AN INVESTIGATION OF SPLANCHNIC BLOOD FLOW IN PATIENTS WITH COLORECTAL CANCER

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DEDICATION

To my Mum and Dad, with thanks to friends and family for their support and encouragement, in particular Jeanine, Claire, Craig and Paige.

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DECLARATION

The material contained in this thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or qualification of this or other university or other institute of learning. All work and practices described herein are original contributions and were performed by myself. All ultrasound scans and measurements of liver blood flow were performed exclusively by myself. The nature and extent of any work contributed by other researchers is acknowledged below. The research was performed at the Glasgow Royal Infirmary.

Dr Ramzi Al-Shaiba performed the majority of the liver tumour volume measurements via Computed Tomography scans.

Dr Paul Glen performed Doppler ultrasound scans for the inter-observer liver blood flow measurement study.

Dr Wilson Angerson assisted in the statistical analysis and design of the project.

SUMMARY

Colorectal cancer is one of the most common forms of cancers worldwide and the second most common cause of cancer death in the European Union. Surgical resection of the tumour is the primary choice of treatment as it is the only option that offers a chance of permanent cure. However, approximately half the patients who undergo apparently curative surgery will die within five years. It has been shown that up to 30% of patients undergoing apparently curative surgery for colorectal cancer harbour occult liver metastases at the time of presentation and it is the presence of these hepatic metastases that determines the likelihood of death from disseminated disease.

The liver possesses a dual blood supply, approximately 75% of the blood coming from the portal vein and 25% from the hepatic artery in the normal subject. There is evidence that these proportions are altered shortly after metastatic seeding in the liver. Previous investigations using Doppler ultrasonography have shown that hepatic arterial blood flow is increased in the presence of liver metastases, while some studies suggest that portal venous blood flow is reduced. The Doppler Perfusion Index (DPI), defined as the ratio of hepatic arterial to total liver blood flow, increases as a result of both these changes. An elevated DPI at the time of apparently curative surgery has been shown to be highly predictive of subsequent recurrence in the liver.

It is not known, however, whether the DPI is the most effective index for quantifying the tumour-induced changes in liver blood flow. There is also only limited evidence on whether the primary colorectal tumour alters liver blood flow. To address these questions, hepatic arterial and portal venous blood flow were measured in a series of patients with colorectal liver metastases and normal control subjects.

There was no significant difference in either component of liver blood flow between metastases patients with and without a primary or recurrent colorectal tumour. This confirms previous reports that the presence of a synchronous primary does not affect metastases driven blood flow changes. Hepatic arterial flow was increased in patients with liver metastases relative to controls, and portal venous flow was reduced. However, the latter difference was attributable to age mismatching, as portal venous blood flow was found to decline significantly with increasing age. An optimised index, the Dual Flow Index (DFI), was developed by logistic regression analysis to distinguish between metastases and control patients on the basis of the blood flow measurements. This was found to only marginally improve on the DPI in accuracy of discrimination (84% vs. 82% after age adjustment of blood flow). It was concluded that the DPI was close to optimal as a diagnostic index, but that the age-dependence of blood flow should be considered in clinical practice.

Doppler ultrasonography is recognised to be an operator-dependent technique, and this may account for conflicting reports in the literature about the blood flow changes associated with liver metastases. It has been suggested that functional Computed Tomography (CT) may provide equivalent information about liver blood flow in a less operator-dependent manner. However, reports on the effectiveness of functional computed tomography in detecting liver metastases are conflicting.

Dual-phase contrast-enhanced spiral CT scans were used to assess the varying parameters of tumour-induced changes in liver blood flow. Abdominal scans were performed in patients with liver metastases, colorectal cancer patients without overt metastatic disease, and patients with small benign hepatic haemangiomas.

No significant differences were found between these patient groups after adjustment for age imbalances. It was concluded that too many variables affect CT parameters in the routine clinical scanning procedure employed in this and other reported studies for them to be useful for studying blood flow changes. Further work is required in this area using a disease-free normal control population, however Doppler ultrasound currently remains the method of choice for the non-invasive assessment of splanchnic blood flow.

Most patients with colorectal liver metastases have evidence of a systemic inflammatory response as manifested by an elevated plasma C-reactive protein concentration. There is evidence that pro-inflammatory cytokines, in particular interleukin-6, are released from colorectal tumours. It is possible that pro-inflammatory agents are associated with a hypermetabolic state in the liver, increasing oxygen demand and blood flow, or that they modulate hepatic arterial or portal venous blood flow in some other manner. It may therefore be that the systemic inflammatory response is important in determining the changes in hepatic haemodynamics that occur in patients with colorectal liver metastases. The relationships between liver blood flow, tumour volume and the systemic inflammatory response were subsequently examined in patients with colorectal liver metastases to shed further light on the mechanisms underlying the blood flow changes.

Hepatic arterial blood flow and total liver blood flow increased significantly, though not strongly, with both increasing tumour volume and increasing C-reactive protein concentration. Tumour volume and increasing C-reactive protein concentration were themselves positively correlated, and it was not possible to determine which of them was more directly related to blood flow. Total liver blood flow was more strongly correlated with plasma interleukin-6 concentration, and this relationship did not appear to be due to a common dependence on tumour size. Portal venous flow increased non-significantly with

tumour volume, C-reactive protein and interleukin-6, while the DPI showed a marked lack of correlation with any of these variables.

It was concluded that, in patients with colorectal liver metastases, hepatic arterial and total liver blood flow may be modulated by circulating interleukin-6, a mediator of the systemic inflammatory response. They also vary with tumour volume, although the basis of this relationship remains unclear. It is unlikely, however, that these mechanisms are responsible for the elevated Doppler Perfusion Index in patients with colorectal liver metastases.

To test these conclusions, and further elucidate the haemodynamic effects of the systemic inflammatory response, a double-blind, randomised, placebo-controlled study was performed to determine the effect of an anti-inflammatory treatment (ibuprofen 1200mg/day for 7 days) on liver blood flow in patients with colorectal liver metastases. The power of the study was limited by a lower recruitment rate and a higher rate of failure to complete the study than expected, largely due to treatment interventions. It was also limited by an unexpectedly low level of systemic inflammation at baseline. The proportion of patients with an elevated DPI fell non-significantly from 67% to 42% in the ibuprofen group and remained static at 73% in the placebo group. None of the other blood flow variables changed significantly from baseline in either treatment group, and none of the changes differed significantly between treatment groups. The low recruitment rate of the study limits its final conclusions to some degree, however it can be seen that the systemic inflammatory response does not wholly explain the abnormalities in liver blood flow in patients with colorectal liver metastases, and that its contribution to the elevated Doppler Perfusion Index in these patients is likely to be small.

Chapter 1 : Introduction

1.1 Colorectal Cancer: Incidence and Mortality

Colorectal cancer is one of the most common forms of cancers worldwide, with an estimated 783,000 new cases diagnosed in 1990, and approximately one million new cases diagnosed in the year 2000. It accounts for 9.7% of all new cancer cases in the world (Parkin et al., 1990; Ferlay et al., 2001). Each year there are approximately 437,000 (8.4% of the world total) cancer deaths from colorectal cancer, making it the second most common cause of cancer death in the European Union (Parkin et al., 1990; CRC CancerStats, 1999).

Colorectal cancer is more common in Westernised countries; the US has the highest incidence with 53 cases per 100,000 (Parkin et al., 1997). In Europe, the highest rates are in the Northern countries of Denmark, Belgium and Germany and the lowest rates are in the Southern countries of Portugal and Spain. More specifically, the UK had over 30,000 new cases of colorectal cancer in 1995 with a higher incidence rate in Scotland, where colorectal cancer accounted for 13.8% of newly diagnosed malignancies (excluding non-melanoma skin cancer). In Scotland the lifetime risk of developing colorectal cancer is 4.6% for men and 3.2% for women (Moir et al., 1999). Overall, the incidence of colorectal cancer has been increasing since 1971 where 20,400 cases were reported, to 1997 where 28,900 cases were reported; an overall increase of 42% (Hayne et al., 2001).

The majority of colorectal cancers in the UK occur in the colon compared with the rectum (2:1). Incidence rates are strongly related to age, over 80% of patients are aged 60 years or more at the time of initial diagnosis. However, colon cancer rates are slightly higher in females and rectal cancers are slightly higher in males (CRC CancerStats, 1999).

1.2 Aetiology

Colorectal cancers can arise from genetic or environmental factors. However the large majority of cases are sporadic in nature, most likely caused by an interaction of these two factors (Wilmink, 1997).

1.2.1 Genetic Factors

Genetic factors account for a relatively small proportion of all colorectal cancers (approximately 3-5%). It is generally accepted from epidemiological and histopathological studies that the majority of colorectal adenocarcinomas originate from premalignant adenomatous polyps. The multi-step process involving complex genetic mutations which underlie the progression from benign disease to a malignant state, is known as the adenoma-carcinoma sequence (Jass, 1989; Fearon & Vogelstein, 1990; Wilmink, 1997). Benign adenomatous polyps can develop from normal colonic mucosa and are present in approximately one third of the European and US population. Tubular adenomas account for the majority of benign polyps (approximately 75%), tubulovillous adenomas and villous adenomas are less frequent (approximately 15%, 10% respectively). The malignant potential of these polyps has an inverse relationship with their incidence. However, approximately 40% of villous adenomas will become malignant (van Stolk et al., 1998; CRC CancerStats, 1999). Overall, approximately 5% of these benign polyps will be malignant over a 5-10 year period (CRC CancerStats, 1999).

There are several types of genetic alterations involved in the advanced stages of carcinoma formation. Mutations on tumour suppressor genes may be involved in the early stages of carcinoma formation. These genes inhibit tumorigenesis by inducing apoptosis or

by interrupting the mitogenic signal transduction pathways. They can promote cancer growth when inactive in both alleles and are often associated with metastatic disease and poor prognosis (Younes & Johnson, 1997). A mutation present on chromosome 5 has been demonstrated in approximately 40% of colorectal cancers, but was not shown on early stage polyps (Vogelstein et al., 1988).

Another early event, may be the mutation in the K-ras oncogene. This mutation has been found in 58% of intermediate and late stage adenomas and 47% of carcinomas. Although it is less common in early stage adenomas, its presence in benign colonic abnormalities indicates that it is unlikely to be the sole cause of malignancy (Fearon et al., 1987; Vogelstein, 1988; Fearon & Vogelstein, 1990; McLeod & Murray, 1999).

1.2.2 Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

HNPCC is the most common familial colorectal cancer syndrome, accounting for approximately 5% of all colorectal cancers (CRC CancerStats, 1999). It results from a DNA mismatch repair gene deficiency leading to either tumour growth in the colon alone at an early age (Lynch I syndrome) or in the colon with additional tumours arising in the stomach, liver, biliary system, urinary tract, endometrium and pancreas (Lynch II syndrome; Wilmink, 1997). Lynch II syndrome is mostly associated with right sided colonic lesions (Lynch & Lynch, 1993).

Currently, five mismatch repair genes have been identified and mutations in two of them account for more than 95% of HNPCC families. The percentage risk of cancer in patients carrying an HNPCC gene is approximately 85-90% (Wilmink, 1997). Despite the characteristic large, proximal and poorly differentiated tumour, HNPCC patients usually have a better prognosis than other colorectal carcinomas. Testing for microsatellite

instability (MSI) from tumour tissue samples taken at the time of surgery can indicate damage to a mismatch repair gene. MSI occurs in nearly all HNPCC and 15% of sporadic cancers. If samples test positive for microsatellite instability, familial screening for HNPCC could be beneficial (Starkey, 2002).

In younger colorectal cancer patients approximately 41% have been shown to be carriers of DNA mismatch repair genes. In Scotland alone there are approximately 12,500 carriers of these DNA mismatch repair genes (90% risk for males, 69% risk females; CRC CancerStats, 1999).

1.2.3 Familial Adenomatous Polyposis (FAP)

FAP accounts for around 1% of all colorectal cancers in Westernised populations. It can be identified by numerous benign polyps in the colon and/or rectum and the percentage risk of malignancy by the age of 40 years is extremely high (CRC CancerStats, 1999). A mutation on the adenomatous polyposis coli (APC) gene has been identified as one of the earliest changes in colorectal cancer and is found specifically in the germline of FAP patients. Currently APC mutations are detectable in 80-90% of FAP cases and APC is mutated in 70% of all colorectal cancers; certain polymorphisms of APC have been shown to give an increased risk for colorectal cancer (Laken et al., 1997; Starkey, 2002).

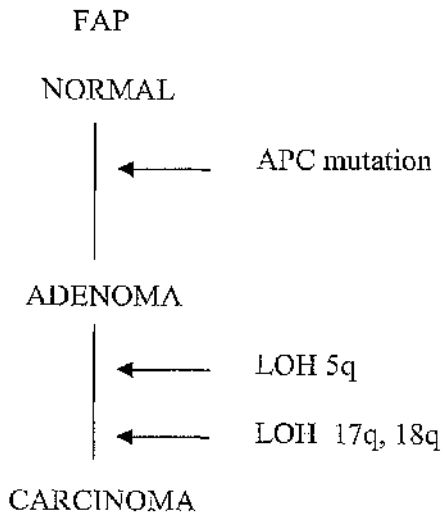

Figure 1.1 Genetic familial adenomatous polyposis (Adapted from Younes & Johnson, 1997).

1.2.4 Environmental Factors

The large majority of colorectal cancers are sporadic and genetic damage is thought to occur primarily through environmental factors. It has been suggested that approximately 80% of cases of colorectal cancer may be preventable by diet (Cummings & Bingham, 1998). However, there is considerable controversy as to which specific dietary constituents contribute to increased risk of colorectal cancer and those which have a protective effect.

Despite growing evidence of an increased risk of colorectal cancer with increased intake of fat or red meat, it is not possible to firmly conclude that there is a modulatory effect on colorectal carcinogenesis (Woutersen et al.,1999). Many studies have failed to demonstrate a direct relationship between incidence and animal fat intake, which may be due to variations in cooking methods, methodological limitations of studies and a varying combination of foods, such as fish in the diet. It has been suggested that the high polyunsaturated fat content (rich in n-3 fatty acids) in fish and fish oil reduces the promotional effects of diets high in animal fats (n-6 fatty acids) and/or unsaturated acids in colorectal or breast cancer. This suggests that the overall type and composition of ingested dietary fat is more important than the total dietary fat intake (Woutersen et al., 1999; Rao et al., 2001).

It has been questioned whether alcohol drinkers are at higher risk due to the lack of protective antioxidant vitamins from vegetables and fruits, or whether there is a carcinogenic effect of alcohol that is independent of dietary deficiencies (Jedrychowski et al., 2002). The effect of tobacco on colorectal cancer incidence has also been presented recently. Giovannucci and co-workers have recently estimated that around one in five colorectal cancers in the US may be associated with tobacco use (Giovannucci, 2001).

Inflammation may play a key role in colorectal tumour development since it has long been recognised that dysplasia found in patients with inflammatory bowel disease, ulcerative colitis and Crohn's disease is associated with increased incidence of colorectal cancer (Glotzer, 1985; Wilmink, 1997).

It was initially observed in 1969 by Burkitt that incidence rates of colorectal cancer are much lower in populations where the diet consists of high fibre and low fat (Burkitt, 1971). This led to the hypothesis that dietary fibre prevents colorectal cancer by diluting or absorbing faecal carcinogens, reducing colonic transit time, altering bile acid metabolism, reducing pH in the colon or increasing the production of short chain fatty acids (Kritchevsky, 1995). However, many studies since Burkitt's initial observation have been unable to find such a link between colorectal cancer and dietary fibre (Platz et al., 1997). For example, in a study of 88,757 women, Fuchs and co-workers (1999) followed the intake of dietary fibre and incidence of colorectal cancer for 16 years. During this period, 787 cases of colorectal cancer and 1012 cases of adenomas of the distal colon and rectum were found in 27,530 of the participants who underwent colonoscopy investigation. This prospective study failed however to find an association with dietary fibre and protection from colorectal cancer or adenoma (Fuchs, 1999).

A recent study into the effect of fruit and vegetable intake on the incidence rate of colorectal cancers, investigated two large cohorts (88,764 women and 47,325 men). No association between consumption and incidence was found (Michels et al., 2000).

In contrast, physical exercise may play a protective role against colorectal carcinogenesis. However, reasons for this remain unclear. The effect of exercise on bile composition, the faster and more regular colonic transit associated with exercise, and the

effect of exercise on insulin levels (hyperinsulinaemia is associated with increased colon cancer) may offer some explanation for decreased risk (Hill, 1999).

Hormone replacement therapy has also been associated with a decrease in colorectal cancer prevalence. There are several studies showing this relationship with some also showing a reduction in risk of death from the disease (McMichael & Potter, 1980; Jacobs et al., 1994; Newcomb & Strorer, 1995; Calle et al., 1995; Chlebowski et al., 2004; Slattery et al., 2005). Further studies are required to explain this effect and investigate the potential for preventative therapy (Boyle & Langman, 2000).

1.3 Clinical Features of Colorectal Carcinoma

Presentation of cancer of the colon and rectum can occur from the effects of the primary, secondary or from the typical symptoms of malignant disease. It is widely accepted that during the early stages, an uncomplicated carcinoma of the colon is usually symptomless.

Commonly recognised symptoms of colorectal cancer include altered bowel habit, bleeding per rectum of a short duration and blood in the stool, however symptoms are dependent on the site and stage of disease (CRC Cancer Stats, 1999; McArdle, 2000).

Right sided tumours in the large bowel wall are often relatively large as they are free to expand in the caecum. They are less likely to cause obstructive symptoms and any bleeding may mix within the stool and can be invisible by the time it reaches the rectum. Therefore right sided tumours are typically more advanced at diagnosis. Left sided colonic tumours are characteristically annular and the intraluminal contents are more solid. Therefore the mode of presentation is more often abdominal pain, constipation or

obstruction. Overt rectal bleeding and altered bowel habit are more often a manifestation of rectal disease (Allum et al., 1994).

1.4 Staging and Prognosis of Colorectal Cancer

The main objectives of staging cancer, defined by the unified American Joint Committee on Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC), are to aid the clinician in the planning of treatment; give an indication of prognosis; help evaluate results of treatment; contribute to continuing investigation of human cancer; and to assist in the exchange of information between centres.

Colorectal cancer spreads by direct extension to adjacent organs, by lymphatic pathways, and by haematogenous routes. Although lymph node metastases represent regional disease, it is also presumed that such disease represents the potential for disseminated disease as well (Enker et al., 1981).

1.4.1 Dukes' Classification

To date, one of the most important and effective methods of staging uses pathological information based at the time of presentation. Following from the first classification system designed by Lockhart Mummery, Dr Cuthbert Dukes (1932) produced a simple, reproducible and clinically helpful prognostic classification system intended for rectal cancer. Classification of Dukes' stage is illustrated in Table 1.1 and is based on the direct and lymphatic spread found in the surgically excised tumour specimen (Dukes, 1932; Turnbull et al., 1967; McArdle & Hole, 2002).

Table 1.1 Dukes' classification and outcome in colorectal cancer (adapted from McArdle & Hole, 2002).

Dukes' Stage	Indication	Proportion of Patients	5-Year Survival
A	tumour limited to bowel wall	7%	85 – 95%
B	tumour extends beyond the wall without metastatic regional lymph nodes	47%	70 – 75%
C1	direct spread in continuity limited to the bowel wall	30%	35 – 45%
C2	highest node contained metastatic tumour		
D	distant metastases or advanced local disease	15%	0 – 5%

1.4.2 TNM Staging

Due to disadvantages of the conventional Dukes' staging, such as lack of precision as it does not assess the exact depth of tumour penetration, number of lymph nodes involved and the extent of spread outside the bowel wall, the TNM classification has now been widely adopted. Originally introduced in the early 1950s, a single version was internationally approved in the late 1980s. The TNM system is now used for carcinomas of the upper gastrointestinal tract, breast, ovary, urinary bladder, prostate and the colon and rectum. The basis for TNM classification includes assessment of tumour size and depth of penetration ('T'), nodal involvement ('N') and the presence of distant metastases ('M'). TNM provides the clinician with more information and it can be easily translated into other staging classifications, such as Dukes' staging (Hermanek et al., 1990). The principles of TNM classification are illustrated in Figure 1.2 and the association between TNM and Dukes' staging is illustrated in Table 1.2.

Primary tumour, clinical (T):

TX Primary tumour cannot be assessed.

T0 No evidence of primary tumour.

Tis Carcinoma in situ.

T1 Tumour invades submucosa.

T2 Tumour invades through muscularis propria.

T3 Tumour invades through muscularis propria into subserosa.

/ or into non-peritonealized pericolic or perirectal tissues.

T4 Tumour invades other organs of structures and/ or perforates visceral

Peritoneum.

Regional lymph nodes (N):

NX Regional lymph nodes cannot be assessed.

N0 No regional lymph node metastasis.

N1 Metastasis in 1 to 3 regional lymph nodes.

N2 Metastasis in 4 or more regional lymph nodes.

Distant Metastasis (M):

MX Distant metastasis cannot be assessed.

M0 No distant metastasis.

M1 Distant metastasis.

Figure 1.2 TNM Classification (American Joint Committee on Cancer · Union Internationale Contre le Cancer, 1995).

Table 1.2 TNM classification in relation to Dukes' staging.

TNM Classification			Dukes' Stage
Tis	N0	M0	
T1	N0	M0	A
T2	N0	M0	A
T3	N0	M0	B
T4	N0	M0	B
Any T	N1	M0	C
Any T	N2	M0	C
Any T	Any N	M1	D

1.5 Treatment of Colorectal Cancer

Surgical resection of the tumour remains the primary choice of treatment as it is the only option that offers a chance of permanent cure. However, approximately half the patients who undergo apparently curative surgery, will die within five years (McArdle, 2000).

The majority of patients who present with colorectal cancer are elderly and many have significant co-morbidity, especially due to cardiovascular or respiratory disease. Nevertheless, approximately 80% of patients will receive a surgical resection of their primary tumour (CRC CancerStats, 1999; McArdle, 2002).

1.5.1 Surgical Treatment

Before surgery is undertaken, histological confirmation of the diagnosis should be obtained, usually by colonoscopy. For rectal cancers, the height of the tumour is determined using a rigid sigmoidoscope and the degree of fixity is established by palpation. Endoluminal ultrasound also plays an important part in assessing the depth of tumour penetration. Additionally, all patients should be screened for liver or lung metastases prior to surgery (Dorudi et al., 2002).

It has been reported recently that survival rates following colorectal surgery have improved over the last two decades. This has been attributed to the development of specialist centres, improvements in techniques and the use of adjuvant and neoadjuvant therapies rather than earlier presentation. If there is a continual increase in the proportion of patients undergoing treatment by specialist colorectal surgeons and oncologists at specialist centres, further improvements in survival are likely (McArdle & Hole, 2002;

McArdle et al., 2005). Moreover, in rectal surgery, if the principles of mesorectal excision are adhered to, overall local recurrence rates should be less than 10%. As local recurrence is difficult to treat and ultimately fatal, good surgical technique lends itself to better long term survival for patients with rectal cancer (Leslie & Steele, 2002).

1.5.2 Chemotherapy and Radiotherapy

Neo-adjuvant Chemotherapy

Neo-adjuvant therapy aims to treat distant micro-metastases as early as possible and downstage primary tumours for more effective and less radical surgical treatment without compromising overall survival or cure. Bismuth and co-workers (1996) were amongst the first to document a successful outcome from neo-adjuvant treatment. It was reported that a small cohort of patients with disseminated disease (53 of 330) were down staged following neo-adjuvant chemotherapy to such a degree that surgical resection of colorectal liver metastases became possible.

More recently, these findings have been supported by Adam and co-workers (2001). The ability to respond to neo-adjuvant chemotherapy demonstrates a significant potential for increased survival however the extent of this may take many years to define (Adam et al., 2001).

Adjuvant Therapy

The benefit of adjuvant chemotherapy in suitable patients with Dukes' C colorectal cancers has been illustrated in several large studies (Wolmark et al., 1990; Moertel et al., 1990; Wolmark et al., 1993). However, adjuvant treatment in Dukes' B colorectal patients is still under debate. It is still generally accepted that adjuvant chemotherapy should only

be offered to those Dukes' B patients presenting with high-risk prognostic factors, due to the toxicity and cost of adjuvant therapy. However, a study reported by Mamounas and co-workers (1999) investigated four protocols of adjuvant treatment in 1,565 patients with Dukes' B and 2,255 patients with Dukes' C colon cancer, with 5-year follow-up. The study showed that survival was increased in both Dukes' B and Dukes' C patients who received some form of adjuvant chemotherapy. Although the study was not large enough to compare which regimen of treatment was the most effective, it was able to confirm the benefits of chemotherapy in all stage B and C patients, irrespective of high-risk prognostic factors.

Post-operative adjuvant therapy usually consists of treatment for 6 months or longer with 5-fluorouracil based regimens. Increasing understanding of the molecular pharmacology of fluorouracil has given rise to the coadministration of other drugs, with the intention of increasing the efficacy (Midgley & Kerr, 2000). However, there is some uncertainty as to which regimen is the most effective. Two of the most promising combinations are fluorouracil plus levimasole and fluorouracil plus folinic acid (QUASAR group, 2000). More recent chemotherapy agents may be more effective. However at this point they are only administered as part of a clinic trial (Leslie & Steele, 2002).

Possible side effects from fluorouracil based regimens include nausea, vomiting, increased susceptibility to infection, oral mucositis, diarrhoea, desquamation of the palms and soles, and less commonly, cardiac and neurological effects (Midgley & Kerr, 2000).

Adjuvant radiotherapy is only suitable for patients with rectal cancer as the morbidity associated with abdominal radiotherapy is prohibitive (Leslie & Steele, 2002). Studies in Europe have mainly focused on the application of preoperative radiotherapy. The Uppsala trial in particular, showed preoperative radiotherapy improved local control

and decreased toxicity in comparison to postoperative radiotherapy (Frykholm et al., 1993). However, studies in North America have shown that postoperative radiotherapy can be more effective when combined with 5-fluorouracil based chemotherapy (Colorectal Cancer Collaborative Group, 2001).

Palliative Therapy

In patients with inoperable advanced colorectal cancer, palliative chemotherapy is the mainstay of treatment, approximately doubling the survival in comparison to controls (Scheithauer et al., 1993; Smyth et al., 1997). The most widely used regimen to date is bolus 5-fluorouracil and folinic acid (Mayo regimen). However, it has been reported that intermittently infused 5-fluorouracil and folinic acid (de Gramont regimen) or continuously infused 5-fluorouracil (Lokich regimen) may be more effective and less toxic (Lokich et al., 1989; Leslie & Steele, 2002).

In patients with inoperable rectal cancer, the main aim of treatment by radiation is to kill the primary gross tumour. Endocavitary irradiation (contact x-ray therapy, brachytherapy) is the main technique used to deliver high doses to the tumour but low doses to normal tissue. External-beam radiotherapy is a complementary technique used to control subclinical disease in the rectal wall and perirectal lymph nodes (Gerard et al., 2003).

In spite of improvements in surgical technique, increased access to chemotherapy and radiotherapy treatments, overall results are still unsatisfactory and long term survival and improvement is disappointing.

1.6 Colorectal Liver Metastases

The liver is the largest organ in the body, some of its main functions are: secretion of bile; storage of glycogen and the production of plasma proteins; the breakdown of proteins and the formation of urea, and the desaturation of fats; the storage of iron and vitamin B_{12}; and the destruction of various toxic substances and the production of heparin and fibrinogen concerned in the clotting of blood. All of these functions depend on the maintenance of an adequate blood supply and are vital to the correct functioning of the body (Woodcock, 1975).

1.6.1 Incidence and Mortality

It has been shown that up to 30% of patients undergoing apparently curative surgery for colorectal cancer are harbouring occult liver metastases at the time of presentation (Finlay & McArdle, 1986) and it is the presence of these hepatic metastases that determines the likelihood of death from disseminated disease. Currently, only half of the patients undergoing curative surgery will survive five years; the majority of deaths are due to disseminated disease within the first three years (McArdle, 2002).

Due to the unique venous drainage of the gastrointestinal tract through the portal vein, the liver is the most common site of distant metastases. Around one quarter of the patients who develop liver metastases will have disease isolated to the liver. The majority of these patients will have unresectable disease and will eventually succumb to liver failure (Silen, 1989; Sasson & Sigurdson, 2002). Factors influencing survival include the number and position of liver metastases and the percentage hepatic replacement by the tumour. If

left untreated, patients with liver metastases have a poor prognosis, with a median survival of less than twelve months (Wood et al., 1976; Stangl et al., 1994).

1.7 Detection of Liver Metastases

1.7.1 Imaging of Liver Metastases

Detection of liver metastases is fundamental in the accurate staging and subsequent management of patients with colorectal cancer. Intraoperative ultrasound has been widely used for the detection of liver lesions for a number of years. This technique may detect solid lesions down to 3-5mm and the sensitivity has been previously reported as high as 98%. In addition, intraoperative ultrasound can detect tumours deep in the liver, which may evade surgical palpation (Choi et al., 1991; Machi et al., 1991; Schmidt et al., 2000).

Currently the most common methods of choice for liver screening in colorectal cancer are computed tomography (CT) or magnetic resonance imaging (MRI). Both of these techniques routinely use contrast media to enhance the liver parenchyma to tumour ratio. There are mixed reports as to which method produces the highest detection and characterisation rates, however it is generally accepted that both methods are highly effective at detecting metastases of 1cm or larger. In contrast, the sensitivity of imaging methods (CT and MRI) for detecting liver lesions smaller than 1cm is approximately 50% when surgery with intraoperative ultrasound is used as the "gold standard". The true accuracy of the reference standard itself is however difficult to establish (Ward et al., 1999; Robinson, 2000).

Conventional ultrasound was previously used for the detection of liver metastases as it is non-invasive, has minimal costs and widespread availability. With the emergence of contrast-enhanced CT and MRI the efficacy of conventional ultrasound has been questioned. However, recent advances in ultrasound technology have improved the sensitivity and specificity of this technique. The development of microbubble contrast

agents allow a safe and simple method for improving the liver to tumour ratio and can provide valuable information on the blood flow through smaller vessels to malignant tumours (Blomley et al., 2001). Additionally a multi-centre trial has recently reported a higher rate of lesion detection for contrast-enhanced ultrasound, than both CT and MRI (Leen et al., 2002).

The growth of previously undocumented liver metastases following apparently curative surgery, may be a result of subsequent seeding and growth of micrometastases following incomplete or palliative resection of the primary tumour. It has also been proposed that the primary tumour inhibits angiogenesis of its metastasis (Peeters et al., 2004; Peeters et al., 2005; Peeters et al., 2006). However, in the majority of cases it is likely that occult liver metastases went undetected at the time of diagnosis. Advances in radiological techniques are therefore focusing on improving the detection and characterisation of lesions to provide a more accurate method of staging patients (Machi et al., 1991).

1.7.2 Liver Haemodynamics

Despite advances in the detection of liver metastases, the current minimum detection size of lesions by CT and MRI is approximately 3-4mm diameter, however, the majority of lesions in this size range are not visualised. It has been suggested that liver blood flow is altered shortly after metastatic seeding in the liver and this has been the focus of many studies designed to improve detection of lesions in the size range 0.1-2mm (Robinson, 2000).

The liver is unique in the fact that it possesses a dual blood supply, approximately 75% of the blood coming from the portal vein and 25% from the hepatic artery. The

hepatic artery is a branch of the coeliac axis coming from the abdominal aorta; the portal vein channels blood from the stomach, spleen and intestines. Hepatic arterial blood is rich in oxygen, whereas blood from the portal vein is deficient in oxygen but rich in nutrients. Both hepatic arterial and portal venous vessels branch and divide until they reach the hepatic sinusoids. Therefore each cell within the liver is supplied with oxygenated and nutrient-bearing blood. Blood is drained from the liver via numerous small hepatic veins into the three main hepatic veins: right, middle and left. The middle vein marks the plane between the right and left lobes of the liver. The hepatic veins connect into the inferior vena cava and then to the right side of the heart (Woodcock, 1975; Fan & Chang, 2002).

It has been shown that metastases are seeded in the liver via the portal venous system and initially derive most of their nutrients via the portal venous flow. However, as they grow and develop a vasculature their blood supply is predominantly derived from the arterial component of liver blood flow, which increases to meet the metabolic demand causing an increase in the ratio of arterial to portal venous blood flow (Ridge et al., 1987).

These alterations in liver blood flow can be exploited for the detection of micrometastases through techniques such as scintigraphy, ultrasound and CT. However, there are technical limiting factors to the measurement of liver blood flow and conflicting reports on the accuracy.

1.7.3 Hepatic Perfusion Research

Human Studies

It is clear that any permanent improvement in survival rate from colorectal cancer will greatly depend on the ability to significantly improve detection of occult metastatic disease to the liver. As technology advances, the ability to image micrometastatic liver

lesions will continue to improve, however, for the foreseeable future there will always be limiting factors preventing 100% specificity and sensitivity. By assessing the alterations in hepatic haemodynamics and combining with routine radiological screening methods, the detection and characterisation rates of liver metastases may be enhanced which will ultimately lead to improvements in survival rates. There have been a number of studies over the last 25 years utilising radiological techniques to exploit hepatic haemodynamics aiming for an improvement in detection and survival rates.

Alterations in the hepatic blood flow of patients with colorectal liver metastases were first measured by Leveson and co-workers (1983). Dynamic flow scintigraphy was used to assess liver perfusion and the ratio of hepatic arterial to total liver blood flow was used as an indicator of the presence of hepatic metastases.

Liver perfusion was studied by injecting a bolus of contrast agent or radiotracer into a peripheral vein where the hepatic arterial component arrives in the liver approximately 6-10 seconds ahead of the start of the portal venous component. If the rate of arrival of the contrast agent or tracer is measured it is possible to derive a measurement of the ratio of arterial to portal or to total hepatic inflow. Results from studies using a radioactive colloid tracer indicated that patients harbouring liver metastases have a higher hepatic arterial to total liver blood flow ratio, termed the Hepatic Perfusion Index (HPI; Leveson et al., 1983; Parkin at al., 1983; Goldberg et al., 1991a; Robinson, 2000).

Initial studies using HPI produced high sensitivity values for the detection of liver metastases. A prospective study in normal healthy controls and patients with gastrointestinal cancers reported that 96% (24/ 25) of patients with metastatic disease at laparotomy had an elevated HPI value, in comparison the sensitivity of isotope scanning of the same cohort was a less remarkable 64% (Leveson et al., 1983).

In addition, approximately half of the colorectal cancer patients with no overt liver disease at laparotomy had abnormally elevated HPI values (Leveson et al., 1983). It was later reported that 18 patients with apparently normal livers at laparotomy, had developed liver metastases at one year follow-up. It is of interest that all of these patients had an abnormal HPI value at the time of initial investigations. In contrast, all of the patients with low HPI values remained free from hepatic disease and it was suggested that dynamic flow scintigraphy had a potential role for the staging and identification of patients with occult liver metastases from gastrointestinal cancers (Leveson et al., 1985).

However, other centres reported less encouraging results and were unable to reproduce the high sensitivity and specificity described by Leveson and co-workers. Ballantyne and co-workers (1987) found raised HPI values in only 68% (17/ 25) of patients with overt liver metastases. Likewise, a study by Laird and co-workers (1987) reported a high number of false-negative HPI values in patients with hepatic tumour involvement.

Despite this, further studies have since confirmed the original reports by Leveson and co-workers. Hemingway and co-workers (1992) reported a diagnostic sensitivity of 96% for the HPI technique compared with 79% for static scintigraphy in patients with colorectal cancer. More recently, Warren and co-workers (1998) reported that an elevated HPI was associated with poor outcome in patients having a potentially curative resection for colorectal cancer.

In response to the reports of poor reproducibility and accuracy, the HPI technique was investigated by Maughan and co-workers (1992) in a multicentre study using a phantom to simulate the dual blood supply of the liver. Furthermore, in order to assess non-instrumental differences, 28 patients underwent HPI measurements at two different centres. There was a generally good agreement between calculated HPI values in the

phantom study, variations occurring only as a result of statistical variations and uncertainty in the gradient calculation. For the patient studies, there were no statistically significant differences in the pooled HPI results from the two centres. Nevertheless there was a poor correlation between the two sets of values between centres, with correspondence in only 79% of patients. It was thought that the 'grey area' between normal and abnormal HPI values was responsible for this result and it was concluded that each centre using the HPI technique should establish its own range of normal values by studying a control population (Maughan et al., 1992).

