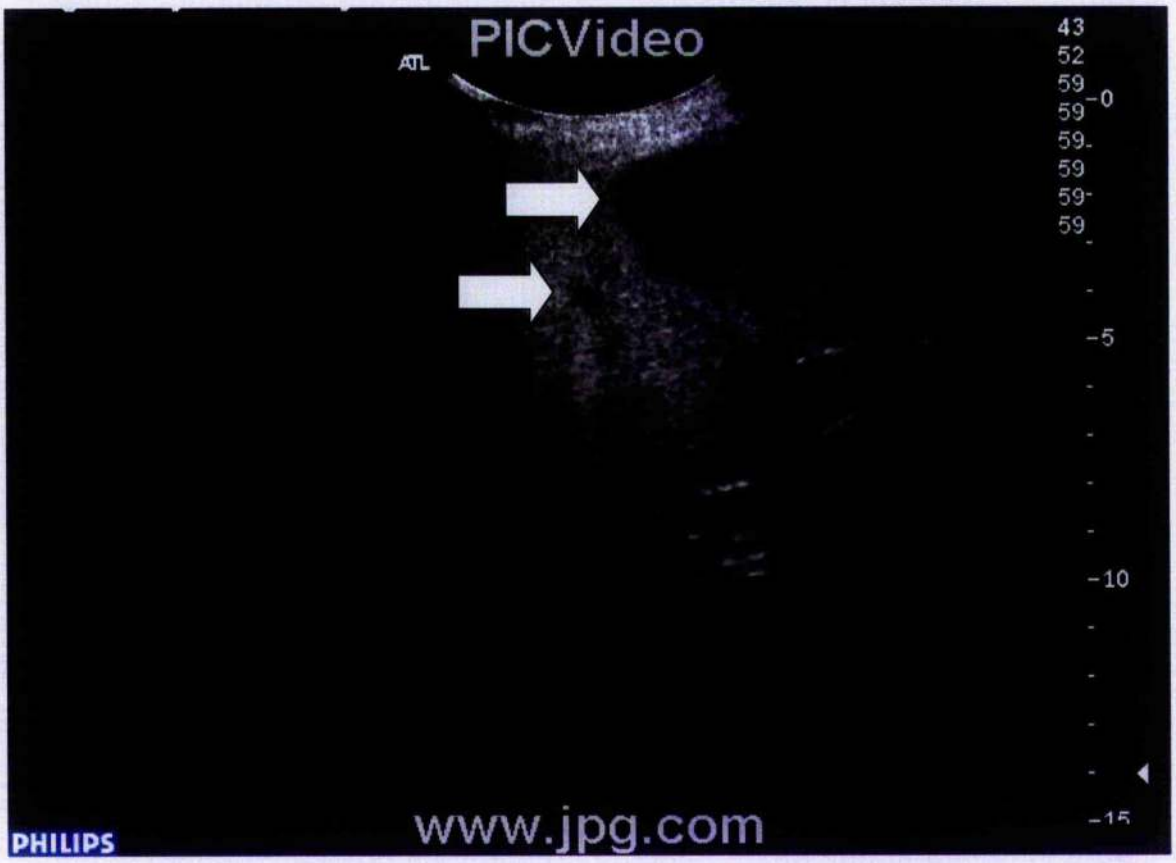
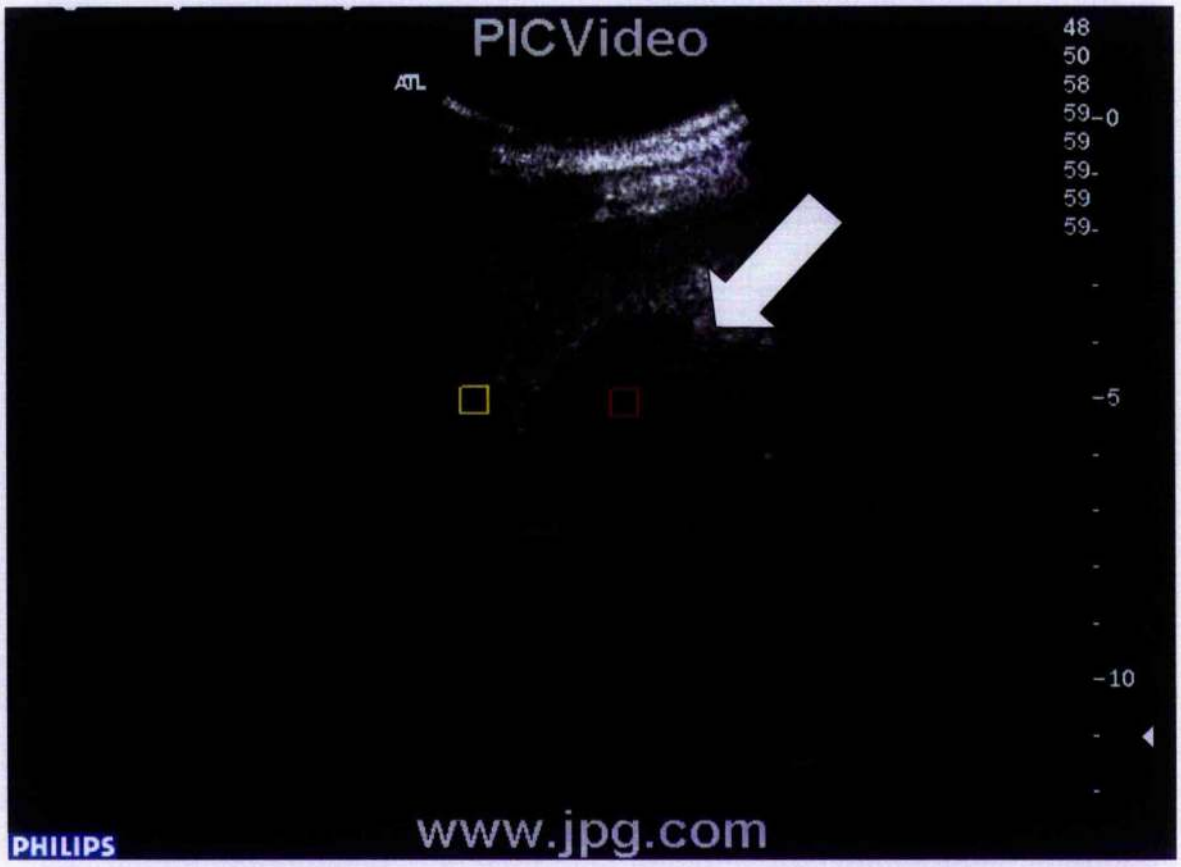


Figure 8: Demonstration of placement of Regions of Interest (ROI) over hepatic parenchyma (yellow) and colorectal liver metastasis (red).



White arrow displays the halo outlining the outer edge of the liver metastasis.

3.1.3 Results

Contrast uptake by colorectal liver metastasis

The uptake of contrast was significantly lower in the colorectal liver metastasis for each of the three doses of Definity® ultrasound contrast agent ($p<0.05$, Mann-Whitney). The disparity in contrast uptake began at sixty seconds after the administration of contrast, with the liver parenchyma having a significantly greater uptake of contrast that was maintained throughout the remainder of the four minute scans. Figures 9-11 graphically display the contrast uptake for each dose of Definity® at each measured time point.

Figure 12 displays the PMR for each doses of Definity® ultrasound contrast agent at each time point. There were no differences between the three doses up to 180s. At 210s and 240s, the PMR was found to be greater in the 0.4mL dose of contrast although this did not achieve statistical significance ($p>0.05$, Mann Whitney).

Detection of colorectal liver metastases using percutaneous contrast enhanced ultrasound

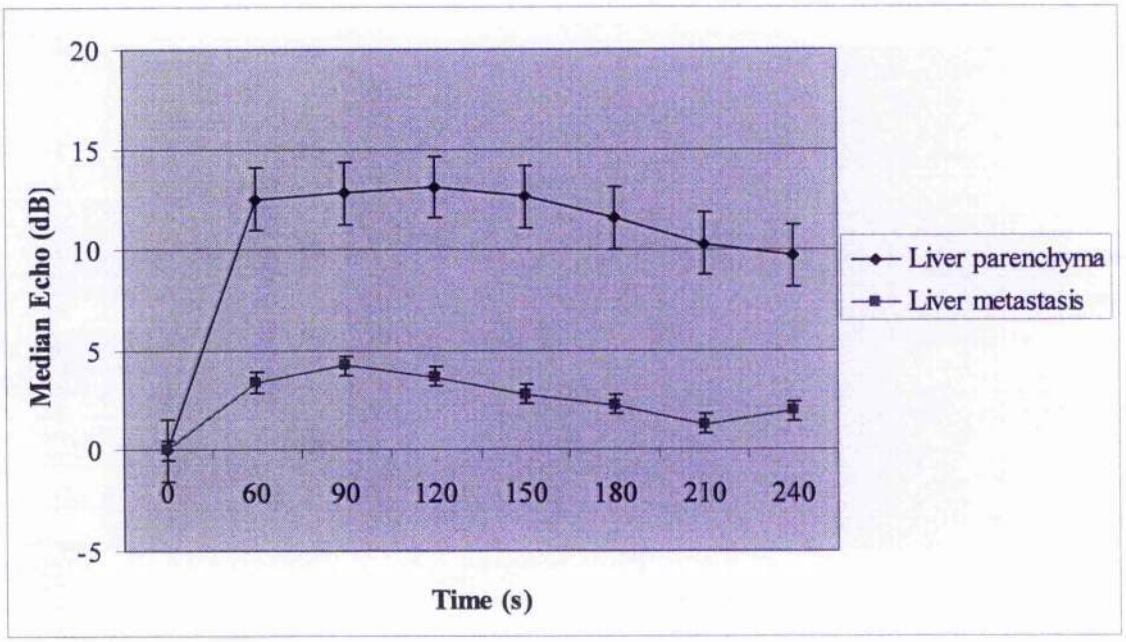
In the twenty three patients studied, a total of 59 colorectal liver metastases were identified on the standard of reference. The mean size of metastasis was 3.24cm (SD 1.84, range 0.5cm to 7cm). The mean number (± standard deviation; SD) of metastases detected per patient for each imaging modality was: CT/MRI 2.57 (± 1.68); US 1.65 (± 1.03) and CE-US 2.83 (± 2.10). CEUS detected significantly more metastases than US ($p<0.0001$) and CT/MRI ($p<0.0001$).

The sensitivity of US in the detection of colorectal liver metastases was 64.4% achieving an accuracy of 64.4% and a positive predictive value of 100%. The sensitivity of CE-US was 100% with an accuracy of 90.8% and a positive predictive value of 90.8%.

Change in surgical management as a result of CEUS

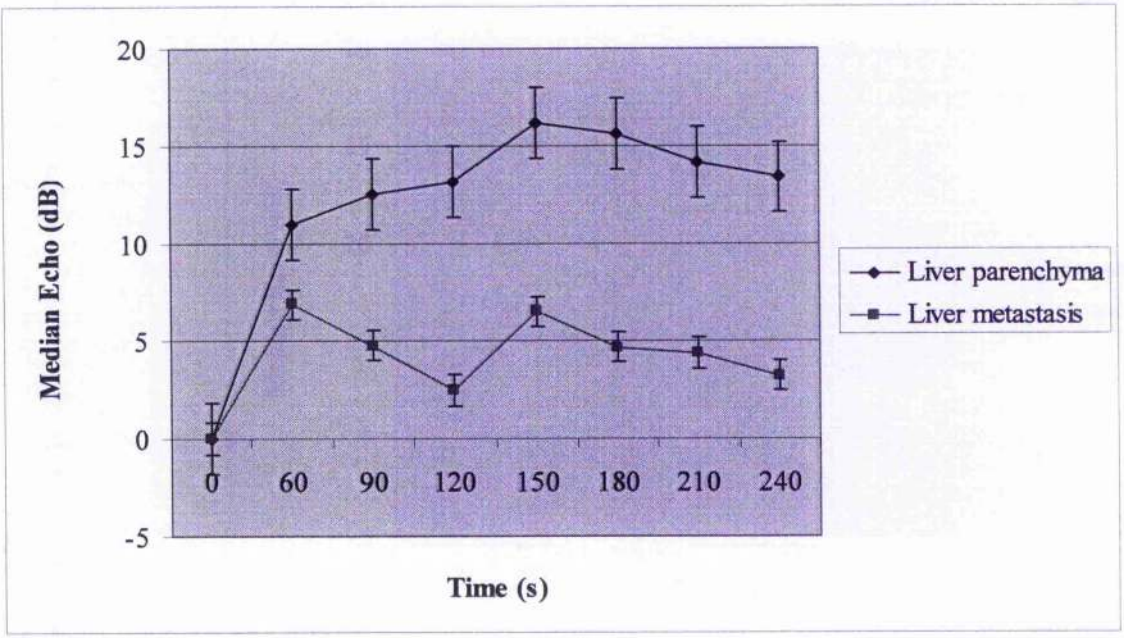
CE-US altered the management in 12 of the 23 patients (52.2%). This was due to: detection of extra metastases in 6 patients and close proximity to major vessels and structures in the remaining 6 patients. As a consequence, 10 patients underwent RF ablation in addition to their planned resection and/or radiofrequency ablation; 1 patient was referred for chemotherapy after undergoing radiofrequency ablation and 1 patient underwent extended hepatic resection.

Figure 9: Comparison of ultrasound contrast uptake by liver parenchyma and colorectal liver metastasis after administration of 0.4mL Definity® ultrasound contrast agent.



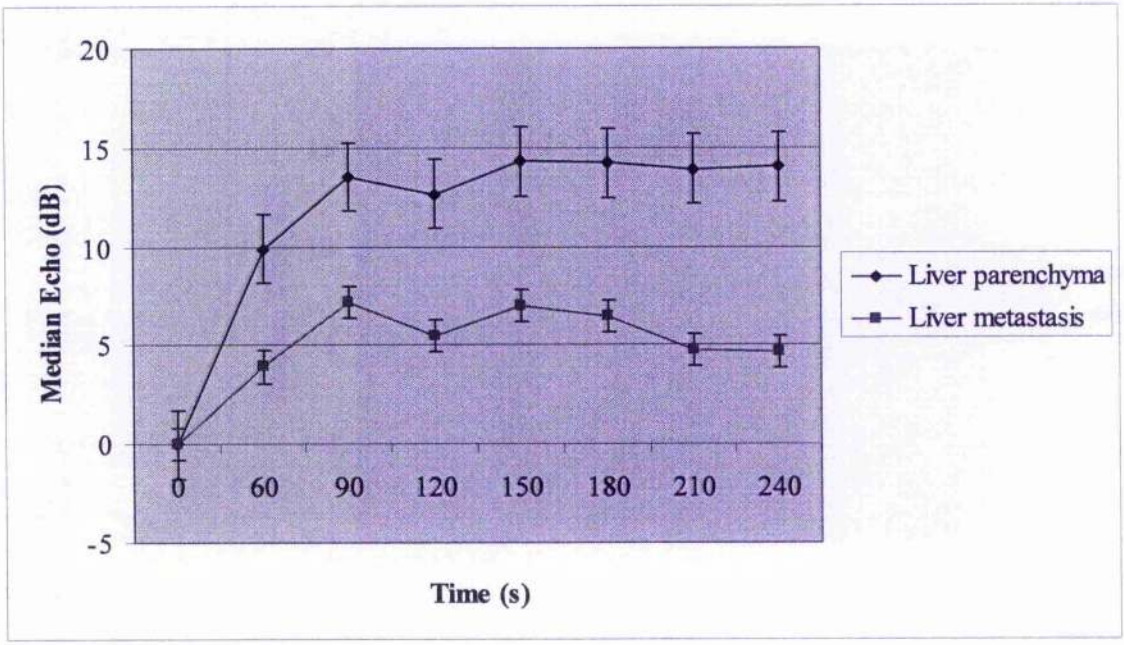
Median echo and standard error displayed

Figure 10: Comparison of ultrasound contrast uptake by liver parenchyma and colorectal liver metastasis after administration of 0.6mL Definity® ultrasound contrast agent.



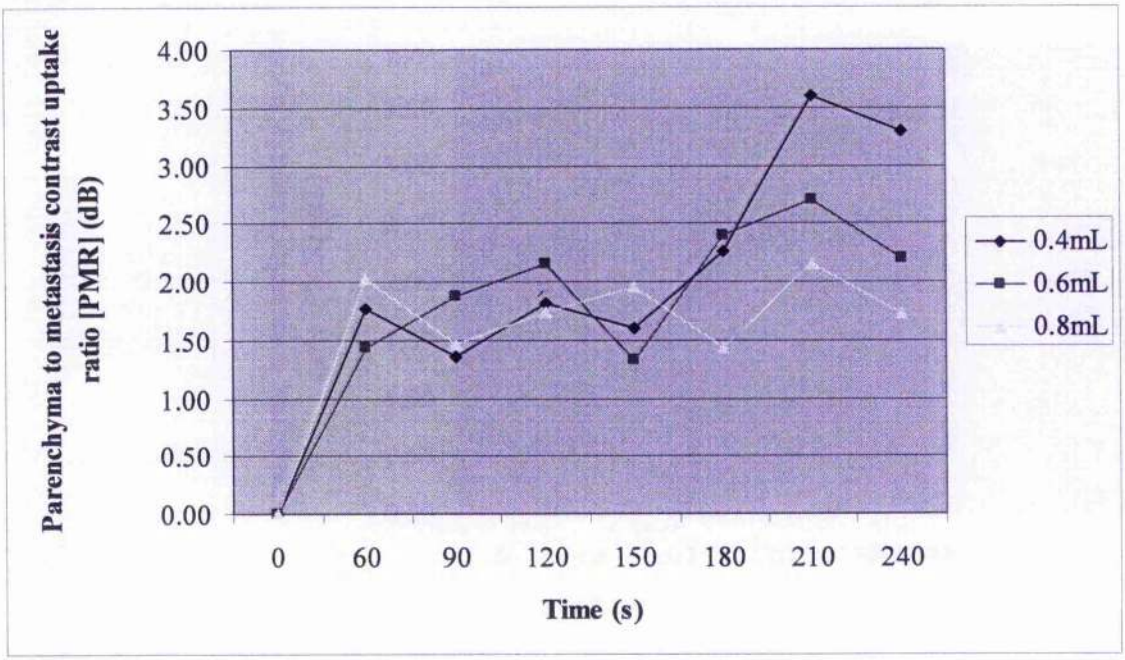
Median echo and standard error displayed

Figure 11: Comparison of ultrasound contrast uptake by liver parenchyma and colorectal liver metastasis after administration of 0.8mL Definity® ultrasound contrast agent.



Median echo and standard error displayed

Figure 12: Comparison of liver parenchyma to liver metastasis contrast uptake ratio (PMR) with three doses of Definity® ultrasound contrast agent.



3.1.4 Discussion

Unenhanced percutaneous ultrasound is accepted to have limitations in the detection of colorectal liver metastases. The introduction of ultrasound intravenous contrast agents coupled with pulse inversion harmonic imaging technology has overcome some of these limitations, resulting in a growing evidence base for the use of CE-US in routine clinical practice. Interest has turned to understanding the complex interaction between ultrasound contrast agents, the ultrasound beam and the hepatic parenchyma in order to optimise CE-US use in the detection of focal liver lesions.

In this study, all three doses of Definity® contrast agent documented early disparity in the uptake of contrast between the colorectal liver metastasis and adjacent parenchyma that was maintained in to the later phases. The CE-US detection of malignant liver lesions occurs in the arterial phase when the lesion appears hypoechoic. Characterisation of this malignant liver lesion as a colorectal liver metastasis relies on the lesion remaining hypoechoic into the late phase. If these criteria are applied to the results after administration of Definity® ultrasound contrast agent, then it would appear that all three doses are appropriate for use in the detection and characterisation of colorectal liver metastases.

To compare the three doses, the parenchyma to metastasis ratio was calculated and initially, there were no significant differences in the ratios of the three doses. However, at 180 seconds after administration of the contrast the two smallest doses recorded greater ratios than the highest dose of 0.8mL. 0.4mL then continued to increase recording the highest ratios at 210 and 240 seconds. The differences in PMR ratio are likely to be a reflection of the microbubbles' interaction with the liver. Not unexpectedly, the 0.4mL

dose of contrast recorded lower individual contrast uptakes for both metastasis and parenchyma when compared to the other higher doses. However, the uptake declines as time progresses, suggesting that the contrast is being washed out of the parenchyma and liver metastasis, accounting for the higher PMR in the late phase of CE-US. This phenomenon is seen partially with the 0.6mls dose with the metastasis and the parenchyma having a slow decline in uptake in the late phase, but is completely absent with the 0.8mls dose. These two higher doses may have saturated the liver, making a greater amount of contrast available to the circulation, indirectly increasing the metastasis contrast uptake and decreasing the PMR.

Much of the research on Definity® ultrasound contrast agent has focused on its effectiveness in cardiac imaging, a role for which is licensed for in the United States and has guidelines for its use in routine clinical practice. This novel study has found that Definity® also has a role in the detection of colorectal liver metastases. In comparison to unenhanced ultrasound, CE-US found significantly more metastases (65 vs 38) as well as having a higher sensitivity (100% vs 64.4%) and accuracy (90.8% vs 64.4%). Furthermore, the use of CE-US changed the management in 52.2% of patients due to not only the finding of additional metastases, but to improved localisation of the metastases defining their proximity to major vessels and structures.

These findings are consistent with other published studies using other ultrasound contrast agents, one of which reported an increased mean weighted sensitivity (using CT and MRI as the reference) from 70% to 91% after injection of ultrasound contrast [94;167-169;175;180;181;319-321;323]. These improvements have resulted in leading researchers in the field

of CE-US to predicted that CE-US will replace unenhanced ultrasound in routine clinical practice, working alongside contrast enhanced CT and MRI in lesion characterisation[166].

In addition to the other findings in this study, CE-US detected 6 additional suspected colorectal liver metastases than the standard of reference of combined CT/MRI. Interpretation of this finding must be tempered as no pathological analysis is currently available to determine the exact nature of these 6 lesions. Future studies should overcome this limitation by taking biopsies for pathological confirmation.

In conclusion, this novel study found that a low dose of Definity® ultrasound contrast agent enhanced the late phase vascular imaging of colorectal liver metastases. In addition, the administration of Definity® was found to result in improved detection of colorectal liver metastases when compared to unenhanced ultrasound. These findings support the potential clinical role of Definity® enhanced ultrasound in the detection and characterisation of colorectal liver metastases.

Section 3.

Chapter 2: A prospective trial determining a new clinical application of contrast enhanced ultrasound in the intraoperative detection of colorectal liver metastases.

3.2.1 Introduction and aims

Partial hepatectomy is the only potentially curative option for patients with colorectal liver metastases. Unfortunately, up to 50% of these patients develop hepatic recurrence with the majority occurring within the first two years after surgery[200;208]. Estimations of the growth rate of colorectal liver metastases have found them to be slow growing[77;211]. Therefore, it is likely that these early recurrences are not new, but are metastases that were occult at the time of initial radiological staging and operation.

Current preoperative radiological staging of colorectal liver metastases consists of contrast enhanced computerised tomography (CT), magnetic resonance imaging (MRI) and/or intraoperative ultrasound (IOUS), the later being the current optimal standard[144;324-326]. Contrast enhanced ultrasound is an evolving technology that is developing an extensive evidence base in the transcutaneous detection of colorectal liver metastases[166;169;180;320]. This chapter assessed a novel application of this developing technology; contrast enhanced intraoperative ultrasound (CE-IOUS).

3.2.2 Patients and methods

Sixty consecutive patients (mean age 66.7 years, range 40-82; 31 females and 29 males) scheduled to undergo partial hepatectomy for colorectal liver metastases were recruited from two centres. All patients had been deemed suitable for hepatic resection based upon: curative resection of the colorectal primary (or was achievable in those undergoing planned synchronous resections); fitness for anaesthesia; no extra-hepatic disease (on imaging of the chest, abdomen and pelvis by CT) and surgically resectable colorectal liver metastases. Ethical approval was achieved from the local research and ethics committees and all patients gave their informed consent. Fifty-one patients had previous resection of their colonic primary, whilst the remaining nine patients were to undergo synchronous colonic and liver resection.

CT and MRI scanning

In all patients, CT and/ or MRI were performed within 6 weeks of the operation (mean 3.5 weeks) according to a standardised protocol. Two phase CT examination was performed using two Multidetector CT scanners (Siemens, Sensation 4 and Sensation 16, Erlangen, Germany) with 2-4mm slice thickness/ 16 x 1.5mm collimation and enhanced with 150mL of Omniscan 300 (Amersham, UK) injected at 3-5mL/second. Scanning was performed at 25-30 seconds for the arterial phases and 55-60 seconds for the portal venous phase following the start of the bolus contrast injection in a peripheral vein.

MRI was performed using two 1.5T MRI scanners (Philips, Gyroscan, Eindoven, Netherlands; Siemens Vision, Erlangen, Germany) with body and phase coils. Two different liver specific MRI contrast agents, MultiHance (Bracco Spa, Milan, Italy; dose

1mL/kg body weight) and Resovist (Schering AG, Berlin, Germany; 8 micromols Fe/kg body weight) were used. Pre contrast axial T2 and T1 weighted 2D scans with and without fat saturation were first performed. MultiHance enhanced axial 3D scans were carried out at 17 seconds, 45 seconds, 120 seconds and 60 minutes following a bolus peripheral venous injection (at 2mL/second followed by 20mL flush at 2mL/second), whilst Resovist enhanced axial T2 weighted scan was performed 10 minutes after a bolus injection.

Preoperative CT scans alone, combined CT and MRI and MRI scans alone were performed in 40, 4 and 16 patients respectively.

Intraoperative Ultrasound and Contrast Enhanced Intraoperative Ultrasound

At laparotomy, all patients underwent thorough abdominal and pelvic exploration for extrahepatic disease. The liver was mobilised from the diaphragm and bimanually palpated to detect the presence of metastases. Unenhanced ultrasound (intraoperative ultrasound; IOUS) was then performed using a HDI 5000 scanner (Philips, Bothell, Washington, USA) with a high frequency finger probe (CT8-4, Figure 12). IOUS was done systematically in axial, sagittal and oblique sweeps to ascertain complete liver coverage. Variables recorded were: number of new metastases detected; number of previously diagnosed metastases detected; overall number of metastases detected and proximity to major vascular and biliary structures.

Following the IOUS scan, contrast enhanced intraoperative ultrasound was performed (CE-IOUS) using the same scanner and finger probe. After changing the scanner setting to pulse inversion harmonic imaging (PIH), a bolus injection of 2.4mL of contrast agent (SonoVue®, Bracco Spa, Milan, Italy) was administered via the central line

followed by a 10mL saline flush (Figure 13). The ultrasound gain, focal zone and output power (Mechanical Index: 0.02-0.04) settings were all standardised during PIH imaging. Again, scanning was performed systematically with recording of the same variables as IOUS.

The number of metastases identified on CT and/or MRI, IOUS and CE-IOUS were counted and mapped according to Couinaud's classification [258]. Benign hepatic cysts were excluded and identified lesions were correlated with the findings from histopathology.

SonoVue® is a second generation microbubble contrast agent containing sulphur-hexafluoride gas stabilised by a phospholipid shell (mean size 2.5 micron in diameter; 90% measuring less than 8 micron) [Figure 13]. It has been extensively researched in transabdominal hepatic imaging and is licensed in the United Kingdom for this purpose[166;320;323;327-330]. SonoVue® was selected as the characteristics, the optimal dose for injection, as well as the optimal pulse inversion harmonics settings for SonoVue® have all been previously assessed.

Data analysis

Non-parametric statistical testing was performed (Mann Whitney and Wilcoxon Sign Rank Tests) with significance taken at the 5% level (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA). Changes in surgical management following IOUS and CE-IOUS were compared (for example: abandoned resection; more extensive resection; limited resection or combined resection with radiofrequency ablation).

Figure 13: Schematic representation of SonoVue® microbubbles structure.[331]

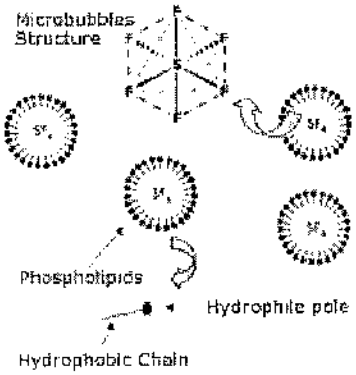


Figure 14: The CT8-4Hz finger probe for intraoperative contrast enhanced ultrasound.

