

Late Gadolinium Hyperintensity (LGH) of Colorectal Liver Metastases (CRLM) on Magnetic Resonance Imaging (MRI)

by

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ABSTRACT (SUMMARY)

Late gadolinium hyperintensity (LGH) of solid focal liver lesions on MRI with extracellular contrast agents is typically considered a “benign” finding seen most commonly with hemangiomas, but has also been observed with colorectal liver metastases (CRLM). In the first part of this thesis, we determined the prevalence of LGH in CRLM on MRI with extracellular contrast agents. In a retrospective cohort of 134 patients with 232 pathology-confirmed CRLMs, the prevalence of LGH in CRLMs was 47.8% (95% CI: 39.7%-56.0%). Some CRLMs demonstrate tumour fibrosis, which is associated with long-term survival. LGH with extracellular contrast agents in CRLMs may be due to leakage of contrast and progressive accumulation in the extracellular space of fibrotic tumours. In the second part of this thesis, we determined whether LGH is associated with tumour fibrosis and overall survival in patients with CRLM using extracellular contrast agents. In a retrospective cohort of 121 surgical patients who received preoperative MRI with extracellular contrast agents after chemotherapy, LGH was associated with tumour fibrosis ($R=0.43$, $p<0.001$) and with improved survival with a hazard ratio of 0.32 (95% CI: 0.14-0.75, $p=0.008$). If LGH occurs due to leakage of extracellular contrast into fibrotic tumour, then LGH would

not occur in CRLM using intravascular contrast agents. Therefore, in the final part of this thesis, we determined the prevalence of LGH with intravascular contrast agents and the diagnostic accuracy of liver MRI using intravascular contrast agents. In a prospective cohort of 48 patients with known colorectal cancer who were referred for an MRI of the liver, we performed MRI with an intravascular contrast agent. The prevalence of LGH was 11.9% (95% CI: 4.0% to 19.9%). The diagnostic accuracy of MRI with the intravascular contrast agent for diagnosing CRLM was high with a per-lesion sensitivity and specificity of 0.99 and 0.91 respectively. In summary, LGH of CRLM on MRI with extracellular contrast agents is common and is associated with tumour fibrosis and overall survival. In contrast, LGH of CRLMs on MRI with intravascular contrast agents is uncommon, leading to an excellent diagnostic accuracy for diagnosing CRLM, and may be a useful diagnostic problem-solving tool.

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LIST OF ABBREVIATIONS

1.5T	1.5 Tesla
3.0T	3.0 Tesla
ADC	apparent diffusion coefficient
APC	adenomatous polyposis coli
AJCC	American Joint Committee on Cancer
AUC	area under the curve
CEA	carcinoembryonic antigen
CE-CT	contrast-enhanced CT
CE-IOUS	contrast-enhanced intraoperative ultrasound
CE-US	contrast-enhanced ultrasound
CIMP	CPG island methylator phenotype
CIS	chromosomal instability
CNR	contrast-to-noise ratio
CRLM	colorectal liver metastases
CT	computed tomography
cTNM	clinical TNM staging
DCE-MRI	dynamic contrast-enhanced MRI
DWI	diffusion weighted imaging
EASL	European Association for the Study of the Liver
EGFR	epidermal growth factor receptor
EPI	echo planar imaging

FOBT	fecal occult blood testing
FAP	familial adenomatous polyposis
fat-sat	fat saturation
FNH	focal nodular hyperplasia
FOLFIRI	fluorouracil, leucovorin, and irinotecan
FOLFOX	fluorouracil, leucovorin, and oxaliplatin
FOLFOXIRI	fluorouracil, leucovorin, oxaliplatin, and irinotecan
FOV	field of view
FSE	fast spin echo
GBCA	gadolinium-based contrast agents
GEE	generalized estimating equation
GIST	gastrointestinal stromal tumour
GRE	gradient echo
HAI	hepatic arterial infusion
HCC	hepatocellular carcinoma
HR	hazard ratio
IOUS	intraoperative ultrasound
irRC	immune-related response criteria
irRECIST	immune-related RECIST
JPS	juvenile polyposis syndrome
LGH	late gadolinium hyperintensity
LR	likelihood ratio
LR+	likelihood ratio given a positive test

LR±	likelihood ratio given an indeterminate test
LR-	likelihood ratio given a negative test
MMR	mismatch repair
mRECIST	modified RECIST
MSI-L	low frequency microsatellite instability
MSS	microsatellite stable
MR	magnetic resonance
MRI	magnetic resonance imaging
MSI-H	high frequency microsatellite instability
NSF	nephrogenic systemic fibrosis
PACS	picture archiving and communication system
PET	positron emission tomography
PET-CT	positron emission tomography/computed tomography
PJS	Peutz-Jeghers syndrome
pTNM	pathological TNM staging
RECIST	Response Evaluation Criteria in Solid Tumours
RF	radiofrequency
RFA	radiofrequency ablation
ROC	receiver operating characteristics
ROI	region of interest
SD	standard deviation
SI	signal intensity
SNR	signal to noise ratio

TACE	transarterial chemoembolization
TE	echo time
THAD	transient increase in hepatic attenuation
THID	transient increase in hepatic intensity
TNM	tumour nodal metastases
TR	repetition time
TTE	target tumour enhancement
US	ultrasound
VEGF	vascular endothelial growth factor
WHO	World Health Organization
XELOX	capecitabine and oxaliplatin

CHAPTER 1 : LITERATURE REVIEW

1.1 COLORECTAL CANCER

1.1.1 Significance and impact

Colorectal cancer is the 2nd leading cause of cancer deaths in men (after lung) and the 3rd leading cause of cancer deaths in women (after lung and breast) in Canada and the United States (1, 2). Global estimates suggest that there were 1.4 million cases diagnosed and 693,900 deaths in 2012 alone (2). The highest incidence rates are in developed nations with a slight male preponderance (3).

The American Cancer Society estimates that in 2017, the number of new cases of colorectal cancer in the US will be around 135,430 people and that 50,260 people will die from their disease (2). The Canadian Cancer Society estimates that in 2017, 26,800 Canadians will be newly diagnosed with colorectal cancer and 9,400 Canadians will die of this disease (1). This translates into an average of 73 Canadians diagnosed and 26 Canadians that die from colorectal cancer every day. The 5-year survival is approximately 64% (1).

In addition to its human cost, colorectal cancer has significant economic costs. A report from the American Cancer Society stated that cancer causes the highest economic loss of all the leading causes of death worldwide with a cost of \$1,037 billions in 2018 inflation-adjusted USD (\$895 billion USD in 2008), nearly 20% higher than heart disease (\$873 billions in 2018 inflation-adjusted USD or \$753 billion USD in 2008) (4). Colorectal cancer ranks second among the cancers that cause the most economic impact globally with an estimated economic impact of \$115 billions in 2018 inflation-adjusted USD (\$99 billion USD in 2008) (4).

The data on the economic impact of colorectal cancer in Canada is somewhat limited. However, one study published in 2003 estimated that the average lifetime medical costs of

colorectal cancer per patient to be \$55,000-\$64,000 in 2018 inflation adjusted Canadian dollars (\$29,000-34,000 in 1988 Canadian dollars) which amounted to \$982 million in 2018 inflation-adjusted Canadian dollars (\$520 million in 1988 Canadian dollars) (5, 6). The cost is higher for cancer diagnosed at a higher stage due to increased costs of treatment and hospitalizations. This is in addition to the cost of lost wages, which in one study published in 2009, was estimated to be \$5.57 billion in 2018 inflation-adjusted Canadian dollars (\$2.95 billion in 2009 Canadian dollars) (5, 6).

1.1.2 Pathophysiology and classifications

Most cases (~75%) of colorectal cancer occur sporadically usually from adenomatous polyps (80-90%) and occasionally from sessile adenomas (10-30%)(7). Tubular adenomas are the most common type of polyps, but have the lowest potential for malignancy. Villous adenomas are the least common type of polyps but have the highest potential for malignancy (7). The malignancy potential of tubulovillous adenomas are somewhere in between (7). In addition to villous pathology, the size of the adenoma or adenomatous polyp is also related to malignant transformation (7). Other types of polyps including hyperplastic, juvenile, hamartomatous, inflammatory, and lymphoid polyps are considered non-neoplastic and are not thought to be precursors to cancer (7).

Approximately 70-85% of colorectal cancer cases are sporadic, meaning there is no apparent family history or genetic syndrome that suggests the cancer was inherited (8). The other 15-30% of cases are related to family history or genetic syndromes. Most inherited cases are related to familial colorectal cancer, a diverse group of genetic mutations with low penetrance. These mutations have additive effects in addition to interactions with environmental risk factors. "Common Familial Risk Colon Cancer" refers to individual with a first-degree relative with colorectal cancer diagnosed over the age of 50 years (7, 8). These individuals have a 2-3x risk of colorectal cancer about the general population. Among individuals who have one first degree relative diagnosed with colorectal cancer under 45 years of age or two first degree relatives with colorectal cancer at any age, then the risk increases to 3-6x that of the general population (8). The minority of cases are due to highly penetrant genetic syndromes such as familial polyposis syndrome, Lynch syndrome, juvenile polyposis syndrome, or Peutz-Jeghers syndrome (8).

Among sporadic cases of colorectal cancer, it is thought that a series of acquired molecular events are responsible for colon carcinogenesis (9). Over time, a series of genetic mutations in normal colon epithelium and/or adenomas lead to malignant transformation (9). There are two major pathways by which this occurs. The majority (~80%) of cases is due to chromosomal instability (CIS) and the minority (~20%) of cases is due to hypermethylation (CpG island methylator phenotype or CIMP) which causes hypermutation (9). The large majority of the CIMP cases involve high frequency microsatellite instability (MSI-H) (9). The MSI-H phenotype colorectal cancers often arise from sessile serrated adenomas, occur in the proximal colon, and are more prevalent in elderly females (9). More detail on the molecular biology of colorectal cancer is described in subsection 1.2.4.

The environment likely influences the propensity for molecular events leading to colon carcinogenesis (10). Environmental risk factors include unhealthy diet (red and processed meat), obesity, physical inactivity, alcohol and smoking. Inflammatory bowel disease (IBD) is associated with increased risk of colorectal cancer(11). In patients with ulcerative colitis, the risk of colorectal cancer is between 7-10% in a patient who have had ulcerative colitis for 20 years (12). This risk increases with the length of the disease. The risk of colorectal cancer in Crohn's disease is not well understood.

Colorectal cancer is divided anatomically between colon and rectal cancer. Due to anatomical considerations, this has important implications for both therapy (surgery and radiation) and prognosis. Due to the more intimate relationship of the rectum to surrounding structures, there is increased risk for invasion of adjacent structures as well as more difficult surgery and higher risk for positive surgical margins. In addition, extra-hepatic disease is more likely with rectal cancer due to the porto-systemic system (see subsection 1.16). As a result, rectal cancer confers a reduced prognosis compared to colon cancer (10). There is also some data to suggest that there may be inherent biological (molecular) differences between rectal cancer and proximal colon cancers, which may confer a worse prognosis for patients with rectal cancer (13, 14).

1.1.3 Screening and diagnosis

Colorectal cancer most commonly presents (1) in asymptomatic patients during colorectal cancer screening, (2) in asymptomatic patients as an incidental finding on imaging, and (3) in the symptomatic patient.

The American College of Physicians recommends colorectal cancer screening in average-risk adults starting at 50 years of age and high-risk adults at 40 years of age or at 10 years younger than the age of diagnosis of the youngest affected first-degree relative (15). Screening should be stopped in patients after the age of 75 years or if the patient's life expectancy is less than 10 years (15). Screening methods include: fecal blood testing, sigmoidoscopy or colonoscopy (15). Alternative methods include CT colonography or double-contrast barium enema colon X-ray for patients with a positive FOBT and an incomplete colonoscopy (15).

Fecal testing involves the detection of blood in stool samples. Sigmoidoscopy and colonoscopy involves direct optical visualization of tumours through a sigmoidoscope or colonoscope (15). Each screening method has its own advantages and disadvantages. Fecal testing is cheap and easily accessible, but is limited by both false negatives (due to tumours that do not cause occult bleeding) and false positives (due to occult blood from other causes) (15). Sigmoidoscopy and colonoscopy provide direct visualization of tumours and the ability to biopsy tumours at the time of visualization; however, it is resource and expertise-intensive (15). The Canadian Cancer Society currently recommends stool testing (either guaiac-based fecal occult blood test or fecal immunochemical test) every 2 years after the age of 50, followed by a colonoscopy (or double contrast barium enema or flexible sigmoidoscopy) if the fecal test is positive (16).

Occasionally, asymptomatic patients will present through incidental findings seen on imaging such as colonic or rectal mass or manifestations of metastatic disease.

Finally, patients may present with clinical symptoms. This can be related to the primary malignancy, including hematochezia, melena, anemia (due to occult blood loss), change in bowel habits, bowel obstruction (7, 12). This can also be related to metastatic disease, usually liver metastases, including: abdominal fullness, hepatomegaly, or jaundice (7, 12).

Definitive diagnosis of colorectal cancer is based on pathology confirmation, usually biopsy of the primary tumour during colonoscopy. Patients need to have a complete colonoscopy (or if this is not possible, then CT colonography may be performed as an alternative) as synchronous primary cancer may occur (7).

1.1.4 Staging

Colorectal cancer is usually staged with the tumour-node-metastasis (TNM) classification, which is used to group cancers into four categories (stages I, II, III, IV) developed by the American Joint Committee on Cancer (17). These are further subdivided into seven stages (stages I, IIa, IIb, IIIa, IIIb, IIIc, and IV) (17).

The TNM classification is based on 3 pieces of information. “T” describes the growth of the primary tumour and the extent of its invasion into nearby structure (17). “N” describes the extent to which the tumour has spread to the regional lymph nodes (17). “M” describes metastases to distant organs in the body, including the liver, bones, or lungs (17). The TNM classification can be based on clinical staging (cTNM), which includes imaging information (17). It can also be based on pathological staging (pTNM) (17).

T-staging for rectal cancer requires MRI (or alternatively endoscopic ultrasound), which should be done prior to any neoadjuvant chemoradiation (9). M-staging for colon and rectal cancer requires imaging of the chest, abdomen, and pelvis. Liver metastases is present in approximately 20% of patients and therefore, liver imaging needs to be performed using CT or MRI (9). Lung metastases are present in 9-18% of patients with rectal cancer (less common in colon cancer) and should be determined using a chest CT (9). There is no evidence to support routine imaging of the bones or brain (9). Data does not currently support routine use of PET-CT (9).

The definitions of the different T, N, and M stages are described in Table 1.1. The corresponding stage categories are described in Table 1.2.

The different stages of colorectal cancer confer different survival rates (17). A study based on the Surveillance, Epidemiology, and End Results (SEER) data, a nation-wide, multicentre American database, involving 119,363 patients demonstrated that the overall

survival rate for all stages of colorectal cancer was 65.2% (17). The 5-year survival rates for Stage I, II, II, and IV colorectal cancers were 93.2%, 82.5%, 59.5%, and 8.1%, respectively (17). In addition to classic TNM staging, histological subtype was also noted to be a significant predictor of survival with 5-year survival rates to be 65.9%, 61.8%, and 36.0% respectively for adenocarcinoma, mucinous adenocarcinoma, and signet ring carcinoma. However, signet ring carcinoma is a rare subtype (approximately 1.0% of all colorectal cancers in this population) (17). The survival differences were consistent across stages II, III, and IV, but not for stage I (17). Note that this data was derived from January 1, 1991 to December 31, 2000 and were based on the older, 6th edition AJCC staging classification. The survival rates have improved significantly in the last decade due to improvements in screening and management.

Table 1.1: Definitions of TNM categories of colorectal cancer as described by the American Joint Committee on Cancer (AJCC), 7th edition

Primary tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Submucosal invasion
T2	Muscularis propria invasion
T3	Pericorectal invasion
T4a	Peritoneal invasion
T4b	Adjacent organ invasion
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	1-3 regional lymph nodes involved
N1a	1 regional lymph node involved
N1b	2-3 regional lymph nodes involved
N1c	Regional nonperitoneal, pericolic/perirectal fat involvement without direct involvement of regional lymph nodes.
N2	4 or more regional lymph nodes involved
N2a	4-5 regional lymph nodes involved
N2b	7 or more regional lymph nodes involved
Distant metastasis (M)	
MX	Distant metastases cannot be assessed
M0	No distant metastases
M1	Distant metastases
M1a	Metastases to 1 organ/site
M1b	Metastases to more than 1 organ/site or to the peritoneum.

*Adapted from AJCC Cancer Staging Manual, 7th edition. 2010. Springer-Verlag New York. Reproduced with permission.

Table 1.2: Stages of colorectal cancer as it relates to TNM categories as described by the American Joint Committee on Cancer (AJCC), 7th edition.

Stage	T	N	M
0	Tis	N0	M0
I	T1	N0	M0
	T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-T2	N1/N1c	M0
IIIB	T3-T4a	N1/N1c	M0
	T2-T3	N2a	M0
IIIC	T1-T2	N2b	M0
	T4a	N2a	M0
	T3-T4a	N2b	M0
	T4b	N1-N2	M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b

*Adapted from AJCC Cancer Staging Manual, 7th edition. 2010. Springer-Verlag New York. Reproduced with permission.

1.1.5 Management

Surgery

For colon cancer, removal of the tumour and corresponding lymphatic vessels (and nodes) are required (9). The degree of resection depends on the location of the tumour and the anatomy of the subsequent blood supply (9). For rectal cancer, total mesorectal excision (removal of the rectum, mesorectum, and mesorectal fascia) is required to remove the primary tumour and local lymph nodes (9). The goal is to obtain a circumferential margin (at least 1mm between the tumour and the resection margin) (9).

Neoadjuvant therapy

In some cases of rectal cancer, patients may benefit from neoadjuvant radiotherapy and/or chemotherapy (9). Neoadjuvant therapy can decrease the local recurrence rate (9). For Stage 1 rectal cancer, no neoadjuvant treatment is recommended due to the low local recurrence rate (9). For Stage 3 rectal cancer, neoadjuvant treatment is recommended as studies have shown a decrease in local recurrence (9). However, for Stage 2 rectal cancer, the benefit is less clear and some people advocate neoadjuvant therapy only for high-risk patients with T4 or advanced T3 disease (9).

There is some controversy on the exact neoadjuvant treatment. There are two major options: short course radiotherapy followed rapidly by surgery (without any significant downstaging effects) vs. long course radiotherapy and chemotherapy (9). The latter option is preferred, particularly with more advanced tumours (9).

Adjuvant therapy

Adjuvant chemotherapy is recommended in patients with Stage 3 colon cancer or in high risk Stage 2 cancer (eg. perforated cancer, T4 disease, bowel obstruction, < 12 lymph nodes removed) (9). The chemotherapy regimen varies and may include fluorouracil, capecitabine, and/or oxaliplatin (9).

Distant metastases

In surgically resectable patients, liver and lung metastases should be treated with surgery (please see following subsections 1.1.6 and 1.1.7 on colorectal liver metastases) (9, 18). Chemotherapy is used either in conjunction with surgery or alone and may include cytotoxic agents and/or targeted chemotherapeutic agents (9, 18).

1.1.6 Colorectal liver metastases

Approximately 50% of patients diagnosed with colorectal cancer will develop colorectal liver metastases (CRLM) (19). This can be diagnosed at the time of the diagnosis of the primary (so-called “synchronous” metastases) or subsequently (so-called “metachronous” metastases) (19). Synchronous metastases occur in approximately one fifth of patients and may reflect different tumour biology, although the literature on this remains unclear (19).

The liver is the most common site of colorectal metastases and is often the first site of metastatic disease involvement (20). This is likely related to the underlying portal anatomy and physiology. The venous drainage of colon and upper rectum is largely via the portal vein, which directly drains into the liver (20). The normal lymphatic drainage system has a similar pattern the venous drainage. Therefore, CRLM are common and metastatic disease is often isolated to the liver (20). The rectum has dual portal and systemic venous and lymphatic drainage; therefore, there is a higher risk of extra-hepatic metastases with rectal cancers (20, 21).

Untreated, the median survival of patients with CRLM is approximately 6-12 months (21). However, with improvements in surgery and chemotherapy techniques, the median survival has improved significantly and is curative in a subgroup of patients who are surgical candidates (see subsection 1.17) (21).

1.1.7 Management of colorectal liver metastases

In the last decade, the number and the quality of the treatment options for colorectal liver metastases (CRLM) have improved significantly. These include surgery, radiation, ablative techniques, systemic chemotherapy (cytotoxic and targeted therapies), and arterial pump chemotherapy.

Surgery

Surgery is the first-line treatment (in conjunction with chemotherapy and other techniques) in patients who are surgical candidates (22). In one meta-analysis, the 5-year and 10-year survival of patients who underwent surgical resection was 38% and 26%, respectively (23). Survival may be higher with recent improvements in surgical

techniques and improved chemotherapy options with recent data suggesting median survival may exceed 5- years(18). Surgery is the only treatment option that has been shown to be curative for patients with CRLM (22). Traditional definitions of resectability include physically fit for surgery, ability to remove all macroscopic disease with clear margins and leave a sufficiently functioning liver (approximately one-third of the standard liver volume), and limited resectable extra-hepatic disease (eg. limited pulmonary metastases amenable to lung resection) (22). Recently, there has been some evidence to suggest that patients with borderline resectable disease may still benefit from surgical resection, including patients that may achieve resectability when surgery is combined with ablative techniques or hepatic functional reserve can be achieved through either portal vein embolization or two-stage hepatectomy (24, 25). Recent evidence suggests that some patients with R1 resection or extrahepatic disease may still benefit surgery and potentially achieve long-term cure (26, 27). Patients who develop recurrent disease may also be considered for repeat resection (28).

Nonsurgical Locoregional Therapy

Surgery is the first-line treatment for patients with resectable CRLM; however, some patients do not meet criteria for resectability either due to the extent of their disease or due to co-morbid conditions that preclude surgery (29). Various regional therapies have been developed for this patient population and may be delivered either for curative or palliative intent either in addition to surgery or as an alternative to surgery (30). These include a range of ablative, radiation, and embolization techniques. The most commonly used nonsurgical locoregional therapies are radiofrequency ablation and stereotactic radiation.

Radiofrequency ablation (RFA) is an ablative technique that involves administering electrical current directly within a metastatic lesion in order to cause burn sites of metastatic disease (30). With the growing realization that local treatment of CRLM improves morbidity and mortality, RFA is becoming an increasingly popular tool for treatment (30). In studies comparing RFA to surgical resection, it has been shown that RFA performs less well than surgery in terms of incidence of recurrence, time to progression, and survival (30). Nevertheless, outcomes are good in well-selected patients (30). Success of RFA depends on the location of the lesion (accessibility for RFA), number of lesions, and size of the lesions (30). In clinical practice, RFA is often used as an alternative technique in patients who are not surgical candidates or used in conjunction

with surgery in order to increase the number of patients who can become surgical candidates (30).

Stereotactic body radiotherapy (SBRT) is a radiation technique that involves delivery of high doses of radiation through multiple radiation beams via a small number of fractions to the site of disease (30). Like RFA, SBRT is typically used in patients who are not surgical candidates (30). In some studies, SBRT has similar outcomes compared to RFA (30). The decision to perform SBRT vs. RFA depends on location of tumour, tumour size, physician expertise, and patient preference (30).

Other less commonly used techniques include microwave ablation (similar to RFA except using electromagnetic radiation in the microwave range), cryoablation (using freezing temperatures less than -40 degrees centigrade to kill tumour cells), and radioembolization (delivery of high dose radiation to tumour cells via radioactive tracers) (30).

Systemic chemotherapy

Significant advances have been made in systemic chemotherapy for metastatic colorectal cancer (18). These include cytotoxic therapies as well as targeted chemotherapies (18). Current first-line treatment typically involves the use of combination cytotoxic therapies (18). Commonly used regimens include the combination of fluorouracil, leucovorin, and irinotecan (FOLFIRI), the combination of fluorouracil, leucovorin, and oxaliplatin (FOLFOX), the combination of capecitabine and oxaliplatin (XELOX), the combination of fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) (18).

A number of targeted chemotherapies have been developed for second-line therapy or in combination with cytotoxic agents for first-line therapy in patients who are appropriate candidates (18). These include anti-angiogenic agents such as bevacizumab, which is a monoclonal antibody that targets vascular endothelial growth factors, and regorafenib, which is a multitargeted tyrosine kinase inhibitor (18). These also include monoclonal antibodies that target the epidermal growth factor receptor (EGFR) including cetuximab and panitumumab (18).

Regional chemotherapy

The liver is the most common single site for metastatic disease from colorectal cancer and liver-only or liver-dominant metastatic disease is common (31). Therefore, chemotherapy

that targets metastatic disease in the liver in unresectable patients may be helpful. The most commonly used and widely studied technique is the use of hepatic arterial infusion (HAI) chemotherapy (31). This involves the placement of an implanted pump that delivers chemotherapy directly to the hepatic metastases via the hepatic arteries (31). Because the predominant blood supply to liver metastases are from the hepatic arteries (compared the portal system for the normal liver parenchyma), this technique allows higher concentrations of chemotherapy to be delivered directly to tumour cells (31). Additionally, it allows for use of chemotherapeutic agents with high hepatic metabolism since it bypasses first-pass hepatic clearance (31). HAI allows for high tumour response rate with minimal systemic toxicity and is often used in patients with liver-only or liver-dominant disease who are not surgical candidates and who have progressed on first-line chemotherapy (31). In some cases, HAI has been able to downsize unresectable patients with CRLM (31).

Other regional chemotherapy techniques include trans-arterial chemoembolization (TACE), which involves intra-arterial delivery of chemotherapeutic agents, and is generally used clinically as a salvage or palliative technique (29).

1.2 BIOLOGY OF COLORECTAL CANCER

1.2.1 Overview

There is a wide range of clinical presentations of colorectal cancer from indolent to aggressive. This is observed clinically as the initial disease presentation, the natural progression of the disease, and response to treatment. The heterogeneity of colorectal cancer biology has important clinical implications for patients: cancer biology is important in determining the best management option for each individual patient.

1.2.2 Clinical features of cancer biology

Clinical and pathological staging of colorectal cancer (described in subsection 1.1.4) likely represents surrogate measures of cancer biology. Colorectal cancer staging is used in

order to risk-stratify patients for treatment including the use of chemotherapy or radiation before or after surgery (17). In addition to classical staging, the level of carcinoembryonic antigen (CEA) in the serum is also used as a marker of disease aggressiveness. Higher CEA level is associated with poorer prognosis (32).

In patients with CRLM, specific clinical criteria are used to measure cancer biology and to estimate which patients are most likely to benefit from surgery. Fong et al studied how various clinical parameters predicted the long-term survival of patients with CRLM after hepatectomy and used the results of these findings to create a widely-used clinical risk score to risk-stratify patients for surgery (32). The clinical risk score allots one point for each of five prognostic variables: size of the CRLM, number of CRLM, CEA level prior to surgery, time from diagnosis of the primary colorectal cancer to diagnosis of liver metastases, and node positivity of the primary colorectal cancer (32). It has been shown that a high clinical risk score (Score = 4-5) confers poor long-term survival post-hepatectomy than a low score (32, 33).

1.2.3 Histopathology features of cancer biology

Histopathology features of colorectal cancer may also confer prognosis. Pathology features included in classical pathological staging include the degree of tumour invasion of surrounding structures and adjacent organs as well as lymph node involvement (17). The degree of differentiation of tumour cells may also represent tumour biology.

Studies have also shown that the degree of antitumoural local immune response may also contribute to cancer biology and prognosis (34). Patients who have increased local immune cell infiltration (CD45R0-positive and CD3-positive lymphocytes) confer a better prognosis than those with poor lymphocyte infiltration, independent of cancer stage (34). Lymphocyte infiltration has also been shown to be associated with some molecular biology markers (MSI-H phenotype)(34).

Several studies have also described the correlation between tumour histopathology and long-term survival in patients with CRLM who have undergone hepatectomy. One study published in 2008 showed that in patients who received cytotoxic chemotherapies prior to surgery, the degree of pathological response was significantly correlated with 5- year survival rates (75% in those with complete pathological response, 56% in those with

major response defined as 1-49% residual tumour cells, and 33% in those with minor response defined as $\geq 50\%$ residual tumour cells) (35). In 2012, different types of pathologic responses and their relationship to long-term survival were evaluated (36). It was noted that tumour fibrosis was correlated with long-term survival, but tumour necrosis was not. Among patients with $\geq 40\%$ tumour fibrosis, the 5-year survival was 87% vs. 51% among patients who had $< 40\%$ tumour fibrosis (36). More recently, it has been shown that tumour fibrosis is associated with long-term survival in both patients who had received prior chemotherapy and in patients who had not (37).

1.2.4 Molecular biology features of cancer biology

As briefly discussed in subsection 1.1.2, colorectal cancer is thought to be due to various genetic mutations. These genetic mutations can be inherited in the form of highly penetrant mutations (eg. familial adenomatous polyposis or hereditary non-polyposis colorectal cancer) ($<5\%$), inherited in the form of low or moderate penetrance mutations (10-25%), or develop sporadically (70-85%) (8).

Adenoma-carcinoma sequence

Colorectal carcinoma is thought to arise from adenomatous polyps, which are dysplastic. Other types of polyps such as hamartomatous polyps and hyperplastic polyps are not dysplastic polyps and are not thought to be precancerous (38). The risk of a 1cm adenoma becoming a carcinoma in 10 years is approximately 10-15% (38). Not all adenomas will become carcinomas (38). Corollaries to this are that removing adenomatous polyps decreases the risk of colorectal cancer and syndromes that predispose to adenomatous polyps will increase the risk of colorectal cancer (38).

APC (Adenomatous Polyposis Coli) gene mutation

The APC gene is a tumour suppressor gene (38). It likely has a number of different downstream effects; however, the most highly studied is its role in binding β -catenin in the canonical Wnt signaling pathway, a pathway that is important in regulating gene transcription (38). It is thought that APC mutations may be the pivotal, rate-limiting step in initiating the adenoma-carcinoma sequence given that APC inactivation is very common and that the frequency of mutations in small adenomas is equal to that of advanced adenomas or carcinomas (38).

Germ-line mutations in APC lead to Familial Adenomatous Polyposis (FAP) or FAP variants such as attenuated FAP and Gardner's syndrome (FAP with extra-colonic features including epidermoid cyst, osteomas, dental anomalies, and desmoid tumours) (8, 38). Patients with FAP develop adenomatous polyposis, have an increased risk of colorectal cancer, and have an increased propensity for proximal colorectal cancer (8, 38). With classic FAP, the average age of diagnosis with colorectal cancer is 39 years of age, and nearly 100% of patients will develop colorectal cancer by mid-life, if untreated (8, 38). Attenuated FAP is less severe with a later age of cancer diagnosis and an overall 69% lifetime risk. (8, 38) Approximately 75% of FAP is autosomal dominant and inherited and approximately 25% are likely due to de novo germ-line mutations (8, 38).

Some rare low or moderate penetrance germ-line mutations in APC can lead to some forms of inherited colorectal cancer. This includes those with the I1307k allele seen in the Ashkenazi Jewish population, who have a 2x risk of colorectal cancer compared to the general population (8, 38).

Somatic mutations in APC can cause sporadic colorectal cancer (8, 38). The vast majority (70-80%) of patients with sporadic CRC have somatic mutations in APC (8, 38). Mutations in this patient population usually involve premature truncation of the APC protein (8, 38). A small fraction of patients with colorectal cancer do not have any APC mutation but have defects in other part of the Wnt pathway (8, 38).

Mutations in DNA mismatch repair (MMR) genes

Various mutations in MMR genes are seen in colorectal cancer. Patients with MMR gene defects have a distinct molecular, pathologic, and clinical phenotype. MMR gene mutations are associated with high frequency microsatellite instability (MSI-H), which is defined as microsatellite instability of greater than 40% (39-41). Microsatellites are repeated sequences of DNA that make up the genetic signature of individuals (39-41). Histologically, patients with MMR gene mutations show increased lymphocytic infiltration, Crohn's disease-like lymphocytic differentiation, mucinous differentiation, and medullary growth pattern (34). Clinically, these patients present with proximal colon cancers, have more rapid tumour progression from adenoma to carcinoma (3-5 years vs. 20-40 years), and have improved prognosis relative to microsatellite stable (MSS) tumours (39-41). MSI-H is seen in approximately 20% of colorectal cancers (39-41).

Germ-line mutations in MMR genes lead to hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome (8). Nearly 100% of HNPCC patients have the MSI-H phenotype (8, 38). The most common underlying mutations are in MSH2 and MLH1 (together make up 70% of HNPCC) but other mutations are implicated as well. Some de novo germ-line mutations in MMR genes may account for some cases of sporadic colorectal cancers, particularly in young patients under 35 years (38).

Defects in MMR may also be implicated in sporadic colorectal cancer (38). It is thought that only a small fraction of these patients have true mutations of MMR genes (38). Most defects are likely due to a phenotype called CIMP (CpG island hypermethylation phenotype) where hypermethylation of the MLH1 gene promotor or other gene sequences leads to decrease in gene expression of MLH1 and other MMR genes (38).

A small group of patients have low frequency microsatellite instability (MSI-L). The significance of MSI-L is unclear and this phenotype may not differ from microsatellite stable patients (38).

Other oncogenes and tumour suppressor genes in hereditary syndromes

There are several germ-line gene mutations responsible for various hereditary syndromes implicated in colorectal cancer.

Juvenile polyposis syndrome (JPS) is characterized by multiple hamartomatous polyps (38). Hamartomatous polyps have abnormal mucosa but are not dysplastic and are not thought to be precancerous (38). For example, patients with Cowden syndrome also develop hamartomatous polyps, but do not have an increased risk of colorectal cancer. It is thought that the underlying mutation in JPS may involve a tumour suppressor gene, which increases the risk of cancer (38). A subset of patients with JPS have been found to have mutations in genes involving TGF- β signaling pathway, which is important in regulating gene transcription (38).

Peutz-Jeghers syndrome (PJS) is also characterized by multiple hamartomatous polyps. However, in PJS, there is also an increase in adenomatous polyps, which increases the risk of colorectal cancer (38). Many patients have been found to have mutations in the LKB1 tumour suppressor gene, which is important in the mTOR pathway involved in cell proliferation and growth (38).

MYH-associated polyposis (MAP) syndrome is an adenomatous polyposis syndrome with autosomal recessive inheritance (38). It involves defects in MYH, which is involved in DNA repair (38). MAP syndrome is characterized by the presence of adenomatous polyps as well as hyperplastic and sessile serrated polyps (38). MAP is associated with polyposis, increased risk of colorectal cancer, and increased propensity for proximal colorectal cancer (38).

Other oncogenes and tumour suppressor genes in sporadic colorectal cancers

Many somatic mutations have been found in any single colorectal cancer. However, only a limited number of genes have been found across colorectal cancers suggesting that these may be the key to driving tumour development and growth. As part of The Cancer Genome Atlas (TCGA) project, recurrently 15 and 17 mutated genes in hypermutated and non-hypermutated cancer were identified, respectively (42). Some commonly studied mutations include KRAS, BRAF, PIK3CA, PTEN, p53, and TGF- β .