The HPI technique is prone to several errors. Selection of 'regions of interest' (ROI) and variations in the time period over which the gradient of the curves are calculated can all affect the accuracy of the HPI. The presence of intrahepatic porto-systemic shunting, sometimes associated with large liver tumours, may affect the HPI, causing false-negative values (Leen, 1999). A further limitation is the interpretation of results and the fact that the hepatic arterial flow and portal venous flow cannot be assessed individually.

Advances in Doppler ultrasonography have provided a more direct method for the measurement of hepatic blood flow. The flow in a particular vessel can be calculated by measuring the flow velocity and multiplying this by the cross sectional area of the vessel. As an analogy to the HPI, the Doppler Perfusion Index was calculated as the ratio of hepatic arterial to the sum of hepatic arterial and portal venous blood flows (DPI: HAF/ HAF + PVF; Leen et al., 1991a). Initial studies showed an increased DPI in patients with overt colorectal liver metastases, compared with normal control subjects. Liver blood flow was measured in 30 patients with colorectal cancer (19 with proven hepatic metastases) and 16 control subjects. It was found that both the hepatic arterial blood flow and portal venous blood flow differed significantly between the metastatic patients and control group.

However, better discrimination was provided by the Doppler Flow Ratio (DFR: HAF/PVF) and the DPI. It was suggested that the DFR and the DPI may have the potential to detect occult metastases below the limits of conventional imaging techniques (Leen et al., 1991a).

Further studies indicated the potential for the DPI technique in the detection of occult liver metastases. A study to compare DPI with intraoperative ultrasound for the detection of liver metastases was performed in 90 patients undergoing an apparently curative operation for colorectal cancer. After one year of follow-up, 23 patients developed liver metastases, all of which had abnormal pre-operative DPI values. Intraoperative ultrasound was abnormal in only six cases and after one year of follow-up, four of these patients had developed liver metastases. Furthermore, all patients with a normal DPI value remained disease free after one year (Leen et al., 1994).

More recently, five-year outcome in 120 patients who underwent potentially curative surgery for colorectal cancer was reported (Leen et al., 2000). Liver metastases occurred in 56 (47%) of patients, 50 (42%) died of recurrent disease and a further six patients died with clinical evidence of recurrence but without pathological confirmation. Of the patients with abnormal DPI, 73% developed recurrent disease. In addition, 89% of patients with a normal DPI remained disease free. Furthermore, all patients who had local recurrence alone had an elevated DPI. The suggested explanation for this observation was that most patients with apparently isolated local recurrence also have occult liver metastases (Gilbert et al., 1984; Leen et al., 2000). It was concluded that the DPI technique can accurately predict recurrence after a potentially curative resection for colorectal cancer and therefore has a potential role in the selection of patients who may benefit from adjuvant chemotherapy (Leen et al., 2000).

A further study was performed to examine the role of the primary tumour in inducing abnormal liver blood flow. Blood flow was measured in 14 patients before and after resection of a colorectal primary tumour. Five patients had normal DPI values before surgery and there was no change in DPI or blood flow in this group after surgery. Nine of the patients had an abnormally elevated pre-operative DPI; there were only minimal changes in DPI following resection of the primary tumour, predominantly due to a decrease in mean hepatic arterial blood flow. It was concluded that the primary tumour plays a relatively minor role in influencing changes in liver blood flow. It was further suggested that these changes may be more closely linked to the presence of occult metastases and the associated host response (Oppo et al., 2000). There is however no data available on patients with overt liver metastases undergoing liver blood flow measurements before and after resection of a primary tumour.

As with the HPI technique the DPI technique is subject to errors. Potential sources of error involved in the DPI technique include non-uniform insonation of the vessel, spectral broadening, angle correction, area estimation and errors in the post processing facilities of the ultrasound system. These errors can be minimized by standardising the technique used for each measurement and the addition of colour Doppler provides greater identification of vessel anatomy.

Patient variables that may alter liver blood flow measurements include variations in hepatic arterial anatomy, which occurs in approximately 30% of patients. A dual hepatic arterial supply may result in an underestimation of the true arterial supply to the liver. Despite this, only a small proportion of these variations are likely to affect the determination of whether the DPI is truly abnormal or normal. Cirrhosis of the liver may result in elevated DPI values due to the increased hepatic arterial flow and decreased portal

venous flow. To distinguish this from metastasis, clinical reports and radiological investigations should be examined. It is also suggested that the portal vein congestive index should be measured, as cirrhosis is associated with increased intrahepatic portal resistance. In order to minimize these sources of error, it is recommended that as with the HPI, each centre should establish their own normal range of DPI values (Leen et al., 1993b; Leen, 1999).

There have been conflicting reports on the accuracy and clinical usefulness of the DPI technique. A comparative study of screening for asymptomatic colorectal liver metastases was performed in curative resection patients. It was found that CT and MRI were the most sensitive techniques and that DPI and HPI techniques had low diagnostic accuracies (CT, sensitivity 0.67, specificity 0.91; DPI, sensitivity 0.58, specificity 0.57; HPI, sensitivity 0.50, specificity 0.55; Glover et al., 2002).

Furthermore, Roumen and co-workers (2005) presented disappointing results in a study of DPI measurements in 133 patients. Numerous problems were encountered during data measurement such as air in the colon, troubling contrast from previous radiological investigations (barium enema), location of common hepatic artery, obesity, scar tissue and insufficient fasting. Subsequently, 29 patients were excluded as the DPI was unreliable. Of the remaining subjects, a trend for higher hepatic arterial blood flow was found in the primary liver metastases group compared to the disease free cohort. However it was not possible to demonstrate any clear separation of DPI or hepatic arterial blood flow values between the three study groups (disease free n=57, metachronous metastases n=11, synchronous metastases n=19).

Despite the potential sources of error and conflicting reports, the clinical results suggest that the DPI technique is a useful method of studying the blood flow changes associated with liver metastases.

The DPI is a function of blood flow in two vessels, the hepatic artery and the portal vein, in which the blood flow changes are in opposite directions. It is unclear whether the DPI combines the effect of these changes in an optimal manner.

The technique of using Doppler ultrasound to investigate alterations in hepatic haemodynamics has several advantages over hepatic scintigraphy. Doppler ultrasound provides a direct measurement of flow in the vessels supplying the liver for example. In comparison, scintigraphy provides perfusion data within a region of interest which is arguably a more speculative method of assessing liver blood flow. Patient safety, cost and accessibility also have major implications when selecting a research method. Ultrasound technology has become more readily available over the last 10 years as a widespread method of screening throughout medical and surgical disciplines, however with the exception of a few centres, scintigraphy is not as readily accessible as a low risk efficient research tool.

As there were serious challenges to the operator dependency of the DPI method, it was crucial to this project that all measurements be carried out to test the technique as a viable and novel source of haemodynamic information.

Animal Studies

In order to clarify the observed alterations in hepatic haemodynamics in humans using the HPI technique, a dynamic hepatic scintigraphy technique was developed in the rat by Nott and co-workers (1987). Micrometastases were induced in Fisher rats using an

intraportal inoculation of Walker 256 carcinosarcoma cells. An additional control group comprised of rats which received normal saline. A significant decrease in portal venous flow occurred in the inoculated rats at 4 and 6 days when compared to controls (Nott et al., 1987).

Following on from this initial report a second study was described in animals by Nott and co-workers (1989). As before, micrometastases were induced using Walker 256 carcinosarcoma cells which were inoculated into the portal vein of anaesthetized male Fisher rats. A control group of rats were similarly inoculated with Walker cells killed by temperature reduction. The rats were then studied at 2, 4 and 6 days after an injection of either viable or dead Walker cells. In addition to the radionuclide studies (HPI technique), microsphere technique (for quantification of absolute changes in both hepatic arterial and portal venous inflow), electromagnetic flowmetry (to determine relative changes in hepatic arterial and portal venous flow), portal pressure studies and measurement of intrahepatic shunting, were all carried out in order to investigate the alterations in hepatic haemodynamics.

It was found that rats bearing live tumour cells demonstrated increased HPI values 4 and 6 days after inoculation. As previously reported in the animal model, it was found that the increased HPI values were the result of decreased portal venous flow and not increased hepatic arterial flow, as previously assumed by Leveson and co-workers (1985). As the liver tumour developed, the portal flow decreased, portal pressure increased and in turn, splanchnic vascular resistance and portal venous resistance were also significantly elevated. It was suggested that the decrease in portal venous flow was due to arteriovenous shunting or a mechanical effect resulting from compression of the portal triad by the tumour.

Furthermore an increased passage of microaggregated albumin through the liver occurred at 4 and 6 days after inoculation of live tumour cells, suggesting that increased portal resistance may be due to the development of new arteriovenous shunts within or surrounding the tumours rather than at pre-existing presinusoidal arterioportal communications (Ackerman, 1974; Nott et al., 1989).

Subsequently, similar studies were carried out in animal models in order to provide a better understanding of altered hepatic haemodynamics. It was described that in humans, colorectal liver metastases rarely exhibit significant arteriosystemic shunting and are relatively hypovascular (Taylor et al., 1978; Goldberg et al., 1987). Therefore, Hemingway and co-workers (1991) utilised the hypovascular HSN sarcoma cells as a more realistic model of human liver metastases than the previously described hypervascular Walker 256 tumour. It was confirmed that the HPI increased when the tumours were at a microscopic stage of development and that this was due to a reduction in portal venous blood flow rather than an increase in hepatic arterial flow. However in contrast to the findings in the Walker 256 model, there was no evidence of a significant increase in portal venous pressure, intrahepatic portal vascular resistance or arteriovenous shunting. The authors concluded that, in this model, the reduction in portal venous blood flow was unlikely to be due to mechanical compression of branches of the portal vein. As there was a substantial increase in vascular resistance in the organs draining into the portal vein, the authors suggested that circulating vasoactive agents that elicit splanchnic vasoconstriction may contribute to the blood flow changes.

This suggestion received further support from a study by Carter and colleagues (1994) who cross-perfused normal rat bowel segments with arterial blood alternately from rats with intrahepatic HSN tumours and from control animals. There was a significant

increase in vascular resistance when the bowel was perfused by blood from tumour-bearing animals, consistent with the hypothesis that circulating vasoconstrictors active in the bowel are increased in the presence of hepatic tumours (Carter et al., 1994).

The link between the haemodynamic alterations observed using colour Doppler ultrasound and the pathophysiology of liver metastases was also investigated in animal models. Yarmenitis and co-workers (2000) implanted Walker 256 carcinosarcoma cells subcutaneously into 30 male Wistar rats. A further 10 rats comprised the control group. The animals were assigned into groups of 10 and liver blood flow measurements were performed at 0, 4, 7 and 15 days after tumour inoculation. Liver histology followed each Doppler measurement.

Both the hepatic arterial flow volume and the DPI were increased in the last three groups (4 – 15 days) compared with the first group (0 days: controls). There was a less prominent decrease in the portal vein flow volume in the 4 – 15 days. Resistive index of the hepatic artery peaked at day 15 and portal venous resistive index was overlapping in all groups. When histological examination of the livers was performed there was a uniform pattern of neoplastic infiltration, and malignant cells were detected in the livers of all rats as early as day 4.

The results of the study indicate that significant haemodynamic changes can occur without the presence of fully developed and well-vascularised metastatic infiltrates. Furthermore it is suggested that changes can be triggered by isolated neoplastic cells in the liver connective tissue, even before the hepatocytes are involved. It is of great interest therefore that alterations in the hepatic arterial flow and DPI occurred as early as day 4 and remained constant until the study ended at day 15.

In conclusion it is clear that the animal model can provide a useful tool to elucidate the altered hepatic haemodynamics in colorectal cancer. It has been shown that both HPI and DPI were increased in rats bearing liver metastases however the mechanisms behind these alterations were contradictory. There was a significant decrease in portal venous flow with HPI (Nott et al., 1987; Nott et al., 1989; Hemingway et al., 1991) and a primary increase in hepatic arterial flow with a less prominent decrease in portal venous flow with DPI (Yarmenitis et al., 2000). It is possible that the alterations in hepatic flow that occur in the rat differ from those that occur in the human liver. The type of carcinoma cell line (Walker 256 or HSN Sarcoma) can lead to a hyper- or hypo-vascular tumour respectively, which can have significant implications on the study design and any conclusions and comparisons drawn to human colorectal cancer.

It is clear that there is a large amount of interesting data presented from centres aiming to define a novel prognostic method for the detection of occult liver metastases. However there are still gaps in the current knowledge, including (i) the clinical accuracy and usefulness of the Doppler Perfusion Index technique (Glover et al., 2002; Roumen et al., 2005); (ii) a clear definition of the alterations in hepatic arterial and portal venous blood flow occurring in patients with colorectal liver metastases (Nott et al., 1987; Leen et al., 1991a; Kruger et al., 2000; Yarmenitis et al., 2000); and (iii) the effect of a primary colorectal tumour on liver metastases driven blood flow changes.

1.7.4 Functional Computed Tomography

Functional computed tomography (CT) scans have also been used for the measurement of changes in liver haemodynamics and the possible detection of occult liver metastases. Using perfusion measurements of single-slice dynamic CT scans, Miles and

co-workers (1993) described a technique similar to the HPI method but one which provides a greater spatial resolution and is applicable to the left lobe of the liver.

In a small study of controls, patients with liver metastases, patients with cirrhosis and patients with miscellaneous disease, dynamic CT was performed at a single level on the liver while a bolus injection of contrast media was administered intravenously. Data acquisition was performed at several time points until 44 seconds after injection. By placing a region of interest over the aorta, spleen and right and left lobes of the liver, enhancement data was used to produce time-density curves for each region of interest. Hepatic arterial perfusion was then calculated by dividing the maximum rate of enhancement of the liver before the splenic peak by the peak aortic enhancement. Similarly, portal perfusion was measured by taking the maximum slope after the splenic peak enhancement and dividing by the aortic enhancement. The HPI was then calculated by a method analogous to that used in dynamic colloid scintigraphy, namely the ratio of arterial perfusion to total liver perfusion.

Initial results indicated that CT-determined HPI was significantly correlated with colloid scintigraphy HPI. Additionally, the CT determined arterial perfusion ratio was elevated in patients with cirrhosis and those with metastases; furthermore the portal perfusion index was lower in the cirrhotic group. Several possible clinical applications of CT perfusion measurements were suggested, namely identification of cirrhosis, screening for liver metastases, vascular assessment in transplant patients and assessment of treatment for cirrhosis. In addition, it was suggested that with some modification, the technique might provide a means to evaluate tumour perfusion characteristics in patients undergoing radiation therapy or chemotherapy (Miles et al., 1993).

Subsequently, Platt and colleagues (1997) investigated the use of dual-phase helical CT scanning to perform hepatic perfusion-related measurements in cancer patients. As the contrast material was delivered under a 50 second infusion rather than a bolus, it was not possible to calculate absolute perfusion values. The enhancement at selected time points during the arterial phase of scanning, and their ratios to peak liver enhancement, were effectively used as a surrogate. All enhancement values and ratios were found to be significantly elevated in those patients who developed liver metastases during an 18-month follow-up compared with those who did not, the overall prognostic accuracy being reported as 89% (Platt et al., 1997). However, a later study using a similar protocol in breast cancer patients failed to identify patients who subsequently developed metastases (Sheafor et al., 2000).

Some of the potential limitations of the functional CT technique include: patient weight, which may affect the degree of liver enhancement when a standard amount of contrast media is used; fatty infiltration and cirrhosis, which may affect baseline liver attenuation measurements; and altered liver blood flow associated with cirrhosis, which may simulate metastatic disease (Platt et al., 1997).

Furthermore, the radiation burden of constant single slice scanning and moreover during triple-phase scanning, cannot be readily justified on a large population cohort as an experimental method. There are also inconsistencies in chemotherapy intervals reported between different studies. Chemotherapy can certainly influence hepatic haemodynamics and potentially reduce the effects of occult disease on measured attenuation and enhancement values. This may explain the differences in study results (Sheafor et al., 2000).

1.7.5 Percentage Hepatic Replacement (PHR)

The extent of metastatic disease within the liver is instrumental in the assessment and prognosis of patients with colorectal cancer. The extent, number and position of liver metastases were previously described in terms of solitary, multiple in one lobe or multiple in both lobes. However, Mansfield and co-workers (1969) suggested that it might be more useful to express the degree of invasion as the ratio of tumour volume to total liver volume. The 'percentage hepatic replacement' (PHR) provides a potentially more sensitive measure of tumour burden and may be used for assessment and staging of patients (Jaffe et al., 1968; Wood et al., 1976; Finan et al., 1985).

The measurement of PHR has been performed using various methods such as isotope scanning, angiography, ultrasound, computed tomography (CT) or laparotomy. It is generally accepted that intraoperative assessment of the liver can underestimate the true extent of tumour replacement, as only the surface of the liver can be truly evaluated, thereby missing lesions deep in the parenchyma. Of the other methods, it has been shown that CT is the most sensitive method for evaluating PHR (Ward et al., 1988; Hunt et al., 1989a).

Using a computer-assisted method to evaluate CT images, Purkiss and Williams (1992) reported a positive correlation between survival and PHR in 23 patients with liver metastases. It was then reported by Dworkin and co-workers (1995) that tumour volume and not PHR, was the most sensitive technique for assessing the extent of disease. The relationship between the liver parenchymal volume and the change in metastases volume is not clear. It was suggested that parenchymal regeneration may occur during metastatic growth in order to sustain liver function, subsequently PHR may underestimate metastases volume change.

It was later reported by Purkiss (1996) that subjective interpretation of CT scans to estimate PHR was inaccurate and should be abandoned for an objective computer assisted method which was more precise, specific and reproducible.

Percentage hepatic replacement has also been investigated with respect to liver blood flow. A study performed by Hunt and co-workers (1989b) used laparotomy, isotope scanning, ultrasound and CT to measure PHR, and dynamic scintigraphy to assess liver blood flow in control subjects and 45 patients with liver metastases. The amount of PHR was separated into three groups: <25%; 25-50%; and >50%. Neither the colloid clearance rate or the 'liver to spleen' ratio demonstrated any significant change with PHR. However, the total hepatic arterial blood flow was significantly raised in the patients with metastases, in accordance with Leveson and co-workers (1985). More importantly, there was an increasing trend with hepatic arterial flow and PHR staging. It was concluded that both tumour growth and prediction of response to therapy may be more closely related to haemodynamic factors than to physical size estimates. Despite this, there was poor separation between the PHR groups preventing the recommendation of dynamic scintigraphy for staging disease.

1.7.6 Mechanisms of Haemodynamic Changes

Metastasis of cancer is a highly selective process consisting of a series of linked steps favouring the survival of a subpopulation of metastatic cells pre-existing within the primary tumour mass. Growth of small micrometastases requires the development of a vascular supply and continuous evasion of host defence cells (Fidler, 1990; Takeda et al., 2002). As growth of metastases occurs, the majority of their blood supply is derived from

the hepatic arterial component of liver blood flow, which increases to meet the higher metabolic demand (Ridge et al., 1987).

Studies investigating the changes in hepatic perfusion index in the Fisher rat inoculated with Walker carcinosarcoma cells, reported a significant decrease in portal venous flow and no alteration in hepatic arterial flow, resulting in increased HPI values. It was reported that these changes were accompanied by increases in the portal and splanchnic vascular resistance and consequently an increased amount of arteriovenous shunting throughout the liver (Nott et al., 1987; Nott et al., 1989; Nott et al., 1991).

In contrast, studies were carried out using technetium-labelled microspheres of serum albumin to measure baseline shunting during hepatic arterial perfusion scintigraphy in a small cohort of patients with colorectal liver metastases. It was reported that the degree of shunting was low (less than 5% in all cases). Furthermore, there was no evidence to suggest that the use of angiotensin II significantly increases baseline shunting (Goldberg et al., 1987; Goldberg et al., 1991b). The low level of shunting was also confirmed in animal studies by Hemingway and co-workers (1991).

Dynamic scintigraphy was performed on male Hooded Lister rats inoculated with HSN sarcoma cells. It was shown that the HPI can change in the absence of arteriosystemic shunting despite an increase in portal vascular resistance. There was no formation of a collateral circulation as portosystemic shunting was not increased. It was concluded that the decrease in portal venous flow, which was accompanied by a significant rise in splanchnic vascular resistance, was unlikely to be due to mechanical obstruction and more likely related to other factors such as the release of vasoactive agents which bring about splanchnic vasoconstriction (Hemingway et al., 1991).

There have been numerous studies into the effects of the vasoconstrictor angiotensin II on liver and tumour blood flow. In particular it has been shown that an infusion of angiotensin II significantly increases tumour:liver blood flow (Hemingway et al., 1992; Leen et al., 1993c; Dworkin et al., 1996). This vasoactive manipulation has been proposed in the past as a targeted method of improving tumour blood flow and drug uptake for hepatic tumours. Carter and co-workers (1992) used a methylene diphosphonate marker, degradable starch microspheres and angiotensin II to test the potential for drug delivery to liver tumour site in animal models. By introducing via the hepatic artery, it was found that degradable starch microspheres alone and degradable starch microspheres with angiotensin II significantly increased the retention in liver and tumour displaying a 12 fold improvement over controls. It has also been confirmed that continuous infusion of angiotensin II increases flow to liver tumour site (Burke et al., 2001) and that this warrants further investigation to enhance tumour targeting in patients with unresectable liver metastases. Furthermore it has been shown that noradrenaline may also improve the blood flow to tumour sites and hence offer an effective method for improving drug delivery to liver metastases (Shankar et al., 1999).

1.8 Splanchnic Flow

The gastrointestinal tract and associated organs require an adequate supply of oxygen and nutrients to meet their metabolic needs. The blood vessels which supply the digestive organs located in the abdomen (and the spleen) comprise the splanchnic circulation. Over 25% of the output from the left ventricle of the heart can flow through the splanchnic circulation. A major function of the splanchnic circulation is to provide fuel to enable the processes of secretion, motility, digestion, absorption and excretion to take place. It also functions as a storage site for a large volume of blood which can be mobilised when the need arises (Smith & Morton, 2001).

The coeliac artery contributes approximately 20% of the blood supply to the liver, providing it with oxygenated arterial blood. The rest of the output of the coeliac artery provides oxygenated blood to the stomach and spleen. The superior mesenteric artery supplies the pancreas and small intestines and provides part of the oxygenated blood supply to the large intestine. The inferior mesenteric artery also supplies the large intestine with oxygenated blood. The venous blood arising from the abdominal organs contains the absorbed nutrients intestines. This constitutes the portal blood which transports the nutrients in the portal vein to the liver (Smith & Morton, 2001).

It has been shown that a reciprocal relationship exists between hepatic arterial and portal venous flow (Leen et al., 1991). Quantitative flowmetry has described a substantial reduction in portal venous flow in response to an increased hepatic artery flow in patients with an elevated perfusion index. This has also been demonstrated in animal models where an increase in Hepatic Perfusion Index (HPI) was due entirely to reduced portal venous

inflow secondary to increased splanchnic vascular resistance (Nott et al., 1989; Hemingway et al., 1991; Hemingway et al., 1993; Carter et al., 1994).

There are numerous factors which are known to alter hepatic haemodynamics by affecting the splanchnic or intra-hepatic circulation.

1.8.1 Serotonin

Serotonin (5-hydroxytryptamine, or 5-HT) is known to have several biological functions. Derived from the amino acid tryptophan, it acts as a neurotransmitter in the central and sympathetic nervous systems and as an activator of blood platelets. There are numerous reports that serotonin production is increased in colorectal cancer in both human and animal models (Quinn et al., 1979; Tutton & Barkla, 1982; Nitta et al., 2001; Seretis et al., 2001). Nitta and co-workers (2001) demonstrated that serotonin content of the small intestine of colon-26 tumour-bearing mice increased significantly 2 weeks post inoculation of tumour cells. This increase was associated with an increase in tryptophan hydroxylase activity and the number of enterochromaffin cells compared to control mice.

It has also been shown that high serotonin levels may be an indicator of neuroendocrine differentiation in colorectal adenocarcinomas (Seretis et al., 2001). Furthermore it has been reported that selective serotonin reuptake inhibitors (SSRI) can reduce the growth of colorectal tumours, possibly occurring through an antipromoter effect or direct cytotoxic effect (Xu et al., 2006).

It is also known that the plasma concentration of serotonin is particularly high in patients with liver cirrhosis and portal hypertension. It has therefore been suggested that serotonin may increase splanchnic blood flow and hence portal pressure. This may also be

linked to the development of liver cirrhosis and portal hypertension (Lebrec, 1990; Hoyer et al., 1994; Li et al., 2006).

1.8.2 Somatostatin

Studies have shown that somatostatin, a peptide hormone, may inhibit the release of some hormones *in vivo* resulting in a reduction in the portal venous pressure. Furthermore, Zhu and co-workers (2000), reported that this decrease in portal venous blood pressure was reduced specifically as a result of decreased blood velocity and blood flow.

Animal studies with somatostatin have also shown a decreased portal venous in-flow and consequently a decrease in portal venous pressure. It has been suggested that this may be attributed to a reduction in the release of glucagon, a vasodilatory gastrointestinal hormone (Hori et al., 1993).

1.8.3 Nitric Oxide

Nitric oxide is known to be a potent vasodilator and inhibitor of platelet function. Since initial investigations into the pharmacological role of nitric oxide in the late 1970s, it has been shown that nitric oxide is involved with almost every aspect of human physiology for example blood pressure, memory and cellular apoptosis (Wallis, 2005).

Nitric oxide is produced in the liver by sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells and hepatocytes. It has recently been shown that nitric oxide can modulate the intrahepatic vascular tone in normal rats (Kawada et al., 1993; Gasull et al., 2001; Moal et al., 2006). Furthermore, it has been shown that portal hypertension in cirrhosis is partly due to a decreased liver nitric oxide production from the liver sinusoidal endothelial cells. This results in an increased vascular tone with a further increase in

hepatic resistance and portal pressure. In contrast to liver nitric oxide level, systemic nitric oxide production is increased in cirrhosis, resulting in splanchnic vasodilatation and subsequent increased portal inflow, which contributes to portal hypertension (Gupta et al., 1998; Rockey et al., 1998; Gonzalez-Abraldes et al., 2002; Moal et al., 2006).

1.8.4 Tumour Necrosis Factor

Studies in animal models have reported that an increased production of tumour necrosis factor-alpha (TNFα), a pro-inflammatory cytokine, may exert an indirect role in the vasodilatation associated with portal hypertension by inducing the production of nitric oxide (Lopez-Talavera et al., 1995; Wang et al., 2004).

1.8.5 Endothelin-1

There is growing evidence that endothelin-1, a potent vasoconstrictive mediator, has a detrimental effect on portal venous and arterial blood flow resulting in an overall increase in portal venous pressure. It has been shown that endothelin-1 causes a reduction in sinusoid diameter and sinusoidal flow as well as increasing total portal resistance in the normal rat liver (Myhre et al., 1993; Hongzhi et al., 2005; Kuro et al., 2006). Endothelin-1 is also produced by colorectal tumours and there is increasing interest into the potential prognostic value of pre-operative serum endothelin-1 levels (Arun et al., 2004; Elahi & Everson, 2004).

1.8.6 Prostaglandin

High concentrations of prostaglandin (an endogenous vasodilator) in human and animal tumour tissues were first documented over 20 years ago. It has since been suggested that an increased biosynthesis of endothelial substances such as nitric oxide and prostacyclin may play a major role in the hyperhaemodynamics of portal hypertension (Fernandez et al., 1996; Laleman et al., 2005). Cyclooxygenase (COX) is a key enzyme involved in the conversion of arachidonic acid to prostaglandins. Two isoenzymes exist in the mammalian body : COX-1 which is expressed constitutively in many cell types and COX-2 which is expressed only in response to certain stimuli such as trauma, growth factors, tumour promoters and cytokines (Smith, 1996; Taketo, 1998a).

Studies with portal hypertensive rats have shown enhanced release of nitric oxide after long-term prostacyclin inhibition. This suggests a link between both vasodilatory substances, nitric oxide and prostacyclin causing increased splanchnic hyperaemia in portal hypertension (Fernandez et al., 1996).

1.8.7 Oestrogen

It is known that serum oestrogen levels are increased in patients with liver cirrhosis (Maruyama et al., 1991; Sakamoto et al., 2005). Oestrogens and progestogens also increase the gene expression of prostacyclin and other important vessel dilating factors (Kuhl, 1996; Mendelsohn & Karas, 1999). Interestingly, Sakamoto and co-workers (2005) reported that treatment of cirrhotic rats with exogenous oestrogen resulted in an increase in hepatic blood flow and a decrease in portal venous pressure. The administration of exogenous oestrogen may increase the number of oestrogen receptors and promote the production of nitric oxide by the sinusoidal endothelial cells (Sakamoto et al., 2005).

1.9 Factors Affecting Liver Blood Flow in Colorectal Cancer

There are a number of factors that have been consistently reported to alter liver blood flow in the healthy subject. These are the fasting/fed state and the systemic inflammatory response.

It is widely accepted that, in the normal subject, there is a physiological hyperaemic response which is thought to be part of the increased metabolic demand during the digestive process. In an early study, Burns and co-workers (1969) described the effects of digestion on intestinal blood. They reported that mesenteric blood flow, measured using electromagnetic flow probes, began to increase within 5 minutes of feeding. This reached a plateau at about 30 minutes and was still above the fasting blood flow at 3 hours. Similarly, Moneta and co-workers (1988) using Duplex sonography and following a standard meal in healthy volunteers, found that mesenteric flow was increased, maximally at 20-30 mins following the meal, and this flow increase persisted for at least 90 mins afterwards. More recently Kelly (1997), using Duplex ultrasound equipment, reported that portal venous blood flow was increased at 1 hour following a test meal, but had returned to normal at 4 hours. Lycklama a Nijeholt and co-workers (1997) using a magnetic resonance velocity mapping technique in volunteers, also found that portal venous flow was increased by approximately two thirds following a test meal. More specifically, both portal venous flow and hepatic venous flow are increased with the onset of digestion (Kelly et al., 1997). Therefore, if the components of liver blood flow are examined in a non-fasting state then there may be an increase in portal venous flow and this will reduce the Doppler perfusion index.

It has long been recognised that splanchnic blood flow is altered as part of the systemic response to injury (Aulick et al., 1981; Dahn et al., 1987). Indeed, there is considerable evidence to show that splanchnic hypermetabolism explains most of the hypermetabolic response to injury (Takala, 1997).

It is therefore of interest that as part of the systemic inflammatory response following surgical injury, portal venous blood flow has been reported to fall whereas hepatic arterial flow as been reported to increase (Souba & Wilmore, 1983). Also, that interleukin-6, an important mediator of the systemic inflammatory response, including the increase in acute phase proteins such as C-reactive protein (Gabay & Kushner, 1999) has been reported to stimulate splanchnic blood flow (Lyngso et al., 2002).

It has been shown that in patients undergoing potentially curative surgery for colorectal cancer, elevated C-reactive protein concentrations either pre- or post-operatively are associated with increased recurrence and poorer survival independent of Dukes' stage (McMillan et al., 1995; Nozoe et al., 1998; Nielsen et al., 2000). It may therefore be that the systemic inflammatory response is important in determining the changes in Doppler Perfusion Index reported in patients with colorectal liver metastases.

1.10 Treatment of Liver Metastases

In spite of improved surgical technique and increased access to chemotherapy and ablative treatments, long term survival and progress is disappointing. Critically, it is the presence of metastatic liver disease that presents a poor prognosis.

The most commonly used liver nomenclature is based on the original classification by Couinaud (1957) which divides the liver into eight functionally independent segments. Each segment has a branch of the portal vein, hepatic artery and bile duct with hepatic veins located in the periphery of each segment for outflow (Strasberg, 1997; Sasson & Sigurdson, 2002).

1.10.1 Surgical Treatment

Surgical resection of hepatic metastases remains the only potentially curative intervention and current techniques are becoming more aggressive, especially in patient selection. Previous indications for the selection of resectable patients included those with a maximum of four lesions in one lobe; the presence of four or more metastases being associated with a poor prognostic factor (Fong et al., 1999; Iwatsuki et al., 1999). However other criteria such as the patient's condition, liver function, the extent of the disease (including extrahepatic disease), suspected primary liver tumour, possible exposure to hepatitis and alcohol use all play a major role in selection for surgery (Fan & Chang, 2002; Penna & Nordlinger, 2002).

In patients with suspected primary liver tumour, thorough laboratory tests and hepatitis panel should be obtained. Also in patients with any degree of cirrhosis,

hemihepatectomy will not be tolerated, a maximum resection of one or two segments is generally accepted (Fan & Chang, 2002).

In the majority of patients with liver metastases, it is generally agreed that liver function will be maintained after an upper limit resection of 75% of the volume of the liver, or six of the eight anatomical segments. Liver function at this level is only maintained if the remaining liver parenchyma is normal, especially in patients who have undergone chemotherapy, which may alter liver parenchyma (Penna & Nordlinger, 2002).

Surgical resection of liver metastases can be divided into two groups: (a) anatomical resections of one or several segments; and (b) atypical or wedge resection removing a portion of liver parenchyma surrounding a tumour (Starzl et al., 1975; Starzl et al., 1982; Bismuth et al., 1982; Makuuchi et al., 1987).

There have been many large studies investigating the outcome after resection of colorectal liver metastases. Mortality and morbidity rates are summarized in Table 1.3.

A significant proportion (60-70%) of patients will have recurrence of liver metastases following liver resection (Scheele et al., 1991; Fong et al., 1999). Although only a small proportion of these patients will be suitable for repeat hepatic resection, rates of death and complications are comparable to those of initial hepatic resections (Petrowsky et al., 2002).

Table 1.3 Mortality and morbidity following liver resection for colorectal liver metastases (Sasson & Sigurdson, 2002).

Study	Mortality (30-day)	Morbidity	Hepatobiliary Complications*	Infectious Complications
Scheele et al.	4%	16%	8%	3%
Iwatsuki et al.	1%**	8%	-	-
Nordlinger et al.	2%	23%	-	-
Cady et al.	4%	-	3%	6%
Fong et al.	3%	24%	4%	6%
Doci et al.	2%	18%	6%	8%

*Hepatobiliary complications include liver failure, biliary fistulas and bile duct injuries.

** 60-day mortality.

The majority of patients with colorectal liver metastases will have unresectable disease. This may be due to tumour burden, involvement of major vascular structures or extrahepatic disease. Treatment options for such patients is usually limited to chemotherapy and more recently, to ablative techniques such as radiofrequency ablation (Solbiati et al., 1997; Sasson & Sigurdson, 2002).

Some studies have indicated that liver resection or hepatectomy is associated with the post-operative growth of residual tumour, however the mechanisms by which this occur are not clearly defined. It has been suggested that surgical resection may remove the source of a variety of growth inhibitors resulting in angiogenesis and the subsequent growth of previously dormant metastases. Alternatively residual tumour cells may exhibit receptors for cytokines that are released in response to surgery (O'Reilly et al., 1994; Folkman J 1995; Whitworth et al., 2006). It has been previously demonstrated that Hepatocyte Growth Factor (HGF) and interleukin-6 are likely to be early markers of postoperative liver failure following hepatectomy (Chijiiwa et al., 2002).

A recent study performed by Whitworth and co-workers (2006) demonstrated an increase in the peritoneal fluid concentration of HGF after cancer and non-cancer laparotomy, suggesting that it is the surgery, rather than the malignancy that is associated with the increased concentrations of the cytokine concentration. Receptors for this cytokine have been detected in a number of tumours but particularly in colorectal carcinoma, suggesting that an increase in concentration of this magnitude might stimulate the growth of residual tumour cells (Whitworth et al., 2006).

Furthermore, it has been shown that the over-expressions of HGF and c-met indicate an adverse prognosis for patients with Hepatocellular Carcinoma. It has been proposed that a sustained high level of serum hepatocyte growth factor after hepatectomy

may be a factor related to early tumour recurrence and metastasis. Liver regeneration may be a main factor leading to a high level of serum HGF in the early postoperative stage (Wu et al., 2006).