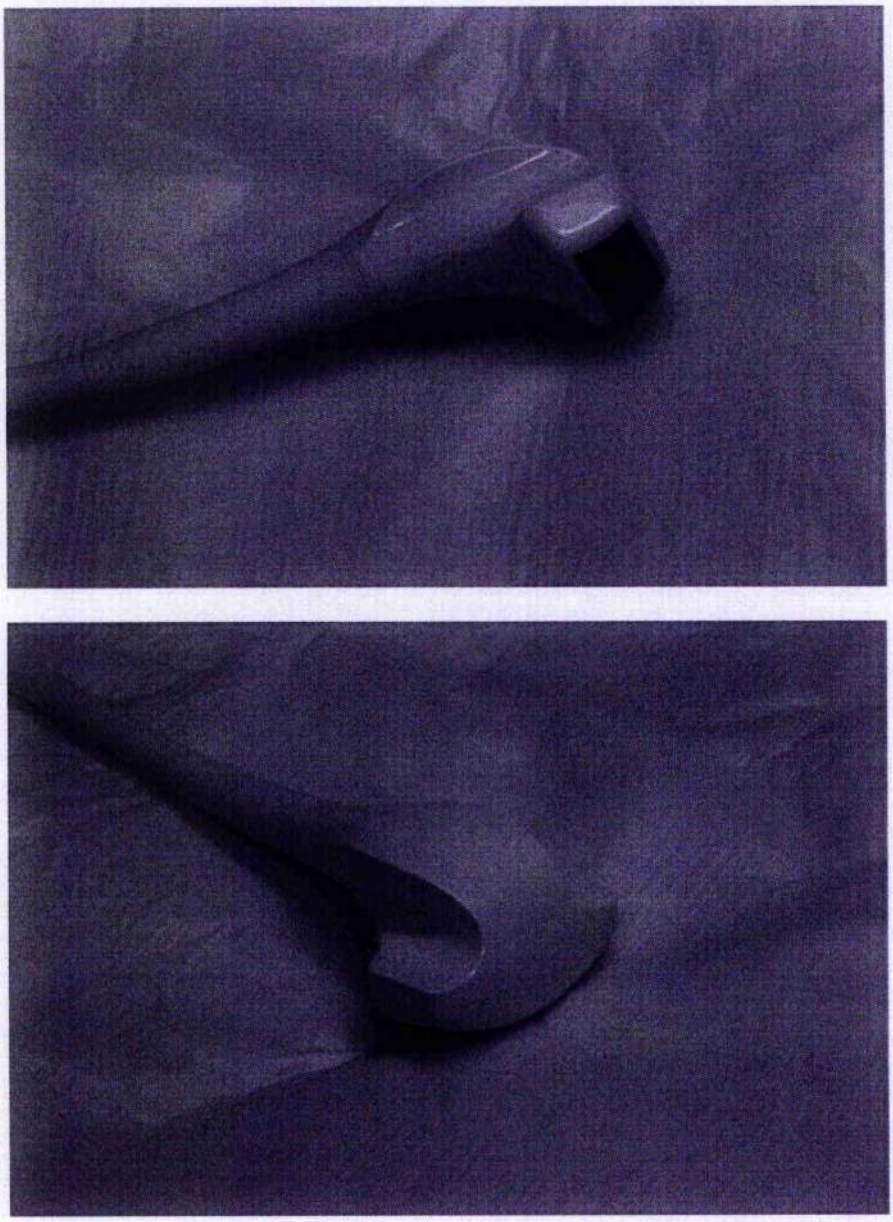
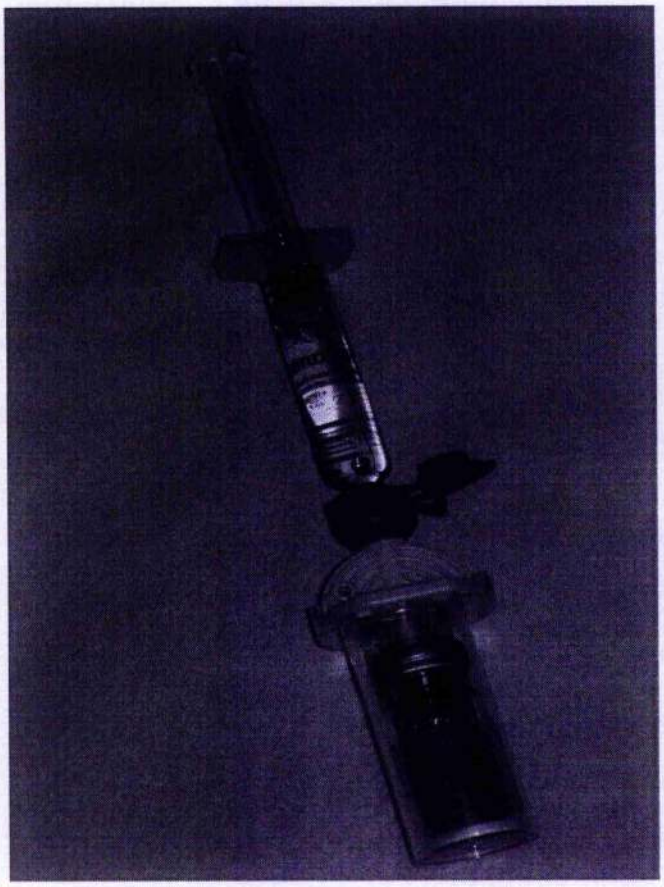


Figure 15: Ultrasound contrast agent SonoVue® being reconstituted with saline prior to intravenous injection.



3.3.3 Results

CE-IOUS was not performed in three patients due to more extensive disease being diagnosed: one patient was found to have more than 10 liver metastases in a background of extensive fatty liver on IOUS and the remaining two patients had peritoneal metastases. All three were excluded from further analysis

Number of metastases detected

A total of 107 lesions were identified on histopathological assessment of biopsies and resected specimens, which was taken as the standard of reference. Of note, biopsies were taken prior to performing radiofrequency ablation on imaged metastases. Of these 107 lesions, 103 were colorectal liver metastases and 4 were haemangiomas. The mean number (± standard deviation; SD) of correctly identified colorectal liver metastases per patient on combined CT /MRI, IOUS and CE-IOUS was 1.54 (± 1.06), 1.65 (±1.19) and 1.95 (±1.79) respectively with CE-IOUS detecting significantly more liver metastases than IOUS (p=0.029) and combined CT/MRI (p=0.047). There were no statistical differences observed in the number of metastases detected between: IOUS and combined CT/MRI (p=0.53) and CE-IOUS and histopathology findings (p=1.00). For combined CT/MRI, IOUS and CE-IOUS: the sensitivity was 76.7%, 81.5% and 96.3%; the accuracy 73.8%, 78.5% and 96.3% and positive predictive values were 95.2%, 95.5% and 98.0% respectively.

Size of lesions

The mean size (± standard deviation; SD) of lesions identified on CT/MRI/IOUS combined compared to CE-IOUS was 2.73cm (±1.46) and 1.71cm (±1.57) respectively. The mean size of the additional lesions identified on CE-IOUS was 0.84cm (±0.27), with the smallest lesion identified being 0.4cm.

Change in surgical management following CE-IOUS

CE-IOUS altered surgical management in 17 of the 57 patients (29.8%). This was due to: additional metastases detected in 11 cases (19.3%); fewer metastases detected in 2 patients (3.5%); benign haemangioma diagnosed in 2 patients that were thought to have metastases on CT or MRI or IOUS (3.5%); additional arteriovenous malformation detected in 1 patient (1.8%); and tumour margin too close in proximity to inferior vena cava in 1 patient (1.8%). Figures 16 and 17 display the ultrasound images obtained in one of the patients that had an additional lesion found on CE-IOUS.

The finding of 11 additional metastases with CE-IOUS changed the surgical plan to: extension to trisegmentectomy in 3 cases; additional non-segmental wedge resection in 2 cases and radiofrequency ablation of the additional lesions as an adjunct to partial hepatectomy, in 6 patients. In the 2 patients that had fewer lesions detected with CE-IOUS, both were scheduled to undergo right hepatectomy. These were changed to trisegmentectomy in one patient and bisegmentectomy (VII, VIII) and subsegmentectomy in the other. In the 3 patients with benign lesions diagnosed on CE-IOUS, all had their planned resections cancelled. The remaining patient with the unresectable metastasis due to vessel proximity underwent RF ablation instead.

Forty of the 57 patients had no alteration in their planned surgical management after CE-IOUS was performed. Within this group, CE-IOUS did not detect any additional lesions in 37 patients. However, in the remaining 3 cases there were changes in staging that did not result in a change in management: 2 patients had additional lesions detected, but they did not entail any extended resection or adjunctive surgical procedures and in 1 patient a lesion was wrongly diagnosed as metastasis on IOUS and CT, that was accurately identified on CE-IOUS as a benign haemangioma. Overall, CE-IOUS altered CT/MRI/IOUS hepatic staging in 20 out of 57 patients (35.1%).

Figure 16: Unenhanced intraoperative ultrasound demonstrating no evidence of colorectal liver metastases in one patient.

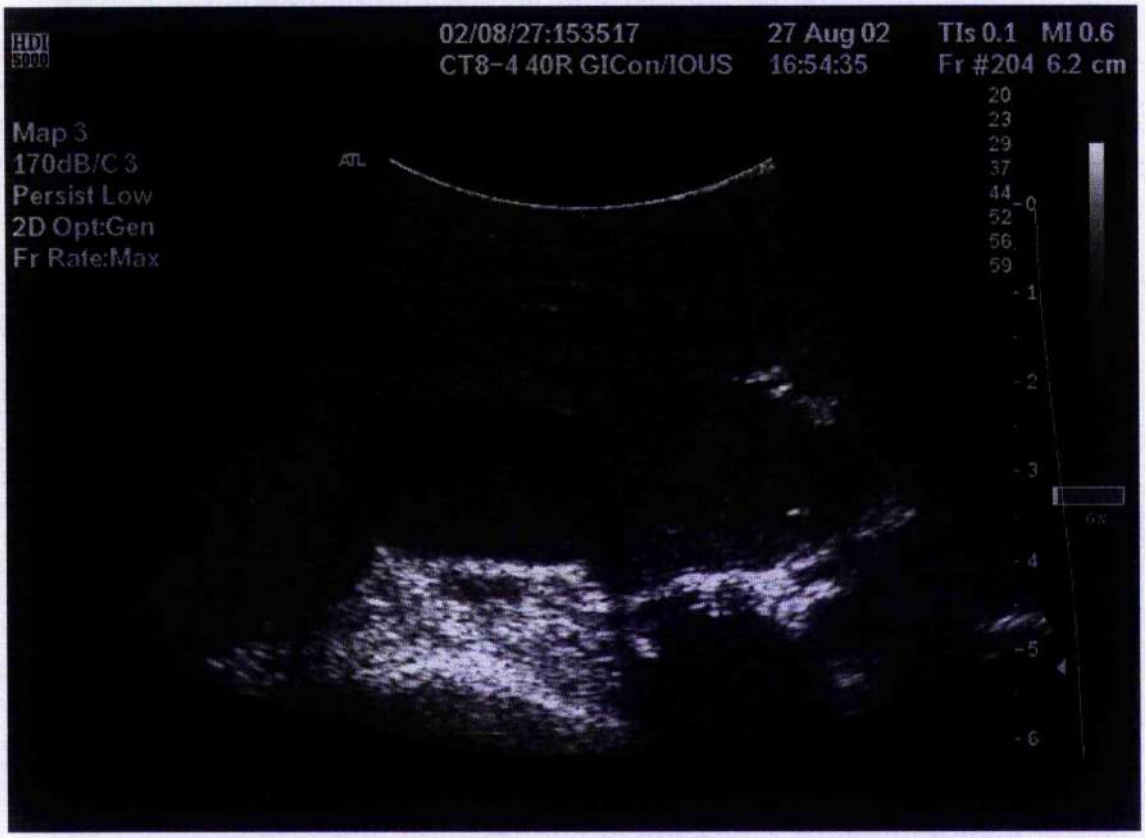
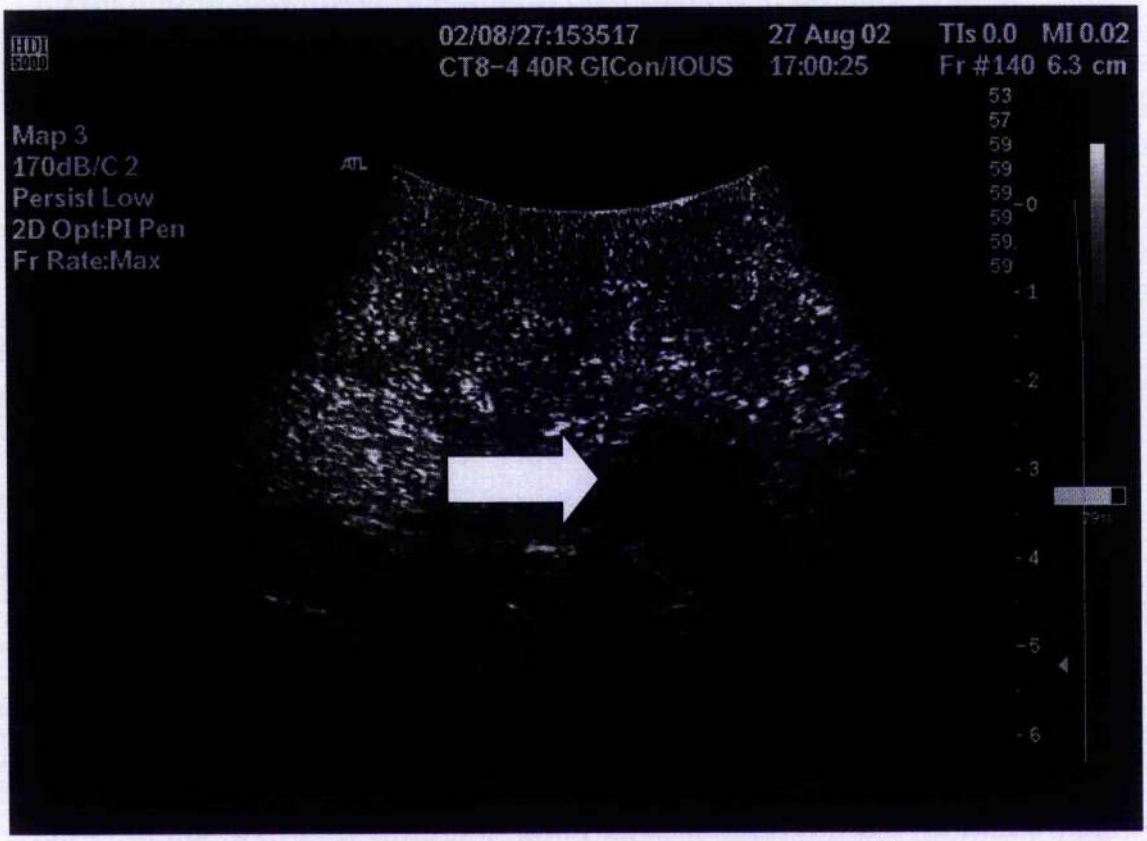


Figure 17: Contrast enhanced intraoperative ultrasound (CE-IOUS) showing colorectal liver metastasis in same patient as figure 16.



3.2.4 Discussion

This paper documents the first description of the use of contrast enhanced intraoperative ultrasound (CE-IOUS) with significant improvements described in the detection of colorectal liver metastases. The addition of contrast agents improves every imaging modality and the value of contrast agents in CT and MRI in the detection of colorectal liver metastases is established in current clinical practice. The application of contrast agents to ultrasound is relatively new, but is gaining increasing support in clinical practice. Therefore, the extension of contrast enhanced ultrasound to the intraoperative setting is a natural progression.

This trial found CE-IOUS changed the staging in 35.1% of patients when compared to preoperative imaging (CT/MRI) and intraoperative imaging (IOUS) combined. The detection of additional lesions, fewer lesions and benign lesions in addition to improved lesion characterisation, altered surgical management in 29.8% of patients. With a positive predictive value of 98%, CE-IOUS is promising and appears capable of detecting metastases that were previously considered occult. This is supported by the smaller size of metastasis that CE-IOUS detected in comparison to other imaging modalities.

The use of microbubbles has several advantages over other imaging modalities. First, it is not radioactive, allowing several doses to be given at one examination if required. Second, it has low protein content with a good allergy and anaphylaxis profile and can be used in renal failure. Third, it produces real-time imaging allowing improved lesion characterisation. As for transabdominal contrast enhanced ultrasound, the typical appearance of a metastasis is of a hypoechoic lesion in the arterial phase that remains devoid of microbubbles in the portal and late phases.

There are some limitations in the CE-IOUS technique. PIH is a prerequisite requirement and although widely available, it is not yet available for many of the intraoperative finger-probes. The finger probe used in this study is ideally shaped for imaging the liver compared to the hand held probes used for percutaneous ultrasound. Another consideration is to the level of mechanical index during scanning. 0.02-0.04 is an extremely low ultrasound output power that renders the ultrasound screen display dark until the arrival of contrast. Although this can be compensated to some extent by increasing the ultrasound gain, there is a degree of observer adaptation with a learning curve. Lastly, the duration of contrast enhancement is only 2-3 minutes which may be related to the use of a high frequency finger probe as well as interaction with general anaesthetics delivered via positive pressure ventilation thereby disrupting the microbubbles. Repeat injections of SonoVue® are feasible to complete the examination and future software developments may be targeted at improving sensitivity to ultrasound contrast agents optimising the interaction.

In conclusion, these preliminary results suggest that CE-IOUS is superior to other imaging modalities in the detection of colorectal liver metastases. Long term outcome studies will determine its true value in clinical practice.

Section 3

Chapter 3: The development of a novel technique for the functional imaging of hepatic perfusion in colorectal liver metastases using contrast enhanced ultrasound.

3.3.1 Introduction and aims

Since the 1950s, the development of altered liver perfusion as a result of the presence of colorectal liver metastases has been widely known. The initial works of Breedis and Young (1954) [121] followed by Ackermann (1969-1974)[123-126] found the hepatic blood supply changed from the predominant portal vein supply seen in healthy liver parenchyma to hepatic arterial supply in the presence of colorectal liver metastases. Although the exact mechanism driving angiogenesis is unknown, the alteration in blood supply could provide a means by which occult colorectal liver metastases could be detected.

The measurement of hepatic blood flow is difficult and, despite there being many techniques available, there are few that accurately quantify blood flow. Non-invasive techniques have the greatest potential to enter routine clinical practice and have provided a source of research interest. Leveson and co-workers developed the Hepatic Perfusion Index (HPI) in 1983 using dynamic flow scintigraphy and static isotope scans and in 1991, Leen et al assessed the use of Doppler ultrasound (DPI) in the measurement of hepatic perfusion [132;133;137-139]. Despite promising results, both the HPI and the DPI suffered from reproducibility problems, with other researchers finding poor predictive values in the detection of occult metastases when the two techniques were replicated in different centres[134-136].

Contrast enhanced ultrasound (CE-US) is an evolving non-invasive technology in the measurement of cardiac perfusion, combining pulse inversion harmonic imaging with ultrasound contrast agents (microbubbles). These gas filled microbubbles are intravascular tracers that possess similar rheology to red blood cells. As a result, measurement of the

transit of these microbubbles through selected areas of myocardium has been shown to allow assessment of regional myocardial perfusion[332;333]. With growing evidence in the diagnosis of both acute and chronic coronary stenosis, (termed myocardial contrast echocardiography, MCE) CE-US is a valuable tool in the assessment of ischaemic heart disease that has become incorporated in to routine practice in the United States[334-340].

To date, there has been little work attempting to quantify liver perfusion using transabdominal CE-US. The aim of this chapter was to develop a novel and reproducible technique using CE-US to measure altered hepatic perfusion in patients with colorectal liver metastases.

3.4.2 Patients and methods

Fifty two consecutive patients (23 female, 29 male) with pathologically confirmed colorectal liver metastases attending one specialist colorectal centre underwent one preoperative percutaneous CE-US. A healthy aged-matched control group (n=26) was recruited for comparison who met none of the following exclusion criteria: evidence of a systemic inflammatory response (e.g. operation in last 3 months; arthritis; long term anti-inflammatory medication); parenchymal liver disease (e.g. cirrhosis; hepatitis; alcohol excess) or suspected/ confirmed malignancy. Ethical approval was obtained from the local research and ethics committee and all subjects gave their written informed consent.

Measurement of liver perfusion: data acquisition

Each subject was placed supine with right upper quadrant, epigastrium and inferior right chest exposed (Figure 18). A cannula with a three way tap was inserted into a peripheral vein in the subject's arm. Fundamental unenhanced ultrasound (US) with a 5-2Hz probe on a HDI 5000 scanner (Philips, Bothell, USA) was performed to target a large area of hepatic parenchyma. This area excluded the portal vein, hepatic artery or visible metastases. Once the area was targeted, the scanner was switched to pulse inversion harmonic imaging (PIH; mechanical index 0.06-0.08) and the subject requested to remain still and keep his/her breathing quiet and shallow, allowing the target area to be maintained as a static image.

A bolus injection of 2.4mL of contrast (SonoVue®, Bracco Spa, Milan, Italy) followed by 2mL bolus saline flush were given and a 45 second video loop was recorded. A second scan was performed if the target area was not maintained as a static image due to

patient movement. The video loop was then saved to CD to allow data analysis.

SonoVue® is a second generation contrast agent consisting of sulphur hexafluoride gas (SF6) stabilised by a phospholipid shell[341]. The mean bubble diameter is 2.5μm and the concentration is between 100 and 500 million bubbles per mL. Due to the high molecular weight of the SF6 and its low solubility in water, SonoVue has good resistance to the pressure changes experienced in the pulmonary and coronary circulations. More importantly it has an outstanding safety profile. Following intravenous administration it is estimated that the elimination half life is 6 minutes with more than 80% of the gas expired via the lungs after 11 minutes.

Measurement of liver perfusion: data analysis

Video loops were analysed by computer quantification software (Q-Lab®, Quantification software, Philips, Bothell, USA). Each loop was replayed and a region of interest (ROI) selected that represented a large area of liver parenchyma (1000-2000mm^2) [Figure 19]. Q-Lab then analysed the ROI and drew a hepatic perfusion curve (signal intensity versus time). The perfusion curve was then cropped to time zero which was defined as the first arrival of contrast i.e. all time points after time zero displayed a sustained increase in signal intensity that was at least 0.2 dB greater than time zero (Figure 20). The perfusion curve was exported to Microsoft Excel (Microsoft, Seattle, WA, USA) in the form of a series of signal intensity measurements at 0.5 second time intervals. In line with previous research, the signal intensity at time zero that corresponded to the background signal from the hepatic parenchyma and other tissues, was subtracted from all subsequent signal intensities on the perfusion curve (Figures 21 and 22)[334;342]. Each

perfusion curve was analysed for four parameters: signal intensity at each 0.5s time point; areas under the curve; gradients of the curve and hepatic perfusion index.

Areas under the curve were calculated by linear interpolation between data points and gradients of the curves were calculated by linear regression analysis. These operations were performed over various time intervals as defined in the results section. The Hepatic perfusion index (HPI) was calculated: HPI = H1 / H1 + H2; where H1 equals the slope of the arterial portion of the time intensity curve and H2 equals the portal venous portion of the time intensity curve[343]. The arterial and portal venous portions of the curve were defined according to the individual subject's perfusion curve as described in the results section.

Statistical analysis of liver perfusion

Signal intensities, peak intensities, areas under the curve, gradients of the curve and hepatic perfusion indices were compared between the patients and controls using the Mann-Whitney test. A p-value of less than 5% was considered to be significant with SPSS used for statistical analysis (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA). To display the distribution of data between the two groups, scatter plots were drawn for each significant parameter. In addition, Receiver Operator Characteristic (ROC) curves were drawn to describe the power of each significant parameter between groups to determine its sensitivity and specificity.

Measurement of liver perfusion: development of CE-US technique

The development of a new technique evolves as each of its components is individually assessed, optimised and validated. The following paragraphs explain the theory and initial work around the development of liver perfusion measured by CE-US.

The majority of work in myocardial perfusion has focused on administering intravenous contrast by continuous infusion[334]. The microbubbles are then destroyed by a single high power ultrasound pulse that is triggered at a specific time point in the cardiac cycle (usually end-systole) with the rate of reappearance of the microbubbles recorded (replenishment kinetics) until attainment of a steady state. Using computer quantification packages, the reappearance rate was calculated to be equivalent to the mean microbubble velocity with the myocardial concentration at steady state indicating microvascular cross sectional area[338]. Initial work was carried out using animal coronary arteries that were incorporated in to a complex tube system that was connected to flow meters, pump infusers and pressure monitors. The results from these studies allowed definition and quantification of myocardial perfusion that went on to be confirmed with by live animal and finally, human studies[332;335-339].

There has also been work done using a bolus administration of contrast. Jayaweera et al (1994) used bolus administration of a contrast agent in to the left anterior descending coronary artery in eighteen mongrel dogs[333]. Using MCE they drew signal intensity versus time curves. For comparison, the transit rates of red blood cells labelled with technetium 99m were recorded. The authors found that the perfusion curves were similar and concluded that using a bolus administration of contrast acted as an excellent intravascular tracer for measuring myocardial blood flow. Wei et al in 1998 directly compared bolus

administration to continuous contrast infusion in the measurement of myocardial perfusion[338]. From this animal study, the authors derived signal intensity versus time curves for both types of contrast administration and found that both approaches correlated well with the severity of the coronary stenosis. However, the authors concluded by favouring continuous contrast infusion as it minimised any influence from contrast recirculation, could be customised to the individual and allowed quantification of myocardial blood flow without any posterior wall attenuation.

For this chapter, it was hypothesised that if microbubbles have been shown to be reliable intravascular tracers, then a bolus administration of the contrast would allow quantification of real time functional imaging of liver blood flow (i.e. first pass analysis). In addition to the known differences between the cardiac and hepatic circulations (the presence of a hepatic artery and portal vein; different autoregulation and the timing of the blood supply is not as complex as the cardiac cycle), some contrast agents (including SonoVue® used in this study) are pooled in the liver parenchyma during the later phases of CE-US which would be likely to bias interpretation of parameters from a steady state. It was also thought that bolus administration had practical advantages: infusion machinery is not required and most contrast enhanced trained sonographers will already be used to administering boluses of contrast for focal liver lesion detection.

To measure the liver blood flow, it was decided to perform CE-US with the static image containing the hepatic artery and portal vein, an approach similar to the Doppler Perfusion Index. The ROI was then placed over each of these two vessels and the signal intensity-time curves drawn. However, after performing this approach in 10 patients, it became apparent that maintaining a static image that contained both vessels for 45 seconds

was significantly affected by respiration. Despite educating the patient to take shallow and regular breaths, it was noted that even the smallest movement of a vessel moved it away from the ROI. Few patients could hold their breath for the full 45 seconds. Subsequently, it was decided to measure the parenchymal microcirculation in an approach similar to MCE, by placing a ROI over a large area of hepatic parenchyma that excluded the portal vein, the hepatic artery and any visible hypoechoic colorectal liver metastases. Exclusion of visible metastases meant that if the developed technique was successful, then the same area could be targeted in a study designed to detect occult liver metastases.

Four parameters were selected to quantify hepatic perfusion: signal intensity at each 0.5s time points (including peak intensity); areas under the curve; gradients of the curve and hepatic perfusion index. To date, there are few publications that have tried to define and quantify hepatic perfusion using CE-US.

In this chapter, signal intensity was selected as it provided an absolute value of contrast uptake at selected time points, including the peak contrast uptake. Peak contrast uptake has previously been shown to be significantly higher in patients with cirrhosis when compared to healthy controls, using contrast enhanced Doppler[344]. The gradients of the curves in continuous infusion techniques have been shown to quantify mean microbubble velocity in myocardium and it is likely that the gradients from a bolus technique reflect the velocity of the microbubbles during the time period analysed[334]. Lastly, area under the curve could reflect blood volume. However, this, like interpretation of curve gradients, is an assumption that would need to be clarified by in-vitro studies using labelled red blood cells and flow meters as described previously[333].

The Hepatic perfusion index (HPI) is ideally suited to a bolus contrast injection technique as it is based on first pass of the contrast agent prior to any influence from contrast recirculation[345]. Hepatic arterial flow is determined by analysis of the integrated liver time intensity curve and the integrated left ventricle time intensity curve as described by Peters that results in the following: HPI = H1 / H1 + H2.

Measurement of liver perfusion: intra- and interobserver variability

Intra- and interobserver reproducibility studies were performed on data acquisition and data interpretation. The first observer (SJ Moug) was aware of the allocation of each subject (patient or control) however, the second observer was blinded to each subject's class. Each observer performed and interpreted their CE-US independently of the other.

Interobserver reproducibility of data acquisition was performed using eight subjects (n=6 patients, n=2 controls). Each observer administered one bolus of contrast and acquired data according to the method described already. On another group of 10 patients, the first observer performed two successive CE-US on each patient.

In relation to time zero, an external blinded investigator selected and coded forty-four scanning loops. Time zero was then calculated independently on 24 scanning loops (n=12 patients, n=12 controls) by the first and second observer. The remaining twenty blinded scanning loops (n=12 patients, n=8 controls) underwent intraobserver reproducibility assessment. For each of these twenty loops, the first observer calculated time zero. Each scan was then re-coded by the external investigator and the first observer repeated the calculation of time zero.

To determine any potential influence the area of ROI may have on data selection, three different regions of interest (ROIs) were drawn on 10 patients: 1000mm^2; 1500mm^2 and 2000mm^2. Hepatic perfusion curves were drawn for each ROI with signal intensities at each 0.5second time interval compared.

Statistical analysis of intra- and interobserver variability and ROI comparison

The intraobserver and interobserver bias for data acquisition and interpretation of time zero were assessed by using the Student paired t-test. Significance was established at a p value less than 5% and SPSS was used for statistical analysis (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA).

Intraobserver and interobserver reproducibility were quantified using the intraclass correlation coefficient (ICCC). ICCC overcomes a major drawback of the conventional Pearson product-moment correlation coefficient which is a measure of linear association rather than of agreement. A high product-moment correlation coefficient does not necessarily imply close agreement. For example, there may be a consistent additive bias between the observers. This contrasts to the ICCC which is a true index of agreement between observers' measurements. The following criteria for agreement were used: poor, ICCC less than 0.40; fair, ICCC from 0.40 to 0.59; good, from 0.60 to 0.74; excellent, greater than or equal to 0.75. The ICCC is derived from a two-way analysis of variance with observers and subjects as factors. The formula used to calculate the ICCC was (MSS – MSE)/ MSS + (k – 1) MSE + k/n (MSO – MSE) where MSS is the mean squared variation between subjects; MSO is the mean squared variation between observers (or

repeated measurements); MSE, the residual mean squared; k, the number of observations and n, the number of subjects.

To quantify the intraobserver and interobserver variability the coefficient of variation (CV) was calculated: CV = 100% x standard deviation (SD)/mean.

In relation to the comparison of ROIs, both the ICCC and CV were calculated for the following: $1000mm^2$ vs $1500mm^2$; $1000mm^2$ vs $2000mm^2$ and $1500mm^2$ vs $2000mm^2$.

Figure 18: The positioning of subject, scan probe and ultrasound machine during contrast enhanced ultrasound measurement of hepatic perfusion.