The Ras proteins are a family of G-proteins that are involved in signal transduction downstream of tyrosine kinase growth factors (eg. EGFR) that are involved in cell growth, differentiation, and survival (38). The most commonly mutated Ras protein is KRAS. KRAS somatic mutations are found in approximately 40% of colorectal cancers (38). KRAS mutations likely affect tumour growth and development but not in initiation since KRAS mutations can be found in noncancerous hyperplastic polyps and are more likely to be found in large adenomas or carcinomas than in small adenomas (38). KRAS mutation is seen in 40-50% of adenomas greater than 1 cm, but only 10% of adenomas less than 1 cm (38). Mutations of other Ras proteins are found in a small number of colorectal cancers (38). Mutations in upstream tyrosine kinase growth factors, such as epidermal growth factor (EGFR) is seen in some colorectal cancers but is rare (EGFR mutations are seen in < 5% of colorectal cancers) (38). BRAF is a protein kinase that is activated by Ras proteins (38). Mutations in BRAF is seen in 10-20% of colorectal cancer and is associated with CIMP and MSI-H phenotype (38).

Phosphatidylinositol-3,4,5-triphosphate (PIP3) are cell membrane phospholipids involved in cell growth and survival (38). PIP3 is activated by PI3Ks (38). Mutations in PIK3CA, one of the subunits of some PI3Ks, is found in 15-25% of colorectal cancers (38, 43). The PTEN protein inhibits PIP3 (38). Mutations of PTEN are found in approximately 10% of colorectal cancers (38). However, PTEN is the underlying mutation in Cowden syndrome, where there is no increased risk of colorectal cancer (38). It is thought that PTEN

mutation may have an additive effect with other mutations (eg. KRAS mutation) in carcinogenesis, but is not implicated on its own (38).

Approximately 70% of colorectal cancers have mutations in p53 (38). p53 is involved in regulating the cell growth, cell death, and angiogenesis (38). Therefore, it is thought to be involved in the transition from adenoma to carcinoma (38).

The TGF- β pathway is important in regulating gene transcription (38). Approximately 25% of colorectal cancers have mutations of the TGF- β type II receptor gene including over 90% of MSI-H cancers and approximately 15% of MSS cancers (38). In addition, mutations of proteins downstream to TGF- β , such as in the SMAD2, SMAD3, and SMAD4 genes is seen in 10-25% of colorectal cancers (38).

1.2.5 Personalized medicine and implications for therapy

Personalized medicine refers to uniquely identifying features of a patient's disease in order to direct individualized therapy. In the context of colorectal cancer, this is generally discussed in the setting of targeted chemotherapeutic agents. The nature of colorectal cancer as a heterogeneous disease combined with chemotherapeutic agents that have significant toxicities lends itself particularly well to personalized treatment.

The ability to perform personalized medicine relies on identifying biomarkers of disease. These may include pathological, molecular, or imaging features. Biomarkers can be subcategorized into diagnostic markers that are used for early detection and/or risk stratification, prognostic markers that predict the nature history of disease, and predictive markers that can predict treatment outcomes (44). In colorectal cancer, most research has been focused on using biomarkers to identify patients for targeted chemotherapy or to identify patients who are expected to have good response or resistance to chemotherapeutic agents.

Several targeted therapeutic agents are used clinically. Bevacizumab is an anti-angiogenic agent that targets vascular endothelial growth factor-A (VEGF-A). Bevacizumab improves progression-free survival when used in combination with other chemotherapeutic agents (45). It is known that some tumours have resistance to bevacizumab (45). However,

there are no well-validated patient or tumour characteristics that can identify which patients will be resistant to bevacizumab prior to therapy.

Panitumumab and cetuximab are monoclonal antibodies that target the epidermal growth factor receptor (EGFR). These agents improve efficacy when combined with other chemotherapeutic agents. It has been shown that the presence of KRAS mutation within tumours predicted lack of response to these agents (46). Although it has been shown that tumours with KRAS mutation do not respond well to treatment, even among those which are KRAS wild-type, the response rate is only ~ 30% (vs. 15% in an unselected population) (46). This highlights the need for further research on determining which additional factors may be important in predicting response. Some evidence has suggested that BRAF mutation, PI3K mutations, and PTEN mutations may also confer resistance to EGFR-targeted agents; however this is less well studied.

One of the major challenges of using the mutation status as biomarkers is that this is often determined using a single biopsy from a single tumour. However, it is known that a single tumour biopsy may underestimate mutation burden due to tumour heterogeneity. Tumour heterogeneity occurs among different tumour sites (primary and metastatic sites) and even within individual tumours (47, 48). Mutation status may also change over time (49). It is thought that tumour heterogeneity may be responsible for treatment failure and chemotherapy resistance in some patients (50, 51). However, logistically, it is impossible to obtain samples of entire tumours at multiple sites across time.

1.2.6 Limitations of current techniques

Although there have been significant developments in methods of evaluating cancer biology, current techniques remain limited. Clinical methods of evaluating tumour biology can be nonspecific and classical staging of colorectal cancer classifies all patients with CRLM as Stage IV disease, even though many patients are highly treatable and even curative.

Evaluation of pathology and molecular biology is limited for several reasons. They require pathological specimens, which may be difficult to obtain. Liver biopsy of CRLM is rarely performed due to technical difficulties in small lesions and in lesions that are in a poorly accessible location (52). There are also serious risks of needle tract seeding (52). Samples

from the primary colorectal cancer are often used as a surrogate to evaluate the molecular features of the subsequent CRLM; however, this can be inaccurate due to tumour heterogeneity and sporadic mutations (47, 50). Even when a liver biopsy is obtained, tissue sampling is an issue since different metastases are often heterogeneous (48). Studies have shown that taking multiple samples from the same tumour often identify different pathological and molecular features (47). This may be a significant limitation for using tissue samples to determine targeted therapeutic options.

A noninvasive technique (such as imaging) that can reliably predict pathological response may have potential implications for risk-stratification prior to surgery or with determining perioperative such as neoadjuvant or adjuvant chemotherapy. Few imaging methods are used clinically to determine tumour morphology, although size-based criteria, such as the Response Evaluation Criteria in Solid Tumours (RECIST), are common (53). Please see subsection 1.7 for further discussion.

1.3 IMAGING OF FOCAL LIVER LESIONS

1.3.1 Overview of liver imaging techniques

Diagnosis or staging of CRLM using imaging involves two components: detection of liver lesions and accurately characterizing lesions as CRLM. For the latter component, understanding the appearances of other focal liver lesions and how that compares to CRLM is crucial.

There are multiple modalities commonly used to image focal liver lesions. These include ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and nuclear medicine (PET and PET/CT) in the clinical setting (54). In this subsection, the most common focal liver lesions and their typical imaging appearances will be described.

1.3.2 Dual blood supply of the liver

The liver has a dual blood supply (55). A normal liver typically receives 70-80% of its blood supply from the portal vein and 20-30% of its blood supply from the hepatic arteries, although this proportion can vary in the presence of portal vein thrombosis or

diffuse liver disease (55). Branches of the portal vein and hepatic arteries are intimately associated in the portal triad. The portal vein branches drains directly into the hepatic sinusoids (55). The microvasculature of the hepatic arterioles is more complex. Some of the blood flow from the hepatic arterioles drains into the hepatic sinusoids via arteriosinus twigs (55). However, some of the blood flow from the hepatic arterioles supplies the peribiliary plexus and the vasa vasorum on the wall of the portal vein (55). There are also variable direct arterioportal anastomoses, which are sometimes seen (55).

Although the portal vein is the dominant blood supply to normal liver parenchyma, liver tumours (both primary and metastatic) are preferentially supplied by the hepatic artery (55). This phenomenon is exploited in multiphase contrast-enhanced CT and MRI. Imaging is performed at different time points (arterial phase, portovenous phase, and delayed phases) in order to exploit the dual blood supply of the liver (56).

The dual bloody supply of the liver is also the basis for intra-arterial chemotherapy such as TACE (transarterial chemoembolization) and HAI (hepatic artery infusion) chemotherapy pumps (30, 31).

1.3.3 Benign liver tumours

Simple Hepatic Cyst

The most common benign lesions in the liver are simple hepatic cysts (57). These are seen as anechoic lesions with posterior acoustic enhancement and no internal vascularity on ultrasound. On CT, cysts are seen as hypodense (water density) lesions with well-defined margins and thin walls that do not enhance on post-contrast imaging (57). On MRI, cysts appear as T1 dark, T2 bright lesions with well-defined margins and thin walls that do not enhance on post-contrast imaging (57).

Hemangioma

Cavernous hemangiomas are the most common type of benign, solid lesions in the liver (58-60). They are composed of dilated vascular channels (58). On ultrasound, the most typical appearance is that of a homogeneous hyperechoic lesion (60). Typically no flow is seen on Doppler ultrasound (60). On CT, the classic appearance is that of a hypodense lesion on noncontrast CT that demonstrates peripheral nodular enhancement with progressive centripetal filling on contrast-enhanced imaging (60). On MRI, hemangiomas are typically T1 hypointense relative to the background liver and T2 hyperintense (58).

On the contrast-enhanced MRI with extracellular contrast agents, hemangiomas classically demonstrate peripheral, nodular enhancement with progressive centripetal filling (58). Due to the tiny vascular channels, the enhancement pattern of hemangiomas on both CT and MRI generally follows that of the blood pool (58).

Focal nodular hyperplasia

Focal nodular hyperplasia, or FNH, is thought to be a congenital vascular malformation of the liver (61). They are often called “stealth” lesions on ultrasound since they have a similar echogenicity to the background liver and can be difficult to visualize (61). On Doppler ultrasound, they may demonstrate a “spoke-wheel” appearance with radiating blood vessels (61). On CT, FNH’s are often isodense relative to the background liver on the noncontrast study and demonstrate arterial enhancement that fades to the level of the background liver on delayed phases (61). On MRI, they are generally isointense on T1 and isointense or slightly hyperintense on T2 and demonstrate homogeneous arterial enhancement and fading to background liver on delayed phases with extracellular contrast agents (61). With hepatobiliary-specific contrast agents, they demonstrate prolonged enhancement on hepatobiliary phase due to the presence of hepatocytes within the lesion (61). FNH’s may have a central scar (61). On CT and MRI, the central scar may demonstrate late enhancement on delayed phase imaging with extracellular contrast agents (61).

Hepatocellular adenoma

Hepatocellular adenomas are a heterogeneous group of benign tumours that are often seen in young women with a high estrogen environment or in men or women on anabolic steroids (62). Multiple adenomas may be seen in the context of glycogen storage disease. Hepatocellular adenomas have been classified into 4 subtypes: (1) L-FABP negative, (2) inflammatory, (3) b-catenin positive, and (4) unclassified (62). The imaging appearances of adenomas are variable and include the presence of fat, calcification, or hemorrhage (62). Some imaging signs are associated with each subtype (62). For example, intratumoural fat is associated with the L-FABP negative adenomas and the atoll sign (rim of T2 hyperintensity) is associated with inflammatory adenomas (62). Although hepatocellular adenomas are benign lesions, they are important to diagnose for several reasons: (1) adenomas (especially large ones) are prone to hemorrhage and should be considered for surgical resection and (2) some adenomas (b-catenin positive subtype) can be premalignant (62).

Biliary Cystadenoma

Biliary cystadenoma is a cystic tumour arising from intrahepatic bile ducts (57). It is premalignant but may transform into its malignant counterpart, biliary cystadenocarcinoma (57). Its typical appearance is that of a multiloculated cystic mass with septations and calcifications (57). The presence of enhancing mural nodules suggests malignancy (57).

1.3.4 Malignant liver tumours

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a primary malignancy of hepatocyte origin (63, 64). They are often seen in the context of cirrhosis (any cause), although they can develop in the absence of cirrhosis, especially in patients with chronic hepatitis B (63, 64). Noncontrast ultrasound is less sensitive and specific than CT or MRI, particularly with a cirrhotic liver (64). Tumours can have variable echogenicity. On CT, the typical appearance of HCC is a hypodense mass that demonstrates arterial enhancement with washout of contrast on the portovenous or delayed phases (64). On MRI, HCCs typically demonstrate arterial phase enhancement with washout on portovenous or delayed phase with extracellular contrast agents (64). "Threshold growth" defined as $\geq 50\%$ size increase of a mass in ≤ 6 months is also considered a suspicious feature in a high risk patient (e.g. cirrhosis or chronic hepatitis B infection, or prior HCC) (65). There are other minor ancillary features that favour malignancy such as mild to moderate T2 hyperintensity, diffusion restriction, or intralesional fat (66). Appearance of HCCs on hepatobiliary phase with hepatobiliary-specific contrast agents varies (64, 67). HCCs may demonstrate diffusion restriction (64). However, the imaging appearances of HCCs may vary considerably: they may be solitary, multifocal, or diffuse and they may demonstrate a variety of features including necrosis, fat, calcification, or hemorrhage (64).

Cholangiocarcinoma

Cholangiocarcinoma is a primary malignancy of biliary origin (68). Intrahepatic cholangiocarcinoma refers to those, which arise within the intrahepatic bile ducts. Intrahepatic cholangiocarcinoma can present as a mass-forming tumour, but can also grow along bile ducts (68). Mass-forming peripheral cholangiocarcinoma is typically seen as a hyperechoic mass on ultrasound, although it may be hypoechoic in a minority of cases (68). On CT, they are hypodense and demonstrate a thick rim enhancement with

progressive enhancement on delayed phases (68). On MRI, they are generally T1 hypointense and may have mixed T2 intensity (68). On contrast-enhanced MRI with extracellular contrast agents, they typically demonstrate rim enhancement with progressive delayed enhancement (68). Other features that are seen in cholangiocarcinoma include capsular retraction (retraction of the smooth liver border near the tumour) and biliary dilatation (68).

Combined Hepatocellular-Cholangiocarcinoma

Combined hepatocellular-cholangiocarcinoma (cHCC-CC) is an extremely rare tumour that has histopathologic features of both HCC and cholangiocarcinoma (69). They are thought to have a substantially worse prognosis than HCC alone and a similar or possibly worse prognosis to cholangiocarcinoma (69, 70). They can occur in patients with or without cirrhosis (71). The imaging appearances are variable and may have features of both HCC and of cholangiocarcinoma (70, 71). Common imaging features include strong arterial (often ring) enhancement and heterogeneous late enhancement with partial washout (70, 71).

Hypovascular metastases

The liver is a common site for metastases. Metastases are often divided as “hypovascular metastases and hypervascular metastases” (72). The terms “hypovascular” and “hypervascular” are misnomers and actually refer to the absence or presence of arterial enhancement on CT or MR imaging, rather than the actual tumour vascularity or blood flow. “Hypovascular metastases” are the group of metastases that do not demonstrate substantial arterial enhancement relative to the background liver (72). These include metastases from lung cancer, colorectal cancer, pancreatic adenocarcinoma, and most breast cancers (72). Imaging appearances are variable. A typical ultrasound finding of hypovascular metastases include the “Bull’s eye” or “target” appearance where a solid mass is seen with a hypoechoic rim or halo (73). On CT, lesions are often hypodense on noncontrast imaging and may demonstrate rim enhancement on contrast-enhanced imaging (74). On MRI, typical imaging appearances include T1 hypointensity, T2 intermediate signal, and continuous rim enhancement on contrast-enhanced imaging. Contrast-enhanced MRI with hepatobiliary specific contrast agents are helpful for determining the presence and number of metastases (ie. detection) as metastases appear conspicuously as hypointense lesions on an enhancing background liver, but is not specific for characterization of lesions (67, 75). CRLMs can have imaging appearances similar to cholangiocarcinoma with one study demonstrating that 16.5% of CRLMs demonstrate

intrahepatic biliary dilatation (76). Although the study did not perform radiologic-pathologic correlation, one possibility for this is the presence of tumour fibrosis (76).

Hypervascular metastases

“Hypervascular metastases” are the group of metastases that demonstrate arterial enhancement relative to the background liver (58). These include metastases from neuroendocrine tumours, renal cell carcinoma, thyroid cancer, melanoma, and some breast cancers (58). Imaging appearances are variable, but hypervascular metastases are characterized by enhancement in arterial or early portovenous phases on contrast-enhanced CT or MRI (58). On MRI, most metastases are T1 hypointense and T2 intermediate to hyperintense (58). Melanoma may be T1 hyperintense due to the presence of melanin (58).

Lymphoma

The imaging appearance of lymphoma of the liver (primary or secondary) is variable. Lymphoma may present diffusely as hepatomegaly or as discrete hypodense, homogeneous masses (72).

1.3.5 Nontumour mimics of liver tumours

Hepatic Abscess

Liver abscesses are collections within the liver due to infections (77). Pyogenic hepatic abscesses are abscesses that arise due to bacterial infection (77). They are often seen as a “cluster” with a dominant abscess surrounding by several smaller satellite abscesses (77). Air may be seen within the abscess (77). The echogenicity of abscesses on ultrasound is variable (77). On Doppler ultrasound, there may be hypervascularity surrounding the abscess (77). On CT, the abscesses are hypodense (78). They may demonstrate rim enhancement in the surrounding liver parenchyma on contrast-enhanced CT or MRI (79).

Amebic hepatic abscesses are abscesses that arise due to parasitic infections (usually from *Entamoeba histolytica*) (80). They are often seen as a unilocular hypoechoic mass on ultrasound (80). On CT, they present as a unilocular mass with rim enhancement, possibly with surrounding edema (80). On MRI, they present as T1 hypointense, T2 hyperintense

with surrounding T2 hyperintensity representing perilesional edema (80). On post-contrast MRI, they may demonstrate rim enhancement (80).

Focal Fat

Hepatic steatosis may present regionally and simulate a mass either as focal fatty accumulation or as focal fatty sparing (81). Signs that suggest a “lesion” is actually regional distribution of fat include (1) the lesion appears as fat on all imaging modalities and imaging sequences, (2) there is no mass effect from the “mass” with vessels running through it without being displaced, and (3) typically locations of focal fatty sparing (81).

Perfusion abnormalities

Transient increase in hepatic attenuation (THAD) or intensity (THID) are perfusion abnormalities seen on arterial phase CT or MRI due to regional differences in perfusion and blood flow of the hepatic arteries and portal veins (58). These are seen as wedge-shaped areas of increased attenuation on arterial phase imaging (58). The pseudolesions are not seen on any other phase or on noncontrast imaging (58).

Regenerative and Dysplastic Nodules

Regenerative nodules are non-neoplastic hepatic nodules that arise in a cirrhotic liver (82). They are made up of largely healthy hepatic tissue (82). They occasionally will accumulate iron within the nodule and are called siderotic nodules (82). On all imaging modalities, they demonstrate imaging characteristics of normal liver (on a background of surrounding liver fibrosis/cirrhosis) (82). They do not demonstrate arterial phase enhancement (82). Siderotic regenerative nodules will be hyperdense to liver on noncontrast CT and are T2 hypointense as they contain iron (82).

Dysplastic nodules are premalignant hepatic nodules arising in a cirrhotic liver (82). Depending on the degree of dysplasia, they can be low-grade and appear similar to regenerative nodules or they can be high-grade and appear similar to hepatocellular carcinoma (82). On contrast-enhanced CT, they may demonstrate arterial enhancement, but do not wash out on portovenous or delayed phase (82). On MRI, they are often seen to contain fat (82). On contrast-enhanced MRI with extracellular contrast agents, they may demonstrate arterial enhancement without the washout seen with HCC (82).

1.4 IMAGING OF COLORECTAL LIVER METASTASES: CURRENT CLINICAL METHODS

1.4.1 Ultrasound

Conventional (noncontrast) ultrasound

On noncontrast ultrasound, the typical appearances of CRLM are hypoechoic lesions that sometimes demonstrate the “target” or “bull’s eye” appearance (56). Technical difficulties limit the detection rate of conventional ultrasound including reduced visibility depending on patient factors (eg. body habitus) or location of lesion (deep lesions) (56).

Noncontrast, conventional ultrasound is inexpensive and widely accessible; however, due to its significantly poorer sensitivity (lower detection rate), it has generally been replaced by cross-sectional imaging (CT or MRI), particularly for CRLM staging (56).

Contrast-enhanced ultrasound

Contrast-enhanced ultrasound (CE-US) is widely used for characterization of CRLM in Europe, Asia, and Canada (83). In many studies, the sensitivity and specificity has been demonstrated to be comparable to CT or MRI (83). CE-US has additional properties, which make them useful for diagnosis of focal liver lesions that is unique from other modalities. Due to the real-time nature of CE-US, the entire enhancement pattern of lesions can be observed (84). This is particularly important for flash-filling hemangiomas, whose enhancement pattern may be difficult to appreciate on contrast-enhanced CT or MRI where the timing of the arterial phase may be after the filling of the hemangioma and for “hypovascular” metastases, which often show an early hypervascular arterial phase that rapidly washes out prior to the typical arterial phase timing on CT or MRI (84-86). In addition, the microbubbles used in CE-US are intravascular and thus follow the vascular blood-pool at all times (84). This is in contrast to extracellular contrast agents with CT or MRI(84). With extracellular contrast agents, many malignant tumours demonstrate persistent contrast enhancement rather than the expected “washout” of contrast due to vascular permeability(84). This is particularly true of lesions, which have significant fibrotic content such as cholangiocarcinoma and may also be true in fibrotic CRLM (84). With CE-US, the intravascular nature of the microbubbles demonstrates washout of contrast for virtually all malignant tumours(84).

Despite the many advantages of CE-US, its technical challenges are similar to conventional, noncontrast ultrasound. Visibility can be limited by body habitus, presence of bowel gas

that may obscure the region of interest, and depth of penetration (85). In addition, only a single lesion may be imaged at a given time, which may limit evaluation in patients with multiple lesions (85). Success is also operator-dependent and may be variable depending on institution-specific expertise (85). For this reason, the use of CE-US at many centres remains limited to use as a problem-solving tool after imaging with CT or MRI.

Intraoperative ultrasound

Intraoperative ultrasound (IOUS) refers to the use of ultrasound during surgery to help detect and characterize lesions (87). IOUS, particularly when done with contrast-enhanced ultrasound techniques (CE-IOUS), improves detection of lesions in as many as 10-50% of patients (87). This leads to changes in surgical management that subsequently improves disease free survival (87). Even when MRI with hepatobiliary-specific contrast agents is used for preoperative imaging, CE-IOUS improves sensitivity from 82% to 99%, according to one recent study (88). IOUS and CE-IOUS is particularly helpful in patients who have undergone preoperative chemotherapy, where response to chemotherapy may cause lesions to “vanish” on preoperative imaging (89).

1.4.2 Computed tomography

Computed tomography (CT) with iodinated intravenous contrast is commonly used in the diagnosis and staging of CRLM (56). Contrast-enhanced CT (CE-CT) is widely available and fast. (56). CE-CT has the added advantage of being useful for detecting metastatic disease at other sites in the body (eg. lung, brain, peritoneal disease) or at evaluating the primary colorectal cancer (or any local recurrence) (56). Therefore, it is ideal for whole body staging (56).

While the sensitivity and specificity of CE-CT for diagnosing CRLM is good, particularly for large lesions, its sensitivity for detecting lesions less than 1 cm falls considerably (31-38%) (56). As a result, CE-CT can be limited particularly in the preoperative setting when accuracy of detection and characterization is crucial for surgical planning, compared to MRI (56). Although on a per scan basis, CE-CT is relatively inexpensive compared to MRI, the improved diagnostic quality of MRI makes it a relatively more cost-effective test, particularly in the pre-operative setting. According to a health economic study performed in Australia, the incremental cost-effectiveness ratio of contrast-enhanced MRI compared to CE-CT was \$40, 548 in Australian dollars (approximately \$37,827 CAD) when the costs

of surgery and quality adjusted life years was included (90). The authors concluded that the relatively minor per scan cost advantage of CE-CT over MRI (\$3311 AUD for CE-CT compared to \$3740 AUD for MRI; incremental cost difference of \$429 AUD) was offset by the superior sensitivity and need for further imaging (90). Although a similar study has not been performed in Canada, we expect that this relationship would likely hold with Canadian data. The cost of cancer imaging in Canada is a minor part of the total net costs of cancer care. A study published on the economic burden of all types of cancer care in Canada demonstrated that diagnostic tests (including imaging studies) represented only \$6.8 million dollars (0.3%) out of the total of \$2,610.4 million dollars spent on direct cancer care costs (91).

Given its many advantages, CE-CT is often used as the first-line tool for staging of metastatic colorectal cancer and diagnosis and staging of CRLM. An additional MRI is used at many institutions to improve sensitivity of diagnosing CRLM in the preoperative patient; however, this is institution-dependent.

1.4.3 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is commonly used in the diagnosis and staging of CRLM in the preoperative setting. In a meta-analysis on the use of various imaging modalities for the diagnosis of chemotherapy-naïve CRLM, it was found that MRI has the best per-lesion sensitivity, particularly for small lesions (< 10mm) (54). The value of MRI is likely greater in patients post-chemotherapy, where other modalities (such as PET-CT) are particularly limited (92).

There are a large number of MRI techniques, including noncontrast and contrast-enhanced sequences, used in the diagnosis of CRLM. Commonly used noncontrast MRI sequences used to diagnose CRLM include: T2-weighted imaging and diffusion-weighted imaging (56). Commonly used contrast-enhanced MRI techniques include MRI with extracellular contrast agents and MRI with hepatobiliary-specific contrast agents. See section 1.5 for more details on MRI techniques.

MRI is often accepted as the current best test for diagnosis and staging of CRLM. It is widely used clinically for this purpose. The use of hepatobiliary-specific contrast agents, in particular, has significantly increased the sensitivity (detection rate) of CRLM (67).

However, there remain significant limitations, including difficulties in characterizing liver lesions, particularly small lesions (< 1cm), lesions treated with chemotherapy, and tumours with significant mucinous content (75, 92, 93).

1.4.4 Nuclear Medicine

The role of Positron-Emission Tomography/Computed Tomography (PET-CT) in the staging of CRLM is controversial (56). Studies have shown that PET-CT is sensitive (78-95%) for detecting CRLM greater than 1 cm; however, its sensitivity (~36%) is low for CRLM less than 1cm due to problems with misregistration (54). In addition, metabolic activity on PET-CT decreases after treatment with chemotherapy, which limits its utility in the post-chemotherapy setting (94). In lesions with significant necrosis or mucinous content, false negatives can occur. Additionally, PET-CT is not available at many institutions and is not routinely offered to patients with CRLM. The greatest role for PET-CT may be in identifying extra-hepatic sites of disease, which can potentially alter criteria for surgical resectability (56). In 2014, a study suggested that PET-CT prior to surgery did not alter surgical management compared to CT alone, raising the question of its clinical utility in this setting (95). For these reasons, the role of PET-CT remains controversial and it is not part of the routine clinical practice at many institutions.

Whole-Body Positron-Emission Tomography / Magnetic Resonance Imaging (PET/MRI) is an emerging technique that some authors have suggested may have a future role in imaging colorectal cancer (96). The superior soft tissue contrast of MRI allows for superior performance in T-staging rectal cancer or M-staging of liver metastases compared to PET/CT or CT alone and PET may be superior to MRI alone in M-staging for extrahepatic disease (96, 97). Potential limitations include technical challenges of misregistration and attenuation correction as well as practical limitations of availability and cost (96). This relatively new technique is not well studied and the data on diagnostic performance of PET/MRI remains limited (96).

1.5 MAGNETIC RESONANCE IMAGING OF THE LIVER

1.5.1 Basics of MRI physics

Electromagnetism

An electromagnetic wave has an electric field component and a magnetic field component, which are perpendicular to each other (98). Any change in the electric field generates a magnetic field and conversely, any change in the magnetic field generates an electric field (98). A spinning charged particle creates an electromagnetic field, which can act like small bar magnets within a larger, external magnetic field (98). Such charged particle will “precess” or spin around its own axis as well as around the axis of the external magnetic field (98). In clinical MRI, these charged particles are mostly charged hydrogen nuclei within water molecules. The external magnetic field (called B_0) is typically at 1.5 Tesla or 3.0 Tesla for most clinical magnets in use today.

The protons will “precess” or spin around its own axis as well as around the axis of the external magnetic field (98). The precessional frequency of the proton is called Lamor frequency and is described as follows:

$$\omega = \gamma B_0$$

(Equation 1.1)

where ω is the Lamor frequency, γ is the gyromagnetic ratio (constant), and B_0 is the external magnetic field (99).

Magnetic susceptibility

Different substances are magnetized to different degrees within a magnetic field due to its own inherent properties. Substances are classified into three categories: diamagnetic, paramagnetic, and ferromagnetic (98). Diamagnetic substances have no unpaired orbital electrons and therefore have no intrinsic magnetic moment (98). They demonstrate a very slight negative magnetic susceptibility within an external magnetic field (98). The most commonly encountered diamagnetic substances in clinical MRI is water (98). Paramagnetic substances have unpaired orbital electrons (98). They become magnetized within an external magnetic field and demonstrate an increase in the effective magnetic field (98). Gadolinium is a rare earth element with seven unpaired electrons, making it a

strong paramagnetic substance (98). Therefore, it is commonly used for MRI contrast agents. Some inherent body tissues are paramagnetic such as hemosiderin within blood products (98). Ferromagnetic substances have a large positive magnetic susceptibility and are permanently magnetized even if the external magnetic field has been turned off (98). Iron, cobalt, and nickel are ferromagnetic substances (98). These are sometimes used within transplanted devices in the body (eg. aneurysm clips, etc.).

Detecting and generating signal in MRI

Protons in the body will tend to spin aligned with the external magnetic field (98). In clinical MRI, hydrogen protons within water line up with the external magnetic field of the MRI scanner. Receiver coils are used to detect magnetization from precessing protons oscillating at Larmor frequency (99). The coils are only sensitive to the transverse (X-Y plane) component of the magnetization (99).

A radiofrequency pulse (RF pulse) is an electromagnetic wave of a specific frequency sent into the patient by a transmitter coil during the MRI scan, which causes application of an alternating magnetic field, called the B_1 field (98). The RF pulse causes a change in the magnetic field, resulting in a change in alignment of the hydrogen protons (98). After the RF pulse, the hydrogen protons return to their original alignment, which generates an electrical signal (98). This electrical signal is measured by the receiving coil (98). The angle by which the RF pulse tips the protons away from the main magnetic field is called the “flip angle” (98). Since receiver coils only detect magnetization in the transverse plane, the RF pulse is most effect at producing signal when it is at 90 degrees to the external magnetic field (99).

1.5.2 Tissue contrast and relaxation parameters

T1 recovery time

After an RF pulse is turned off, the protons will realign with the axis of the main external magnetic field, or the z-direction (98). The time it takes to realign with the main magnetic field along the longitudinal axis to $1 - e^{-1}$ or 63% of the equilibrium longitudinal magnetization is called the T1 relaxation time (98). T1 is an inherent property of tissues (98). The differences in T1 between tissues can be exploited in order to obtain contrast between tissues in an image (98).

T2 relaxation time

After an RF pulse is turned off, the magnetization caused by the RF pulse in the x-y direction will decay with time (98). This is referred to as “relaxation” and the time it takes for the decay to $1/e \sim 37\%$ of its maximal value is called the $T2^*$.

$T2$ refers to the time-varying interactions within the magnetic field, also known as “spin-spin relaxation” and largely depends on the free diffusion or motion of water molecules within the body (98). Therefore, the larger the water content of a tissue, the longer the $T2$ decay (98). $T2'$ refers to the decay related to inhomogeneities in the magnetic field, which are fixed in space and time (98). $T2'$ inhomogeneities can be related to the external magnetic field or to inherent tissue properties. $T2$ relaxation can be isolated from $T2'$ using spin-echo imaging whereas gradient recall echo imaging depicts $T2^*$ decay (see subsection 1.5.5). Like $T1$, $T2$ decay also represents an inherent property of the tissue and can be exploited in order to obtain contrast between tissues in an image (98).

TR and TE

By exploiting the differences in $T1$ or $T2$ properties of different tissues, contrast can be obtained between tissues in an image. Changing the parameters of the MRI acquisition can alter the relative $T1$ or $T2$ weighting of an image.

The repetition time (TR) is the time between application of RF pulses (98). With a longer TR time, the $T1$ recoveries of different tissues become more similar (98). Therefore, a long TR reduces the $T1$ effect (98). Generally speaking, a short TR enhances the $T1$ contrast (making it a so-called “ $T1$ -weighted image”); however, too short of a TR can lead to little to no signal (98). Therefore, a TR close to the $T1$ of the tissue of interest is generally the best balance between signal and $T1$ contrast. (98)

The echo time (TE) is the time between the application of the RF pulse to the time of MR signal sampling (98). With a longer TE, the $T2$ relaxation is greater between different tissues. Therefore, a long TE increases the $T2$ effect (making it a so-called “ $T2$ -weighted image”) (98).

1.5.3 Data Acquisition and K-Space

Gradients and Spatial Encoding

In order to form images, it is necessary to determine the location of the signal (i.e. spatial encoding) (98). In order to solve this problem, we apply time-dependent magnetic field gradients superimposed on top of the main external magnetic field (99). These alter the resonant frequency spatially, allowing for spatial encoding. The magnitude of the gradient refers to the rate at which the magnetic field changes per unit distance (99). The direction of the gradient can be applied in the x-direction (frequency encoding), the y-direction (phase-encoding), or the z-direction (slice selection) (99).

Applying a gradient in the z-direction (slice selection) allows for selective excitation of a slab of transverse magnetization within a selected location and thickness (99). The selection gradient will cause magnetization of the slab of interest to have a particular range of Larmor frequencies (99). An RF pulse can then be applied to a matching range of frequencies, which allows for transverse magnetization to be limited to the desired slab (99).

Fourier Transformation and K-Space

A Fourier transformation is a mathematical transformation that decomposes signal into a series of frequencies (98). The Fourier space in MRI is called “k-space” (99). Each point in k-space has x and y coordinates as well as amplitude. The points near the centre of k-space contain low spatial frequency information, which provides data on image contrast and shape. The points near the periphery (or “edge”) of k-space contain high spatial frequency information, which provides data on fine resolution (98).

The area under a gradient profile (determined by the magnitude of the gradient and the duration of the gradient application) in the x-direction and in the y-direction defines the spatial frequencies within k-space, K_x and K_y , respectively (99). Therefore, it is possible to determine the spatial frequency at any point in k-space by applying appropriate x and y gradients. Data in k-space can be sampled in any order, although classic methods involve line-by-line acquisition (Cartesian method) (see subsection 1.5.5) (99).

1.5.3 Signal-to-Noise Ratio

In any image, there is a level of background noise caused by random fluctuations. The ability to determine useful information from an image is related to the ratio of useful signal within an image vs. the background noise, which is termed “signal-to-noise ratio” or SNR (100).

A number of parameters can affect the SNR. The strength of the external magnetic field increases the SNR (100). Although noise increases proportionally with B_0 , the signal increases by square of B_0 , such that the overall SNR is increased by B_0 , assuming noise from the patient dominates electronic noise (100). The signal is also proportional to pixel size (or resolution) (100). Therefore, several size parameters including field of view and slice width will affect the SNR (100). Signal averaging will increase signal without corresponding increasing noise. Therefore, the number of averages or number of excitations will improve SNR, although this has negative consequences by increasing scan time (100). Noise is proportional to the square root of the bandwidth; therefore, an increase in the bandwidth will increase the noise and decrease the SNR (100).

The SNR can also be affected by the use of phased array coils (100). Phased array coils are multichannel surface coils (100). Surface coils have the advantage of producing high SNR by limiting the spatial extent of the excitation at the region of interest (100). However, single channel surface coils are limited by reduced penetration depth and field of view (100). Multichannel surface coils contain multiple coil elements, which are designed such that each element receives uncorrelated (de-coupled) noise information (100). In this way, multichannel surface coils are able to produce images with high SNR (like single-channel small surface coils), but over a large field of view (like an equivalent larger single coil) (100).