There is evidence that the effect of anaesthesia and surgery can inhibit the immune system to a degree that residual tumour can grow and progress more rapidly. Doenicke and Kropp (1976) showed that halothane-nitrous oxide can depress the phagocytic activity of the reticulo-endothelial system. In addition it has been shown that halothane and isoflurane can inhibit the function of the nitric oxide signalling pathway in blood vessels and the brain (Zuo & Johns, 1997). Surgical stress can also rapidly inhibit neutrophil phagocytic activity and have a detrimental effect on the immune system from the early period of surgery (Kawasaki et al., 2007). This may in turn have implications on the body's ability to cope with infection and metastatic seeding and growth in colorectal cancer.

1.10.2 Chemotherapy

For patients with advanced disease, it is clear that systemic chemotherapy with fluorouracil still remains the therapeutic mainstay 40 years after it was first introduced. This treatment has greater survival and quality of life benefits than the best supportive care, however long-term survival after treatment by intravenous chemotherapy alone is rare (Nicum et al., 2000; Ruo et al., 2001).

Results of a large randomised trial investigating three regimens in metastatic colorectal cancer have recently been published. The study compared overall survival between the de Gramont regimen (fluorouracil bolus and infusion with folinic acid given over two consecutive days every two weeks; de Gramont et al., 1988), the Lokich regimen (protracted fluorouracil infusion; Lokich et al., 1989) and raltitrexed (Jackman et al., 1991).

An average survival of 10 months and a 2-year survival of approximately 15% was reported for all three groups; however, progression free survival and quality of life were superior in the de Gramont and Lokich groups. Overall, the study confirmed the benefits of the de Gramont regimen with low toxicity despite the high financial costs (Maughan et al., 2002).

Hepatic Artery Infusion

The rationale for chemotherapy delivery via the hepatic artery is based on both anatomical and pharmacological factors. It is known that established liver metastases derive the large majority of blood flow via the arterial component and in addition, the liver extracts a high proportion of certain drugs during their first pass through the arterial system. Therefore, when administered via the hepatic artery, the regional exposure to chemotherapy drugs is increased and systemic toxicity is limited (Ruo et al., 2001).

1.10.3 Ablative Treatment

Ablative therapies for both liver metastases and primary liver tumours are becoming a popular treatment option in the case of unresectable lesions. Methods of physical ablation include: percutaneous ethanol injection, focused ultrasound, hyperthermia, direct current fulguration, laser photocoagulation, cryoablation and radiofrequency ablation (Dick et al., 2002).

Percutaneous ethanol injection has shown effective response in patients with small Hepato Cellular Carcinomas (HCC) but there have been no studies to date showing any positive impact on colorectal liver metastases (Amin et al., 1993).

Treatment of liver metastases using cryoablation involves freezing the tumour area with the aid of circulating liquid nitrogen through a probe placed within the tumour. Cell

death and microvascular thrombosis occurs due to the freeze-thaw process of the tumour tissue and tumour destruction is complete (Ruers & Bleichrodt, 2002). Survival rates for one and two years after cryoablation have been reported to be 76% and 61% respectively (Ruers et al., 2001).

The technique of radiofrequency ablation is based on the principle of converting radiofrequency waves into heat using a high-frequency alternating current. In a study of 117 patients by Solbiati and co-workers (2001a,b) an overall 2-year survival rate of 69% was reported. One of the major advantages of radiofrequency ablation for treatment of liver metastases is the relatively low morbidity in comparison to liver resection or other ablative therapies. The procedure can be performed as a minimally-invasive percutaneous or laparoscopic operation, reducing harm to normal liver. Patient selection is less restricted and can be offered to patients undergoing partial resection of liver metastases for residual disease or un-resectable lesions (Solbiati et al., 1997; Pearson et al., 1999; Bilchik et al., 2000; Solbiati et al., 2001a,b; Parikh et al., 2002; Ruers & Bleichrodt, 2002).

In conclusion, these observations indicate that major improvements in survival will most likely result from better detection of colorectal primary tumours and metastases at an earlier stage in their growth and development. By exploiting the alterations in hepatic haemodynamics as a marker of metastatic spread to the liver, there may be significant improvements in colorectal cancer survival rates.

1.11 The Role Of Cyclooxygenase in the Pathogenesis of Colorectal Cancer

It has been reported that the presence of an inflammatory response plays a major role in the progression of several solid organ tumours (Coussens & Werb, 2002). Studies have also shown that non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin synthesis, may reduce the incidence of colorectal cancers (Taketo, 1998a). It is suggested that NSAIDs exert their chemopreventive actions by inhibition of cell growth and proliferation, and induction of apoptosis (Smith et al., 2000). The mechanisms by which these processes are achieved are still not fully understood. One commonly studied mechanism is via the COX-2 dependent pathways.

There is increasing evidence of a link between overexpression of COX-2 in colorectal cancer tumorigenesis and other solid organ tumours (Taketo, 1998a, b; Hwang et al., 1998; Uefuji et al., 2000). The overexpression of COX-2 in colorectal cancer is also associated with a several-fold increase in concentration of prostaglandin E_2. One possible mechanism may be that colorectal cancer cell proliferation and growth is promoted through transactivation of epidermal growth factor receptor and its mitogenic signalling. However the mechanism by which prostaglandins promotes colorectal cancer cell invasiveness remains unknown (Pai et al., 2002; Pai et al., 2003; Huang et al., 2006).

There have been numerous retrospective and prospective studies into the use of non-steroidal anti-inflammatory drugs and COX-2 inhibitors for the prevention of colorectal cancer. Huang and co-workers (2006) have suggested that a nonselective COX inhibitor and a COX-2 selective inhibitor trigger apoptosis in human colon cancer cells through at least two known pathways, extrinsic death receptor-transmembrane pathway and an intrinsic mitochondrial pathway. Members of the tumour necrosis factor (TNF) ligand and

receptor superfamily are involved in the transmembrane pathway of apoptosis, such as TNFα, Fas ligand and TNF-related apoptosis-inducing ligand. These ligands when coupling to their respective receptors, trigger a number of intracellular events that lead to apoptotic cell death. Studies have shown that NSAIDs may induce apoptosis in human colorectal cancer cells by up-regulating genes of the TNF ligand and receptor pathway, which may be mediated by TNF receptors and TNF-related apoptosis-inducing ligand receptors (Huang et al., 2006).

Despite increasing evidence of the potential positive effects of COX-2 inhibitors and NSAIDs in the prevention of colorectal cancer, chemoprevention in average-risk individuals is still not favoured. Cardiovascular events and gastrointestinal risks along with cost-effectiveness of chemoprevention need to be considered carefully when comparing with other strategies, such as colorectal cancer screening alone (Rostom et al., 2007).

It has also been reported that pro-inflammatory cytokines such as interleukin-6 are released from colorectal tumours (Kinoshita et al., 1999; Miki et al., 2004). Furthermore there is increasing evidence of an association between raised circulating concentrations of C-reactive protein, tumour growth and poor outcome of patients with colorectal cancer (McMillan et al., 2001; McMillan et al., 2003; Erlinger et al., 2004).

The role of the systemic inflammatory response in altered hepatic haemodynamics in colorectal cancer has yet to be defined. There may be an association between altered metabolic rate in normal liver tissue induced by pro-inflammatory cytokines, or that alterations in liver blood flow are manipulated by inflammatory mediators through other pathways.

AIMS

The aims of this project were to:

1. Establish the effects of colorectal liver metastases and primary or recurrent colorectal tumours on hepatic arterial and portal venous blood flow, and examine the potential for an optimised index to express the haemodynamic changes.

2. Examine the potential value of functional Computed Tomography for the assessment of liver blood flow alterations in patients with colorectal liver metastases.

3. Evaluate the effect of tumour volume and the systemic inflammatory response on liver blood flow in patients with colorectal liver metastases.

4. Investigate the effect of anti-inflammatory treatment on the components of liver blood flow in patients with colorectal liver metastases.

Chapter 2 : The effect of liver metastases on liver blood flow measured by Doppler ultrasound in patients with colorectal cancer.

2.1 Introduction and Aims

Advances in Doppler ultrasound have provided the means to investigate altered liver blood flow and measure hepatic arterial and portal venous flow independently. The Doppler Perfusion Index (DPI) provides a measure of changes in both hepatic arterial and portal venous blood flow in a single index. This has been shown in a number of studies to be useful for the detection of occult metastases and may play a role in the selection of patients for adjuvant chemotherapy (Leen et al., 1991a; Leen et al., 1993a; Leen et al., 2000). However, the DPI is a blood flow ratio, and does not make use of all the information present in absolute blood flow measurements. It was defined in analogy with the earlier Hepatic Perfusion Index (HPI), which was based on scintigraphic measurements that yielded only relative rather than absolute measurements of blood flow. It is therefore unclear whether the DPI combines the arterial and portal blood flow measurements in the optimum manner from the point of view of detecting liver metastases.

The first aim of the present study was to examine the relationship between ultrasound-measured hepatic arterial and portal venous liver blood flow in order to determine the most appropriate way of utilising these measurements to discriminate between patients with and without liver metastases. The model derived from this analysis was then compared with the DPI to determine whether a significant improvement in discriminatory power could be obtained.

It has been shown that a colorectal primary tumour may inhibit growth of its own metastasis (Peeters et al., 2004; Peeters et al., 2006). The role of the primary tumour in inducing changes in liver blood flow has previously been investigated in a small cohort of patients undergoing potentially curative surgery for colorectal carcinoma (Oppo et al.,

2000). However, the influence of the primary tumour has not previously been examined in patients with overt liver metastases. A further aim of the present study was therefore to investigate whether the presence of the primary or recurrent colorectal tumour alters the relationship between hepatic arterial and portal venous blood flow in patients with liver metastases.

Null Hypotheses

There have been conflicting reports on the clinical accuracy of the Doppler Perfusion Index technique (Glover et al., 2002; Roumen et al., 2005). Therefore, the first part of the study was designed to test the null hypothesis that the Doppler Perfusion Index does not discriminate between patients with colorectal liver metastases and normal subjects.

The second null hypothesis states that altered Doppler Perfusion Index in patients with liver metastases, is due to a reduction in portal venous blood flow (Leen et al., 1991a; Di Giulio et al. 1997; Kopljar et al. 2004; Oktar et al., 2006).

Finally, it has been shown that the presence of a primary colorectal tumour may inhibit the growth of liver metastases (Peeters et al., 2004; Peeters et al., 2006). Therefore the null hypothesis states that the presence of a colorectal primary tumour influences liver metastases driven hepatic blood flow alterations.

2.2 Patients and Methods

2.2.1 Patients

Patients with colorectal cancer attending the Glasgow Royal Infirmary between April 2000 and April 2003 were studied. Blood flow measurements were performed before surgery in patients presenting with colorectal cancer.

Inclusion criteria consisted of : Histologically proven colorectal cancer; Liver metastases confirmed histologically, by progression on CT, Ultrasound or intraoperative investigation.

Exclusion criteria consisted of : Anticancer treatment during the study period or during the preceding month; Patients currently receiving or who have had non-steroidal anti-inflammatory drugs in the last two weeks; Patients receiving warfarin; Diagnosis of other liver disease or suspected cirrhosis.

The presence or absence of overt liver metastases in these patients was determined on the basis of pre-operative CT and ultrasound scanning and intraoperative findings. Measurements were also performed in patients known to have liver metastases who had previously been treated and were attending a follow up clinic. None of these patients had received chemotherapy within four months prior to the blood flow measurements. Clinical records were consulted to establish whether or not the primary tumour had been completely resected in these patients. Patients who had no evidence of residual tumour at the time of surgery or of local recurrence at the time of scanning were deemed to have isolated liver metastases.

Normal healthy control subjects were recruited from patients who had negative investigations including abdominal CT scans; and from healthy members of staff in the

Department of Surgery. Clinical details, Dukes' stage and where relevant, details from ultrasound and CT scans were recorded.

The study was approved by the research ethics committee of North Glasgow University Hospitals NHS Trust. All subjects were informed of the purpose and procedure of the study and were given a patient information sheet. Informed consent was given by all subjects in the study, a small number of patients (n=5) declined to take part in the study.

2.2.2 Methods

Hepatic blood flow measurements were performed using an HDI 5000 (Philips-ATL, Bothell, USA) ultrasound system with duplex-colour Doppler and a 3.5 MHz curvilinear scanhead. The angle between the Doppler beam and the vessel was steerable and an angle of 50° to 68° was used for velocity measurements. Using software loaded on the ultrasound scanner it was possible to compute the time averaged mean velocity (the time-average of the weighted mean velocities).

All subjects were fasted for 12 hours prior to examination. Each subject was scanned in the supine position and all measurements were performed during respiratory suspension. A transverse scan was obtained at the epigastrium to locate the common hepatic artery on its longitudinal axis. Hepatic arterial blood velocity was measured by placing the Doppler cursor over the lumen of the common hepatic artery, close to the origin at the coeliac axis at the point where the artery becomes horizontally straight. The time average mean velocity was calculated automatically by placing callipers over a minimum of four cardiac cycles. A minimum of three measurements of the hepatic artery cross-sectional area were then taken at the same point at right angles to the vessel and the mean was calculated off-line.

Portal venous flow was measured using a right intercostal approach. The time average mean blood velocity was measured as above, in the intra-hepatic section of the portal vein before it branches into the right and left portal trunks. Cross sectional area measurements of the vessel were taken using a right subcostal approach, with the patient in suspended inspiration.

Hepatic arterial and portal venous blood flow (HAF, PVF; ml/ min) were calculated from the product of time average mean velocity (cm/s) and average cross sectional area of the vessel (cm^2) multiplied by sixty. Total liver blood flow (TLBF) was calculated as the sum of hepatic arterial and portal venous flow. The Doppler Perfusion Index (DPI) was then calculated as the ratio of hepatic arterial flow to total liver blood flow.

2.2.3 Intra-observer and Inter-observer Variation

Intra-observer Variation

Repeat liver ultrasound scans were performed by myself as described in section 2.2.2 on 18 subjects with a minimum 10 minutes rest between each scan. Measurement values were saved directly to the ultrasound scanner to avoid unconscious bias during scans and data was analysed at the end of the procedure.

Inter-observer Variation

Ultrasound scans of 14 patients, chosen at random, were performed by Dr Paul Glen and myself. Two studies were excluded by one observer due to problems locating the common hepatic artery and hence DPI values were unobtainable, a third patient was excluded due to incomplete examination. Each subject was scanned using the same Ultrasound scanner and methods as previously described in section 2.2.2. As the scans

were performed separately, each observer was blind to the placement of Doppler callipers and cross sectional area measurements.

2.2.4 Statistical Analysis

As the distribution of most measured variables was skewed, the summary statistics reported are the median and interquartile range. Differences between groups in raw data were tested for significance using the Mann-Whitney test. For comparison with published data, means and standard deviations of some variables were also calculated.

As there was a substantial imbalance in age between patient groups, the age-dependence of blood flow values was investigated to determine whether it could bias comparisons of blood flow between groups. Spearman rank correlation analysis demonstrated a significant association between portal venous blood flow and age, therefore age-adjusted blood flow values were calculated to allow unbiased comparisons between groups. Exploratory analysis showed that logarithmically transformed portal venous blood flow had an approximately linear relationship with age, with homogeneous, normally distributed residuals, and a gradient that that was similar in patients with and without liver metastases. Hence, age adjusted blood flow values were calculated using linear regression analysis of logarithmically transformed data, and the age-adjusted comparison of blood flow between patient groups was by analysis of covariance.

The relationship between hepatic arterial and portal venous blood flow within each patient group was also expressed in a simple parametric form using linear regression analysis after logarithmic transformation to normalise residuals. The relationship between hepatic arterial and portal venous blood flow and the presence of liver metastases was also analysed using a logistic regression model, in order to derive a blood-flow-dependent index

reflecting the odds of a patient having metastases. The appropriateness of the logistic regression model was confirmed using the Hosmer-Lemeshow goodness-of-fit test.

A p-value of 0.05 or less was defined as significant. All statistical analysis was performed using SPSS software (SPSS Inc., Chicago, USA).

2.3 Results

2.3.1 Intra-observer and Inter-observer Variation

The correlation of duplicate Doppler Perfusion Index (DPI) measurements for a single observer are shown in Figure 2.1. The mean difference and standard deviation between the two replicate observations was -0.01 (0.13). The intraclass correlation coefficient was 0.63, Spearman rank correlation coefficient was 0.65 (p=0.004 that a significant correlation did not exist) and conventional (Pearson) correlation coefficient was 0.62 (p=0.006), 95% Confidence Intervals were -0.07 and 0.05.

The mean difference (standard deviation) between the DPI values measured by both observers was -0.01 (0.07). The Intraclass correlation coefficient was 0.71, Spearman rank correlation coefficient was 0.75 (p=0.008) and conventional (Pearson) correlation coefficient was 0.70 (p=0.016), 95% Confidence Intervals were -0.05 and 0.03. The inter-observer variation is shown in Figure 2.2.

2.3.2 Blood Flow Measurements in Patients with Liver Metastases and Control Subjects

Sixty-nine patients with colorectal liver metastases and 37 control subjects were studied; their demographic characteristics and liver blood flow parameters are shown in Table 2.1.

The cancer patients were significantly older than the control subjects ($p<0.001$). There was a significant increase in hepatic arterial velocity ($p<0.001$), hepatic artery cross sectional area ($p<0.001$), hepatic arterial flow ($p<0.001$) and Doppler perfusion index ($p<0.001$), and a significant reduction in portal vein cross sectional area ($p=0.016$) and portal venous blood flow ($p=0.002$) in patients with liver metastases compared to normal subjects.

2.3.3 *Effect of Colorectal Tumours on Liver Blood Flow in Patients with Metastases*

Twenty-six of the 69 patients with liver metastases also had primary or recurrent colorectal tumours, while 43 had apparently isolated liver metastases. The demographic characteristics and liver blood flow parameters of these two subgroups are shown in Table 2.2.

There was no significant difference in any of the demographic or blood flow parameters between these two groups of patients. Therefore, metastases patients with and without colorectal tumours were pooled in all subsequent analyses.

2.3.4 *Liver Blood Flow and Age*

Because of the difference in age between controls and patients with liver metastases, it was necessary to examine the relationship between blood flow values and age before considering the differences in blood flow in greater detail. The relationship between hepatic arterial and portal venous flow with age, are shown in Figures 2.3 and 2.4 respectively.

Linear regression models of the following form were separately fitted to the data for hepatic arterial and portal venous blood flow:

$$\log(\text{blood flow}) = A_0 + (A_1 \times \text{group}) + (A_2 \times \text{age})$$

where group is an indicator variable equal to zero for controls and one for metastases patients, and A_0, A_1 and A_2 are the coefficients determined by the model fitting procedure. A_1 is a measure of the difference in flow between groups adjusted for age differences, and A_2 is the slope of the relationship between blood flow and age within each group. A preliminary analysis showed that there was no evidence that this slope differed between

groups for either hepatic arterial ($p=0.79$) or portal venous ($p=0.48$) blood flow, and so a common value of A_2 was assumed to apply to both groups.

The results of the analysis are shown in Table 2.3. Hepatic arterial flow did not vary significantly with age ($p=0.47$), but differed significantly between controls and metastases patients ($A_1>0$, $p<0.001$). By contrast, portal venous flow decreased significantly with age within each group ($A_2<0$, $p=0.027$), and there was no significant difference in flow between groups after adjusting for age ($p=0.37$).

For the purpose of further analysis, an age-adjusted value for portal venous flow was calculated for each patient using the following equation:

$$\log(PVF_{70}) = \log(PVF) + A_2 \times (70 - \text{age})$$

where PVF_{70} and PVF are the adjusted and the originally measured values of portal venous flow respectively, and A_2 is the slope coefficient calculated for PVF. This equation adjusted the flow for each patient to an equivalent age of 70 years, an arbitrarily chosen value equal to the median age of patients with metastases. Although the relationship between hepatic arterial flow and age was not significant, age-adjusted hepatic arterial flow values (HAF_{70}) were calculated on a similar basis so that flow in both vessels was treated in a consistent manner. This procedure allowed the relationship between portal venous and hepatic arterial flow to be studied for the two groups as if they had similar ages. An age-corrected DPI_{70} was also calculated using the standard formula with age-corrected blood flow values. In most of the following analyses, both uncorrected and age-corrected blood flow values are reported so that the effect of age-correction can be assessed.

2.3.5 *Relationship Between Hepatic Arterial and Portal Venous Blood Flow and the Presence of Liver Metastases*

Figures 2.5 and 2.6 show the relationship between hepatic arterial (HAF) and portal venous (PVF) flow in the presence and absence of liver metastases, before and after age adjustment. There was a significant, and similar, positive correlation between log(HAF) and log(PVF) in both metastases patients ($r=0.32$, $p=0.007$) and control subjects ($r=0.34$, $p=0.04$). These correlations were not altered by age correction.

After verifying that the slope of the regression line did not differ significantly between groups, a linear regression model of the following form was fitted to the data for hepatic arterial and portal venous blood flow, with and without age correction:

$$\log(HAF) = B_0 + (B_1 \times \text{group}) + (B_2 \times \log(PVF))$$

where B_0, B_1 and B_2 are the regression coefficients. The results are shown in Table 2.4. Age correction made very little difference to the regression coefficients, which confirmed a significant association between HAF and PVF within groups ($B_2>0$, $p=0.001$) and a significant difference in HAF between groups for a given level of PVF ($B_1>0$, $p<0.001$).

Substituting the age-corrected values of the coefficients from Table 2.4 into the above equation and taking antilogs yields the following relationships:

$$HAF_{70} = 10.2 \times (PVF_{70})^{0.42} \text{ in control subjects}$$

$$HAF_{70} = 25.5 \times (PVF_{70})^{0.42} \text{ in metastases patients}$$

Hence, the presence of metastases is associated with a 2.5-fold increase in hepatic arterial flow for a given level of portal venous flow.

2.3.6 *Derivation of a Potential Diagnostic Index*

To derive an optimised index to distinguish between subjects with and without metastases, the following equation was fitted to the hepatic arterial (HAF) and portal venous (PVF) blood flow data using logistic regression analysis:

$$P/(1-P) = \exp[C_0 + C_1 \log(HAF) + C_2 \log(PVF)]$$

where P is the probability of a subject having metastases, and C_0, C_1 and C_2 are the calculated coefficients of the model.

The results of the logistic regression analysis for both age-adjusted and non-adjusted blood flow values are shown in Table 2.5. Both C_1 and C_2 were significantly different from zero, indicating that a combination of hepatic arterial flow and portal venous flow was the best predictor of group membership. Despite the fact that, after age correction, only hepatic arterial flow differed significantly between the two groups, flow in both vessels needs to be taken into account to distinguish between groups. This corresponds to the fact that, in Figures 2.5 and 2.6, the line that best separates the groups is not a horizontal line (i.e. a fixed level of HAF) but a sloping one (i.e. a function of flow in both vessels).

P/(1-P) is the odds of a subject having metastases. The log of the odds, which we term the Dual Flow Index (DFI), was calculated by taking the logarithm of the above equation:

$$DFI = \log(P/(1-P)) = C_0 + C_1 \log(HAF) + C_2 \log(PVF)$$

Using the coefficients in Table 2.6, the age uncorrected DFI is given by:

$$DFI = 6.05 + 3.21 \log(HAF) - 3.53 \log(PVF)$$

and the age-corrected DFI is:

$$DFI_{70} = -0.37 + 3.10 \log(HAF_{70}) - 2.48 \log(PVF_{70})$$

The difference between the DFI and the DPI is illustrated schematically in Figure 2.7, which shows the hypothetical population distribution of log(HAF) and log(PVF) in metastases and control patients. There is a boundary zone where the groups overlap, and where the odds of a patient having metastases is close to one. Lines of constant DFI have the equation:

$$\log(HAF) = (DFI-C_0)/C_1 - (C_2/C_1) \log(PVF)$$

Hence they are straight lines with a gradient of $-C_2/C_1$ and an intercept that varies with the DFI. Since they are lines of constant odds by definition, they are parallel to the boundary zone.

The corresponding equation for the DPI can be derived from its definition:

$$DPI = HAF / (HAF + PVF)$$

Rearranging this equation and taking logs:

$$\log(HAF) = \log[DPI/(1-DPI)] + \log(PVF)$$

Hence lines of constant DPI have a fixed gradient of one, and an intercept that varies with the DPI. Unlike the DFI, their gradient does not necessarily parallel the boundary zone, and so the DPI may not provide optimal discrimination between groups.

In practice, the optimised gradient $-C_2/C_1$ was close to one for both uncorrected ($-C_2/C_1$ = 1.1) and age-corrected data ($-C_2/C_1$ = 0.8). This implies that the DPI would be expected to provide close to optimal discrimination.

2.3.7 *Comparison of Diagnostic Indices*

The distributions of the DFI and the DPI in control subjects and patients with liver metastases are shown in Figures 2.8 and 2.9 respectively. Despite the theoretical superiority of the DFI, its ability to distinguish the groups was very similar to that of the DPI, as predicted above. Age correction somewhat increased the overlap in values of both indices between control subjects and patients with liver metastases, reducing their discriminatory power.

Figure 2.10 shows the receiver-operator characteristic (ROC) curve for the DFI, in which sensitivity in predicting the presence of metastases is plotted against specificity for varying threshold values. Also shown for comparison are the ROC curves for hepatic arterial and portal venous blood flows in isolation. The ROC curve for the DPI was very similar to that for the DFI and is not plotted. The DFI was more efficient than hepatic arterial flow alone, which in turn was more efficient than portal venous flow, although all three variables were better than chance alone, as represented by the diagonal line.

Figure 2.11 shows ROC curves for the corresponding age corrected variables, DFI_{70}, HAF_{70} and PVF_{70}. Age-correction reduced the efficiency of portal venous flow as a diagnostic parameter to the point where it was not significantly better than chance alone. Hepatic arterial flow was little affected, and the DFI and DPI were somewhat less efficient, becoming only slightly better than hepatic arterial flow alone.

Table 2.6 summarises the sensitivity and specificity for all the indices considered. The threshold values were chosen so that sensitivity and specificity were approximately equal. The results confirm that the DFI provides only a marginal increase in accuracy when compared with the DPI. Without age correction, 88% of patients were correctly classified

using the DFI, as compared with 86% using the DPI. With age-correction, the accuracy of the two indices was reduced to 84% and 82% respectively.

2.3.8 Blood Flow Measurements in Patients Undergoing Apparently Curative Resection

Blood flow measurements were performed pre-operatively in 38 patients with colorectal cancer who, on the basis of imaging investigations and intraoperative findings, had no evidence of liver metastases at the time of investigation. The median age of the patients was 72 years (interquartile range 62-79, range 42-88) and 24 were male. Thirty patients had colonic and 8 had rectal tumours; and 2, 11 and 25 were classed as Dukes' stage A, B and C respectively.

Figures 2.12 to 2.15 show the hepatic arterial blood flow, portal venous blood flow, the DPI and the DFI in these patients in comparison to non-cancer control subjects and patients with liver metastases. All values are age-corrected. The box-and-whisker plot format shows the median, the upper and lower quartiles and the range, with extreme values being plotted individually. Patients undergoing apparently curative resection had intermediate values of these variables that overlapped the values in the other two groups. With or without age correction, none of the variables differed significantly from control values ($p>0.05$), but hepatic arterial flow, the DPI and the DFI were all significantly lower than in patients with metastases ($p\leq0.001$). Twelve patients (32%) had a DFI that exceeded the threshold in Table 2.6, and 13 (34%) had a DPI that exceeded the corresponding threshold.

2.4 Discussion

Doppler ultrasound measurements of liver blood flow are recognised to be subject to several sources of error. Errors in mean blood velocity measurement can arise from non-uniform insonation of the vessel, spectral broadening, inaccurate angle correction and errors in the processing facilities of the system. The estimation of mean blood vessel cross sectional area is subject to errors associated with ultrasound machine resolution limits, non-perpendicularity of the beam-to-vessel angle and the variation of cross sectional area with time. In addition, the technique is dependent on the operator's accuracy and consistency in selecting the locations for velocity and area measurements, and the ability of the patient to cooperate with respiratory suspension. Some of these errors were minimized by standardizing the technique used for each measurement and by the use of colour Doppler to give precise assessment, geometry and identification of the vessel (Leen, 1999).

It is reported that anatomical variation in the hepatic artery are present in approximately 30% of the population (Leen et al., 2000); however only a small proportion of these variations are likely to affect the determination of whether the actual blood flow is truly normal or abnormal. During the study, accessory hepatic arteries were only identified in two such patients. In order to avoid underestimation of the DPI, the total flow in each vessel was added together in order to give the total hepatic arterial supply.

In subjects with normal arterial anatomy, the hepatic artery becomes the hepatic artery proper after the right gastric artery branch. However, this branch is rarely visualised on Doppler ultrasound images, hence it is difficult to ensure accurate flow measurements from the hepatic artery proper. Measurements were therefore performed in the common hepatic artery, accepting that this would result in a small overestimation of arterial flow to

the liver. All subjects were fasted for 12 hours prior to the Doppler ultrasound examination to ensure that blood flow through the right gastric and gastroduodenal arteries was minimized and to standardise conditions for flow measurements (Oppo et al., 1998).

Hepatic blood flow values may also be altered by the presence of liver cirrhosis (Leen et al., 1993b); however none of the patients in the study had clinical evidence of cirrhosis at the time of measurement. It has been previously shown by Leen and co-workers (1993b) that the changes in hepatic haemodynamics between patients with metastases and those with cirrhosis was clearly differentiated by the measurement of portal-vein congestive index (ratio of portal-vein cross sectional area to velocity averaged over time) which was only elevated in the cirrhotic patients.

Approximately 40% of the patients in this study with liver metastases had either a primary or recurrent colorectal tumour. There was no evidence that either hepatic arterial or portal venous blood flow differed between these patients and those in whom tumour was apparently confined to the liver. This suggests that the presence of a colorectal tumour does not affect metastases driven blood flow changes, and is consistent with the results of Oppo and colleagues who studied patients with colorectal cancer before and after they underwent apparently curative resection (Oppo et al., 2000).

The results of the present study confirm that hepatic arterial blood flow, as measured by Doppler ultrasound, is increased in patients with colorectal liver metastases, as has been shown by several other research groups (Leen et al., 1991a, 1991b; Di Giulio et al., 1997; Guadagni et al., 2000; Kruger et al., 2000; Kopljar et al., 2004; Oktar et al., 2006). Increases primarily in both blood velocity but also, to a lesser extent, in vessel cross sectional area contributed to the change in blood flow, which was more than doubled relative to non-cancer control subjects. Portal venous blood flow was lower in patients

with liver metastases relative to controls. However, portal venous flow was negatively correlated with age and the difference in blood flow between groups after adjustment for differences in age was not statistically significant. Previous reports in the literature are in conflict on whether portal venous flow is reduced (Leen et al. 1991a, 1991b; Di Giulio et al. 1997; Kopljar et al. 2004; Oktar et al., 2006) or unaltered (Kruger et al. 2000; Guadagni et al. 2000) in patients with liver metastases. In the present study, the DPI was significantly increased in patients with metastases, mainly because of the increase in hepatic arterial blood flow.

These results are largely consistent with those of Leen and colleagues (1993a), with which they are compared in Table 2.7. Hepatic arterial blood flow values in the two studies were very similar in control subjects, but were some 30% lower in the present study in patients with metastases. Conversely, portal venous blood flow values were similar in cancer patients but 30% lower in the present study in controls. As a consequence, differences between groups in hepatic arterial and portal venous blood flow and the DPI were all smaller in the present study. The reason for these discrepancies is unclear, but they are not associated with any single patient group, blood vessel or type of measurement (velocity or area). Hence, it is likely that a combination of factors is responsible, including the composition of the patient samples and the different ultrasound scanners and operators.

Leen and co-workers (1991b) found no significant correlation between age and blood flow in either vessel. However, an inverse relationship between age and liver blood flow has previously been demonstrated in normal subjects using Doppler ultrasonography (Zoli et al., 1989; Zoli et al., 1999). In particular it has been documented that portal venous flow and total liver blood flow are reduced without any additional intrahepatic shunting and this is particularly evident after 75 years, which may explain the well-known age-related

reduction in liver function and ability to metabolise drugs. Furthermore, Zoli and co-workers (1999) reported that a decrease in portal flow, which was partly counterbalanced by an increase in hepatic artery flow, was observed in subjects between the ages of 45 and 75.

Age related reductions in liver weight and volume may also be attributed to reduced liver blood flow. It was shown by Zoli and co-workers (1999) that serum concentrations of liver enzymes (such as alkaline phosphatase, alanine and aspartate aminotransferase) and serum albumin concentrations were all within the normal range in elderly subjects. It has also been shown that with age, there is a reduction in hepatocyte mass rather than a reduction in morphological liver size (Wynne et al., 1989; Wakabayashi et al., 2002).

It is significant that there was a negative correlation between portal venous blood flow and age in this study. This suggests that age should be considered when interpreting liver blood flow measurements in patients with colorectal cancer. Furthermore patient age may play a role in the conflicting DPI data presented from various centres so far. This may have positive implications for the selection of younger, fitter patients undergoing assessment for more aggressive chemotherapy treatment. For example, approximately one third of patients with Dukes' stage B tumour will have recurrent disease, but are generally denied chemotherapy. Also, around one third of patients with a Dukes' stage C tumour survive 5 years following curative resection, but are exposed to the unnecessary toxic side-effects of adjuvant therapy (Leen et al., 2000).

The differences in age between the metastases group and the control subjects in this study were mainly due to difficulties in recruiting healthy volunteers over the age of sixty. A large cohort of healthy controls was measured throughout the study consisting of relatives, colleagues and known healthy patients with small benign liver haemangioma.

There was a significant correlation between hepatic arterial and portal venous blood flow in both control subjects and metastases patients. This may simply reflect a common dependence of both components of liver blood flow on tissue mass. A consequence of this relationship is that the "normal range" of hepatic arterial blood flow varies with the level of portal venous flow. Hence, for the purpose of detecting the presence of liver metastases on the basis of blood flow measurements, both hepatic arterial and portal venous flow should be taken into account regardless of whether the latter is altered by the presence of metastases.

The DPI is a function of the ratio of hepatic arterial to portal venous blood flow. Leen and co-workers (1993a) found complete separation in DPI values between metastases and control patients (i.e. 100% sensitivity and specificity), the minimum value in the former group being 0.30 and the maximum control value being 0.25 (Leen et al., 1993a). In the present study the DPI without age correction had a sensitivity of 84% and specificity of 89% using a threshold value of 0.25. As discussed above, the greater overlap in DPI values between patient groups in the present study, and hence the lower sensitivity and specificity, cannot be ascribed to any single cause. Nonetheless, the DPI remains a reasonably powerful diagnostic index.

The DFI is an alternative index, similarly based on hepatic arterial and portal venous blood flow measurements, which was optimised to discriminate between metastases and control patients according to a logistic regression model. However, it provided only a marginal increase in accuracy when compared with the DPI. This suggests that the DPI, or equivalently the ratio of hepatic arterial to portal venous blood flow, reflects essentially all the diagnostic information present in the two blood flow measurements, and that there is little to be gained from the use of a more complex index such as the DFI.

The potential clinical value of the DPI and similar blood flow indices lies in their ability to predict outcome in colorectal cancer patients undergoing apparently curative resection, possibly by reflecting the presence of occult liver metastases. It has been reported that 91% of such patients survived five years if they had a normal DPI at the time of surgery, as compared with 29% of patients with an abnormal DPI (Leen et al. 2000). It was not an aim of the present study to investigate the relationship of the DPI or DFI with survival, but the distribution of pre-operative DPI and DFI values in patients undergoing apparently curative resection overlapped the ranges found in control and metastases patients. Approximately one third of these patients had high values of the DPI and DFI, as compared with less than 20% of control subjects.

The first null hypothesis states that the Doppler Perfusion Index does not discriminate between patients with liver metastases and normal subjects. This study rejects the hypothesis and accepts the alternative. Secondly, it was stated that the Doppler Perfusion Index is increased as a result of reduced portal venous blood flow. This null hypothesis cannot be supported due to the primary increase in hepatic arterial blood flow and age dependent alterations in portal venous blood flow observed in this study. Finally, it was proposed that the presence of a colorectal primary tumour influences liver metastases driven hepatic blood flow alterations. This hypothesis can also be rejected as there were no significant differences observed in relation to the presence of a primary tumour.