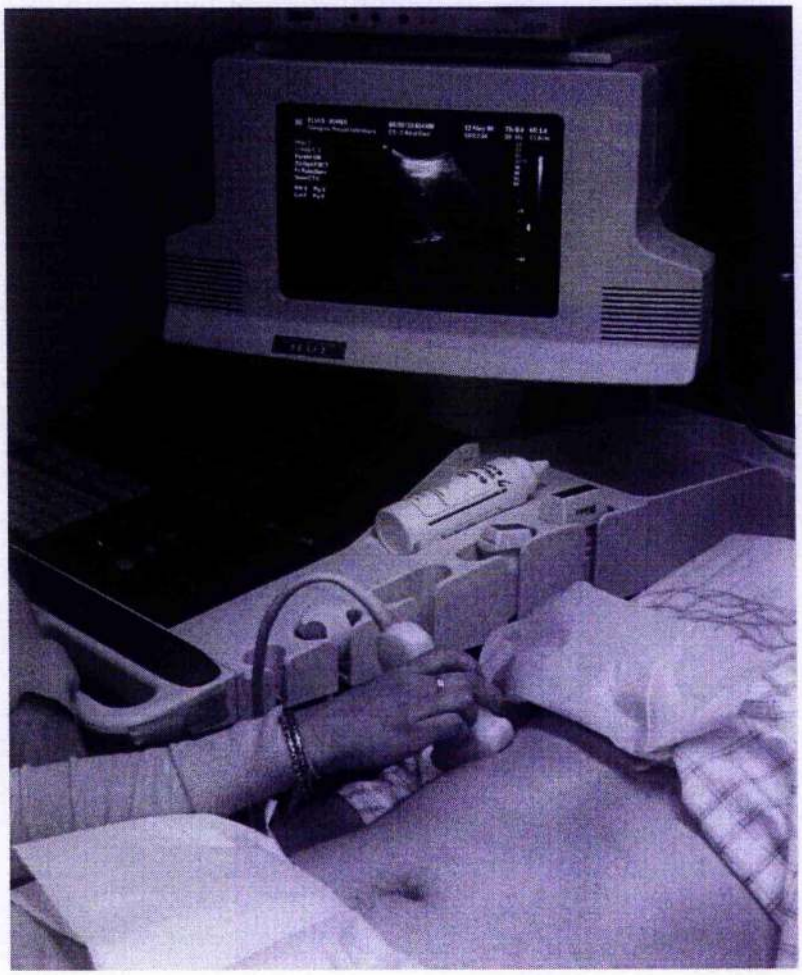


Figure 19: Frame from Q-Lab quantification software showing loop of contrast filled hepatic parenchyma with selected region of interest in red.

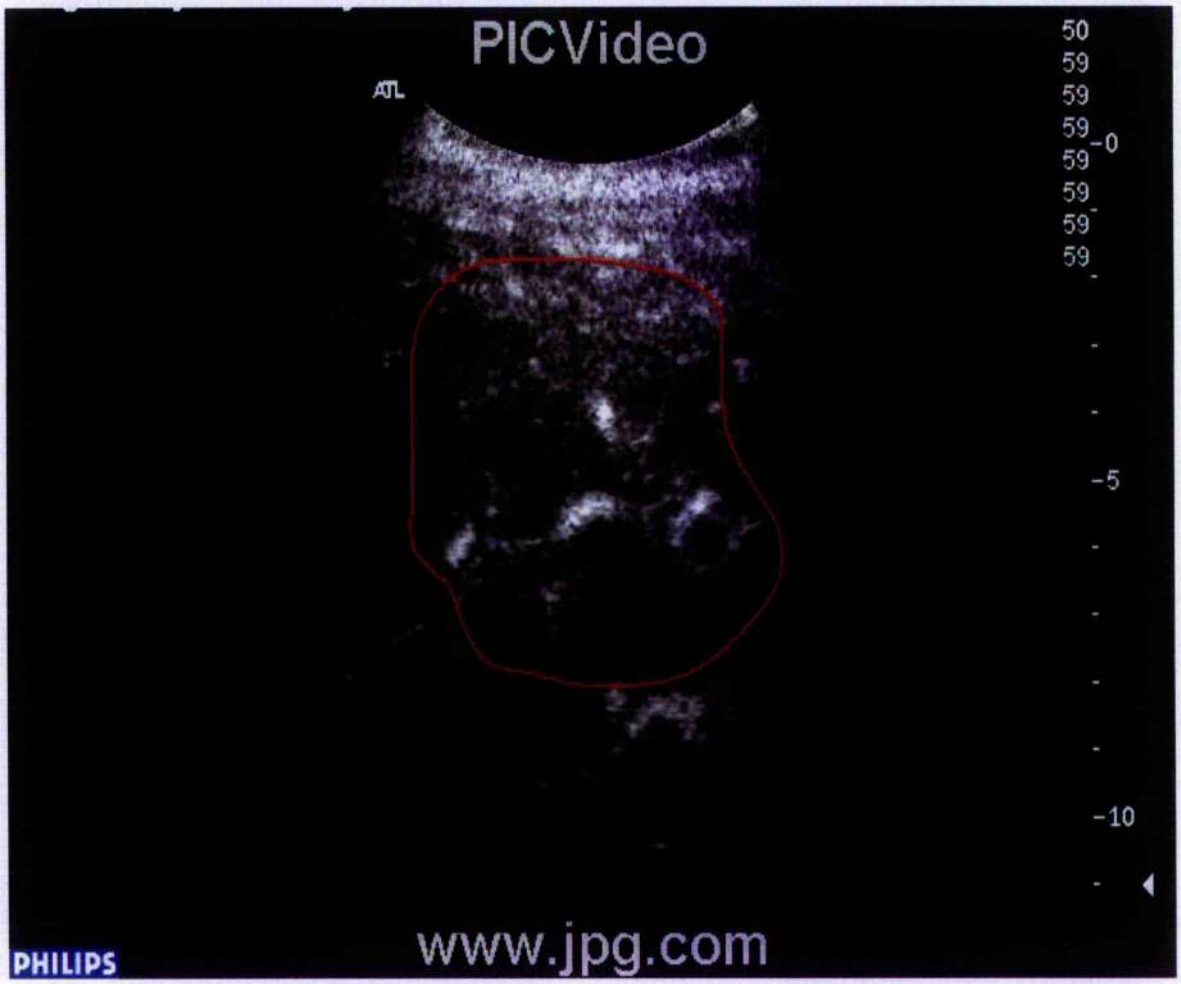
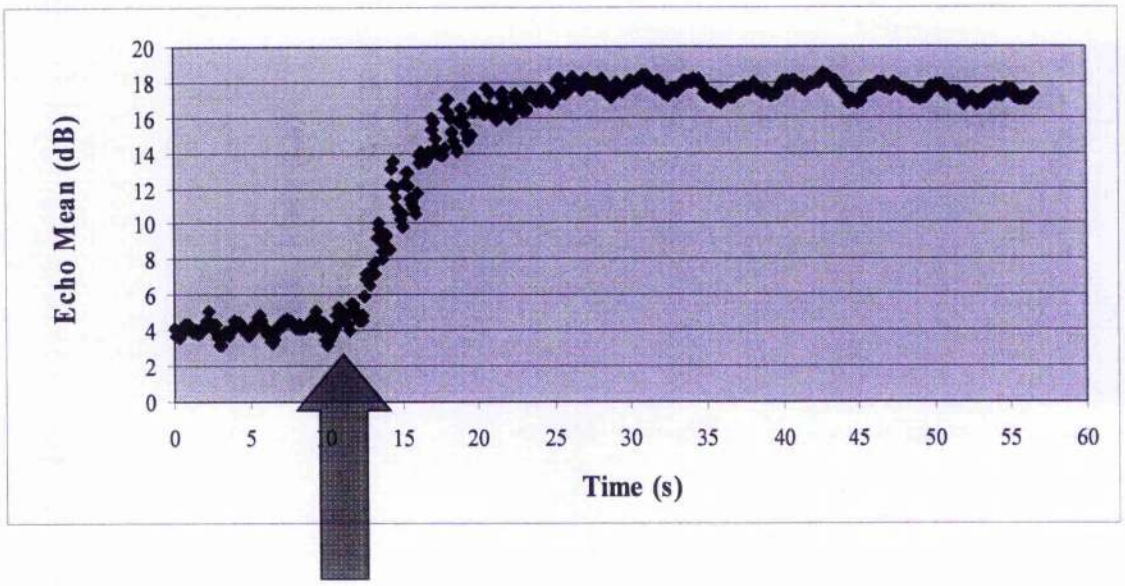
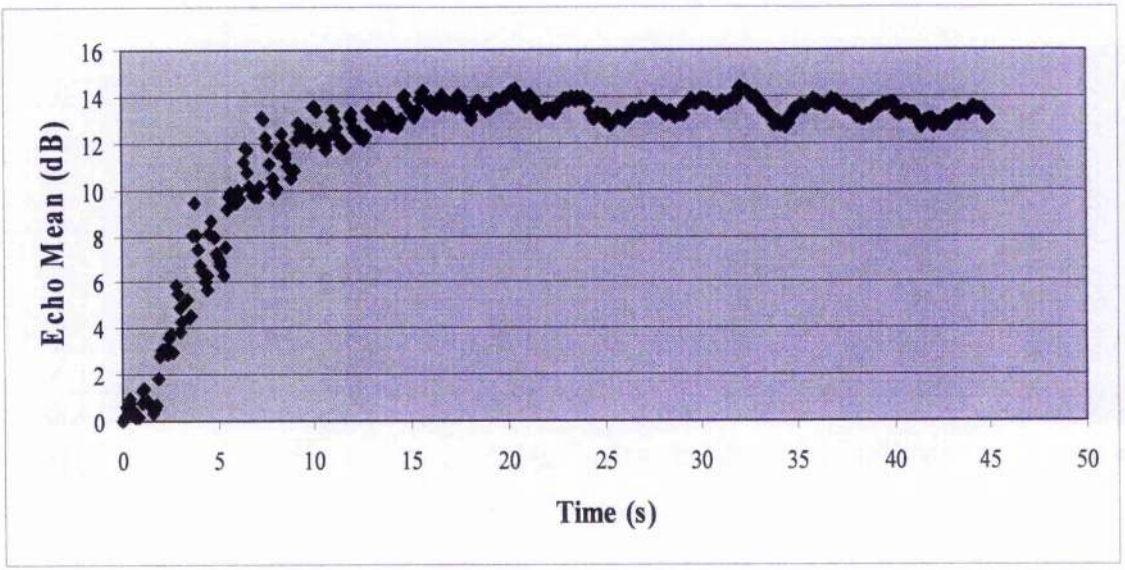


Figure 20: Hepatic perfusion (signal intensity-time) curve achieved with CE-US in one patient with pathologically confirmed colorectal liver metastases.



It can be seen that the echo mean in the first 10 seconds after contrast administration fluctuates around 4dB which equates to the signal from background tissues in this patient. The grey arrow displays time zero, representing the first arrival of contrast.

Figure 21: The hepatic perfusion (signal intensity-time) curve seen in Figure 20 with the curve now starting at time of contrast arrival (time zero) with the background signal subtracted.



3.4.3 Results

The mean age of the patients with colorectal liver metastases was 65 years (SD 11.2, range 22-82 years), which did not differ significantly from the mean age of the control group (61 years, SD 7.6, range 50-84 years; Student t-test $p>0.05$). Percutaneous CE-US was completed in all recruited patients and controls successfully with no adverse reactions.

Figure 22 displays the median liver perfusion curves for the patient group and control group. Patients had a significantly higher signal intensity during the early phase of the perfusion curve for each 0.5s time point from 1.0s to 19.5s ($p<0.05$, Mann-Whitney). Table 7 displays the comparison in signal intensities between the groups at each 0.5s time point during this time period.

The significant time period was subdivided in to four time intervals according to the level of significance of the difference between groups at individual time points, allowing areas under the curve to be calculated (Table 8). From 1.0s to 19.5s, area under the curve was significantly greater in the patient group (patients median area 179.0 dB.s vs controls median area 119.7 dB.s; $p=0.001$). 4.5s to 8.5s was the time period of greatest disparity between the groups (i.e. $p \leq 0.001$ for each 0.5s time point) and area under the curve during this period was also significantly higher in patients with colorectal liver metastases (patients median area 193.1 dB.s vs controls median area 125.8 dB.s; $p=0.001$).

Visual assessment of the median liver perfusion curves for the patients and controls (Figure 22) allowed selection of four time periods where the gradients appeared to differ between the two groups: 1s to 6s; 4.5s to 8.5s; 4.5s to 11.5s and 20s to 30s. The calculated gradients during 4.5s to 8.5s and 4.5s to 11.5s were not significantly different between the

two groups (p=0.109 and 0.314 respectively). The gradient of the curve from 1s to 6 s was significantly greater in the patients when compared to the controls: patients median gradient was 1.31 dB/s (0.83 – 1.74) versus controls median gradient of 0.74 dB/s (0.43 – 1.27) [p=0.002]. In addition, the gradient of the curve from 20s to 30s was significantly lower in the patient group when compared to the controls: patients median gradient 0.05 dB/s (-0.02 – 0.125) versus controls median gradient 0.13 dB/s (0.04 – 0.18) [p=0.006].

Two hepatic perfusion indices (HPI 1 and HPI 2) were calculated according to the statistically significant curve gradients. For HPI 1, the arterial gradient taken was from 4.5s to 8.5s and the venous gradient was 20s to 30s. For HPI 2, the same venous gradient was used, but the arterial gradient was from 1s to 6s. The patient group had significantly higher HPIs than the controls for both indices. For HPI 1, the patients' median HPI was 0.96 versus the controls' median HPI of 0.86 (p=0.003). For HPI 2, the patients median HPI was 0.97 versus the controls' median HPI of 0.87 (p=0.001).

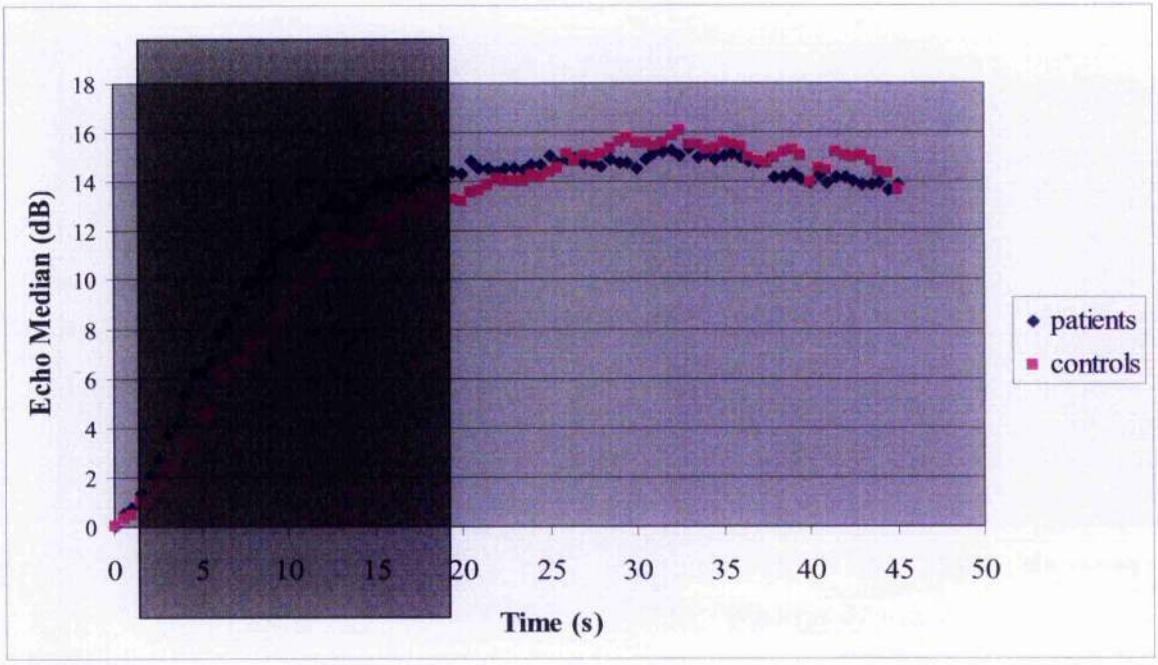
Figures 23, 24 (a, b, c, d), 23 (a, b, c) and 25 display the scatter plots for absolute signal intensities, areas under the curve, gradients of the curve and HPI. It can be seen that there is significant overlap of data between the patients and the controls, especially for areas under the curve and gradients of the curve. The absolute signal intensities selected (4.5s, 5.0s, 5.5s and 7.5s) occurred within the most significant time period (4.5s to 8.5s) of the liver perfusion curve and although there is still significant overlap of data, it can be seen that the higher signal intensities occurred in the patient groups, especially at the 4.5s and 5s time points.

ROC curves for all four parameters were drawn with results displayed in Table 9. To allow comparison between parameters, a cut off point was established. It was thought

that a moderate specificity with a high sensitivity would be the most clinically relevant approach i.e. using 60% specificity would minimise the number of false positives as well as reflecting accepted specificities for current imaging modalities like percutaneous ultrasound.

The highest areas under the curve and subsequent highest sensitivities (with specificity established at 58%) were achieved with the signal intensity parameter: 7.5s had an area of 0.77 and sensitivity of 85% and 5.5s had an area of 0.76 with 81% sensitivity. The areas under the curve parameter achieved lower areas on ROC curve analysis (range 0.72 to 0.74) when compared to the signal intensity parameter, with the highest sensitivity being 79%. The gradients of the curve had the lowest area results as well as the lowest sensitivity at 70% (20s to 30s curve gradient). Figure 25 (a, b, c, d) displays the ROC curves for the highest sensitivities from each parameter.

Figure 22: Comparison of liver perfusion between patients with colorectal liver metastases and healthy controls.



The shaded area displays the region of statistically greater signal intensity (1.0s to 19.5s) in the patients with colorectal liver metastases ($p<0.05$). Each data point represents the median signal intensity at that time point within each group.

Table 7: Comparison of liver perfusion at individual time points in patients with colorectal liver metastases versus healthy controls during contrast enhanced ultrasound.

	signal intensity at time point (dB)		
time point	**patients with colorectal liver metastases**	**controls**	**p value**
1.0	0.72 (0.49-0.98)	0.47 (0.14-0.69)	0.018
1.5	1.28 (0.81-2.63)	0.82 (0.42-1.43)	0.009
2.0	2.17 (0.98-3.08)	0.92 (0.60-2.02)	0.005
2.5	2.64 (1.49 – 4.7)	1.57 (0.86 – 2.43)	0.002
3.0	3.68 (1.80 – 4.89)	1.81 (0.91 – 3.19)	0.003
3.5	4.06 (2.17 – 6.46)	2.25 (1.30 – 3.84)	0.01
4.0	5.15 (2.81 -7.41)	2.29 (1.47 – 5.38)	0.002
4.5	6.26 (3.38 – 8.21)	3.25 (2.11 - 4.44)	0.000
5.0	6.20 (3.92 – 8.03)	3.26 (1.91 – 5.79)	0.000
5.5	6.60 (4.84 – 9.54)	4.09 (2.39 -6.07)	0.000
6.0	7.60 (4.83 – 10.03)	4.84 (2.14 -6.95)	0.001
6.5	8.05 (5.26 – 10.61)	5.04 (2.62 – 7.71)	0.001
7.0	8.80 (6.28 – 11.38)	5.07 (3.33 – 7.84)	0.000
7.5	9.63 (6.26 – 11.38)	5.32 (3.14 – 8.76)	0.000
8.0	9.83 (6.78 – 12.01)	5.06 (3.76 – 8.76)	0.001
8.5	10.49 (6.76 – 12.24)	5.36 (4.47 – 9.95)	0.001
9.0	10.70 (7.49 – 12.72)	6.08 (3.96 – 10.18)	0.002
9.5	11.11 (7.27 – 13.05)	6.44 (4.98 – 10.18)	0.001
10.0	11.20 (7.66 – 13.65)	6.78 (4.86 – 10.06)	0.000
10.5	11.32 (7.80 – 13.74)	7.27 (5.05 – 10.46)	0.001
11.0	11.45 (8.39 – 13.89)	7.87 (5.83 – 11.26)	0.002

	signal intensity at time point (dB)		
time point	patients with colorectal liver metastases	controls	p value
11.5	11.84 (8.65 – 14.55)	7.68 (5.45 – 12.10)	0.003
12.0	12.71 (8.72 – 14.20)	7.68 (5.89 – 12.10)	0.002
12.5	12.94 (8.63 – 14.95)	8.48 (6.56 – 12.08)	0.003
13.0	13.09 (8.97 – 14.80)	9.14 (6.85 -12.50)	0.004
13.5	12.79 (9.22 – 15.11)	9.57 (6.37- 12.94)	0.001
14.0	12.84 (9.79 – 15.21)	9.37 (6.97 – 13.07)	0.004
14.5	13.16 (9.88 – 15.39)	9.51 (7.06 – 13.10)	0.002
15.0	13.60 (9.91 – 15.40)	9.93 (7.55 – 13.38)	0.005
15.5	13.73 (10.11 – 15.14)	10.16 (7.76 – 14.05)	0.005
16.0	13.76 (10.46 – 15.53)	10.48 (7.88 – 14.05)	0.010
16.5	14.04 (10.69 – 15.55)	10.53 (8.17 – 14.04)	0.012
17.0	13.59 (10.85 – 15.71)	11.08 (8.46 -14.20)	0.028
17.5	13.86 (11.13 – 15.79)	11.60 (8.62 -14.40)	0.027
18.0	13.81 (11.45 – 15.68)	11.71 (8.69 -14.74)	0.037
18.5	14.17 (11.34 – 16.20)	11.90 (8.69 -14.74)	0.026
19.0	13.95 (11.32 – 15.88)	11.84 (8.90 – 15.10	0.043
19.5	14.15 (11.58 -16.21)	12.25 (8.95-15.13)	0.031

Results expressed in decibels (dB) and shown as median (interquartile range).

Table 8: Comparison of area under curve for selected time periods in patients with colorectal liver metastases versus healthy controls during contrast enhanced ultrasound.

		area under curve during time period		
time period (s)	**level of significance of each 0.5s time point during time period**	**patients with colorectal liver metastases**	**controls**	**p value**
1 to 19.5	p≤0.043	179.0 (125.3 – 215.0)	119.7 (91.4 – 170.9)	0.001
4.0 to 15.5	p≤0.005	186.1 (130.9 – 223.1)	121.4 (93.3 177.7)	0.001
4.0 to 11.5	p≤0.002	199.9 (142.0 -237.4)	130.9 (90.5 – 192.7)	0.001
4.5 to 8.5	p≤0.001	193.1 (136.5 – 230.8)	125.8 (86.2 – 185.2)	0.001

Results expressed in dB.s and shown as median (interquartile range).

Figure 23a: Scatter plot displaying distribution of signal intensities at 4.5 seconds after time zero in patients with colorectal liver metastases and healthy controls.

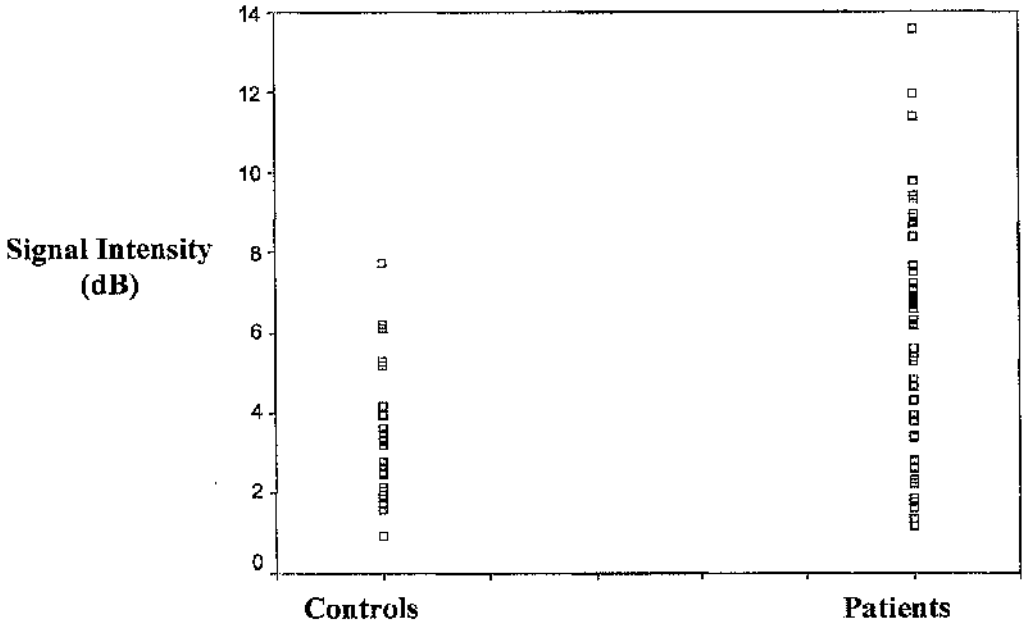


Figure 23b: Scatter plot displaying distribution of signal intensities at 5.0 seconds after time zero in patients with colorectal liver metastases and healthy controls.

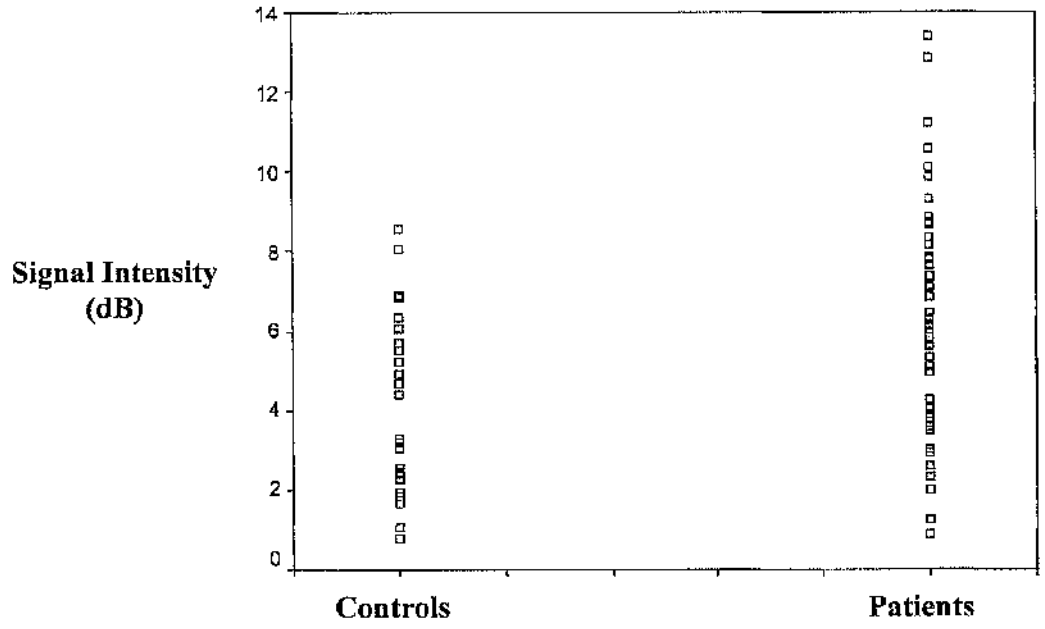


Figure 23c: Scatter plot displaying distribution of signal intensities at 5.5 seconds after time zero in patients with colorectal liver metastases and healthy controls.

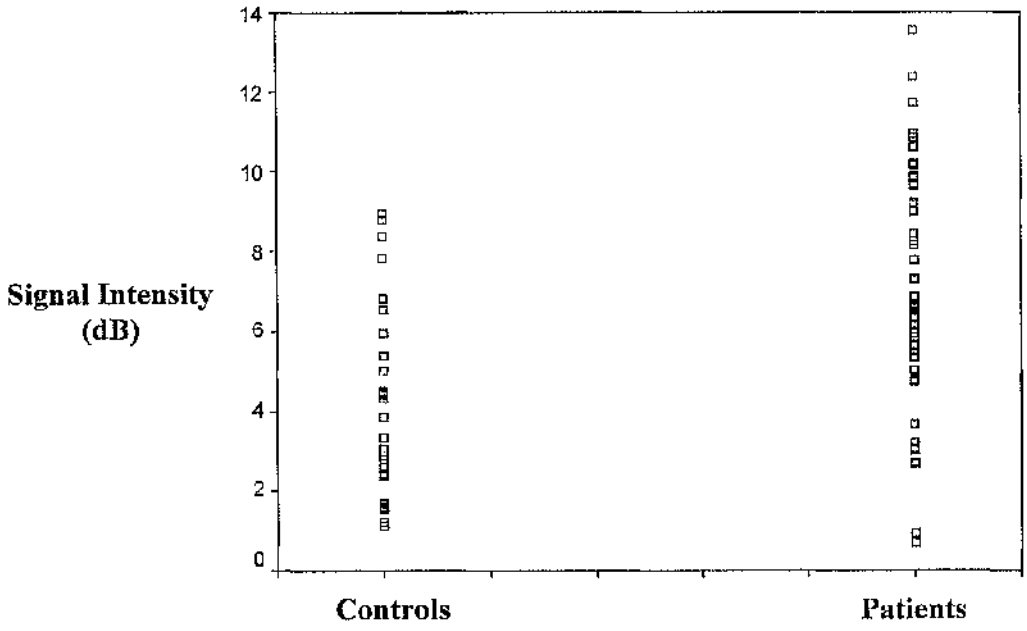


Figure 23d: Scatter plot displaying distribution of signal intensities at 7.5 seconds after time zero in patients with colorectal liver metastases and healthy controls.

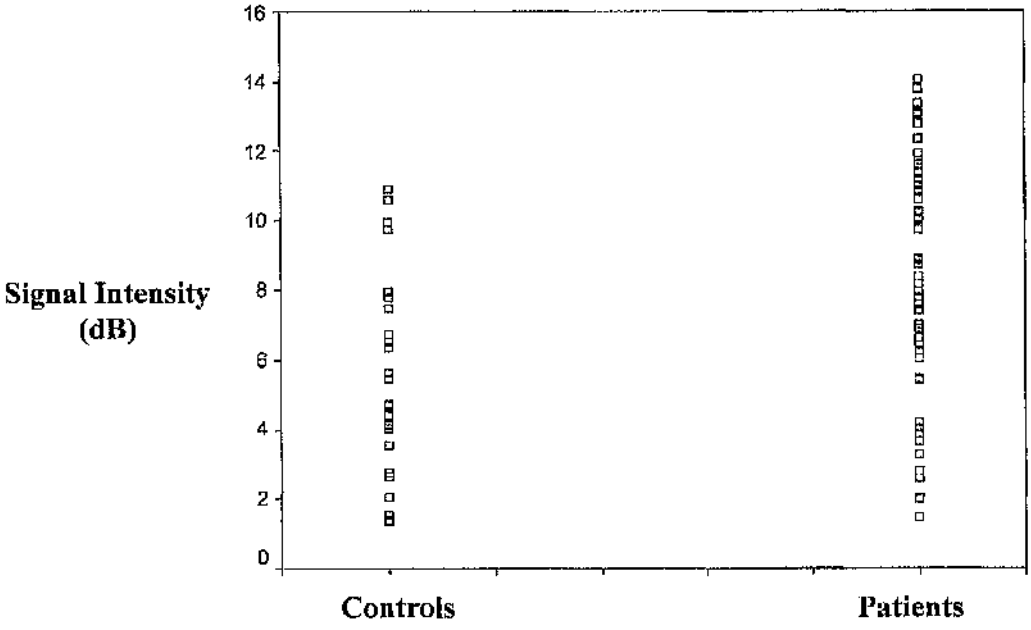


Figure 24a: Scatter plot displaying distribution of area under curve 1-19.5s in patients with colorectal liver metastases and healthy controls.