Phased array coils also allow for the use of parallel imaging techniques. Parallel imaging is a technique in which k-space is deliberately undersampled, which decreases scan time (101). Normally, undersampling of k-space leads to spatial aliasing since different frequencies cannot be resolved (101). However, with a phased array coil, each coil element receives a different set of data based on the coil characteristics as well as the location of the coil (ie. each coil is most sensitive to the volume of tissue nearest in distance to the coil) (101). With knowledge of the coil sensitivities of the individual coil elements, the image can be reconstructed in order to resolve the different frequencies causing spatial aliasing due to undersampling (101). In essence, the spatial information provided by the coil sensitivities of individual elements can be used to partially replace the spatial information obtained through phase encoding (102). The maximum acceleration factor of parallel imaging is dependent on the number of coil elements (101).

Contrast-to-Noise Ratio

The contrast-to-noise ratio (CNR) is the difference in signal between two tissues relative to the noise (102). SNR is important in order to resolve useful information from background noise; however differences in SNR between different adjacent tissues are also required for clinical MR imaging (102). For example, a focal liver lesion with high SNR cannot be detected if the SNR is the same as the background liver. In this case, the CNR (difference in SNR between the background liver and the focal liver lesion) is required for lesion conspicuity.

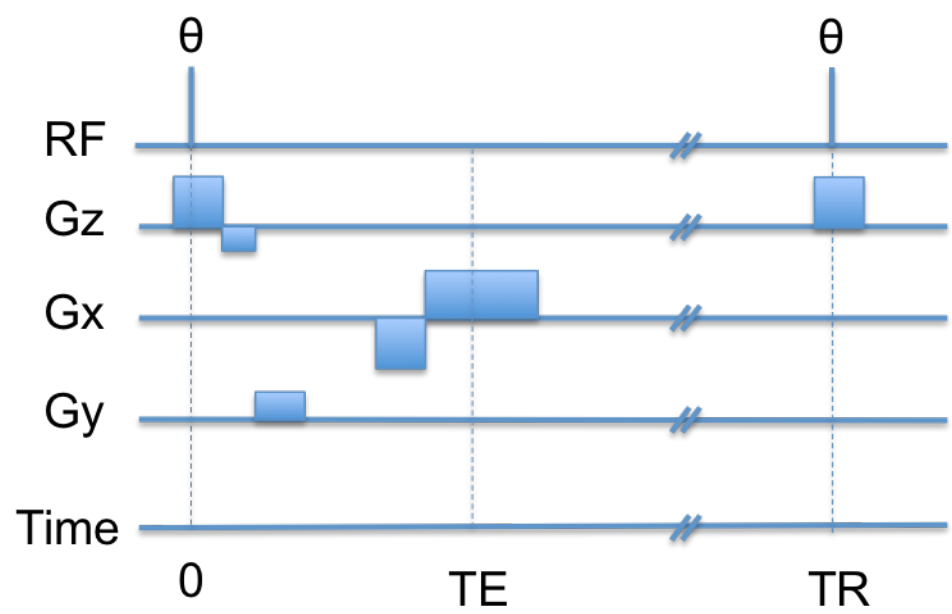
1.5.5 Pulse sequences

A pulse sequence is a series of operations (including RF pulses and gradients) that are applied during an MR study in order to obtain an image. Different pulse sequences are used in order to obtain different MR images that optimize the scan for individual clinical purposes (tissue contrast, speed, artifacts, etc.). These are depicted by pulse sequence diagrams. Some commonly used pulse sequences are described below.

Gradient echo sequences

In a gradient echo (GRE) sequence, an RF pulse is applied (98). A negative x-gradient is used to move out to the desired edge of k-space (98). A positive x-gradient is then applied in order to acquire data in k-space (98). In order to move in the y-direction, gradients in the y-direction are used to move between each line of k-space (98). The pulse sequence diagram is shown in the figure below.

Figure 1.1: Pulse sequence diagram for gradient echo



The advantage of gradient echo is that it is a relatively fast sequence (98). However, there can be dephasing of adjacent proton spins, which can lead to increased magnetic susceptibility (98). These artifacts can be a disadvantage of GRE sequences, although in some situations they are exploited in order to increase sensitivity to pathologies (98).

Chemical shift artifact is a phenomenon in GRE techniques that exploits the de-phasing of fat and water protons in order to obtain information about the fat content of tissues (100). Fat and water precess at slightly different frequencies. Immediately after application of the RF pulse, the fat and water are “in-phase” because the protons are both tipped in the transverse plane (100, 103). However, because fat and water spin at slightly different speeds, over time, the spins of fat and water will “de-phase” (100, 103). When fat and water protons are “in-phase” there will be addition of signal, but when fat and water protons are “out-of-phase” there will be cancellation of signal (100, 103). This causes a phenomenon called the “boundary effect” or “India Ink artifact” where there is a dark line surrounding abdominal organs due to the loss of signal due to adjacent water (within the organ) and fat (visceral fat) (100). The phenomenon can also be exploited in order to determine the fatty content of an organ, typically the liver, since a fatty liver will have signal loss on the “out-of-phase” image compared to the “in-phase” image (100, 104). For fat fractions < 50%, the fatty content of the liver can be estimated using the fat fraction equation as follows (105):

$$Fat\ Fraction = \frac{SI\ (in\ phase) - SI\ (out\ of\ phase)}{SI\ (in\ phase)} \times 100\%$$

(Equation 1.3)

where *SI (in phase)* is the SI on in-phase imaging and *SI (out of phase)* is the SI on out-of-phase imaging.

A “spoiled” gradient echo sequence refers to a GRE sequence that utilizes a method called “spoiling”, which eliminates the magnetization in the transverse direction (100, 106). Therefore, it eliminates the T2 and T2* weighting of an image, so that the image is more T1-weighted (100, 106). An image is naturally “spoiled” using a long TR; however, the advantage of gradient and RF spoiling methods is that a shorter TR can be used to obtain a T1-weighted gradient image (100, 106). Gradient spoiling uses a series of variable gradients whereas RF spoiling uses additional phase offsets in order to cancel out the

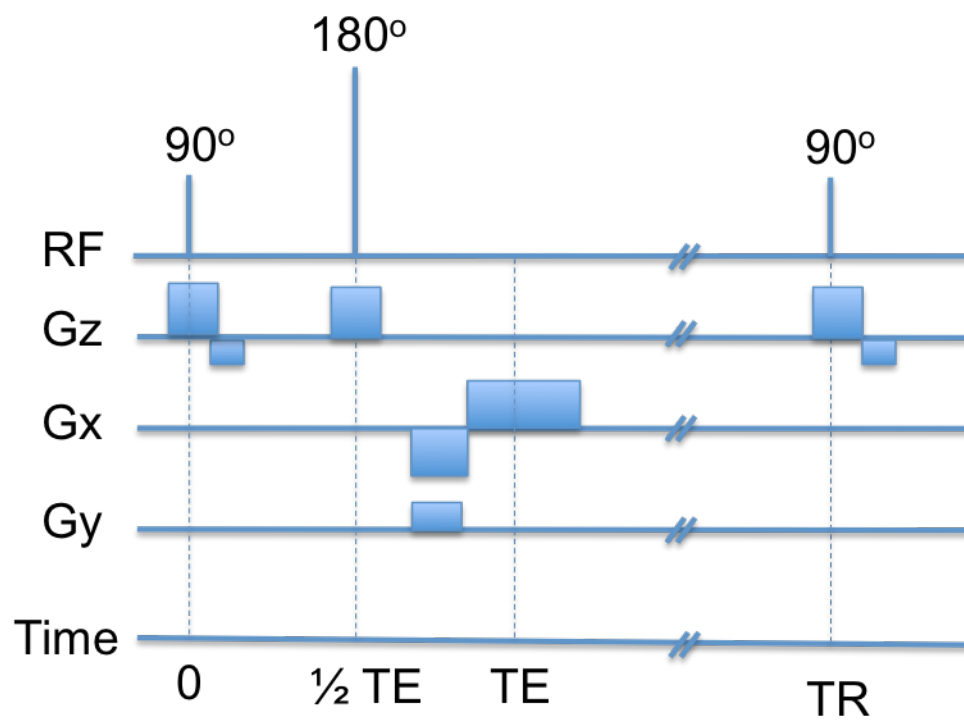
transverse magnetization vector. (100, 106, 107). Spoiled GRE sequences are particularly important in T1-weighted images that need to be acquired quickly (eg. timed contrast-enhanced images) (100, 106, 107).

Spin echo sequences

Gradient echo sequences are highly sensitive to magnetic susceptibility (98). Although this is sometimes useful as described above, this can be a disadvantage in many cases. Spin echo sequences reduce the sensitivity to magnetic susceptibility.

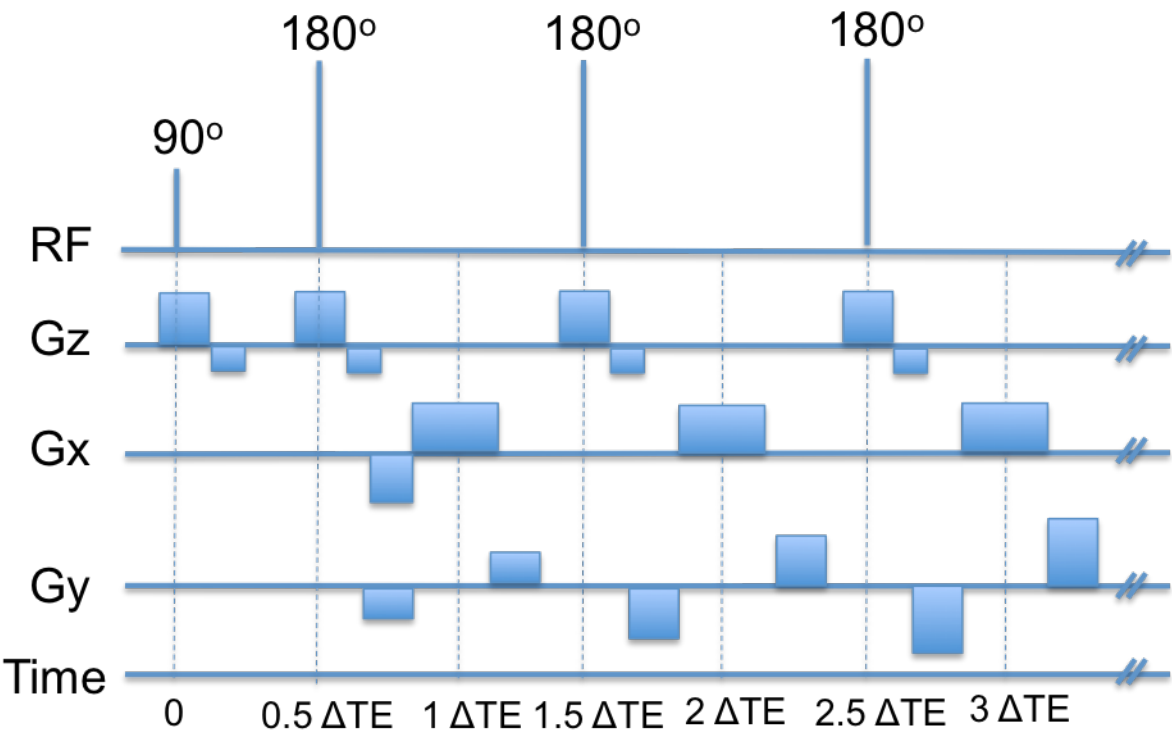
In a conventional spin echo (SE) sequence, an initial 90 degree RF pulse is applied. The spins are allowed to de-phase naturally (98). A second 180 degree refocusing pulse is then applied after a period of de-phasing (98). This corrects for inhomogeneities in the magnetic field since it reverses the phase angles (98). This allows for the acquisition of T2-weighted images with reduction of T2* effect (98). The major disadvantage of the spin echo sequences is that the scan time is longer due to the requirement to wait for the spins to de-phase (98).

Figure 1.2: Pulse sequence diagram for conventional spin echo



In order to reduce scan time, the fast spin echo (FSE) sequence has been developed (98, 108). In FSE, multiple 180 degree refocusing pulses are performed after a single 90 degree initial RF pulse (98, 108). After each refocusing pulse, a line of k-space is acquired such that multiple lines of k-space are acquired for each repetition time (98, 108). The 180 degree refocusing pulses correct for inhomogeneities in the magnetic field and the multiple pulses allows for reduction in scan time (98, 108).

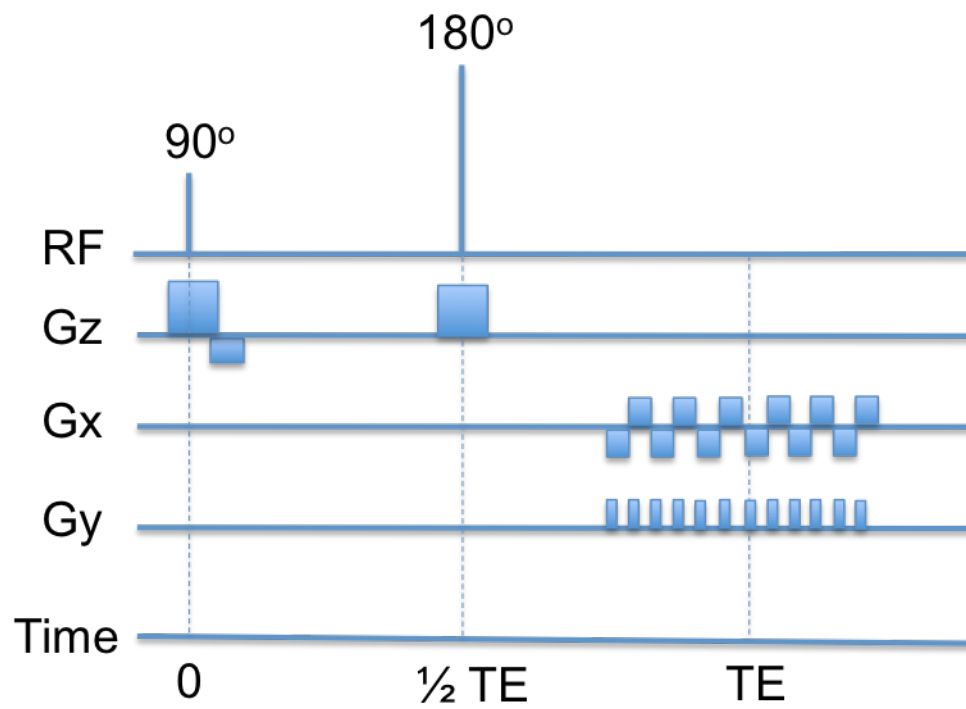
Figure 1.3: Pulse sequence diagram for fast spin echo



Echo planar imaging

Echo-planar imaging (EPI) is a very fast MR acquisition technique (98). In EPI, high performance gradients are used in order to rapidly turn on and off gradients and acquire lines of k-space in both positive and negative directions within a single RF pulse, which increases the speed of imaging relative to spin-echo or fast spin-echo sequences (98).

Figure 1.4: Pulse sequence diagram for echo planar imaging



The major advantage of EPI is speed (98). Therefore, it is most commonly used in MR imaging that is highly sensitive to motion (eg. diffusion-weighted imaging – see below). The disadvantage of EPI is that these sequences are highly sensitive to magnetic field inhomogeneities and demonstrate considerable artifacts (98). Ghosting artifacts called can occur due to eddy currents, imperfect gradients, field inhomogeneities or mismatched timing, which cause phase errors during negative and positive gradient readouts (98). These are N/2 ghosts since they are derived from half the data (98). EPI is also subject to susceptibility artifacts since it is highly sensitive to frequency and phase errors (98). Chemical shift artifacts can also occur for the same reason (98).

Diffusion-weighted imaging (DWI)

Diffusion is the process of random motion of water molecules (98, 100, 109, 110). Diffusion-weighted imaging measures the amount of free motion of water molecules within a tissue (98, 100, 109, 110). This is often used to infer differences in cellularity, permeability, viscosity, and/or perfusion between tissues, based on the extent of water diffusion. In order to do this, opposite polarity gradients are applied to a tissue with a 180 degree refocusing pulse (98, 100, 109, 110). The gradients have no effect on stationary particles but produce significant effects on moving particles (ie. tissues with significant diffusion) (98, 100, 109, 110). The amount of diffusion “weighting” can be changed by applying different strengths of the gradients and different timing between gradients. Combined, these effects on “diffusion weighting” are represented by the b-value (98, 100, 109, 110). Images at multiple b-values are obtained (typically 2-3 b-values for liver imaging) and signal intensity on these various acquisitions are used to quantify and verify diffusion weighting by calculation of the apparent diffusion coefficient (ADC) (98, 100, 109, 110).

Because the motion being measured is the tiny movement of water particles within tissues any gross movement within the body will degrade the image (98, 100, 109-111). Therefore, DWI often involves the use of EPI, although other types of sequences can be used.

Fat saturation techniques

There are several fat saturation techniques that are commonly used. Frequency-selective fat saturation (usually simply known as “fat-sat”) involves the addition of a flip angle and gradient prior to the regular sequence (112). The RF frequency is at a very narrow range of frequencies centered around the Larmor frequency of fat such that fat is selectively

excited (112). This is most commonly used for fat saturation in basic sequences for liver MRI (100). The disadvantage is that it is sensitive to field and RF inhomogeneities, which can cause incomplete fat suppression (112).

Inversion recovery is another commonly used fat saturation technique (112). It is a technique that exploits the short T1 value of fat (112). An additional 180 degree pulse is used to refocus the spin echo at the null point of fat such that the signal from fat is removed (112). Inversion recovery is advantageous in that it is less sensitive to inhomogeneities in the magnetic field (100). However, it cannot be used in post-gadolinium images since gadolinium-containing tissues often have similar relaxation times (112).

1.5.6 Contrast agents in liver MRI

Gadolinium

Commonly used contrast agents for MR imaging are gadolinium-based. Gadolinium is a highly paramagnetic substance with seven unpaired electrons (113). Chelates are compounds containing a ligand bonded to a central metal atom at 2 or more points. Gadolinium-chelates shorten T1, which causes signal enhancement on T1-weighted images (113). The different types of gadolinium-chelates have different pharmacokinetic properties; however, they all produce signal enhancement through their properties (113).

Chemical structure and properties

Gadolinium-based contrast agents (GBCAs) are gadolinium chelates used in MRI that are classified based on their chemical structure and properties.

GBCAs can be linear or macrocyclic (113). Linear GBCAs have a linear structure as the name implies whereas macrocyclic GBCAs have a cyclical structure with the central gadolinium molecule within the centre (113). Because of differences in structure, macrocyclic GBCAs tend to be more stable and it is less likely for the gadolinium to dechelate from the ligand and bind to other molecules that lead to tissue deposition (113).

GBCAs can also be nonionic or ionic. Ionic GBCAs are salified (typically with sodium or meglumine) and will dissolve into charged particles in blood (113). Ionic GBCAs are more stable and therefore have a better safety profile relative to non-ionic GBCAs (113).

GBCAs in Liver Imaging

In liver imaging, contrast-enhanced MRI is generally performed on axial, spoiled gradient-echo, T1-weighted imaging with precontrast, arterial phase, portovenous phase, and delayed phases.

Gadolinium-based contrast agents enter the liver via the hepatic artery and portal vein. The distribution and pharmacokinetics of the agents vary depending on the class of agent. Two classes of contrast agents are typically used in contrast-enhanced MRI for diagnosing and staging CRLM in clinical practice: extracellular contrast agents and hepatobiliary specific contrast agents.

Extracellular contrast agents

Extracellular contrast agents are a class of MRI contrast agents. Extracellular contrast agents circulate in the intravascular space upon intravenous injection and then distributes into the extracellular fluid compartment, as their name implies (114). Some commonly used extracellular contrast agents include: gadobutrol (*Gadavist*), or gadopentetate dimeglumine (*Magnevist*), gadodiamide (*Omniscan*), gadoteridol (*ProHance*), gadoversetamide (*Optimark*), and gadoterate meglumine (*dotarem*).

The extracellular contrast agent used in this thesis is gadobutrol, which is a macrocyclic, nonionic gadolinium-based contrast agent. It is known by the trade names Gadavist® and Gadovist® (Bayer). The chemical structure of gadobutrol is shown in Figure 1.5a.

MRI with extracellular contrast agents can help to distinguish the appearance of many different types of focal liver lesions (see section 1.2) by their differing enhancement patterns (72). With extracellular contrast agents, CRLM are often “hypovascular” relative to the background liver, meaning that they are hypointense relative to the background liver on all phases, including delayed phases (72). They may demonstrate a characteristic continuous rim that helps to distinguish them from other benign lesions (72). However, in a subgroup of lesions, they may demonstrate delayed retention of contrast, which may make them difficult to distinguish from benign hemangiomas (73).

Hepatobiliary-specific contrast agents

Hepatobiliary-specific contrast agents are a relatively newer class of MRI contrast agents that demonstrate uptake by hepatocytes and (partial) elimination via the biliary system (67, 115). Some commonly used hepatobiliary-specific contrast agents include: gadoxetic acid (*Primovist/Eovist*), managafodipir (*Teslascan*), and gadobenate dimeglumine (*MultiHance*). As a result, normal liver parenchyma (and any other tissue containing significant hepatocytes) demonstrates retention of contrast on hepatobiliary phase (67). The exact timing of the hepatobiliary phase depends on the agent used. Because hepatobiliary-specific contrast agents typically have combined extracellular and hepatobiliary-specific properties, the arterial and portovenous phases often appear similar to MRI with extracellular contrast agents (67).

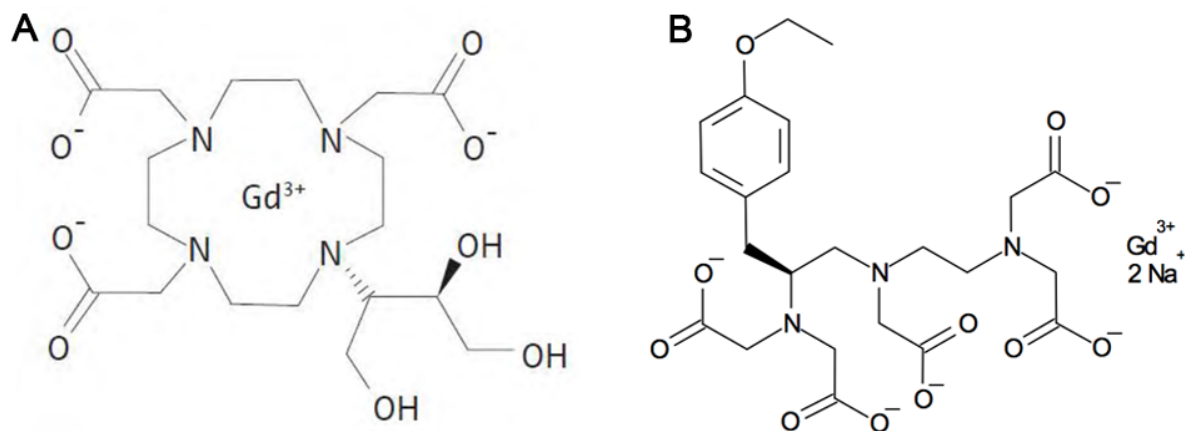
Hepatobiliary-specific contrast agents are useful in MR imaging of CRLM because on hepatobiliary phase, the background liver parenchyma is hyperintense due to contrast taken up by the hepatocytes, whereas the metastases (which do not contain hepatocytes) remain hypointense (67). This contrast allows for excellent sensitivity for detecting CRLM (67). However, since most benign lesions also do not contain hepatocytes (with the exception of focal nodular hyperplasia), benign lesions are also hypointense relative to the enhancing background liver parenchyma (75). Therefore, characterization of the lesions as benign or malignant is not improved compared to MRI extracellular contrast agents. In some cases, characterization is reduced since other helpful findings typically seen on delayed phase and portovenous phase MRI with extracellular contrast agents may be obscured.

Intravascular contrast agents

Gadofosveset trisodium (Ablavar® or Vasovist®) is a gadolinium-based intravascular (blood-pool) contrast agent that binds (reversibly) to albumin and therefore stays largely within the intravascular space. According to one in vivo study in rabbits, the intravascular concentration of gadofosveset was 61% and 41% at 1 minute and 5 minutes post contrast injection, compared to 38% and 18% at 1 minute and 5 minutes post contrast injection for gadobenate dimeglumine (116). Gadofosveset is FDA-approved for vascular imaging and

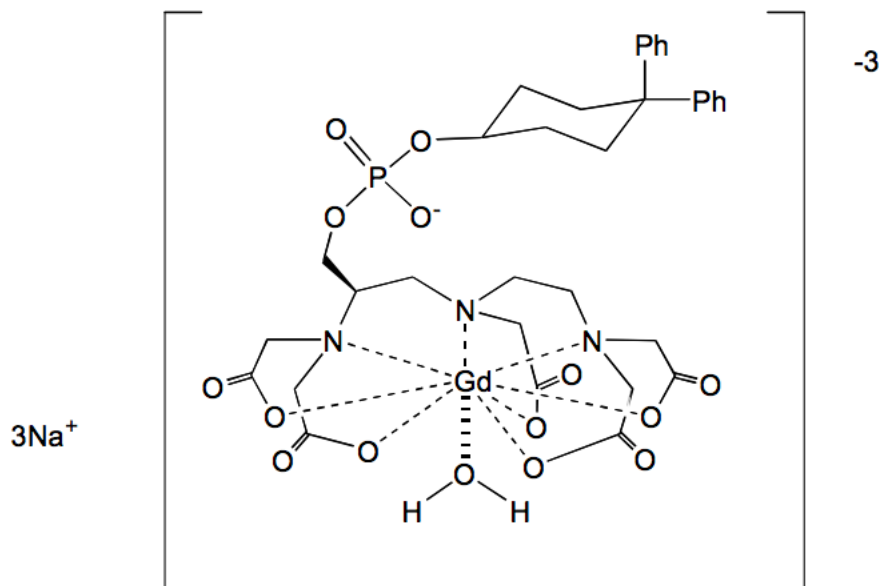
is typically used for MR angiography in patients with a contraindication to CT or CT contrast agents (117). Gadofosveset was recently discontinued in Canada in 2017 and is no longer commercially available. Gadofosveset is a linear, ionic gadolinium-based contrast agent with chemical structure shown in Figure 1.6.

Figure 1.5: Chemical structures of (a) macrocyclic, nonionic, extracellular contrast agent, gadobutrol, and (b) linear, ionic, hepatobiliary-specific contrast agent, gadoxetic acid.



Figures adapted from Product Monograph for Gadovist® 1.0 and Product Monograph for Primovist® (114, 115).

Figure 1.6: Chemical structure of gadofosveset, a linear, ionic, blood-pool, gadolinium-based MRI contrast agent



Figures adapted from Product Monograph for Ablavar® (118).

Safety of GBCAs

Immediate adverse reactions, including acute toxicity and hypersensitivity reactions, with GBCAs are uncommon and serious adverse reactions are extremely rare. According to Fraum et al (2017), in over 200 millions administrations of GBCAs, there have only been 614 case reports of severe adverse reactions, including only 54 cases of death or permanent disability (119).

Nephrogenic systemic fibrosis (NSF) is a clinical entity involving deposition of gadolinium ion in tissues in patients with end-stage renal failure (113, 119, 120). Due to the inability to eliminate gadolinium in patients with renal insufficiency, there is increased displacement of gadolinium ions from their chelates (119). Free gadolinium forms gadolinium-phosphate complexes that precipitate in tissues, are engulfed by microphages, and induce a fibrotic response (119). This results in skin thickening and fibrosis, which can cause contractures and loss of mobility (119). Fibrosis can also occur in other tissues including muscles, liver, lungs, and heart (119). Although NSF is rare, the morbidity and disability of NSF can be severe. The incidence of NSF is increased with GBCAs that have a linear structure, since they are less stable (119). Linear extracellular GBCAs, gadodiamide, gadopentetate, and gadoversetamide, account for over 99% of reported GBCA-specific NSF (119). Only five unconfounded cases have been attributed to macrocyclic extracellular GBCAs gadoteridol, gadoteridol, and gadobutrol (119). NSF may also be less common in hepatobiliary specific contrast agents due to partial hepatobiliary excretion (119). There have only been two reported unconfounded cases of NSF with hepatobiliary-specific contrast agents, gadobentate and gadoxetate (119).

Beyond NSF, gadolinium deposition has been described in multiple sites in the body, including the brain, bones, and liver, and in patients with normal renal function (119, 121). The presence of gadolinium deposition does not in itself appear to produce a definite clinical entity; however, this topic is an ongoing area of research and debate (119).

1.5.7 Typical sequences used in MR imaging of CRLM

T2-weighted imaging is helpful because it can easily distinguish T2-hyperintense cysts as well as hemangiomas (the most common solid lesion in the liver), which are generally T2 hyperintense (60). Most CRLM are intermediate signal on T2-weighted imaging. However, this finding can be inaccurate since CRLM that have been treated with chemotherapy can

be T2 hyperintense, mimicking hemangiomas (92). CRLM with mucinous content can also be T2 hyperintense, since mucin is T2 bright (93).

T1-weighted “in-phase” and “out-of-phase” sequences are helpful for determining T1 characterization as well as determining the fatty content of the liver or liver lesions that contain fat such as adenomas or hepatocellular carcinomas and distinguishing focal fat from tumour (81, 104).

Diffusion-weighted imaging (DWI) is helpful in the diagnosis of CRLM because many CRLM demonstrate restricted diffusion, whereas benign lesions do not. However, this finding can be inaccurate in CRLM that have been treated with chemotherapy, since the apparent diffusion coefficient (ADC) increases in many CRLM post-chemotherapy (122). In addition, diffusion-weighted imaging is subject to artifact and misregistration (111).

T1-weighted gadolinium-enhanced images (usually spoiled gradient echo images) are helpful for determining the enhancement characteristics of tumours (123).

1.5.8 Dynamic-contrast enhanced MRI and perfusion imaging

Dynamic-contrast enhanced MRI (DCE-MRI) involves obtaining multiple acquisitions at various time points post-contrast injection. In the liver, images are typically obtained in the precontrast, arterial, portovenous, and a variety of delayed phases. The timing of the delayed phases depends on the type of contrast agent used and institutional protocols. These are commonly used in the liver in order to obtain information regarding the differential perfusion of different tissues (e.g. tumours) due to the dual blood supply of the liver (see subsection 1.3.2)

Quantitative DCE-MRI is a quantitative technique that measures the physiologic perfusion of contrast within tissues (124-126). It involves specialized MR sequences, typically multiple, rapid and repeated T1-weighted, 3D spoiled gradient echo images performed at multiple flip angles in order to enable T1 mapping (quantification) (124). This allows for quantification of the time evolution of contrast agents within the liver tissues, which provides models of vascular behavior in the microcirculation (124). However, this requires specialized imaging that is not typically performed in clinical MRI scans of the

liver. Due to the need for multiple rapid and repeated images, quantitative DCE-MRI is also highly susceptible to respiratory motion (124).

DCE-MRI quantification can include model-free approaches versus model-based approaches (127). Model-based approaches can be challenging due to the complexity of the liver vascular anatomy and physiology. Models can include arterial input modeling (hepatic artery and portal vein), compartmental modeling (intravascular space, extravascular extracellular space, and extravascular intracellular space), and kinetic modeling (mixing within the microcirculation) (127).

This allows for quantification of various vascular and perfusion parameters. In model-free approaches, the hepatic perfusion index (ratio of hepatic arterial to total liver blood flow) is most commonly used. In model-based approaches, some of the more commonly used parameters include: K^{trans} (transfer from plasma to extravascular volume), k_{ep} (transfer from extravascular to plasma volume), and v_e (extracellular fluid volume).

In the liver, quantitative DCE-MRI has been used for assessment of systemic liver condition such as liver fibrosis or cirrhosis; for example, some studies have demonstrated that K^{trans} and v_e been decrease with increasing fibrosis (128). There are also some early studies looking at quantitative DCE-MRI to determine early response to treatment, particularly to anti-angiogenic chemotherapeutic agents (129-132).

1.6 MRI OF COLORECTAL LIVER METASTASES WITH INTRAVASCULAR CONTRAST AGENTS

1.6.1 MR intravascular contrast agents for liver imaging

Gadofosveset-enhanced MRI is not typically used for liver imaging (off-label use) and literature on its use in liver imaging is extremely limited. Two papers have currently been published describing its use in liver imaging. One paper published by our group compared the appearance of 12 patients with focal liver lesions using MRI with gadofosveset with MRI with gadobutrol (133). This study provided preliminary evidence that hemangiomas may accumulate gadofosveset on delayed-phase imaging whereas metastases do not (133). We recently published a paper (see subsection 1.6.4) describing the appearance of 11

different types of benign and malignant focal liver lesions using gadofosveset-enhanced MRI compared to gadobutrol-enhanced MRI (134).

1.6.2 Theoretical mechanisms of action of different MR contrast agents in hemangiomas vs. colorectal liver metastases

MRI with extracellular contrast agents

Hemangiomas consist of many blood channels; therefore, their imaging appearance will follow the blood-pool using contrast-enhanced MRI with extracellular contrast agents such as gadobutrol. As a result, they demonstrate peripheral nodular enhancement on arterial phase with subsequent fill-in and retention of contrast on delayed phase (Figure 1.7-1.8). Colorectal liver metastases are so called “hypovascular” metastases (56). Therefore, they show minimal enhancement of contrast due to perfusion (56). However, some (not all) metastases do show some retention of contrast on delayed phase imaging (Figure 1.9) (73). This is hypothesized to be due to leakage of contrast into the lesion via the interstitium (Figure 1.7) (73). Although this phenomenon is commonly seen in clinical practice, it is not well described in the literature and the mechanism by which this occurs has not been elucidated.