In summary, this study has confirmed that hepatic arterial blood flow and the DPI are increased in patients with colorectal liver metastases, and has shown that the presence of a primary or recurrent colorectal tumour does not significantly alter liver blood flow in patients with metastases. The DFI, an optimised index of abnormal liver blood flow, only marginally improved on the DPI as a diagnostic test. Portal venous blood flow, and derived indices such as the DPI and DFI, are age-dependent, and this should be considered when interpreting haemodynamic data in patients with colorectal cancer.

Table 2.1 Demographic characteristics and Doppler ultrasound based liver blood flow parameters in control subjects and patients with colorectal liver metastases.

	Control subjects (n=37)	**Liver metastases (n=69)**	**P-value**
Demographics			
Age (years)	49 (29, 59) (34, 56)	70 (64, 75) (66, 71)	<0.001
Sex (male/female)	20 / 17	42 / 27	0.54
Hepatic artery			
Time-average velocity (cm/s)	15.7 (12.4, 19.5) (13.5, 17.6)	24.8 (18.7, 36) (21.5, 28.9)	<0.001
Cross sectional area (cm^2)	0.17 (0.14, 0.24) (0.15, 0.19)	0.23 (0.18, 0.30) (0.20, 0.25)	<0.001
Blood flow (ml/min)	154 (120, 224) (135,193)	334 (238, 535) (286, 428)	<0.001
Portal vein			
Time-average velocity (cm/s)	13.5 (9.8, 16.2) (10.2, 15.6)	11.4 (9.0, 14.1) (10, 12.5)	0.084
Cross sectional area (cm^2)	0.95 (0.82, 1.2) (0.87, 1.08)	0.81 (0.64, 1.2) (0.73, 0.89)	0.016
Blood flow (ml/min)	762 (515, 1014) (615, 886)	542 (403, 727) (432, 608)	0.002
Whole liver			
Blood flow (ml/min)	930 (686, 1203) (783.1, 1120.96)	933 (708, 1183) (803,1024)	0.93
Doppler Perfusion Index	0.17 (0.13, 0.21) (0.14, 0.20)	0.37 (0.28, 0.49) (0.33, 0.42)	<0.001

Data given as median (interquartile range); (95% confidence intervals).

Table 2.2 Demographic characteristics and Doppler ultrasound liver blood flow parameters in patients with colorectal liver metastases, with and without the presence of colorectal tumour.

	Liver metastases + colorectal tumour (n=26)	**Liver metastases alone (n=43)**	**P-value**
Demographics			
Age (years)	69 (62, 73) (64, 73)	70 (65, 74)	0.38
Sex (male/female)	18 / 8	24 / 19	0.32
Hepatic artery			
Time-average velocity (cm/s)	26.7 (12, 89.1) (20.1, 42)	24.2 (8.3, 104) (20.1, 31.5)	0.281
Cross sectional area (cm^2)	0.24 (0.07, 0.73) (0.19, 0.28)	0.22 (0.07, 0.71) (0.19, 0.26)	0.281
Blood flow (ml/min)	394 (173, 1216) (307, 524)	313 (68, 1798) (248, 408)	0.189
Portal vein			
Time-average velocity (cm/s)	11.1 (5.4, 21) (9.9, 13.7)	11.9 (4.8, 31.5) (9.1, 12.8)	0.139
Cross sectional area (cm^2)	0.81 (0.40, 2.02) (0.70, 1.06)	0.81 (0.08, 1.8) (0.68, 0.95)	0.504
Blood flow (ml/min)	530 (337, 2115) (419, 724)	542 (112, 1455) (423, 642)	0.310
Whole liver			
Blood flow (ml/min)	958 (579, 2851) (803,1180)	900 (327, 3148) (724,1008)	0.809
Doppler Flow Ratio	0.70 (0.18, 1.99) (0.49, 0.91)	0.55 (0.17, 8.2) (0.41, 0.79)	0.620
Doppler Perfusion Index	0.41 (0.15, 0.67) (0.33, 0.48)	0.36 (0.14, 0.89) (0.29, 0.44)	0.488

Data given as median (interquartile range); (95% confidence intervals).

Table 2.3 Coefficients of the regression equations relating hepatic arterial and portal venous blood flow to age and patient group.

Dependent variable	Independent variable	Coefficient		95% CI	P-value
Log(HAF)	Constant	A_0	5.26	4.77, 5.75	<0.001
	Group	A_1	0.87	0.54, 1.21	<0.001
	Age (yr)	A_2	-0.004	-0.014, 0.006	0.43
Log(PVF)	Constant	A_0	6.99	6.62, 7.37	<0.001
	Group	A_1	-0.12	-0.37, 0.14	0.37
	Age (yr)	A_2	-0.008	-0.016, -0.001	0.027

CI, confidence interval; HAF, hepatic arterial blood flow (ml/min); PVF, portal venous blood flow (ml/min). For definition of regression equation and coefficients, see text.

Table 2.4 Coefficients of the regression equations relating hepatic arterial flow to portal venous flow and patient group, with and without age correction.

Dependent variable	Independent variable	Coefficient		95% CI	P-value
Uncorrected					
Log(HAF)	Constant	B_0	2.31	0.76, 3.87	0.004
	Group	B_1	0.91	0.66, 1.16	<0.001
	log(PVF)	B_2	0.42	0.18, 0.66	0.001
Age-corrected					
$Log(HAF_{70})$	Constant	B_0	2.32	0.76, 3.88	0.004
	Group	B_1	0.92	0.68, 1.16	<0.001
	$log(PVF_{70})$	B_2	0.42	0.18, 0.66	0.001

CI, confidence interval; HAF, hepatic arterial blood flow (ml/min); PVF, portal venous blood flow (ml/min). For definition of regression equation and coefficients, see text.

Table 2.5 Coefficients of the logistic regression model of the relationship between the presence of liver metastases and hepatic arterial and portal venous blood flow.

	Independent variable	Coefficient		95% CI	P-value
Uncorrected	Constant	C_0	6.05	-2.81, 14.92	0.18
	log(HAF)	C_1	3.21	1.93, 4.49	<0.001
	log(PVF)	C_2	-3.53	-5.26, -1.80	0.001
Age-corrected	Constant	C_0	-0.27	-8.93, 8.39	0.95
	$\log(HAF_{70})$	C_1	3.10	1.89, 4.31	<0.001
	$\log(PVF_{70})$	C_2	-2.48	-4.10, -0.86	0.003

CI, confidence interval; HAF, hepatic arterial blood flow (ml/min); PVF, portal venous blood flow (ml/min). For definition of regression equation and coefficients, see text.

Table 2.6 Sensitivity, specificity and accuracy of diagnostic indices derived from blood flow measurements.

		No. (%) of subjects correctly classified		
Variable	**Threshold**	**Metastases (sensitivity)**	**Control (specificity)**	**All subjects (accuracy)**
Uncorrected				
PVF (ml/min)	<620	45/69 (65%)	24/37 (65%)	69/106 (65%)
HAF (ml/min)	>230	53/69 (77%)	28/37 (76%)	81/106 (76%)
DPI	>0.25	58/69 (84%)	33/37 (89%)	91/106 (86%)
DFI	>0.40	61/69 (88%)	32/37 (86%)	93/106 (88%)
Age-corrected				
PVF_{70} (ml/min)	<550	39/69 (57%)	22/37 (59%)	61/106 (58%)
HAF_{70} (ml/min)	>205	55/69 (80%)	29/37 (78%)	84/106 (79%)
DPI_{70}	>0.25	57/69 (83%)	30/37 (81%)	87/106 (82%)
DFI_{70}	>0.50	57/69 (83%)	32/37 (86%)	89/106 (84%)

PVF, portal venous blood flow; HAF, hepatic arterial blood flow; DPI, Doppler perfusion index; DFI, Doppler flow index.

Table 2.7 Comparison of blood flow parameters in the present study with previously reported values.

Variable	Leen et al. 1993a	Guadagni et al. 2000	Present study
Control patients	n=50	n=18	n=37
HA time-average velocity (cm/s)	19.0 (5.0)		18.3 (12.8)
HA cross sectional area (mm^2)	17.0 (10.0)		17.8 (6.2)
HA blood flow (ml/min)	194 (125)	245 (87)	187 (109)
PV time-average velocity (cm/s)	15.0 (6.0)		13.2 (3.9)
PV cross sectional area (mm^2)	129.0 (36.0)		102.3 (40.3)
PV blood flow (ml/min)	1200 (570)	503 (153)	797 (318)
Total liver blood flow (ml/min)	1390 (640)		984 (361)
DPI	0.14 (0.06)	0.33 (0.10)	0.19 (0.10)
Metastases patients	n=67	n=19	n=69
HA time-average velocity (cm/s)	33.0 (12.0)		29.5 (16.7)
HA cross sectional area (mm^2)	32.0 (14.5)		25.2 (12.8)
HA blood flow (ml/min)	630 (330)	362 (107)	440 (337)
PV time-average velocity (cm/s)	11.2 (4.0)		12.0 (4.7)
PV cross sectional area (mm^2)	99.0 (26.6)		88.4 (36.1)
PV blood flow (ml/min)	670 (340)	655 (414)	625 (364)
Total liver blood flow (ml/min)	1300 (580)		1065 (601)
DPI	0.49 (0.13)	0.38 (0.11)	0.40 (0.15)

Data given as mean (SD). HA, hepatic arterial; PV, portal venous; DPI, Doppler perfusion index.

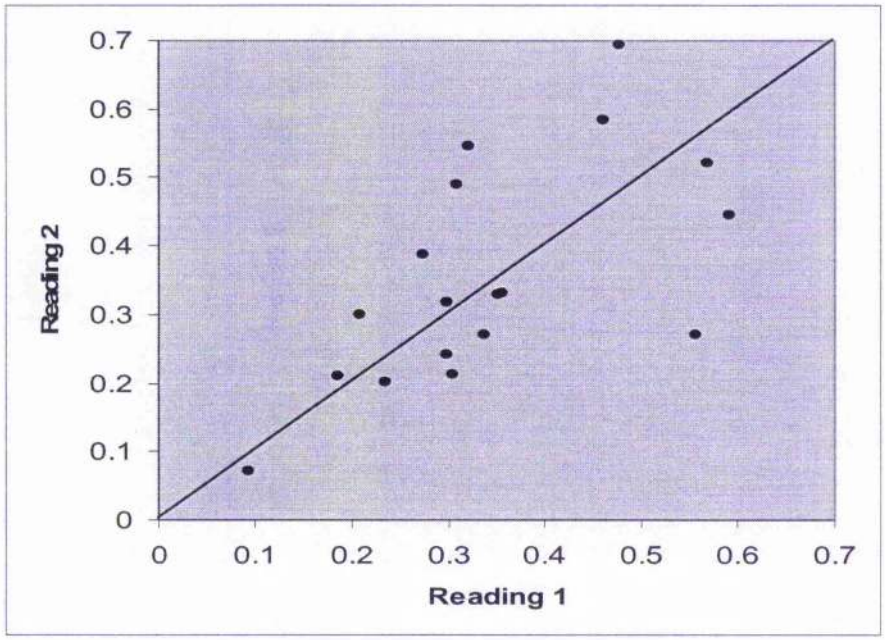

Figure 2.1 Correlation of duplicate Doppler Perfusion Index (DPI) for a single observer.

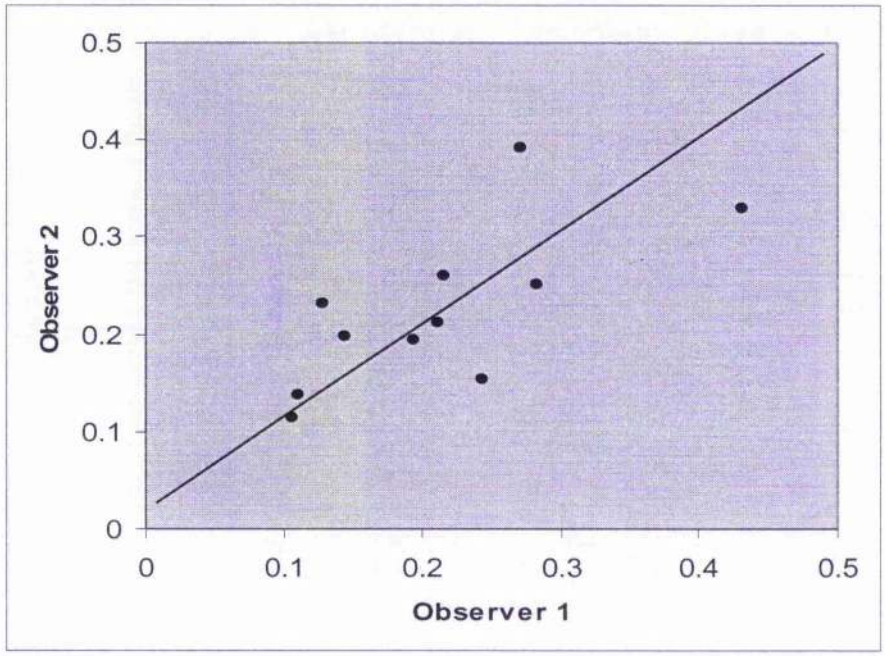

Figure 2.2 Correlation of Doppler Perfusion Index (DPI) for two observers.

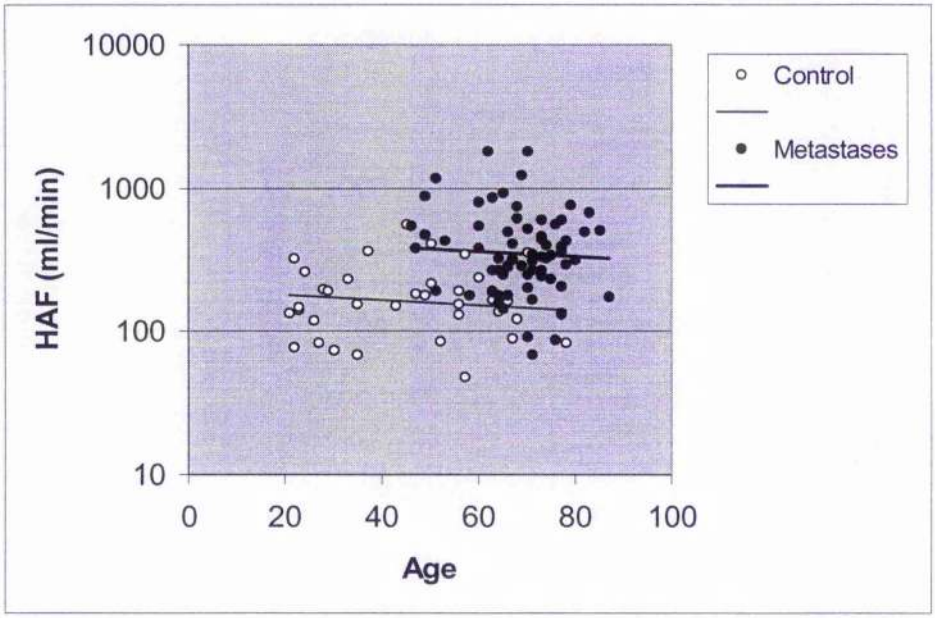

Figure 2.3 Relationship between hepatic arterial blood flow (HAF) and age in control subjects and patients with liver metastases. Regression lines for the two groups are also shown.

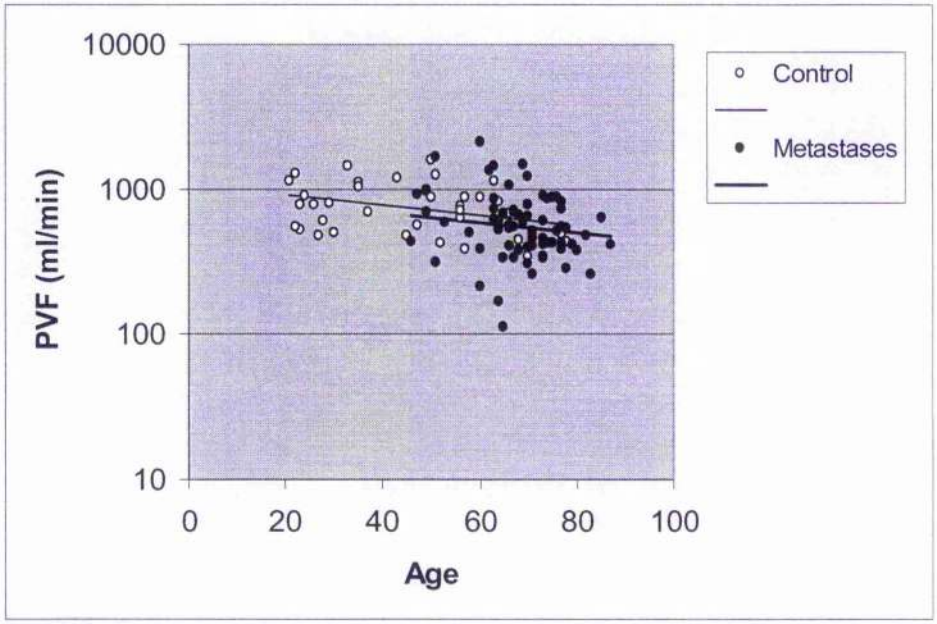

Figure 2.4 Relationship between portal venous blood flow (PVF) and age in control subjects and patients with liver metastases. Regression lines for the two groups are also shown.

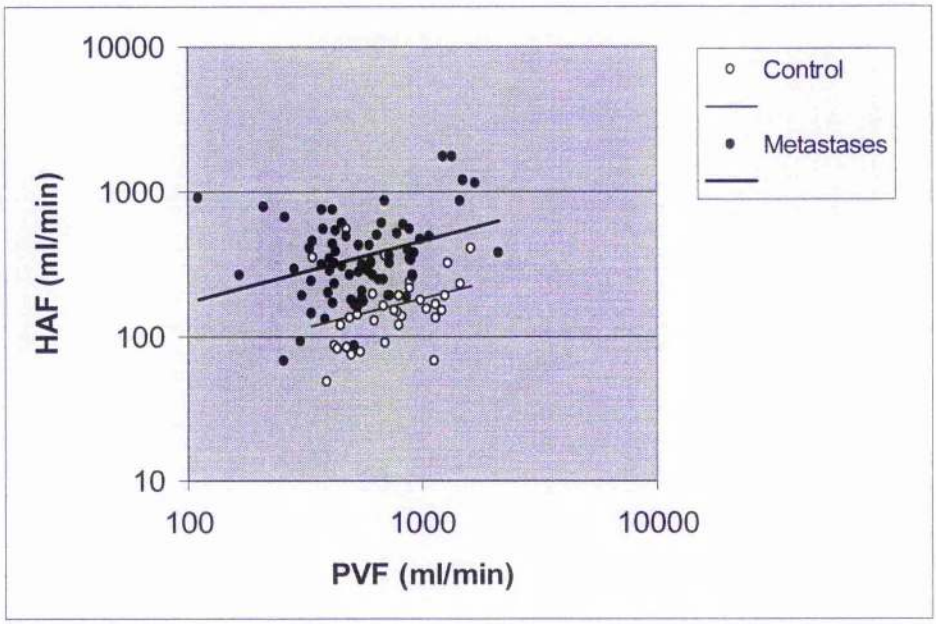

Figure 2.5 Relationship between raw hepatic arterial (HAF) and portal venous blood flow (PVF) in control subjects and patients with liver metastases. Regression lines for the two groups are also shown.

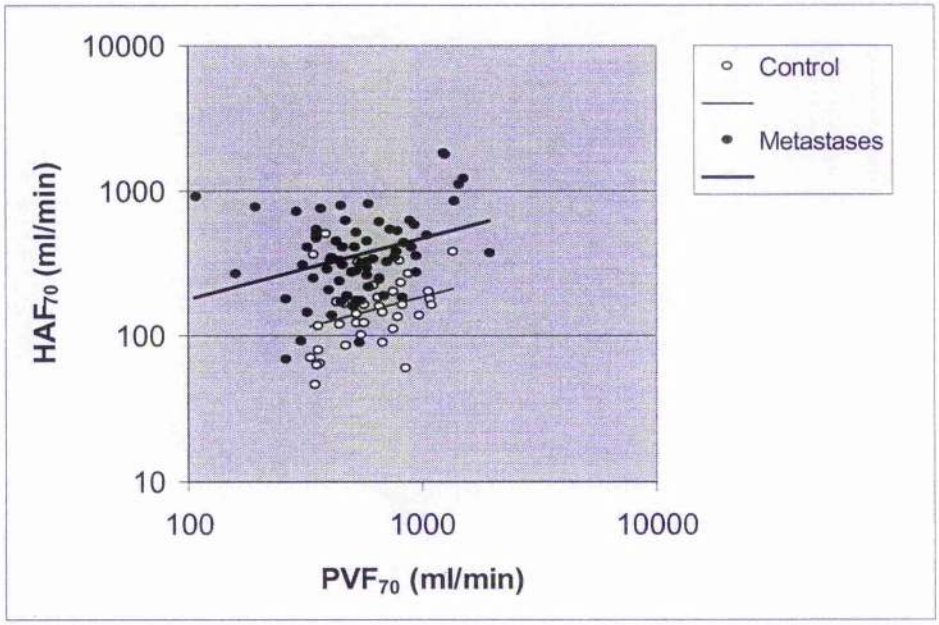

Figure 2.6 Relationship between age-corrected hepatic arterial (HAF) and portal venous blood flow (PVF) in control subjects and patients with liver metastases. Regression lines for the two groups are also shown.

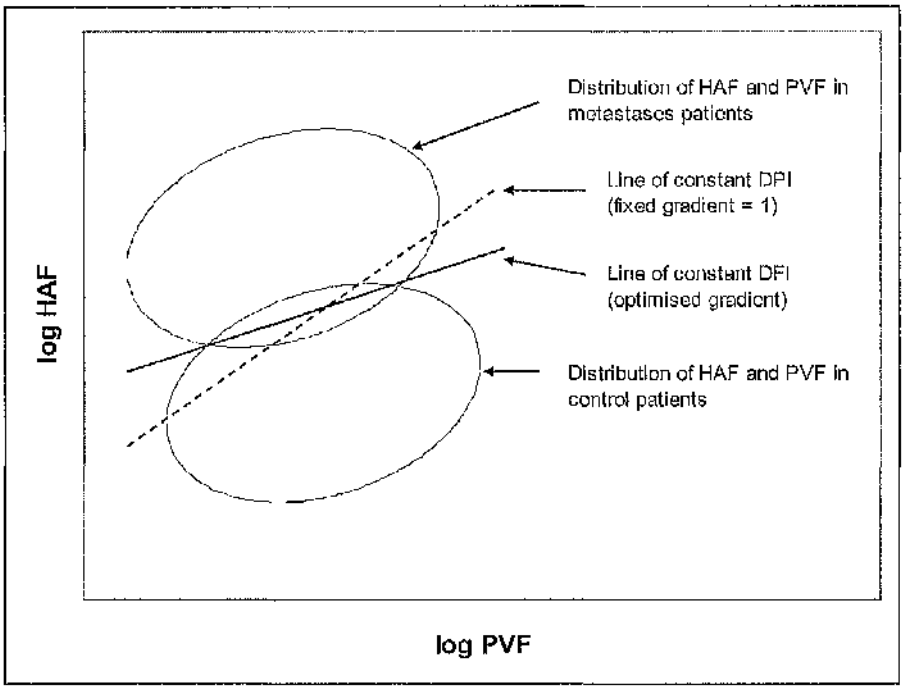

Figure 2.7 Schematic diagram illustrating how the DFI can potentially improve on the DPI in discriminating between patient groups. The ellipses represent the distribution of hepatic arterial (HAF) and portal venous (PVF) blood flow in the two groups. Lines of constant DFI have a gradient similar to the boundary zone between groups. Lines of constant DPI have a gradient of one, and do not necessarily parallel the boundary zone.

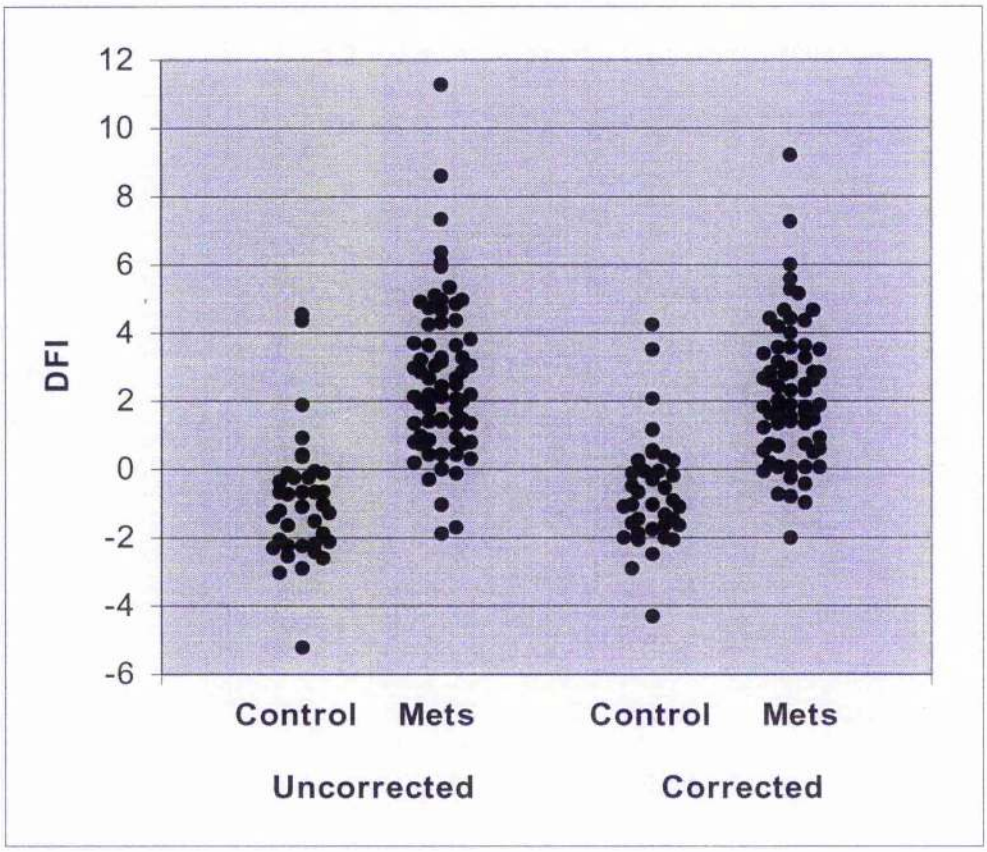

Figure 2.8 Distribution of Dual Flow Index (DFI) in control subjects and patients with liver metastases, with and without age correction.

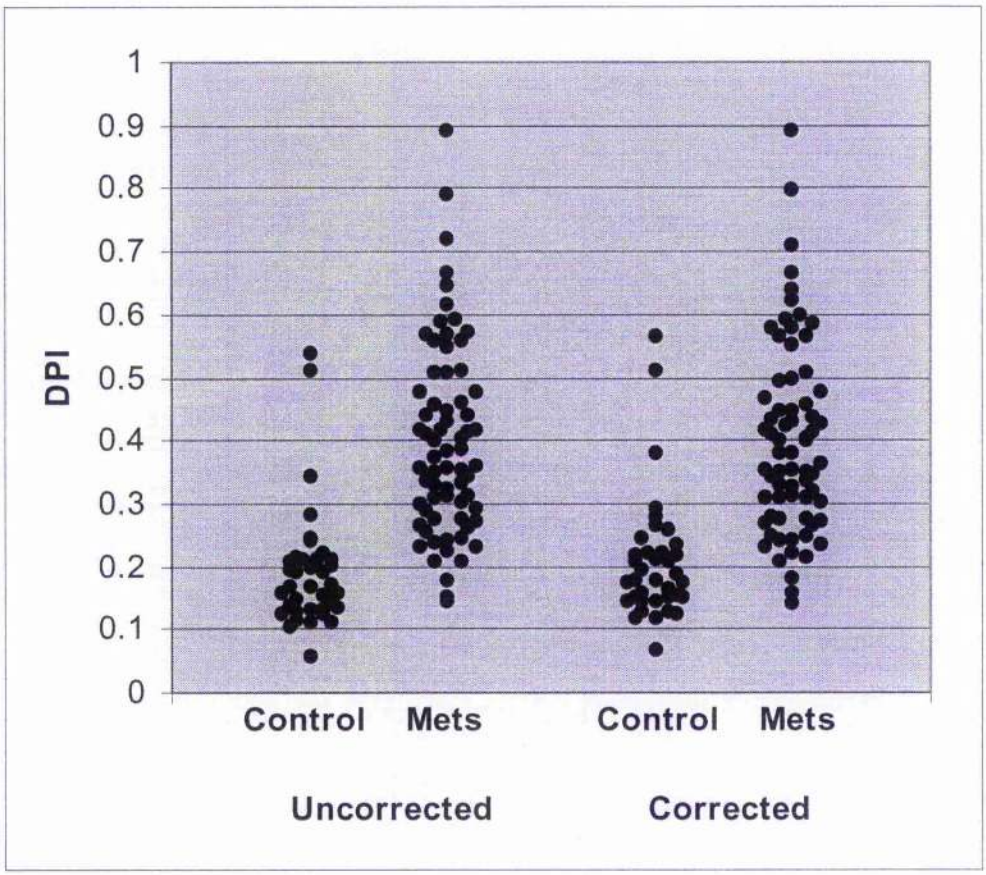

Figure 2.9 Distribution of Doppler Perfusion Index (DPI) in control subjects and patients with liver metastases, with and without age correction.

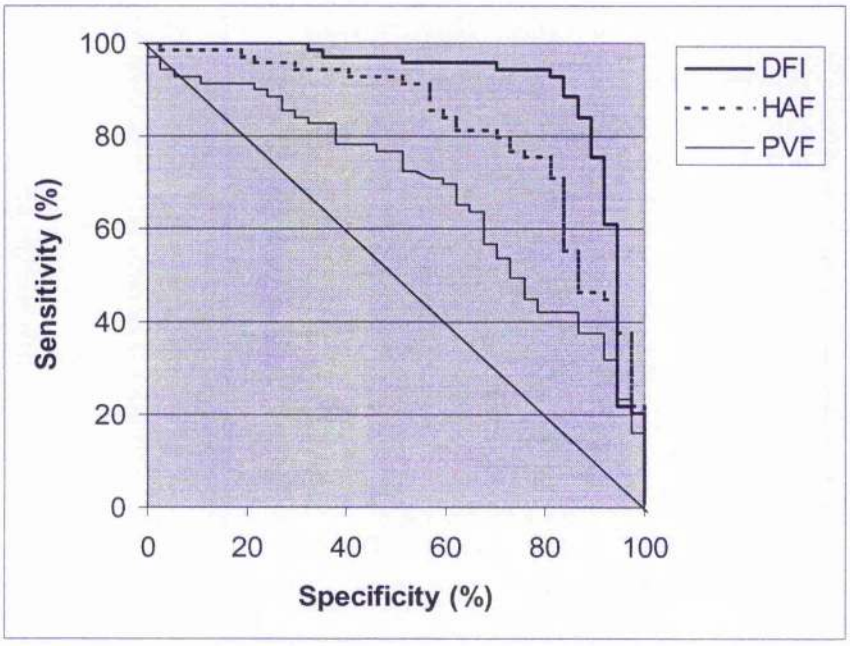

Figure 2.10 Receiver-operator characteristic (ROC) curves for the raw Dual Flow Index (DFI), hepatic arterial flow (HAF) and portal venous flow (PVF) distinguishing between control subjects and patients with liver metastases.

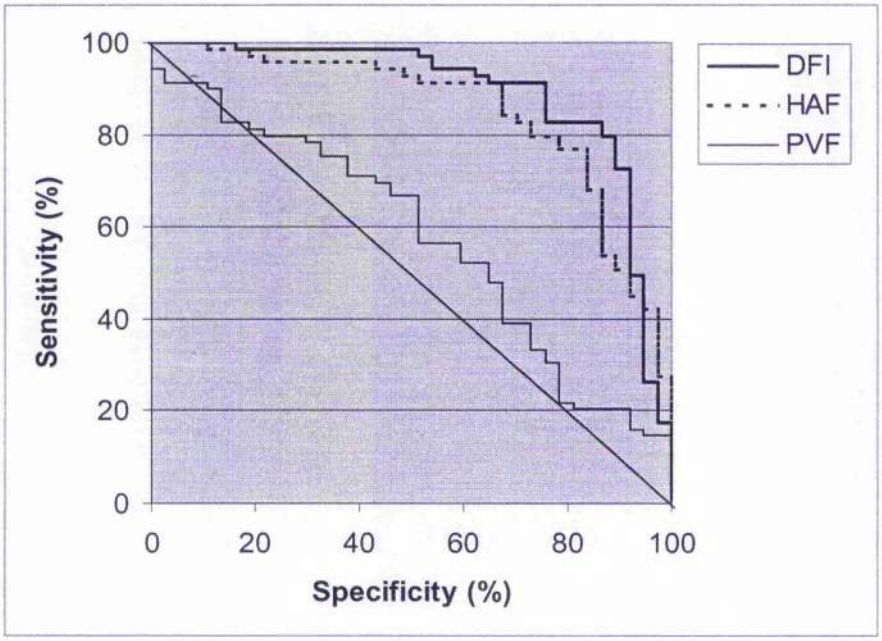

Figure 2.11 Receiver-operator characteristic (ROC) curves for the age-corrected Dual Flow Index (DFI), hepatic arterial flow (HAF) and portal venous flow (PVF) distinguishing between control subjects and patients with liver metastases.

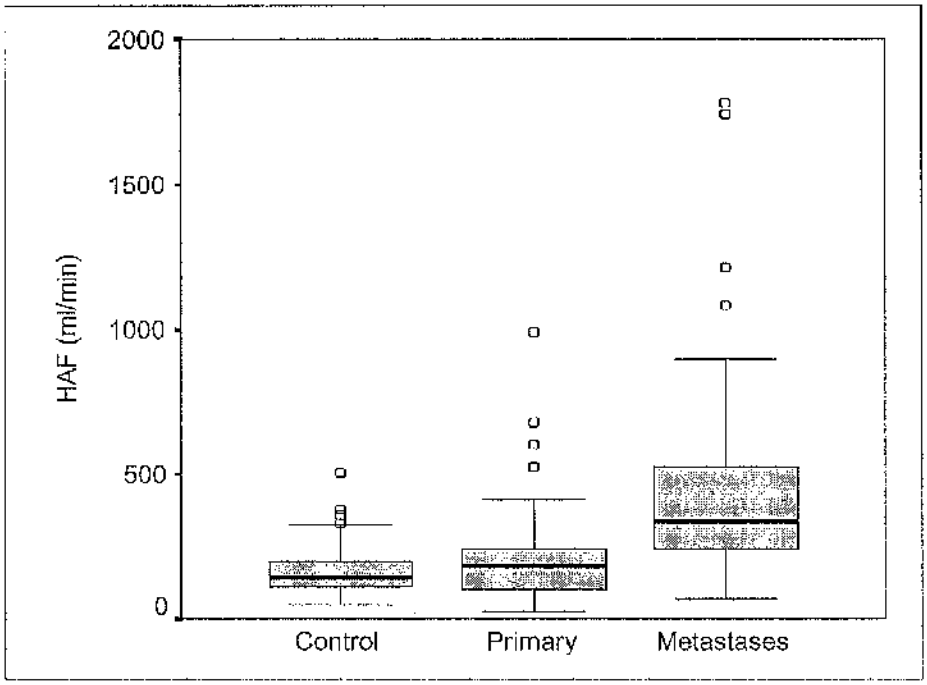

Figure 2.12 Hepatic arterial blood flow (HAF) in control subjects, patients with primary colorectal cancer and patients with liver metastases (age-corrected data).