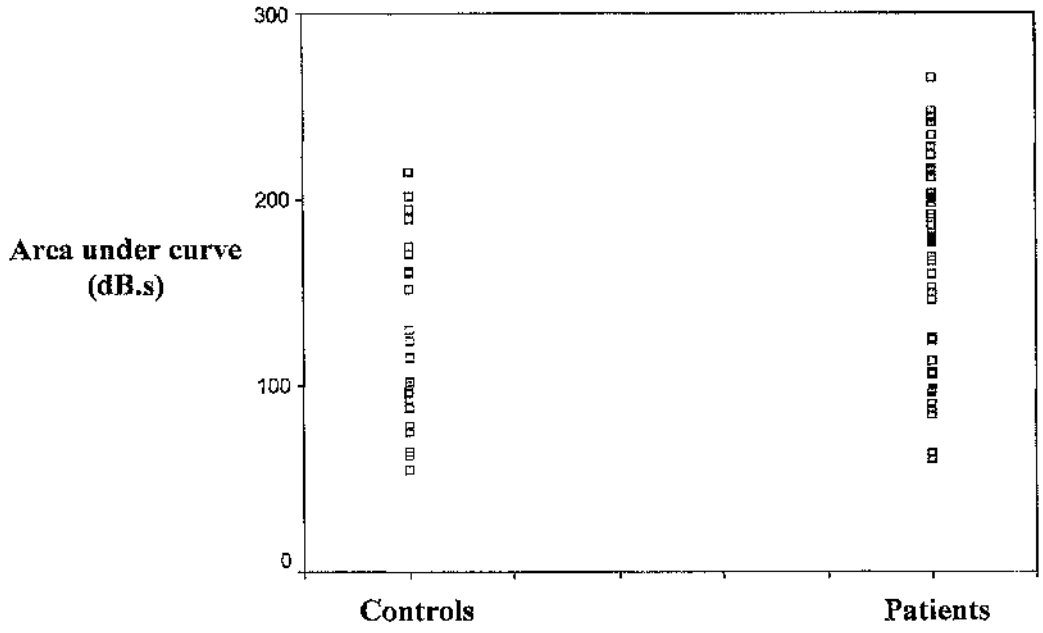


Figure 24b: Scatter plot displaying distribution of area under curve 4-15.5s in patients with colorectal liver metastases and healthy controls.

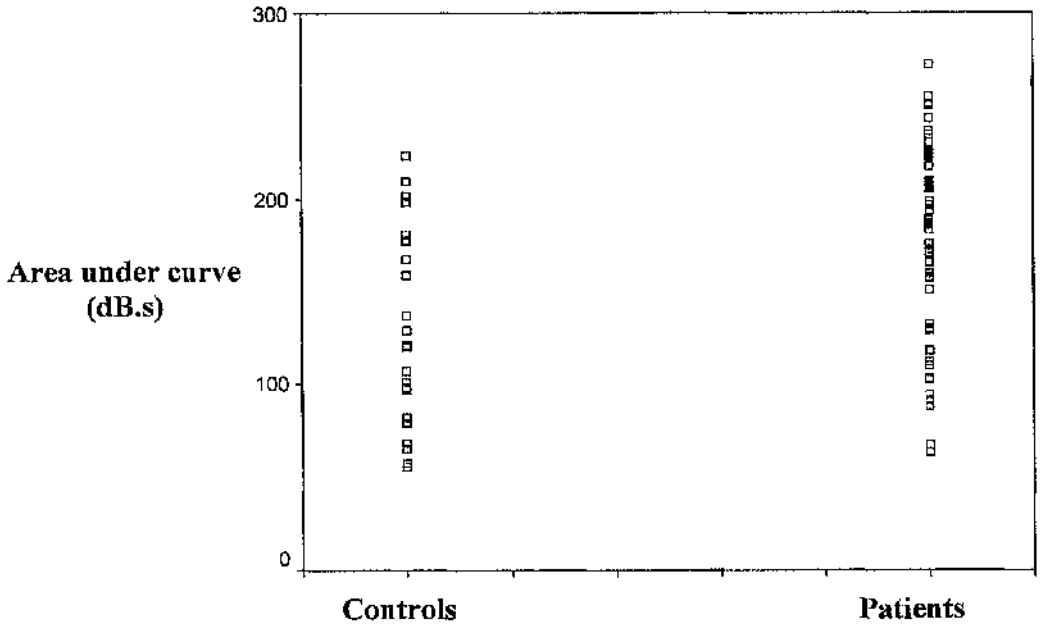


Figure 24e: Scatter plot displaying distribution of area under curve 4-11.5s in patients with colorectal liver metastases and healthy controls.

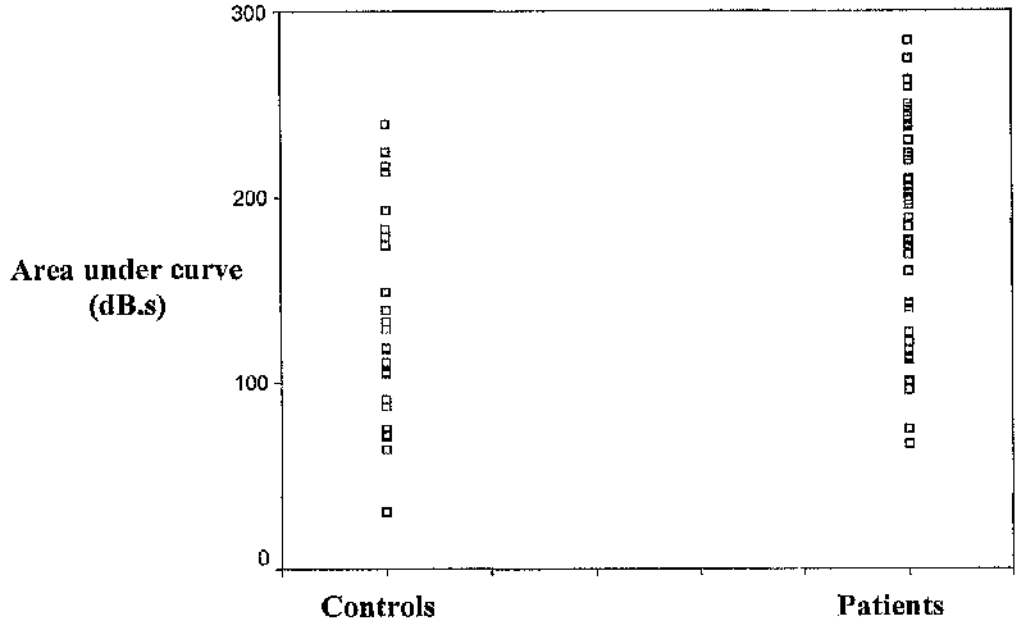


Figure 24d: Scatter plot displaying distribution of area under curve 4-8.5s in patients with colorectal liver metastases and healthy controls.

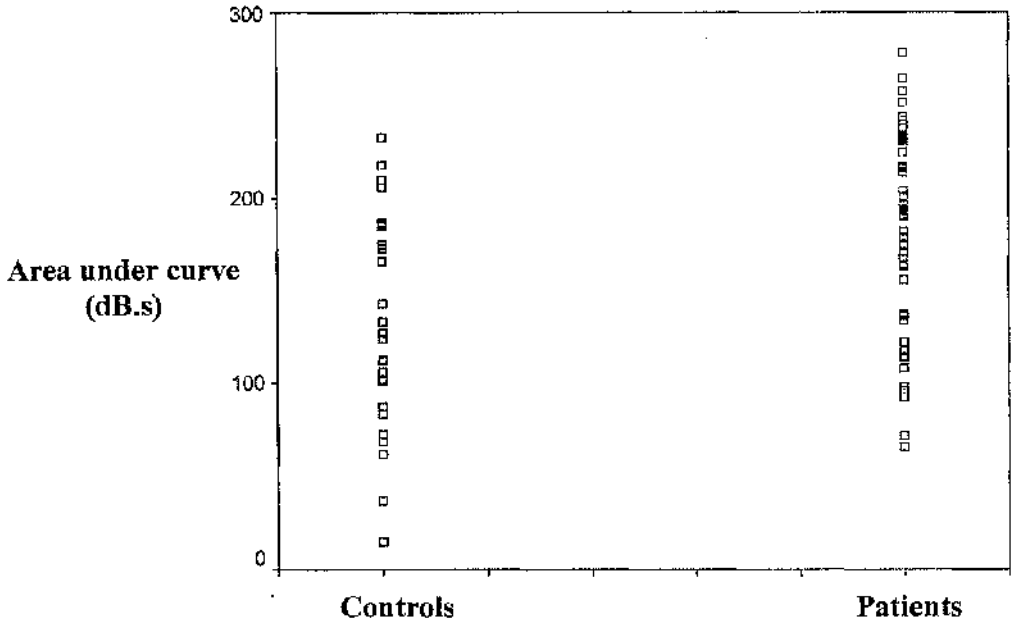


Figure 25a: Scatter plot displaying distribution of gradient of curve from 1.0s to 6.0s in patients with colorectal liver metastases and healthy controls.

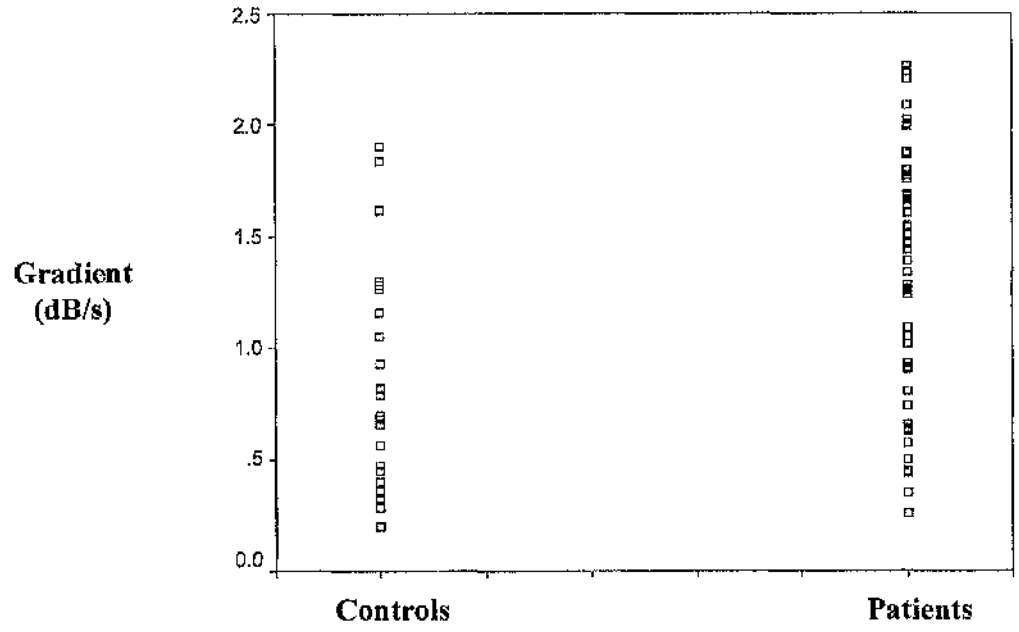


Figure 25b: Scatter plot displaying distribution of gradient of curve from 4.5s to 8.5s in patients with colorectal liver metastases and healthy controls.

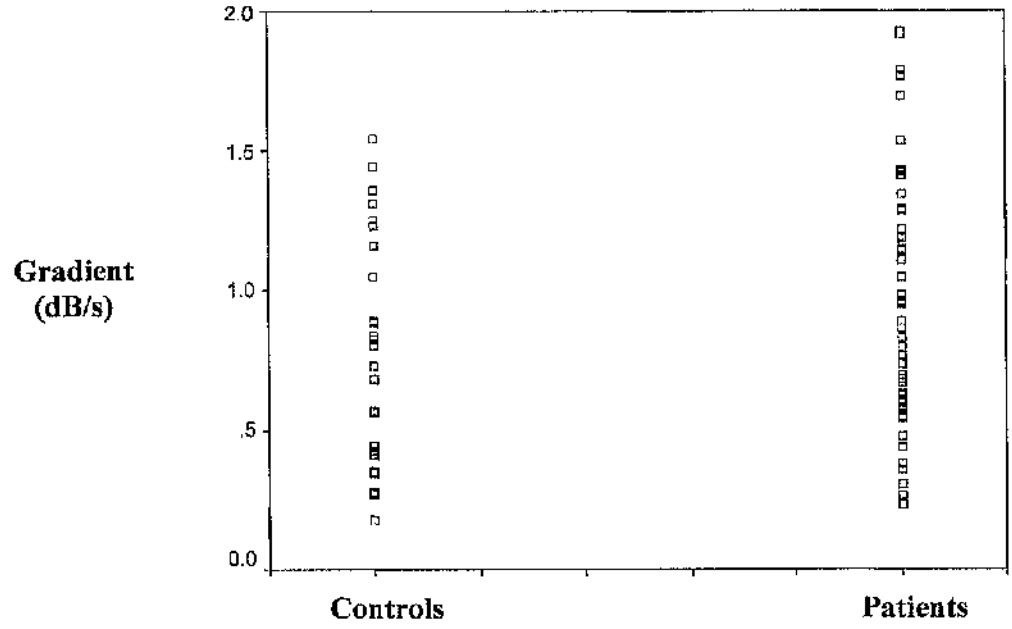


Figure 25c: Scatter plot displaying distribution of gradient of curve from 4.5s to 11.5s in patients with colorectal liver metastases and healthy controls.

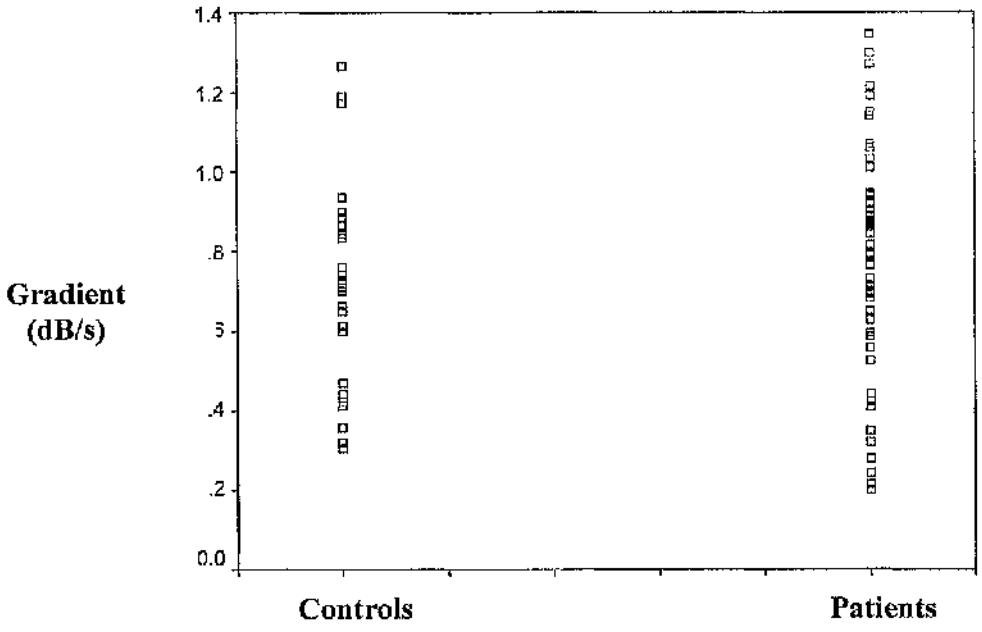


Figure 25d: Scatter plot displaying distribution of gradient of curve from 20s to 30s in patients with colorectal liver metastases and healthy controls.

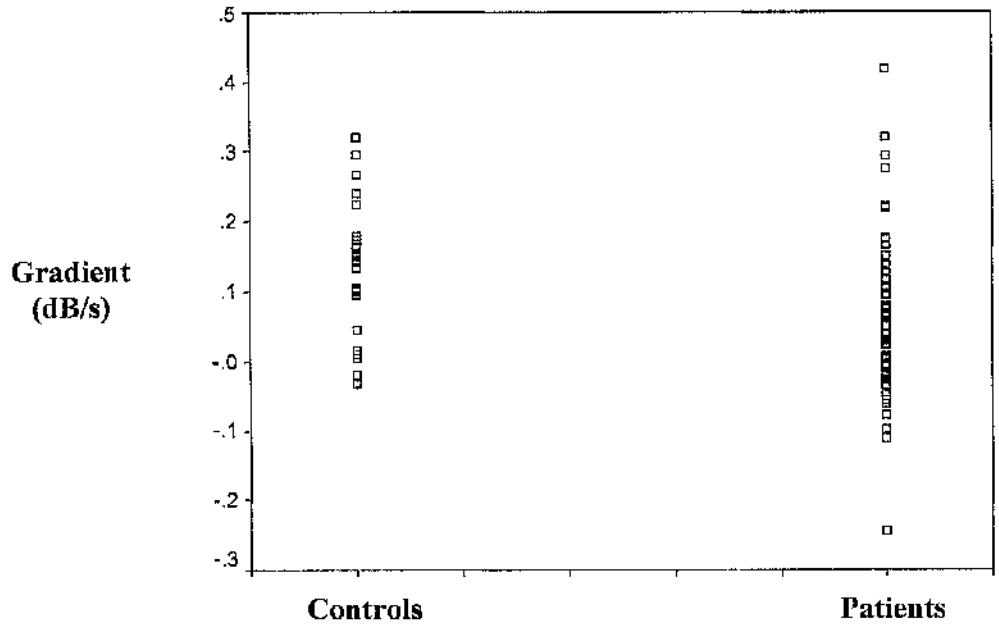


Figure 26a: Scatter plot displaying distribution of Hepatic Perfusion Index in patients with colorectal liver metastases and healthy controls (arterial: gradient of curve from 1 to 6 seconds; venous: gradient of curve from 20 to 30 seconds).

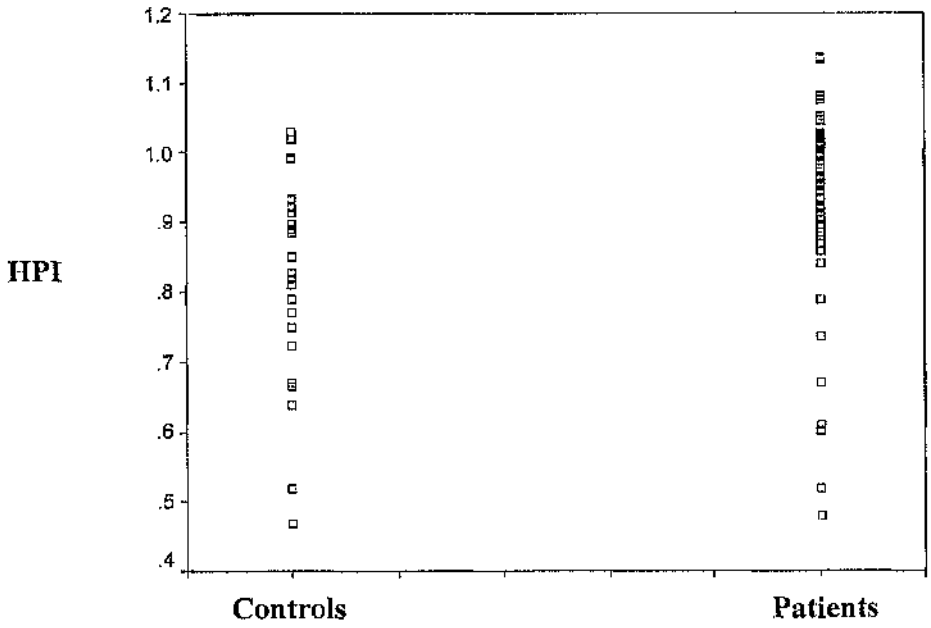


Figure 26b: Scatter plot displaying distribution of Hepatic Perfusion Index in patients with colorectal liver metastases and healthy controls (arterial: gradient of curve from 4.5 to 8.5s seconds; venous: gradient of curve from 20 to 30 seconds).

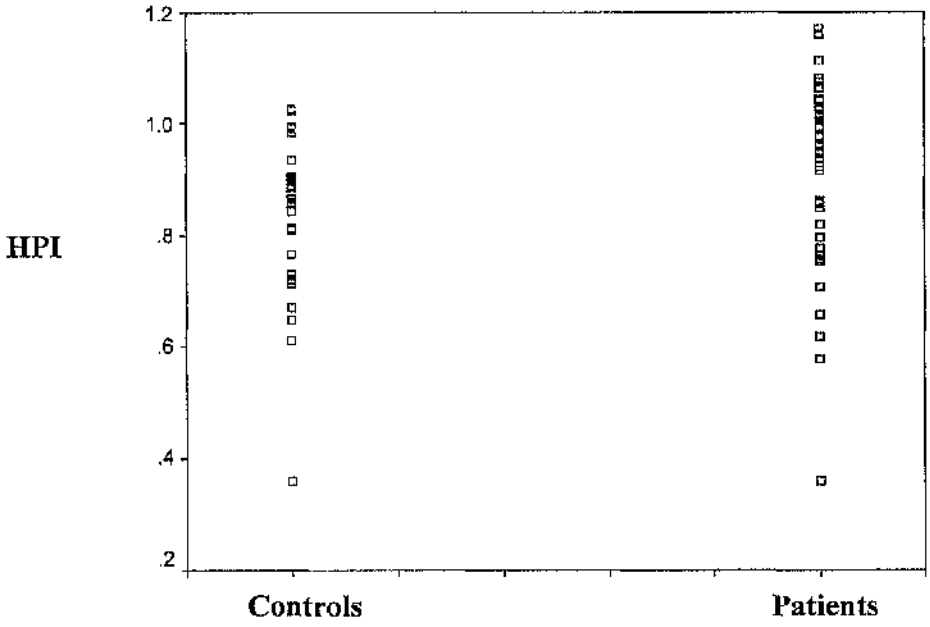


Figure 27a: ROC curve for measurement of liver perfusion (signal intensity at 7.5 seconds) in patients with colorectal liver metastases and healthy controls.

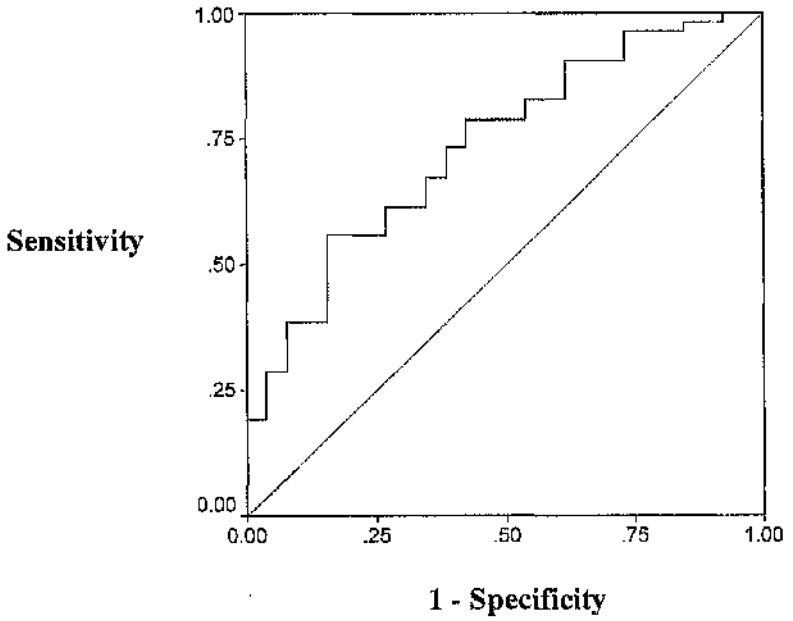


Figure 27b: ROC curve for measurement of liver perfusion (area under curve 4.5 to 8.5 seconds) in patients with colorectal liver metastases and healthy controls.

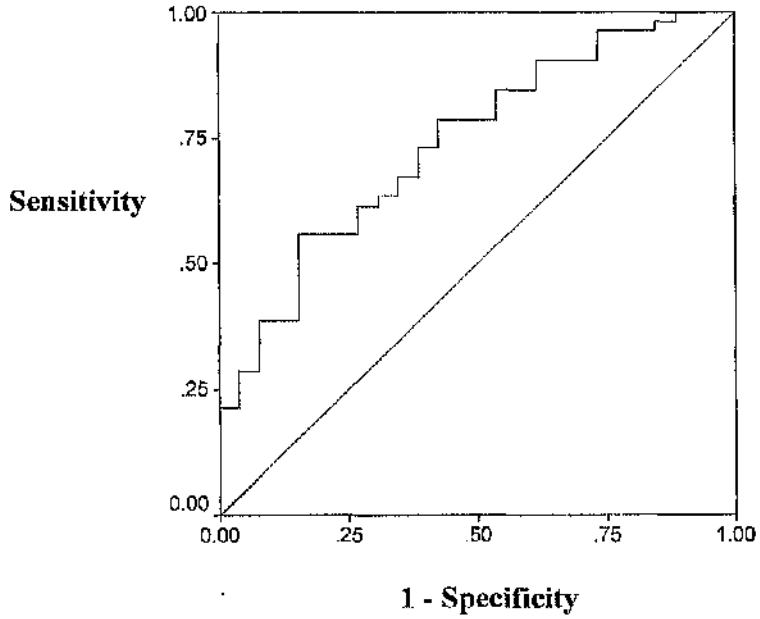


Figure 27c: ROC curve for measurement of liver perfusion (gradient of curve at 20 to 30 seconds) in patients with colorectal liver metastases and healthy controls.

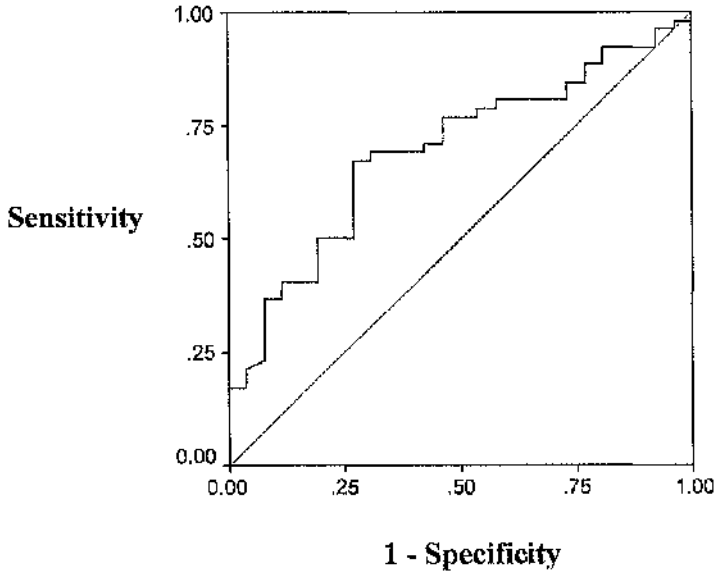


Figure 28d: ROC curve for measurement of Hepatic Perfusion Index in patients with colorectal liver metastases and healthy controls (arterial: gradient of curve from 1 to 6 seconds; venous: gradient of curve from 20 to 30 seconds).

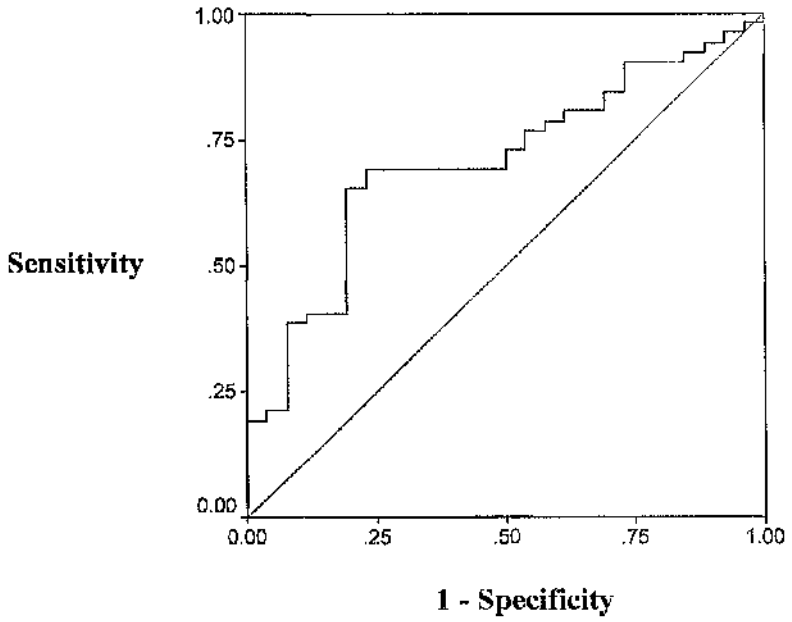


Table 9: Sensitivity and specificity of four liver perfusion parameters at detecting patients with colorectal liver metastases using ROC curve analysis.

Parameter		area under ROC curve	95% CI	p value	sensitivity	specificity
signal intensity	4.5s	0.76	0.65 -0.86	0.000	77%	58%
	5.0s	0.74	0.63 -0.86	0.000	71%	58%
	5.5s	0.76	0.65 – 0.87	0.000	81%	58%
	7.5s	0.77	0.67 – 0.88	0.000	85%	58%
area under the curve	1 to 19.5	0.72	0.61 – 0.84	0.001	73%	58%
	4 to 15.5	0.73	0.62 – 0.85	0.001	79%	58%
	4 to 11.5	0.74	0.62 – 0.85	0.001	79%	58%
	4.5 to 8.5	0.74	0.63 – 0.85	0.001	79%	58%
gradient	1.0s to 6.0s	0.72	0.60 – 0.84	0.002	75%	58%
	4.5s to 8.5s	0.61	0.48 – 0.75	0.109	56%	58%
	4.5s to 11.5s	0.57	0.44 – 0.70	0.314	56%	58%
	20s to 30s	0.69	0.57 – 0.81	0.006	70%	58%
HPI (1)	H1/ H1 +H2	0.71	0.59 - 0.82	0.003	69%	58%
HPI (2)	H1/H1 + H2	0.73	0.61-0.84)	0.001	77%	58%

The parameters are defined according to absolute time points (signal intensity) or a specific time interval (area under the curve, gradient and HPI). Selection of time points for each parameter is defined in results section.