Figure 1.7: Theoretical mechanism of action of MRI enhancement with extracellular contrast agents with hemangiomas and with colorectal liver metastases in the (a) noncontrast, (b) arterial), (c) portovenous, and (d) delayed phases

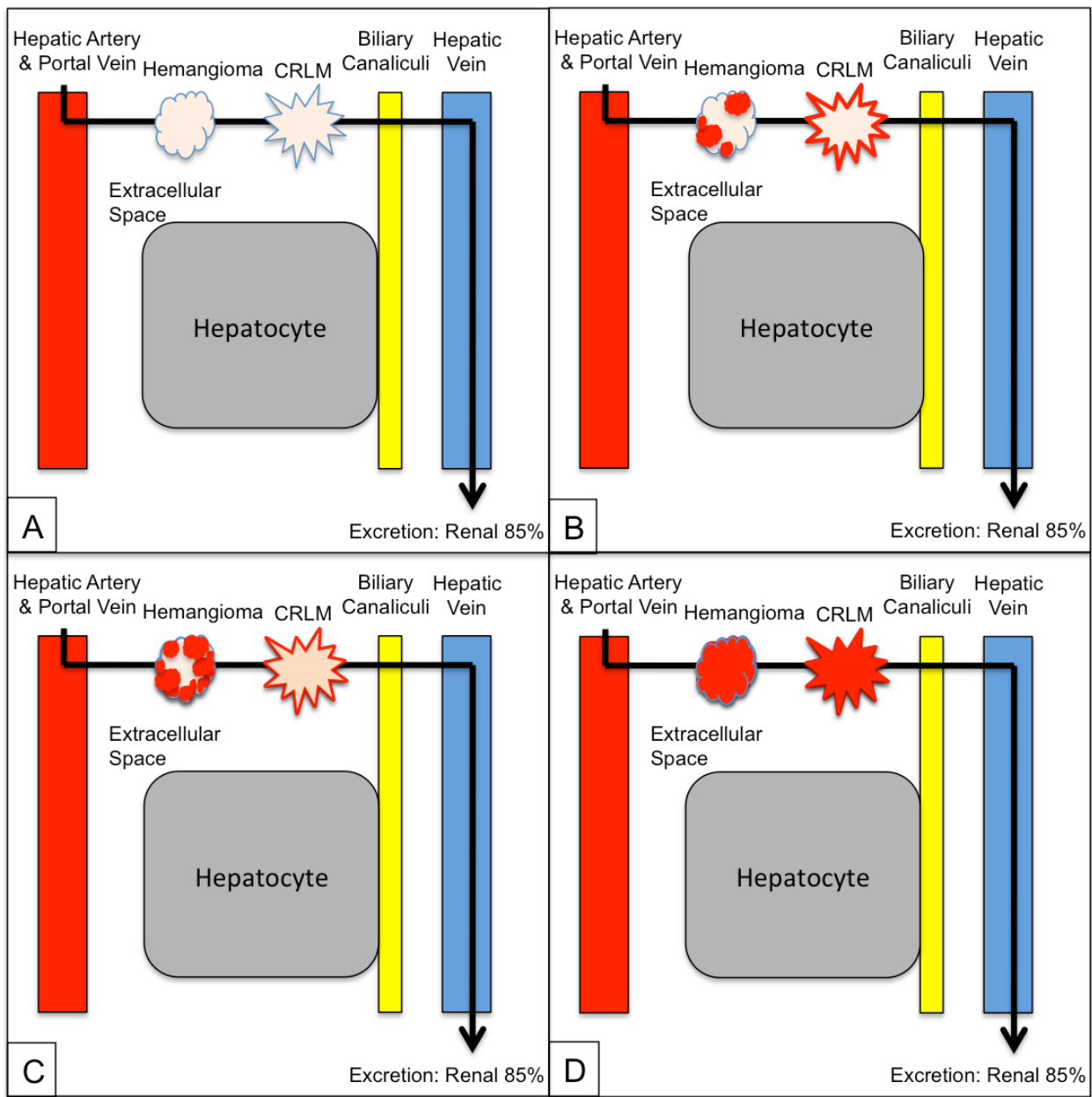
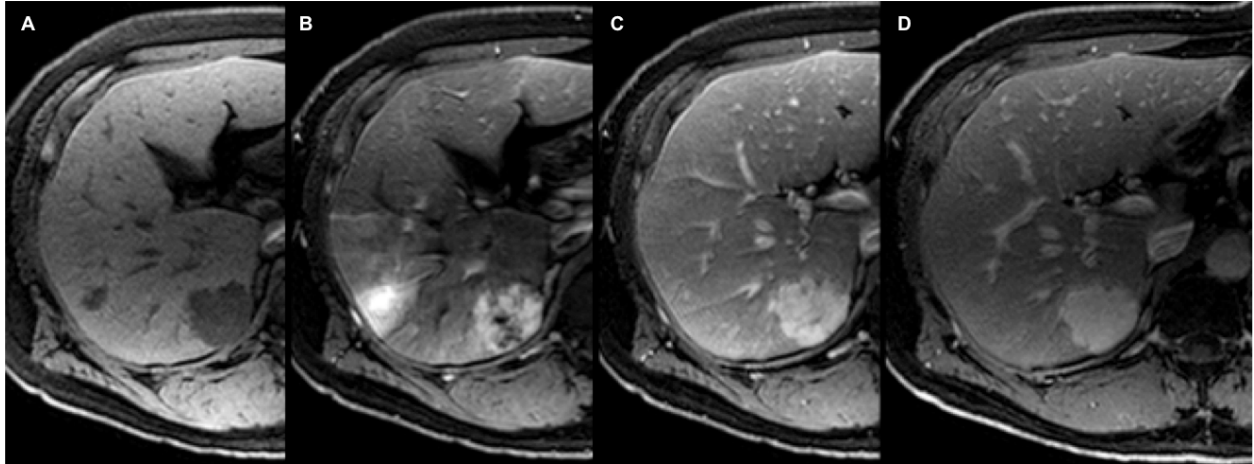
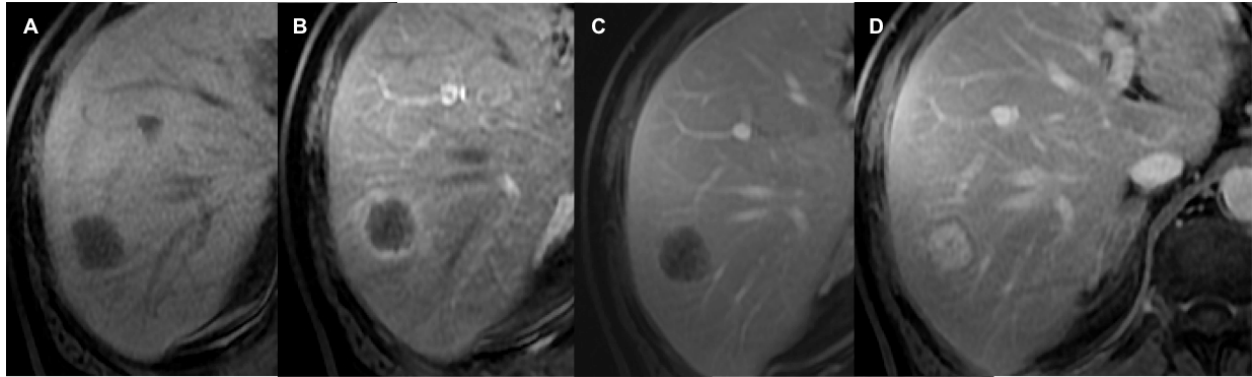


Figure 1.8: MRI appearance of a hemangioma with extracellular contrast agent, gadobutrol, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases



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Figure 1.9: MRI appearance of a colorectal liver metastasis with extracellular contrast agent, gadobutrol, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases



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MRI with hepatobiliary-specific contrast agents

Hepatobiliary-specific contrast agents demonstrate active uptake of contrast into hepatocytes (67). This is best seen on the hepatobiliary phase, which is seen at approximately 20 minutes for gadoxetate, one of the more commonly used hepatobiliary-specific contrast agents (67). Because of this active uptake into hepatocytes, the background liver is bright and all liver lesions that do not contain hepatocytes will be dark (including both hemangiomas and colorectal liver metastases) (67) (Figure 1.10-1.11). Liver lesions that do contain hepatocytes such as focal nodular hyperplasia will appear bright on hepatobiliary phase imaging (67). Because of this phenomenon, it can be difficult to distinguish hemangiomas from colorectal liver metastases on hepatobiliary (delayed) phase imaging (75) (Figure 1.11-1.12). In arterial and portovenous phases, the appearances of lesions are similar to that of extracellular contrast agents since there is an extracellular component, although hemangiomas can demonstrate early washout, which may be seen in the portovenous phase (135).

Figure 1.10: Theoretical mechanism of action of MRI enhancement with hepatobiliary-specific contrast agents with hemangiomas and with colorectal liver metastases in the (a) noncontrast, (b) arterial), (c) portovenous, and (d) delayed phases.

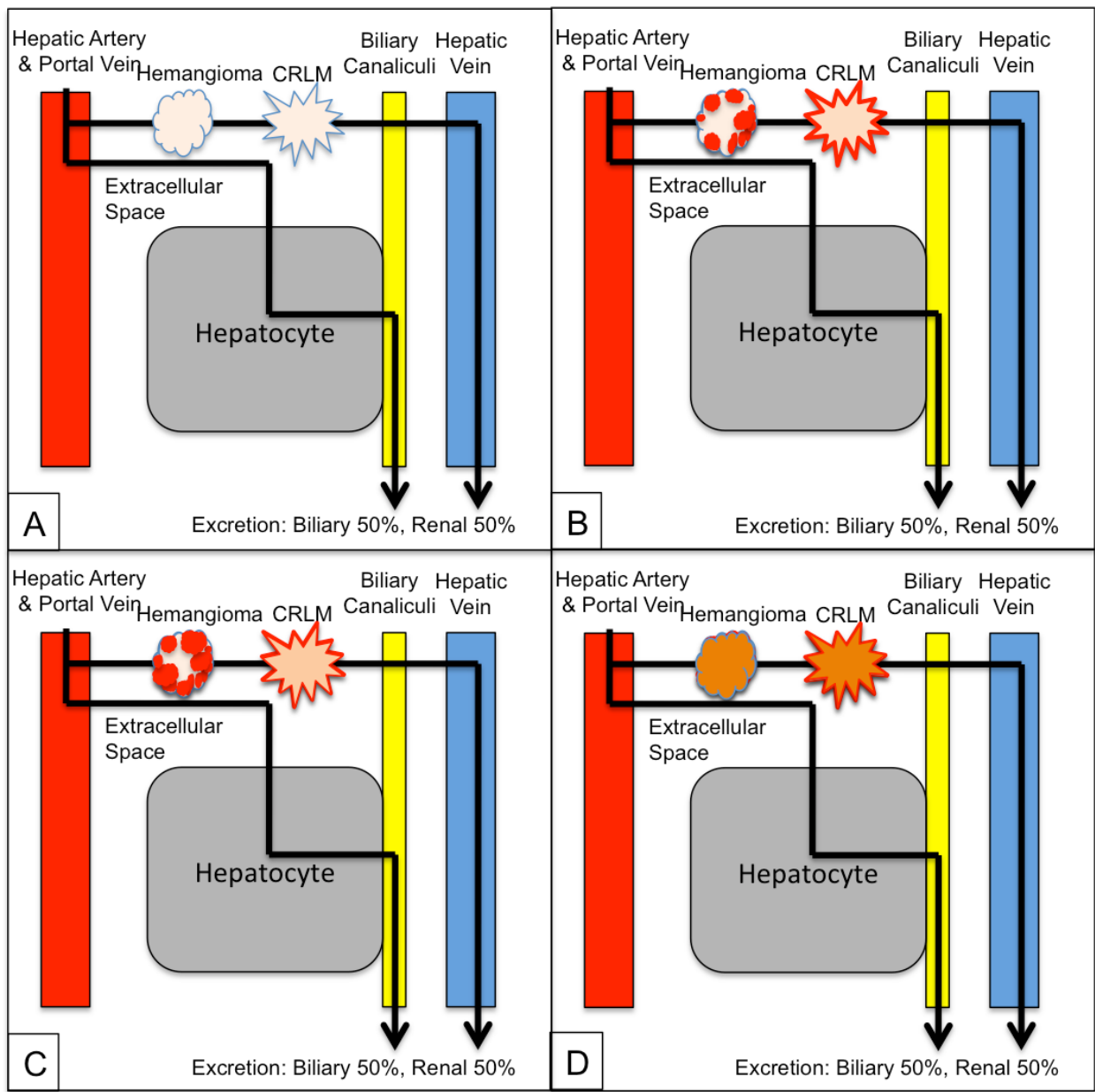
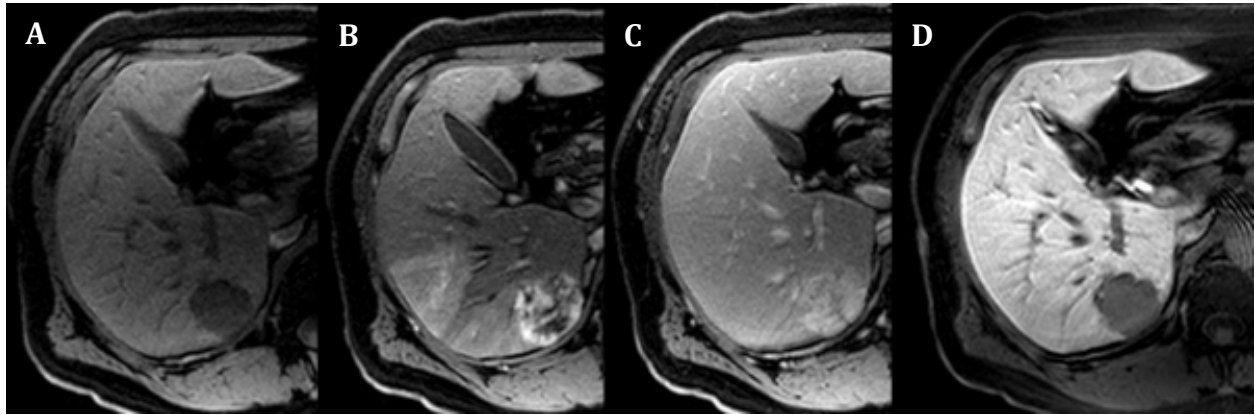
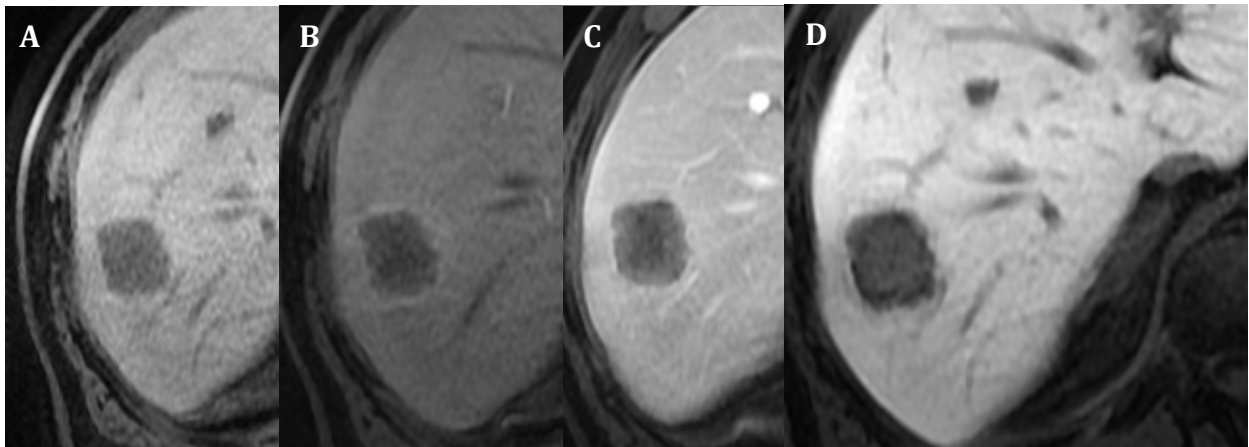


Figure 1.11: MRI appearance of a hemangioma with hepatobiliary-specific contrast agent, gadoxetate, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases.



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Figure 1.12: MRI appearance of a colorectal liver metastasis with hepatobiliary-specific contrast agent, gadoxetate, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases.



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MRI with intravascular contrast agents

Few studies have been done on the appearance of focal liver lesions using MRI with intravascular contrast agents (see subsection 1.5.6). However, in theory the appearance of hemangiomas with intravascular agents will be similar to that of extracellular contrast agents because their imaging appearance will follow the blood-pool (133, 134) (Figure 1.13-1.14).

With colorectal liver metastases, we will expect that the lesions will be hypoenhancing on arterial and portovenous phase with intravascular agents as with extracellular contrast agents because they are “hypovascular” and have less perfusion compared to the background liver (133, 134). On delayed phase imaging, it is expected that, unlike extracellular contrast agents, there will be minimal leakage of contrast into the lesion via the interstitium, since the contrast will be largely in the intravascular rather than the extravascular space (133, 134) (Figure 1.13, 1.15).

Our group has described this finding in a limited number of patients. However, no mechanistic studies have been performed to specifically assess this.

Figure 1.13: Theoretical mechanism of action of MRI enhancement with intravascular contrast agents with hemangiomas and with colorectal liver metastases in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases.

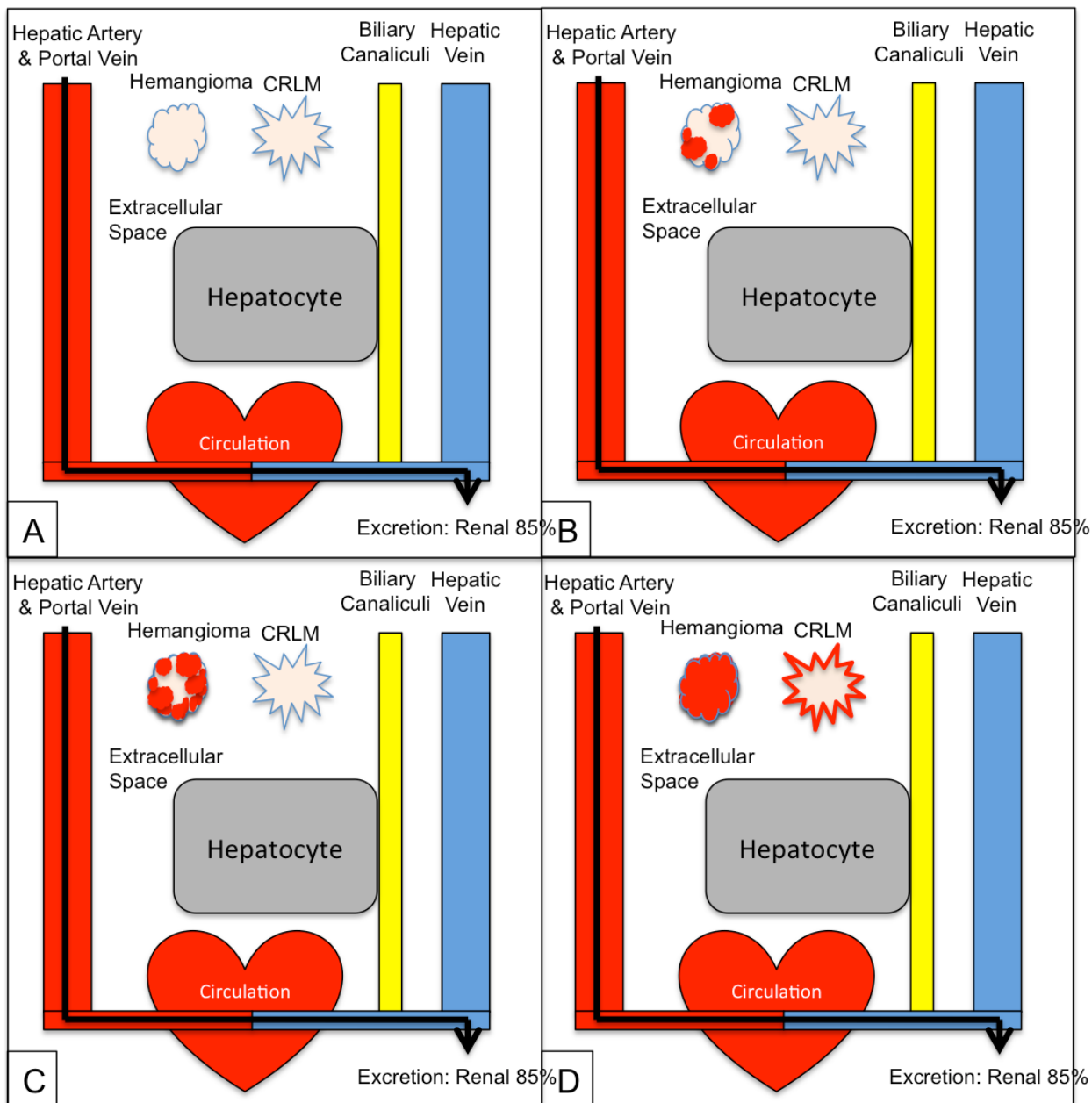
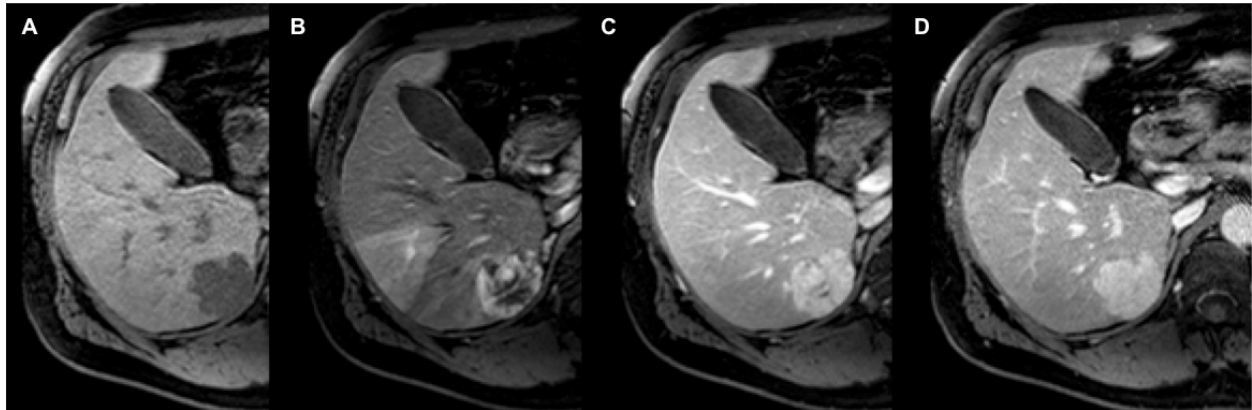
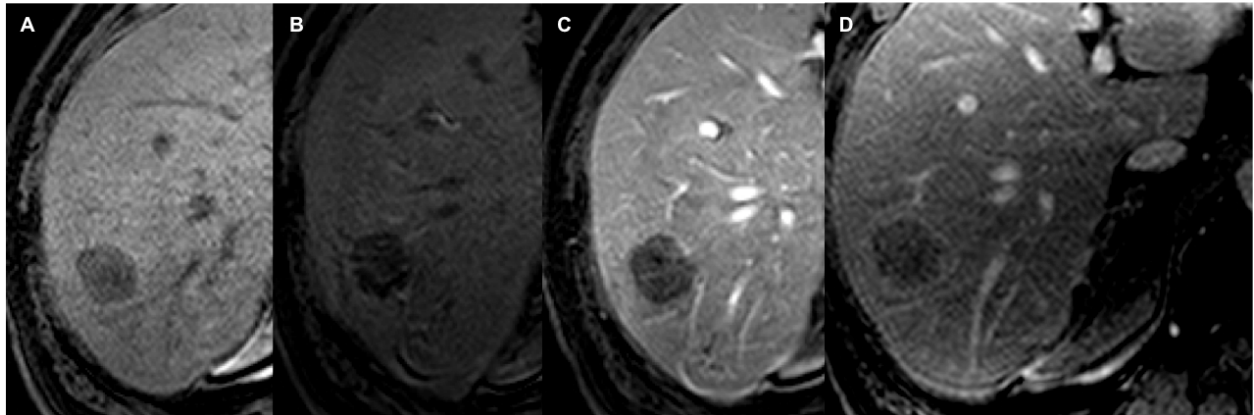


Figure 1.14: MRI appearance of a hemangioma with intravascular contrast agent, gadofosveset, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases.



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Figure 1.15: MRI appearance of a colorectal liver metastasis with intravascular contrast agent, gadofosveset, in the (a) noncontrast, (b) arterial, (c) portovenous, and (d) delayed phases.



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1.6.3 Intravascular contrast agents in contrast-enhanced ultrasound (CE-US)

Although intravascular contrast agents are not currently used for MR imaging of focal liver lesions, they are commonly used in the setting of contrast-enhanced ultrasound (CE-US) (83) (subsection 1.4.1). Presumably, the enhancement pattern of focal liver lesions on MRI with intravascular contrast agents would parallel the enhancement pattern of focal liver lesions on CE-US with intravascular contrast agents. CE-US with intravascular contrast agents is clinically used to distinguish benign from malignant lesions (73, 83, 85). Benign lesions such as hemangiomas retain contrast on delayed phase (73, 83, 85). It is well known that hypovascular hepatic metastases including CRLM typically demonstrate very brief arterial enhancement followed by rapid washout (73, 83, 85). The peak enhancement occurs earlier than the time at which arterial phase imaging on CT or MRI is performed, which is likely why CT and MRI rarely show enhancement of hypovascular metastases (73, 83, 85). Ultrasound with intravascular contrast agents show complete washout (73, 83, 85). This is in contrast to CT or MRI with extracellular contrast agents, which demonstrate leakage of contrast into the interstitium of some metastases (possibly due to fibrotic tissues) on delayed phase imaging (73, 83, 85). This feature of complete washout is used to distinguish benign from malignant lesions, particularly in the setting when lesions are indeterminate based on CT or MRI techniques (73, 83, 85). Sustained enhancement on CEUS virtually excludes hepatic metastases (73, 83, 85).

However, as discussed previously in subsection 1.4.1, ultrasound is limited because they are operator-dependent and it can be difficult to visualize certain lesions. In addition, we are only able to visualize one liver lesion at a time with CE-US and evaluation can be time-consuming, particularly if there are multiple lesions.

1.6.4 Appearance of other focal liver lesions with gadofosveset-enhanced MRI

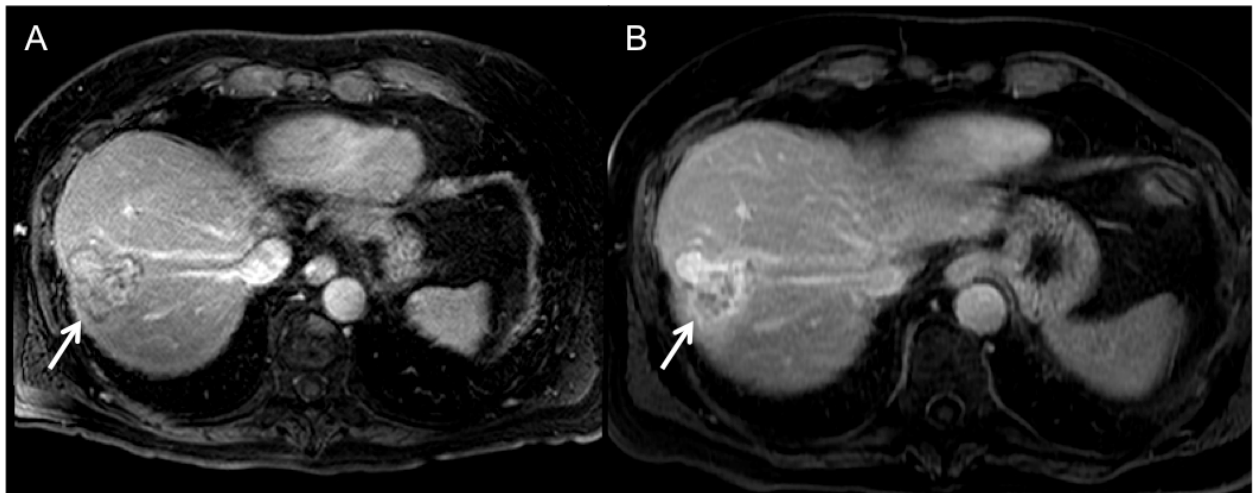
In order to use gadofosveset-enhanced MRI in the diagnosis of colorectal liver metastases, we need to know the appearance of a wide-range of commonly encountered focal liver lesions with gadofosveset-enhanced MRI. In a pictorial essay published by our group, we described the appearance of a series of focal liver lesions using gadofosveset-enhanced MRI compared to gadobutrol-enhanced MRI (134). The focal liver lesions described in the review included benign lesions (hemangioma, focal nodular hyperplasia, and adenoma) and both primary and secondary malignant lesions (hepatocellular carcinoma,

cholangiocarcinoma, metastases from colorectal carcinoma, pancreatic, adenocarcinoma, breast carcinoma, renal cell carcinoma, and neuroendocrine tumours).

The MRI appearance of the benign lesions (hemangioma, focal nodular hyperplasia, and adenoma) with gadofosveset-enhanced was similar to the appearance with extracellular contrast agents (134). The MRI appearances of hepatocellular carcinoma, pancreatic adenocarcinoma metastases, and renal cell carcinoma metastases were similar with both gadobutrol- and gadofosveset-enhanced MRI (134).

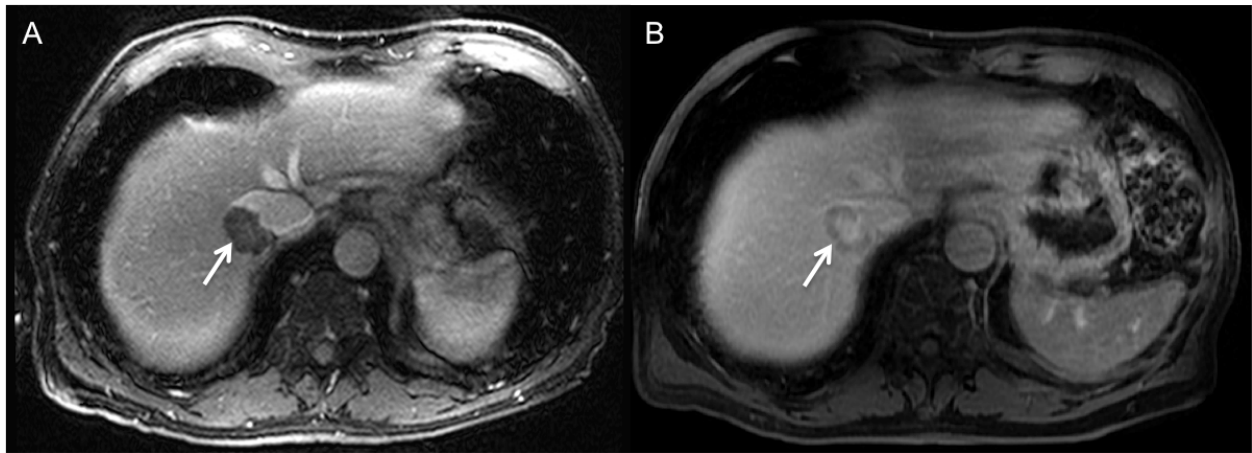
With cholangiocarcinoma, colorectal metastases, and breast metastases, the degree of delayed MRI enhancement with gadofosveset was less pronounced than with gadobutrol (Figures 1.16-1.18). Although no mechanistic studies are available, it has been suggested that the delayed enhancement seen on MRI with extracellular contrast agents in these types of tumours may reflect diffusion of contrast in regions of tumour fibrosis via the interstitium during the extravascular phase (73, 134). This effect may be less pronounced with gadofosveset due to its intravascular properties. With neuroendocrine metastases, the degree of delayed phase MRI enhancement is greater with gadofosveset than with gadobutrol (Figure 1.19) (134). Again, no mechanistic studies are available; however, it is known that many neuroendocrine tumours contain vascular channels and it would be expected that with an intravascular contrast agent such as gadofosveset, we would expect a greater degree of enhancement in tumours with rich vascular channels (134, 136).

Figure 1.16: Cholangiocarcinoma seen (a) on 10-minute delayed phase imaging using gadofosveset-enhanced MRI and (b) on 10-minute delayed phase imaging using gadobutrol-enhanced MRI. There is central enhancement on delayed phase imaging and associated capsular retraction. Note that the degree of central enhancement on delayed phase imaging appears greater with gadobutrol than with gadofosveset.



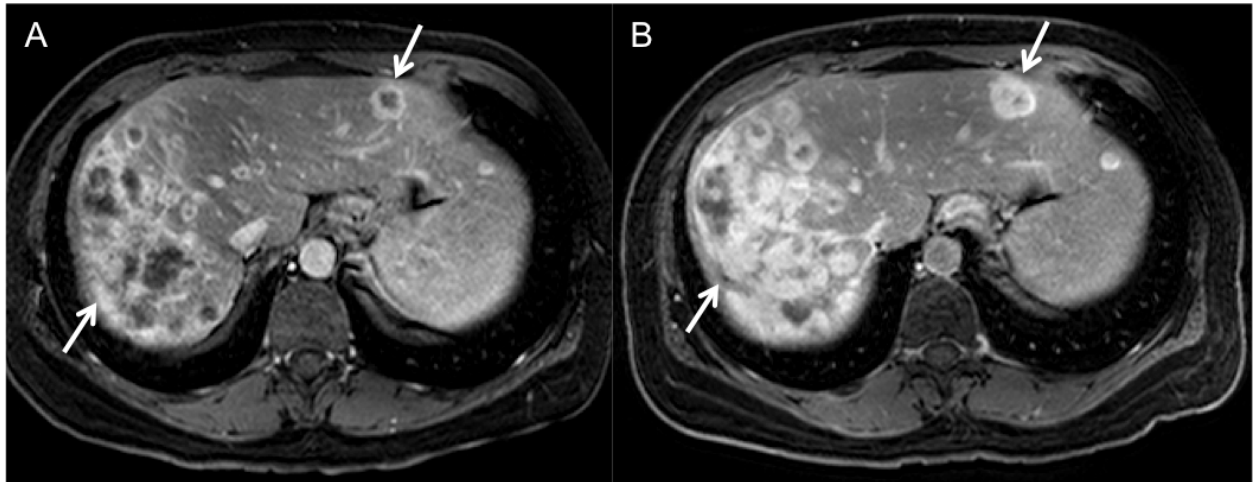
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Figure 1.17: Liver metastasis from colorectal cancer seen on (a) on 10-minute delayed phase imaging using gadofosveset-enhanced MRI and (b) on 10-minute delayed phase imaging using gadobutrol-enhanced MRI. The lesion appears hypoenhancing with gadofosveset. There is central enhancement and peripheral hypointense rim seen on delayed phase imaging with gadobutrol that is not seen with gadofosveset.



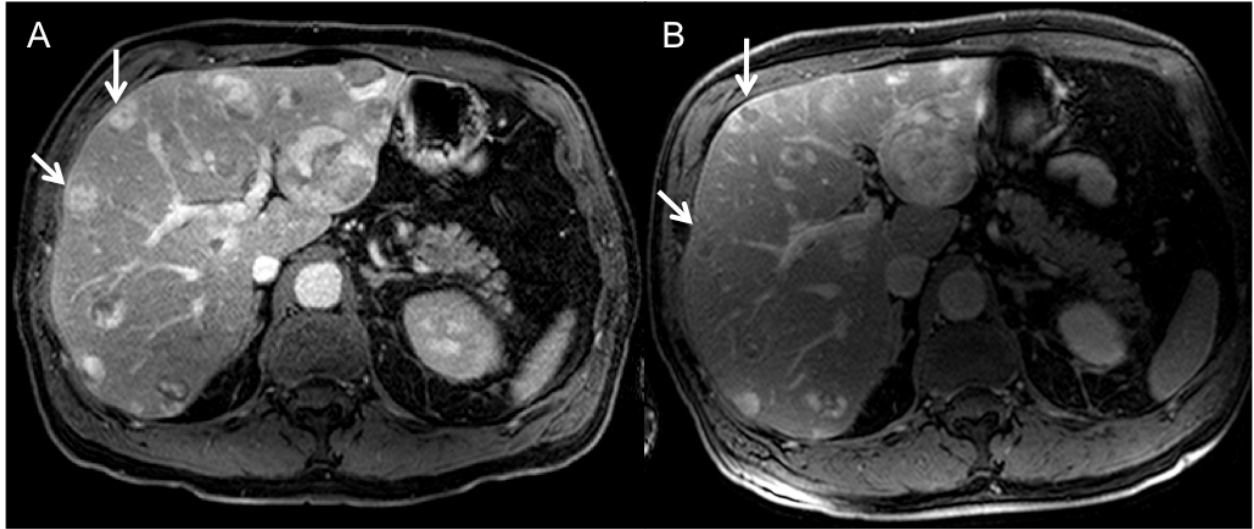
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Figure 1.18: Multiple liver metastases from breast cancer seen (a) on 10-minute delayed phase imaging using gadofosveset-enhanced MRI and (b) on 10-minute delayed phase imaging using gadobutrol-enhanced MRI. There is rim-enhancement of the metastases. Enhancement is less pronounced on delayed phase imaging with gadofosveset than with gadobutrol.



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Figure 1.19: Multiple liver metastases from a carcinoid tumour seen (a) on 10-minute delayed phase imaging using gadofosveset-enhanced MRI and (b) on 10-minute delayed phase imaging and with gadobutrol-enhanced MRI. There is persistent enhancement in delayed phase imaging. Note that there is a greater degree of enhancement on delayed phase imaging with gadofosveset than with gadobutrol.