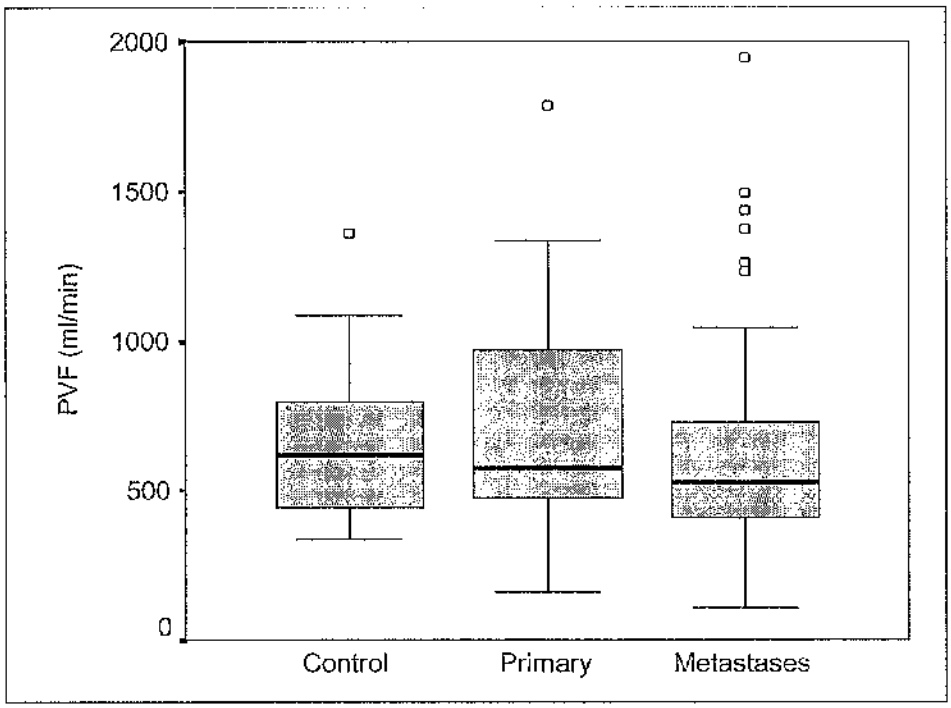

Figure 2.13 Portal venous blood flow (PVF) in control subjects, patients with primary colorectal cancer and patients with liver metastases (age-corrected data).

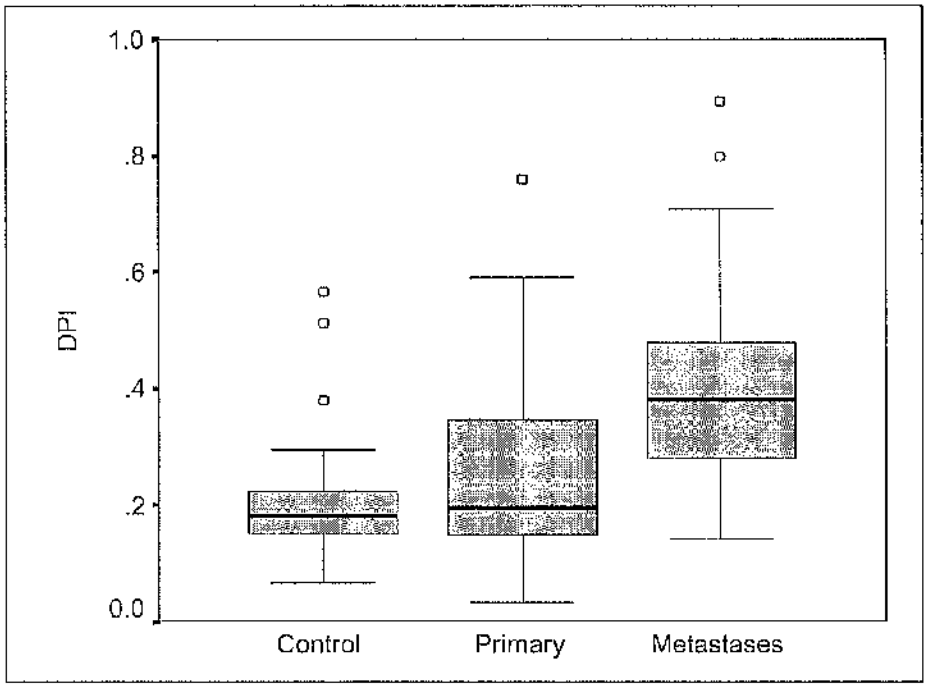

Figure 2.14 Doppler Perfusion Index (DPI) in control subjects, patients with primary colorectal cancer and patients with liver metastases (age-corrected data).

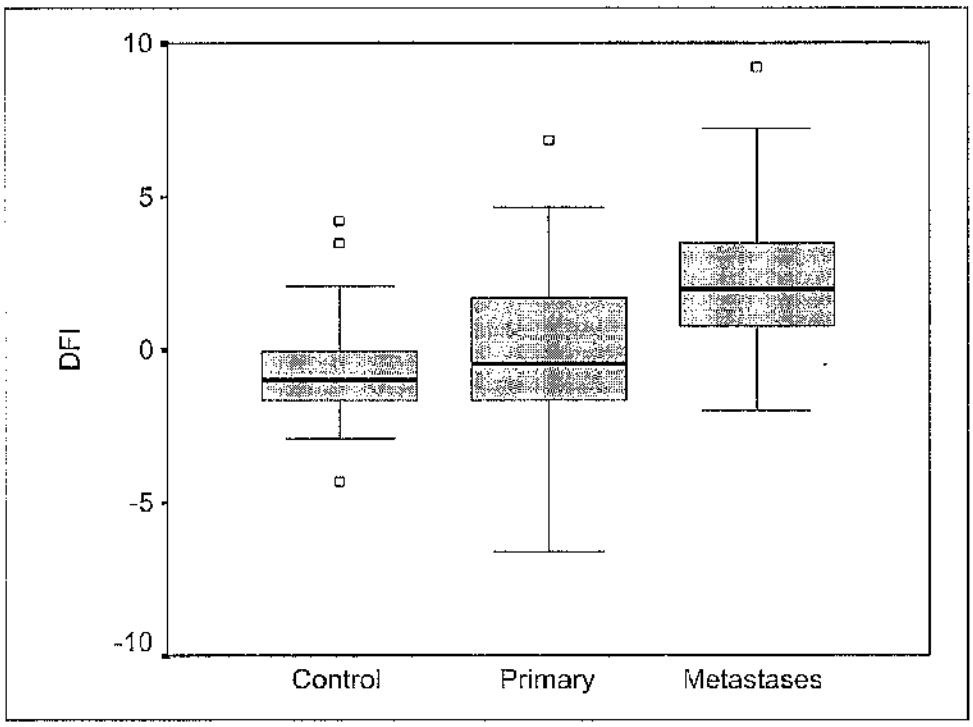

Figure 2.15 Dual Flow Index (DFI) in control subjects, patients with primary colorectal cancer and patients with liver metastases (age-corrected data).

Chapter 3 : Liver blood flow in patients with colorectal liver metastases as measured by Computerised Tomography (CT).

3.1 Introduction and Aims

The measurement of liver blood flow by Doppler ultrasound is generally recognised to be an operator-dependent procedure that requires considerable training, and is probably unsuitable for routine use outside specialist centres. There is therefore an interest in developing alternative methods of measurement that are less operator-dependent.

Miles and co-workers (1993) described a method based on single-slice dynamic computerised tomography (CT) scanning. Measurements of enhancement were performed after a bolus injection of contrast medium by placing regions of interest over the liver, aorta and spleen. Estimates of hepatic arterial and portal venous liver perfusion were obtained by dividing the maximum slope of the liver enhancement curve by the peak aortic enhancement, the splenic peak being used to separate the arterial and portal venous phases of enhancement. Significant changes in perfusion were detected in patients with various conditions, including liver metastases. However a limitation of the technique was that it required a different protocol from standard diagnostic imaging CT scans, and hence imposed an additional burden on hospital resources as well as increasing the exposure of patients to ionising radiation.

Subsequently, Platt and colleagues (1997) described a method based on dual-phase spiral CT scanning using a protocol that was also appropriate for routine diagnostic imaging studies, and similar to that used for clinical CT scanning of the liver in Glasgow Royal Infirmary. The ratio of liver attenuation at specific time points during the arterial phase to peak liver attenuation was found to be significantly increased in colorectal cancer patients who went on to develop liver metastases within 18 months relative to those who did not, with an overall prognostic accuracy of 89%. It was suggested that this could be

attributed to increased hepatic arterial flow associated with occult liver metastases (Platt et al., 1997).

The aim of the present study was to evaluate perfusion-related measurements similar to those described by Platt and colleagues (1997), derived from clinical dual-phase CT scans of the liver in patients with and without liver metastases.

Null Hypotheses

It has previously been shown that CT liver perfusion data has a high prognostic accuracy in determining patients who are harbouring occult liver metastases (Miles et al., 1993; Platt et al., 1997). This study was designed to test the null hypothesis that liver perfusion data, as measured by dual-phase CT scans, can be used to discriminate between patients with colorectal liver metastases and normal subjects.

3.2 Patients and Methods

3.2.1 Patients

Patients with colorectal cancer who underwent dual-phase spiral CT scanning of the abdomen at Glasgow Royal Infirmary between April 1999 and April 2003 were studied. They were classed as having either (a) overt liver metastases or (b) a clear liver on the basis of radiological and surgical findings. Those with liver metastases were further classified according to whether or not the primary tumour was still present. The control group comprised of (c) patients with small hepatic haemangiomas who underwent a similar CT scanning protocol.

Inclusion criteria consisted of : (a) Histologically proven colorectal cancer; Liver metastases confirmed histologically, by progression on CT or Ultrasound; (b) Histologically proven colorectal cancer; No evidence of liver metastases on CT, Ultrasound or intraoperative investigation; (c) Radiologically confirmed liver haemangioma of insignificant size and position relative to the liver parenchyma.

Exclusion criteria consisted of : Anticancer treatment during the study period or during the preceding month; Patients currently receiving or who have had non-steroidal anti-inflammatory drugs in the last two weeks; Patients receiving warfarin; Diagnosis of other liver disease or suspected cirrhosis.

All subjects were informed of the purpose and procedure of the study and were given a patient information sheet. Informed consent was given by all subjects included the study.

3.2.2 Methods

CT scanning of the abdomen was performed using a Somatom Plus 4 spiral scanner (Siemens AG, Munich, Germany). Patients received 30 ml of iodine contrast medium (Gastrografin, Schering AG, Berlin, Germany) prior to the scan. A dual-phase (arterial and venous) scan was performed during and after the infusion of 150 ml of a non-ionic contrast medium (Omnipaque; Nycomed Imaging AS, Oslo, Norway) which was administered intravenously at 3ml/s using a pump injector (Medrad Inc., Indianola, PA, USA). Arterial phase scanning was started 27 seconds after the start of contrast injection and portal phase scanning was started at 60 seconds. Contiguously reconstructed sections (pitch 1:0.5) were obtained through the liver with 5 mm collimation, with a table speed adapted to cover the entire liver with a single breath hold and a reconstruction interval of 5-10 mm. All scans were saved to Magneto Optical (MO) disk and were analysed offline using a Magic-View workstation (Siemens AG, Munich, Germany).

Circular regions of interest (ROI) were mapped over the lumen of the aorta and over the right lobe of the liver using a mouse-controlled cursor, avoiding any major intrahepatic vessels or visible lesions. A further region was drawn around the inside edge of the liver encompassing the whole organ. Mean attenuation in Hounsfield units (HU) was measured for each ROI in each section that included part of the liver.

The parameters for evaluation were selected to be similar to those of Platt and co-workers (1997), who measured attenuation 25 and 40 seconds after the start of contrast injection. However, in the present study, acquisition of images did not start until 27 seconds after the start of injection, and in several patients liver tissue was not imaged until more than 30 seconds after the start of the injection. The time points selected for analysis were therefore adjusted to 35 and 45 seconds. At each of these time points, liver

attenuation in Hounsfield Units was measured for both the selected ROI and the whole liver. The peak attenuation attained over both phases of the scan was also determined for the liver ROI, the whole liver and the aorta.

As baseline unenhanced scanning was not available in the present study, it was not possible to perform measurements of enhancement by subtraction of baseline attenuation measurements.

3.2.3 Statistical Analysis

Demographic data were analysed using the Kruskal-Wallis test and the chi squared test. Liver attenuation data were also initially analysed using the Kruskal-Wallis test. As there was a substantial age imbalance between patient groups, liver attenuation variables were adjusted for age using the same method (i.e. analysis of covariance) as was used for the blood flow variables in Chapter 2. A P-value of 0.05 or less was defined as significant. All statistical analysis was performed using SPSS (SPSS, Chicago, USA).

3.3 Results

Forty four patients with liver metastases, 14 colorectal cancer patients with apparently clear livers and 10 haemangioma (control) patients were studied. Their demographic and clinical characteristics are shown in Table 3.1. The cancer patients were significantly older than the control patients ($p<0.01$).

The results of the attenuation measurements in the liver ROI in these patient groups are shown in Table 3.2. Attenuation at both time intervals was lower in both cancer groups than in controls whether expressed in absolute units, as a percentage of the liver peak or as a percentage of the aortic peak. This was statistically significant before age adjustment. However, analysis of covariance showed that these differences were largely due to the effect of age, and, after adjusting for age, none of the measures of liver attenuation differed significantly between patient groups.

The results of the whole liver attenuation measurements in the same three groups are shown in Table 3.3. They were similar to those of the regional liver measurements, and showed no significant differences between groups after age adjustment.

Liver ROI attenuation measurements in metastases patients with ($n=14$) and without ($n=30$) a colorectal tumour are compared in Table 3.4. Without age adjustment, absolute attenuation measurements at 35 seconds ($p<0.05$) and 45 seconds ($p<0.01$) were significantly higher in patients without colorectal tumours. After age adjustment, only the difference at 45 seconds remained significant ($p<0.05$). Attenuation expressed as a percentage of the liver or aortic peak did not differ significantly between groups with or without age adjustment.

The corresponding measurements in the whole liver are shown in Table 3.5. Only the difference in absolute attenuation at 45 seconds was significantly different between these two patient groups before age adjustment, and none differed significantly when adjusted for age.

3.4 Discussion

In this study of dual-phase contrast-enhanced CT scanning, hepatic attenuation at 35 seconds after the start of contrast injection was found to be substantially lower in colorectal cancer patients with and without liver metastases than in a considerably younger group of patients with hepatic haemangiomas, who were treated as controls. This difference became more marked when attenuation was expressed as a percentage of the liver or aortic peak attenuation.

Selection of a control group for this study was problematical since very few patients undergo abdominal CT scanning without a condition that could potentially affect splanchnic blood flow, while the associated radiation dose precluded the use of normal volunteers. Patients with small haemangiomas were chosen because these benign tumours are not known to significantly alter total perfusion to the liver. Haemangiomas, which are present in 5-20% of the population, produce a vascularity that is different from normal liver parenchyma. Under procedures such as contrast enhanced ultrasound for example, these benign lesions may demonstrate peripheral nodules, an increased arterial and a less common increased venous enhancement within the lesion compared to normal liver parenchyma (Brannigan et al., 2004). It is important to note that attenuation measurements were performed on both normal liver parenchyma (region of interest) avoiding any major vessels, visible lesions or surrounding nodules, and separately on the surrounding whole liver perimeter. This was designed to minimise any unexpected false representation of altered perfusion to the whole liver in such patients. By selecting patients with small and few haemangiomas it could be expected that relatively normal perfusion characteristics would be displayed.

The limited numbers of patients with small benign lesions made the lack of age-matching unavoidable, but this had important consequences. In an analysis which corrected for the effect of age, no differences in liver attenuation between the three patient groups were statistically significant.

It is unclear why liver attenuation as measured in this study should decrease with increasing age. In Chapter 2 it was found that hepatic arterial blood flow did not vary significantly with age. However, the delivery of contrast material to the liver does not depend solely on hepatic arterial blood flow but also on cardiac output and its distribution, and on the amount of contrast that reaches the liver via the portal vein. Although the hepatic arterial route would be expected to be dominant during the first 35 seconds after the start of contrast injection, a substantial portal venous contribution cannot be excluded. Hence it may be that the greater attenuation in the younger haemangioma group reflects a higher rate of early contrast delivery via the portal vein. As baseline unenhanced scanning was not performed, it is unknown whether age-dependent differences in the attenuation of the liver tissue itself also contributed to the observed results. There are currently no reports in the literature on the effect of age correcting during functional CT perfusion studies.

As normal control subjects could not be studied, a sub-population of patients with colorectal cancer and no overt liver metastases were used as a reference group. It was expected that these patients would have lower hepatic arterial flow than patients with overt liver metastases (Leen et al., 1993a), with whom they were well matched in age. The presence of occult liver metastases in this sub-population could not be ruled out, however it was expected that a difference in attenuation would be observed between the two groups in general. However, there was little apparent difference in liver attenuation between these

two groups at either time point. This may have been due to the presence of occult liver metastases or the lack of sensitivity of the method as a whole.

Unexpectedly, the presence of a primary tumour in patients with liver metastases was found to reduce absolute liver attenuation 45 seconds after the start of contrast injection, even after age correction. No difference in hepatic arterial or portal venous blood flow was found between patient subgroups with and without a colorectal tumour using Doppler ultrasound (Chapter 2). Also, unlike the attenuation differences discussed above, this difference was reduced when attenuation was expressed as a percentage of the peak liver or aortic attenuation. A possible mechanism that could explain such a finding would be the accelerated arrival of contrast via the portal vein due to arterioportal shunting in patients who have undergone surgery to remove the primary tumour. However, in view of the large number of comparisons performed in this study, the atypical features of this difference and its marginal significance level, it is equally likely to represent a Type I statistical error.

The results of the present study appear to contradict those of Platt and co-workers (1997), who used a similar procedure to study patients with a variety of primary tumours in whom liver metastases were not detectable at the time of scanning. They reported a significantly elevated ratio of liver attenuation to peak liver attenuation at both 25 and 40 seconds after the start of contrast injection in those patients who developed overt liver metastases over the following eighteen months. If their findings reflected increased hepatic arterial blood flow in patients who developed metastases, a similar increase in attenuation ratios would be expected in patients with overt liver metastases relative both to normal controls and to colorectal cancer patients without liver metastases. The different time

points selected in the present study are unlikely to explain the difference in results as there was a substantial overlap in the time intervals studied.

A study performed in breast cancer patients by Sheafor and co-workers (2000) is in closer agreement with the present study. The authors reported a higher ratio of hepatic attenuation to peak hepatic attenuation at both 25 and 30 seconds in those patients who subsequently developed liver metastases. However, when the results were corrected for the time since chemotherapy using a method similar to the age correction in the present study, all statistically significant differences were lost. The authors concluded that there were too many variables that could potentially affect hepatic haemodynamics, and therefore the CT enhancement technique was not sufficiently robust to identify those at risk of developing liver metastases.

There are several technical factors that may help to explain these contradictory findings. Basic differences in attenuation can occur between CT scanners' fields and within each individual scanners' fields (McCullough et al., 1983); and variations in patient weight or cardiac output may affect the overall rate and intensity of enhancement (Kormano et al., 1983; Chambers et al., 1993). To minimise the effect of these limitations, a similar protocol to Platt and co-workers (1997) was strictly adhered to, including the contrast amount and injection rate, and the CT scanner was calibrated as routine every 24 hours. Measurements in the aorta were taken to provide a reference point specific to each patient, and normalising liver attenuation with respect to the aortic peak reduced variation within patient groups. The effect of variations in body weight and cardiac output were assumed to be of limited importance as similar variations were present in patients in the Doppler ultrasound liver studies.

Another factor which may bias the results of CT liver perfusion studies is the selection of regions of interest. It is thought that each patient may have inherent differences in attenuation in different hepatic regions (Chambers et al., 1993). To minimise this effect, regions were drawn over the entire liver parenchyma and within a relatively constant single area of the right lobe, avoiding any vessels or lesions. Measurements were performed by a single observer, reducing the degree of variation and both sets of data (ROI and entire liver) were then analysed separately.

Alternative protocols using a bolus injection of contrast and imaging in a single scanning plane appear to be more promising for the study of liver blood flow changes (Miles et al. 1993; Cuenod et al. 2001). Delivery of the contrast agent as a bolus allows better separation of the arterial and portal phases, while single plane imaging reduces the effect of regional variations in attenuation throughout the liver. However, such protocols impose a substantial radiation burden and cannot replace conventional CT scanning for clinical purposes; hence they are suitable only for experimental use.

The present study utilised CT scanning data which had been acquired primarily for clinical purposes. If it had yielded information about liver perfusion similar to that provided by Doppler ultrasound it would have had major advantages over the latter technique in being less operator-dependent as well as being routinely performed in the relevant patient groups. However, this study failed to confirm its reported usefulness in this respect. Therefore, the null hypothesis cannot be rejected and further studies are required to compare the potential roles of functional CT and Doppler ultrasound in assessing liver perfusion for the detection of occult liver metastases.

Future Studies

Measuring liver perfusion using functional CT scans is currently very time consuming and produces mixed results between centres that are difficult to interpret for the early detection of liver metastases. The technique may be strengthened by a study protocol that includes baseline unenhanced CT scans, and corresponding liver blood flow measurements using ultrasound, thus providing valuable reference information. Automated software that can map a region of interest automatically throughout a whole CT scan would also have a positive impact on the technique. Finally, research into the differences between single-slice and volumetric CT perfusion would also provide further insight into the potential for this innovative technique.

Interest in this area has recently turned to magnetic resonance imaging (MRI). In comparison to CT, the lack of ionizing radiation, use of smaller volumes and safer contrast media indicate that MRI may be used as a safer research tool. Initial studies by Totman and co-workers (2005) have reported promising results. A significantly reduced portal perfusion and increased HPI were reported in 12 patients with colorectal liver metastases compared with 14 control subjects (referred for routine contrast spine imaging with no neoplastic disease). Unfortunately the initial report failed to investigate the differences in age between the colorectal cancer patients (mean 65 years) and the control subjects (mean 45 years).

Whilst the potential for this technique is interesting, its success will rely on establishing a standard scanning protocol from normal perfusion studies, and through long term follow-up scans on a large cohort of patients following excision of their primary tumour (Harvey & Blomley, 2005; Totman et al., 2005).

Table 3.1 Demographic and clinical data in CT study.

	Control (n= 10)	**Clear liver** (n= 14)	**Liver metastases** (n= 44)	**P-value**
Age (years)	52.5 (37-70) (39, 66)	75.5 (38-81) (57, 81)	72 (49-87) (68, 77)	0.003
Sex (male/female)	2 / 8	7 / 7	25 / 19	0.11
Site (colon/rectum)		9 / 5		
Dukes' Stage (AB/C)		5 / 9		

Age expressed as median (range), (95% confidence intervals).

Table 3.2 CT attenuation measurements in liver region-of-interest (ROI) in patients with haemangioma (control), non-metastatic colorectal cancer (clear liver) and colorectal liver metastases.

	Control (n = 10)	**Clear liver** (n = 14)	**Liver metastases** (n = 44)	**Raw P-value**	**Adjusted P-value**
Absolute attenuation (HU)					
35 secs	82.8 (13.1)	74.4 (11.3)	72.0 (11.7)	0.05	0.30
	(65.8, 94.2)	(64.4, 88.0)	(67.1, 74.1)		
45 secs	96.1 (23.4)	83.2 (21.5)	85.6 (20.3)	0.36	0.78
	(64.4, 117.7)	(60.5, 109.1)	(73.8, 88.3)		
Percentage of peak liver attenuation					
35 secs	57.0 (7.6)	47.8 (7.1)	48.3 (7.7)	0.013	0.38
	(42.7, 78.6)	(40.5, 52.8)	(46.7, 51.1)		
45 secs	56.27 (24.13)	54.2 (11.8)	57.4 (10.5)	0.28	0.49
	(38.5, 93.8)	(43.8, 68.6)	(53.4, 63.3)		
Percentage of peak aortic attenuation					
35 secs	27.4 (5.7)	21.4 (4.6)	21.0 (5.0)	0.009	0.35
	(18.8, 42.3)	(40.5, 52.8)	(18.8, 22.4)		
45 secs	30.1 (6.9)	24.2 (7.4)	25.2 (6.7)	0.11	0.58
	(0, 31.7)	(43.8, 68.6)	(21.2, 28.6)		

Results expressed as mean (SD); (95% confidence intervals). P-values reported are for difference between groups before (raw) and after adjustment for age.

Table 3.3 CT attenuation measurements in whole liver in patients with haemangioma (control), non-metastatic colorectal cancer (clear liver) and colorectal liver metastases.

	Control (n = 10)	Clear liver (n = 14)	Liver metastases (n = 44)	Raw P-value	Adjusted P-value
Absolute attenuation (HU)					
35 secs	83.7 (14.5) (64.0, 95.1)	74.4 (11.7) (63.2, 88.5)	72.1 (11.2) (68.4, 77.8)	0.06	0.18
45 secs	98.2 (22.9) (75.0, 116.2)	85.0 (21.7) (62.0, 111.3)	83.6 (19.4) (72.4, 88.7)	0.19	0.51
Percentage of peak liver attenuation					
35 secs	58.9 (8.1) (44.2, 85.6)	48.0 (7.2) (40.3, 68.3)	50.9 (11.1) (45.0, 54.4)	0.008	0.34
45 secs	65.3 (9.4) (45.8, 72.1)	55.6 (11.8) (0, 62.5)	58.4 (12.4) (0, 59.0)	0.15	0.49
Percentage of peak aortic attenuation					
35 secs	27.6 (5.6) (18.8, 42.2)	21.4 (4.7) (16.9, 29.0)	20.7 (4.5) (17.8, 22.0)	0.003	0.21
45 secs	30.7 (6.1) (21.0, 31.2)	24.7 (7.5) (0, 33.7)	24.2 (6.3) (0, 24.8)	0.04	0.54

Results expressed as mean (SD); (95% confidence intervals). P-values reported are for difference between groups before (raw) and after adjustment for age.

Table 3.4 CT attenuation measurements in liver region-of-interest (ROI) in patients with colorectal liver metastases with and without the presence of a primary tumour.

	Liver metastases + colorectal tumour (n = 14)	Liver metastases alone (n = 30)	Raw P-value	Adjusted P-value
Absolute attenuation (HU)				
35 secs	66.4 (9.9) (60.6, 65.2)	74.7 (11.7) (70.8, 84.1)	0.031	0.10
45 secs	72.3 (13.0) (67.8, 76.1)	92.9 (20.1) (85.3, 109.5)	0.002	0.03
Percentage of peak liver attenuation				
35 secs	47.8 (11.1) (38.4, 45.3)	48.6 (5.7) (48.6, 52.8)	0.59	0.78
45 secs	54.5 (12.8) (45.6, 54.9)	58.9 (9.0) (59.3, 70.9)	0.30	0.86
Percentage of peak aortic attenuation				
35 secs	21.5 (6.1) (15.8, 18.28)	20.8 (4.5) (21.2, 25.4)	0.50	0.46
45 secs	24.4 (7.4) (19.4, 23.7)	25.6 (6.4) (27.0, 31.9)	0.80	0.99

Results expressed as mean (SD); (95% confidence intervals). P-values reported are for difference between groups before (raw) and after adjustment for age.

Table 3.5 CT attenuation measurements of whole liver in patients with colorectal liver metastases with and without the presence of a primary tumour.

	Liver metastases + colorectal tumour (n = 14)	Liver metastases alone (n = 30)	Raw P-value	Adjusted P-value
Absolute attenuation (HU)				
35 secs	67.4 (11.2) (56.2, 66.7)	74.2 (10.7) (74.9, 78.9)	0.08	0.26
45 secs	73.7 (13.5) (63.5, 75.3)	88.3 (20.3) (83.6, 102.2)	0.04	0.25
Percentage of peak liver attenuation				
35 secs	49.6 (10.8) (35.1, 42.9)	51.4 (11.3) (50.0, 61.0)	0.45	0.79
45 secs	56.9 (13.2) (0, 39.4)	59.1 (12.4) (53.7, 65.2)	0.66	0.96
Percentage of peak aortic attenuation				
35 secs	20.8 (5.4) (12.8, 17.1)	20.6 (4.2) (21.0, 23.6)	0.71	0.57
45 secs	23.7 (7.1) (0, 17.4)	24.4 (6.0) (24.0, 28.5)	0.88	0.72

Results expressed as mean (SD); (95% confidence intervals). P-values reported are for difference between groups before (raw) and after adjustment for age.

Chapter 4 : The relationship between tumour volume, the systemic inflammatory response and liver blood flow in patients with colorectal liver metastases.

4.1 Introduction and Aims

There are a number of factors that are known to influence changes in liver blood flow. Serotonin (5-hydroxytryptamine or 5-HT) has been shown to increase the splanchnic blood flow and portal pressure (Lebrec, 1990; Hoyer et al., 1994; Li et al., 2006). The vasodilator nitric oxide, which is produced in the liver, is associated with an inverse relationship with hepatic resistance and portal pressure (Rockey et al., 1998; Moal et al., 2006). In addition, the vasodilator prostaglandin has also been linked with nitric oxide in portal hypertensive rats (Fernandez et al., 1996). It has also been shown that tumour:liver blood flow is enhanced with the use of angiotensin II, noradrenaline and more recently with bradykinin receptor agonist (Hemingway et al., 1992; Dworkin et al., 1996; Shankar et al., 1999; Emerich et al., 2001).

More recently, there has been evidence to show that the presence of an inflammatory response plays a significant role in the progression of a number of solid tumours (Coussens & Werb, 2002, Erlinger et al., 2004). Increased circulating concentrations of C-reactive protein, a marker of the systemic inflammatory response, are associated with decreased survival in patients with locally-advanced and metastatic gastrointestinal cancer (O'Gorman et al., 2000; McMillan et al., 2001, McMillan et al., 2003). It has also been shown that albumin concentrations are independently associated with C-reactive protein levels, but not with percentage hepatic replacement, in patients with colorectal liver metastases (Al-Shaiba et al., 2004). Elahi and co-workers (2004) have also reported that a prognostic score combining elevated C-reactive protein concentrations and hypoalbuminemia may be of use for patients with gastrointestinal cancers.

It is also known that C-reactive protein can be upregulated by pro-inflammatory cytokines, interleukin-1, interleukin-6, and tumour necrosis factor, all of which have been linked to the malignant potential of tumours (Nozoe et al., 1998; Miki et al., 2004). Furthermore, that pro-inflammatory cytokines, in particular interleukin-6, are released from colorectal tumours (Kinoshita et al., 1999; Miki et al., 2004). It is possible that pro-inflammatory agents are associated with a hypermetabolic state in the liver, increasing oxygen demand and blood flow.

It remains unclear to what extent changes in liver blood flow in patients with colorectal liver metastases can be explained simply by the metabolic demands of growing tumour tissue, whether they are related to changes in metabolic rate in normal liver tissue induced by pro-inflammatory cytokines, or whether they are influenced by inflammatory mediators in some other manner. The aim of the present study was therefore to examine the relationship between liver blood flow, tumour volume and the systemic inflammatory response in patients with colorectal liver metastases to shed further light on these issues.

Null Hypothesis

The study was designed to test the null hypothesis that increased Doppler Perfusion Index, observed in patients with colorectal liver metastases, is related to tumour volume and the systemic inflammatory response.

4.2 Patients and Methods

4.2.1 Patients

Patients who had undergone resection of a primary colorectal cancer, who then developed liver metastases without evidence of extrahepatic disease and were referred for assessment for liver resection, were included in the study. None of the patients had received recent chemotherapy.

Inclusion criteria consisted of : Histologically-proven colorectal cancer; Liver metastases confirmed histologically or by progression on CT.

Exclusion criteria consisted of: Anticancer treatment during the study period or during the preceding month; Patients currently receiving or who have had non-steroidal anti-inflammatory drugs in the last two weeks; Patients receiving warfarin; Diagnosis of other liver disease or suspected cirrhosis.

All subjects were informed of the purpose and procedure of the study and were given a patient information sheet. Informed consent was given by all subjects included the study.

4.2.2 Outline of Procedure

As part of their clinical workup, patients underwent contrast-enhanced dual-phase spiral computed tomography (CT) of the abdomen as described in Chapter 3, and these scans were used for the measurement of tumour and liver volume as described below. A blood sample was taken for the routine measurement of C-reactive protein, and in some patients an aliquot of serum was retained for the measurement of interleukin-6 (see Section 4.2.4).

Within one week of the CT scan, hepatic arterial and portal venous blood flow were measured using Doppler ultrasound as described in Chapter 2, and total liver blood flow and the Doppler Perfusion Index calculated. The blood flow measurements were performed without knowledge of the tumour and liver volume measurements.

The study was approved by the research ethics committee of North Glasgow University Hospitals NHS Trust.

4.2.3 Measurement of Tumour and Liver Volumes and Percentage Hepatic Replacement

Volume measurements were performed using a similar approach to that previously described (Purkiss & Williams, 1992; Dworkin et al, 1995). Contrast enhanced CT-scans were performed using a Somatom plus 4 spiral-CT scanner (Siemens AG, Munich, Germany). Sections were viewed on a Magic-View workstation (Siemens AG, Munich, Germany), and the cross-sectional areas of all the metastatic lesions in each 5 mm slice were measured by mapping their perimeters. The total tumour volume was calculated as the sum of the tumour area in all slices multiplied by the slice thickness. The total liver volume was measured in a similar manner by outlining the whole liver in each section. Normal liver volume was calculated by subtracting the tumour volume from the total liver volume. The percentage hepatic replacement was calculated as the ratio of tumour volume to total liver volume multiplied by 100.

To assess the reproducibility of the method used, two observers had previously independently measured tumour and liver volumes and percentage hepatic replacement in 10 randomly selected patients from the above cohort. The intra-class correlation coefficient for agreement between observers was 0.97 or greater for all comparisons. Three quarters of

the patient measurements were performed by Dr Al-Shaiba, as stated in the acknowledgements.

4.2.4 Measurement of Interleukin-6 Concentrations

Interleukin-6 concentrations were measured using a sensitive solid-phase enzyme-linked immunosorbent assay (R&D Systems Europe Ltd, Abingdon, UK). The lower level of detection was 2.0 ng/l and the intra-assay variability was less than 6% over the sample concentration range.

4.2.5 Statistical Analysis

The relationships between blood flow variables and demographic, biochemical and tumour characteristics were analysed using the Mann-Whitney test and Spearman's rank correlation analysis as appropriate. In addition, linear regression analysis was used to obtain further information about these relationships. Variables were logarithmically transformed to normalise their distributions in the regression analysis. SPSS for Windows (SPSS Inc., Chicago, USA) was used for analysis.

4.3 Results

Characteristics of the patients with colorectal liver metastases (n=52) are shown in Table 4.1. The majority of patients were male, over the age of 65 years and had multiple liver tumours. The majority of patients had a percentage hepatic replacement of less than 25% and a C-reactive protein concentration greater than 10mg/l.

Table 4.2 compares hepatic arterial flow, portal venous flow, total liver blood flow and DPI in patients grouped by sex and by number and localisation of metastases. There were no significant differences in any of the blood flow variables between each grouping.

Correlation coefficients reflecting the strength of association between blood flow variables and continuous demographic, biochemical and tumour-related variables are shown in Table 4.3. Hepatic arterial blood flow was correlated with tumour volume (R_s = 0.30, p=0.03, Figure 4.1), percentage hepatic replacement (R_s = 0.30, p=0.03) and C-reactive protein (R_s = 0.29, p=0.04, Figure 4.2). Portal venous blood flow was not significantly correlated with either measure of tumour volume or with C-reactive protein. Total liver blood flow was correlated with tumour volume (R_s = 0.31, p=0.03), percentage hepatic replacement (R_s = 0.33, p=0.02) and C-reactive protein (R_s = 0.28, p=0.05). There was no correlation between the blood flow variables and normal liver volume.

In patients whom interleukin-6 was measured (n=32), there was also a significant correlation between total liver blood flow and interleukin-6 (R_s = 0.44, p=0.01, Figure 4.3). The correlation between hepatic arterial flow and interleukin-6 was not significant (R_s = 0.34, p=0.06).

Correlations between the Doppler Perfusion Index and the tumour-related and biochemical variables were all statistically non-significant, and were substantially weaker than those between hepatic arterial blood flow and the same variables.

Age-adjustment of the blood flow variables as described in Chapter 2 had minimal effect on the above correlation coefficients and did not alter any of the conclusions regarding statistical significance.