Reproducibility of data acquisition

The interobserver CV was 7.3% with an ICCC of 0.698. There was no evidence of bias between observers' data acquisition (p=0.960). In relation to intraobserver reproducibility of data acquisition, the CV was 14.2%, ICCC 0.829 with no significant differences between measurements 1 and 2 (p=0.300). Figures 28 and 29 display the spread of the data.

Reproducibility of data analysis

No significant differences were found in the calculation of time zero between the first and second observers (t-test p=0.716) with Figure 30 graphically displaying the results. The interobserver CV was 2.5% with an ICCC of 0.992. The intraobserver CV was 1.7% with an ICCC of 0.996 with no evidence of bias between first and second measurements (p=0.516) [Figure 31].

Influence of region of interest

In the comparison of the three ROIs: 1000mm^2 against 1500mm^2, the mean CV was 3.2% (range 1.5 – 6.1) with an ICCC of 0.989. Analysis of 1500mm^2 versus 2000mm^2, the mean CV was 2.7% (1.1 – 6.1) and an ICCC of 0.993. The mean CV was 5.2% (1.9 – 11.8) with an ICCC of 0.971 when ROI 1000mm^2 was compared to 2000mm^2.

Figure 28: Interobserver variability in data acquisition between the first (1) and second observer (2).

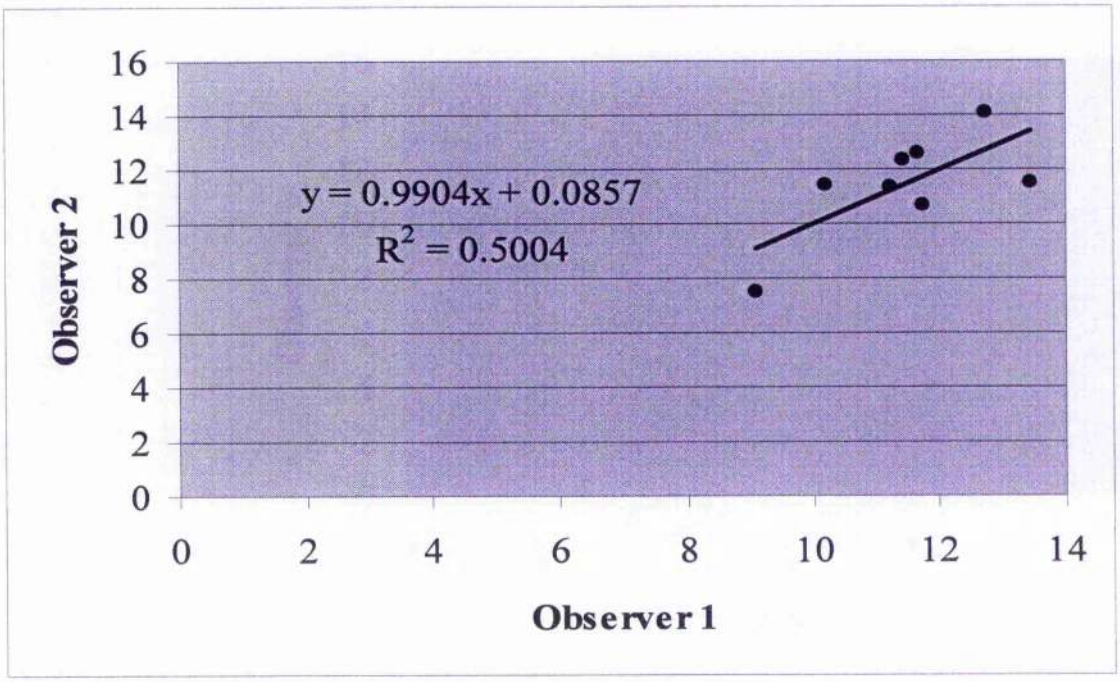


Figure 29: Intraobserver variability in calculation of data acquisition with the first observer.

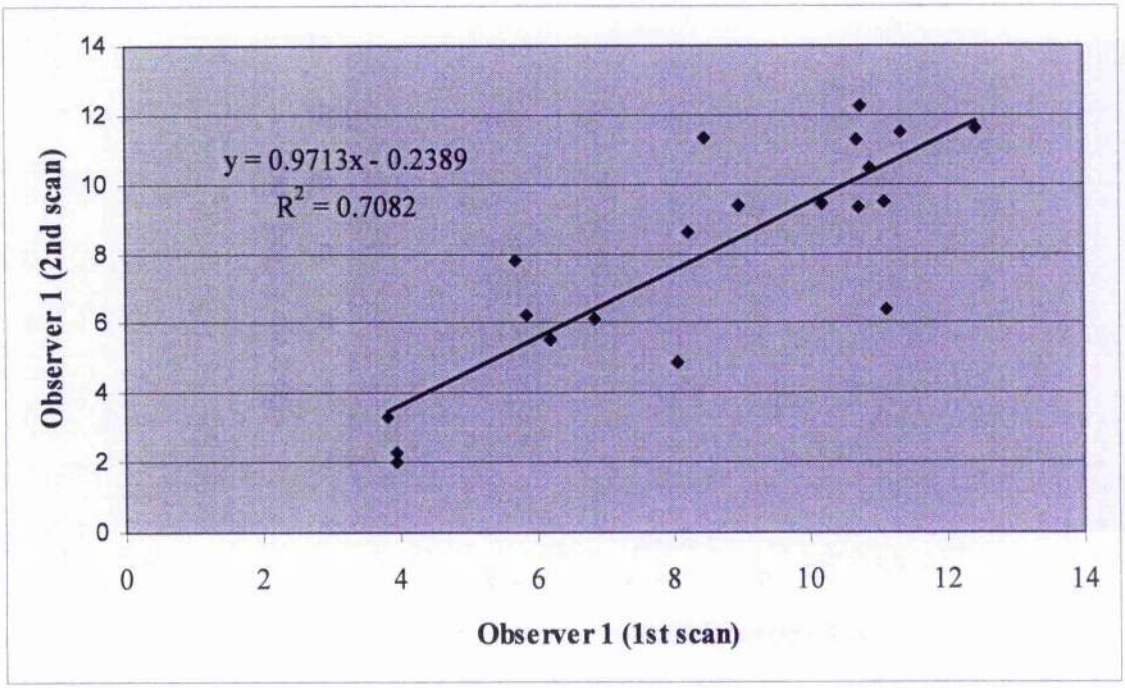


Figure 30: Interobserver variability in calculation of time zero between the first (1) and second observer (2).

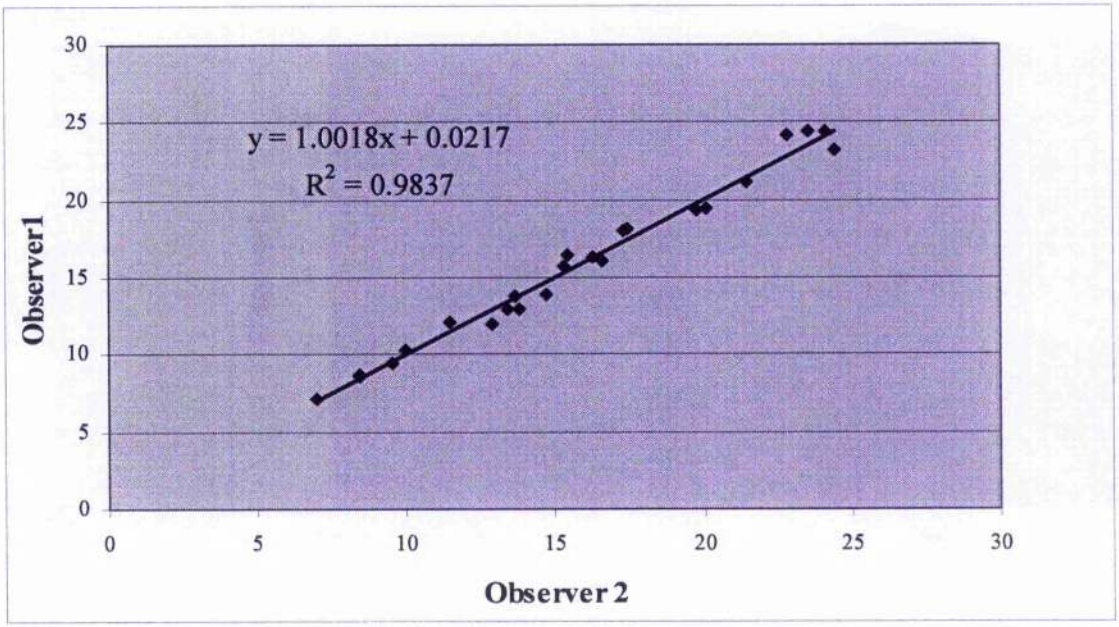
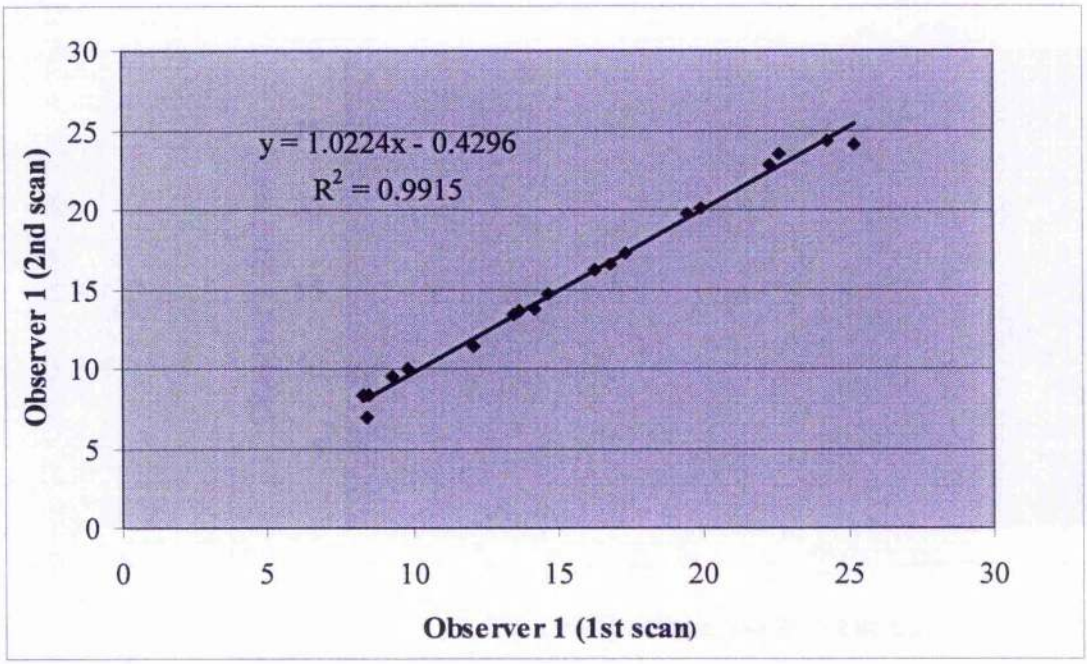


Figure 31: Intraobserver variability in calculation of time zero by the first observer.



3.4.4 Discussion

Liver blood flow is an important physiological marker of liver function that can reflect the liver's response to injury, regeneration and the presence of malignant disease[132;138;346]. Despite advancing imaging technologies, accurate measurement of liver perfusion remains technically difficult due to the dual blood supply and the changing contributions of the hepatic artery and the portal vein. The development of a technique that can reliably measure liver perfusion could potentially detect early angiogenesis caused by colorectal liver metastases, allowing early diagnosis and initiation of treatment.

This study has developed a novel and reproducible technique for measuring liver perfusion that detected altered liver perfusion in patients with colorectal liver metastases. Using a bolus injection of intravenous contrast with low power pulse inversion harmonic imaging, the uptake of contrast through the liver allowed signal intensity-time curves to be drawn. With previous research documenting that the microbubbles are mimicking red blood cell flow, then measurement of the liver's contrast uptake allows quantification of hepatic perfusion. Furthermore, the targeting of a large area of hepatic parenchyma in the ROI means that perfusion at the level of the hepatic microcirculation is being measured.

Initial visual comparison of the signal intensity-time curves displayed three arbitrary time phases that are likely to reflect the three vascular phases of hepatic imaging with contrast enhanced ultrasound: early (arterial); intermediate (portal) and late (sinusoidal). The early phase began at the arrival of contrast and involved a steep gradient during the first 10 to 15 seconds. The intermediate phase started at approximately 15 seconds with a flatter slope and continued to approximately 30 seconds before reaching a plateau that was maintained through the late phase.

The majority of differences in hepatic perfusion were found in the early phase with the patient group having significantly greater signal intensities, gradients and areas under the curve when compared to the control group. The remaining difference was in the intermediate phase when the patient group experienced a significantly smaller gradient. The differences in the early and intermediate gradients between patients and controls resulted in the HPI being significantly elevated in the patient group.

The increased perfusion displayed by the patients during the early phase of the perfusion curve is likely to be the result of the predominant hepatic arterial blood supply. Since pathologists first documented an altered blood supply in the presence of colorectal liver metastases, many authors using different technologies and approaches have confirmed their findings[132;134;138;347;348]. The initial steeper gradient in the patients' perfusion curve reflects the earlier arrival of the microbubbles carried by the dominant hepatic artery, with the increased area under the curve suggesting that this increased velocity results in a greater blood volume. Later on in the perfusion curve, the portal vein begins to exert its influence. A steeper gradient in the controls in the intermediate phase suggests that although the patients have an increased arterial supply, they also have a reduction in their portal vein contribution. This is a finding that other authors have documented, with some concluding that a balance between the two arterial components exists as the overall blood supply (and subsequently volume) to the liver does not change. This study would support this theory, as it found no significant differences in total area under the curve and in peak intensity, suggesting that the overall blood supply and microbubble concentration remains unchanged in the presence of colorectal liver metastases.

There were no differences in any of the perfusion parameters between the groups in the late phase. It is highly likely that recirculation of the intravenous contrast agent is occurring during this time frame, dampening the separation of the arterial and venous components. The liver is unusual in that recirculation of a contrast agent occurs through two routes: via the splenic circulation and via the splanchnic recirculation. As a result, measurement of perfusion parameters in the late stage using a bolus technique may not yield any worthwhile information.

Unlike other types of radiological imaging, ultrasound imaging is dependent on the experience of the sonographer using it. As a result, the development of any new technique using ultrasound needs reproducibility studies to ensure that the technique is transferable between sonographers, a limitation that prevented the HPI and DPI from entering routine clinical practice. This study performed reproducibility analysis on both acquisition of data and interpretation of data and found that contrast enhanced measurement of liver perfusion is reproducible.

Data acquisition requires the sonographer to target a large area of liver parenchyma that will remain stationery during the 45 seconds of the ultrasound scan. There was a learning curve present as the sonographer had to administer the contrast bolus with one hand whilst keeping the probe in position with the other. In addition, some subjects have difficulty maintaining their breathing shallow and regular during the ultrasound loop. However, both these difficulties can be overcome with practice from the sonographer (the second observer underwent 20 training scans) and clear directions to the patients.

The arrival of the contrast to the hepatic parenchyma (time zero) was extremely important as it determined the start of the liver perfusion curve. If there was found to be

wide variation in the calculation of time zero, then any statistical interpretation of the perfusion curves would be substantially flawed. However, there were no significant differences demonstrated by the intraobserver and interobserver reproducibility studies.

The only remaining potential bias was selection of the region of interest. Q-Lab allowed any area to be chosen of any shape the observer requested and it was seen that the smaller areas (50-100mm^2) resulted in substantial variations in data, either due to them sitting on a large vessel, or the patient's respiration moving the region of interest. As a result, they were disregarded as they were not reflecting perfusion of the hepatic microcirculation. Large areas of hepatic parenchyma (i.e. greater than 1000mm^2) were then selected that did not include any tumour, the hepatic artery or the portal vein. Reproducibility studies found the three selected areas (1000 mm^2, 1500 mm^2, 2000 mm^2) did not significantly affect the acquisition of data and 1000 mm^2 was taken as the minimal accepted area of the region of interest.

These initial results are promising, making it important to determine the clinical applicability of liver perfusion measured by contrast enhanced ultrasound. From the scatter plots for each of the four significant parameters (signal intensities; areas under the curves; gradients of the curves and hepatic perfusion indices), it can be seen that there is no clear separation of data between the two groups. As a result, a cut off point cannot be established that confidently differentiates between healthy controls and patients with metastatic disease. This is reflected in the range of sensitivities and specificities achieved by the four variables. Interestingly, the highest sensitivity of 85% was achieved by signal intensity at a single time point (7.5s). Using a single time point to differentiate between groups requires the technique to be highly specific, leaving little margin for error and it

was anticipated that the other three parameters would perform better at differentiating between the two groups as they covered a larger data range.

Unfortunately, these results appear to limit the clinical applicability of the contrast enhanced ultrasound measurement of liver perfusion. With no parameter capable of accurately defining the presence of colorectal liver metastases in patients with overt disease, it is extremely likely that this technique will not be capable of detecting occult disease. There are several potential explanations for this. First, targeting and measuring the hepatic blood flow at the level of the microcirculation may dampen the individual contributions of the hepatic artery and portal vein. As a result, it is possible that the significant differences found by measuring perfusion at the level of the microcirculation would become magnified if the individual vessel's components could be measured. This study set out to measure the perfusion of the individual vessels, but found that the patients' breathing made this difficult and even impossible in some cases. However, in the future it may become possible to track the vessels, minimising the influence of breathing and there are early reports of this advanced computer software in the literature[336].

Another potential factor is the physics of the microbubbles. We know what gas the microbubbles contain and how they interact with ultrasound waves as part of pulse inversion harmonic imaging. What is uncertain is how the microbubbles interact with the hepatic parenchyma on their passage. It has been documented that SonoVue® eventually pools in the liver, which suggests that analysis of the arrival of the contrast agent would provide the most information, including perhaps the time to arrival. However, the contrast agent could be interacting with the hepatic parenchyma upon arrival via the hepatic artery

and not accurately representing blood flow. In addition, there could be interaction with other organs via the portal vein and subsequent recirculation.

Both these limitations could be addressed by starting with laboratory based studies where parameters of hepatic blood perfusion are defined. There are obvious differences in myocardial and hepatic perfusion and what applies to CE-US in cardiac imaging may not be relevant in hepatic imaging. The first step would be a comparison between signal intensity versus time curves for CE-US against labelled red blood cells. If good correlation was found then this would establish that microbubbles are intravascular tracers for liver perfusion. Subsequent experiments would be a combination of animal and human studies where the relationship between microbubble transit rates, hepatic blood volume and hepatic blood flow would be established, leading to optimal interpretation of signal intensity-time perfusion curves. Decisions could then be made as to whether direct measurement of hepatic artery and portal vein provides the best data or whether measuring the perfusion of the microcirculation is more relevant. In addition, continuous infusion of contrast with replenishment kinetics (potentially including a mathematical model) could be analysed.

One final discussion point is the evidence on which this chapter is based on: the alteration in hepatic blood supply in the presence of colorectal liver metastases. The fact the hepatic circulation changes is unquestioned. What is less clear is to what extent this change occurs, what factors are influencing the change and whether the change is a static phenomenon. For example, tumour size appears to be an influencing factor. Ackermann found that small tumours (1-2mm) had a variable blood supply that could be arterial, venous or both in origins. Moderate sized tumours (3-7mm) had well developed arterial circulations whilst massive tumours (7-33mm) returned to a varied pattern[123-126]. It is

likely that there are other influencing factors (including: age and sex; hepatic pressure, portal hypertension, portosystemic shunting; catacholamines, hormones; inflammation, cytokines, prostaglandins; growth factors; nitric oxide) and that hepatic vascularity is a changing phenomenon.

In conclusion, measurement of altered hepatic perfusion in colorectal liver metastases can be reliably performed by percutaneous contrast enhanced ultrasound. Further work analysing the individual contributions of the hepatic blood supply alongside improved understanding of the physics of the microbubbles in the hepatic circulation may develop a clinically applicable technique for the detection of occult colorectal liver metastases.

Section 3.

Chapter 4: Perioperative changes in hepatic perfusion in patients undergoing curative partial hepatectomy for colorectal liver metastases using contrast enhanced ultrasound.

3.4.1 Introduction and Aims

Previous work has proposed that altered hepatic perfusion present in patients with colorectal liver metastases may have a predictive role in the detection of occult liver metastases[132;133;349;350]. Two main approaches have been documented using dynamic scintigraphy and Doppler ultrasound, with both groups finding that their individual perfusion indices reliably detected the presence of both overt and occult colorectal liver metastases. Leveson et al calculated HPI using dynamic scintigraphy in 50 patients that underwent laparotomy for colorectal cancer [133]. None of the group had hepatic metastases and were followed up for one year, during which 18 patients developed hepatic metastases. Every one of these 18 patients had an elevated HPI prior to resection of their primary leading the authors to conclude that calculation of HPI can detect occult liver metastases.

Using Doppler ultrasound, Leen et al (1991) also found that altered hepatic perfusion in patients undergoing resection for colorectal cancer had the potential to detect metastases below the limits of conventional imaging techniques [137;138]. However, both approaches suffered from poor reproducibility when other centres attempted to validate them[141]. As a result, neither approach has been accepted into routine medical practice.

We have developed a novel technique using contrast enhanced ultrasound that reliably measures hepatic perfusion. Using this technique the principal aim of this chapter was to document perioperative hepatic perfusion changes in patients undergoing curative partial hepatectomy for colorectal liver metastases. The secondary aim was to assess any relationship between perioperative hepatic perfusion and long-term patient outcomes.

3.4.2 Patients and methods

17 consecutive patients (10 female, 7 male) with a mean age 68 years (range 42 – 82 years, SD 10.1 years) undergoing partial hepatectomy for pathologically confirmed colorectal liver metastases were recruited from one specialist colorectal centre. Ethical approval was obtained from the local research and ethics committee and all patients gave their written informed consent. Using contrast enhanced ultrasound (CE-US), serial measurements of hepatic perfusion were taken: pre-operative; intra-operative; post-operative days 1, 2 and 3 and at 3 months.

Hepatic perfusion technique consisted of fundamental unenhanced ultrasound (5-2Hz probe; HDI 5000 scanner, Philips, Bothell, USA) to target a large area of hepatic parenchyma (region of interest $1000mm^2$-$2000mm^2$). This area excluded the portal vein, hepatic artery and visible metastases and was maintained as a static image throughout a 45 second loop of CE-US (peripheral bolus injection of 2.4mL SonoVue® with 2mL saline flush; pulse inversion harmonic imaging and mechanical index 0.06-0.08). Signal-time intensity curves were derived by Q-Lab® (Quantification software, Philips, Bothell, USA). Three parameters were selected to allow data interpretation: area under the curve (AUC), peak intensity and peak gradient calculated for each curve. The time period for area under the curve was from 1.0s to 19.5s, a time period that has been shown to be significant from previous analysis (chapter 3). Peak intensity was the highest contrast uptake recorded within 20 seconds after contrast administration, a time period found to be significant from previous work (chapter 3). It was expressed as a percentage change from baseline [peak intensity = (peak intensity/ intensity at time zero) x 100]. Peak gradient was the highest gradient calculated during the early phase of the time intensity curve (from 0s to 10s).

Medians (interquartile range) were calculated and Wilcoxon's signed ranks test performed to compare variables on each post-operative day with pre-operative values.

All patients were followed up regularly as part of the routine surveillance performed by the specialist centre. CT and/ or MRI scanning was/ were performed at 6 months then at yearly intervals up to 5 years after their partial hepatectomy. If patients became symptomatic, then earlier investigations were instigated. Each patient's disease free survival and overall survival were recorded with comparison made to perioperative hepatic perfusion.

3.4.3 Results

AUC experienced a decrease intra-operatively (242.7dB.s vs 181.5dB.s, p=0.07), increased significantly on post-operative day 1 (242.7dB.s vs 262.8dB.s, p=0.025) and remained elevated on post-operative days 2 and 3 (242.7dB.s vs 255.4 dB.s, p=0.056 and 248.6 dB.s, p=0.036 respectively). AUC returned to pre-operative values at 3 months (242.7dB.s vs 221.4 dB.s, p>0.05). Figure 32 graphically displays the changes.

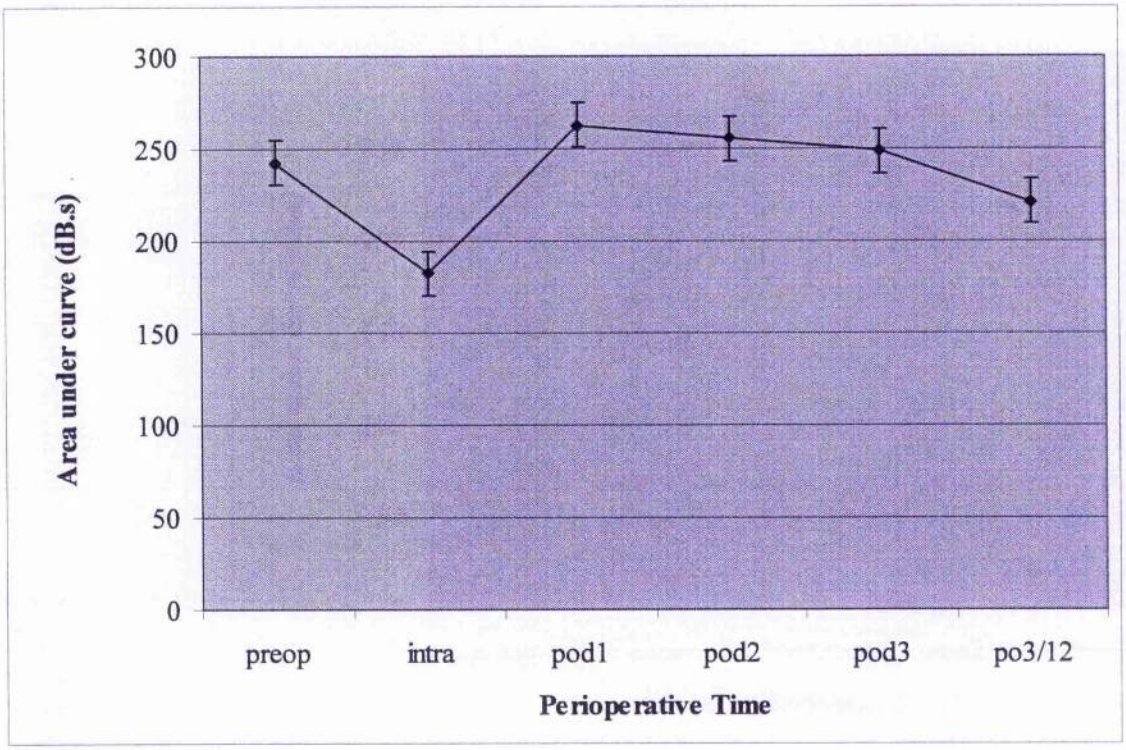
Peak intensity experienced a significant decrease intra-operatively (835.0dB vs 384.4dB, p=0.001), returned to pre-operative values on day 1 and remained unchanged throughout the series (Figure 33).

Peak gradient decreased intraoperatively, but not to significance (2.32dB/s vs 2.15dB/s, p>0.05). It remained unchanged through the remainder of the study time points (Figure 34).

The median time of follow up was 16.2 months (range 3.0 – 27.2 months). Six patients developed recurrence: liver metastases in 5 patients and lung metastases in the remaining patient. During follow up, only one patient with confirmed recurrent liver metastases had died due to progression of their disease. Median time to hepatic recurrence was 11 months (95% CI 0.9 – 21.1) with an overall survival at 2 years of 87.5%.

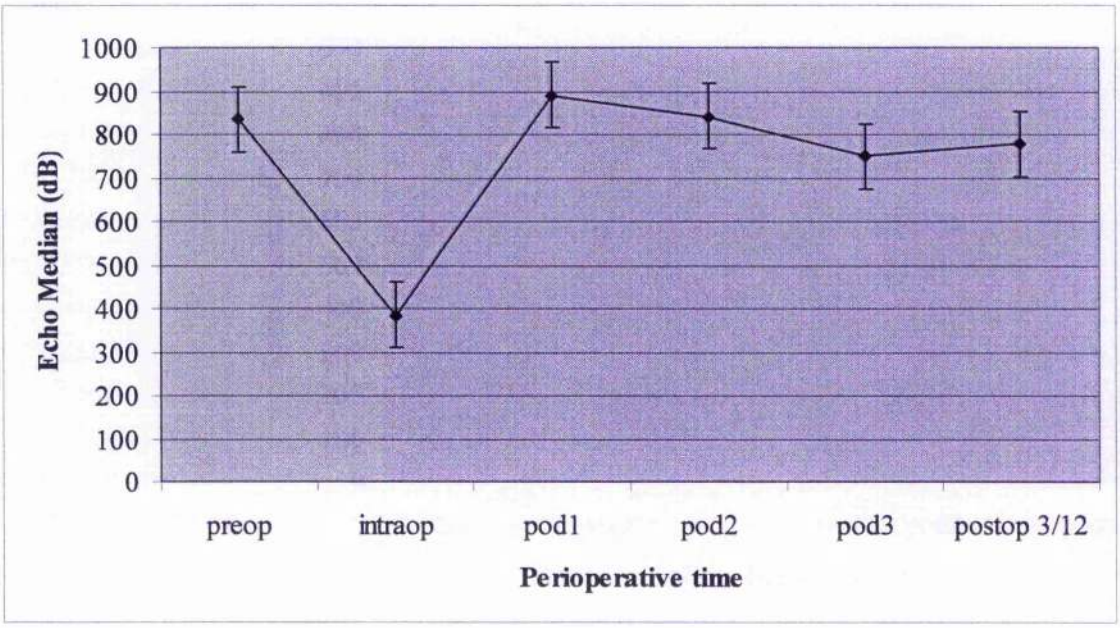
Comparison of hepatic perfusion between patients that had local recurrence (n=5) and those that were disease free (n=11) found no significant differences in AUC and peak gradient throughout the perioperative time period. In relation to peak intensity, there were no differences between the groups preoperatively and on postoperative days 1 to 3. However, at 3 months the recurrence group had a significantly greater peak intensity than the patients that had not recurred (1269.9 dB vs 505.7 dB, p=0.003), shown by figure 35.