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1.7 IMAGING OF COLORECTAL LIVER METASTASES TO ASSESS TREATMENT RESPONSE AND PROGNOSIS

1.7.1 Size-based tumour response criteria

In the 1970s to the 1990s, there was growing awareness that standardized criteria were necessary to determine response to chemotherapy, particularly in chemotherapy clinical trials. The first widely used imaging tumour response criteria was published by the World Health Organization (WHO) in 1979. The WHO criteria were size-based bi-dimensional criteria, which took the products of bi-dimensional measurements for individual tumours and summed them to obtain a baseline sum. This was then compared over time on follow-up measurements.

In 2000, the Response Evaluation Criteria in Solid Tumours (RECIST) criteria version 1.0 was published in 2000, which effectively replaced the WHO criteria (53). RECIST 1.0 was also a size-based criteria (53). The most significant changes from the WHO criteria involved use of unidimensional size-based criteria, specific guidelines on the number of lesions and the minimum size of lesions that were included, specific definitions of progressive disease, and specific criteria on how to incorporate newer CT and MRI technologies (53).

In 2009, RECIST 1.0 was updated to RECIST 1.1 (137). The major changes from RECIST 1.0 to RECIST 1.1 include decreasing the number of lesions assessed and specific criteria for pathological lymph nodes (137). The guidelines for RECIST 1.1 are summarized in Table 1.3.

Based on RECIST 1.1, all malignant lesions are identified (137). Lesions are then classified into “measurable” or “nonmeasurable” (137). Measurable lesions include any tumour $\geq 10\text{mm}$ in longest diameter on CT or MRI (minimum slice thickness = 5mm) or any lymph node $\geq 15\text{mm}$ in short axis diameter (perpendicular to longest diameter) (137). Any other lesion is considered nonmeasurable (137). Among the measurable lesions, “target” lesions are chosen up to a maximum of 5 per patient and up to 2 per organ (137). All measurable lesions not chosen as target lesions and nonmeasurable lesions are then considered “nontarget” lesions (137). Only the soft tissue components of bony lesions may be considered and cystic lesions may be considered but are not preferred as potential target lesions (137). The “sum of the longest diameters” is determined by adding up the longest

diameter of all tumours and the short axis of all lymph nodes chosen as target lesions (137). The baseline sum is followed quantitatively and non-target lesions are followed qualitatively (137). Progressive disease is defined as $\geq 20\%$ increase from smallest value of the sum of the longest diameters, unequivocal progression of existing non-target lesions, or new disease (137).

The WHO criteria and subsequently RECIST 1.0 and RECIST 1.1 represent major breakthroughs in the use of imaging to determine response to chemotherapy. Prior to the advent of these criteria, there were no standardized criteria available for this purpose. Accurately determining response to chemotherapy is crucial to both clinical practice in order to determine when to continue or discontinue treatment and to research in order to establish endpoints for clinical trials. However, size-based tumour response criteria have significant limitations. Size alone does not necessarily reflect pathological response or the actual tumour burden. One study showed that RECIST was not able to accurately predict the viable tumour burden seen on pathology (138). A tumour may not change in size (or may even increase in size), but may have less viable tumour, which has subsequently been replaced by fibrosis, necrosis, or mucin (138). Growing awareness of these limitations has led to the advent of newer, morphology-based tumour response criteria, which will be further discussed in subsection 1.7.2.

1.7.2 Morphology-based tumour response criteria

As described in the previous subsection, tumour size alone may not reflect pathological response or actual tumour burden (138). Size-based criteria may be particularly inaccurate in tumours that demonstrate “pseudoprogression” where lesions responding to treatment initially do not decrease in size and may even increase in size (eg. due to extensive necrosis) (139). This has been described in a number of different tumour types, particularly in patients on targeted or immune-based chemotherapeutic agents. Several morphology-based response criteria have been developed to address this problem.

Among patients with hepatocellular carcinoma (HCC), it was recognized that size-based criteria alone did not sufficiently predict treatment response, particularly among those patients who received molecular-targeted therapies or locoregional treatments (140). In 2000, the European Association for the Study of the Liver (EASL) developed a response guideline that included the degree of tumour necrosis (140). These guidelines were

revised and combined with the RECIST guidelines in order to develop the modified RECIST (mRECIST) criteria for HCC (140). Using the mRECIST criteria, only the enhancing components of HCC target lesions were measured (140). These criteria led to improvements in tumour response prediction in HCC and were an improvement on techniques based on size-alone.

The Choi response criteria are a CT-based tumour response criteria that was first developed in the setting of imatinib in patients with metastatic gastrointestinal stromal tumour (GIST) (141). Using the Choi criteria, the attenuation of the tumour as measured by the CT Hounsfield units (HU) is used to determine tumour in addition to size (141). Target lesions are chosen in the same manner as RECIST except that measurable lesions are defined as $\geq 15\text{mm}$ rather than $\geq 10\text{mm}$ (141). The Choi criteria were better correlated with disease-specific survival among patients with imatinib-treated GIST than RECIST (141).

The Choi criteria have subsequently been adapted for other tumour types. In the setting of patients with metastatic renal cell cancer treated with sunitinib, the Choi criteria had a better predictive value for progression-free survival and overall survival than RECIST (141). In patients with advanced soft tissue sarcoma treated with trabectedin, patients who progressed based on RECIST but not based on Choi criteria had an improved survival compared to patients who progressed on Choi criteria alone (142). In melanoma treated with ipilimumab and bevacizumab, early response based on Choi criteria was seen, but this was not shown to correlate with progression-free survival or overall survival (143).

Several criteria for immune-based chemotherapeutic agents have been explored. The immune-related response criteria (irRC) and the related immune-related RECIST (irRECIST) criteria have been developed to account for the phenomenon of pseudoprogression followed by delayed shrinkage or decreasing size in existing target lesions in the presence of new lesions that is often seen in patients on immunotherapeutic agents (144). Preliminary evidence suggests that they may be more predictive of treatment response than RECIST.

1.7.3 Morphology-based imaging criteria for colorectal liver metastases

In the setting of CRLM, morphological criteria have been used either for tumour-response to chemotherapy as well as for predicting long-term survival.

The mRECIST criteria, described in subsection 1.7.2, have been adapted for CRLM. However, mRECIST was not predictive of viable tumour burden in this setting (138). The Choi criteria described in subsection 1.7.2, has also been adapted for CRLM and was shown to be a better predictor of time to tumour progression than RECIST alone (145).

One study published by Chun, Vauthey, et al in JAMA developed CT morphological criteria in the setting of CRLM treated with bevacizumab (146). The CT criteria were based on three morphological characteristics: heterogeneity of attenuation, tumour-lesion interface, and peripheral rim enhancement (146). They found that the morphological criteria were associated with pathologic response and overall survival (146).

Several MRI-based techniques have also been developed to assess tumour morphology in CRLM. Several studies have related the appearance of tumours on diffusion-weighted imaging (DWI) to chemotherapy response; however, this has not been shown to be related to long-term survival (122). Quantitative dynamic contrast-enhanced MRI (DCE-MRI) has been shown to be a marker of early response to bevacizumab, but the role of quantitative DCE-MRI is limited, as specialized sequences need to be acquired (129, 132).

Several MRI-based techniques have been developed to assess tumour morphology in CRLM. One study correlated high-resolution pre-contrast T1 and T2 appearances of CRLM on MRI to pathological findings (147). This study showed that intraacinar necrosis was T1 hyperintense and T2 hypointense and fibrosis was T1 hypointense and T2 hyperintense (147). This was a small study performed on 6 patients with 9 freshly resected ex-vivo CRLM specimens (147). No contrast-enhanced imaging study was performed and this study did not look at long-term survival (147).

Quantitative dynamic contrast-enhanced MRI (DCE-MRI) can be a marker of early response to bevacizumab, an antiangiogenic chemotherapeutic agent (129, 132). However, the role of quantitative DCE-MRI in other situations has not been evaluated. However, quantitative DCE-MRI may be limited in clinical practice in that it requires specialized sequences not routinely used in preoperative MRI.

PET and PET-CT have also been evaluated in some studies to assess for tumour response in the setting of CRLM (148). Metabolic activity on PET or PET-CT appears to be correlated with pathological response and long-term outcomes (148-152). However, currently PET-CT is limited due to technical limitations: mis-registration limits utility of PET and PET-CT for small lesions (under 1 cm) (148). PET-MRI may combine the advantages of tissue contrast with MRI and the metabolic information of PET; however, it is a relatively new technique and data on its potential uses remains limited (96).

1.8 FRAMEWORK FOR IMAGING BIOMARKERS

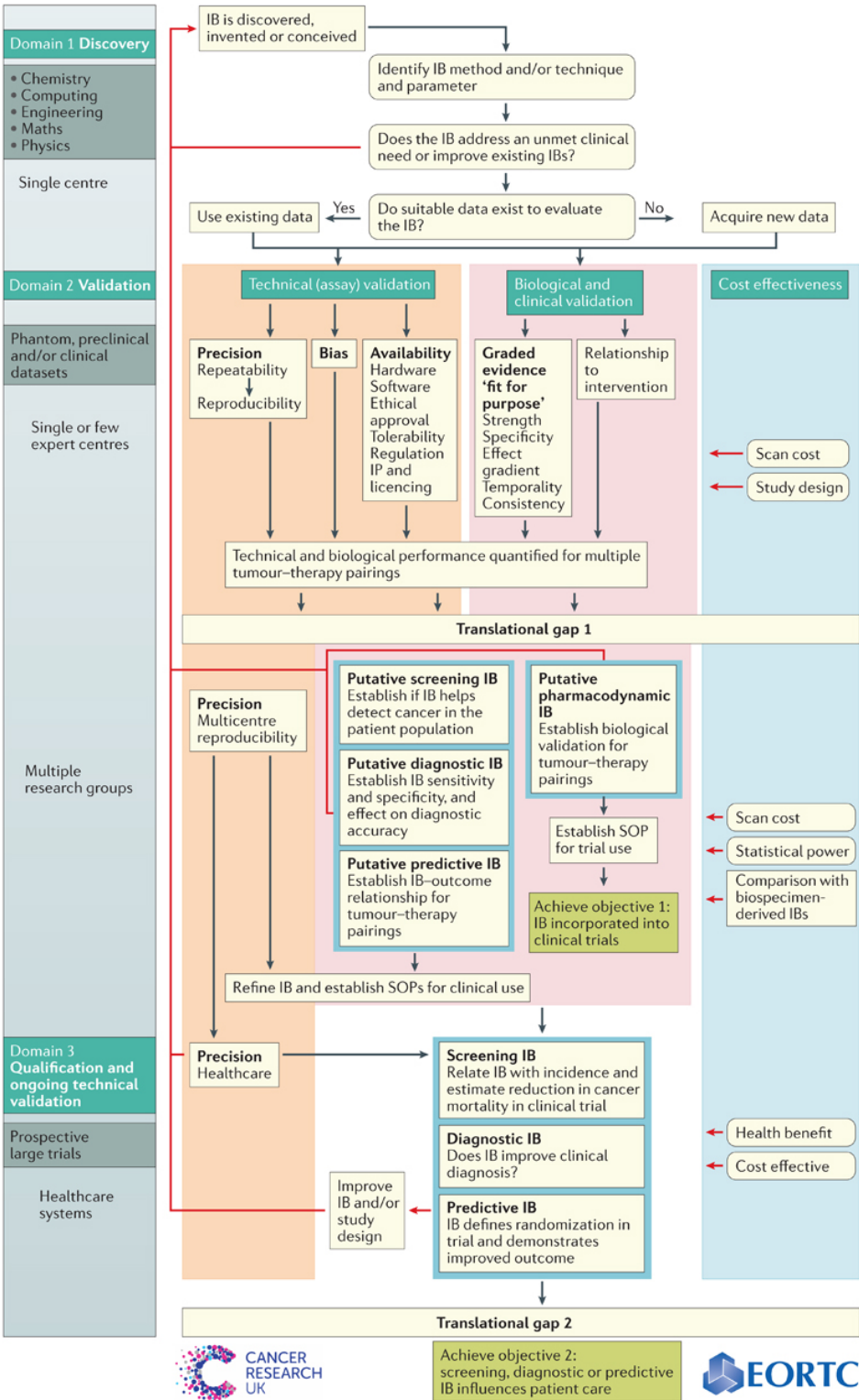
A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of a normal biologic process, pathogenic process, or pharmacologic response to a therapeutic intervention” (153). An imaging biomarker is type of biomarker that is based on an imaging test and can be quantitative (parametric mapping), semi-quantitative or qualitative (153). Biomarkers can be classified into prognostic, predictive, response, or monitoring biomarkers (153). A prognostic biomarker forecasts a disease course in the absence of treatment (154). A predictive biomarker forecasts whether a treatment will be beneficial or not (154). A response biomarker is a biomarker, which changes after treatment and this change determines whether treatment will lead to a beneficial outcome (153). A monitoring biomarker is used to detect relapse or emergence of toxicity (153).

There are multiple frameworks that have been proposed in the process of development and validation of imaging biomarkers (153-156). Although the details of these frameworks differ somewhat, they generally involve 3 components: technical validation, biological and clinical validation, and feasibility for utilization (155). The technical validation component requires a demonstration of accuracy, precision, and feasibility of biomarker measurement (154). It involves determining the limits of detection, limits of quantification, and establishing reference normal values. It also requires assessment of repeatability and reproducibility and determination of technical performance and measurement error (154). The qualification component involves determining whether the biomarker is associated with a clinical endpoint and the ability of the biomarker to serve as a measurable indicator of biological process, pathologic process, or response to an intervention (154). The utilization component requires an assessment of biomarker

performance in the specific context of proposed use, including its usefulness in clinical decision-making in a real-world context, practical issues around biomarker incorporation into routine workflows, and cost-effectiveness (154).

Each of these components forms a parallel track that progresses through 3 domains of biomarker development: discovery, validation, and qualification and ongoing technical validation (155). The discovery domain is typically performed at a single centre and involves discovery or invention of the biomarker (155). Specifically, at this stage, the imaging biomarker is identified and the technique is defined, the clinical need for the biomarker is identified, and existing data is used to evaluate the biomarker(155). In the validation domain, initial work is typically performed either at a single institution or at a few expert centres and subsequent work is performed at multiple site settings (155). Technical validation includes determining repeatability and reproducibility of results, determination of sources of bias, and establishment of technical parameters including hardware and software requirements, licensing, and patient tolerability as first steps and then multicentre reproducibility and refining of standardized operating practices in subsequent steps (155). Initial studies on biological and clinical validation include determining the relationship of the biomarker to the biological and clinical parameters (155). Subsequent studies include determining relationships in a multi-site setting and establishing standardization guidelines and incorporation into clinical trials (155). Utilization includes determining scan cost (155). Finally, in the qualification domain, large prospective studies are performed at the healthcare system level and the focus is on ongoing improvement of the imaging biomarker, evaluation of the clinical outcomes at the system level and determination of health benefit and cost-effectiveness (155). This is summarized in Figure 1.20.

Figure 1.20: Framework for development of imaging biomarker for cancer studies.



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1.9 SUMMARY OF LITERATURE REVIEW

Colorectal liver metastases (CRLM) are common and have significant human and economic costs. In recent years, there have been major breakthroughs in management of CRLM including surgical and non-surgical techniques. However, use of these techniques requires early, accurate diagnosis and staging, which remains limited.

Contrast-enhanced MRI is generally accepted to be the best test for diagnosis and staging. However, there remain significant limitations, particularly in diagnosing small lesions, although diagnosis of these lesions is crucial for surgical management. Distinguishing benign hemangiomas from colorectal liver metastases based on delayed phase imaging is challenging as retention of contrast seen with colorectal liver metastases on delayed phase imaging with extracellular contrast agents can mimic hemangiomas. However, the prevalence of colorectal liver metastases with delayed retention of contrast is not well known. Small preliminary studies suggest that contrast-enhanced MRI with gadofosveset, an intravascular contrast agent currently used for vascular imaging but not for liver imaging, may not demonstrate this pitfall, but literature remains extremely limited with no studies in this specific population and no studies looking at diagnostic accuracy.

The mechanism by which delayed enhancement with gadobutrol occurs is also not well understood. Fibrosis is seen in other disease processes as delayed enhancement. Fibrosis within tumours is a known prognostic marker in CRLM. If delayed enhancement does in fact represent the tumour fibrosis, then delayed enhancement may represent an imaging biomarker of prognosis.

CHAPTER 2 : HYPOTHESES AND AIMS

2.1 HYPOTHESES AND AIMS

2.1.1 Hypothesis and Aim 1

Conventional literature states that colorectal liver metastases (CRLM) are hypoenhancing relative to the background liver on delayed phase MR imaging with extracellular contrast agents. This is helpful in distinguishing malignant CRLM from hemangiomas, which are the most common solid, benign lesions in the liver. However, we have observed from clinical practice that there are some CRLMs that demonstrate late gadolinium hyperintensity (LGH) on 10-minute delayed phase MRI with extracellular contrast agents, although this has rarely been described and never previously systematically studied. A “good” diagnostic test typically has a sensitivity of at least 80% and preferably greater than 90% (157). If LGH is to be a useful sign in excluding malignancy, then the prevalence of LGH in CRLMs should be less than 20% (or sensitivity of the sign is >80%).

We hypothesize that the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is greater than 20%.

We aim to determine the prevalence of LGH in CRLMs on 10-minute delayed phase gadobutrol-enhanced MRI in a retrospective cohort of patients with pathology-confirmed CRLM who received preoperative gadobutrol-enhanced MRI prior to hepatectomy.

2.1.2 Hypothesis and Aim 2

Based on the first research aim, we expect to demonstrate that a subset of CRLMs demonstrates LGH and a subset of CRLMs do not demonstrate LGH. This leads us to speculate on the underlying histological or biological reasons for these differences. Tumour fibrosis within CRLMs is a common pathological finding and this has previously been shown to be associated with good prognosis (survival) post-hepatectomy (36). In other disease processes, fibrosis is associated with delayed enhancement on MRI with

extracellular contrast agents (68). This is thought to be due to leakage of contrast from the extracellular space into the regions of fibrosis via the interstitium.

We hypothesize that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is significantly correlated with the percentage of tumour fibrosis on post-hepatectomy specimens. We additionally hypothesize that LGH on 10-minute delayed phase MRI is significantly associated with overall survival, after adjusting for known confounders.

We aim to determine the association between LGH of CRLM on 10-minute delayed phase gadobutrol-enhanced MRI and post-hepatectomy tumour fibrosis. We will determine this association in a retrospective cohort of patients with pathology-confirmed CRLM who received gadobutrol-enhanced MRI after chemotherapy and prior to hepatectomy. In addition, we aim to determine the association between LGH of CRLM on 10-minute delayed phase gadobutrol-enhanced MRI in this cohort with overall survival post-hepatectomy.

2.1.3 Hypothesis and Aim 3

From hypothesis 1, we expect to show that some CRLMs demonstrate LGH on MRI with extracellular contrast agents. From hypothesis 2, we expect to show that LGH of CRLM on gadobutrol-enhanced MRI is correlated to tumour fibrosis since contrast will leak into fibrotic tumours from the extracellular space. We would expect that the leakage of contrast would not occur with an intravascular contrast agent, such as gadofosveset. As a result, we would expect that fewer CRLMs demonstrate LGH. We would also expect that the diagnostic accuracy of MRI with intravascular contrast agents for characterization of CRLM would be excellent (previously described in the literature as a sensitivity > 90%, specificity > 90%, LR+ > 10, and LR- < 0.1) (157).

We hypothesize that the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with intravascular contrast agents is less than 20% and significantly less than the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents. We additionally hypothesize that the diagnostic accuracy of reader interpretation of MRI with intravascular contrast agents is excellent (sensitivity > 90%, specificity > 90%, LR+ > 10, and LR- < 0.1) (157). We additionally hypothesize that there is significant added

value of delayed phase imaging on MRI with intravascular contrast agents in reader interpretation.

We aim to determine the prevalence of LGH in CRLM on gadofosveset-enhanced MRI in a prospective cohort of patients with known colorectal cancer and a suspected focal liver lesion who were referred for a clinical liver MRI for diagnosis and/or staging. In addition, we aim to determine the diagnostic accuracy of a trained radiologist using gadofosveset-enhanced MRI in the diagnosis of CRLM in this cohort and to determine the added value of delayed phase imaging (5-minutes and 10-minutes post contrast injection) on MRI with gadofosveset-enhanced MRI.

CHAPTER 3 : PREVALENCE OF LATE GADOLINIUM HYPERINTENSITY (LGH) OF COLORECTAL LIVER METASTASES ON MRI WITH EXTRACELLULAR CONTRAST AGENTS

3.1 ABSTRACT

3.1.1 Introduction

Hypointensity on delayed phase MRI with extracellular contrast agents is an imaging sign often used to diagnose colorectal liver metastases (CRLM). This sign is often used to distinguish CRLM from solid benign lesions, such as hemangiomas. However, based on our clinical observations, we believe that some CRLM may demonstrate late gadolinium hyperintensity and that this sign in isolation has poor sensitivity (< 80%) contrary to some sources in the literature. Therefore, we hypothesize that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is greater than 20%.

3.1.2 Methods

A retrospective study on patients with resected, pathologically confirmed CRLMs who had a preoperative gadobutrol-enhanced MRI at our institution between January 1, 2006 and December 31, 2012 was performed. The prevalence of LGH in CRLMs was determined using qualitative visual analysis, weighted by patient to account for the effect of clustering. Semi-quantitative measurements of lesion-liver contrast-to-noise ratios (CNRs) were also performed. A Mann-Whitney test was used to determine if there was a significant difference in the prevalence of CRLMs that demonstrate LGH between patients who had received chemotherapy and those who had not.

3.1.3 Results

There were 134 patients with 232 CRLMs who met inclusion/exclusion criteria. The overall prevalence of LGH in CRLMs was 47.8% (95% CI: 39.7% to 56.0%) using visual analysis determined by a reader. The mean CNR was -8.6 (95% CI: -11.7 to -5.6) among CRLMs that did not demonstrate LGH based on visual analysis versus +13.6 (95% CI: +11.2 to +16.1) among CRLMs that did demonstrate LGH based on visual analysis. At a cutoff of CNR = +2.6, the prevalence of LGH in CRLMs was equal for visual analysis and semi-quantitative analysis. We did not detect a difference in prevalence of LGH of CRLMs among those who had received chemotherapy prior to MRI and those who did not.

3.1.4 Conclusions

Late gadolinium hyperintensity (LGH) of CRLMs is common (47%) on delayed-phase MRI with extracellular contrast agents. Therefore, LGH should not be used to exclude malignancy in the absence of other diagnostic features.

3.2 INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths in men (third in women) in North America (2). Most deaths are related to metastatic disease, often to the liver. Colorectal liver metastases (CRLM) occur in approximately 50% of all patients with colorectal cancer. Although the survival of untreated CRLM is poor (median survival approximately 9 months), this has improved significantly with surgery and chemotherapy (23). In patients who are surgical candidates, the 5-year and 10-year survivals are 38% and 26%, respectively (23).

Preoperative imaging for detection and characterization of CRLM is crucial for surgical planning. Magnetic resonance imaging (MRI) is generally considered the best imaging modality for detection and characterization (54). Although advances in techniques using hepatobiliary-specific contrast agents and diffusion-weighted imaging have led to excellent detection of lesions, per-lesion characterization remains challenging (123). This is particularly true for small lesions (<10mm) and in patients who have received prior chemotherapy (54, 92).

One of the signs used to characterize CRLM includes hypointensity on delayed phase MRI with extracellular contrast agents (72, 158). This sign is used because hemangiomas, the most common solid benign lesion in the liver, rarely demonstrate this sign. Contrary to conventional literature, we have observed in clinical practice that not all CRLM demonstrate this sign. There are several other reports of the same phenomenon (84). CRLM that demonstrate late gadolinium hyperintensity (LGH) on delayed phase MRI with extracellular contrast agents may be more difficult to diagnose given the overlap between benign and malignant findings; however, the prevalence of these CRLM has not been previously studied. A “good” diagnostic test typically has a sensitivity of at least 80% and preferably greater than 90% (157). If LGH is to be a useful sign in excluding malignancy, then the prevalence of LGH in CRLMs should be less than 20% (or sensitivity of the sign is >80%).

Therefore, we hypothesize that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is greater than 20%.

We aim to determine the prevalence of LGH in CRLMs on 10-minute delayed phase gadobutrol-enhanced MRI in a retrospective cohort of patients with pathology-confirmed CRLM who received preoperative gadobutrol-enhanced MRI prior to hepatectomy.

3.3 METHODS

3.3.1 Patient population

We performed a retrospective study on patients with CRLM who received a preoperative MRI with an extracellular contrast agent (gadobutrol) at our institution between January 1, 2006 and December 31, 2012 prior to hepatectomy. All CRLM analyzed in this study were confirmed on pathology, post-hepatectomy. All CRLMs on MRI were matched on a per-lesion basis by lesion size and location with pathology, based on clinical pathology reports. Exclusion criteria included patients whose imaging was unavailable or image quality was unacceptable for analysis. If multiple MRI studies that met our inclusion/exclusion criteria were performed in the same patient, then the study performed closest to the surgical date was chosen. Any CRLM that could not be confirmed on pathology on a per-lesion basis were also excluded.

As part of the standard clinical liver imaging protocol at our institution, patients receive delayed phase contrast-enhanced MRI with three-dimensional, fat-suppressed, spoiled gradient-echo axial T1-weighted imaging approximately 10-minutes post-contrast injection with an intravenous dose of gadobutrol at 0.1mL/kg body mass up to 10mL at 1.0mmol/mL. All studies were performed on 1.5T (GE Twinspeed™, TE: 2.2ms, TR: 4.5ms, flip angle 15 degrees) or 3.0T (Philips Achieva™, TE: 1.4ms, TR: 3.0ms, flip angle 10 degrees) magnets with an eight-channel body phased array coil covering the entire liver.

The following baseline demographic information was obtained: age, sex, and whether the patient received chemotherapy prior to MRI.

3.3.2 Imaging analysis

Imaging analysis was performed on standard picture archiving and communication system (PACS) software at our institution (Agfa Impax 6.3.1, AGFA HealthCare N.V., Belgium^{1™}). CRLM were identified by a reader.

Visual analysis of the lesions was performed by a single reader (HMCC, 5 years of experience) who determined whether the CRLMs demonstrated LGH, which was defined as lesions that appeared visually hyperintense relative to the background liver on 10-minute delayed phase imaging on MRI with extracellular contrast agents. The reader was not blinded as all lesions were pathology-confirmed, resected CRLMs.

Semi-quantitative analysis of the lesions was performed by measuring the liver-lesion contrast-to-noise ratio (CNR) of each CRLM on 10-minute delayed phase MRI with extracellular contrast agents (159). Measurements were obtained on the single axial slice where the lesion had the largest long-axis diameter. An oval region of interest (ROI) most closely approximating the entire tumour was drawn to determine the lesion's mean signal intensity (SI). The mean SI of five 1-2 cm ROIs drawn in the surrounding background liver parenchyma (avoiding tumour or major blood vessels) on the same slice as the tumour was determined. The standard deviation (SD) of the background noise was calculated from taking the mean SD of eight 1-2 cm ROIs drawn in the background four quadrants, taking care to exclude banding surrounding the patient due to motion artifact.

The CNR of each CRLM was calculated according to the following formula:

$$CNR = \frac{\text{Signal intensity (lesion)} - \text{Signal intensity (liver)}}{\text{Standard deviation (background air)} \sqrt{\frac{2}{4 - \pi}}}$$

(Equation 3.1)

The correction factor for standard deviation:

$$\sqrt{\frac{2}{4 - \pi}}$$

(Equation 3.2)

was applied to correct for the use of multichannel coil and parallel imaging (160).

Semi-quantitative analysis was performed by the same reader as the visual analysis, 6 months apart from the visual analysis, to reduce recall bias.

3.3.3 Statistical analysis

Based on the visual analysis, the prevalence of lesions that demonstrated LGH on 10-minute delayed phase MRI with extracellular contrast agents was determined.

Based on the semi-quantitative analysis, the mean CNR of the CRLMs was calculated, after weighting by patient to account for clustering due to multiple lesions per patient. Multiple lesions from the same patient may have a similar imaging appearance (likely due to similar biology). If there is a single patient with a large number of lesions, then this could skew the results. This was done by assigning a weight of 1 to each patient such that the CNR from each CRLM from a patient with x CRLM would have a weight of $1/x$ (161). The mean CNR for lesions that demonstrated LGH and did not demonstrate LGH on visual analysis was determined and compared using the Student's t -test. The cutoff CNR at which the prevalence of hyperintense lesions based on semi-quantitative analysis was the same as the prevalence of lesions that demonstrated LGH based on visual analysis was determined. This was obtained by determining the prevalence of LGH using a range of cutoffs for CNR. We defined lesions that had a CNR greater than this cutoff as demonstrating LGH for the remainder of the paper.

A Mann-Whitney test was used to compare the difference in prevalence of CRLMs that demonstrate LGH between patients who had received chemotherapy and those who had not received chemotherapy, weighted per patient in the manner as previously described. A p -value < 0.05 was taken as statistically significant.

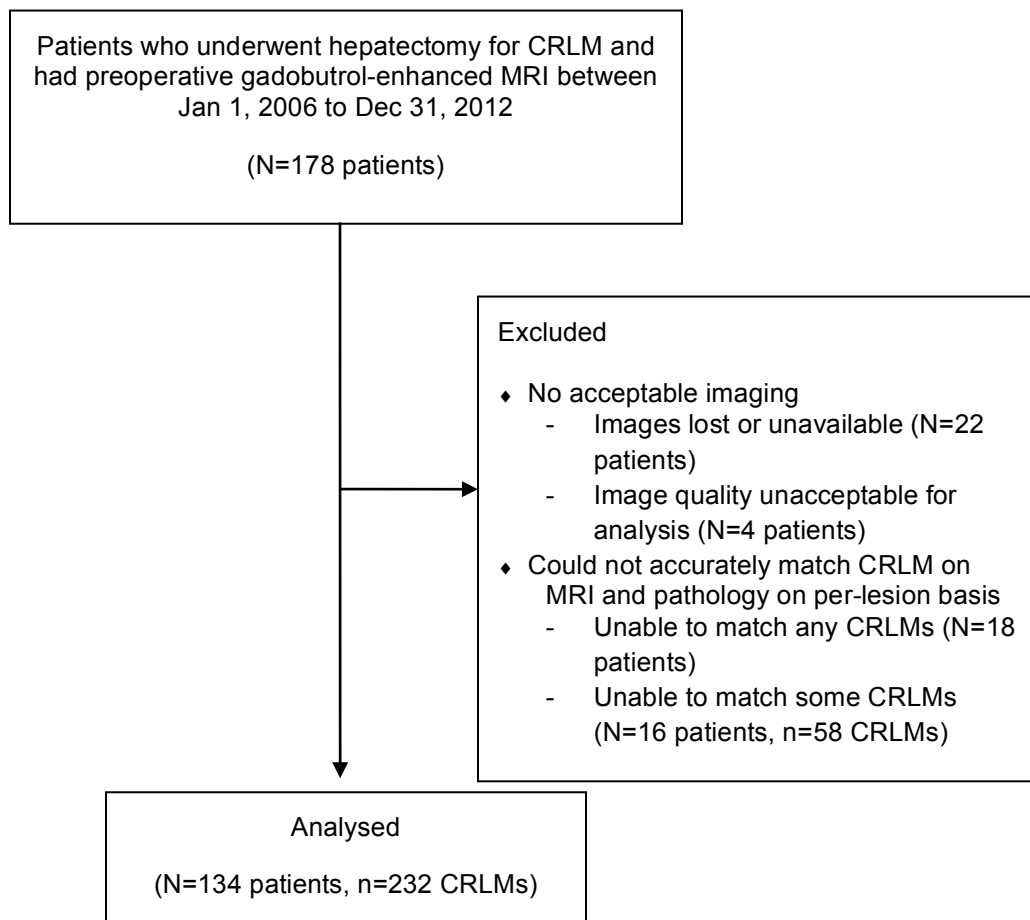
3.4 RESULTS

3.4.1 Patient demographics

There were a total of 178 patients who met inclusion criteria for our study (Figure 3.1). We excluded 26 patients (22 where images were not available and 4 where image quality was unacceptable for analysis). There were 18 patients who were excluded, as it was not possible to accurately match any CRLMs on MRI and pathology. There were an additional 16 patients where only some of the CRLMs identified on MRI could be matched on a per lesion basis with pathology. There were 58 lesions from these patients, which could not be matched on a per-lesion basis and were excluded from analysis. Therefore, there were 134 patients with 232 CRLMs that were included in the study.

Among the 134 patients, 79 (59.0%) patients were male and 55 (41.0%) were female. The mean age was 63.7 years (SD: 11.2 years, range: 37-86 years).

Figure 3.1: Flowchart of inclusion/exclusion criteria



3.4.2 Statistical analysis

The prevalence of LGH based on visual analysis was 47.8% (95% CI: 39.7% to 56.0%).

The mean CNR of CRLMs on 10-minute delayed phase MRI with extracellular contrast agents was +2.2 (95% CI: -0.7 to +5.0) (N=134 patients, n=232 CRLMs). The mean CNR of CRLMs that demonstrated LGH based on visual analysis was +13.6 (95% CI: +11.2 to +16.1). The mean CNR of CRLMs that did not demonstrate LGH based on visual analysis was -8.6 (95% CI: -11.7 to -5.6). There was a significant difference in the mean CNR between CRLMs that demonstrated LGH based on visual analysis compared to those that did not ($p < 0.001$) (Figure 3.2).

At a CNR cutoff of +2.6, the prevalence of LGH based on CNR was 47.4% (95% CI: 39.5% to 55.4%), which was approximately the same as the prevalence of LGH based on visual analysis. Therefore, LGH was defined as $\text{CNR} > +2.6$ on 10-minute delayed phase MRI for the remainder of the study (Figure 3.3).

The prevalence of LGH (based on CNR) of CRLMs was 49.0% (95% CI: 39.0% to 59.0%) for patients who had received chemotherapy prior to MRI (N=85 patients, n=149 lesions) and 41.9% (95% CI: 28.3% to 55.5%) for patients who had not received chemotherapy prior to MRI (N=45 patients, n=78 lesions). The prevalence of LGH of CRLMs was not statistically different among those who had received chemotherapy prior to MRI and those who had not based on the Mann-Whitney test ($p=0.365$).

Figure 3.2: Mean contrast-to-noise ratio (CNR) of CRLMs with or without late gadolinium hyperintensity (LGH) based on visual analysis. Error bars represent 95% confidence intervals.