Among the biochemical and tumour variables, interleukin-6 was strongly correlated with C-reactive protein (R_s = 0.60, $p<0.001$) but not with tumour volume (R_s = 0.17, $p=0.35$). However, C-reactive protein was significantly correlated with tumour volume (R_s = 0.36, $p=0.01$).

Linear regression analysis was used to attempt to determine which biochemical and tumour characteristics were independently associated with hepatic arterial and total liver blood flow. Associations between hepatic arterial flow and predictor variables were all of a similar magnitude, which failed to reach significance even in a univariate analysis ($0.05<p<0.10$). Total liver blood flow was significantly related to C-reactive protein ($p=0.05$) and interleukin-6 ($p=0.01$) on univariate analysis. Interleukin-6 was the strongest predictor of total liver blood flow, and no other variable added significant additional information on multivariate analysis ($p>0.1$).

4.4 Discussion

In the present study, hepatic arterial blood flow and total liver blood flow increased significantly, though not strongly, with increasing tumour volume. This is consistent with the results of Hunt and co-workers (Hunt et al., 1989b), who found a positive association between an index of hepatic arterial blood flow, derived from dynamic hepatic scintigraphy, and estimated percentage hepatic replacement in patients with colorectal liver metastases.

A number of mechanisms could in principle explain such an association. Ridge and co-workers (1987) proposed the theory that metastases are initially seeded in the liver via the portal venous system. As they grow they develop a vasculature derived from the hepatic arterial supply which increases to meet the metabolic demand. In addition, Purkiss (1998) proposed two theories of metastatic volume growth in the liver. The first suggests that metastatic growth replaces normal liver parenchyma and the second theory proposes normal parenchymal volume is maintained or displaced as metastases enlarge. However it is likely that a combination of these factors occurs within a more complicated process and the growth may vary according to factors within the tumour or from factors within or outside the liver (Purkiss SF, 1998). Larger tumours would be expected to require a greater arterial blood supply to meet the requirements of their own growth and metabolism. In addition, they might induce a progressive increase in the metabolic rate of normal liver tissue as an element of the host response. Furthermore, if the primary factor altering liver blood flow is the requirement for tumour growth and metabolism, it would be expected that liver blood flow would be more strongly correlated with tumour volume than with systemic biochemical variables. However, the lack of correlation between hepatic arterial blood

flow and the volume of normal liver tissue suggests that tissue volume is not necessarily a reliable predictor of blood flow requirements. The contribution of tumour blood flow to the variations in hepatic arterial and total liver blood flow therefore remains uncertain.

It may also be possible that as growth occurs, the liver metastases compress the portal venous channels and induce a compensatory increase in arterial blood flow (Hunt et al., 1989b). This seems unlikely to be a major contributor in the present study as it would not explain the observed positive association between tumour volume and total liver blood flow. Indeed, increasing tumour volume tended to be associated with increased rather than reduced portal venous blood flow, although the relationship was not statistically significant.

An alternative theory is that larger tumours might alter the production of local or systemic vasoactive agents.

There are numerous reports that serotonin production is increased in colorectal cancer (Quinn et al., 1979; Tutton & Barkla, 1982; Nitta et al., 2001; Seretis et al., 2001). Further evidence has shown a link between serotonin and liver cirrhosis and portal hypertension which suggests serotonin may in fact be influencing the splanchnic blood in these subjects (Lebrec, 1990; Hoyer et al., 1994; Li et al., 2006).

There has been increasing interest in the role of cyclooxygenase in the progression of colorectal cancer, specifically, there is increasing evidence of a link between overexpression of COX-2 in colorectal cancer tumorigenesis and other solid organ tumours (Taketo, 1998a, b; Hwang et al., 1998; Uefuji et al., 2000). Cyclooxygenase plays a key role in the production of prostaglandin. As an endogenous vasodilator its influence on liver blood flow has been shown to be linked with nitric oxide and portal hypertension (Fernandez et al., 1996; Laleman et al., 2005). It is clear that substances such as

prostaglandin, nitric oxide and serotonin may influence the liver blood flow in relation to tumour volume and further studies are required to clarify this.

Hepatic arterial and total liver blood flow had a positive association with plasma C-reactive protein concentration, and this was of a similar strength to their correlation with tumour volume. As C-reactive protein is synthesised exclusively in the liver, this association might reflect an increased metabolic rate in normal liver tissue. However, C-reactive protein concentration was itself correlated with tumour volume, as was also found by Al-Shaiba and colleagues (Al-Shaiba et al., 2004), and it was not possible to determine whether blood flow was independently related to this inflammatory marker.

There are few factors produced by liver metastases which are known to evoke an acute phase protein response and that also have an effect on liver blood flow. The primary candidate is interleukin-6 since it is produced by colorectal tumours, is related to tumour size and the presence of metastases (Kinoshita et al., 1999) and is a prime mediator of C-reactive protein production from the liver (Gabay & Kushner, 1999). In order to examine the above relationships in more detail, circulating interleukin-6 concentrations were analysed.

Interleukin-6 was most strongly correlated with total liver blood flow, which is consistent with the observations of Lyngso and co-workers (2002) who reported that an infusion of interleukin-6 in healthy subjects was associated with an increase in splanchnic blood flow. In their study, circulating interleukin-6 concentrations reached 35ng/l compared with approximately 10ng/l in the present study and a value of <2 ng/l in healthy subjects (Wallace et al., 2002). Therefore, elevated circulating concentrations of interleukin-6 may have contributed to increased total liver blood flow in the present study. This conclusion is strengthened by the fact that, unlike C-reactive protein, interleukin-6 had

little or no association with tumour volume, suggesting that its relationship with total liver blood flow was not due to a common dependence on tumour size.

Circulating interleukin-6 was more weakly correlated with hepatic arterial and portal venous blood flow individually, and these relationships did not reach statistical significance. This may be a consequence of limited numbers as there was not a large difference in the correlation coefficients, and a relationship with total liver blood flow logically implies a relationship with one or both of its two components. Lynsgo and colleagues concluded that the major effect of interleukin-6 on splanchnic blood flow was in the organs draining into the portal vein (Lynsgo et al., 2002).

It is noteworthy that the Doppler Perfusion Index had virtually no association with tumour size, C-reactive protein or interleukin-6. This reflects the fact that both hepatic arterial and portal venous blood flow tended to increase in tandem with all of these factors. It is therefore likely that the mechanisms responsible for the relationships between liver blood flow, tumour size and the systemic inflammatory response within the population of patients with colorectal liver metastases are different from those that cause an elevation of the Doppler Perfusion Index in these patients relative to normal controls, as described in Chapter 2. The fact that total liver blood flow varied with tumour volume in the present study, but did not differ significantly between metastases and control patients in Chapter 2, as in previous studies (Leen et al., 1991b), further supports the concept that other influences on blood flow are involved.

This study cannot reject the null hypothesis, as increased Doppler Perfusion Index had no association with tumour volume or the systemic inflammatory response. It is most likely that splanchnic blood flow is being influenced by a combination of these factors produced during the inflammatory process or directly by the primary tumour itself. Future

studies investigating relationships between substances such as serotonin, bradykinin and prostaglandins would help further elucidate the reasons for altered splanchnic haemodynamics in patients with colorectal liver metastases.

Study Limitations

The method of percentage hepatic replacement of tumour volume has yet to be accepted as a useful and accurate tool for the assessment of liver metastasis growth. Liver tumour volume is comprised of a number of components such as neoplastic cells, a vascular component and other stromal supporting tissues. This is also true for normal liver parenchyma which may consist of a number of components such as intrahepatic biliary system. A change in the size of a tumour expressed as either volume or percentage hepatic replacement could therefore be influenced by these other components that may or may not reflect the underlying tumour cells (Purkiss SF, 1998).

A possible confounding factor in the above study is that vasoconstrictors and vasodilators may influence the observed alterations in liver blood flow. Further work is required in order to provide firm conclusions on the effects of these substances.

Unfortunately interleukin-6 measurements were only available in 32 patients due to blood sample collection problems and time constraints. If this could have been avoided, a larger data set may have produced more significant results, for example stronger associations between hepatic arterial blood flow and interleukin-6 may have been observed.

It is also important to note that the tumour may not be the only source of interleukin production. Interleukin is also secreted by phagocytic cells which in the liver are principally the Kuppfer cells of the reticuloendothelial system. However, it has been previously shown that serum interleukin-6 concentrations are strongly correlated with

interleukin-6 concentration in the tumour tissue and with the primary tumour size (Kinoshita et al., 1999).

In summary, hepatic arterial and total liver blood flow may be modulated by circulating interleukin-6, a mediator of the systemic inflammatory response, in patients with colorectal liver metastases. They also vary with tumour volume, although the basis of this relationship remains unclear. It is unlikely that these mechanisms are responsible for the elevated Doppler Perfusion Index in patients with colorectal liver metastases.

Table 4.1 Characteristics of patients with colorectal liver metastases (n= 52).

Age	71 (65–77) (68, 74)
Sex (male / female)	30 / 22
Single / multiple tumours	14 / 38
Single / multiple lobes	33 / 19
Tumour volume (cm^3)	75.4 (16.3–401.9) (26.7, 178)
Percentage hepatic replacement	3.9 (1.1–16.8) (2.5, 9.5)
(≤25 / >25%)	43 / 9
C-reactive protein (mg/l)	22 (<5–87) (8, 48)
(≤10 / >10 mg/ l)	21 / 31
Interleukin-6 (ng/l)*	8.9 (4.3–13.9) (5.3, 11.7)
Hepatic arterial flow (ml/min)	325 (210–502) (269, 428)
Portal venous flow (ml/min)	543 (416–718) (460, 615)
Total liver blood flow (ml/min)	927 (691–1172) (762, 1024)
Doppler Perfusion Index	0.36 (0.28–0.48) (0.31, 0.44)

Data given as median (interquartile range), (95% Confidence Intervals). * n=32;

Table 4.2 Blood flow parameters in patients with liver metastases grouped according to demographic and tumour characteristics.

	N	HAF (ml/min)	PVF (ml/min)	TLBF (ml/min)	DPI
Sex					
Male	30	325	524	916	0.34
		(197, 495)	(404, 695)	(691, 1103)	(0.26, 0.48)
		(248, 390)	(428, 652)	(744, 1028)	(0.28, 0.46)
Female	22	324	550	930	0.39
		(223, 601)	(421, 754)	(643, 1436)	(0.32, 0.56)
		(233, 599)	(423, 728)	(656, 1432)	(0.32, 0.56)
P-value		0.52	0.63	0.63	0.22
No. metastases					
Single	14	290	552	947	0.35
		(188, 571)	(478, 754)	(683, 1248)	(0.24, 0.42)
		(179, 599)	(416, 833)	(677, 1432)	(0.24, 0.44)
Multiple	38	330	523	916.26	0.36
		(248, 497)	(412, 675)	(691, 1087)	(0.30, 0.51)
		(270, 439)	(428, 615)	(761, 1024)	(0.31, 0.46)
P-value		0.63	0.39	0.74	0.36
Location of metastases					
1 Lobe	33	325	558	938	0.35
		(185, 499)	(407, 781)	(602, 1212)	(0.29, 0.44)
		(203, 471)	(475, 700)	(685, 1145)	(0.30, 0.42)
2 Lobes	19	325	493	858	0.40
		(258, 493)	(426, 662)	(756, 1013)	(0.30, 0.50)
		(248, 546)	(423, 672)	(751, 1049)	(0.28, 0.51)
P-value		0.61	0.64	0.98	0.41

Data given as median (interquartile range), (95% Confidence Intervals). HAF, hepatic arterial blood flow; PVF, portal venous blood flow; TLBF, total liver blood flow; DPI, Doppler Perfusion Index.

Table 4.3 Correlations between blood flow parameters and demographic, biochemical and tumour characteristics (n=52).

	HAFV	PVFV	TLBF	DPI
Age				
R_s	0.21	-0.13	0.07	0.23
P-value	0.14	0.34	0.62	0.11
NLVol				
R_s	0.07	0.08	0.13	-0.01
P-value	0.60	0.58	0.35	0.92
Tvol				
R_s	0.30	0.19	0.31	0.16
P-value	0.03	0.17	0.02	0.25
PHR				
R_s	0.30	0.22	0.33	0.16
P-value	0.03	0.12	0.02	0.27
CRP				
R_s	0.29	0.13	0.28	0.19
P-value	0.04	0.37	0.05	0.18
IL-6*				
R_s	0.34	0.28	0.44	0.11
P-value	0.06	0.13	0.01	0.56

Data given as Spearman rank correlation coefficient and p-value. * n=32; HAF, hepatic arterial blood flow; PVF, portal venous blood flow; TLBF, total liver blood flow; DPI, Doppler perfusion index; NLVol, normal liver volume; TVol, tumour volume; PHR, percentage hepatic replacement; CRP, C-reactive protein; IL-6, interleukin-6.

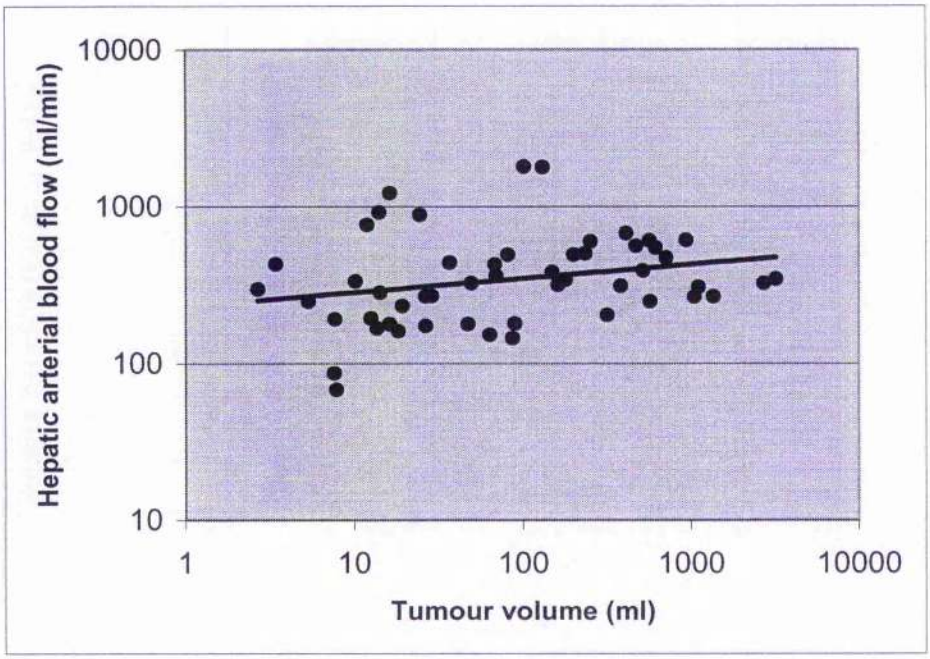

Figure 4.1 Relationship between hepatic arterial blood flow (HAF) and tumour volume (TV) in patients with liver metastases. The equation of the regression line is HAF = 230 x $TV^{0.09}$ ml/min.

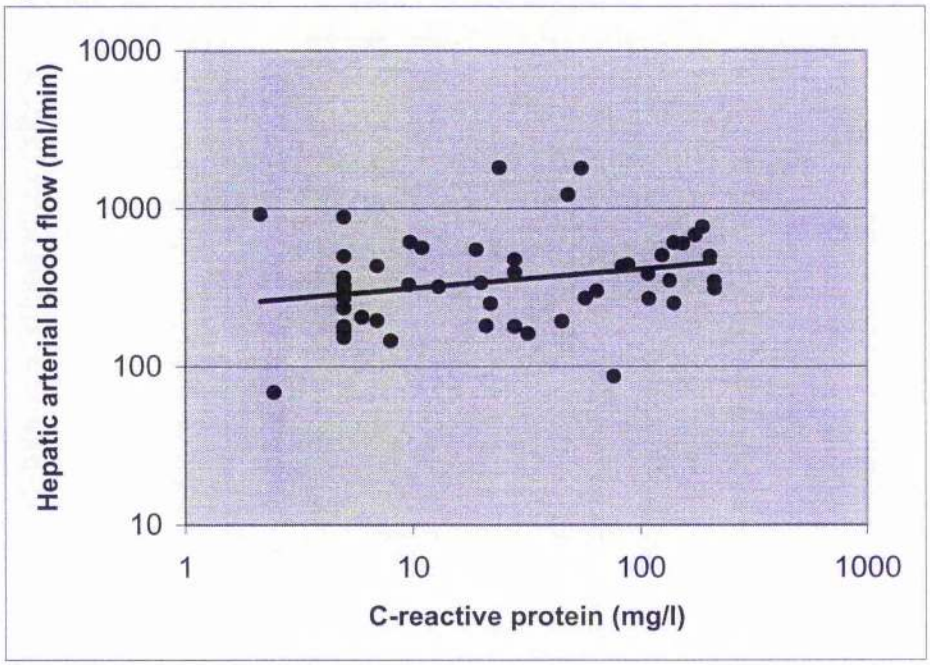

Figure 4.2 Relationship between hepatic arterial blood flow (HAF) and plasma C-reactive protein (CRP) in patients with liver metastases. The equation of the regression line is HAF = 233 x $CRP^{0.12}$ ml/min.

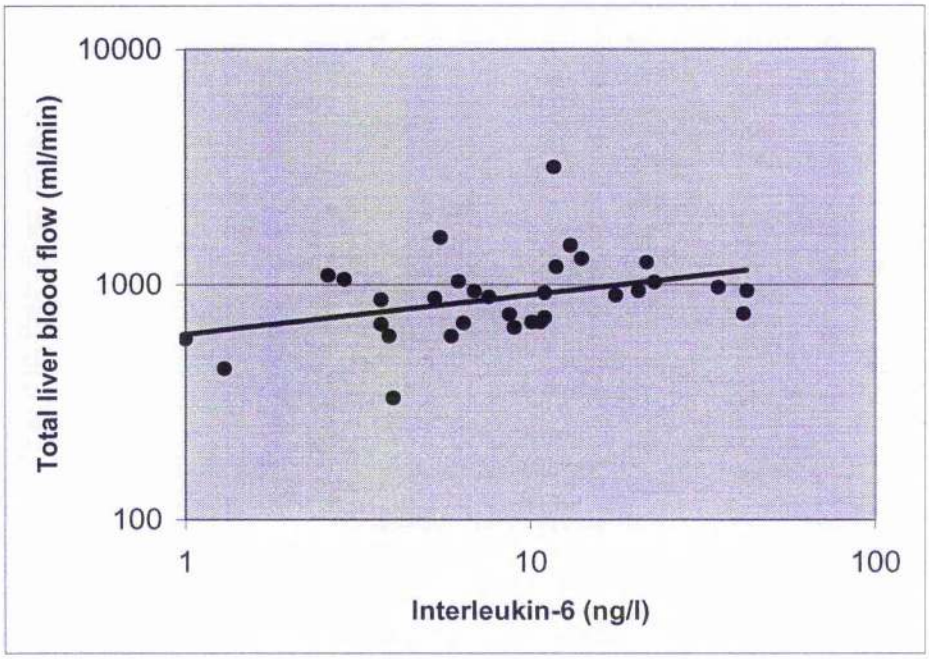

Figure 4.3 Relationship between total liver blood flow (TLBF) and plasma interleukin-6 (IL-6) in patients with liver metastases. The equation of the regression line is TLBF = 614 x IL-$6^{0.17}$ ml/min.

Chapter 5 : The effect of anti-inflammatory treatment on liver blood flow in patients with colorectal liver metastases.

5.1 Introduction

The mechanisms by which the presence of metastatic disease might stimulate both alterations in liver blood flow and a systemic inflammatory response have not as yet been clearly defined. As noted in the previous chapter, there is increasing evidence that pro-inflammatory cytokines, in particular interleukin-6, are released from the tumour (Kinoshita et al., 1999; Miki et al., 2004). It may be that this release of pro-inflammatory cytokines is associated with a hypermetabolic state in the liver increasing oxygen demand within the liver. Indeed, interleukin-6, the principal regulator of the acute phase protein response (Gabay & Kushner, 1999), is associated with an increase in liver blood flow (Lyngso, 2002). It is therefore possible that changes in liver blood flow are at least partly secondary to a systemic inflammatory response in patients with colorectal cancer.

In Chapter 4 it was suggested that, although hepatic arterial and total liver blood flow were correlated with circulating levels of C-reactive protein and interleukin-6, the influence of inflammatory mediators on blood flow was unlikely to account for the increase in DPI in patients with colorectal liver metastases. The aim of the present study was to examine the effect of an anti-inflammatory treatment on liver blood flow in patients with colorectal liver metastases in order to further elucidate the haemodynamic effects of the systemic inflammatory response. Therefore the study was designed to test the null hypothesis that there is no relationship between alterations in liver blood flow, increased Doppler Perfusion Index, and the systemic inflammatory response in patients with colorectal liver metastases.

5.2 Patients and Methods

5.2.1 Patients

Patients who had undergone resection of a primary colorectal cancer, who then developed liver metastases without evidence of extrahepatic disease and were referred for assessment for liver resection, were included in the study. None of the patients had received recent chemotherapy.

Inclusion criteria consisted of : Histologically-proven colorectal cancer; Liver metastases confirmed histologically or by progression on CT; Baseline C-Reactive protein greater than 10mg/l; Baseline Doppler Perfusion Index greater than 0.30.

Exclusion criteria consisted of : Anticancer treatment during the study period or during the preceding month; Patients currently receiving or who have had non-steroidal anti-inflammatory drugs in the last two weeks; Patients receiving warfarin; Previous adverse reaction to non-steroidal anti-inflammatory drugs; Prior history of peptic ulceration; Diagnosis of other liver disease or suspected cirrhosis.

5.2.2 Methods

Hepatic arterial and portal venous blood flow was measured using Doppler ultrasound, and total liver blood flow and the Doppler Perfusion Index calculated as described in Chapter 2. In addition a blood sample was taken for the routine measurement of C-reactive protein, and an aliquot of serum was retained for the measurement of interleukin-6 as described in Chapter 4. Percentage hepatic replacement was measured in the majority of patients, also as described in Chapter 4.

Patients were then randomly assigned to receive either ibuprofen (1200mg/day) or placebo for approximately one week. Liver blood flow and C-reactive protein and interleukin-6 measurements were then repeated. The study was conducted in a double-blind manner, and the treatment each patient received was revealed only after all data had been recorded.

The principle outcome measures were Doppler Perfusion Index, absolute and change from baseline value; and C-reactive protein to confirm efficacy of treatment.

The study was approved by the research ethics committee of North Glasgow University Hospitals NHS Trust. All subjects were informed of the purpose and procedure of the study and were given a patient information sheet. Written informed consent was given by all subjects included the study.

5.2.3 Power Calculation

A power calculation was performed to determine the number of patients to be recruited. The change in the Doppler Perfusion Index from baseline level was selected as the most important outcome measure. On the basis of pilot data, it was estimated that the standard deviation of this measure within a treatment group would be of the order of 0.10 units. A standard power calculation showed that 16 patients per group would be required to detect a mean difference between the two groups of 0.10 units at the 5% significance level with 80% power. Allowing for a 10% dropout rate and a 5% increase in numbers to compensate for the use of non-parametric statistical analysis, it was planned to recruit 40 patients in total.

5.2.4 Statistical Analysis

Comparisons between the two treatment groups were performed using the Mann-Whitney test and Fisher's exact test. Comparisons between baseline and post-treatment data within each group were performed using the Wilcoxon signed rank test. SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis.

5.3 Results

Recruitment was slower than expected, and within the time available only 29 patients entered the study. Post-treatment blood flow measurements could not be performed in 6 of these patients as they subsequently underwent procedures such as surgical resection, tumour ablation or the administration of chemotherapy. Of the 23 patients who completed the study, 12 received ibuprofen and 11 received placebo.

Baseline demographic, blood flow and biochemical characteristics for the two treatment groups are shown in Table 5.1. There were no significant differences in any of these variables between the groups before starting treatment. Twenty patients in total had a Doppler Perfusion Index of greater than 0.25 at baseline. However only 8 patients (67%) in the ibuprofen group and 8 patients (73%) in the placebo group that had a DPI of greater than 0.25, completed the study. Unexpectedly, however, only 11 patients in total (3 in the ibuprofen group and 6 in the placebo group of which completed the study) had a baseline C-reactive protein concentration of greater than 10 mg/l, and only 8 patients (one in the ibuprofen group and 5 in the placebo group of which completed the study) had a baseline interleukin-6 level above the detection threshold of 2 ng/l.

The second set of measurements was performed after a median of 7 days of treatment in each group (range 5-8 days for ibuprofen and 4-8 days for placebo). Changes from baseline values in the blood flow and biochemical variables are shown in Table 5.2. There were no statistically significant changes from baseline values in any of the variables in either group. There were also no significant differences between groups in the changes in any of the variables. After treatment, 5 patients (42%) in the ibuprofen group and 8 patients (73%) in the placebo group had a Doppler Perfusion Index of greater than 0.25.

5.4 Discussion

In the present study, there was no significant alteration in circulating levels of C-reactive protein or interleukin-6 following treatment with either ibuprofen or placebo. However, only 9 of the 23 patients entered into the study had evidence of a systemic inflammatory response at baseline, and only 3 of these received ibuprofen. This was surprising, since in the previous chapter as well as in previous studies (Kinoshita et al. 1999; Al-Shaiba et al. 2004) the majority of patients with colorectal liver metastases had elevated concentrations of interleukin-6 and/or C-reactive protein. It would appear that, in this treatment study, a subpopulation with a low prevalence of systemic inflammation was recruited. This is consistent with the low median percentage hepatic replacement, approximately 2%, compared with a median of 7% in the study by Al-Shaiba and colleagues, for example (Al-Shaiba et al., 2004). Possible explanations for this pattern include an unconscious bias towards the selection of fitter patients for this study, or a greater tendency of patients with more inflammation to fail to complete it.

In view of the low level of inflammation at baseline, it is perhaps not surprising that treatment with ibuprofen, as with placebo, did not result in a significant change in hepatic arterial, portal venous or total liver blood flow. It was therefore not possible to confirm that any component of liver blood flow is modulated by mediators of the inflammatory response in patients with liver metastases.

Approximately 70% of patients in each treatment group had a baseline Doppler Perfusion Index greater than 0.25, the threshold identified in Chapter 2 as providing the best discrimination between patients with liver metastases and normal controls. After treatment with ibuprofen the proportion with an elevated Doppler Perfusion Index fell non-

significantly to 42%, while in the placebo group it remained static. This is consistent with the conclusion reached in Chapter 4 that the systemic inflammatory response is unlikely to be primarily responsible for the increase in the Doppler Perfusion Index in patients with liver metastases, although the low level of inflammation in the present study limits its power to test that conclusion.

The power of this study was also limited by a lower than expected rate of recruitment and the failure of 20% of patients recruited to complete the study. The effect of this was to increase the minimum difference between groups in the change in Doppler Perfusion Index that could be detected with 80% power from 0.10 to 0.12. Hence a large treatment effect, for example a reduction of the Doppler Perfusion Index in the ibuprofen group to normal control level, would still have been detectable.

It is therefore reasonable to conclude that this study confirms that the systemic inflammatory response does not wholly explain the abnormalities in liver blood flow in patients with colorectal liver metastases, and that its contribution to the elevated Doppler Perfusion Index in these patients is likely to be small. The null hypothesis cannot be rejected on this basis and further work is required in this area.

Future Studies

It is clear that further work in this area is required to provide more conclusive data. In particular, a similar investigation with a larger patient population and a longer duration of study would be beneficial. One limiting factor was the availability of time to study each patient. Ideally, a 14 day period at least would have provided the opportunity for each patient to act as their own control. For example, administering placebo for 7 days, performing study measurements and then administering ibuprofen for a further 7 days before performing the final set of measurements. This study time frame was not possible within the range of this project as, understandably, medical or surgical intervention often occurred within a very short period from diagnosis.

Table 5.1 Baseline characteristics and liver blood flow in patients with colorectal liver metastases receiving ibuprofen or placebo, including subjects who did not complete the trial.

	Ibuprofen (n=14)	Placebo (n=15)	P-value
Age (years)	74 (66, 77) (66, 75)	72 (64, 74) (65, 80)	0.33
Sex (male/female)	8/ 6	9/ 6	
Percentage hepatic replacement (%)*	1.6 (0.9, 10.1) (0.7, 13)	3.0 (0.6, 62.9) (0.5, 90)	0.76
C-reactive protein (mg/l)**	7.0 (1.6, 33) (6.0, 25)	14.0 (-2.0, 99) (6.0, 160)	0.63
Interleukin-6 (ng/l)**	2.0 (1.0, 6.9) (2.0, 12)	3.75 (-4.8, 43) (2.0, 34)	0.13
Hepatic arterial blood flow (ml/min)	280 (187, 469) (107, 495)	277 (206, 332) (167, 346)	0.16
Portal venous blood flow (ml/min)	516 (439, 667) (374, 700)	537 (403, 843) (318, 817)	1.0
Total liver blood flow (ml/min)	803 (702, 1059) (691, 1096)	762 (630,1154) (584, 1073)	0.69
Doppler Perfusion Index	0.42 (0.27, 0.46) (0.14, 0.51)	0.31 (0.26, 0.37) (0.24, 0.40)	0.49

Data given as median (interquartile range), (95% confidence intervals). * n=9 in each group; ** n=10 Ibuprofen; n=12 Placebo.

Table 5.2 Changes in blood flow and biochemical data following treatment in patients receiving ibuprofen or placebo.

	Ibuprofen (n=12)	**Placebo (n=11)**	**P-value**
C-reactive protein (mg/l)	0.0 (-3.5, 0.0) (-9, 0.0)	0.0 (-1.5, 0.0) (-5, 24)	0.51
Interleukin-6 (ng/l)	0.0 (0.0, 0.0) (-2, 0.0)	0.0 (0.0, 11.5) (-4.5, 16.3)	0.72
Hepatic arterial blood flow (ml/min)	-28 (-157, 95) (-257, 126)	13 (-55, 126) (-55, 152)	0.41
Portal venous blood flow (ml/min)	-3 (-122, 173) (-139, 362)	-25 (-192, 125) (-198, 142)	0.76
Total liver blood flow (ml/min)	-121 (-214, 127) (-253, 194)	56 (-157, 262) (-252, 317)	0.45
Doppler Perfusion Index	-0.03(-0.12, 0.01) (-0.28, 0.02)	0.09 (-0.05, 0.13) (-0.06, 0.18)	0.14

Data given as median (interquartile range), (95% confidence intervals). Positive and negative changes represent increases and decreases respectively relative to baseline values. None of these changes was significantly different from zero ($p>0.05$). Tabulated p -values are for comparison of changes between ibuprofen and placebo.

Chapter 6 : Discussion and Conclusions

Since its development in 1991, the Doppler Perfusion Index has been evaluated by a number of research groups interested in the blood flow changes associated with colorectal liver metastases. The majority of studies agree that the Doppler Perfusion Index is increased in patients with colorectal liver metastases relative to normal controls, and that this is due partly or wholly to an increase in hepatic arterial blood flow (Leen et al., 1991a; Guadagni et al., 2000; Kruger et al., 2000). There is conflicting evidence however, on whether portal venous blood flow is reduced or unchanged (Di Giulio et al. 1997; Oktar et al., 2006). The results of Chapter 2 confirm the increase in hepatic arterial blood flow and Doppler Perfusion Index, but suggest that portal venous blood flow would not have differed significantly had a fully age-matched group of controls been studied. Although a reduction in portal venous blood flow and total liver blood flow with age has been reported in the gerontological literature (Zoli et al., 1989; Zoli et al., 1999), it has not previously been noted in studies of liver blood flow in cancer, and it is unclear whether the conflicts in the literature noted above reflect differences in the degree of age matching. It is clear therefore, that age should be considered when interpreting the Doppler Perfusion Index and similar indices in clinical practice. Furthermore, it is known that colorectal tumours are more aggressive when occurring in younger patients (Leff et al., 2007). For example, young patients with a Duke's stage B tumour are more likely to be treated aggressively with chemotherapy than older patients. Age correcting the Doppler Perfusion Index for this reason may have positive implications on prognostic accuracy.

The strongest evidence for a reduction in portal venous blood flow associated with liver metastases comes from animal studies. Hepatic Perfusion Index was previously measured in rats inoculated with hypervascular Walker 256 carcinosarcoma cells. It was shown that an increased HPI was as a result of decreased portal venous flow, resulting in

elevated portal pressure, splanchnic vascular resistance and portal venous resistance (Nott et al., 1987; Nott et al., 1989). Subsequently, studies utilising the hypovascular HSN sarcoma cells, which are more representative of human metastasis, were performed in rats. A significant increase in HPI was reported again as a result of decreased portal venous flow. However, in contrast to previous findings there was no evidence of an increase in portal venous pressure or arteriovenous shunting (Hemingway et al., 1991). Furthermore, Yarmenitis and co-workers (2000) reported increased Doppler Perfusion Index, increased hepatic arterial flow and a less prominent decrease in portal venous flow in rats bearing Walker 256 carcinosarcoma cells.

It can be concluded that the Hepatic Perfusion Index and Doppler Perfusion Index are increased in animals bearing liver metastases, however the mechanisms behind these changes are contradictory. The hypervascular tumour model has been shown to alter liver blood flow as early as 4 days from inoculation (Yarmenitis et al., 2000) and cause a degree of arteriovenous shunting (Nott et al., 1987; Nott et al., 1989) however, the growth pattern of this tumour type is atypical of the majority of human colorectal liver metastases (Taylor et al., 1978; Goldberg et al., 1987; Brannigan et al., 2004). It has also been shown that the blood vessels supplying colorectal liver metastases are typically immature and lacking in both smooth muscle cells and immunoreactive nerves (Ashraf et al., 1997). These observations led to the alternate hypothesis that systemic humoral vasoactive agents are responsible for mediating the changes in liver blood flow through vasoconstriction in the splanchnic system, however exactly which substances are linked remains to be seen. The results of Chapter 2 do not exclude the possibility that such agents play a role, but refocus attention on the increase in hepatic arterial blood flow, which cannot be attributed entirely to a response to reduced portal venous blood flow.

It is known that the hepatic buffer response maintains total hepatic flow, in particular, hepatic arterial flow is autoregulated to compensate for any alterations in portal venous flow. Portal venous flow is also regulated by splanchnic blood flow and hepatic arterial resistance (Lautt, 1985; Lautt, 1996). It could be expected therefore that discrete alterations in hepatic arterial or portal venous flow are less effective in highlighting trends compared to haemodynamic ratios such as the Hepatic Perfusion Index and Doppler Perfusion Index. That provides no guarantee, however, that the Doppler Perfusion Index is an optimum index. Logistic regression analysis yielded the Dual Flow Index (DFI), which was objectively derived to maximise discriminatory power. However, the gain in accuracy was not enough to justify its greater complexity in practical use.

There was no evidence in this study that liver blood flow was mediated by the presence of a primary colorectal tumour. This is consistent with a previous report by Oppo and co-workers (2000). It is of interest that the presence of a primary colorectal tumour in humans has been shown to inhibit angiogenesis of its liver metastasis and that peritumoural and intratumoural vascular density are increased significantly following subsequent removal of the primary tumour (Peeters et al., 2004). Further work in this area may yield interesting results by studying liver blood flow in patients with liver metastases before and after removal of a primary tumour; moreover by documenting hepatic alterations in patients pre- and post-resection/ ablation of liver metastases.

Currently there is no reliable method for the detection of occult liver metastases and clinicians rely on a number of prognostic factors when treating patients. The Doppler Perfusion Index however has not gained wide acceptance as a technique for routine use in the evaluation of patients with colorectal cancer, and this must be partly attributable to its perceived operator dependence (Glover et al., 2002; Roumen et al., 2005). As contrast-

enhanced CT imaging has become more widely used, it potentially offers the opportunity to utilise information regarding blood flow encoded in the contrast uptake curves in the liver. There is strong evidence in the literature that single-slice bolus contrast injection CT protocols can provide that information in an accessible form (Miles et al., 1993), but it is far less certain that it can be obtained as a by-product of CT studies designed to image the whole liver (Sheafor et al., 2000). The results of Chapter 3 suggest that this technique is not as robust as the Doppler ultrasound method in detecting differences in blood flow between different patient groups, and that further studies are required using age matched normal subjects as a reference.