Figure 32: Perioperative changes in area under the curve (AUC) in patients undergoing curative partial hepatectomy for colorectal liver metastases.



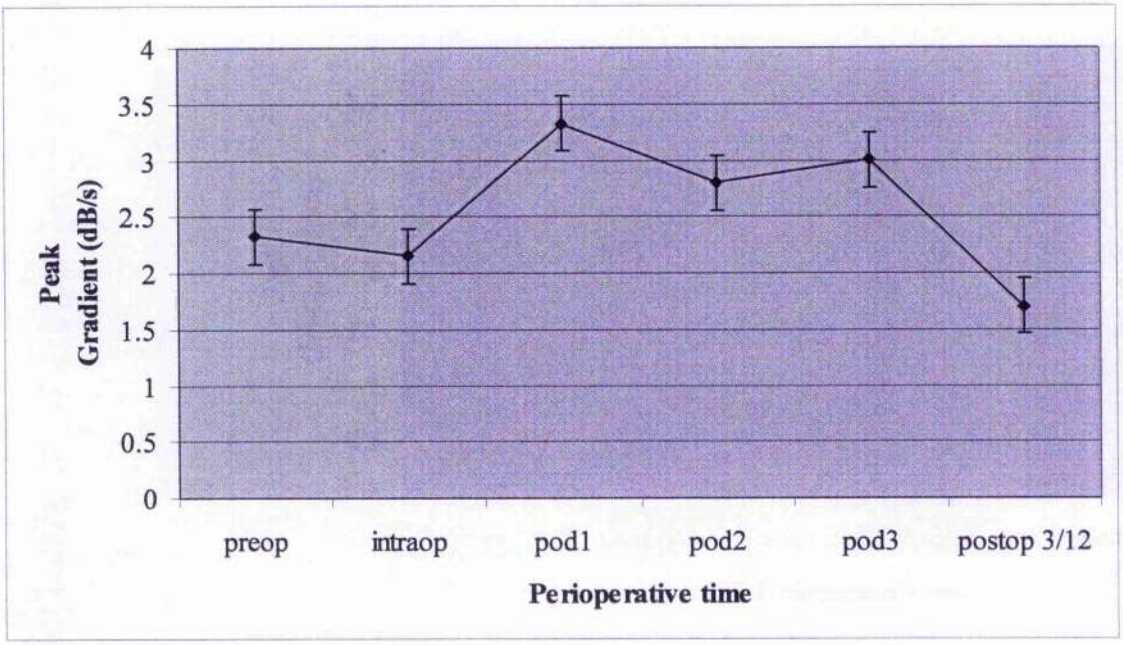
Results expressed in dB.s and displayed as median (standard error)

Figure 33: Perioperative changes in Peak Intensity in patients undergoing curative partial hepatectomy for colorectal liver metastases.



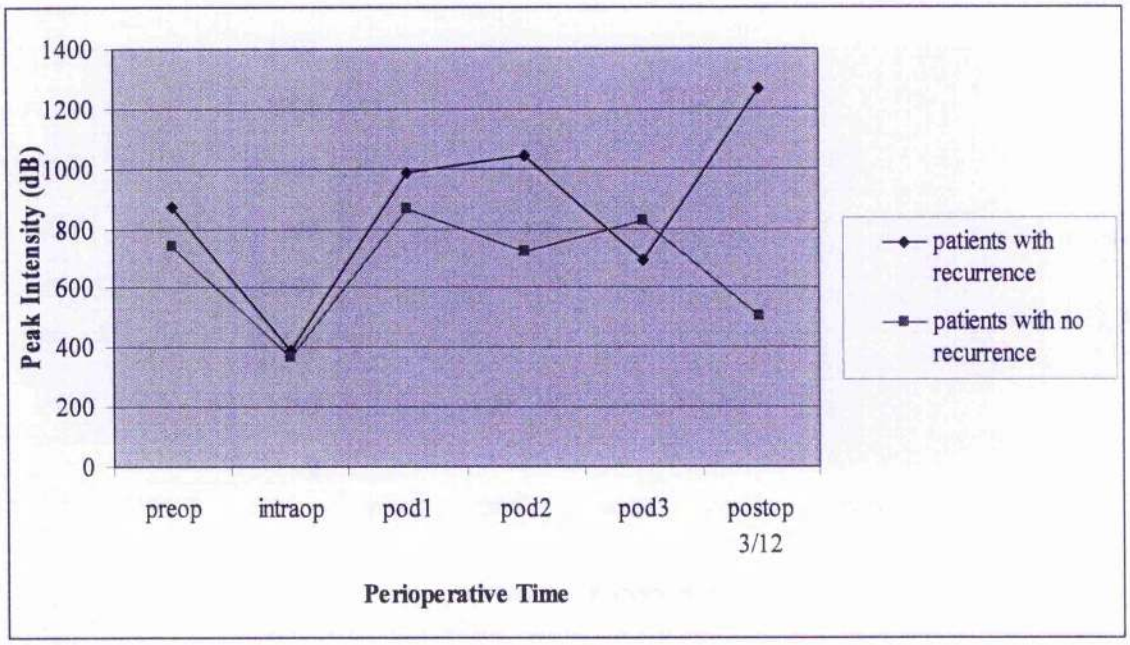
Results expressed in percentage change in decibels and displayed as median (standard error). See methods for full explanation.

Figure 34: Perioperative changes in Peak Gradient in patients undergoing curative partial hepatectomy for colorectal liver metastases.



Results expressed in dB/s and displayed as median (standard error)

Figure 35: Comparison of perioperative changes in peak intensity in patients with and without locally recurrent metastatic disease.



Results expressed in percentage change in decibels and displayed as median (standard error). See methods for full explanation.

3.4.4 Discussion

This is first study to document perioperative hepatic perfusion in patients undergoing curative hepatic resection for colorectal liver metastases using contrast enhanced ultrasound.

Of the three parameters, AUC experienced the most fluctuation during the time period studied. The first change happened intraoperatively and this decrease in AUC was accompanied by decreases in both peak intensity ($p>0.05$) and peak gradient ($p<0.05$). It is likely that these changes are a result of the general anaesthetic. Indeed, all patients had anaesthetic agents administered that are known vasodilators, decreasing blood pressure and subsequently, hepatic perfusion. Furthermore, all were intubated and were receiving positive pressure ventilation that has previously been shown to destruct the microbubbles[351].

Whilst the other two parameters returned to normal on postoperative day 1, AUC continued to increase up to day 3. This may be a reflection of the acute systemic inflammatory response syndrome (SIRS), increasing the blood supply to the liver remnant. This response to surgery is physiological, indicating an adequate host response to surgical trauma and is well documented in the literature in patients undergoing various types of major surgery [352-354]. If the postoperative recovery is uncomplicated, then SIRS declines over the following weeks. Indeed, AUC had returned to preoperative values by the follow up scan performed at three months.

To assess if hepatic perfusion measured by CE-US had the capacity to predict occult hepatic metastases, the three parameters were compared between two groups: disease free versus the group that had disease recurrence. AUC and peak gradient were not

statistically different between the groups at any of the time points. However, peak intensity was found to be significantly higher at 3 months after surgery in the patients that had recurred than in the disease free group. This allows the tentative suggestion that peak intensity may have a potential predictive role although the small patient numbers disallow any stronger conclusions.

AUC is easily defined and calculated and may well be the most sensitive parameter of quantifying perioperative hepatic perfusion using CE-US. Perioperative changes in AUC are likely to reflect the perioperative SIRS, and future work analysing blood markers of SIRS (e.g. C-reactive protein and interleukin-6) would provide further information. Unfortunately, AUC does not appear to be indicative of occult hepatic disease. This could be a reflection of the small study numbers and certainly increased recruitment would allow definite conclusions to be drawn. In addition, it is still not understood what aspect of hepatic perfusion AUC is representing. It may well be blood volume or capacitance and if so, it is not known if this is the best way to interpret hepatic perfusion with CE-US. As discussed in the previous chapter, the definition and quantification of hepatic perfusion using CE-US needs further research to improve our understanding of the complex interactions between the microbubbles, hepatic parenchyma and computer technology.

This study has documented perioperative changes in hepatic perfusion using CE-US. Increased numbers with longer follow up are required in addition to improved understanding of the interpretation of CE-US to allow definite conclusions to be drawn.

Section 3.

Chapter 5: Short and long term outcomes in patients with colorectal liver metastases undergoing partial hepatectomy with a novel triphasic approach.

3.5.1 Introduction and aims

Traditionally, hepatic resection has been associated with a significant risk of intraoperative haemorrhage with some authors documenting losses up to 10 litres[237]. Improved surgical and anaesthetic techniques have evolved over the last decades, reducing the risk of haemorrhage. However, with recent research continuing to document intraoperative blood loss and subsequent blood transfusions as major determinants of patient morbidity and mortality after hepatic resection, the ideal aim of bloodless hepatic resection remains elusive[218;235,237;355;356].

Since Hogarth Pringle (1908) first demonstrated that hepatic inflow vascular occlusion could minimise hepatic bleeding, several vascular occlusion techniques have evolved: hepatic inflow with or without outflow occlusion; total or selective and intermittent or continuous[244;246]. Each technique varies in level of technical difficulty, prevention of haemorrhage, intraoperative patient tolerance and hepatic tolerance, with the risk of hepatic failure being foremost[246]. Indeed, with the documented incidence of hepatic dysfunction and/ or failure being as high as 18%, some surgeons have disregarded hepatic occlusion techniques altogether [247;248].

Any of the aforementioned surgical techniques can be combined with low central venous pressure (CVP) anaesthesia. Maintenance of CVP less than 5cmH_20 has been shown to minimise back bleeding, intraoperative blood loss and subsequently, blood transfusions when compared to higher intraoperative CVP[226;242;243]. However, maintenance of a low CVP intraoperatively can be problematic, requiring administration of intravenous nitroglycerins that could result in haemodynamic instability. Furthermore, prolonged low CVP may lead to renal hypoperfusion with subsequent permanent renal compromise.

As surgical and anaesthetic techniques have evolved and improved patient outcomes, the traditional patient selection criteria for partial hepatectomy have extended. As a result, there has been interest in developing a prognostic score with the Clinical Risk Score having received the most attention[198]. The CRS allocates 1 point for each of the following: node positive primary; disease free interval <12 months; >1 tumour; tumor size >5cm and CEA >200ng/mL. Patients scoring 0-2 have the best outcomes and should be considered for resection. In contrast, patients scoring 4-5 have poorer outcomes and surgery should be carefully considered alongside adjuvant therapies. This clinical risk score has recently been validated by two other centres and requires further research in different populations to determine its widespread applicability[206;357].

The presence of an elevated systemic inflammatory response has been shown to predict reduced disease free survival and long term survival in several different types of malignant disease, including primary colorectal cancer[29;32-38]. The exact mechanism behind the increased C-reactive protein is unclear, and to date no studies have assessed any prognostic value elevated C-reactive protein may have on metastatic colorectal cancer.

The primary aim of this chapter was to evaluate patient outcomes after partial hepatectomy for colorectal liver metastases with a novel triphasic approach: preoperative dehydration; intraoperative low CVP anaesthesia and selective continuous vascular occlusion. Particular attention was paid to blood loss, hepatic function and renal function. The secondary aim was to determine the prognostic value of the clinical risk score in our population and to compare it to any prognostic influence C-reactive protein may have.

3.5.2 Patients and methods

Patients

From 1998 to 2006, 91 consecutive patients were referred to Glasgow Royal Infirmary to undergo planned partial hepatectomy for colorectal liver metastases. Ethical approval was obtained from the local research and ethics committee of North Glasgow University Hospitals Trust and all subjects gave their written informed consent.

All operations were performed in one surgical unit by a single consultant surgeon and anaesthetist. All patients underwent routine cardiorespiratory assessment to assess fitness for general anaesthesia. The only absolute contraindication to surgery was extrahepatic disease. Patients being considered for resection underwent radiological imaging within 8 weeks of their planned operation: transabdominal ultrasound; contrast enhanced computed tomography (of chest, abdomen and pelvis) and/or magnetic resonance imaging. Intraoperatively, unenhanced and contrast enhanced ultrasonography were routinely performed to confirm preoperative staging and to ensure satisfactory resection margins.

Methods

Anaesthetic technique

Preoperatively, patients were given a light meal 12 hours before their operation, and then fasted. They received their routine medication, including diuretics, and 3 litres of oral bowel preparation (Klean Prep, Norgine Ltd, Harefield, Middlesex, UK). No intravenous

fluids were administered unless the patient was diabetic in which case they received intravenous 5% dextrose at 83mL/hour with insulin administered via a sliding scale.

In the anaesthetic room, intravenous and intra-arterial access was achieved. An epidural was inserted at the level of T9/10 and the patient given 12-15 mL of 0.5% levobupivacaine. Pre-intubation, patients were pre-oxygenated with 100% oxygen and given 150-200μg of fentanyl. Anaesthesia was induced with a propofol infusion at a rate of 1.5-2.0 μg/mL and increased as required. Once unconscious, 35-50mg of rocuronium was administered and the patient was then intubated.

During surgery, general anaesthesia was maintained by propofol infusion with boluses of rocuronium or 0.5% levobupivacaine via epidural if required. Patients were monitored by: electrocardiogram (ECG); intra-arterial blood pressure; fraction of inspired oxygen (FiO_2); pulse oximetry ($Sp0_2$); CVP; tidal volume; respiratory rate; nasopharyngeal temperature and blood loss.

Intraoperative fluid management was divided into two phases: hepatic and posthepatic. The hepatic phase began at the induction of anaesthesia and finished when the specimen was removed with full haemostasis. During this phase 65mL/hr of Hartmann's solution was administered and urine output was recorded, but did not influence fluid management. There was no administration of mannitol or dopamine. Metaraminol (0.5-1.0mg) or ephedrine (3-9mg) boluses were given if required for prolonged hypotension (systolic blood pressure less than 90mmHg). CVP was maintained less than $5cmH_20$, without administration of intravenous nitroglycerins. After delivery of the specimen, the posthepatic phase began with 500-1000mL of intravenous crystalloid (Hartmann's

solution) administered to increase CVP followed by 500mL of colloid (Gelofusine) prior to the patient leaving theatre.

Postoperatively, the propofol infusion was discontinued when the abdomen was closed and the patient was then extubated. The patient spent a short time in recovery where the urine output was monitored. Intravenous fluids were administered as alternate 0.9% saline and 5% dextrose at a rate of 83-100mL/hr and adjusted to maintain urine output greater than 30mL/hr. Patients were then transferred to the surgical high dependency unit where the epidural infusion was continued for 3 days (0.125% bupivacaine/diamorphine 50μg/mL, rate of 8-12 mL/hr as required).

Surgical technique

The technique for hepatic resection with vascular inflow occlusion has been described previously[358;359]. Patients were placed in the 15° head-down tilt position to obviate the risk of air embolism. Bilateral subcostal laparotomy with a xiphoid extension as appropriate was performed to provide surgical access. The liver was mobilised by division of the appropriate triangular ligaments and the vena cava exposed. Selective continuous vascular control was achieved without dissection by application of a vascular clamp to the appropriate left or right portal structures (Half-Pringle)[358]. This occlusion resulted in a visible demarcation line along the proposed line of resection for a hemihepatectomy that was marked on the liver surface by diathermy [Figures 36, 37, 38]. The appropriate right or left hepatic vein outflow (including the middle vein if back bleeding was present after clamping of the left vein) was occluded with a bulldog clamp. Parenchymal dissection was performed by an ultrasonic dissector (CUSA, Valley Lab,

London, UK)[359]. Bridging vessels and bile ducts were repeatedly electrocauterised using Ligasure (Autosuture, United States Surgical Corp., Norwalk, CT, USA) and divided with scissors. Large vessels were controlled with suture, clip or vascular stapling device. After removal of the specimen, the exposed edges were sealed by argon beam coagulation (CONMED Corporation, Utica, NY, USA) to achieve haemostasis. Thereafter, inflow and outflow occlusion was removed.

Outcome variables and statistical analysis

Patients' casenotes and the prospective surgical hepatic database were analysed for: age, sex and American Society of Anaesthesiologists grading (ASA). The type of hepatic resection was classified as: right lobectomy (Couinaud's segments[258] V, VI, VII and VIII ±I); left lobectomy (segments II, III and IV ±I); trisegmentectomy (3 segments); bisegmentectomy (2 segments); monosegmentectomy (1 segment) and subsegmentectomy/ wedge (<1 segment). For RF ablation, the number of segments ablated and their position were recorded.

Thirty day morbidity and mortality were recorded and to standardise reporting a published grading system was applied[360]. Grade 0 represents no complications. Grade 1 complications resolve spontaneously or with minimal intervention i.e. antibiotics, bowel rest. Grade 2 requires moderate intervention i.e. intravenous medication, chest drain insertion. Grade 3 graded complications require surgical or radiological intervention or readmission to hospital. Grade 4 is scored when patients are left with a long standing disability, organ resection or enteral division. Grade 5 is death. Overall, minor complications are grouped as grades 1 to 2 and major complications 3 to 5.

Figure 36: Selective placement of a vascular clamp to the portal pedicle allows selective control of the hemi-liver for resection with visible demarcation.

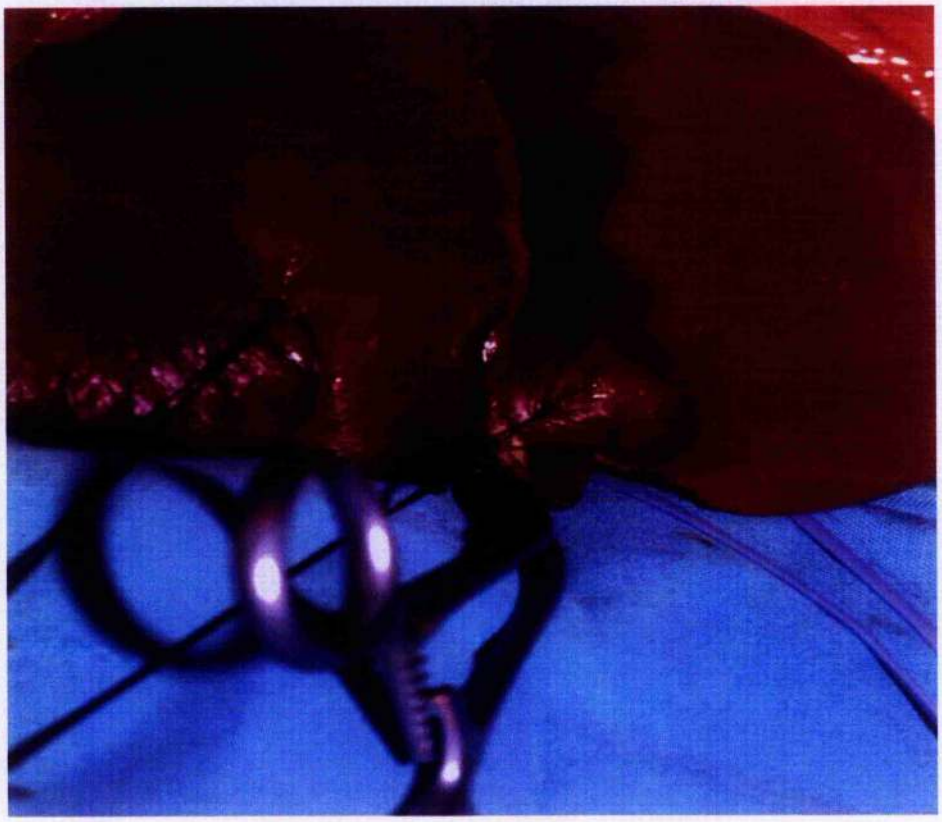


Figure 37: Doppler image illustrating normal flow from both sides of the liver before clamp application.

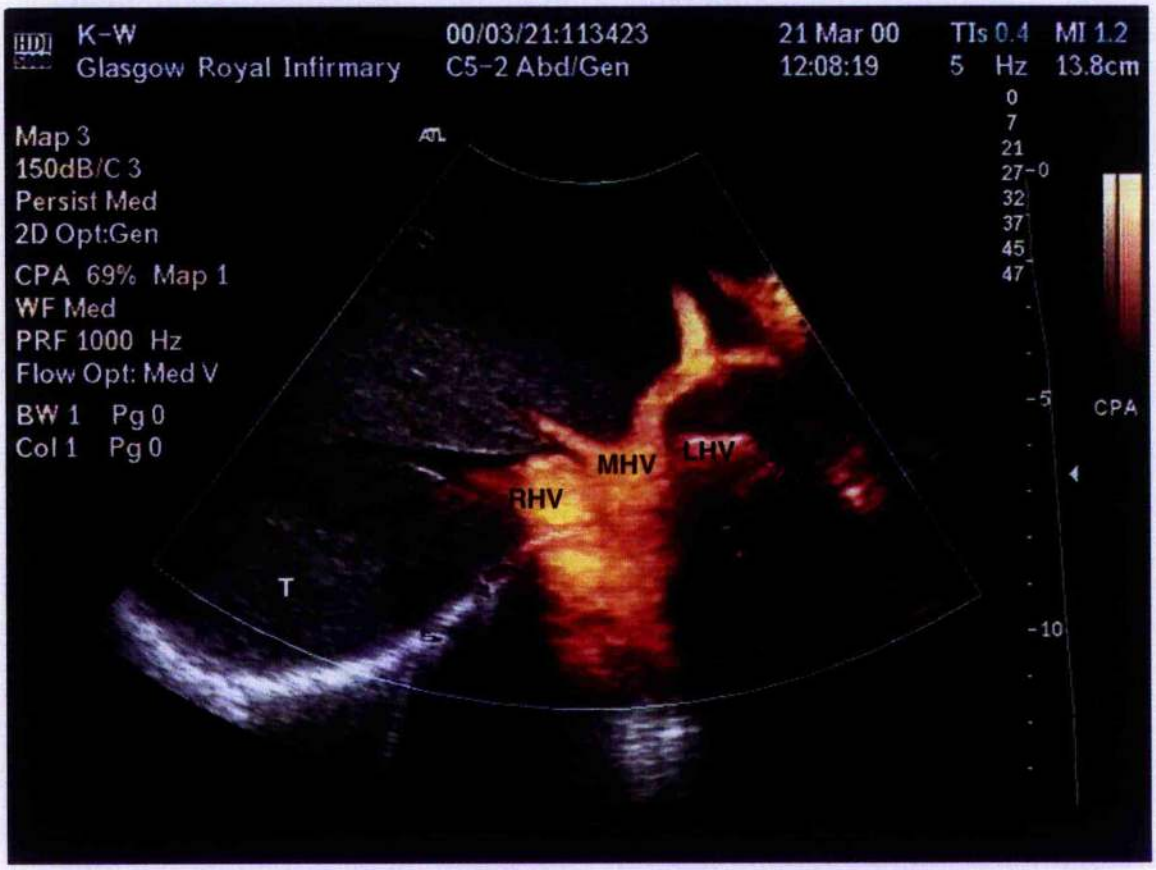
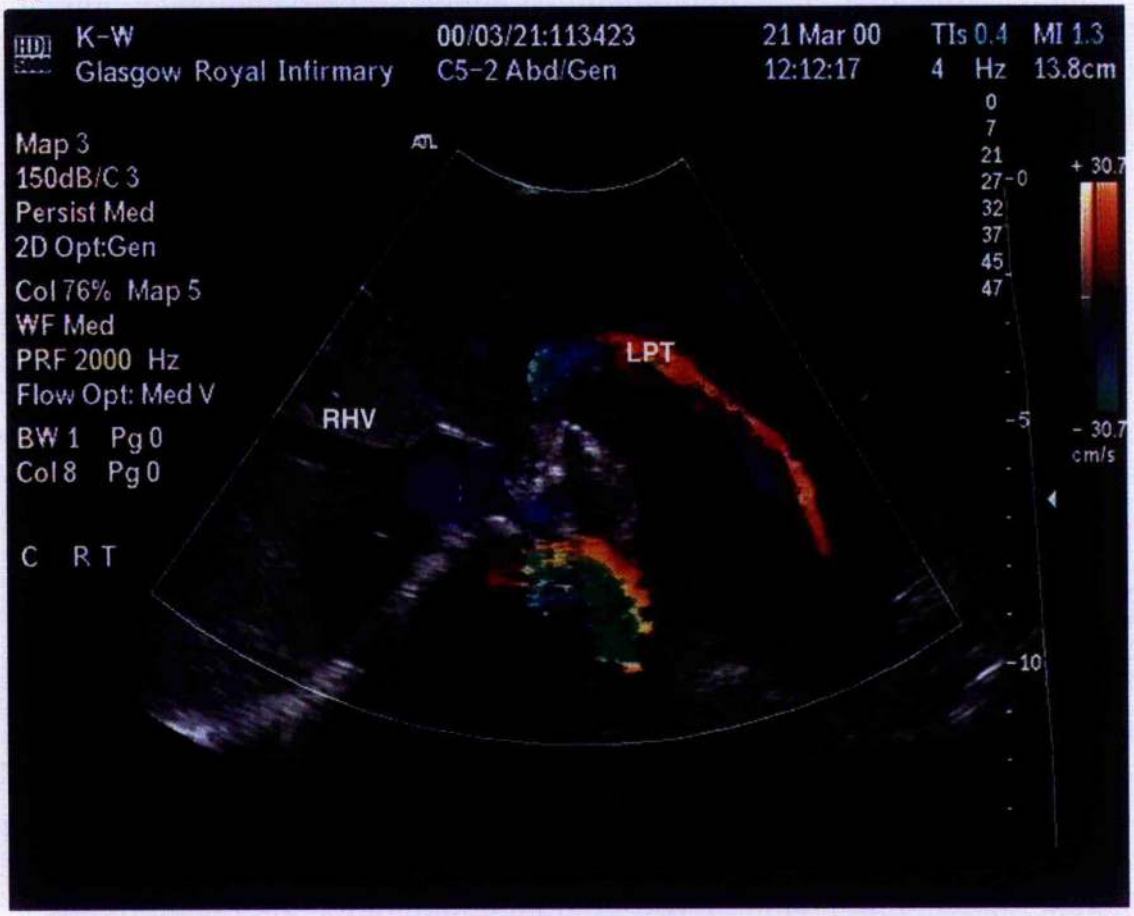


Figure 38: Doppler image showing no flow from the right liver following clamp application in same patient as Figure 37.



Intraoperative blood loss was measured by adding the suction bottle volume to the increase in weight of surgical packs/swabs. The indication for intraoperative blood transfusion was determined by haemodynamic instability and/or myocardial ischaemia in the presence of significant blood loss (>1000mL). Postoperative blood loss was taken as the difference between preoperative and postoperative day one haemoglobin. Patients were transfused in concordance with post-operative hospital guidelines which are based on SIGN guidelines[232]. In those patients undergoing synchronous colonic resection or radiofrequency ablation, blood loss included the loss from both these procedures.

To determine perioperative hepatic function after selective continuous vascular occlusion, serial measurements of liver function in the last 50 patients with colorectal liver metastases were analysed. Blood samples were taken: preoperative; postoperative days 1 to 7 and one follow up sample at 4-6 weeks. Samples were analysed for: hepatic parenchymal enzymes (aspartate transaminase, AST; alanine transaminase, ALT; alkaline phosphatase, Alk Phos; gamma-glutamyltransferase, GGT); hepatic physiological markers (bilirubin) and hepatic synthetic markers (prothrombin time, PT; activated partial thromboplastin time, APTT; thrombin time, TCT; albumin; total protein).

Hepatic function variables on each postoperative day were compared with preoperative values using Wilcoxon's signed rank test. Comparisons between patients undergoing major resections (at least 3 lobes resected) and those undergoing minor hepatic resections were performed using the Mann-Whitney test. Statistical significance levels are reported without adjustment for multiple comparisons to err on the side of over- rather than understating changes of possible clinical significance.

Perioperative renal function was initially assessed retrospectively in 50 consecutive patients undergoing partial hepatectomy. Intraoperative urine output was recorded and serial blood sampling of serum urea and creatinine taken: 24 hours preoperatively; postoperative days 1, 2 and 3. After analysing the data, a prospective controlled trial was performed to assess changes in glomerular filtration rate (GFR) in patients undergoing partial hepatectomy with perioperative fluid restriction and low intraoperative CVP. Twenty nine consecutive patients undergoing hepatic resection for colorectal liver metastases (preoperative bowel preparation with no supplementary intravenous fluids plus intraoperative CVP <5cmH_20 = fluid restricted group) were compared to thirty controls undergoing colonic resection for primary colorectal cancer or diverticular disease (preoperative bowel preparation with supplementary intravenous fluids and no fluid restriction intraoperatively = not fluid restricted group). Renal function was again assessed by serial blood sampling: 24 hours and 1 hour preoperatively; 6 hours postoperatively; postoperative days 1 to 5 and at 3 months. Samples were analysed for: urea; creatinine; serum osmolality; beta 2 microglobulin and cystatin C.

The most commonly used marker of GFR in clinical practice is plasma (or serum) creatinine. Measurement of creatinine is performed routinely by biochemistry laboratories and is convenient as well as being low in cost. Unfortunately, plasma creatinine has limitations. Not only is there documented variation in measurements on the same sample between laboratories, but creatinine levels can vary considerably between individuals with levels influenced by gender, age, body composition and dietary factors[361;362]. Furthermore, when renal function changes rapidly, (e.g. after ischaemic acute renal failure), creatinine

shows a poor correlation with glomerular filtration rate (GFR) reducing its clinical applicability [363].

Beta-2 microglobulin and cystatin C are low molecular weight proteins that do not appear to have the same limitations as creatinine in the measurement of GFR. Beta 2 microglobulin is present on the surface of all nucleated cells and becomes elevated in proximal renal tubular injury and according to two recent reports, may be superior to creatinine for estimation of GFR[364;365].