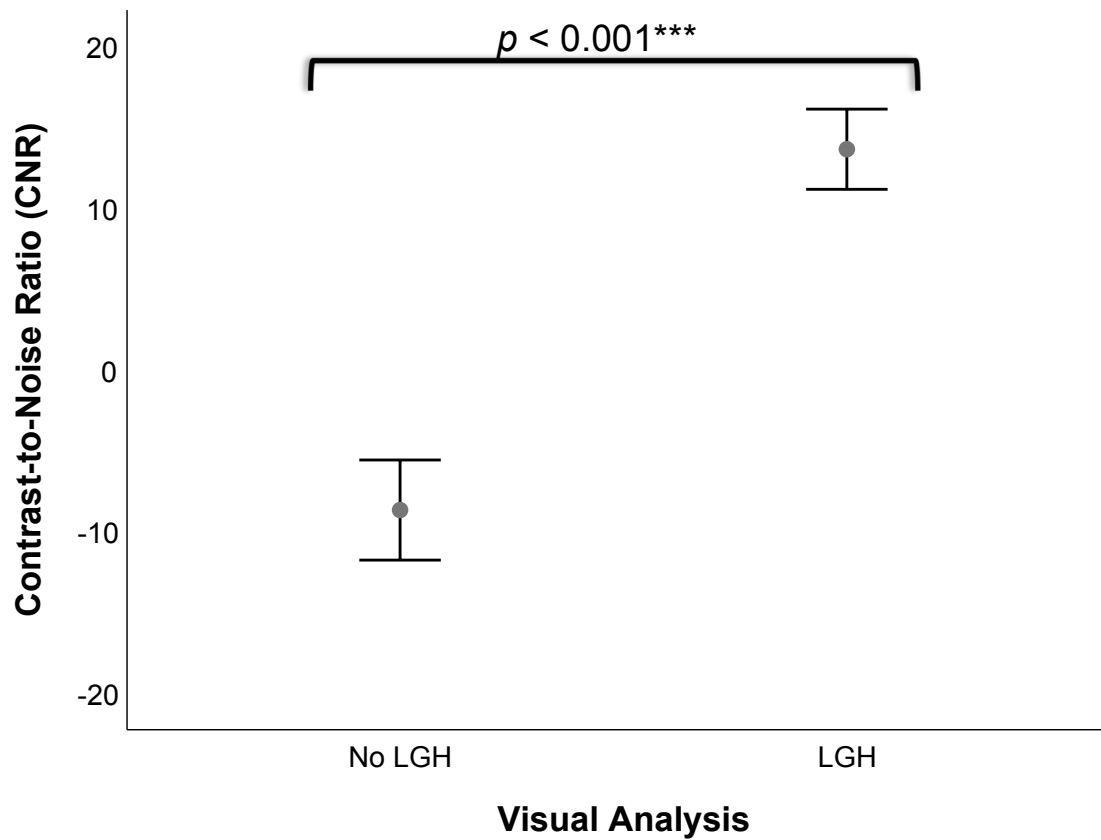
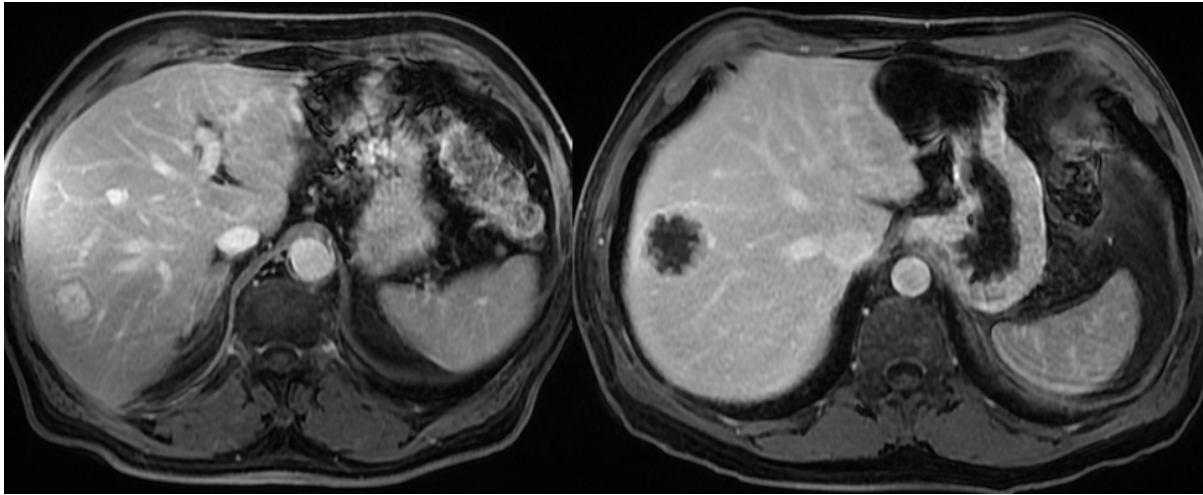


Figure 3.3: Examples of pathology-confirmed colorectal liver metastases on 10-minute delayed phase MRI with an extracellular contrast agent that (a) demonstrates late gadolinium hyperintensity (LGH) in a 76-year-old male (left) and (b) does not demonstrate LGH in a 60-year-old male (right)



3.5 DISCUSSION

This study demonstrates that close to half of CRLMs are hyperintense relative to the background liver on 10-minute delayed phase MRI with extracellular contrast agents using semi-quantitative and visual analysis. We did not detect differences in LGH due to chemotherapy prior to MRI. To our knowledge, this is the first study to systematically investigate the prevalence of CRLMs that demonstrate hyperintensity on delayed phase imaging with gadobutrol-enhanced MRI.

Although the phenomenon of delayed enhancement has been previously described in the literature, most conventional literature sources suggest that the vast majority of colorectal liver metastases are hypoenhancing and that this feature can be used to distinguish between benign and malignant lesions in patients with colorectal cancer (72). Mahfouz and colleagues first described the concept that metastases demonstrate delayed washout of contrast in 1994 (162). However, the paper described the finding of peripheral washout of contrast as a specific sign of malignancy. They did not suggest that delayed, heterogeneous washout of the overall lesion suggests malignancy, although this has occasionally been interpreted as such. To our knowledge, no study-to-date has described the overall enhancement pattern of lesions. Given the results of our study, close to half of CRLM demonstrate LGH on delayed phase imaging. Therefore, LGH cannot be used to exclude malignancy in the absence of other features.

Based on results from the visual analysis, the optimal cutoff for CNR to define LGH was $CNR = +2.6$. At this CNR cutoff, there approximately the prevalence of LGH on visual analysis was equal to the prevalence of LGH on CNR analysis.

In our cohort, 65% of patients received chemotherapy prior to MRI. We did not detect a difference in LGH due to chemotherapy prior to MRI in our analysis. However, our study was not powered to specifically answer this question. This remains a potential confounder.

This study looked at a single imaging sign – it is unclear how this sign would be interpreted by a radiologist in the context of other imaging features. Other features are used in distinguishing benign vs. malignant liver lesions in the setting of colorectal cancer. Features such as rim enhancement and peripheral washout as signs of malignancy, but are

difficult to determine in small lesions (54). Hyperintensity on T2-weighted imaging is typically a feature of benign hemangiomas, but T2 signal often increases in CRLM post-chemotherapy (92). Therefore, T2 hyperintensity may be seen in CRLM post-chemotherapy. Diffusion-weighted imaging can be helpful to distinguish benign from malignant lesions; however, restricted diffusion is known to decrease in CRLM post-chemotherapy, making it more difficult to distinguish CRLM from benign lesions (92, 122). Diffusion-weighted imaging is also subject to artifact, which can make characterization of small lesions particularly challenging (111).

This study involves a retrospective cohort of patients who had received surgery and where CRLMs could be matched by size and location on a per-lesion basis based on pathology reports. CRLMs that could not be matched with pathology were excluded in order to ensure that all lesions were definitively CRLMs. However, this could lead to a bias in the types of lesions chosen for this study. For example, it may be possible that CRLMs in patients with multiple lesions where per-lesion matching was not possible or non-surgical patients where post-surgical pathology was unavailable have a different proportion of CRLMs that demonstrate LGH. Further studies looking at a prospective cohort and nonsurgical cohorts are required. This study also did not assess inter-rater reliability for either the visual analysis or the semi-quantitative analysis, which is an additional limitation of this study.

Because MRIs were not obtained for the purpose of measuring CNR, several technical confounders including magnetic field strength, use of phased-array surface coil, and presence of diffuse liver disease may have affected our results (163, 164). Magnetic field strength may affect the delayed tumour enhancement since the relaxivity of gadolinium varies with field strength, although we did not demonstrate a difference in delayed tumour enhancement with field strength in our cohort (163). The multichannel surface coil used to acquire images and the use of parallel imaging techniques will also affect CNR measurements (160, 165). We attempted to partially correct the effects of multichannel surface coil on noise; however, further studies using more robust methods of measuring noise such as through repeated acquisitions, which could be acquired prospectively, are required (160). Prospective validation studies are required to standardize techniques for measuring delayed tumour enhancement, assess for the reproducibility, and control for technical MRI parameters. Future prospective studies with fixed MRI parameters are required. Further studies are required in order to determine the reliability of measuring LGH using these methods.

This study showed CRLM's demonstrate a wide range of CNRs on delayed phase MRI with extracellular contrast agents. This leads us to wonder what are the underlying histological or biological reasons for these differences. This question could not be answered based on the current study; however, this will be further discussed in the following chapters (Chapter 4 and 5).

3.6 CONCLUSION

In conclusion, nearly half (47%) of CRLMs are hyperintense relative to the background liver on 10-minute delayed phase MRI with gadobutrol. Therefore, late gadolinium hyperintensity (LGH) on MRI with extracellular contrast agents is should not be a feature used to exclude CRLM.

CHAPTER 4 : LATE GADOLINIUM HYPERINTENSITY OF COLORECTAL LIVER METASTASES POST- CHEMOTHERAPY IS ASSOCIATED WITH TUMOUR FIBOSIS AND OVERALL SURVIVAL POST-HEPATECTOMY

This chapter is modified with permission from the following: Cheung HMC, Karanickolas PJ, Hsieh E, et al. *Eur Radiol.* 2018; 28(8): 3505-3512.

4.1 ABSTRACT

4.1.1 Introduction

Late gadolinium hyperintensity (LGH) is a common finding in colorectal liver metastases (CRLM). LGH is associated with fibrosis in other disease processes and pathological-evidence of tumour fibrosis is a known predictor of survival. We hypothesize that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is significantly correlated with the percentage of tumour fibrosis on post-hepatectomy specimens and that LGH on 10-minute delayed phase MRI is significantly associated with overall survival, after adjusting for known confounders.

4.1.2 Methods

The institutional review board approved this retrospective cohort study and waived the requirement for informed consent. A cohort of 121 surgical patients who received preoperative MRI after chemotherapy between 2006-2012 were included in this study. Target tumour enhancement (TTE), defined as the mean contrast-to-noise ratio of up to 2 target lesions on late-phase gadobutrol-enhanced MRI, was determined by two independent raters. The average TTE was correlated with tumour fibrosis on post-hepatectomy specimens using Spearman correlation and with survival post-hepatectomy

using Kaplan-Meier and Cox-Regression. Inter-rater reliability was determined using relative intra-class correlation coefficients.

4.1.3 Results

In the surgical cohort (mean age: 63.0 years; male: 58%), TTE was associated with tumour fibrosis ($R=0.43$, $p<0.001$). Strong TTE was associated with improved survival compared to weak TTE (3-year survival: 88.4% vs. 58.8%, $p=0.003$) with a hazard ratio of 0.32 (95% CI: 0.14-0.75, $p=0.008$), after taking into account known prognostic variables. Inter-rater reliability was very good with a relative intraclass correlation of 0.84 (95% CI: 0.77-0.89)

4.1.4 Conclusion

LGH of CRLM post-chemotherapy using gadobutrol-enhanced MRI is associated with tumour fibrosis and survival.

4.2 INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths in the developed world (3). Approximately half of patients develop liver metastases and most deaths are related to metastatic disease (23). The median survival of patients with colorectal liver metastases (CRLM) without treatment is 7.5 months (21). With advancements in surgical and chemotherapy techniques, the survival of patients with CRLM has significantly improved. In a meta-analysis by Kanas et al (2012), the 5-year and 10-year survival of patients with resected CRLM was now 38% and 26%, respectively (23). This is likely even higher with more recent data and with improving surgical and chemotherapy techniques.

The ability to predict prognosis informs treatment recommendations, including surgery and/or chemotherapy. Several prognostic indicators stratify risk for patients with CRLM including clinical, pathology, and molecular prognostic biomarkers (3-4). However, the use of magnetic resonance imaging (MRI) to stratify risk in patients with CRLM is relatively unexplored. MRI is routinely used clinically for diagnosis, staging, and operative planning in patients being considered for liver resection, so information gained from MRI could be easily translated into clinical practice.

Several studies have demonstrated that tumour fibrosis in post-hepatectomy CRLM specimens is associated with overall survival (36, 166). This may be related to pathological response to chemotherapy. Pathologically, tumour fibrosis in CRLM closely resembles the appearance of tumour fibrosis in cholangiocarcinoma. In cholangiocarcinoma, late gadolinium enhancement on MRI with extracellular contrast agents is correlated with tumour fibrosis (68). This association has also been reported with CRLM, although this is less well-studied (68). Based on this, we hypothesize that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is significantly correlated with the percentage of tumour fibrosis on post-hepatectomy specimens. We additionally hypothesize that LGH on 10-minute delayed phase MRI is significantly associated with overall survival, after adjusting for known confounders.

We aim to determine the association between LGH of CRLM on 10-minute delayed phase gadobutrol-enhanced MRI and post-hepatectomy tumour fibrosis in a retrospective cohort of patients with pathology-confirmed CRLM who received gadobutrol-enhanced MRI after chemotherapy and prior to hepatectomy. In addition, we aim to determine the association

between LGH of CRLM on 10-minute delayed phase gadobutrol-enhanced MRI in this cohort with overall survival post-hepatectomy.

4.3 METHODS

This study was an institutional-REB approved, retrospective study.

4.3.1 Participants

The retrospective cohort included all patients at a single tertiary cancer centre with CRLM who had received a gadobutrol-enhanced MRI after treatment with chemotherapy (variable regimens as determined by standard of care, clinical treatment) and prior to hepatic resection for curative intent between January 1, 2006 and December 31, 2012. Preoperative MRI is performed as part of the routine imaging workup for diagnosis and staging at this institution. All patients met institution guidelines for hepatic resection with curative intent (no extrahepatic disease at time of MRI) and were deemed fit for major surgery. The typical workflow in our institution is as follows: patients are referred to the hepatobiliary surgeons with outside ultrasound or CT imaging suggestive of CRLM. Patients who are possible surgical candidates are then referred for MRI by the hepatobiliary surgeons for preoperative MRI prior to surgery.

Exclusion criteria included patients who did not have 10-minute delayed phase imaging, MRIs with image quality unacceptable for analysis, or patients that did not have measurable target lesions. Patients who died within 30 days of surgery were also excluded in order to exclude deaths due to perioperative mortality. If multiple gadobutrol-enhanced MRIs were performed, the MRI closest to the surgical date was used for analysis. In other words, if there were multiple MRIs (most commonly before and after surgery), then the MRI after the longer duration of chemotherapy was preferentially selected.

Clinical and demographic information was obtained from electronic patient records as well as publicly-available obituary databases, including age, sex, chemotherapy prior to MRI, and a validated clinical risk score, developed by Feroci and Fong (33). The clinical risk score is calculated as a five-point scale, with one point for each of the following:

number of tumours > 1, size of largest tumour ≥ 5 cm, metachronous metastases (time from diagnosis of primary to time of diagnosis of metastases ≤ 12 months), primary colorectal cancer with ≥ 5 lymph nodes positive, and preoperative carcinoembryonic antigen level ≥ 200 ng/mL (33). A high preoperative clinical risk score is a validated predictor of poor long-term, postoperative survival (33).

The clinical end-point for this study was overall survival. Follow-up data was collected up to January 1, 2016.

4.3.2 Magnetic resonance imaging (MRI) protocol and analysis

All patients received a gadobutrol-enhanced MRI for diagnostic and staging purposes as part of their routine clinical workup using standard clinical liver imaging protocols at our institution. As part of the contrast-enhanced series, delayed 3D Axial T1 imaging was routinely performed 10-minutes post-intravenous injection of gadobutrol (0.1 mL/kg body mass up to 10 mL at 1.0 mmol/mL). All studies were performed on 1.5T (GE Twinspeed™, TR~4.5, TE~2.2, Flip Angle~15, Slice thickness=5 mm, Spacing=2.5 mm, FOV=380 mm, Matrix = 320x192) or 3.0T (Philips Achieva™, TR~3.0, TE~1.4, Flip Angle~10, Slice thickness=3 mm, Spacing=1.5 mm, FOV~380, Matrix ~ 250x250) magnets with an eight-channel body phased array coil covering the entire liver and with parallel imaging.

Imaging analysis was performed on standard picture archiving and communication system (PACS) software at our institution (Agfa Impax 6.3.1, AGFA HealthCare N.V., Belgium™). Up to two target lesions were identified as per Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria (137). If there were multiple CRLMs that met criteria for target lesions, then the two largest measurable lesions were chosen. Patients were excluded from the study if there were no measurable lesions as defined by RECIST 1.1. Target lesions were confirmed as CRLMs based on post-operative pathology reports.

For all target lesions, the contrast-to-noise ratio (CNR) on 10-minute delayed phase was calculated (133). Measurements were obtained on the single axial slice where the lesion had the largest long-axis diameter. An oval region of interest (ROI) most closely approximating the entire tumour was drawn to determine the lesion's mean signal intensity (SI). The mean SI of five 1-2 cm ROIs drawn in the surrounding background liver parenchyma (avoiding tumour or major blood vessels) on the same slice as the tumour

was determined. The standard deviation (SD) of the background noise was calculated from taking the mean SD of eight 1-2 cm ROIs drawn in the background four quadrants, taking care to exclude banding surrounding the patient due to motion artifact.

The CNR of each CRLM was calculated according to the following formula:

$$CNR = \frac{\text{Signal intensity (lesion)} - \text{Signal intensity (liver)}}{\text{Standard deviation (background air)} \sqrt{\frac{2}{4 - \pi}}} \quad (\text{Equation 4.1})$$

The correction factor for standard deviation:

$$\sqrt{\frac{2}{4 - \pi}} \quad (\text{Equation 4.2})$$

was applied to correct for the use of multichannel coil and parallel imaging (160).

The target tumour enhancement (TTE) was calculated as the mean of the CNR of the target lesion(s). Two separate readers (HMCC; TM with 6 and 1 years of experience) independently determined the TTE. The mean TTE between the two readers was used for radiologic-pathologic and survival analysis.

4.3.4 Pathology analysis

Gross tumour sizes were determined based on the largest diameter post-fixation (10% buffered formalin). Hematoxylin and eosin stained slides were prepared from representative paraffin blocks. A single pathologist (HE, with 6 years of experience) qualitatively assessed the approximate percentage of fibrosis, necrosis, acellular mucin, and viable tumour cells on each representative slide. The CRLMs identified on the pathology specimens were matched to the target CRLMs identified on imaging by matching the location of the tumour and the size of the tumour. Patients were excluded

from radiologic-pathologic analysis if the specimens were not available for analysis or if there were multiple CRLMs of similar size in the same location that could not be matched on a per-lesion basis.

All imaging and pathology analyses were performed by readers blinded to all clinical information (other than the history of CRLM).

4.3.5 Statistical analysis

Patients were dichotomized into weak and strong TTE (Figure 4.1). The cutoff point was determined using the surgical cohort using the Youden Index for three-year survival (167).

The Youden Index (or “J-statistic”) is defined as follows:

$$J = sensitivity + specificity - 1$$

(Equation 2).

The biomarker with the maximum J-statistic is the point of maximal potential effectiveness based on combined sensitivity and specificity (167). With the Youden Index, the importance of sensitivity and specificity is equally weighted (167).

A Chi-Square test was used to determine if there were differences in demographic data between strong and weak TTE groups.

The median target percentage fibrosis, necrosis, and viable tumour cells were determined for both high TTE and low TTE. Spearman correlations were used to determine whether there was a correlation between TTE and the mean percentage fibrosis, necrosis, acellular mucin, and viable tumour cells of the matched target lesions determined on pathology analysis. Spearman correlation is a non-parametric test that uses rank-orders for two sets of ranks to determine a correlation coefficient, r (168, 169). We chose to use Spearman correlation because our data was not definitely normally distributed. (For normally distributed data, a parametric test, the Pearson correlation, can be used).

The association between the patient's TTE and survival was determined using Kaplan-Meier statistics. Kaplan-Meier is a non-parametric, univariate survival function that is based on a time-to-event model (in this case, the event is overall survival) (168, 170). At each time point, the proportion of subjects surviving is calculated as a survival proportion based on the number of patients who are available for analysis ("patients at risk") (168, 170). The survival proportion at each time point is a conditional probability since the patients who have died are not included in the calculation (168, 170). Patients are considered "censored" at a given time point, if they did not die, but no survival data is available beyond that time point (168, 170). The cumulative survival proportion is the probability of surviving to a given time and this is calculated by multiplying the conditional probabilities of each time point up to this time v. One of the commonly used tests to compare Kaplan-Meier curves is the log rank test (168). The log rank test statistic is a nonparametric test that involves computing the hazard function at each observed event time and adding these to obtain an overall statistic across all time points where there is an event (171).

Multivariable Cox-Regression statistics were used to assess the association between TTE and survival after taking into account clinical risk score. Cox Regression (also known as proportional hazard regression) is a regression method that incorporates a censored time-to-event dependent variable (168, 172). This regression model determines the effect of the covariates at increasing or decreasing the proportionate hazard, which is the hazard ratio based on the conditional probability of the event at each time point (168, 172).

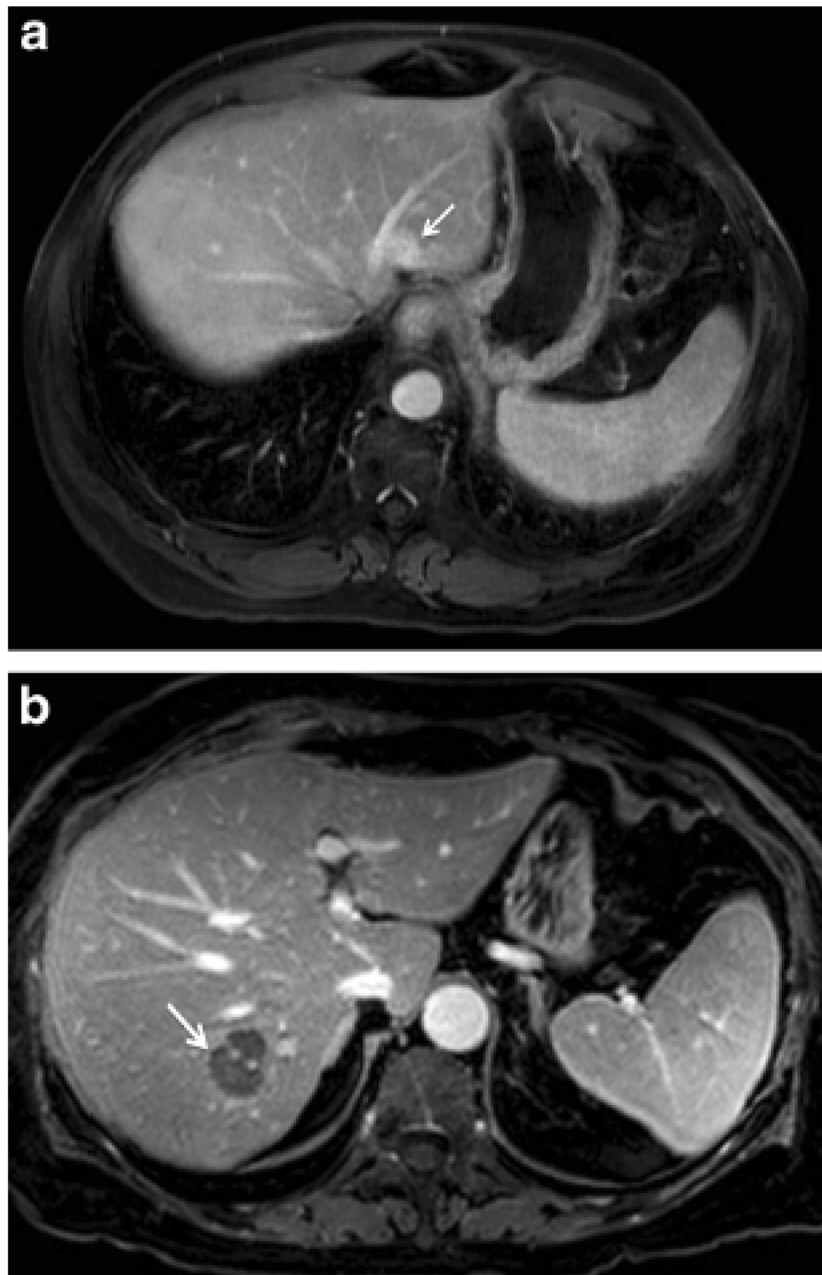
Post-hoc sensitivity analyses were performed using Cox Regression for time from MRI to surgery as well as for any demographic variables that demonstrated significant differences between strong and weak TTE (Table 4.1).

Additional post-hoc analyses were also performed to determine the proportion of patients with heterogeneous target lesions (1 lesion with $CNR < +7$ and 1 lesion with $CNR > +7$). Sensitivity analysis was performed excluding patients with heterogeneous target lesions in order to determine whether heterogeneity affected our results.

The TTE determined by each rater was compared using relative intra-class correlation coefficients (ICC) to determine inter-rater reliability (two-way mixed model). Intra-class correlation is a correlation method used to determine reliability and is often used to determine inter-observer reliability in imaging studies. An ICC value of less than 0.4 is

considered poor reliability, an ICC value between 0.40 and 0.59 is considered fair reliability, an ICC value between 0.60 and 0.74 is considered good reliability, and an ICC value between 0.75 to 1.00 is considered excellent reliability (173).

Figure 4.1: Colorectal liver metastases seen on 10-minute delayed phase, gadobutrol-enhanced MRI (a) in a 75 year-old man with strong target tumour enhancement and (b) in a 60 year-old man with weak target tumour enhancement.



4.4 RESULTS

Among the 121 patients who met inclusion/exclusion criteria for the study (Figure 4.2), the mean age was 63.0 years (SD: 11.2 years) with 70 (57.9%) men and 51 (42.1%) women (Table 4.1). The median time from MRI to surgery was 2.7 months (range: 0.1-10.5 months). There were a total of 40 deaths during the follow-up period.

Based on the Youden Index, the optimal cutoff for weak and strong TTE was a CNR = +7. There were 74 patients (61.1%) who had weak TTE and 47 patients (38.8%) who had strong TTE.

Patients with strong TTE were more likely to have smaller tumours ($p=0.019$). No other demographic data was significantly different between the MRI groups (Table 4.1).

It was possible to match lesions between MRI and pathology using size and location for 91 patients with 126 target CRLM. The Spearman correlations between TTE and the mean target percentage of fibrosis, necrosis, acellular mucin, and viable tumour cells were 0.43 ($p < 0.001$), -0.22 ($p=0.036$), 0.02 ($p=0.84$), and -0.05 ($p=0.63$), respectively. The median target percentage of fibrosis for high TTE and low TTE were 15.0% (interquartile range: 3.0% to 30.0%) and 37.5% (interquartile range: 15.0% to 51.3%), respectively (Figure 4.3a). The median target percentage necrosis for high TTE and low TTE were 30.0% (interquartile range: 15.0% to 50.0%) and 10.0% (interquartile range: 3.8% to 35.0%), respectively (Figure 4.3b). The median target percentage viable tumour cells for high TTE and low TTE were 40.0% (interquartile range: 10.0% to 50.0%) and 32.5% (interquartile range: 16.5% to 50.0%) respectively (Figure 4.3c). Most patients did not have tumours that contained acellular mucin (only 12 patients); therefore, the median target percentage acellular mucin was 0% for both high TTE and low TTE.

Strong TTE was associated with survival on univariate analysis ($p=0.003$). At 3 years, 88.4% of patients with strong TTE on the preoperative MRI were alive vs. 58.8% in patients with weak TTE (Figure 4.4).

There were 112 patients (with 34 events) with complete data available for the multivariable analysis. TTE had an adjusted hazard ratio of 0.32 (95% CI: 0.14-0.75,

p=0.008). The adjusted hazard ratio of clinical risk score was 2.41 (95% CI: 1.19-4.90) (Table 4.2).

Post-hoc sensitivity analyses were performed for time from MRI to surgery and tumour size ≥ 5 cm. None of these variables were found to be significant contributing variables on our sensitivity analysis. When time from MRI to surgery was included in the Cox-Regression model, TTE had an adjusted hazard ratio of 0.33 (95% CI: 0.14-0.75, p=0.009). When tumour size was included in the Cox-Regression model, TTE had an adjusted hazard ratio of 0.33 (95% CI: 0.14 to 0.77, p=0.010).

For reader 1, there were 62 patients (51.2%) that had only 1 target lesion, 45 patients (37.2%) with 2 target lesions with homogeneous CNR (CNR $< +7$ for both lesions or CNR $> +7$ for both lesions), and 14 patients (11.6%) with 2 target lesions with heterogeneous CNR (1 lesion with CNR $< +7$ and 1 lesion with CNR $> +7$). When the 14 patients with heterogeneous target lesions were excluded, there was no significant difference in our results with TTE having an adjusted hazard ratio of 0.36 (95% CI: 0.15 to 0.90, p=0.029).

For reader 2, there were 58 patients (47.9%) that had only 1 target lesion, 45 patients (37.2%) with 2 target lesions with homogeneous CNR (CNR $< +7$ for both lesions or CNR $> +7$ for both lesions), and 18 patients (14.9%) with 2 target lesions with heterogeneous CNR (1 lesion with CNR $< +7$ and 1 lesion with CNR $> +7$). When the 18 patients with heterogeneous target lesions were excluded, there was no significant difference in our results with TTE having an adjusted hazard ratio of 0.29 (95% CI: 0.11 to 0.76, p=0.012).

Inter-rater reliability was very good with a relative intraclass correlation of 0.84 (95% CI: 0.77-0.89).

Figure 4.2: Flow charts of inclusion and exclusion criteria

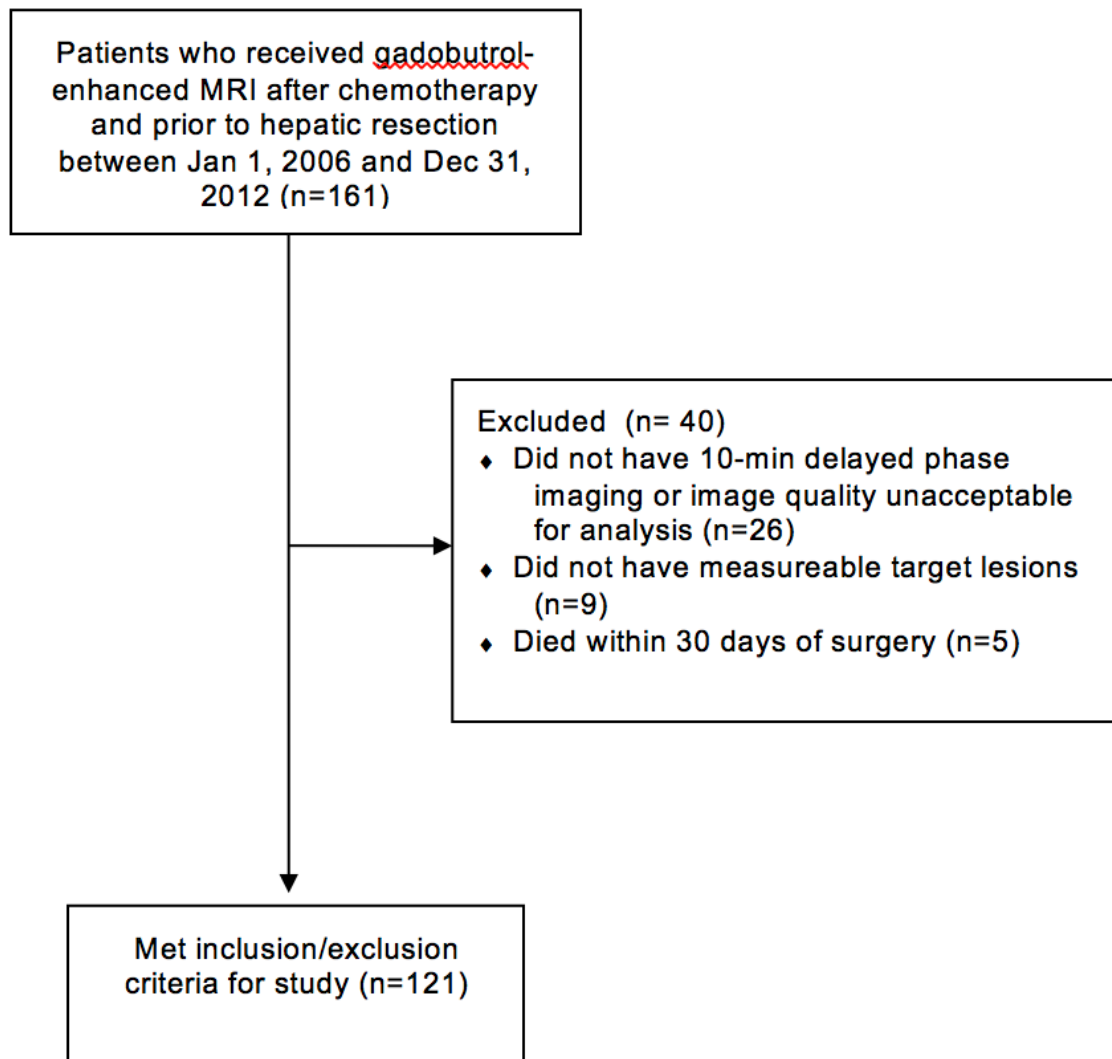


Table 4.1: Baseline demographics of patient population (n=121, entire cohort)

	Weak Target Tumour Enhancement (n=74)	Strong Target Tumour Enhancement (n=47)	p-value*
Age			
< 65 years	40 (54.1%)	24 (51.1%)	P=0.75
≥ 65 years	34 (45.9%)	23 (48.9%)	
Sex			
Male	43 (58.1%)	27 (57.4%)	P=0.94
Female	31 (41.9%)	20 (42.6%)	
Clinical Risk Score			
< 3	52 (76.5%)	34 (77.3%)	P=0.92
≥ 3	16 (23.5%)	10 (22.7%)	
Number of tumours			
= 1 tumour	36 (48.6%)	17 (36.2%)	P=0.18
> 1 tumour	38 (51.4%)	30 (63.8%)	
Tumour size			
< 5cm	55 (74.3%)	43 (91.5%)	P=0.019*
≥ 5 cm	19 (25.7%)	4 (8.5%)	
Time from diagnosis of primary to diagnosis of metastasis			
≤ 12 months	30 (40.5%)	13 (27.7%)	P=0.149
> 12 months	44 (59.5%)	34 (72.3%)	
Number of positive lymph nodes			
< 5 nodes positive	53 (73.6%)	36 (76.6%)	P=0.71
≥ 5 nodes positive	19 (26.4%)	11 (23.4%)	
Data not available	2	0	
Preoperative CEA level			
< 200ng/mL	60 (95.2%)	43 (95.6%)	P=0.94
≥ 200ng/mL	3 (4.8%)	2 (4.4%)	
Data not available	11	2	
Magnet			
1.5 Tesla	45 (60.8%)	30 (63.8%)	P=0.74
3.0 Tesla	29 (39.2%)	17 (36.2%)	

Figure 4.3: Boxplots demonstrating median target percentage (a) fibrosis, (b) necrosis, and (c) viable tumour cells among patients with strong TTE and weak TTE (n=91, for histologic analysis).