There were difficulties in obtaining appropriate control data for this CT study that to some extent limit the conclusion. Most incidental patients with no abnormality were filtered out at the first stages of clinical investigation such as ultrasound or X-ray. Patients with small benign liver haemangiomas who required dual-phase CT for lesion characterisation were chosen as one control group, however, numbers were limited and they were poorly age matched with patients with liver metastases. Differences in CT parameters were found, but they were in the opposite direction to what was expected, and they disappeared after age adjustment. Patients who had a negative follow up scan after resection of a primary colorectal tumour provided an alternative study cohort, as they were both more numerous and well matched for age. Although the presence of occult micrometastases in these patients could not be ruled out, liver blood flow was expected to differ from patients with overt liver metastases. However, no differences in their CT parameters were observed. This may have been a consequence of micrometastatic disease or a lack of sensitivity of the protocol and technique as a whole.

In future, alternative methods such as Magnetic Resonance Imaging (MRI) may prove more useful. MRI can provide the means of assessing liver perfusion without the radiation burden associated with CT, allowing valuable data to be collected on normal control subjects. Furthermore, software developments in both MRI and CT may provide a faster and more automated means of measuring regions of interest in every slice, throughout a whole scan. Initial studies using MRI by Totman and co-workers (2005) have reported promising results. A significantly reduced portal perfusion and increased HPI was reported in patients with colorectal liver metastases compared to control subjects (referred for routine contrast spine imaging with no neoplastic disease).

There is likely to remain some degree of conflict between the requirements of conventional imaging studies and protocols optimised for obtaining haemodynamic information. Further work investigating liver blood flow by Doppler ultrasound concurrently with liver perfusion studies by CT or MRI, may generate interesting data and help produce a standardised technique for the early detection of occult liver metastases.

The mechanisms underlying the blood flow changes associated with colorectal liver metastases remain a subject of interest. There is growing evidence that the presence of a systemic inflammatory response is an important stage-independent prognostic factor in colorectal cancer (O'Gorman et al., 2000; McMillan et al., 2001, McMillan et al., 2003). Interleukin-6, a mediator of this response, is known to alter splanchnic blood flow (Gabay & Kushner, 1999; Lyngso et al., 2002). It is also of interest that, as part of the systemic inflammatory response following surgical injury, portal venous blood flow has been reported to fall whereas hepatic arterial flow is increased (Souba & Wilmore, 1983). The studies in Chapters 4 and 5 were designed to clarify the role of the systemic inflammatory response in the blood flow changes associated with colorectal liver metastases. The results

of the first study suggest to some degree, that total liver blood flow is modulated by interleukin-6, probably by an effect on both the hepatic arterial and portal venous components. It is also possible that numerous other factors may be influencing liver blood flow, for example, nitric oxide, serotonin and endothelin-1 (Quinn et al., 1979; Myhre et al., 1993; Hongzhi et al., 2005; Kuro et al., 2006). The modulation of liver blood flow by interleukin-6 cannot however account for the differences in hepatic arterial flow and Doppler Perfusion Index observed in patients with colorectal liver metastases and controls.

The hypothesis that hepatic arterial blood flow is increased simply to meet the metabolic demands of growing hepatic tumours was one of the earliest to be proposed (Ridge et al., 1987; Purkiss SF, 1998). The observed correlation between hepatic arterial blood flow and tumour volume is consistent with this mechanism playing some part in the increase, but it seems unlikely to account for it entirely. It would be expected that total liver blood flow would be more strongly correlated with tumour volume than with systemic biochemical variables. The contribution of tumour blood flow to the variations in hepatic arterial and total liver blood flow therefore remains uncertain

Hence, the tumour volume effect, like the interleukin-6 effect, may influence hepatic haemodynamics to some degree but does not elucidate the alterations in liver blood flow observed to date. The hypothesis that the increase in hepatic arterial blood flow is secondary to compression of portal venous channels by tumour is likewise unsupported by this study.

Despite the limitations of the intervention study with ibuprofen, the results indicate consistency with the conclusion that the systemic inflammatory response cannot fully account for the changes in liver blood flow in patients with colorectal liver metastases. Unfortunately recruitment was lower than expected and the time frame in which to study

each patient was restrictive. Furthermore, unexpected low levels of baseline C-reactive protein and Doppler Perfusion Index values in the study patients limit the power to draw firm conclusions. Possible explanations for this include the unconscious bias to select fitter patients for the study. Further work in this area would benefit from a similar study using a large cohort of liver metastases patients irrespective of status, with a longer period of investigation for each patient.

Future studies should also be directed towards alternative mechanisms that might alter hepatic arterial blood flow without a substantial alteration in portal venous blood flow. For example, it is possible that the presence of liver metastases disrupts the set point of the hepatic arterial buffer response. It is known that adenosine and ATP (adenosine-5-triphosphate) are key mediators in the hepatic arterial buffer response, influencing hepatic artery dilation to maintain total hepatic blood flow at a constant level (Lautt, 1985; Lautt, 1996; Dominic et al., 2003). Indeed, there is evidence that patients with liver metastases have a relative decrease in liver ATP levels, as measured by Magnetic Resonance Spectroscopy, compared with normal subjects (Brinkmann et al., 1997; Bell & Bhakoo, 1998).

A comparison of the changes in liver blood flow, as measured by colour Doppler ultrasound, and changes in liver adenosine, ATP and nitric oxide levels, would be of considerable interest in patients with colorectal liver metastases.

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Appendices

Appendix 1 Reproducibility studies

Table A Intra-observer variation between Doppler Perfusion Index (DPI) measurements.

Patient No.	1st Observation	2nd Observation	Mean	Difference
1	0.19	0.21	0.20	-0.02
2	0.59	0.44	0.52	0.15
3	0.35	0.33	0.34	0.02
4	0.31	0.49	0.40	-0.18
5	0.57	0.52	0.54	0.05
6	0.30	0.24	0.27	0.06
7	0.36	0.33	0.34	0.03
8	0.34	0.27	0.30	0.07
9	0.56	0.27	0.41	0.29
10	0.24	0.20	0.22	0.04
11	0.30	0.21	0.26	0.09
12	0.46	0.58	0.52	-0.12
13	0.32	0.55	0.43	-0.22
14	0.48	0.69	0.59	-0.22
15	0.09	0.07	0.08	0.02
16	0.21	0.30	0.25	-0.09
17	0.28	0.39	0.33	-0.11
18	0.30	0.32	0.31	-0.02
Mean	**0.35**	**0.36**	**0.35**	**-0.01**
SD	**0.14**	**0.16**	**0.13**	**0.13**

Table B Inter-observer variation between liver blood flow values measured by Doppler ultrasound.

No.	TLBF 1	TLBF 2	HAF 1	HAF 2	PVF 1	PVF 2	HA TAM 1	HA TAM 2	HA XSA 1	HA XSA 2	PV TAM 1	PV TAM 2	PV XSA 1	PV XSA 2
1	750.00	670.50	324.00	346.50	426.00	706.92	22.50	27.50	0.24	0.21	10.00	13.70	0.71	0.86
2	762.00	585.84	207.00	378.84	555.00	590.64	15.00	15.40	0.23	0.41	12.50	10.70	0.74	0.92
3	685.23	354.03	166.83	187.20	518.40	1035.72	8.30	12.00	0.34	0.26	7.20	12.60	1.20	1.37
4	602.49	359.76	86.52	273.24	515.97	1109.76	10.30	20.70	0.14	0.22	9.10	13.60	0.95	1.36
5	990.96	524.40	191.76	332.64	799.20	1382.40	18.80	19.80	0.17	0.28	14.80	18.00	0.90	1.28
6	439.20	146.52	48.60	97.92	390.60	615.60	13.50	10.20	0.06	0.16	9.30	10.80	0.70	0.95
7	915.54	386.76	193.38	193.38	722.16	722.16	29.30	29.30	0.11	0.11	10.20	10.20	1.18	1.18
8	1233.90	716.58	348.30	368.28	885.60	1098.36	21.50	19.80	0.27	0.31	18.00	16.20	0.82	1.13
9	626.40	459.00	135.00	324.00	491.40	922.32	9.00	18.00	0.25	0.30	9.00	12.60	0.91	1.22
10	1203.66	475.44	153.90	321.54	1049.76	1069.20	17.10	23.30	0.15	0.23	16.20	16.20	1.08	1.10
11	1276.38	308.04	135.24	172.80	1141.14	1350.00	9.80	14.40	0.23	0.20	14.30	18.00	1.33	1.25

TLBF Total liver blood flow (ml/min); HAF Hepatic artery flow volume (ml/min); PVF Portal venous flow volume (ml/min); HATAM Hepatic artery time-averaged velocity (cm/s); HAXSA Hepatic artery cross sectional area (cm^2); PVTAM Portal vein time-averaged velocity (cm/s); PVXSA Portal cross sectional area (cm^2).

Table C Inter-observer variation between Doppler Perfusion Index (DPI) measurements.

Patient No.	1st Observer (JM)	2nd Observer (PG)	Mean	Difference
1	0.43	0.33	0.38	0.10
2	0.27	0.39	0.33	-0.12
3	0.24	0.15	0.20	0.09
4	0.14	0.20	0.17	-0.05
5	0.19	0.19	0.19	0.00
6	0.11	0.14	0.12	-0.03
7	0.21	0.21	0.21	0.00
8	0.28	0.25	0.27	0.03
9	0.22	0.26	0.24	-0.04
10	0.13	0.23	0.18	-0.10
11	0.11	0.11	0.11	-0.01
Mean	**0.21**	**0.22**	**0.22**	**-0.01**
SD	**0.10**	**0.08**	**0.08**	**0.07**

1st Observer JM, John MacQuarrie; 2nd Observer PG, Paul Glen.

Appendix 2 Liver blood flow values as measured by Doppler ultrasound

Table A Control Subjects.

No	M0 F1	Date DPI	Age	DPI	TLBF	HAF	PVF	HATAM	PVTAM	HA XSA	PVXSA
1	1	13-Jul-00	50	0.20	2016.75	409.41	1607.34	23.78	8.90	0.29	3.01
2	1	08-Aug-00	60	0.21	1120.96	236.44	884.52	25.10	16.80	0.16	0.88
3	1	08-Aug-00	38	0.06	1201.30	68.33	1132.97	15.60	17.05	0.07	1.11
4	0	10-Aug-00	25	0.20	1619.53	321.30	1298.23	19.13	19.23	0.28	1.13
5	0	07-Sep-00	68	0.19	845.10	162.00	683.10	10.80	9.00	0.25	1.27
6	1	12-Dec-00	29	0.15	563.87	83.87	480.00	13.10	10.00	0.11	0.80
7	0	13-Jan-01	25	0.21	671.93	140.45	531.48	8.67	10.30	0.27	0.86
8	1	19-May-01	54	0.17	506.52	85.32	421.20	7.90	15.60	0.18	0.45
9	1	20-Jun-01	33	0.14	1681.56	233.28	1448.28	16.20	16.20	0.24	1.49
10	0	21-Jun-01	24	0.12	627.62	78.08	549.55	8.68	10.07	0.15	0.91
11	1	10-Jul-01	28	0.13	915.77	119.02	796.76	16.53	13.69	0.12	0.97
12	1	16-Aug-01	65	0.14	963.84	137.28	826.56	17.60	16.80	0.13	0.82
13	0	17-Aug-01	25	0.16	929.94	147.90	782.04	14.50	13.30	0.17	0.98
14	0	08-Sep-01	57	0.17	915.30	153.00	762.30	12.75	12.10	0.20	1.05
15	0	11-Oct-01	37	0.34	1068.12	368.28	699.84	34.10	16.20	0.18	0.72
16	1	01-Feb-02	28	0.24	812.08	197.44	614.64	15.67	7.88	0.21	1.30
17	1	01-Feb-02	30	0.13	573.41	74.38	499.03	10.33	9.56	0.12	0.87
18	0	01-Feb-02	25	0.22	1173.09	260.48	912.61	14.97	12.89	0.29	1.18
19	0	21-May-02	47	0.25	742.68	182.70	559.98	17.40	15.30	0.18	0.61
20	1	14-Oct-02	57	0.28	1233.90	348.30	885.60	21.50	18.00	0.27	0.82
21	1	14-Oct-02	43	0.11	1359.90	151.20	1208.70	18.00	19.75	0.14	1.02
22	0	14-Oct-02	77	0.22	626.40	135.00	491.40	9.00	9.00	0.25	0.91
23	0	15-Oct-02	34	0.13	1203.66	153.90	1049.76	17.10	16.20	0.15	1.08
24	1	15-Oct-02	21	0.11	1276.38	135.24	1141.14	9.80	14.30	0.23	1.33
25	0	18-Oct-02	49	0.15	1155.42	177.24	978.18	21.10	13.70	0.14	1.19
26	0	18-Oct-02	50	0.20	1101.96	214.92	887.04	19.90	17.60	0.18	0.84
27	1	18-Oct-02	56	0.17	761.40	129.60	631.80	12.00	13.50	0.18	0.78
28	1	24-Oct-02	29	0.19	990.96	191.76	799.20	18.80	14.80	0.17	0.90
29	1	29-Oct-02	58	0.11	439.20	48.60	390.60	13.50	9.30	0.06	0.70
30	0	29-Oct-02	56	0.21	915.54	193.38	722.16	29.30	10.20	0.11	1.18
31	1	29-Oct-02	49	0.13	1448.76	192.96	1255.80	13.40	16.10	0.24	1.30
32	0	05-Mar-01	88	0.16	521.94	82.38	439.56	13.73	11.10	0.10	0.66
33	0	17-Jan-02	66	0.12	783.10	90.21	692.89	9.70	10.13	0.16	1.14
34	0	19-Nov-02	63	0.13	1318.80	165.12	1153.68	17.20	21.85	0.16	0.88
35	0	17-Jan-02	68	0.21	569.76	120.96	448.80	14.40	9.35	0.14	0.80
36	0	08-Oct-01	70	0.51	699.96	357.96	342.00	31.40	6.00	0.19	0.95
37	0	30-Apr-01	45	0.54	1034.00	555.92	478.08	84.23	8.30	0.11	0.96

M Male F Female; DPI Doppler perfusion index; TLBF Total liver blood flow (ml/min); HAF Hepatic artery flow volume (ml/min); PVF Portal venous flow volume (ml/min); HATAM Hepatic artery time-averaged velocity (cm/s); HAXSA Hepatic artery cross sectional area (cm^2); PVTAM Portal vein time-averaged velocity (cm/s); PVXSA Portal cross sectional area (cm^2).

Table B Patients with liver metastases alone.

No	M0 F1	Date DPI	Age	DPI	TLBF	HAF	PVF	HATAM	PVTAM	HA XSA	PVXSA
1	1	22-Aug-00	72	0.59	3015.52	1782.27	1233.25	103.50	18.03	0.29	1.14
2	1	06-Oct-00	78	0.39	1448.13	561.96	886.17	44.60	10.90	0.21	1.36
3	0	23-Nov-00	62	0.79	1007.45	795.90	211.55	37.90	4.83	0.35	0.73
4	0	08-Dec-00	35	0.22	1185.90	266.28	919.62	31.70	9.00	0.14	1.70
5	1	11-Dec-00	81	0.44	971.88	429.48	542.41	17.60	11.23	0.41	0.81
6	1	28-Feb-01	79	0.42	1432.42	599.26	833.16	35.67	13.10	0.28	1.06
7	0	12-Mar-01	85	0.72	935.64	675.24	260.40	33.10	7.00	0.34	0.62
8	1	22-Mar-01	68	0.36	871.10	313.34	557.76	31.65	16.60	0.17	0.56
9	1	12-Apr-01	77	0.36	656.07	233.07	423.00	22.85	14.10	0.17	0.50
10	0	09-May-01	68	0.55	741.83	407.75	334.08	25.17	8.70	0.27	0.64
11	1	29-May-01	67	0.34	825.48	282.96	542.52	13.10	13.70	0.36	0.66
12	1	30-May-01	65	0.21	921.41	193.46	727.96	16.97	16.62	0.19	0.73
13	0	11-Jun-01	78	0.33	1089.48	363.48	726.00	23.30	10.00	0.26	1.21
14	1	22-Jun-01	79	0.26	517.86	132.30	385.56	31.50	12.60	0.07	0.51
15	1	23-Jul-01	65	0.37	2319.56	864.26	1455.30	49.67	31.50	0.29	0.77
16	0	25-Jul-01	86	0.44	1145.34	503.82	641.52	46.65	12.15	0.18	0.88
17	0	14-Aug-01	68	0.31	1573.74	495.00	1078.74	25.00	23.05	0.33	0.78
18	0	20-Aug-01	67	0.27	933.24	247.80	685.44	23.60	11.90	0.18	0.96
19	0	03-Sep-01	65	0.24	723.84	177.12	546.72	14.40	6.70	0.21	1.36
20	1	12-Oct-01	70	0.23	394.20	91.80	302.40	9.00	14.40	0.17	0.35
21	1	17-Jan-02	74	0.36	938.31	333.96	604.35	24.20	12.75	0.23	0.79
22	0	07-Feb-02	65	0.23	690.60	160.20	530.40	26.70	13.00	0.10	0.68
23	0	04-Mar-02	70	0.34	602.16	202.80	399.36	16.90	6.40	0.20	1.04
24	1	04-Mar-02	52	0.38	500.94	191.52	309.42	16.80	5.40	0.19	0.96
25	1	25-Mar-02	50	0.56	1581.18	881.34	699.84	39.70	14.40	0.37	0.81
26	1	16-Apr-02	63	0.30	883.82	269.06	614.76	37.37	9.40	0.12	1.09
27	0	25-Apr-02	72	0.46	751.13	345.58	405.55	33.88	12.07	0.17	0.56
28	0	21-May-02	65	0.89	1027.74	915.90	111.84	21.50	23.30	0.71	0.08
29	0	24-May-02	83	0.51	969.78	494.52	475.26	31.70	8.90	0.26	0.89
30	0	03-Jul-02	73	0.21	327.24	68.04	259.20	16.20	9.00	0.07	0.48
31	0	04-Jul-02	80	0.46	691.20	317.40	373.80	23.00	7.00	0.23	0.89
32	0	09-Jul-02	70	0.28	899.55	247.50	652.05	18.75	16.10	0.22	0.68
33	0	09-Jul-02	65	0.30	483.12	144.72	338.40	20.10	9.40	0.12	0.60
34	0	14-Aug-02	59	0.26	677.07	178.92	498.15	21.30	10.25	0.14	0.81
35	0	19-Aug-02	75	0.28	1237.86	341.46	896.40	27.10	18.00	0.21	0.83
36	0	05-Sep-02	78	0.51	584.15	297.00	287.15	19.80	9.03	0.25	0.53
37	0	10-Sep-02	47	0.29	1308.12	378.84	929.28	28.70	12.80	0.22	1.21
38	1	17-Oct-02	62	0.57	3147.72	1797.72	1350.00	42.20	12.50	0.71	1.80
39	1	05-Sep-02	73	0.57	1066.98	606.90	460.08	28.90	7.20	0.35	1.07
40	0	14-Jan-03	71	0.24	685.23	166.83	518.40	8.30	7.20	0.34	1.20
41	1	14-Jan-03	75	0.14	602.49	86.52	515.97	10.30	9.10	0.14	0.95
42	1	16-Dec-02	65	0.62	437.70	270.00	167.70	18.00	6.50	0.25	0.43
43	0	10-Feb-03	78	0.27	762.00	207.00	555.00	15.00	12.50	0.23	0.74

M Male F Female; DPI Doppler perfusion index; TLBF Total liver blood flow (ml/min); HAF Hepatic artery flow volume (ml/min); PVF Portal venous flow volume (ml/min); HATAM Hepatic artery time-averaged velocity (cm/s); HAXSA Hepatic artery cross sectional area (cm^2); PVTAM Portal vein time-averaged velocity (cm/s); PVXSA Portal cross sectional area (cm^2).

Table C Patients with liver metastases + primary colorectal tumour.

No	M0 F1	Date DPI	Age	DPI	TLBF	HAF	PVF	HATAM	PVTAM	HA XSA	PVXSA
1	0	12-Jul-00	69	0.45	2725.61	1216.05	1509.56	58.24	16.03	0.35	1.57
2	0	12-Jul-00	60	0.15	2496.84	381.90	2114.94	19.00	17.45	0.34	2.02
3	0	28-Aug-00	70	0.40	1307.79	524.31	783.48	12.03	9.93	0.73	1.32
4	1	09-Oct-00	53	0.42	1023.48	428.40	595.08	42.00	11.40	0.17	0.87
5	0	03-Nov-00	63	0.18	1052.88	186.12	866.76	14.10	11.65	0.22	1.24
6	0	12-Feb-01	77	0.48	818.34	390.26	428.09	89.10	6.75	0.07	1.06
7	1	28-Feb-01	79	0.65	1180.35	764.82	415.53	60.70	12.15	0.21	0.57
8	0	20-Mar-01	46	0.56	977.58	546.06	431.52	47.90	11.60	0.19	0.62
9	1	21-May-01	73	0.42	579.12	241.92	337.20	16.80	14.05	0.24	0.40
10	0	30-May-01	73	0.51	858.18	438.96	419.22	23.60	13.70	0.31	0.51
11	1	25-Jun-01	87	0.29	587.52	172.80	414.72	19.20	10.80	0.15	0.64
12	0	08-Aug-01	66	0.24	744.12	177.12	567.00	16.40	10.50	0.18	0.90
13	0	13-Aug-01	51	0.41	2850.75	1167.48	1683.27	42.30	17.70	0.46	1.59
14	0	30-Aug-01	64	0.35	932.97	325.47	607.50	28.55	12.50	0.19	0.81
15	1	15-Oct-01	60	0.59	937.44	552.96	384.48	28.80	8.01	0.32	0.80
16	0	17-Oct-01	71	0.35	760.74	267.84	492.90	18.60	10.60	0.24	0.78
17	0	08-Nov-01	67	0.31	1048.81	325.13	723.67	20.07	15.87	0.27	0.76
18	0	08-Nov-01	73	0.57	803.14	457.75	345.40	24.61	5.38	0.31	1.07
19	1	10-Jan-02	66	0.41	692.01	285.66	406.35	20.70	10.75	0.23	0.63
20	0	01-May-02	68	0.48	1285.20	613.20	672.00	36.50	7.00	0.28	1.60
21	1	01-May-02	49	0.32	1461.39	470.67	990.72	54.10	19.20	0.15	0.86
22	1	05-Sep-02	71	0.40	762.06	307.02	455.04	30.10	9.60	0.17	0.79
23	0	04-Jul-02	74	0.31	1267.53	398.13	869.40	28.85	21.00	0.23	0.69
24	0	07-Nov-02	69	0.33	877.68	290.16	587.52	24.80	9.60	0.20	1.02
25	0	10-Feb-03	74	0.43	750.00	324.00	426.00	22.50	10.00	0.24	0.71
26	0	12-Feb-03	68	0.67	1131.00	753.00	378.00	50.20	9.00	0.25	0.70

M Male F Female; DPI Doppler perfusion index; TLBF Total liver blood flow (ml/min); HAF Hepatic artery flow volume (ml/min); PVF Portal venous flow volume (ml/min); HATAM Hepatic artery time-averaged velocity (cm/s); HAXSA Hepatic artery cross sectional area (cm^2); PVTAM Portal vein time-averaged velocity (cm/s); PVXSA Portal cross sectional area (cm^2).

Table D Patients with primary colorectal tumour.

No	M0 F1	Date DPI	Age	DPI	TLBF	HAF	PVF	HATAM	PVTAM	HA XSA	PVXSA
1	1	01-Jun-00	59	0.14	564.84	77.76	487.08	5.40	12.30	0.24	0.66
2	0	26-Jul-00	56	0.33	997.54	329.48	668.06	19.10	8.75	0.29	1.27
3	0	07-Aug-00	51	0.15	1341.42	202.56	1138.86	21.10	17.10	0.16	1.11
4	0	14-Aug-00	63	0.38	1612.66	620.05	992.61	18.13	26.90	0.57	0.62
5	0	01-Sep-00	72	0.07	1105.56	75.24	1030.32	11.40	16.20	0.11	1.06
6	1	07-Sep-00	85	0.10	746.62	71.44	675.18	13.23	17.05	0.09	0.66
7	0	08-Sep-00	68	0.17	1040.49	180.27	860.22	20.03	12.15	0.15	1.18
8	0	08-Nov-00	56	0.14	1444.67	201.60	1243.07	12.00	19.13	0.28	1.08
9	0	19-Feb-01	78	0.77	1251.21	958.65	292.56	17.50	18.40	0.91	0.27
10	1	21-Feb-01	82	0.60	1079.81	650.15	429.66	31.87	11.55	0.34	0.62
11	0	12-Mar-01	54	0.19	1057.68	196.56	861.12	12.60	14.95	0.26	0.96
12	1	14-Mar-01	72	0.21	1003.82	212.78	791.04	27.28	10.30	0.13	1.28
13	0	22-Mar-01	79	0.09	1081.44	99.18	982.26	27.55	15.30	0.06	1.07
14	1	26-Apr-01	42	0.17	775.32	128.04	647.28	19.40	11.60	0.11	0.93
15	0	03-May-01	81	0.18	584.33	106.92	477.41	8.10	12.63	0.22	0.63
16	0	13-May-01	88	0.21	1448.28	301.32	1146.96	16.20	16.20	0.31	1.18
17	1	21-May-01	73	0.42	579.12	241.92	337.20	16.80	14.05	0.24	0.40
18	0	20-Jun-01	74	0.24	681.06	165.06	516.00	19.65	17.20	0.14	0.50
19	0	11-Jul-01	79	0.27	1491.21	396.00	1095.21	27.50	14.15	0.24	1.29
20	1	11-Jul-01	71	0.48	480.02	231.00	249.02	22.00	8.47	0.18	0.49
21	1	27-Aug-01	79	0.16	528.81	87.21	441.60	8.55	4.60	0.17	1.60
22	0	08-Oct-01	78	0.03	979.85	31.70	948.15	5.87	10.50	0.09	1.51
23	0	21-Oct-01	80	0.22	656.21	145.91	510.30	17.37	9.45	0.14	0.90
24	0	03-Jan-01	72	0.22	819.27	180.48	638.79	18.80	10.70	0.16	1.00
25	0	17-Jan-02	76	0.12	596.58	70.08	526.50	14.60	9.75	0.08	0.90
26	0	21-Jan-02	43	0.39	570.78	223.02	347.76	20.65	8.40	0.18	0.69
27	0	24-Jan-02	82	0.13	167.28	22.08	145.20	4.60	5.50	0.08	0.44
28	0	14-Mar-02	66	0.28	559.02	154.02	405.00	15.10	12.50	0.17	0.54
29	0	19-Mar-02	73	0.49	1067.88	520.38	547.50	41.30	12.50	0.21	0.73
30	1	21-Mar-02	63	0.18	1141.31	207.24	934.07	15.70	16.65	0.22	0.94
31	1	01-May-02	49	0.42	874.92	368.16	506.76	23.60	10.30	0.26	0.82
32	1	27-May-02	54	0.38	936.36	354.24	582.12	24.60	12.60	0.24	0.77
33	0	10-Jun-02	71	0.16	576.30	90.30	486.00	21.50	10.80	0.07	0.75
34	1	10-Jun-02	85	0.10	1118.22	115.68	1002.54	24.10	21.70	0.08	0.77
35	1	03-Jul-02	79	0.04	1732.11	77.76	1654.35	14.40	26.90	0.09	1.03
36	0	13-Jun-02	63	0.33	508.77	169.65	339.12	21.75	7.85	0.13	0.72
37	0	15-Oct-02	71	0.21	907.17	191.88	715.29	16.40	10.55	0.20	1.13
38	1	15-Oct-02	67	0.16	1397.61	228.75	1168.86	15.25	16.10	0.25	1.21

M Male F Female; DPI Doppler perfusion index; TLBF Total liver blood flow (ml/min); HAF Hepatic artery flow volume (ml/min); PVF Portal venous flow volume (ml/min); HATAM Hepatic artery time-averaged velocity (cm/s); HAXSA Hepatic artery cross sectional area (cm^2); PVTAM Portal vein time-averaged velocity (cm/s); PVXSA Portal cross sectional area (cm^2).

Appendix 3 Example of ultrasound images for the measurement of the Doppler Perfusion Index (DPI)

Figure A Baseline intercostal ultrasound image of the right lobe of liver.

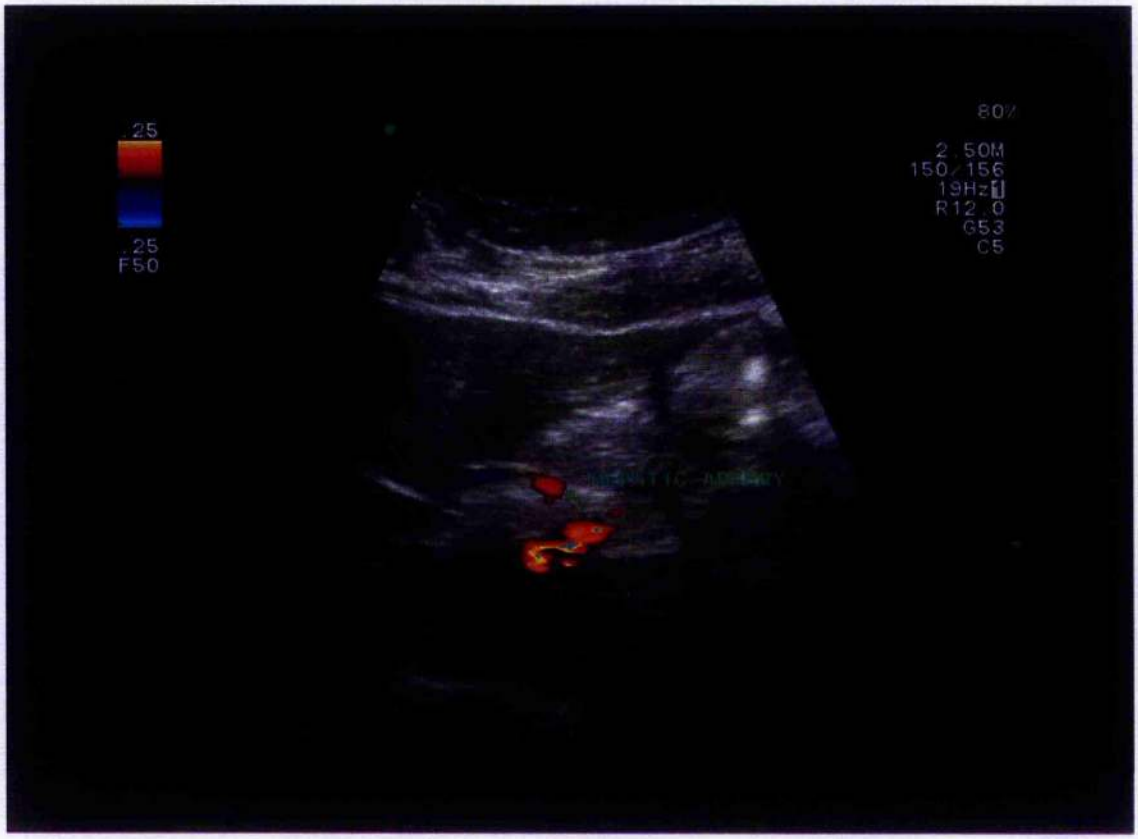

Figure B Colour Doppler ultrasound image showing hepatic artery and liver.

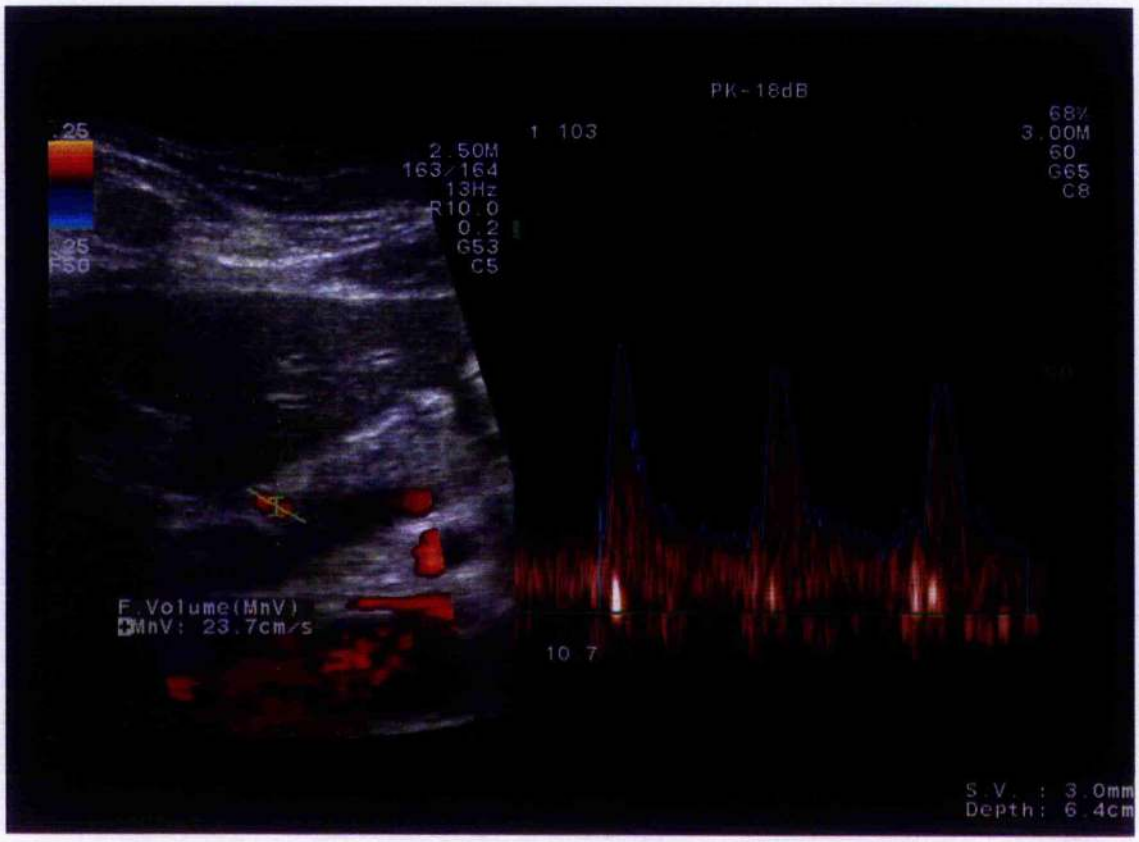

Figure C Colour Doppler ultrasound image and corresponding Doppler trace of hepatic artery. Example of time average mean velocity shown (F. Volume MnV : 23.7cm/s).

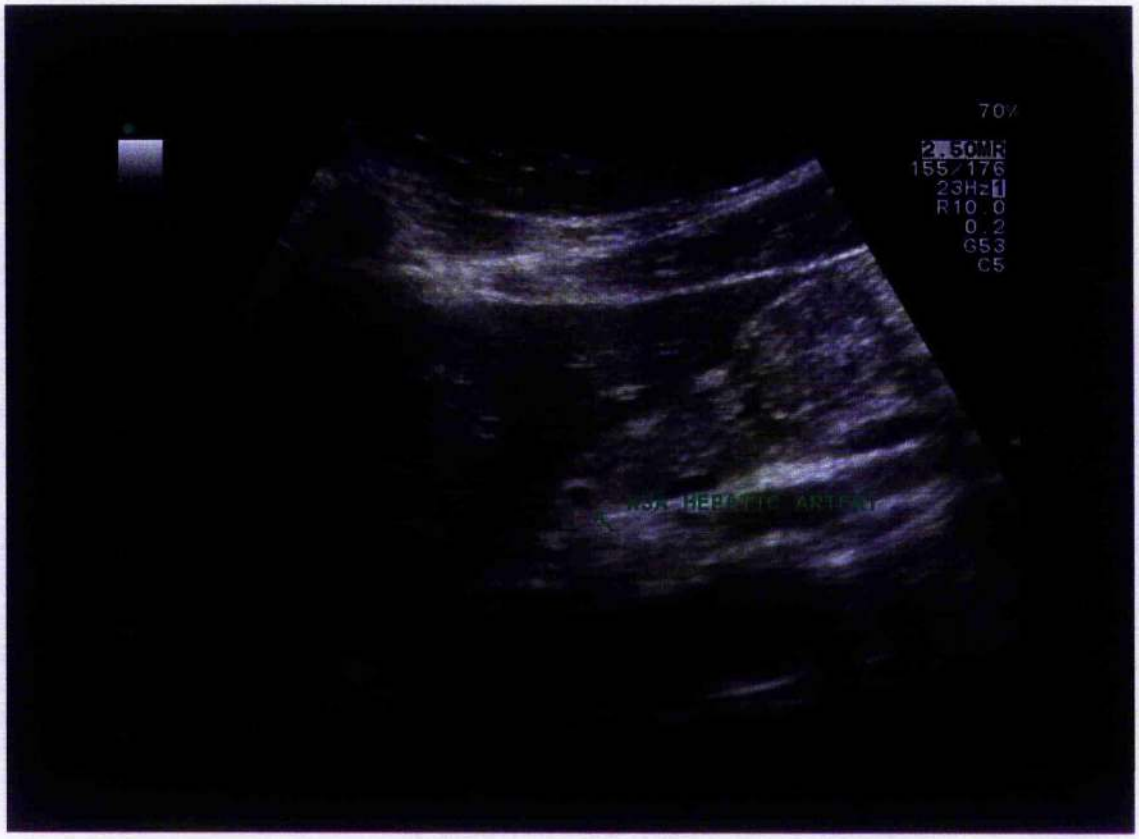

Figure D Baseline ultrasound image showing cross section of hepatic artery.