Cystatin C is metabolised by the proximal renal tubular epithelial cells and has a relatively constant plasma concentration that is independent of gender and age. Again, there is evidence that cystatin C is more sensitive in detecting a reduction in GFR than creatinine in patients with renal diseases and those that have undergone renal transplanation[366;367].

Patients were followed up as part of the routine surveillance programme performed by the surgical department: clinic appointments at 3 and 6 months postoperative, then further clinic appointments with surveillance CT scans at yearly intervals up to 5 years after their operation. This follow up allowed time to local recurrence and long term survival to be calculated (Kaplan-Meier analysis, SPSS for Windows, SPSS Inc., Chicago, Illinois, USA).

All patients had their clinical risk score calculated prior to undergoing partial hepatectomy. One point was given for each of the following factors: node positive primary tumour; preoperative carcinoembryonic antigen (CEA) >200ng/mL; number of liver tumours >1; largest liver tumour >5cm and disease free interval from primary colorectal surgery to metastases <12 months. The primary outcome measure was survival.

Routine preoperative laboratory measurements of C-reactive protein and albumin were recorded in all patients. The limit of detection of the C-reactive protein assay was <6mg/l. The coefficient of variation, over the range of measurement, was less than 5% as established by routine quality control. The Glasgow Prognostic Score (GPS) was calculated as previously described [368]: one point for C-reactive protein >10mg/L and one point for albumin <35g/L. Patients that had both abnormalities present were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1 and a score of 0 was given when both parameters were within normal limits. The primary outcome measure was survival.

For both prognostic scores, multivariate survival analysis was performed using a stepwise backward procedure to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.10. Analysis was performed using SPSS software.

Synchronous bowel resection and/or hepatic procedures

In patients with synchronous disease, the colon or rectum was resected prior to the hepatic resection. In patients with multiple liver metastases, the hepatic resection was performed first, then radiofrequency ablation (RF ablation). Each patient that had undergone a synchronous procedure was then case matched according to liver procedure with a patient who had undergone partial hepatectomy only. Comparison between groups was made in relation to: patient demographics; blood loss; morbidity; mortality; hospital duration; recurrence and overall survival.

3.5.3 Results

Patient demographics

51 men and 40 women, mean age 63.8 years (range 22-82), underwent hepatic resection in this series. The median ASA grade was 2 (range 1 to 3). Of the 91 patients, 80 (88%) underwent their planned hepatic procedure with no alteration in management. The remaining 11 patients did not undergo hepatic resection due to more advanced disease found at laparotomy: 7 patients had their procedure abandoned and 4 underwent open RF ablation. These eleven patients were excluded from further analysis.

Type of hepatic resection performed

The types of hepatic resections performed are displayed in Table 10. It can be seen that 51% were major resections with concomitant extra and intrahepatic procedures performed in 32 patients. Synchronous bowel resection and/or RF ablation accounted for the majority of the synchronous procedures (n=28) with the remaining 4 cases: excision of diaphragm; splenectomy; closure of ileostomy and choledochotomy with extraction of stones. Cholecystectomies were not included.

Postoperative complications

Table 11 displays the type, number and grade of complications recorded after 80 partial hepatectomies. Postoperative complications occurred in 19 patients (24%) with no mortality recorded. Cardiac complications were the overall commonest complications with new onset atrial fibrillation requiring intravenous medication in all cases. In addition, one patient developed cardiac failure that required a period of intubation and ventilation in

intensive care. The most frequent surgery-related complication were bile leaks (n=5) all of which were managed conservatively. One patient had transient hepatic dysfunction developing jaundice that resolved spontaneously during their hospital admission. Only one patient was readmitted to hospital. She presented with severe abdominal pain after an uneventful postoperative recovery that was secondary to pneumonia and subsequently resolved with appropriate intravenous antibiotics. Overall, the majority of complications were classified as minor (n=16; 84%).

Postoperative blood loss and transfusion rate

Median blood loss at surgery was 400mL (range 50 - 3000) with a mean blood loss of 535 ± 465mL. Ten patients had a blood loss greater than 1000mL with only three patients losing more than 1500mL. No patient was transfused intraoperatively and no patient returned to theatre with postoperative haemorrhage. Major resections experienced a significantly greater blood loss than minor resections (500mL *vs* 400mL; Mann Whitney p=0.02). The mean decrease in haemoglobin on postoperative day 1 was 2.0 g/dL (SD 1.2) and three patients received units of homologous concentrated red cells (1, 2 and 4 units respectively) after dropping their haemoglobin and developing myocardial ischaemia on ECG monitoring.

Perioperative hepatic function

Within the group of 50 patients undergoing resection, no incidence of hepatic failure was recorded. Four time points selected to provide an adequate description of changes in all hepatic function variables, are shown in Table 12.

On postoperative day 1, AST and ALT levels rose dramatically, falling rapidly on postoperative day 2 and returning to normal clinical levels by day 7 and follow up respectively. Alk Phos and GGT had biphasic responses, falling significantly on day 1, recovering to cross the baseline on days 3 and 4 then continuing to rise to peak at day 6. They started to decline on day 7 and reached normal values at follow up. Bilirubin peaked on day 1 then returned to normal range on day 2, remaining within normal limits during the remainder of the study. Figure 39 graphically displays the key changes. PT and APTT fluctuated during postoperative period, but remained within normal clinical ranges throughout. Albumin and total protein fell immediately postoperatively, remaining low for next 6 days [Figure 40]. By the end of the first week both had resumed normal levels.

Comparison of hepatic function changes between major and minor resections found greater increases in AST and ALT on postoperative days 2 to 7 ($p<0.05$) after major resections. Although there was no difference in alkaline phosphatase between the two groups, GGT was significantly higher in the major resection group on day 7 ($p=0.016$). There were no other significant differences between the two groups.

Perioperative renal function

Preoperatively, six of the 50 patients had pre-existing mild renal impairment (urea 8-12mmol/L, creatinine 130-230mmol/L). During the hepatic phase, the median urine output was 28.4 mL/hr (range 13.3 - 40.0) with a median CVP of 4 (range 0 - 11). No patient required intravenous vasodilators and metaraminol or ephedrine boluses were administered in only 10 patients. The median urine output during the first postoperative hour was 110mL (range 30 - 370). Postoperatively the median serum urea and creatinine

values fluctuated but remained within normal limits. Of the six patients that had mild renal impairment preoperatively, five had improved urea and creatinine postoperatively whilst the remaining patient returned to preoperative values.

In the prospective trial, the two groups were age matched: fluid restricted patient group mean age 61.6 years, S.D. 12.5 and control unrestricted group mean age 62.2 years, S.D. 17.4. Preoperatively, 1 patient and 5 controls had mild renal impairment (urea 8-12mmol/L, creatinine 130-230mmol/L). Both groups experienced transient fluctuations in all parameters when compared to their 24 hour preoperative values with the median value at each time point remaining within normal limits for each parameter. Figures 41-44 graphically display the perioperative changes in urea, creatinine, beta-2 microglobulin and cystatin C in both groups. No patient or control developed renal failure and at 5 days postoperatively, only 1 patient and 1 control had mild renal impairment.

There were few differences in all parameters between the fluid restricted group and the unrestricted group. Beta-2 microglobulin was greater in the control group 24 hours preoperatively: median 2.2 (range 1.5-3.5) versus median 2.0 (range 1.3-5.4) [p=0.034]. Serum urea at 6 hours postoperatively was significantly higher in the fluid restricted patient group: median 5.1 (range 2.2-8.0) versus median 4.2 (range 1.5-10.1) [p=0.019]. At 3 month follow up, cystatin C was elevated in the control group: median 1.13 (range 0.8-1.6) versus median 0.92, (range 0.7-1.9) [p=0.019]. For all three parameters, the medians were within normal laboratory limits.

Disease free and overall survival

Of the 80 patients that underwent partial hepatectomy for colorectal liver metastases, 70 were pathologically confirmed curative procedures. Minimum follow up in those patients was 3 months with a median follow up of 44 months. Within this curative group, 33 patients (47%) had recurrent disease during follow up with the majority recurring in the liver (n=25) in the liver. The Kaplan-Meier estimate of the median time to local recurrence was 514 days (17.1 months). Median overall survival was 1307 days (43.6 months) with 1, 3 and 5 year survivals of 91.7% (95% CI 84.7-98.7%), 50.4% (95% CI 33.8-67.0%) and 25.6% (95% CI 7.3-43.9%) respectively. Figure 45 displays the survival curve.

Clinical Risk Score

Of the 70 patients that had pathologically confirmed curative procedures, 68 had their Clinical Risk Score calculated (CRS). The CRS were then divided into 3 prognostic groups: good prognosis scores 0-1; intermediate prognosis scores 2-3 and poorer prognosis scores 4-5. On univariate analysis, CRS was found to be a significant predictor of survival (hazard ratio 2.9, 95% CI 1.16-7.19%; p=0.026). Figure 46 displays the survival curves for the 3 groups. On univariate analysis of the individual components of the CRS, tumour size was found to be a significant predictor of survival with preoperative CEA level and disease free interval displaying a trend towards significance (Table 13). Node positive primary and tumour number were found not to be significant. All 5 variables underwent multivariate analysis with tumour size found to be the only significant independent predictor of

survival. Again, CEA level and disease free interval displayed trends towards significance (Tables 14).

Glasgow Prognostic Score

Within the curative hepatic resection group, 56 patients had their Glasgow Prognostic Score (GPS) calculated. The majority of patients scored a GPS of 0 (n=34) with 16 patients scoring 1 and 6 patients scoring 2. In all patients scoring a GPS of 1, an elevated C-reactive protein was found. In the 6 patients with hypoalbuminaemia, all had an elevated C-reactive protein and subsequently a GPS of 2. On univariate survival analysis, GPS was not found to be significant (hazard ratio 0.93, 95% CI 0.50-1.74%, p=0.817). Individually, neither C-reactive protein nor albumin achieved significance (hazard ratio 0.50, 95% CI 0.16-1.60, p=0.242 and hazard ratio 2.23, 95% CI 0.54-9.17, p=0.265 respectively).

The level of C-reactive protein that indicated an inflammatory response was changed to ≥6mg/L. GPS changed to: 0 in 23 patients; 1 in 27 patients and 2 in 6 patients. Neither GPS nor C-reactive protein was found to be significant (p=0.586 and p=0.6 respectively, log rank test)

Synchronous procedures

Of the twenty eight patients that underwent bowel and/ or RF ablation in addition to partial hepatectomy, twenty seven were case matched according to liver procedure with twenty seven patients who had undergone partial hepatectomy only. The remaining case

was excluded as 3 segments of small bowel had been resected for metastatic peritoneal metastases.

Synchronous procedures performed were: bowel resection and partial hepatectomy (n=14); bowel resection, RF ablation and partial hepatectomy (n=8) and RF ablation and partial hepatectomy (n=5). Types of bowel resection performed were: anterior resection (n=12); sigmoid colectomy (n=4); left hemicolectomy (n=2) and right hemicolectomy (n=4). Major hepatic resections accounted for 4 out of the twenty seven cases (13%).

Comparison with the partial hepatectomy only group found both were age matched (mean age 64.7 *vs* 65.6 years; p=0.76) and sex matched (male n=14; female n=13 in each group). There were no significant differences in ASA grade and number of curative operations performed. Mean and median blood losses did not differ significantly between the synchronous group and the partial hepatectomy only group: mean 419mL (range 50-850mL) *vs* 514mL (range 50-3000mL), p=0.49 and median 400mL *vs* 373mL, p=0.60.

The synchronous group experienced higher 30 day morbidity with 9 patients developing postoperative complications: atrial fibrillation (n=3); bile leak (n=2); chest infection (n=1); wound infection (n=1); urinary tract infection (n=1) and ileus (n=1). The partial hepatectomy only group recorded 2 postoperative bile leaks (p=0.03). The majority of complications were grades 1 and 2 (n=10) with the remaining one being grade 3 due to hospital readmission for a chest infection in a patient that had undergone synchronous resection. All complications were managed either conservatively or with intravenous medication. No patient required further laparotomy, re-intubation or a period in intensive care. There was no recorded mortality. The synchronous group had longer duration of

hospital stay than the partial hepatectomy only group (median 11 *vs* 9 days respectively; p=0.04).

Liver recurrence occurred in 8 synchronous procedure patients and in 11 partial hepatectomy only patients with no significant difference in time to recurrence: synchronous group mean 15.1 months (95% CI 10.8-19.5 months) *vs* 20.9 months (95% CI 15.1-26.7 months; p=0.83). Only one patient in the partial hepatectomy group developed recurrence of the primary colonic tumour. Overall survival for both groups at 3 years was 43% (95% CI 17%-69%) with no significant difference between the synchronous and partial hepatectomy only groups: 59% *vs* 44% respectively, p=0.84 Kaplan-Meier log-rank test).

Table 10: Type of hepatic resections performed and associated synchronous procedures.

type of resection	number	synchronous procedures	type of synchronous procedure			
			bowel resection	RF ablation only	bowel resection plus RF ablation	other organ
right lobectomy	22	3	2	0	0	1
left lobectomy	18	6	3	3	0	0
trisegmentectomy	1	1	0	0	0	1
bisegmentectomy	14	10	3	1	4	2
monsegmentectomy	18	11	6	1	4	0
Subsegmentectomy /wedge	7	1	1	0	0	0
	80	32 (40%)	15	5	8	4

Table 11: Postoperative complications after 80 partial hepatectomies for metastatic colorectal cancer.

type of complication	Grade of complication					
	0	1	2	3	4	5
transient biliary leak		5				
arrhythmias/ myocardial infarction			6	1		
respiratory infection/ collection				2		
urinary retention/ infection		1				
transient hepatic dysfunction		1				
ileus		1				
wound infection			2			
Total number of complications	19 (24%)					

Table 12: Changes in hepatic function after partial hepatectomy in 50 patients with colorectal liver metastases.

	Preop	Day 1	Day 7	Follow up
Bilirubin (μmol/L)	10 (7, 14)	23 (16, 27)***	14 (9, 19)	12 (8, 16)
AST (U/L)	24 (19, 34)	260 (85, 409)***	37 (24, 50)***	27 (21, 40)
ALT (U/L)	21 (17, 28)	277 (95, 459)***	70 (39, 91)***	24 (17, 31)
Alk Phos (U/L)	228 (174, 285)	175 (130, 224)***	352 (293, 537)***	242 (195, 314)
GGT (U/L)	35 (21, 53)	32 (21, 55)*	135 (77, 192)***	50 (29, 75)
PT (s)	15 (14, 16)	17 (16, 18)***	16 (14, 17)	15 (14, 16)
APTT (s)	36 (34, 40)	36 (34, 40)	40 (38, 43)*	38 (34, 41)
TCT (s)	10 (10, 11)	11 (10, 12)*	11 (10, 12)	10 (10, 11)
Albumin (g/L)	43 (40, 45)	30 (28, 34)***	33 (31, 36)***	41 (37, 44)*
Total Protein (g/L)	72 (68, 75)	54 (48, 56)***	58 (52, 62)***	71 (66, 74)

Results shown as median (interquartile range).
*p<0.05, ***p<0.001 relative to preoperative value by Wilcoxon signed rank test.

Hepatic marker normal ranges:-
bilirubin 2-22μmol/L; AST 12-48U/L; ALT 3-55U/L; Alkaline phosphatase 80-280 U/L; GGT 5-90U/L; PT 12-16s; APTT 32-44s; TCT 10-13s; albumin 33-55g/L and total protein 62-82g/L.

Figure 39: Changes in hepatic function in patients with colorectal liver metastases after partial hepatectomy.

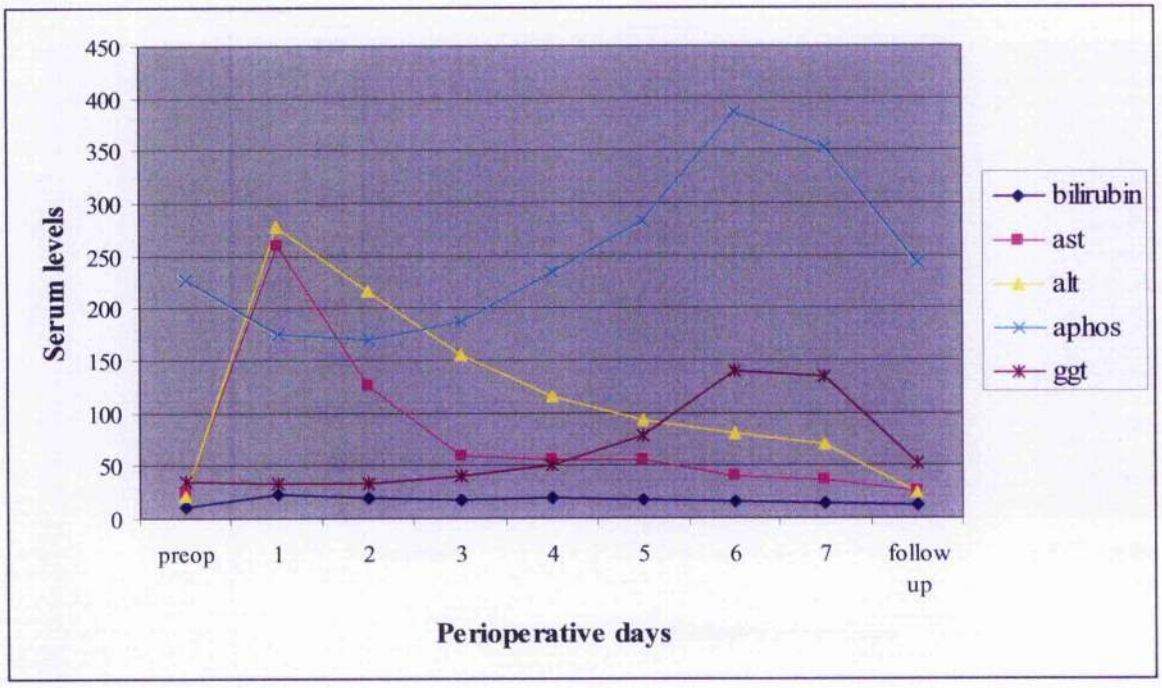


Figure 40: Perioperative changes in remaining hepatic function tests in patients with colorectal liver metastases after partial hepatectomy.

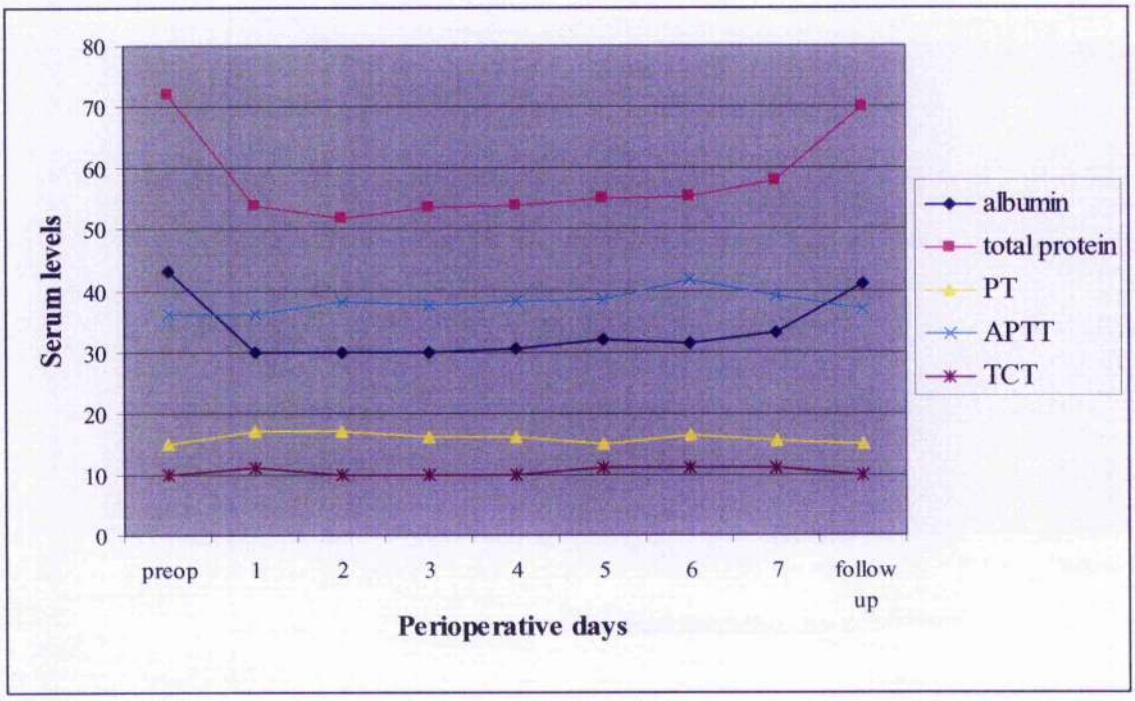


Figure 41: Perioperative changes in serum urea in patients that were fluid restricted against an unrestricted fluid control group.

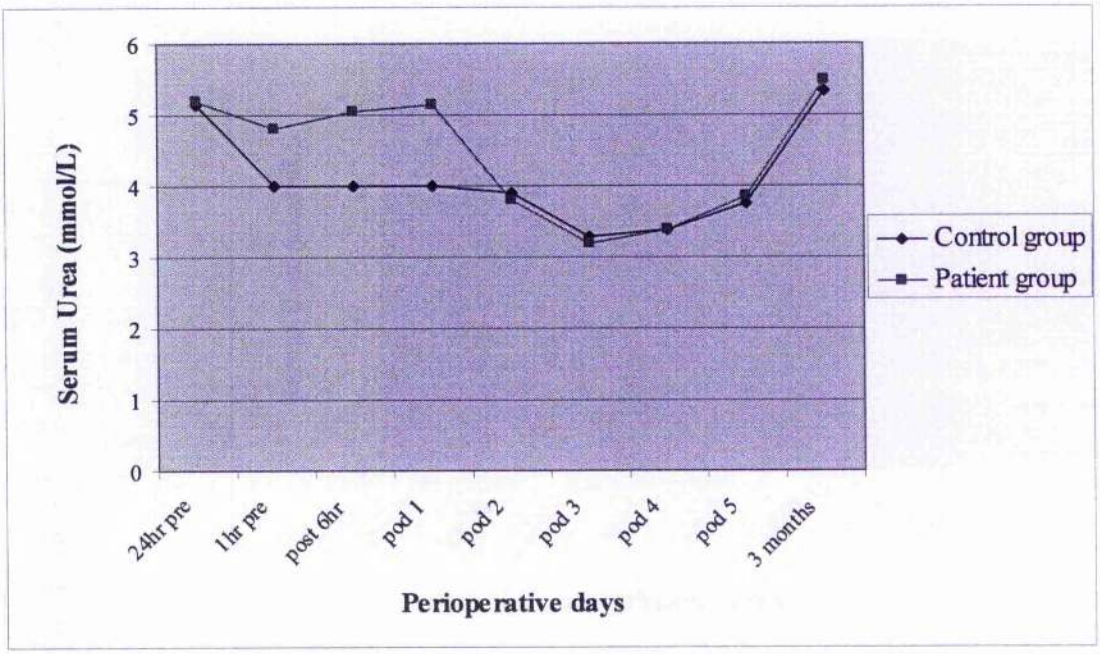


Figure 42: Perioperative changes in serum creatinine in patients that were fluid restricted against an unrestricted fluid control group.

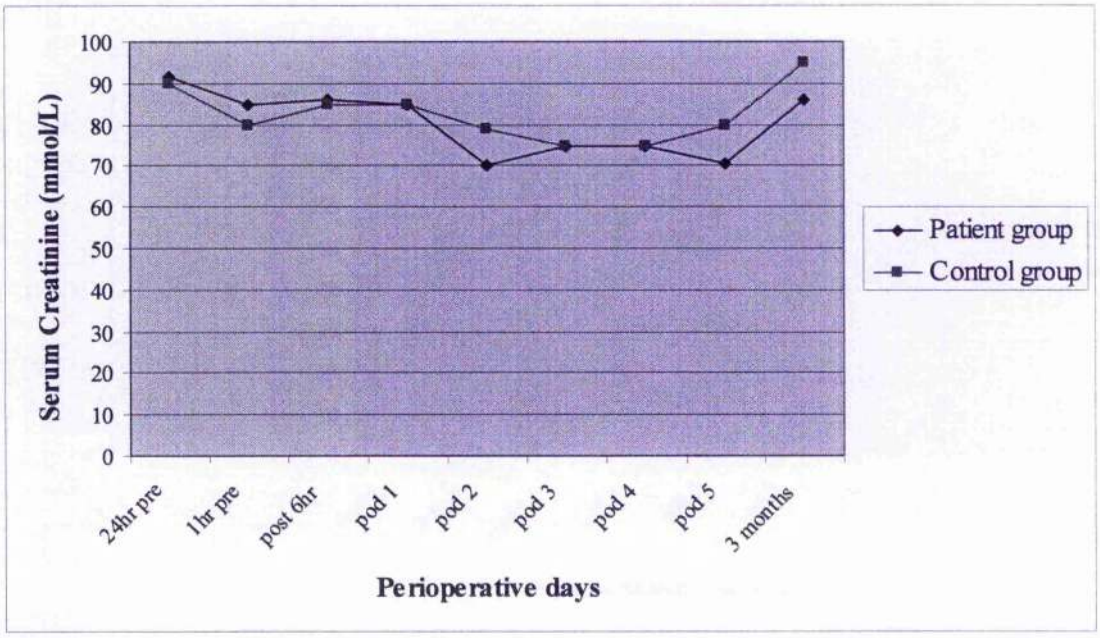


Figure 43: Perioperative changes in beta-2 microglobulin in patients that were fluid restricted against an unrestricted fluid control group.

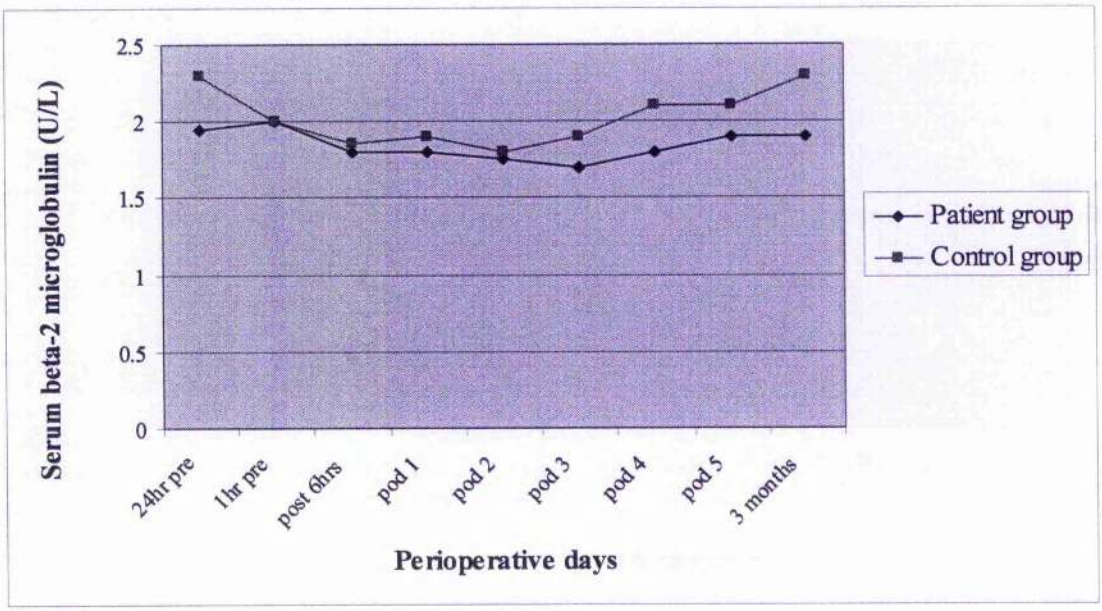


Figure 44: Perioperative changes in cystatin C in patients that were fluid restricted against an unrestricted fluid control group.

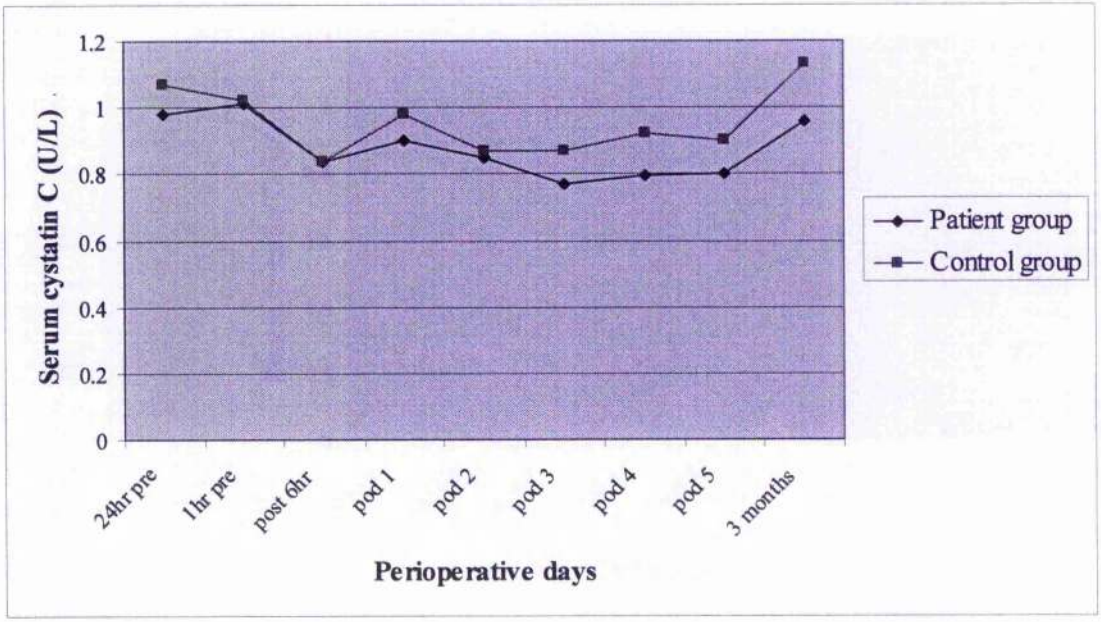


Figure 45: Survival curve for patients undergoing curative partial hepatectomy for colorectal liver metastases.