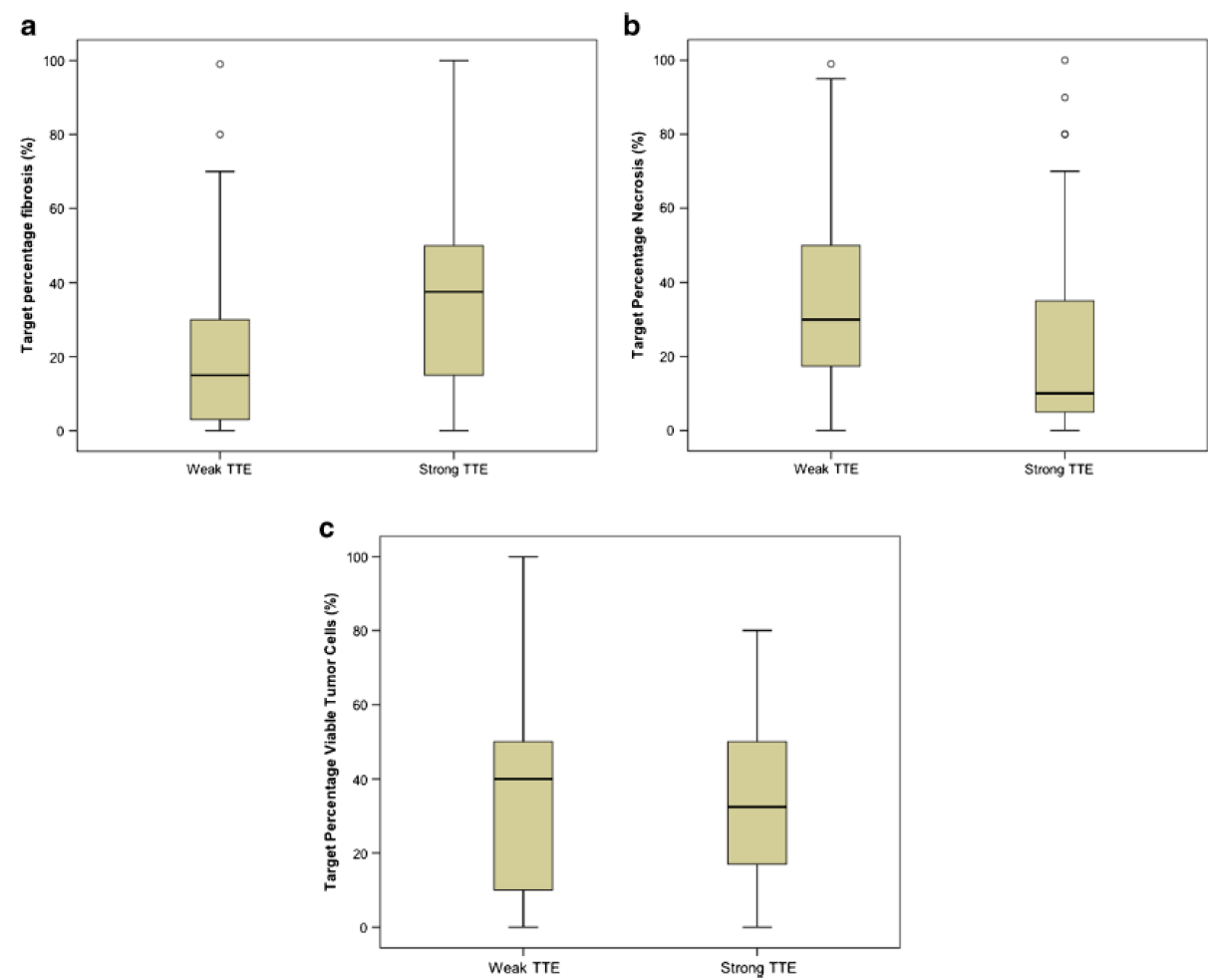


Figure 4.4: Kaplan Meier survival curves showing the association between target tumour enhancement of colorectal liver metastases post-chemotherapy and overall survival in patients who received a gadobutrol-enhanced MRI prior to liver resection (n=121, for univariate analysis).

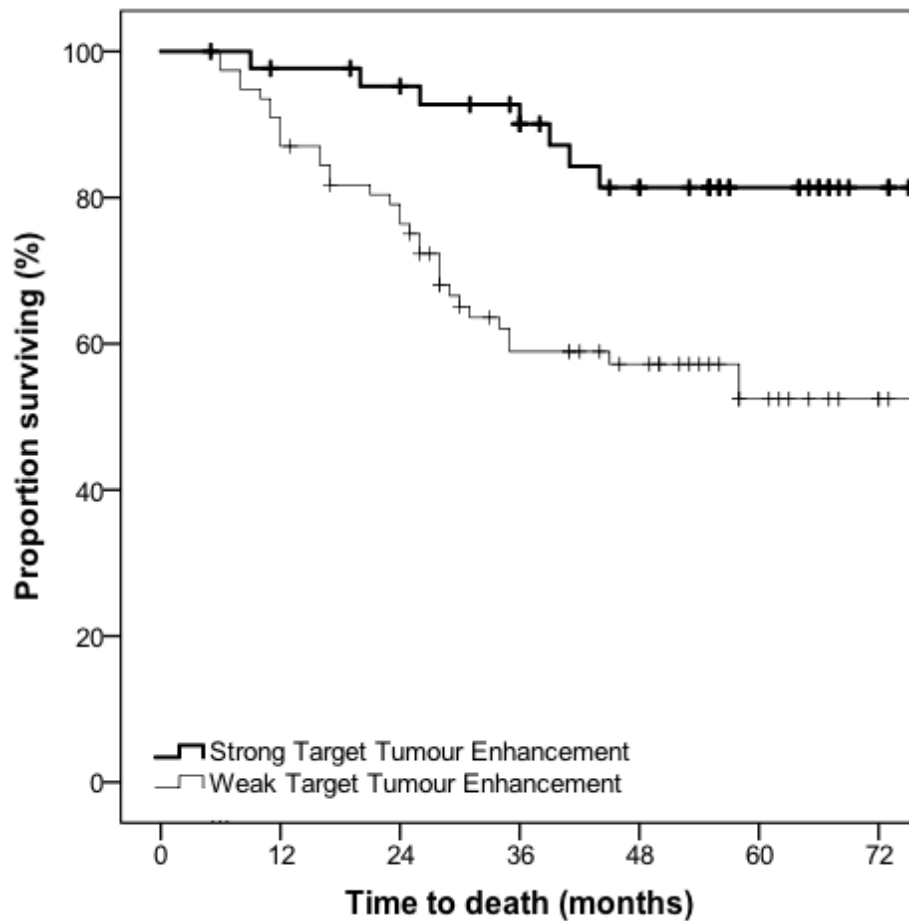


Table 4.2: Cox-Regression model of surgical cohort for the association of target tumour enhancement (TTE) and overall survival (n=112, for multivariate analysis)

	Adjusted Hazard Ratio (95% Confidence Interval)	<i>P</i>-value
Target tumour enhancement		
Weak	Reference	<i>P</i>=0.008**
Strong	0.32 (0.14-0.75)	
Clinical Risk Score		
< 3	Reference	<i>P</i>=0.015*
≥ 3	2.41 (1.19-4.90)	

4.5 DISCUSSION

In this study, we demonstrated that late gadolinium enhancement of CRLM post-chemotherapy on gadobutrol-enhanced MRI post-hepatectomy is associated with tumour fibrosis and with overall survival, after taking into account known clinical prognostic factors. The absolute difference in 3-year survival was 29.6% less in patients who had weak TTE than those who had strong TTE on preoperative MRI, with an adjusted hazard ratio of 0.32.

TTE on preoperative MRI was positively correlated with tumour fibrosis and negatively correlated with tumour necrosis on post-hepatectomy specimens, which may be the physiological explanation for this MRI phenomenon. No prior studies have specifically correlated the late gadolinium enhancement in CRLM with tumour fibrosis, although studies have looked at the correlation between noncontrast MRI signal characteristics of CRLM and tumour fibrosis (147). It is well-established in the pathology literature that tumour fibrosis in CRLM is one of the major pathological responses to chemotherapy and the predominant pathological response associated with treatment response and long-term outcomes (35, 36, 166). Specifically, tumour fibrosis and not tumour necrosis post-chemotherapy is associated with good long-term prognosis (36). Tumour necrosis is known to be poorly enhancing on contrast-enhanced MRI, which could be a confounding factor for measurement of TTE (174). In addition, tumour necrosis would be inversely correlated with tumour fibrosis since these variables may demonstrate collinearity.

Patients with strong TTE were more likely to have smaller tumours ($p=0.019$). If strong TTE represents “good” biology, then these tumours may be less aggressive and therefore tend to be smaller. It is also possible that small tumours have better blood supply compared to larger tumours and therefore demonstrate strong TTE. However, tumour size was not confounding variables in our post-hoc sensitivity analyses, which suggests that TTE may reflect “good” biology independent of tumour size.

RECIST is the most commonly used technique for evaluation of chemotherapy response (137). However, it is a size-based technique that has been shown to poorly correlate with pathological response or long-term survival (138). Several imaging criteria have been developed to address these limitations, including CT-based morphological criteria, which showed good association with pathological response and survival in the setting of CRLM

treated with bevacizumab-containing chemotherapy (146). Some authors have assessed the role of imaging techniques in assessing tumour biology, such as quantitative DCE-MRI and PET, although these techniques are expensive and time-consuming and are less routinely performed in the clinical setting (129, 146, 148, 175).

Our study had several limitations, mostly related to its retrospective nature. There was variability in the timing of MRI in relationship to the administration of chemotherapy, the type of chemotherapy administered, and the duration of chemotherapy. If tumour fibrosis is a chemotherapy response, then increasing the duration of chemotherapy could affect TTE. Radiologic-pathologic correlation was limited by sampling error, which could decrease Spearman correlation particularly in tumours with significant heterogeneity, and not all lesions could be matched on a per-lesion basis, which could lead to selection bias. Additionally, tumour fibrosis can be seen in CRLM even in patients without chemotherapy (37). These confounding factors may contribute to the relatively weak correlation between tumour fibrosis and TTE observed in our study.

Although 10-minute delayed-phase imaging is part of our institution's routine clinical liver MRI protocol, the addition of a 10-minute delayed phase scan may impede workflow and be a limitation at institutions that only perform imaging to 3- or 5-minutes post-contrast. We performed TTE analysis at 10-minute delayed phase based on the cardiac MRI literature, which has shown that fibrosis is best seen between 10 and 30 minutes (176, 177). However, it is unclear whether this is also the case for tumour fibrosis within CRLM and further studies should be performed in order to determine whether 3-5 minute delayed is also sufficient.

Because MRIs were not obtained for the purpose of measuring CNR, several technical confounders including magnetic field strength, use of phased-array surface coil, and presence of diffuse liver disease may have affected our results (163, 164). Magnetic field strength may affect the delayed tumour enhancement since the relaxivity of gadolinium varies with field strength, although we did not demonstrate a difference in delayed tumour enhancement with field strength in our cohort (163). The multichannel surface coil used to acquire images and the use of parallel imaging techniques will also affect CNR measurements (160, 165). We attempted to partially correct the effects of multichannel surface coil on noise; however, further studies using more robust methods of measuring noise such as through repeated acquisitions, which could be acquired prospectively, are required (160). Prospective validation studies are required to standardize techniques for

measuring delayed tumour enhancement, assess for the reproducibility, and control for technical MRI parameters. Future prospective studies with fixed MRI parameters are required.

Our study, while important, demonstrates the need for additional prospective studies to confirm the results, for external validation and to determine its potential clinical impact. Prospective studies are also required to determine the role of specific chemotherapy regimens analyzing pre- and post-treatment MRI scans, to confirm correlation between MRI signal and fibrosis using registered, high-resolution, radiologic-pathologic techniques, to optimize selection of target lesions, and to optimize measurement of TTE through T1 signal mapping. In some patients with multiple CRLMs, the enhancement pattern of different lesions can be heterogeneous. The presence of heterogeneous target lesions did not affect our results on our post-hoc sensitivity analysis, likely due to the relatively small proportion of patients with heterogeneous target lesions (approximately 12-15% of our patient cohort). However, further studies are required in order to determine the optimal method of measuring TTE in these patients. Although reliability between raters in our study was very good, development of standardized semi-automated techniques may further improve reliability.

As many patients are now staged using MRI with hepatobiliary-specific contrast agents, investigating the relationship between late phase enhancement of CRLM and tumour fibrosis and survival may also be valuable (67).

4.6 CONCLUSION

In conclusion, this paper presents the first study to provide evidence that late gadolinium MRI enhancement of tumours post-chemotherapy is associated with tumour fibrosis and overall survival post-hepatectomy in patients with CRLM. Target tumour enhancement on MRI may be a useful tool for risk-stratification. Further studies are required for external validation and to assess its potential clinical impact.

CHAPTER 5 : COLORECTAL LIVER METASTASES (CRLM) ON MRI WITH INTRAVASCULAR CONTRAST AGENT, GADOFOSVESET

5.1 ABSTRACT

5.1.1 Introduction

Late gadolinium hyperintensity (LGH) of colorectal liver metastases (CRLM) on MRI with extracellular contrast agents is common and may be due to leakage of contrast into the interstitium in fibrotic tumours. LGH in CRLM can be difficult to distinguish from benign hemangiomas on conventional MRI with extracellular contrast agents. We would expect that the leakage of contrast would not occur with an intravascular contrast agent, such as gadofosveset, resulting in fewer CRLMs that demonstrate LGH. We hypothesize that the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with intravascular contrast agents is significantly less than the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents, the diagnostic accuracy of reader interpretation of MRI with intravascular contrast agents is excellent, and there is significant added value of delayed phase imaging on MRI with intravascular contrast agents in reader interpretation.

5.1.2 Methods

The institutional research ethics board (REB) and Health Canada approved this prospective study and informed consent was obtained. Patients with known colorectal cancer who were referred for an MRI of the liver were recruited into the study and received a liver MRI with the intravascular contrast agent, gadofosveset. The mean contrast-to-noise ratio (CNR) and the prevalence of LGH (CNR > +2.6) of CRLMs were determined on 10-minute delayed phase gadofosveset-enhanced MRI. This was compared to the mean CNR and the prevalence of LGH of CRLMs on 10-minute delayed phase gadobutrol-enhanced MRI determined in Chapter 3 using a nonpaired Student's t-test. Per-lesion diagnostic accuracy of gadofosveset-enhanced MRI in diagnosing CRLM was

determined. The added value of delayed phase imaging was determined using a multinomial logistic regression model.

5.1.3 Results

There were 48 patients who met inclusion/exclusion criteria. The mean CNR of CRLMs on 10-minute delayed phase gadofosveset-enhanced MRI was -8.3 (95% CI: -12.4 to -4.1) and the prevalence of LGH was 11.0% (95% CI: 3.4% to 18.6%). Both the mean CNR ($p=0.001$) and the prevalence of LGH ($p<0.001$) with gadofosveset-enhanced MRI were significantly different than with gadobutrol-enhanced MRI. The per-lesion sensitivity and specificity of gadofosveset-enhanced MRI was 0.99 and 0.91 when interpreted by an academic body radiologist. There was added value of delayed phase imaging to the diagnostic accuracy of reader interpretation ($p<0.001$).

5.1.4 Conclusions

Late gadolinium hyperintensity (LGH) of colorectal liver metastases (CRLM) is uncommon with gadofosveset-enhanced MRI compared to with gadobutrol-enhanced MRI. Gadofosveset-enhanced MRI has an excellent per-lesion diagnostic accuracy for diagnosing CRLM, partly due to the added value of delayed phase imaging. Gadofosveset-enhanced MRI may be helpful as a problem solving tool for indeterminate or difficult-to-diagnose lesions on conventional MR imaging.

5.2 INTRODUCTION

Colorectal cancer is the second leading cause of cancer deaths in the developed world (3). Most deaths are related to metastatic disease, most commonly to the liver. Advances in surgery and chemotherapy have significantly improved survival outcomes 5-year and 10-year survival for surgical candidates is 38% and 26%, respectively, according to one meta-analysis (23).

As surgery is now standard of care for all resectable candidates, preoperative imaging for detection and characterization of CRLM has become increasingly crucial. As discussed in Chapter 3, late gadolinium hyperintensity (LGH) is conventionally seen as a sign of benignity since hemangiomas (the most common solid benign lesion in the liver) demonstrates this sign (72, 158). However, as demonstrated in Chapter 3, LGH is a common finding in colorectal liver metastases (CRLM) on MRI with gadobutrol, an extracellular contrast agent, and is present in close to half of CRLMs. Given that the overlap in this sign between benign hemangiomas and malignant CRLMs, this may represent a significant imaging pitfall. This has important clinical implications particularly in the era of aggressive surgical resection.

In Chapter 4, we demonstrated that LGH of CRLM on gadobutrol-enhanced MRI is associated with tumour fibrosis on pathology. Although the mechanism by which this association occurs is not well understood, it has been postulated that this may be due to leakage of extracellular contrast into fibrotic tissues via the interstitium (84). If this is the case, then CRLMs would not demonstrate delayed enhancement with intravascular contrast agents, such as gadofosveset. A proof-of-concept, pilot study (done by our group) in a small group of patients with various types of focal liver lesions suggests that hemangiomas demonstrate retention of contrast whereas CRLM do not on gadofosveset-enhanced MRI (133). If this is true, then we expect that MRI with intravascular contrast agents may have excellent diagnostic accuracy in the setting of CRLM. Excellent diagnostic accuracy has been previously described in the literature as a sensitivity > 90%, specificity > 90%, LR+ > 10, and LR- < 0.1 (157).

Based on this, we hypothesize that the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with intravascular contrast agents is less than 20% and significantly less than the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with extracellular

contrast agents. We additionally hypothesize that the diagnostic accuracy of reader interpretation of MRI with intravascular contrast agents is excellent (sensitivity > 90%, specificity > 90%, LR+ > 10, and LR- < 0.1) (157). We additionally hypothesize that there is significant added value of delayed phase imaging on MRI with intravascular contrast agents in reader interpretation.

Therefore, the aim of this study was to determine the prevalence of LGH in CRLM on gadofosveset-enhanced MRI in a prospective cohort of patients with known colorectal cancer and a suspected focal liver lesion who were referred for a clinical liver MRI for diagnosis and/or staging. In addition, we aim to determine the diagnostic accuracy of a trained radiologist using gadofosveset-enhanced MRI in the diagnosis of CRLM in this cohort and to determine the added value of delayed phase imaging (5-minutes and 10-minutes post contrast injection) on MRI with gadofosveset-enhanced MRI.

5.3 METHODS

5.3.1 Patient Population

This study was an institutional REB-approved, Health Canada-approved, prospective clinical imaging trial. A total of 50 patients were actively recruited for participation in this study between August 31, 2013 and March 31, 2016. The sample size was determined based on a desired confidence interval of 95% and a maximum margin of error of 10%. We assumed the prevalence of CRLM to be approximately 80% and the sensitivity and specificity to be approximately 90%. Based on these assumptions, the number of lesions required is 172, using the formula for sample size calculation described by Hajan-Tilaki in 2014 (178). However, we expect an average of 3-5 lesions/patient. Therefore, we need a patient population of 35 to 58.

Inclusion criteria for the study included patients with pathology-confirmed colorectal cancer (either biopsy or resection of the primary colorectal cancer) with suspected liver lesions seen on ultrasound or CT who were referred for a clinical MRI of the liver by a hepatobiliary surgeon for diagnosis and/or staging of CRLM.

Patient recruitment was initiated by the referring hepatobiliary surgery, but final recruitment and informed consent was obtained by a researcher not directly involved in the patient's clinical care.

Patients received standard of care noncontrast MRI, including T1 in-/out-of-phase imaging, T2-weighted imaging, and diffusion-weighted imaging as part of their routine clinical MRI. They also received standard of care contrast-enhanced MRI with either extracellular contrast agent, gadobutrol, or hepatobiliary specific contrast agent, gadoxetic acid, depending on the clinical indication and radiologist and surgeon preference. They then received an additional contrast-enhanced MRI with intravascular contrast agent, gadofosveset, within 1 month of the clinical MRI as part of the research protocol. All patients were recruited into the study prior to acquiring the clinical MRI in order to ensure that the results of the clinical MRI did not bias recruitment.

5.3.2 Imaging protocol

All imaging (both clinical and research MRIs) were performed on a 3.0 Tesla (Philips Achieva™) magnet with an eight-channel body phased array coil covering the entire liver.

As part of the standard clinical MRI, patients receive T2-weighted (axial T2 non-fat saturated imaging: TE 80, TR~2000, Flip angle 90 degrees; axial T2 fat saturated imaging: TE 90, TR~2200, Flip angle 90 degrees; axial T2 fat saturated imaging: TE 200, ~TR 2700, Flip angle 90 degrees), and diffusion-weighted imaging (TE 60, TR ~2500, Flip angle 90 degrees, B values 0, 50, and 1000).

As part of the gadofosveset-enhanced research protocol, all patients receive a 10mL intravenous dose of gadofosveset at 0.25mmol/mL. Three-dimensional, fat-suppressed, spoiled gradient-echo axial T1-weighted (TE: 1.4ms, TR: 3.0ms, flip angle 10 degrees) contrast-enhanced imaging with short breath holds was performed. Parallel imaging was used. Images were acquired in the precontrast, arterial, portovenous, 2-min, 5-min, and 10-min delayed phases. Gadofosveset (Ablavar®, Lantheus) was commercially available in Canada at the time the patients were being recruited and scanned in this study. However, it has subsequently been withdrawn from the Canadian market as of 2017.

5.3.3 Gold Standard

The gold standard for determining whether a lesion was benign or malignant was pathology post-surgery, or if unavailable, follow-up imaging. If patients underwent hepatectomy, then the final diagnosis of lesions was confirmed using pathology. If follow-up imaging is taken as the gold standard, then malignancy was defined as either $\geq 20\%$ increase in size or $\geq 30\%$ decrease in size with chemotherapy with a change in size of at least 5mm, as per RECIST 1.1 definitions of response to chemotherapy. Benignity was defined as no change in size (allowing for 5mm or 5% measurement error, whichever is greater) over a follow-up interval of at least 6 months in the absence of chemotherapy treatment. The follow-up interval can be taken as before or after the research MRI (eg. if a lesion was stable for 6 months prior to the research study in the absence of chemotherapy, this can be considered a benign lesion as per the study definition). Any lesions that could

not be confirmed as benign or malignant based on the gold standard definitions were excluded from the study.

5.3.4 Prevalence LGH on gadofosveset-enhanced MRI

The lesions-liver contrast-to-noise ratio (CNR) of all solid focal liver lesions (both solid benign lesions and CRLMs) was determined on 10-minute delayed phase imaging on MRI with intravascular contrast agent, gadofosveset. The mean CNR as well as the prevalence of lesions demonstrating LGH (defined as $\text{CNR} > +2.6$, based on Chapter 3) were calculated for both CRLMs and for solid benign lesions on 10-minute delayed phase imaging was calculated using the same methods as described in Chapter 3.

We compared the mean CNR and the prevalence of LGH on 10-minute MRI with gadofosveset in this prospective cohort with the mean CNR and the prevalence of LGH on 10-minute MRI with gadobutrol previously established in the retrospective cohort in Chapter 3 using a non-paired Student's t-test.

The ability of CNR on 10-minute delayed phase MRI with intravascular contrast agent, gadofosveset, as a predictor of malignancy was determined over a range of CNR cutoffs (from $\text{CNR} = -65$ to $\text{CNR} = +65$). In order to account for the effects of clustering, calculations were weighted by patient. This was done using the technique described by Genc et al (2005) where each lesion was given a weight equivalent to $1/(\text{number of solid lesions included for each given patient})$, such that the total weight of all the lesions in any given patient is equal to 1 (7).

Receiver operating characteristics (ROC) curves for CNR at 10-minute delayed phase gadofosveset-enhanced MRI as a predictor of CRLM were plotted. An ROC curve is a plot of 1-specificity (x-axis) versus sensitivity (y-axis) (179). The area under this curve (AUC) represents the probability that a randomly chosen disease subject will be correctly rated as diseased than a randomly chosen non-disease subject (179). Therefore, the AUC under an ROC curve is a measure of the accuracy of the test (179). The trapezoid method was used to approximate the area under the curve (AUC) in this study.

Statistical analyses were performed on SPSS (IBM SPSS Statistics for Macintosh, Version 22.0, 2013. Armonk, NY: IBM Corp.). Results were considered statistically significant at $P < 0.05$.

5.3.5 Reader diagnostic accuracy of gadofosveset-enhanced MRI in the diagnosis of CRLM

In order to detect and select lesions for characterization, information on the noncontrast series (T1 in-phase and out-of-phase, T2, and diffusion) as well as the gadofosveset-enhanced series were used. All lesions, excluding simple cysts (which do not enhance on contrast-enhanced imaging), were measured in their largest axial diameter on a single 2D axial image showing the lesion at its largest diameter. All lesions less than or equal to 10mm identified in this manner were assigned a number. Up to 5 lesions are randomly chosen for characterization using a random number generator. The same procedure is performed for all lesions greater than 10mm. Therefore, up to 5 small lesions ($\leq 10\text{mm}$) and up to 5 large lesions ($> 10\text{mm}$) were chosen for characterization. These lesions are labeled on the portovenous phase as “Lesion 1”, “Lesion 2”, etc. in order of their appearance from most superior to most inferior on axial imaging. We chose to limit the number of lesions to be characterized to improve feasibility for the radiologist interpreting the study. We chose to stratify for small and large lesions to account for the known greater diagnostic accuracy with larger lesions.

A board-certified, expert (> 10 years of experience reporting body MRIs), academic body radiologist interpreted the MRI's using standard PACS software. For each patient, the reader was asked to fill out a form characterizing each labeled lesion as benign, malignant, or indeterminate. The reader performed two interpretations for each patient. The first interpretation was performed using the noncontrast imaging (T1, T2, and in-phase and out-phase imaging, and diffusion-weighted imaging) as well as the precontrast arterial and portovenous phase of the gadofosveset-enhanced imaging. The reader then performed a second interpretation using the same sequences, but with the additional delayed phase gadofosveset-enhanced imaging (5-minutes and 10-minutes post-contrast). This was done in order to determine the added value of the delayed phase images.

Per-lesion sensitivity, specificity, and diagnostic accuracy of MRI with intravascular contrast agent, gadofosveset, for diagnosing CRLM were determined. This was done using conservative methods assuming that lesions classified as indeterminate were incorrect.

The likelihood ratio of CRLM for lesions classified as malignant (LR+), benign (LR-), or indeterminate (LR±) with MRI with intravascular contrast agent, gadofosveset, was determined. This was done using methods previously described in the literature (180).

Multinomial logistic regression was performed to determine the added value of delayed phase imaging. Multinomial logistic regression is a type of regression model that allows for multiple nominal discrete outcomes, unlike a binary logistic regression model (181). The multinomial logistic regression model was used in this study because the reader interpretation of the gadofosveset-enhanced MRI had three possible outcomes (benign, indeterminate, malignant). The odds ratio of a lesion being classified differently with delayed phase compared to without delayed phase was determined based on the model.

A generalized estimating equation was performed to determine whether the effect of clustering was significant. If the effect of clustering was significant based on the generalized estimating equation, then this would be included in the model. If the effect of clustering was not significant based on the generalized estimating equation, then this would not be included in the model.

All statistical analyses were performed using R statistical software for Mac OS X GUI (R Foundation for Statistical Computing, 2016, Version 3.4.3 GUI 1.70 El Capitan build).

5.4 RESULTS

5.4.1 Patient Demographics

There were 50 patients who were included in the study. The average age was 59 years (SD: 12 years, Range: 32 -70 years). There were 32 men and 18 women. Among the 50 patients who met inclusion criteria for the study, 2 patients did not have any focal liver lesions (1 patient did not have any lesions at all and 1 patient had multiple peritoneal deposits on the liver surface, but no true liver lesions). These patients were excluded from analysis. Therefore, there were 48 patients for whom analysis was performed. The patients had a combined total of 253 CRLMs and 29 benign solid lesions available for

analysis. The mean time between standard of care clinical MRI and research MRI with gadofosveset was 11 days.

5.4.2 LGH on MRI with intravascular contrast agent, gadofosveset

The mean lesion-liver CNR on 10-minute delayed phase MRI with intravascular contrast agent, gadofosveset was -8.3 (95% CI: -12.4 to -4.1) for CRLMs versus $+10.6$ (95% CI: $+4.7$ to $+16.6$) for solid benign lesions. The mean CNR of CRLMs on 10-minute delayed phase MRI with the intravascular contrast agent, gadofosveset, in this prospective cohort was significantly different from the mean CNR of CRLMs on 10-minute delayed phase MRI with extracellular contrast agent, gadobutrol, previously established in Chapter 3 ($p=0.001$).

The prevalence of LGH of CRLMs was 11.0% versus 76.1% for solid benign lesions. (Table 5.1, Figure 5.1-5.2). The prevalence of LGH of CRLMs on 10-minute delayed phase MRI with the intravascular contrast agent, gadofosveset, in this prospective cohort was significantly different the LGH of CRLMs on 10-minute delayed phase MRI with extracellular contrast agent, gadobutrol, previously established in Chapter 3 ($p<0.001$).

The ROC curve for CNR on 10-minute delayed phase MRI with gadofosveset as predictor of malignancy using MRI is shown in Figure 5.3. The AUC was 0.85 for the gadofosveset as determined using the trapezoid method. The sensitivity and specificity of LGH (CNR $> +2.6$ on 10-minute delayed phase MRI with gadofosveset) as a predictor of malignancy were 0.88 and 0.76.

Figure 5.1: Example of hemangioma in 51-year-old male on 10-minute delayed phase MRI with (a) extracellular contrast agent, gadobutrol, and (b) intravascular contrast agent, gadofosveset. The MRI scans were obtained 9 days apart.

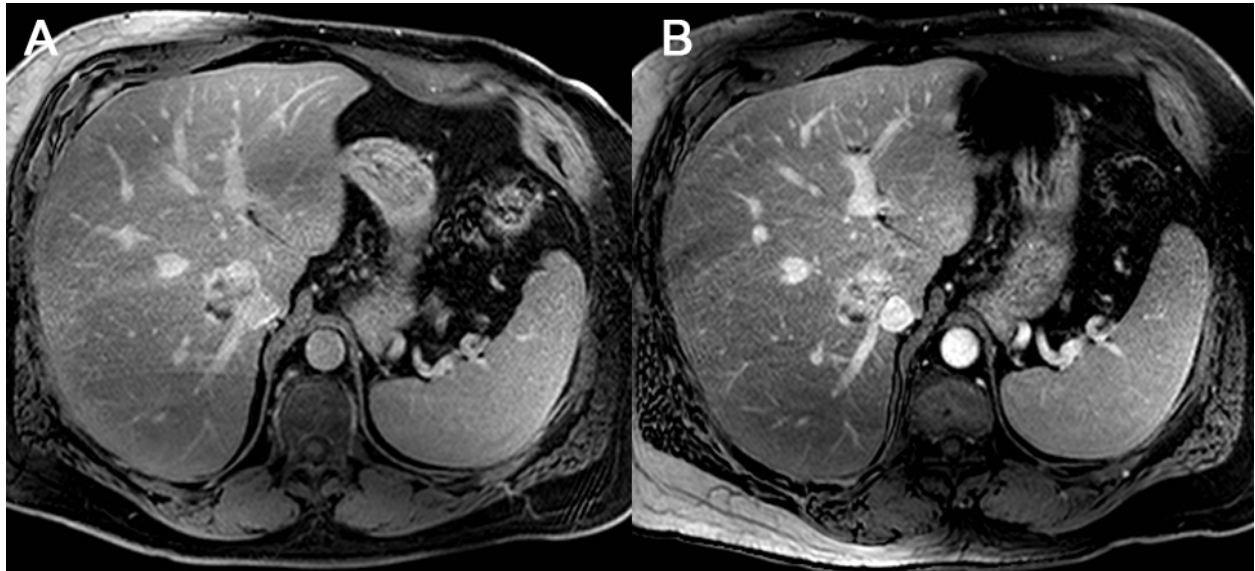


Figure 5.2: Example of colorectal liver metastasis in 43-year old female on 10-minute delayed phase MRI with (a) extracellular contrast agent, gadobutrol, and (b) intravascular contrast agent, gadofosveset. The MRI scans were obtained 6 days apart.

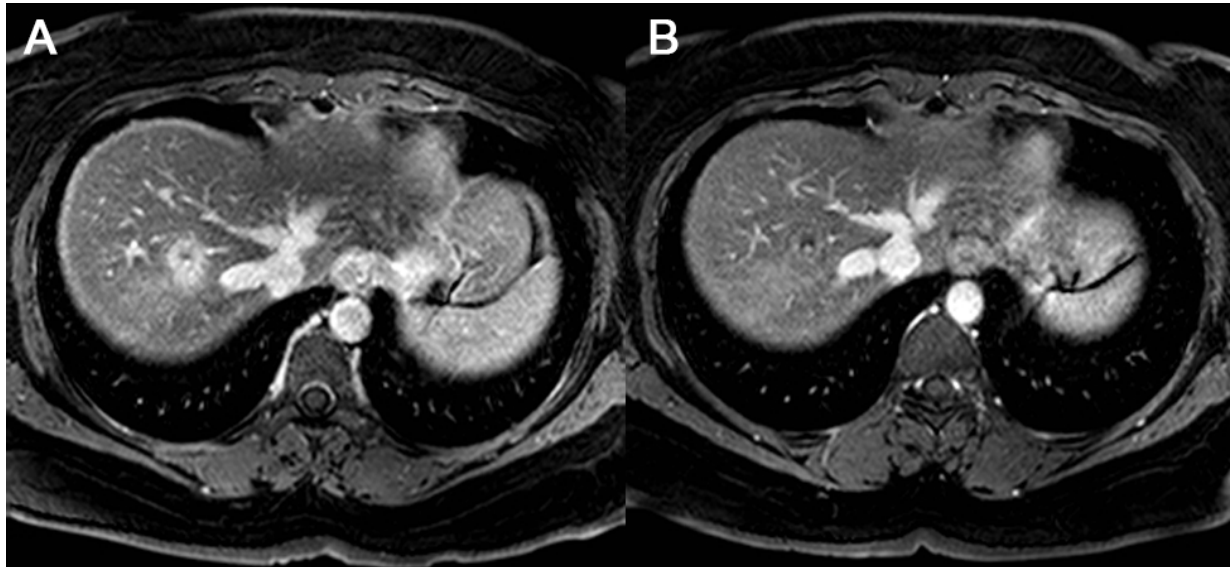
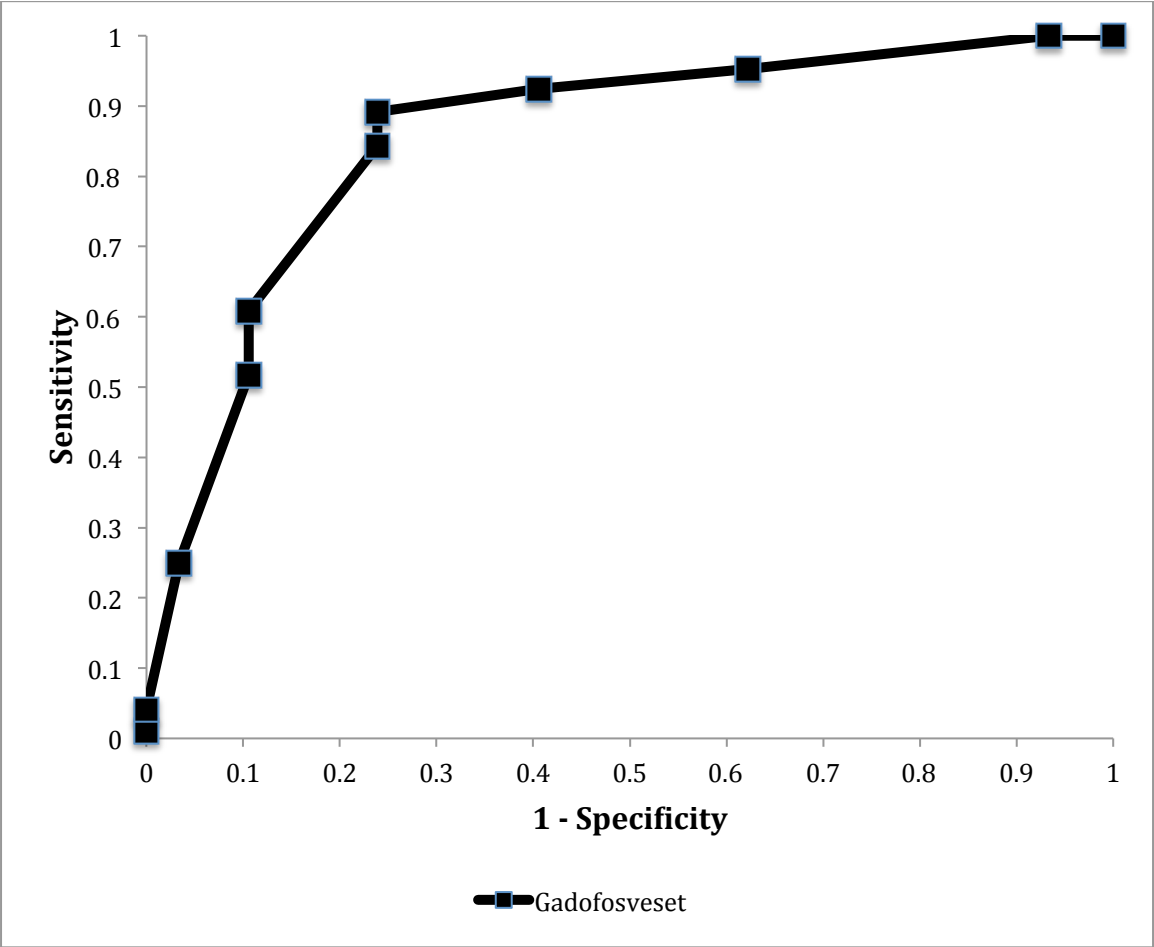


Table 5.1: Mean contrast-to-noise ratio (CNR) and prevalence of late gadolinium hyperintensity (LGH) on 10-minute delayed phase MRI with intravascular contrast agent, gadofosveset, for solid benign and CRLM lesions in prospective cohort of patients with known colorectal cancer

	CRLMs (N=38 patients, n=253 lesions)	Solid Benign Lesions (N= 29 patients, n=15 lesions)
Mean CNR	-8.3 (95% CI: -12.4 to -4.1)	+10.6 (95% CI: +4.7 to +16.6)
Prevalence of LGH	11.0% (95% CI: 3.4% to 18.6%)	76.1% (95% CI: 59.2% to 100.0%)

Figure 5.3: Receiver operating characteristics (ROC) curves for contrast-to-noise ratio (CNR) on 10-minute delayed phase as a predictor of colorectal liver metastases using MRI with intravascular contrast agent, gadofosveset. The area under the curve is 0.85. The sensitivity and specificity of LGH as a predictor of malignancy at CNR = +2.6 is 0.88 and 0.76 respectively.