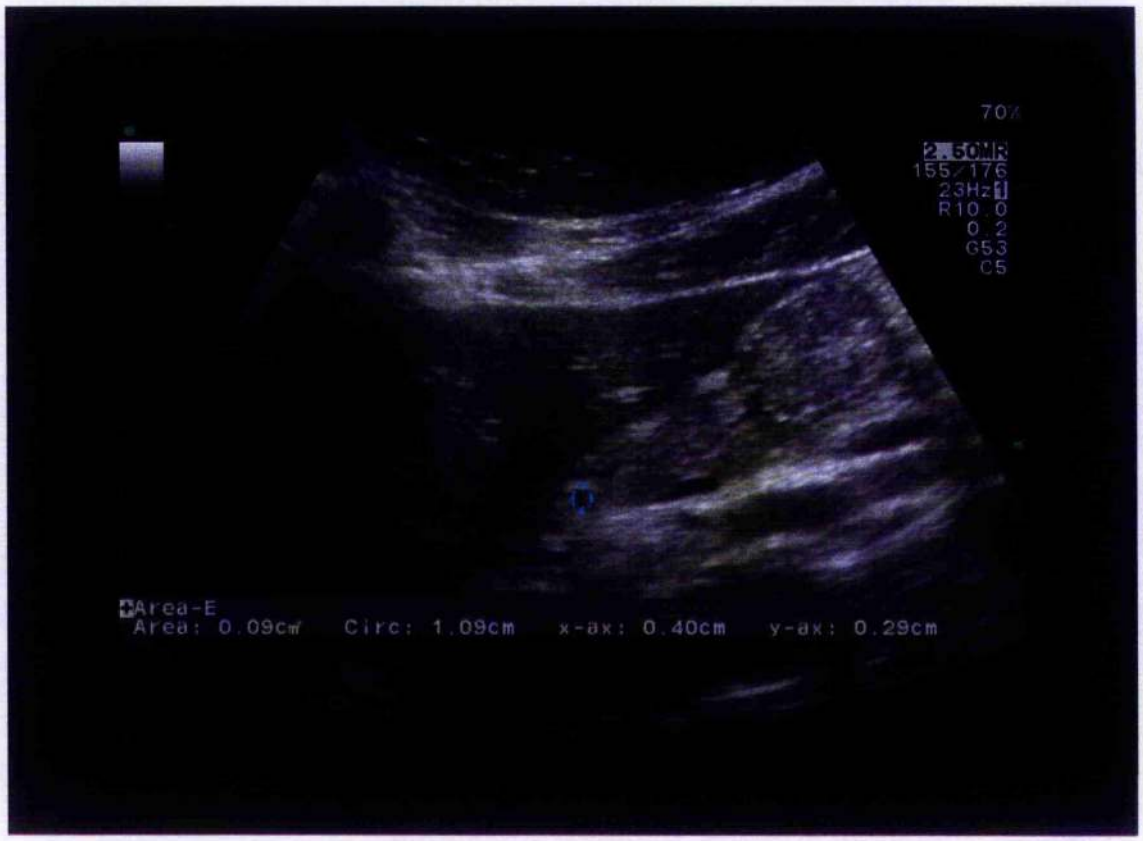

Figure E Cross sectional area measurement of hepatic artery. Example of ellipse measurement ($0.09cm^2$).

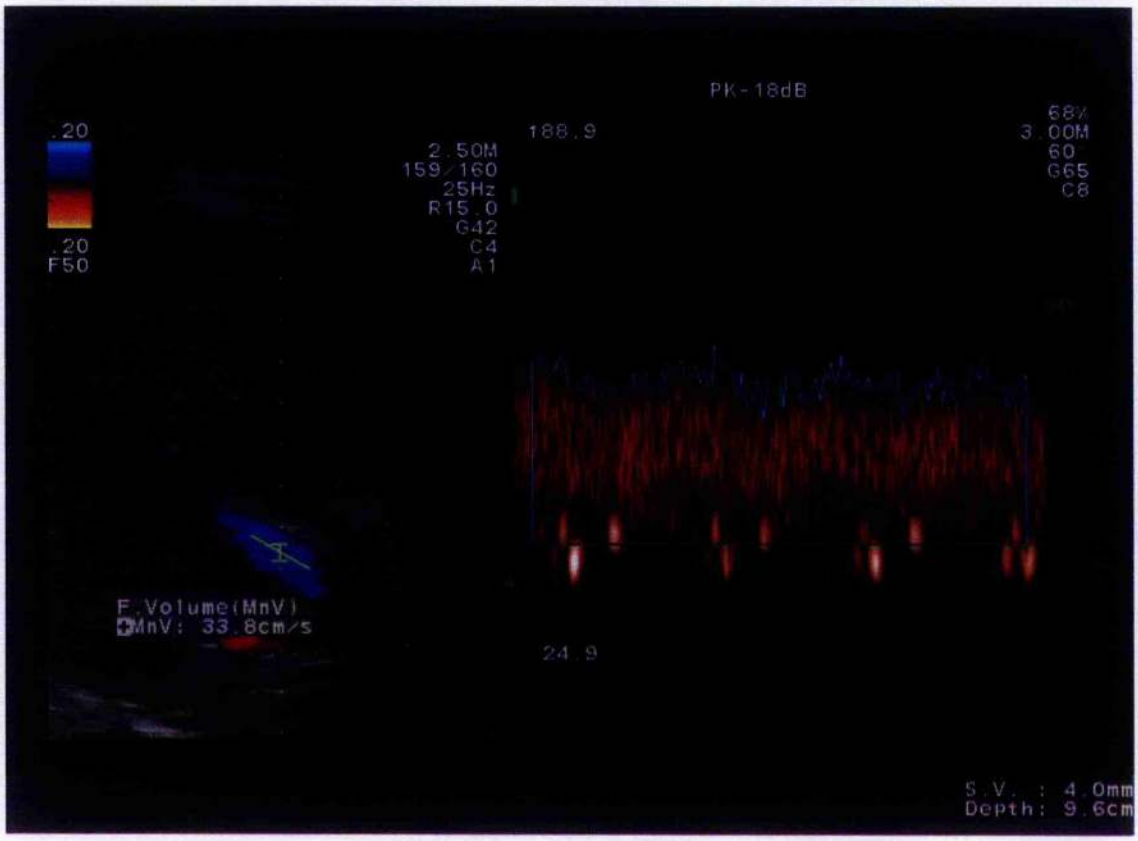

Figure F Colour Doppler ultrasound image and corresponding Doppler trace of portal vein. Example of time average mean velocity shown (F. Volume MnV : 33.8cm/s).

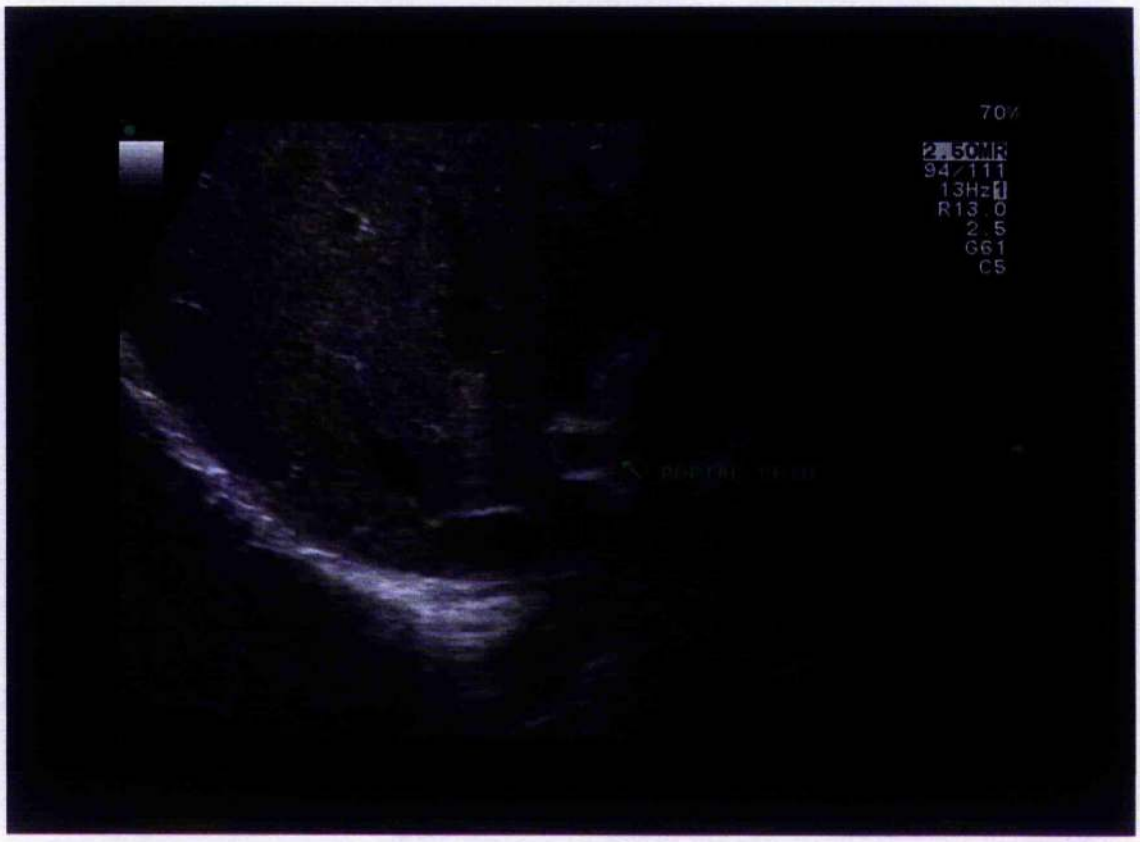

Figure G Baseline ultrasound image showing cross section of portal vein and right lobe of liver.

Figure H Cross sectional area measurement of portal vein. Example of ellipse measurement ($0.38cm^2$).

Appendix 4 Computed Tomography (CT) attenuation measurements

Table A CT attenuation measurements in liver region-of interest (ROI) in control subjects.

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	86.18	92.36	59.83	64.13	32.62	34.96
2	65.80	64.40	45.85	44.88	18.38	17.99
3	94.16	117.67	50.28	62.83	24.35	30.43
4	72.60	72.01	56.99	56.53	22.88	22.69
5	61.46	-	62.43	-	30.51	-
6	81.70	90.30	51.61	57.04	25.29	27.96
7	105.01	140.02	57.43	76.57	26.08	34.78
8	87.35	102.48	50.48	59.22	23.45	27.51
9	85.28	104.96	69.23	85.21	33.07	40.70
10	88.50	81.00	66.24	60.63	37.12	33.98

Table B CT attenuation measurements in liver region-of interest (ROI) in non-metastatic colorectal cancer (clear liver).

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	87.98	-	51.18	-	24.27	-
2	64.37	65.59	35.34	36.01	13.67	13.93
3	70.95	80.99	52.32	59.72	20.38	23.26
4	70.07	65.88	50.92	47.87	20.82	19.58
5	75.14	-	48.70	-	22.59	-
6	80.20	102.30	46.52	59.34	19.93	25.42
7	65.40	72.52	40.46	44.87	15.30	16.97
8	90.09	109.06	59.15	71.61	32.40	39.22
9	76.49	92.50	41.76	50.50	20.03	24.22
10	92.45	120.27	52.76	68.64	25.69	33.42
11	75.51	-	42.75	-	18.98	-
12	58.90	54.80	49.17	45.74	20.43	19.01
13	78.80	90.80	58.63	67.56	25.12	28.94
14	55.00	60.50	39.83	43.81	19.85	21.83

Table C CT attenuation measurements in liver region-of interest (ROI) in patients with colorectal liver metastases.

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	45.20	55.43	33.14	40.63	9.06	11.11
2	48.64	-	48.64	-	14.03	-
3	63.10	70.30	40.04	44.61	18.95	21.12
4	71.48	-	44.29	-	20.95	
5	78.69	73.76	48.63	45.58	21.25	19.92
6	66.82	77.78	57.13	66.50	25.30	29.45
7	58.64	67.25	32.07	36.77	12.46	14.29
8	74.02	85.29	49.05	56.51	25.75	29.67
9	57.14	61.16	53.70	57.48	25.44	27.23
10	71.98	-	41.37	-	19.62	-
11	63.75	71.72	43.40	48.83	20.61	23.19
12	67.85	-	40.27	-	16.22	-
13	61.92	62.10	71.82	72.03	30.96	31.05
14	64.32	68.28	51.69	54.87	24.32	25.82
15	85.25	102.75	63.02	75.96	29.79	35.91
16	67.10	-	38.35	-	16.20	-
17	87.30	88.25	55.08	55.68	26.03	26.32
18	84.74	-	52.63	-	23.28	-
19	72.50	80.07	48.38	53.44	21.49	23.73
20	80.74	94.47	49.21	57.58	20.50	23.99
21	80.06	86.78	54.89	59.50	22.38	24.26
22	60.95	-	47.25	-	18.83	-
23	65.17	67.82	46.70	48.60	19.17	19.95
24	84.59	-	51.05	-	21.95	-
25	72.69	109.50	47.09	70.94	21.17	31.89
26	95.69	131.56	50.31	69.17	19.62	26.98
27	70.30	122.50	36.71	63.98	15.75	27.44
28	80.85	127.44	45.30	71.40	23.28	36.70
29	60.62	75.76	44.56	55.69	15.76	19.70
30	72.52	83.87	51.24	59.26	21.90	25.33
31	85.70	-	49.35	-	23.60	-
32	63.57	72.44	39.98	45.56	15.84	18.05
33	62.55		47.70	-	17.89	-
34	67.17	76.13	47.37	53.69	18.72	21.22
35	70.84	-	46.09	-	22.23	-
36	-	-	-	-	-	-
37	69.48	74.41	47.03	50.37	14.03	15.03
38	69.18	-	48.72	-	18.28	-
39	99.75	126.87	61.74	78.52	31.64	40.24
40	67.32	78.20	38.28	44.47	16.69	19.38
41	84.05	86.78	52.76	54.47	28.99	29.93
42	68.24	83.71	51.59	63.29	17.82	21.86
43	80.54	91.67	52.32	59.55	28.26	32.17
44	93.04	100.60	58.90	63.69	26.41	28.55

Table D CT attenuation measurements in whole liver in control subjects.

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	85.10	92.40	60.86	66.08	32.21	34.98
2	64.00	66.80	44.91	46.88	17.88	18.66
3	95.06	116.20	51.22	62.61	24.58	30.05
4	72.60	75.00	56.67	58.55	22.88	23.63
5	61.79	-	66.55	-	30.67	-
6	85.80	99.60	55.50	64.42	26.56	30.84
7	110.51	142.15	60.51	77.83	27.45	35.31
8	89.14	112.53	54.02	68.20	23.93	30.20
9	88.29	94.32	72.61	77.57	34.24	36.58
10	85.10	85.00	65.92	65.84	35.70	35.65

Table E CT attenuation measurements in whole liver in non-metastatic colorectal cancer (clear liver).

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	88.51	-	51.03	-	24.41	-
2	63.24	69.05	35.19	38.42	13.43	14.66
3	70.76	81.83	51.27	59.29	20.33	23.51
4	68.78	65.69	50.28	48.02	20.44	19.52
5	75.90	-	49.63	-	22.82	-
6	81.50	105.20	46.41	59.91	20.25	26.14
7	67.31	75.03	41.38	46.13	15.75	17.55
8	91.10	111.34	60.73	74.23	32.76	40.04
9	72.32	95.03	40.14	52.75	18.93	24.88
10	94.88	120.68	54.39	69.18	26.37	33.54
11	75.26	-	43.07	-	18.92	-
12	59.30	55.30	50.25	46.86	20.57	19.18
13	76.90	93.80	57.65	70.31	24.51	29.90
14	55.50	62.00	41.26	46.10	20.03	22.37

Table F CT attenuation measurements in whole liver in patients with colorectal liver metastases.

Patient No.	Absolute Attenuation (HU)		% of Peak Liver Attenuation		% of Peak Aortic Attenuation	
	35 secs	45 secs	35 secs	45 secs	35 secs	45 secs
1	50.26	60.15	36.46	43.64	10.07	12.06
2	50.26	60.15	36.46	43.64	14.50	17.35
3	68.40	74.90	43.29	47.41	20.55	22.50
4	75.05	-	46.88	-	22.00	-
5	76.29	75.26	47.95	47.30	20.60	20.32
6	66.67	78.83	58.63	69.33	25.25	29.85
7	59.16	68.18	33.28	38.35	12.57	14.49
8	74.94	84.21	55.66	62.55	26.07	29.29
9	52.22	52.43	70.79	71.07	23.25	23.34
10	78.15	-	44.55		21.30	-
11	60.61	72.38	41.95	50.09	19.60	23.40
12	70.18	-	47.92	-	16.78	-
13	-	-	-	-	-	-
14	56.23	68.20	51.99	63.05	21.26	25.79
15	87.97	102.21	65.84	76.50	30.74	35.72
16	68.48	-	39.92	-	16.54	-
17	78.48	83.61	52.03	55.43	23.40	24.93
18	86.13	-	53.45	-	23.66	-
19	78.30	70.08	55.85	49.99	23.21	20.77
20	82.34	97.76	50.45	59.90	20.91	24.82
21	77.80	-	93.79	-	21.75	-
22	63.21	-	52.98	-	19.53	-
23	63.30	70.34	45.87	50.97	18.62	20.69
24	77.53	-	53.99	-	20.12	-
25	74.93	108.55	48.87	70.80	21.82	31.61
26	97.09	132.51	52.59	71.78	19.91	27.17
27	75.50	122.35	39.81	64.51	16.91	27.41
28	78.92	99.14	47.40	59.54	22.73	28.55
29	60.27	75.46	44.74	56.02	15.67	19.62
30	75.15	87.59	52.29	60.94	22.70	26.45
31	83.40	-	48.11	-	22.97	-
32	60.12	70.24	37.69	44.03	14.98	17.50
33	64.98	-	49.80	-	18.58	-
34	67.97	81.17	76.85	91.77	18.94	22.62
35	-	-	-	-	-	-
36	68.35	63.52	52.24	48.55	21.73	20.19
37	72.18	74.13	49.86	51.21	14.57	14.97
38	70.13	-	48.24	-	18.53	-
39	100.33	127.44	64.90	82.44	31.82	40.42
40	69.16	82.36	39.79	47.39	17.14	20.41
41	83.32	88.73	52.31	55.71	28.74	30.61
42	66.46	89.90	49.97	67.59	17.36	23.48
43	79.10	90.56	51.32	58.76	27.76	31.78
44	78.64	78.77	50.39	50.47	22.32	22.36

Appendix 5 Example of dual-phase CT scans for the measurement of hepatic perfusion

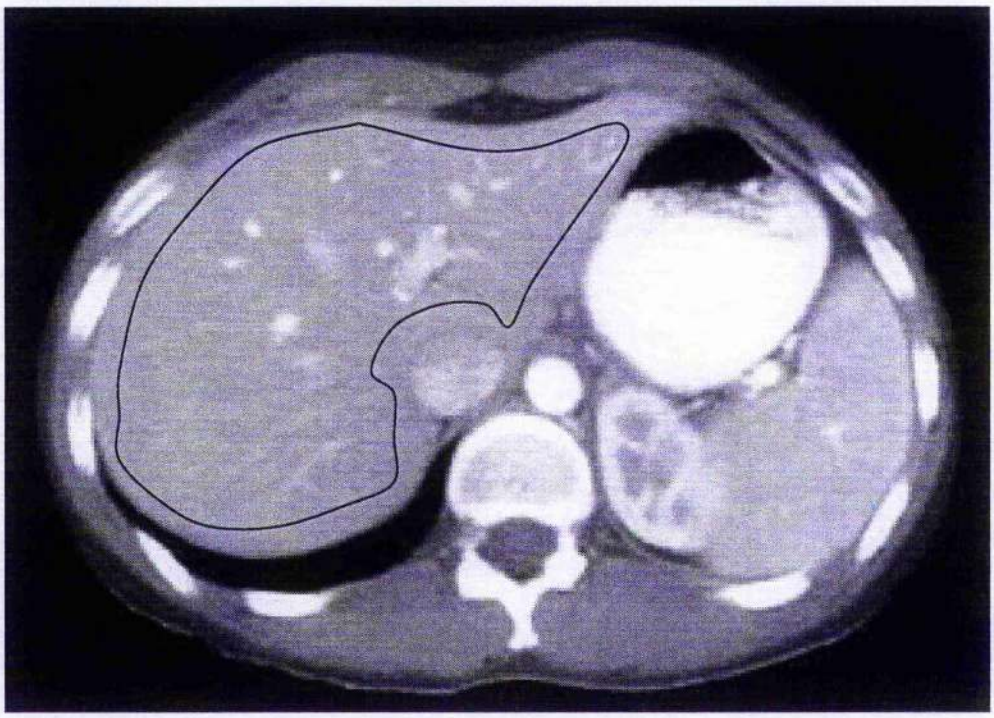

Figure A Dual-phase CT scan of abdomen, showing whole liver perimeter for attenuation measurement.

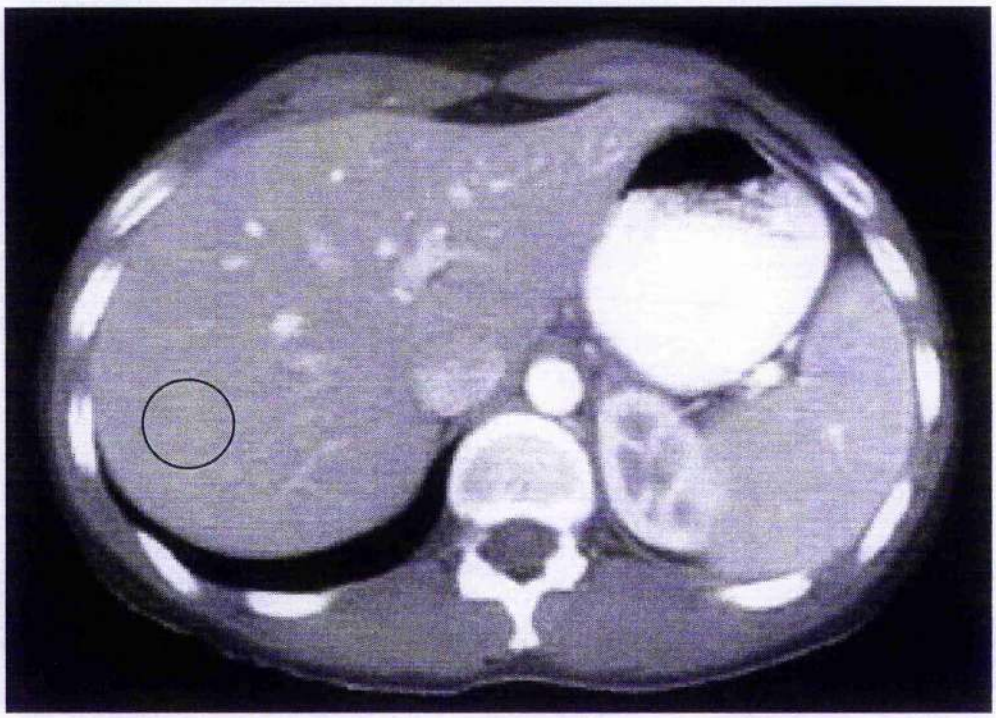

Figure B Dual-phase CT scan of abdomen, showing liver Region of Interest (ROI) for attenuation measurement.

Appendix 6 The relationship between tumour volume, the systemic inflammatory response and liver blood flow in patients with colorectal liver metastases

Table A Characteristics of patients with colorectal liver metastases (n=52).

Patient No.	Tumour Volume	PHR	CRP	IL-6	HAF	PVF	TLBF	DPI
1	709.40	35.90	28.00	13.10	470.67	990.72	1461.39	0.32
2	63.85	3.00	5.00	-	151.62	353.66	505.28	0.30
3	26.65	3.80	5.00	1.00	172.80	414.72	587.52	0.29
4	14.29	0.96	5.00	-	282.96	542.52	825.48	0.34
5	16.39	1.11	48.00	-	1216.05	1509.56	2725.61	0.45
6	1052.40	35.19	109.00	11.90	266.28	919.62	1185.90	0.22
7	563.26	10.70	141.00	-	606.90	460.08	1066.98	0.57
8	568.25	21.60	141.00	-	247.80	685.44	933.24	0.27
9	14.14	0.70	2.15	6.20	915.90	111.84	1027.74	0.89
10	315.50	14.91	6.00	3.90	202.80	399.36	602.16	0.34
11	7.90	0.50	2.47	4.00	68.04	259.20	327.24	0.21
12	69.42	3.91	5.00	2.60	363.48	726.00	1089.48	0.33
13	47.35	2.52	5.00	11.00	177.12	546.72	723.84	0.24
14	10.22	0.70	20.00	42.70	333.96	604.35	938.31	0.36
15	24.61	1.56	5.00	5.50	881.34	699.84	1581.18	0.56
16	130.80	6.00	55.00	-	1782.27	1233.25	3015.52	0.59
17	148.85	7.68	108.00	-	381.90	2114.94	2496.84	0.15
18	16.27	1.09	28.00	8.70	177.12	567.00	744.12	0.24
19	36.94	2.51	88.00	3.70	438.96	419.22	858.18	0.51
20	19.38	1.32	5.00	9.00	233.07	423.00	656.07	0.36
21	49.71	2.96	5.00	6.90	325.47	607.50	932.97	0.35
22	199.95	13.05	204.00	35.30	494.52	475.26	969.78	0.51
23	3223.80	90.00	135.00	41.70	345.58	405.55	751.13	0.46
24	613.39	21.80	19.00	-	546.06	431.52	977.58	0.56
25	89.50	3.75	21.00	3.70	178.92	498.15	677.07	0.26
26	101.69	5.87	24.00	11.70	1797.72	1350.00	3147.72	0.57

Tumour volume (cm^3); PHR, percentage hepatic replacement; CRP C-reactive protein (mg/l); IL-6 Interleukin-6 (ng/l); HAF hepatic arterial flow volume (ml/min); PVF portal venous flow volume (ml/min); TLBF total liver blood flow (ml/min); DPI Doppler perfusion index.

Table A cont./

Patient No.	Tumour Volume	PHR	CRP	IL-6	HAF	PVF	TLBF	DPI
27	233.23	15.84	125.00	-	503.82	641.52	1145.34	0.44
28	87.00	4.80	8.03	-	144.72	338.40	483.12	0.30
29	2741.76	90.00	9.64	2.90	325.13	723.67	1048.81	0.31
30	2.70	0.30	64.00	-	297.00	287.15	584.15	0.51
31	7.74	0.52	45.00	-	191.52	309.42	500.94	0.38
32	13.75	1.00	5.00	6.40	166.83	518.40	685.23	0.24
33	26.63	1.19	5.00	7.60	269.06	614.76	883.82	0.30
34	250.83	16.98	155.00	-	599.26	833.16	1432.42	0.42
35	28.88	3.00	5.00	1.30	270.00	167.70	437.70	0.62
36	68.24	3.60	83.00	22.90	428.40	595.08	1023.48	0.42
37	939.78	26.10	9.78	14.10	613.20	672.00	1285.20	0.48
38	12.61	1.00	7.00	11.00	193.46	727.96	921.41	0.21
39	1111.82	55.80	214.00	-	307.02	455.04	762.06	0.40
40	81.30	4.40	5.00	-	495.00	1078.74	1573.74	0.31
41	11.98	1.05	189.00	-	764.82	415.53	1180.35	0.65
42	7.65	0.70	76.00	5.90	86.52	515.97	602.49	0.14
43	468.09	30.03	11.00	-	561.96	886.17	1448.13	0.39
44	516.70	16.20	28.00	-	390.26	428.09	818.34	0.48
45	409.25	20.90	176.00	20.60	675.24	260.40	935.64	0.72
46	3.46	0.24	7.00	-	429.48	542.41	971.88	0.44
47	18.32	1.05	32.00	10.10	160.20	530.40	690.60	0.23
48	161.30	7.14	13.00	10.70	317.40	373.80	691.20	0.46
49	1366.60	34.27	57.00	-	267.84	492.90	760.74	0.35
50	5.40	0.16	22.00	17.70	247.50	652.05	899.55	0.28
51	379.66	27.91	5.00	5.30	313.33	557.76	871.10	0.36
52	178.00	9.47	213.00	21.80	341.46	896.40	1237.86	0.28

Tumour volume (cm^3); PHR, percentage hepatic replacement; CRP C-reactive protein (mg/l); IL-6 Interleukin-6 (ng/l); HAF hepatic arterial flow volume (ml/min); PVF portal venous flow volume (ml/min); TLBF total liver blood flow (ml/min); DPI Doppler perfusion index.

Appendix 7 The effect of anti-inflammatory treatment on liver blood flow in patients with colorectal liver metastases

Table A Patient details and liver blood flow measurements pre- and post-treatment of placebo or non-steroidal anti-inflammatory drugs.

Patient No.	Age	Placebo 0 Ibuprofen 1	Exclude 0 Include 1	HAF 1	PVF 1	TLBF 1	DPI 1	HAF 2	PVF 2	TLBF 2	DPI 2
1	67	0	1	325.13	723.67	1048.81	0.31	669.60	695.75	1365.35	0.49
2	73	1	1	457.75	345.40	803.14	0.57	-	-	-	0.52
3	66	0	1	136.21	318.17	454.37	0.30	81.00	248.64	329.64	0.25
4	74	0	1	333.96	604.35	938.31	0.36	-	-	-	0.33
5	-	1	0	-	-	-	0.47	-	-	-	-
6	70	0	1	202.80	399.36	602.16	0.34	180.41	466.60	647.01	0.28
7	50	1	1	881.34	699.84	1581.18	0.56	297.36	810.54	1107.90	0.27
8	63	1	1	269.06	614.76	883.82	0.30	145.32	538.20	683.52	0.21
9	77	1	1	184.68	597.24	781.92	0.24	269.04	1070.88	1339.92	0.20
10	72	0	1	345.58	405.55	751.13	0.46	290.70	208.08	498.78	0.58
11	74	1	1	613.20	672.00	1285.20	0.48	779.76	345.60	1125.36	0.69
12	50	0	1	486.90	990.72	1477.62	0.33	316.26	262.44	578.70	0.55
13	65	1	1	103.20	992.58	1095.78	0.09	67.68	875.76	943.44	0.07
14	83	1	1	494.52	475.26	969.78	0.51	237.60	837.00	1074.60	0.22
15	65	1	1	144.72	338.40	483.12	0.30	124.56	269.10	393.66	0.32
16	80	1	1	317.40	373.80	691.20	0.46	-	-	-	0.18
17	72	0	1	68.04	259.20	327.24	0.21	169.92	401.58	571.50	0.30
18	70	0	1	247.50	656.88	904.38	0.27	399.90	635.04	1034.94	0.39
19	71	0	0	307.02	455.04	762.06	0.40	-	-	-	-
20	-	0	0	-	-	-	-	-	-	-	-
21	78	0	0	297.00	287.15	584.15	0.51	-	-	-	-
22	72	0	1	166.83	518.40	685.23	0.24	115.92	637.20	753.12	0.15
23	76	1	1	86.52	515.97	602.49	0.14	106.59	579.36	685.95	0.16
24	75	1	0	324.00	426.00	750.00	0.43	-	-	-	-
25	78	0	1	207.00	555.00	762.00	0.27	255.00	828.36	1083.36	0.24
26	80	1	1	279.60	415.80	695.40	0.40	165.66	276.48	442.14	0.37
27	50	0	1	256.02	817.32	1073.34	0.24	372.84	627.30	1000.14	0.37
29	71	1	1	107.04	718.20	825.24	0.13	233.28	786.24	1019.52	0.23
30	75	0	0	384.18	1727.16	2111.34	0.18	-	-	-	-

HAF 1, Baseline hepatic arterial flow volume (ml/min); HAF 2, Post-treatment hepatic arterial flow volume (ml/min); PVF 1, Baseline portal venous flow volume (ml/min); PVF 2 Post-treatment portal venous flow volume (ml/min); TLBF 1, Baseline total liver blood flow volume (ml/min); TLBF 2, Post-treatment total liver blood flow volume (ml/min); DPI 1, Baseline Doppler perfusion index; DPI 2 post-treatment Doppler perfusion index.

Table B Patient details, liver tumour details and biochemical measurements pre- and post-treatment of placebo or non-steroidal anti-inflammatory drugs.

Patient No.	Placebo 0 Ibuprofen 1	Exclude 0 Include 1	Total Liver Volume	Tumour Volume	PHR	CRP1	CRP2	IL-6 1	IL-6 2
1	0	1	3046.40	2741.76	90.00	15.00	13	2.00	17.30
2	1	1	-	-	-	6.00	-	2.00	-
3	0	1	2104.54	63.85	3.03	37.00	6	2.00	2.00
4	0	1	1433.12	10.22	0.71	259.00	-	33.90	-
5	1	0	-	-	-	160.00	-	-	-
6	0	1	2115.94	315.50	14.91	16.00	-	136.90	-
7	1	1	1582.75	24.61	1.55	6.00	6	2.00	2.00
8	1	1	2240.28	26.63	1.19	6.00	6	2.00	2.00
9	1	1	1466.46	19.38	1.32	-	6	2.00	2.00
10	0	1	3582.00	3223.80	90.00	-	-	22.00	-
11	1	1	3607.13	939.78	26.05	-	100	-	8.40
12	0	1	1976.60	709.40	35.89	6.00	40	15.40	10.90
13	1	1	2021.27	14.14	0.70	13	6	2.00	2.00
14	1	1	1532.43	199.95	13.05	6	25	-	95.90
15	1	1	1827.36	87.00	4.76	51	6	2.00	2.00
16	1	1	2258.60	161.30	7.14	14.00	9	11.60	7.30
17	0	1	1580.03	7.90	0.50	-	6	2.00	2.00
18	0	1	1743.56	5.40	0.31	6.00	6	2.00	2.00
19	0	0	-	-	-	8.00	-	-	-
20	0	0	-	-	-	6.00	-	-	-
21	0	0	-	-	-	-	-	-	-
22	0	1	1358.28	13.75	1.01	6.00	6	2.00	2.00
23	1	1	1089.55	7.65	0.70	-	6	2.00	2.00
24	1	0	-	-	-	6.00	60	11.90	23.70
25	0	1	-	-	-	18.00	8	5.50	21.80
26	1	1	-	-	-	6.00	6	-	2.00
27	0	1	-	-	-	76	6	2.00	2.00
29	1	1	-	-	-	-	21	2.00	3.20
30	0	0	-	-	-	25	29	8.70	8.40

Liver Volume (ml); Tumour Volume (ml); PHR, Percentage Hepatic Replacement, CRP1 pre-treatment C-reactive Protein (mg/l); CRP2 post-treatment C-reactive Protein (mg/l); IL-6 1 Pre-treatment Interleukin-6 (ng/l); IL-6 2 Post-treatment Interleukin-6 (ng/l).