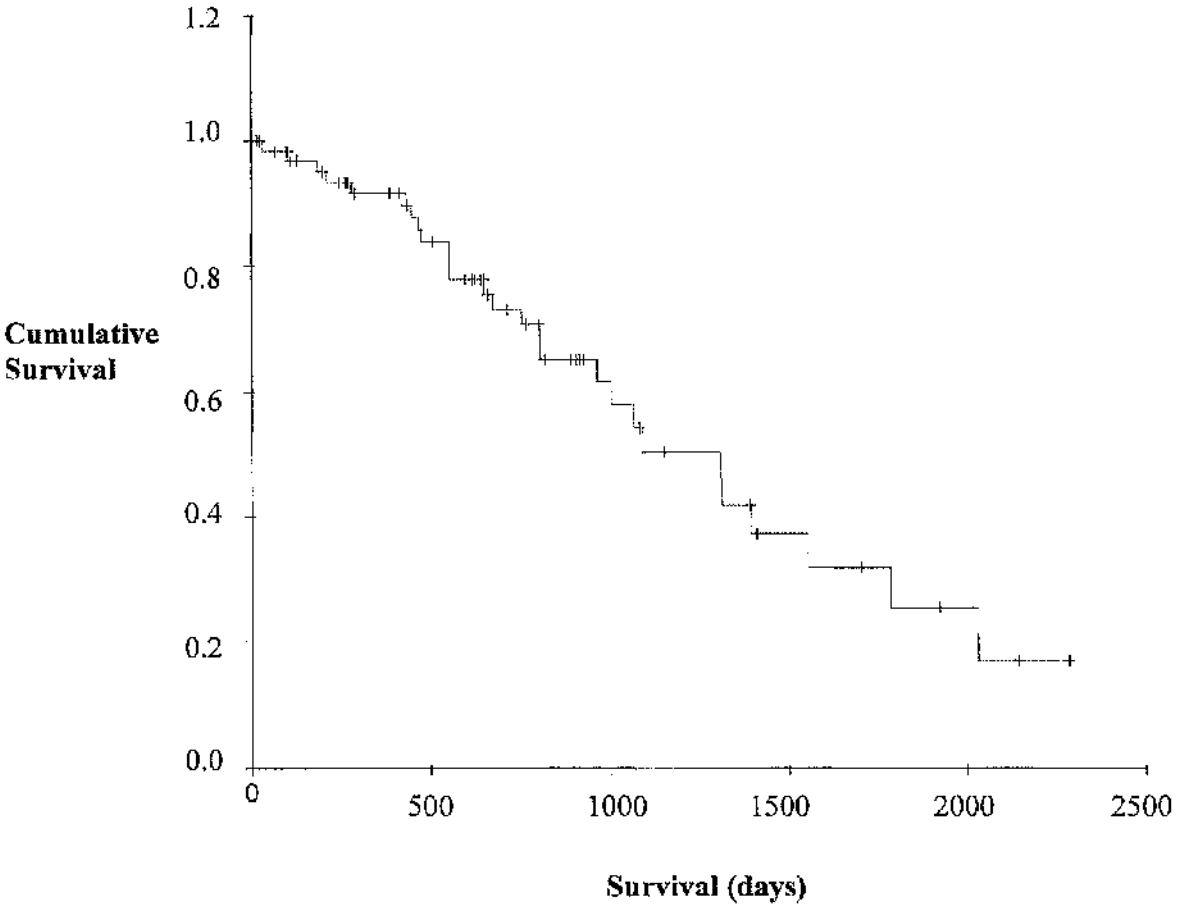
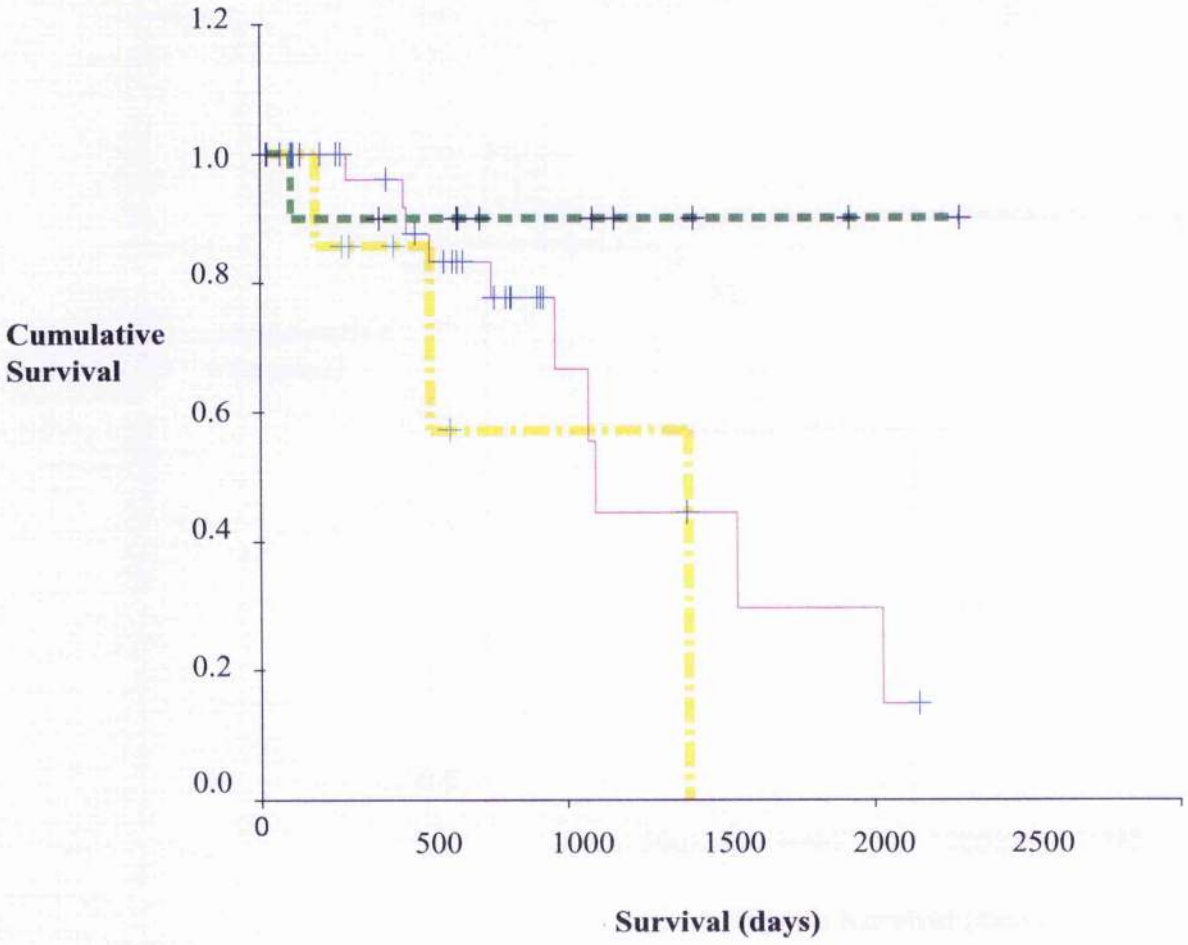


Figure 46: Survival after hepatic resection according to Clinical Risk Score.



Legend

Green dashed line – CRS 0-1

Pink intact line – CRS 2-3

Yellow dot-dashed line – CRS 4-5

Table 13: Univariate predictors of Survival within the Clinical Risk Score.

	Hazard ratio	**95% CI**	**p**
Liver tumour size >5cm	5.48	1.63-18.46	0.006
Disease free interval <12 months	5.34	0.89-32.11	0.068
CEA >200ng/mL	4.34	0.89-21.08	0.069
Node positive primary	2.15	0.44-10.39	0.342
liver tumour >1	0.58	0.15-2.23	0.431

Table 14: Multivariate predictors of Survival within the Clinical Risk Score.

	Hazard ratio	**95% CI**	**p**
Liver tumour size >5cm	5.26	1.57-17.68	0.007
Disease free interval <12 months	4.32	0.78-23.89	0.093
CEA >200ng/mL	3.91	0.86-17.79	0.078
liver tumour >1	0.58	0.15-2.23	0.431
Node positive primary	1.73	0.38-7.75	0.476

3.5.4 Discussion

Hepatic vascular occlusion techniques have evolved since the work of Pringle[244], widening both surgical and anaesthetic options for hepatic resection. This study has documented patient outcomes after hepatic resection with perioperative fluid restriction and the Half-Pringle. This approach consists of three phases: preoperative administration of bowel preparation with no supplementary fluids; maintenance of low CVP anaesthesia and selective continuous hepatic vascular occlusion.

Triphasic approach

It is this department's experience that by actively dehydrating the patient preoperatively, maintenance of a low intraoperative CVP is easily achieved with no risk to the patient's haemodynamic status. Indeed, no patients required administration of intravenous nitroglycerins as some authors have previously reported[199;369] and only occasionally was metaraminol or ephedrine administered to maintain the systolic blood pressure with no operation being interrupted for haemodynamic instability. Selective continuous hepatic vascular occlusion has the advantage of requiring minimal dissection, achieving easy and effective vascular control, which has been confirmed by Doppler studies [358].

Blood loss

Of the three components, it is hard to assess which one makes the greatest contribution, however, it is clear that this triphasic approach is effective at minimising blood loss. With a documented median blood loss of only 400mL, it should be

highlighted that this result included blood losses from synchronous procedures, suggesting that synchronous procedures can be safely performed in conjunction with partial hepatectomy. As expected the blood loss was significantly greater with major resections compared to minor[252].

Although direct comparisons with other studies are difficult and must be interpreted in the context of the surgical/anaesthetic approach (i.e. major versus minor resection, type of surgical occlusion technique used, whether low intraoperative CVP was maintained and whether patients with cirrhosis were included in the study's population), the low blood loss and transfusion rate with this triphasic approach remains.

Postoperative morbidity and mortality

Published literature states that a low blood loss results in improved patient outcome. For a small volume department this study has reported low incidences of morbidity and mortality (24% and zero respectively) with only 3 complications being classified as major. These results compare favourably to large hepatobiliary centre studies with documented morbidity ranging between 8-46% and mortality up to 5% [227;228;280;356;370-372]. In agreement with large centres, biliary leaks were the commonest complication, which all resolved with conservative management during the hospital admission.

Perioperative hepatic function

Hepatic failure or severe dysfunction after partial hepatectomy has been documented to occur within the range 3-18% with the cirrhotic liver at greater

risk[247;248;252;253]. No incidence of hepatic failure was recorded in this study after eighty resections providing evidence that the triphasic approach does not compromise the residual hepatic remnant. Furthermore, a clear pattern was demonstrated in liver function enzymes' changes during the perioperative period.

The immediate increases in AST and ALT after surgery are likely to reflect the degree of hepatic trauma. This phenomenon has been documented in both human and animal studies where the rapid increase is followed by an immediate decline that may return to normal values or remain mildly elevated for up to two weeks after resection[373-377]. With the major hepatic resections experiencing significantly greater increases in AST and ALT than minor resections, it becomes apparent that elevated hepatic enzymes are related to increasing tumour mass excised and subsequently, decreasing remnant liver volume[248;255].

Alkaline phosphatase and GGT experienced significant decline one day after surgery, but then began to rise steadily, peaking at day 6 postoperative. Limited evidence suggests that as alkaline phosphatase and GGT reside in bile canalicular cells the initial drop may be due to the excision of these cells[255;376]. In contrast, the rise of these markers may be an indicator of proliferation of the bile ducts and hepatic regeneration starting[247;252;376]. Based on this assumption, this study found that regeneration started at day four post hepatectomy.

Another potential prognostic indicator is bilirubin[252;253]. As demonstrated in this study, an initial increase in serum bilirubin is likely as it indicates diminished physiological function of the residual hepatic remnant with inability to clear bilirubin.

Clearly, if this increase persisted over the next few postoperative days, the risk of hepatic failure would be high.

Substantial initial decreases in albumin and total protein are expected after partial hepatectomy. Furthermore, they can still not have fully recovered by 2-3 months which led Aronsen et al[378] to suggest that the explanation for these decreases may be multifactorial. Fluid retention, haemodilution, intraoperative blood loss, protein leakage, impaired protein synthesis and increased amino acid requirement for hepatic regeneration have all been suggested and it is beyond the scope of this study to provide any definitive explanation.

Taking into account these hepatic function changes, this study proposes serial blood samples should be taken after hepatic resection to monitor adequate hepatic physiological function and regeneration. The following trends should be expected: rapid, transient elevations of AST and ALT accompanied by significant decreases in alkaline phosphatase and GGT on postoperative days 1 and 2; mild elevation of bilirubin that resolves by day 4 and is accompanied by significant and sustained increases in alkaline phosphatase and GGT.

Perioperative renal function

With patients being actively dehydrated and fluid restricted to minimise blood loss, particular attention was paid to analysing perioperative renal function. Initially a retrospective study was performed that used everyday laboratory markers to assess renal function as well as assessing the perioperative urine output. Despite preoperative dehydration and low intraoperative CVP, no patient developed renal impairment

postoperatively. With intraoperative urine output less than the recommended 1mL/kg/hr, this study strongly suggested that perioperative fluid restriction does not compromise renal function. However, it was thought that caution should be exercised in patients that have mild renal impairment preoperatively exemplified by six patients in this study who had slightly greater fluctuations in postoperative urea and creatinine that resolved or improved by day 3. To build on these findings, a prospective study was performed over a 3 month period that aimed to directly assess renal function and more specifically, GFR. When compared to a non-fluid restricted control group, routine biochemical markers and specific GFR serum markers were not significantly different suggesting that the kidneys are able to accommodate the transient fluid restriction and then respond as normal when fluid is reintroduced. Both these studies confirm that perioperative fluid restriction used as part of the triphasic approach to performing partial hepatectomy did not compromise renal function.

Disease free and overall survival

In relation to long term outcome after partial hepatectomy for colorectal liver metastases, this department has similar figures to large volume specialist centres [280] with a median time to local recurrence of approximately 17 months and a 3 year survival of 50% . This confirms that the combined surgical and anaesthetic approach is not oncologically compromising patients with metastatic colorectal disease and longer follow up with greater numbers will provide long term evidence.

Prognostic indicators

As the number of patients being considered for surgical resection increases, it is important that prognostic indicators are established to help both the surgeon and the multidisciplinary team make the best decision on an individual's treatment. The Clinical Risk Score (CRS) has been developed by one of the world's largest hepatobiliary units performing large numbers of hepatic resection for colorectal liver metastases. Their point scoring system of allocating 1 point for each of the 5 predetermined variables (through univariate and multivariate analysis) creates a final score that is easy to calculate, allowing preoperative treatment decisions to be made. Furthermore, unlike other proposed scores in the literature, there is growing evidence that the CRS is valid in different patient populations[206;357]. Indeed, our own study has found the CRS to be clinically applicable within a socioeconomically deprived population in the West of Scotland. On univariate and multivariate analysis, tumour size greater than 5cm was found to be the most significant independent predictor of survival, with an elevated preoperative CEA and disease free interval less than 12 months close to significance. Node positive primary and tumour number were not significant, but this may be a reflection of low patient numbers.

The CRS has its limitations and in the case of synchronous resections, the CRS can only be calculated postoperatively, once the nodal status of the primary tumour is known. This may not make much of a prognostic difference between scores 0 to 2, but if a patient with a score of 3 preoperatively is found to be node positive postoperatively, then their 5 year survival has decreased from 40% to 20%. Another limitation is that the CRS has no definite exclusion criteria. Certainly the authors' state that the presence of

disseminated disease and/or a positive surgical resection margin are absolute contraindications to surgical resection, but the CRS itself does not determine whether a patient is unresectable. It is possible that some surgeons will consider patients scoring 5 as unsuitable for resection. There have been advances in chemotherapy since 1999 when the paper was published, and chemotherapy may now provide better long term survival than the 14% 5 year survival for patients with CRS 5 as stated in Fong's paper.

There is increasing evidence that the presence of an on-going systemic inflammatory response is associated with poor outcome in patients with advanced cancers. A number of studies have reported that an elevated C-reactive protein had prognostic value independent of clinico-pathological stage in primary operable oesophageal [379], colorectal [36] and pancreatic cancer [34]. In addition, it has been shown that an elevated C-reactive protein and hypoalbuminaemia, may be combined to form the Glasgow Prognostic score (GPS), which has prognostic value, independent of stage and performance status, in patients with inoperable non-small cell lung cancer [380]. Despite this, the presence of a systemic inflammatory response, as evidenced by either C-reactive protein or GPS, did not predict cancer specific survival in this study's group of patients undergoing curative resection for colorectal liver metastases. The small numbers of patients (n=56) in the study is a limiting factor and may go some way to explaining why inflammation was not found to be significant. However, it should be highlighted that the majority of patients scored 0 (n=34), making it possible that the patient selection process for curative hepatic resection is already and unknowingly self-selecting patients with an absent inflammatory process. Indeed, only 6 patients within the group scored a GPS of 2.

Comparison to a group of patients with unresectable colorectal liver metastases should provide more information and is already underway.

Synchronous resections

Colorectal liver metastases present a unique opportunity to deal simultaneously with the primary and secondary disease. A synchronous approach has two potential advantages. First, cost effectiveness by reducing anaesthetic and theatre costs in addition to ward costs if shorter stays in hospital result. Second, less upset for the patients with only one stay in hospital and one recovery period. Despite these advantages, there is widespread reluctance to perform synchronous resections due to the suspected greater increase in postoperative complications with associated poorer long term outcomes.

It has been the choice of this surgical department to perform combined primary and secondary resections, with RF ablation as appropriate. By comparing the synchronous procedures to patients undergoing partial hepatectomy alone, we have gained insight into patient outcomes. In addition to matching for case performed, both groups were matched for age, sex and ASA grade excluding many potentially confounding variables.

It had been previously documented that synchronous procedures result in significantly greater blood losses[277]. Despite the blood losses of the synchronous procedures including the bowel and/ or RF ablation losses, this study found no difference between synchronous resections and partial hepatectomy alone groups. As blood losses, and subsequent transfusion, have been related to poorer long term survival in colorectal liver metastases this finding suggests that synchronous resections are not compromising

patients' long term survival. Indeed, with no significant differences in number of recurrences, disease free survival and long term survival between the groups, synchronous procedures are performing effective oncological surgery.

The main differences between the two groups were significantly higher postoperative complications in the synchronous group (33.3% *vs* 7.4%, p=0.03), associated with a longer hospital stay (median 11 days *vs* 9 days, p=0.04). These findings are consistent with some previous studies, but should be interpreted in the correct context to allow full interpretation. First, they are a reflection of a limitation of the study design. Complications and hospital stays from the staged groups' primary colonic resection have not been included, mainly because the resections were performed at another hospital with the information not being available and/or limited. As a result the complication rate would be expected to be higher in synchronous resection than with partial hepatectomy alone. In addition, ten of the eleven complications were minor, requiring conservative management or intravenous medication. All resolved during the hospital stay and only one resulted in readmission to hospital. Furthermore, there were no anastomotic colonic leaks. Finally, the incidence of postoperative complications in the synchronous group compares favourably to large centre studies with Chua et al documenting 53% complication rate for synchronous and 41% for staged resections and Martin et al 49% for synchronous and 67% for staged resections[277;278] .

Some surgeons might consider the department's approach aggressive. However, we feel that the combination of major colonic resections with synchronous partial hepatectomy and /or RF ablation extends the patient population that is being considered for surgical intervention. Patients can have their primary and secondary disease dealt

with at the same operation eliminating the need for two anaesthetics, two laparotomies and two recovery periods. Patients with bilobar disease can be considered for resection, with either removal of multiple segments (e.g. segment 2, 3 and 7) and/ or the application of RF ablation which depending on the needle size used can ablate up to 7cm easily. It is hoped that greater patient numbers will build on these initial findings.

Limitations of study

This study did not contain large number of patients with cirrhosis, which is a reflection of the population who present with colorectal liver metastases. It is widely accepted that patients with underlying liver disease are at greater risk of significant blood loss and complications than patients with healthy residual liver parenchyma[250]. Indeed, the authors acknowledge that resection of greater numbers of these patients will provide further insight into how the combined approach fares with underlying hepatic disease.

Conclusions

With blood loss minimised and outcomes optimised, the indications for hepatic resection are widening. This study has shown that resection of colorectal liver metastases using a novel triphasic approach, with each phase being validated, can be safely performed with low blood loss, morbidity and mortality as well as improving long term survival. In patients that have metastatic colorectal disease at time of diagnosis, synchronous resections can be performed safely potentially reducing rehabilitation time and hospital costs. In addition, the developing role of RF ablation should be acknowledged in that is allows patients with bilobar or multiple metastases that were

previously deemed unresectable, to be considered for resection and ablation. Finally, prognostic scores, including the clinical risk score, will fulfil an increasingly important role in determining the optimal treatment route for patients with colorectal liver metastases.

Section 3

Chapter 6: Conclusions and future research.

Conclusions

Radiological imaging plays an essential role in the detection and characterisation of colorectal liver metastases. Accurate staging of the disease allows patient selection for hepatic surgery, which remains the only potentially curative option. Despite recent and significant technological advances in radiological imaging, up to 50% of patients that have undergone curative partial hepatectomy will develop hepatic recurrence in the first two years after surgery. Evidence from growth rate studies has shown that colorectal liver metastases are slow growing and that these recurrences were present at the time of initial staging. Therefore, the problem of occult liver metastases remains.

This thesis has examined the potential clinical role of contrast enhanced ultrasound. The intravenous administration of an ultrasound contrast agent that has been used primarily in cardiac imaging was found to enhance late phase vascular imaging in percutaneous contrast enhanced ultrasound. The persistence of a hypoechoic liver lesion in to the late phase is typical of a colorectal liver metastasis and an agent that optimises the late phase would allow improved characterisation of colorectal liver metastases. Indeed, the administration of Definity® was found to result in improved sensitivity and accuracy in the detection of colorectal liver metastases when compared to percutaneous unenhanced ultrasound (sensitivity 100.8%, accuracy 90.8% versus 64.4% and 64.4% respectively). Furthermore, the optimal late phase imaging (quantified by comparing the contrast uptake by the hypoechoic metastasis to the hepatic parenchyma) was achieved by the lowest dose of agent, 0.4mL that would allow repeated injections before the maximal dose was reached.

These findings support the growing evidence base for CE-US and it is likely that in the future, percutaneous unenhanced ultrasound will be replaced by CE-US as first line imaging for the staging of colorectal liver metastases, working alongside CT, MRI and PET.

Unenhanced intraoperative ultrasound (IOUS) is widely regarded as the superior imaging modality for colorectal liver metastases and this thesis has documented the first prospective trial using CE-IOUS. The results are promising, with CE-IOUS being capable of detecting significantly more lesions than IOUS and preoperative CT/MRI. In addition, CE-IOUS detected smaller liver metastases than the other imaging modalities (mean lesion size 1.71cm versus 2.73cm for combined CT/MRI/IOUS), with the smallest lesion detected being only 0.4cm in size. With smaller lesions being detected, alterations in staging and surgical management would be expected and indeed this occurred in 35.1% and 29.8% of patients respectively. Inclusion of more patients with long term follow up will confirm if CE-IOUS is the superior imaging modality, creating a strong case for CE-IOUS to be accepted into widespread clinical practice. There is also the potential as CE-US technology advances for CE-IOUS to be performed laparoscopically removing the need for a laparotomy.

The presence of colorectal liver metastases alters the hepatic blood supply, providing an alternative route to the detection of colorectal liver metastases. Using CE-US, this thesis has developed a novel and reproducible technique to measure hepatic perfusion that documented significant differences between healthy controls and patients with overt colorectal liver metastases. Further analysis assessed the ability of this novel technique to differentiate accurately between healthy controls and patients with colorectal

liver metastases, with a view to determining the role of perfusion in the detection of occult liver metastases. The highest sensitivity and specificity achieved was only 85% and 58%, limiting the technique's clinical applicability as a screening tool for occult colorectal liver metastases.

Hepatic perfusion using CE-US was then used to assess changes in perfusion in patients undergoing curative partial hepatectomy with perioperative fluctuations occurring that were potentially reflecting the SIRS after surgical trauma. Perioperative perfusion changes were analysed for any relationship to hepatic recurrence with one parameter significantly higher in the recurrence group 3 months after the operation (peak intensity).

These two trials have shown that altered hepatic perfusion in colorectal liver metastases can be performed reliably by percutaneous contrast enhanced ultrasound. However, measurement of preoperative or postoperative hepatic perfusion does not accurately predict the likelihood of hepatic recurrence. Further work analysing the relationship between angiogenesis and colorectal liver metastases, alongside improved understanding of the physics of the microbubbles in the hepatic circulation may develop a clinically applicable technique for the detection of occult colorectal liver metastases.

Earlier and increasingly accurate detection of colorectal liver metastases is likely to increase the number of patients being considered for surgical resection. Hepatic surgery has evolved greatly during the last two decades, but there is no widely accepted optimal approach. This thesis has documented a novel approach to performing partial hepatectomy for colorectal liver metastases. The triphasic approach consists of: preoperative dehydration (using bowel preparation with no supplementary fluids);

intraoperative fluid restriction (to allow CVP <5cmH_20) and continuous selective vascular occlusion (Half-Pringle). Preoperative dehydration has been shown to be well tolerated by patients as well as allowing easy maintenance of a low intraoperative CVP. The Half-Pringle renders the hemi liver under resection ischaemic whilst protecting the liver remnant. All three components combined to minimise blood loss with a median blood loss of 400mL. As a result, postoperative morbidity and mortality were low with disease free survival and long term survival comparing favourably to the published literature from larger volume centres.

Particular attention was paid to the perioperative changes in renal and hepatic function with the triphasic approach. Dysfunction in these organs is likely after undergoing partial hepatectomy and very few studies have examined potential changes in depth. Using specific markers for GFR, in addition to routine biochemical blood markers, it was found that the perioperative dehydration of the triphasic approach did not compromise renal function. Hepatic function displayed a distinct pattern in liver function tests which can be used as a guide postoperatively.

Colorectal cancer with hepatic metastases presents a unique opportunity to deal with the primary and secondary disease at the same laparotomy, especially as morbidity and mortality are declining after hepatic resection. It has been the choice of this surgical department to perform combined primary and secondary resections, with RF ablation as appropriate. Using a case matched study this thesis has found that patient outcomes were similar for staged versus synchronous resections. There were no significant differences in: blood loss; number of recurrences; disease free survival and long term survival. Increased morbidity and longer hospital stay was found in the synchronous

group, but both results are likely to be limitations of the study design rather than actual differences. The results suggest that the combination of major colonic resections with synchronous bowel and /or RF ablation is safe and does not compromise long term patient outcomes. It is hoped that greater patient numbers will build on these initial findings.

As a result of improved outcomes after hepatic resection for colorectal liver metastases, interest has increased in the development of prognostic scores to optimise patient treatment. This thesis assessed the potential clinical application of preoperative C-reactive protein as a predictor of outcome after partial hepatectomy. Although a significant predictor in other malignancies, C-reactive protein was not found to be an influence in this cohort. Few patients underwent resection with an elevated C-reactive protein and the study will be continued to include greater numbers. The clinical risk score (CRS) was developed by one of the largest volume centres for hepatic resections in the world. This study found the CRS to be predictive of poor outcome, validating it in another population and supporting its use in routine clinical practice.

This thesis has investigated the potential clinical role of contrast enhanced ultrasound in the detection of colorectal liver metastases. Improved percutaneous and intraoperative detection of metastases has led to optimised disease staging with significant alterations in surgical management. Partial hepatectomy using the triphasic approach has resulted in excellent short and long term patient outcomes and with the inclusion of synchronous colonic and hepatic procedures, could extend the number of patients considered for surgical resection.

Future Research

The evidence for the integration of percutaneous CE-US in the routine detection and characterisation of colorectal liver metastases is growing. The majority of studies are based on SonoVue® meaning further work using Definity® is required to determine its application for three phase vascular imaging. This would extend from the work started in this thesis ideally taking the form of a prospective multicentred trial with large numbers, and using a healthy control group to allow validation of this diagnostic test.

The next progressive step would be a direct comparison between SonoVue® and Definity®. It is likely that the two interact with the hepatic parenchyma and colorectal liver metastases differently allowing their individual advantages to be manipulated to improve the role of CE-US. The initial work could be done as a prospective trial using blinded sonographers performing CE-US using the two agents, on the same group of patients. Determining the exact mechanisms of the contrast-liver interaction would require the development of a hepatic perfusion model. This could take the form of a laboratory based model with the involvement of physicists and/or mathematicians. Indeed, the role of replenishment kinetics in hepatic perfusion measurement could also be assessed.

If the contrast-liver interaction becomes better understood, then the microbubbles could be used as a carrier shell with the gas inside being replaced. We know that by increasing the mechanical index (MI) on the ultrasound machine that we can control the bursting of the microbubbles, subsequently releasing the contents inside. The addition of chemotherapy agents to inside the shell would allow the sonographer to inject a bolus,

then burst the bubbles when the contrast appeared in the liver, thus targeting the liver (and also the colorectal liver metastases) specifically.

Chemotherapy agents are not the only possible intra-microbubble additions: steroids are a possibility as are antibodies for angiogenesis factors (i.e. vascular endothelial growth factor or fibroblast growth factor).

In this thesis, hepatic resection for colorectal liver metastases using a novel approach has been shown to be safe and effective. Attention should now turn to improving long term outcomes. One such area is the role of RF ablation. RF ablation is currently working alongside resection in our hospital and long term follow up data on RF ablation is required as there is little in the literature. Previous authors have been confident that it will challenge surgical outcomes, however, there is evidence that a higher rate of recurrence might occur with RF ablation. As a result we are currently analysing our long term follow up in both our percutaneous RF ablation only group and our combined hepatic resection and RF ablation patient group. Another area involved in determining long term outcomes is the role of chemotherapy in colorectal liver metastases that are suitable for resection. The results of a multicentred trial are expected this year.

This thesis found low morbidity and mortality in patients undergoing pathologically confirmed curative hepatic resections for colorectal liver metastases. Despite these, the long term survival was, although reasonable, not markedly different from previous studies, suggesting that other influences are at work. One possibility is that it is our inability to detect micrometastases preoperatively. If this is the case, then we would expect improved disease free and overall survival in the patients that had their

surgical management altered as part of the CE-IOUS study, something we are currently analysing. If this long term follow up data does not show any improvements, then the molecular biology and theories of metastasis need further evaluation to improve our understanding and to allow improved treatment of colorectal liver metastases.

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