5.4.4 Reader diagnostic accuracy of gadofosveset-enhanced MRI for CRLM and the added value of delayed phase imaging

Among the 50 patients who met inclusion criteria for the study, 2 patients did not have any focal liver lesions (1 patient did not have any lesions at all and 1 patient had multiple peritoneal deposits on the liver surface, but no true liver lesions). These patients were excluded from analysis. Therefore, there were 48 patients for whom analysis was performed. There were 216 lesions that were selected for per-lesion characterization.

The per-lesion sensitivity, specificity, and accuracy of gadofosveset-enhanced MRI for diagnosing colorectal liver metastases were 0.99, 0.91, and 0.95 respectively (Table 5.2). The likelihood ratio of CRLM among lesions that were classified as malignant, indeterminate, and benign were 10.73, 0.53, and 0.007, respectively.

Using multinomial logistic regression, there was a difference in lesion classification with delayed phase imaging compared to without delayed phase imaging (Type 3 analysis of effects: Wald Chi-square = 18.4, df = 2, $p < 0.001$). The odds ratio of a lesion being classified as benign compared to indeterminate with delayed phase imaging compared to without delayed phase imaging was 5.80 (95% CI: 2.18 to 15.44). The odds ratio of a lesion being classified as malignant compared to indeterminate with delayed phase imaging compared to without delayed phase imaging was 6.92 (95% CI: 2.44 to 19.63). Based on the generalized estimating equation, the clustering effect in the model was not significant (alpha value: 0.247, $p=0.322$). Therefore, no additional adjustment for clustering was made.

Table 5.2: Per-lesion sensitivity, specificity, diagnostic accuracy, and likelihood ratios of gadofosveset-enhanced MRI in diagnosing colorectal liver metastases in a prospective cohort of patients with known colorectal cancer (N = 48 patients, n = 216 lesions).

	Without Delay	With Delay
Sensitivity	0.97	0.99
Specificity	0.85	0.91
Accuracy	0.82	0.95
LR+	6.44	10.73
LR±	0.24	0.53
LR-	0.04	0.007

*LR+, LR±, and LR- represent the likelihood ratio of disease given a positive test, an indeterminate test, and a negative test.

Table 5.3: Multinomial logistic regression of gadofosveset-enhanced MRI with and without delayed phase imaging in diagnosing colorectal liver metastases in a prospective cohort of patients with known colorectal cancer (N = 48 patients, n = 216 lesions).

	Odds Ratio (95% confidence interval)	<i>P</i>-value
Reader classification		
Benign	5.80 (2.18-15.44)	P< 0.001***
Indeterminate	Reference	
Malignant	6.92 (2.44 – 19.63)	

5.5 DISCUSSION

The prevalence of CRLMs that demonstrate LGH on MRI with intravascular contrast agent, gadofosveset, is 11.0%. This is significantly less than the prevalence of CRLMs (47.4%) that demonstrate LGH on MRI with extracellular contrast agent, gadobutrol, established in Chapter 3. The per-lesion reader diagnostic accuracy of MRI with intravascular contrast agent, gadofosveset, was excellent (sensitivity and specificity of 0.99 and 0.91, respectively). In particular, there was added value of the delayed phase in the reader interpretation of MRI with intravascular contrast, gadofosveset.

Aside from a few case reports performed by our group describing LGH in focal liver lesions with gadofosveset-enhanced MRI (133, 134), this is the first study to systematically describe the use of gadofosveset-enhanced MRI in the diagnosis of CRLM among patients with known colorectal cancer. Specifically, this is the first study to demonstrate that LGH is less prevalent with gadofosveset than with gadobutrol, that the presence of LGH of solid focal liver lesions has high sensitivity and specificity for excluding malignancy, and that the use of delayed phase gadofosveset-enhanced MRI may have utility in the diagnosis of CRLMs in patients with colorectal cancer.

With aggressive surgery for CRLM becoming increasingly common, there is a growing need for preoperative liver imaging with highly accurate per-lesion characterization. Although detection and sensitivity for diagnosis of CRLM is very high with newer hepatobiliary-specific contrast agents, distinguishing CRLM from benign lesions (ie. specificity) remains challenging (67, 182). This is particularly true with smaller lesions. Distinguishing CRLM from hemangiomas may be difficult with hepatobiliary-specific contrast agents due to the “pseudo-washout effect” (75, 123). Distinguishing CRLM from hemangiomas with extracellular contrast agents may be difficult due to the high prevalence of CRLMs that demonstrate LGH. The high diagnostic accuracy of gadofosveset-enhanced liver MRI for per-lesion diagnosis of CRLMs may be due to the ability to distinguish benign hemangiomas from CRLMs based on delayed phase imaging. The finding in our study that delayed phase gadofosveset-enhanced MRI provides significant added value to diagnostic accuracy corroborates this.

Combining the high sensitivity of hepatobiliary-specific contrast agents and the high specificity of gadofosveset-enhanced MRI may be the best method of optimizing both

sensitivity and specificity. Two recent studies described the use of liver MRI with a combined injection of gadofosveset and gadoxetic acid (183) (184). One of these studies demonstrated that readers were more likely to detect metastases on MRI using combined gadofosveset and gadoxetic acid than with gadoxetic acid alone and that this technique may have improved ability to differentiate metastases from hemangiomas. This may be due to the same phenomenon observed in our study: LGH of gadofosveset is prevalent with benign hemangiomas but not with metastases.

The exact mechanism by which LGH of CRLMs occur on MRI with either extracellular or intravascular contrast agents has never been directly studied. It has been postulated that CRLMs demonstrate late gadolinium enhancement on MRI with extracellular contrast agents due to leakage of contrast into fibrotic tumours via the extracellular space (73, 133). Since gadofosveset largely remains in the intravascular space, this leakage of contrast would not occur as much with gadofosveset (133, 134). This may be the mechanism by which CRLM demonstrate LGH with gadobutrol but not with gadofosveset. Nevertheless, there was still 11% of CRLMs, which demonstrated LGH. Future radiologic-pathologic correlation studies would be helpful in determining the mechanism for LGH in these patients.

Intravascular contrast agents are not generally used for diagnosis of focal liver lesions on MRI. However, intravascular contrast agents are routinely used for diagnosis of focal liver lesions on contrast-enhanced ultrasound. Contrast-enhanced ultrasound with intravascular contrast agents is commonly performed for characterization of lesions that are indeterminate on cross-sectional imaging due to its excellent sensitivity and specificity (73, 83). CRLM demonstrate washout of contrast in contrast on delayed phase imaging, compared to benign hemangiomas, which demonstrate retention of contrast (73, 83, 85). This same phenomenon may also be occurring with gadofosveset on MRI. Contrast-enhanced ultrasound can accurately distinguish hemangiomas from CRLMs. However, MRI has significant advantages over ultrasound: multiple lesions can be visualized at a time with MRI and some lesions that cannot be easily visualized on ultrasound due to lesion location and/or size can often be visualized on MRI (73). Gadofosveset-enhanced MRI may be a helpful tool for diagnosis, particularly in indeterminate or difficult-to-diagnose cases.

There are number of limitations of this study. Measurement of CNR is limited in this study. The use of phased array coil and parallel imaging will affect CNR measurements (165). In

addition, the dose of gadofosveset was not standardized based on weight, which could affect the concentration of contrast and therefore CNR. In addition, this study represents a small, single-institutional study. Further, larger studies are required for external validation and to determine whether gadofosveset-enhanced MRI can be used to improve clinical outcomes in this population. Direct comparison of the diagnostic accuracy of gadofosveset-enhanced MRI with CT and standard-of-care MRI with extracellular contrast agents and/or hepatobiliary-specific contrast agents are also required. Further studies are also required in order to determine how gadofosveset-enhanced MRI could potentially be used in clinical algorithms for diagnosis and staging.

Patients received the research MRI within 1 month of the standard of care MRI with a mean time between MRI scans of 11 days. No therapy was initiated between the two MRI scans. Therefore, we do not believe that the order of the MRI scans would be a potential source of bias in our study.

The patient population in this study was patients with known colorectal cancer referred for an MRI from a hepatobiliary surgery clinic. As such, the pre-test probability of CRLM was high in this population, which may improve the diagnostic accuracy, including the sensitivity and specificity, in this study (185). Further studies are required in populations with different disease prevalence (such as patients with a new diagnosis of colorectal cancer) in order to determine the diagnostic accuracy in other populations.

Given that there is high sensitivity and specificity of LGH for excluding malignancy, this specific sign may be useful as a quantitative tool for diagnosis. If this is the case, further work to develop computer-aided tools such as automated or semi-automated methods of determining LGH may be helpful. Other potential solutions may be to use gadofosveset-enhanced MRI as a problem-solving tool in indeterminate or difficult-to-diagnose cases.

At the time of study enrollment and performance MRI scans in this study, gadofosveset was a commercially available contrast agent in Canada. It has subsequently been removed from the market. It has also been removed from the market in the United States and in Europe. Unfortunately, this limits the practical applicability of gadofosveset-enhanced MRI for diagnosis of colorectal liver metastases in the future.

5.6 CONCLUSION

The prevalence of CRLMs that demonstrate LGH on MRI with intravascular contrast agent, gadofosveset, is 11.0%, which is significantly less than the prevalence of CLRLMs (47.4%) that demonstrate LGH on MRI with extracellular contrast agents determined in Chapter 3. The per-lesion reader diagnostic accuracy of MRI with intravascular contrast agent, gadofosveset, was excellent (sensitivity and specificity of 0.99 and 0.91, respectively) and there was significant added value of the delayed phase in the reader interpretation of MRI with intravascular contrast, gadofosveset.

CHAPTER 6 : CONCLUSIONS

6.1 REVIEW OF HYPOTHESES

6.1.1 Hypothesis 1

Conventional teaching is that late gadolinium hyperintensity (LGH) of colorectal liver metastases (CRLM) on MRI with extracellular contrast agents, such as gadobutrol, is uncommon and that the presence of this sign favours benign etiology such as hemangiomas (72). Although it is understood that some CRLM do not follow this pattern, the prevalence of CRLMs that demonstrate LGH has not been previously described (84).

We had hypothesized that LGH in CRMs on 10-minute delayed phase MRI with extracellular contrast agents is greater than 20% and had limited utility in excluding CRLM based on this sign alone.

In Chapter 3, we determined that LGH in CRLM on 10-minute delayed phase MRI with extracellular contrast agents is extremely common, making up close to half (47.4%) of all pathology-confirmed CRLM in a retrospective cohort of patients who received gadobutrol-enhanced MRI prior to hepatectomy. Therefore, LGH on MRI with extracellular contrast agents has limited utility in excluding CRLM.

6.1.2 Hypothesis 2

Given the results of our findings in Chapter 3, we were interested in knowing what was the association between LGH on MRI with extracellular contrast agents in CRLM and pathological findings. Tumour fibrosis is commonly described in the pathology literature in the setting of CRLM and is known to be the primary driver of pathological response and predictor of long-term outcomes post-hepatectomy in surgically resected patients (36, 45). Tumour fibrosis is known to be associated with long-term outcomes both among patients who have received prior chemotherapy and among patients who are chemotherapy naïve (37).

We had hypothesized that LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents is significantly correlated with the percentage of tumour fibrosis on post-hepatectomy specimens. We had additionally hypothesized that LGH on 10-minute delayed phase MRI is significantly associated with overall survival, after adjusting for known confounders.

In Chapter 4, we demonstrated that LGH of CRLM is significantly associated with tumour fibrosis and overall survival post-hepatectomy.

6.1.3 Hypothesis 3

It has been suggested that delayed enhancement of fibrotic tissues (including CRLM) on MRI with extracellular contrast agents may be due to leakage of contrast from the extracellular space into the fibrotic tumour via the interstitium, although no mechanistic studies to-date have been performed to confirm this in the setting of CRLM (73, 133, 134). If this is true, then an intravascular contrast agent, such as gadofosveset, should not demonstrate this phenomenon and LGH of CRLM on MRI with intravascular contrast agents would be rare (133, 134). Since there would be less overlap of imaging findings between CRLM and benign hemangiomas, the diagnostic accuracy of MRI with intravascular contrast agents for the diagnosis of CRLM may be excellent (133, 134).

We had hypothesized that the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with intravascular contrast agents would be less than 20% and significantly less than the prevalence of LGH in CRLMs on 10-minute delayed phase MRI with extracellular contrast agents. We had additionally hypothesized that the diagnostic accuracy of reader interpretation of MRI with intravascular contrast agents would be excellent (sensitivity > 90%, specificity > 90%, LR+ > 10, and LR- < 0.1) (157). We had additionally hypothesized that there is significant added value of delayed phase imaging on MRI with intravascular contrast agents in reader interpretation.

In Chapter 5, we determined that the prevalence of LGH of CLRM on MRI with intravascular contrast agents is uncommon (11.0%) compared to MRI with extracellular contrast agents (47.4%) determined in Chapter 3. The per-lesion reader diagnostic accuracy of MRI with intravascular contrast agent, gadofosveset, was excellent (sensitivity=0.99, specificity=0.91, LR+=10.73, LR-=0.007). There was significant added

value of the delayed phase in the reader interpretation of MRI with intravascular contrast, gadofosveset.

6.2 SIGNIFICANCE OF FINDINGS

6.2.1 Diagnosis of colorectal liver metastases

In Chapter 3, we determined that LGH of CRLM on gadobutrol-enhanced MRI is common and is present in close to half of CRLMs. Since this finding overlaps with benign hemangiomas, this may be a diagnostic pitfall (60). Radiologists interpreting MRI's of the liver in the setting of colorectal cancer should not use the presence of LGH on gadobutrol-enhanced MRI to exclude malignancy in the absence of other imaging features that suggest benignity.

In Chapter 5, we demonstrated that LGH of CRLM on MRI with gadofosveset is uncommon compared to MRI with gadobutrol. This may be useful in diagnosing CRLM, particular as a problem-solving tool in indeterminate lesions where accuracy diagnosis is crucial for surgical decision making. We demonstrated that delayed phase imaging with gadofosveset has added value over imaging with noncontrast sequences and the arterial and portovenous phases alone.

6.2.2 Prognostication of colorectal liver metastases

In Chapter 4, we demonstrated that LGH of CRLM on preoperative gadobutrol-enhanced MRI is associated with survival in both patients who underwent surgical resection and in patients who did not receive surgery. Therefore, LGH represents a noninvasive prognostic imaging biomarker of survival. Gadobutrol-enhanced MRI is commonly used for diagnosis and staging of CRLM and is a technique readily available at almost all major cancer centres. Measurement of LGH using contrast-to-noise ratio (CNR) is easily done on standard clinical PACS software. Therefore, this prognostic biomarker could be easily translated for use in clinical practice if its clinical utility can be established.

6.2.3 Mechanism of delayed enhancement in colorectal liver metastases

In Chapter 4, we demonstrated that LGH of CRLM on preoperative gadobutrol-enhanced MRI is associated with tumour fibrosis. In Chapter 5, we demonstrated that LGH of CRLM is seen with gadobutrol-enhanced MRI but not with gadofosveset-enhanced MRI, suggesting that the mechanism of LGH is via the extracellular leakage of contrast with gadobutrol. This supports the hypothesis that leakage of contrast within fibrotic tissues may be the mechanism by which LGH of CRLM occurs.

CHAPTER 7 : DISUCSSION AND FUTURE DIRECTIONS

7.1 NOVELTY OF THE WORK

In Chapter 3 of this thesis, we present the first study to describe the prevalence of LGH of CRLM on gadobutrol-enhanced MRI. Although LGH of CRLM tumours has been previously described in the literature and is a phenomenon familiar to many abdominal radiologists, the prevalence has never previously established.

In Chapters 4, we present the first study to show the association of LGH of CRLM with tumour fibrosis and with overall survival. Prior studies have demonstrated the association between tumour fibrosis (on pathology specimens post-hepatectomy) and survival (36). However, no previous studies have described the association between LGH and tumour fibrosis or the association between LGH and survival.

In Chapter 5, we present the first study to describe the prevalence of LGH of CRLM with gadofosveset-enhanced MRI and the diagnostic accuracy of gadofosveset-enhanced MRI for the diagnosis of CRLM in patients with colorectal cancer. Prior pilot studies performed by our group have described a few cases of gadofosveset-enhanced MRI in the setting of CRLM (133, 134). However, this is the largest cohort of patients with CRLM who have received gadofosveset-enhanced MRI to date and the only study to systematically study the prevalence of LGH or the diagnostic accuracy of gadofosveset-enhanced MRI in this population.

7.2 DISCUSSION OF RELATED WORK

7.2.1 Diagnosis of colorectal liver metastases

The work presented in Chapter 3 and 5 represent the first studies to demonstrate that LGH of CRLM on gadobutrol-enhanced MRI is common (47% of CRLM), LGH of CRLM on gadofosveset-enhanced MRI is not common (12% of CRLM), and gadofosveset-enhanced MRI may be useful in diagnosis of CRLM.

There have been two studies to date describing the use of gadofosveset-enhanced MRI for the diagnosis of focal liver lesions. One study was a small proof-of-concept study previously performed by our group and described 12 patients with a range of focal liver lesions, which included CRLM but also included other metastases, adenomas, hemangiomas, and focal nodular hyperplasias (133). Another study was a pictorial essay published by our group, which described the appearance of 10 benign and malignant focal liver lesions (hemangioma, focal nodular hyperplasia, adenoma, hepatocellular carcinoma, cholangiocarcinoma, and metastases from colorectal, pancreatic, breast, renal cell, and neuroendocrine tumours) (134). The study presented in Chapter 5 of this thesis represents the largest cohort of patients who have received gadofosveset-enhanced MRI for the diagnosis of CRLM in patients to-date.

Two additional studies have described the use of combined gadoxetate and gadofosveset-enhanced MRI for the diagnosis of focal liver lesions. The first study was a small technical study by Bannas et al (2016) describing the feasibility and parameter optimization of combined injection gadoxetate and gadofosveset for liver MRI (183). In this study, 11 healthy volunteers received a liver MRI with a dual injection of gadoxetic acid and gadofosveset. The patients in this group had several liver lesions including cysts and metastases. The authors noted that cysts and metastases demonstrated increased conspicuity on delayed phase imaging, as they are hypointense relative to the brightly enhancing liver on hepatobiliary phase. The same group recently published a larger study on a group of 91 patients (184). Based on this larger cohort, they found that metastases were hypoenhancing relative to the background liver on delayed phase whereas hemangiomas were isoenhancing (184). They determined that the sensitivity for correct differentiation of metastases from hemangiomas was improved compared to gadoxetic acid alone (184). These findings are consistent with the results presented in this thesis.

7.2.2 Prognostication of colorectal liver metastases

In Chapter 4, we determined that LGH of CRLM on gadobutrol-enhanced MRI is associated with survival post-hepatectomy. No prior study has demonstrated a relationship between LGH and prognosis in this population.

There have been other studies looking at the association of other imaging features and either response to chemotherapy or survival. For response to chemotherapy, the most widely used imaging criteria is the Response Evaluation Criteria in Solid Tumours (RECIST). RECIST uses size-based criteria to assess response pre- and post-administration of chemotherapy (137). There are a number of limitations to this technique. First, it can only be used pre- and post-chemotherapy and does not provide any prognostic or response information at the baseline study. Even in the setting of chemotherapy response, it is known that size does not always correspond to response, particularly with newer targeted chemotherapeutic agents and/or immunomodulating agents where the morphology of the tumours change but the size does not. In some cases the size of the tumour can increase (“pseudo-progression”) even when the tumour has responded well to chemotherapy (139, 144). Some studies have shown that RECIST does not correlate well with tumour viability(138, 186).

The modified RECIST (mRECIST) criteria was developed to incorporate some information about morphology in addition to the size-based criteria (140). Again, this is only used in the pre-/post-chemotherapy setting. However, mRECIST still shows poor correlation with tumour viability, although this may be better than with conventional RECIST alone (138).

Several morphological based techniques have been developed to address these issues. One study used CT-based morphological features such as arterial enhancement and the appearance of tumour-liver interface and demonstrated an association with long-term outcomes in patients who had received bevacizumab chemotherapy (146). The advantage of this technique is that it uses CT-based criteria, which may be more widely available, particularly in centres that do not have ready access to MRI (146). However, it requires qualitative assessment by an expert reader, which may limit its widespread use and possibly its intra-rater reliability (146). In addition, it has not yet been studied outside of the setting of CRLM treated with bevacizumab (146).

Several studies have looked at the ability of quantitative dynamic contrast-enhanced MRI (DCE-MRI) as a prognostic biomarker of CRLM (129, 132, 187). Some of these studies have shown that quantitative DCE-MRI may have the ability to predict response to chemotherapy with bevacizumab (129, 132, 187). However, these studies have been small in scale, largely because they require specialized imaging sequences that are not routinely performed as part of clinical management of CRLM (129, 132, 187). The use of specialized

imaging and experts who are able to process and interpret the results limits the clinical applicability of these techniques (129, 132, 187).

Some studies have looked at the ability of diffusion-weighted imaging (DWI) for prognosis of CRLM (122, 188). These studies have shown that apparent diffusion coefficient (ADC) will decrease with chemotherapy response (122, 188). However, this is not associated with long-term survival. DWI is also limited by technical difficulties including artifact and misregistration (111).

Some studies have shown that the change in metabolic activity seen on PET or PET-CT after chemotherapy correlates with pathological response and predicts outcomes after surgery (148, 189). However, PET or PET-CT does not predict long-term outcomes independent of chemotherapy (151). PET-CT is limited by technical difficulties due to misregistration and has limited utility in small lesions (under 1 cm) (28). An additional limitation is that PET and PET-CT is not always part of the routine imaging workup for patients with CRLM prior to hepatectomy (95).

A number of clinical, histopathology, and molecular biomarkers of prognosis in CRLM have been evaluated. There is increasing evidence to suggest that the “biology” of individual tumours is what determines prognosis and response to chemotherapy. For example, patients with “good” biology may respond well to surgery even with an R1 resection or with extrahepatic disease, clinical features that were historically considered contraindications to surgery (26, 27). Patients with “good” biology may also respond differently to chemotherapeutic agents, particularly targeted chemotherapies (46).

7.2.3 Mechanism of delayed enhancement in colorectal liver metastases

In Chapter 4, we demonstrated that LGH of CRLM with extracellular MRI contrast agents is associated with tumour fibrosis. Although some authors have postulated that LGH of CRLM on MRI may be due to tumour fibrosis, this has never been demonstrated in a larger study. However, late LGH is known to be associated with fibrosis in other disease processes. One study demonstrated that increasing tumour fibrosis in intrahepatic cholangiocarcinoma was associated with increasing LGH (68).

7.3 LIMITATIONS OF WORK

7.3.1 Diagnosis of colorectal liver metastases

In Chapter 3, we showed that the prevalence of CRLM that demonstrate LGH with gadobutrol is high. Although we suspect that this may be a diagnostic pitfall due to the overlap of imaging findings with hemangiomas, we did not directly measure the change in diagnosis, change in management or change in outcomes based on this finding. Further studies may be helpful in order to better understand how this imaging pitfall affects patient management.

Due to the retrospective nature of the study in Chapter 3, we were unable to control for confounders such as chemotherapy. Chemotherapy may affect the delayed enhancement of CRLMs. This needs to be addressed in prospective studies with MRIs performed pre- and post-chemotherapy.

In Chapter 5, we demonstrated that the prevalence of CRLM that exhibit LGH is lower with gadofosveset than with gadobutrol. We also demonstrated that diagnostic accuracy of CRLM with gadofosveset-enhanced MRI is good. However, we did not directly measure its clinical utility or how this finding may be used to change management and patient outcomes. For example, the clinical utility of gadofosveset-enhanced MRI may be most appreciated as a problem-solving tool if lesions are indeterminate on current stand-of-care imaging. Further studies are required in order to determine how gadofosveset-enhanced MRI may best be applied in clinical management algorithms.

Another limitation of the current thesis is that we did not directly compare the diagnostic accuracy of gadofosveset-enhanced MRI with standard of care contrast-enhanced MRI (with either extracellular contrast agents or with hepatobiliary-specific contrast agents) or contrast-enhanced CT. Future studies are required in order to compare gadofosveset-enhanced MRI with contrast-enhanced CT and contrast-enhanced MRI with standard of care extracellular and hepatobiliary-specific contrast agents.

Gadofosveset is expensive relative to conventional extracellular contrast agents and has less availability compared to conventional agents. In the time between the enrollment and obtaining the MRI scans in this study and the time of writing this thesis, gadofosveset has been removed from the market in Canada. This may limit knowledge translation in the

future. Cost-effective analyses as well as partnerships between clinical centres and industry are required in order to decrease costs and improve availability.

Chapter 5 represents a single institution, prospective study. Further larger studies involving multiple centres are required for external validation. Based on the initial results presented in this thesis, our group is currently working on a larger, multicentre retrospective study for external validation of our results.

7.3.2 Prognostication of colorectal liver metastases

In Chapter 4, we demonstrated an association between delayed enhancement of CRLM on MRI and long-term survival in a discovery cohort of surgical patients and a validation cohort of nonsurgical patients. However, this was a single-institution, retrospective study. Larger studies are required for external validation of these results.

In addition, prospective studies are required to control for confounding variables including both clinical confounders, such as type of chemotherapy, surgery, radiation, portal vein embolization, etc. as well as technical confounders such as magnet strength, dose of contrast agent, flip angle, fatty liver, etc.

Another limitation of this study is that we did not study how this prognostic biomarker can be used to change clinical management. Further studies are required in order to determine how LGH of CRLM can be used to optimize treatment including surgery and/or chemotherapy regimens. This would require prospective studies.

Further work is required to optimize techniques for measurement of LGH. In the current studies, delayed enhancement was measured as contrast-to-noise ratio (CNR) on 10-minute delayed phase gadobutrol-enhanced MRI. Further studies are required to optimize techniques. Specifically, studies should be performed to determine optimal timing of delayed phase sequences as well as measurement techniques for delayed enhancement.

One of the potential advantages of this technique is that it utilizes an imaging test that is already routinely performed in the clinical setting. Contrast-enhanced MRI is the standard

of care at many institutions, including our institution. Other imaging features may also have potential utility in prognostication. We recently performed a pilot project demonstrating that high T2 values of CRLM are also associated with good long-term outcomes (190). Combining multiple prognostic imaging variables to form an imaging prognostication score may provide greater risk stratification than a single imaging feature alone.

Hepatobiliary-specific contrast agents are now increasing in popularity for use in some patients with CRLM. As discussed in Chapter 1, hepatobiliary-specific contrast agents can be beneficial due to increased detection of metastases as a result of increased conspicuity of hypoenhancing metastases on a brightly enhancing background liver on hepatobiliary phase imaging (67). The work presented in this thesis would not be directly applicable to patients who receive MRIs with hepatobiliary-specific contrast agents. However, most hepatobiliary-specific contrast agents have a combined hepatobiliary-specific and extracellular mechanism. Therefore, we would expect that delayed enhancement with gadoxetate-enhanced MRI may also be associated with survival. Given this, we recently performed a preliminary study looking at late enhancement of CRLM in gadoxetate-enhanced MRI and survival. In this study, we demonstrated that increased enhancement of CRLM on preoperative gadoxetate-enhanced MRI was associated with survival (191).

7.3.3 Mechanism of delayed enhancement in colorectal liver metastases

Due to the retrospective nature of our cohort, we were unable to obtain imaging pre- and post-chemotherapy in all of our patients. Therefore, we were unable to determine how chemotherapy modulated delayed enhancement of CRLM. It is known from the pathology literature that tumour fibrosis is the main driver of pathological response to chemotherapy (36). Given the association of tumour fibrosis with delayed enhancement, we can hypothesize that delayed enhancement will increase post-chemotherapy in patients who demonstrate a good chemotherapy response. Further prospective studies are required in order to confirm this hypothesis.

Further studies are also required to determine the underlying pathophysiology and cancer biology that may be responsible for the observed phenomenon of delayed enhancement of CRLM. Although we demonstrated that tumour fibrosis is correlated to delayed enhancement, this was not done in a registered manner due to the retrospective nature of

the study. Further studies are required. Ideally, registered, high-resolution in-vivo and ex-vivo MRI should be registered and correlated to high-resolution whole-mount histology.

In addition, correlation with molecular biology features may also be performed. A recent paper demonstrated that decreased tumour fibrosis is related to increased molecular heterogeneity (ie. increase in mutational status) (48). Another recently published abstract suggests that tumour fibrosis may be related with KRAS wildtype tumours (192). In a pilot study performed by our group, the absence of LGH of CRLM on 10-minute delayed phase gadobutrol-enhanced MRI may correlate with an increasing number of somatic APC mutations. Other studies have determined that increasing number of APC mutations may be associated with poor prognosis and may partially explain the association between LGH and survival that we demonstrated in Chapter 4. If LGH of CRLM is reflective of tumour fibrosis as our findings in Chapter 4 suggest, then LGH may also reflect mutational status of tumours (193).

Finally, the work from this current thesis does not address how these findings can be used in the algorithms for patient management. Further studies are required in order to demonstrate how these findings may be used for decision-making in the clinical setting. For example, it may be possible that patients with CRLM that demonstrate delayed enhancement should receive different surgical and/or chemotherapy regimens than patients who do not.

7.4 FUTURE STUDIES AND DIRECTIONS

7.4.1 Technical Validation of LGH

An important next step would be to perform technical validation studies. We would need to optimize imaging parameters for measuring LGH in CRLM, including optimization of MRI parameters such as TE, TR, flip angle, use of multichannel coils, and parallel imaging. The pharmacokinetics of gadolinium in LGH in CRLM also needs further evaluation to better understand issues related to optimal contrast agent dose and optimal timing of MRI acquisition post-contrast injection. This would eventually lead to a prospective study

using standardized imaging parameters that can be reliably compared across different centres and different scanners.

Future studies are also needed to optimize measurement of LGH. In order to improve reliability, as well as possibly accuracy and precision, we would develop semi-automated or automated techniques of measuring LGH in tumours. In the work presented in this thesis, CNR measurements were performed using ROI analysis on a single axial slice. Three-dimensional measurements of LGH in a volume-based analysis may be helpful particularly with larger tumours that have internal heterogeneity.

7.4.2 Biological Validation of LGH

In this thesis, we performed an early retrospective radiologic-pathologic correlation, which provided some evidence that LGH of CRLM on MRI with extracellular contrast agents may be related to tumour fibrosis. However, the retrospective nature of the study has its limitations. Future studies using high-resolution, prospectively matched radiologic-pathologic correlation is required for improved spatial-resolution and to reduce sampling bias.

In addition, we did not perform radiologic-pathologic correlation in the cohort of patients who received MRI with intravascular contrast agents. Future studies to determine the histopathologic correlate of LGH in CRLM on MRI with intravascular contrast agents would also be helpful.

There is growing evidence that the genomics of tumours may have important clinical implications including natural history, response to treatment, and prognosis (40, 42, 43, 46, 50, 51). It is possible that LGH of CRLM on MRI with extracellular contrast agents may correlate with particular genomic signatures of tumours. A pilot study performed by our group suggests that absence of CRLM may be related to increasing numbers of APC mutations within the tumours (193).

7.4.3 Clinical Validation of LGH

Future studies should address the role of chemotherapy in LGH of CRLM. In particular, LGH of CRLM should be compared pre- and post-chemotherapy in order to determine how chemotherapy affects LGH. Chemotherapy regimens should be standardized and different

types of chemotherapy regimens should be compared. For example, cytotoxic chemotherapy regimens such as FOLFOX may have different effects on LGH than regimens that include anti-angiogenic agents such as bevacizumab. Although the pathology literature has traditionally assumed that tumour fibrosis may be related to response to chemotherapy, some newer studies have described pathologic evidence of tumour fibrosis in CRLM among patients that are chemotherapy naïve (37). Radiologic-pathologic studies pre- and post-chemotherapy would be helpful to shed light on this.

We demonstrated that LGH of CRLM in patients who received MRI prior to surgery was common and was associated with tumour fibrosis and overall survival. However, it is unclear whether this is also true in other patient populations. Future studies are required in order to determine this. Our group has recently performed a study in patients who were not surgical candidates. Based on the preliminary findings from this study, it appears that there is also an association between LGH of CRLM with extracellular contrast agents and overall survival among non-resectable candidates (194). Interestingly, the prevalence of LGH of the CRLM with extracellular contrast agents in this nonsurgical patient population was only 26%, which is less than the prevalence of LGH in the surgical population (Chapter 3) of 47% (194). We speculate that the difference in prevalence of LGH may be due to underlying biologic difference between the surgical and non-surgical population groups, with surgical patients likely having “better biology” than the non-surgical patients. Future studies involving different patient populations are required. Future multicentre studies would be helpful for external validation.

In Chapter 5 of this thesis, we presented the results of a diagnostic accuracy study for the use of gadofosveset-enhanced MRI in the diagnosis of CRLM among patients with known colorectal cancer referred from a hepatobiliary surgery clinic. This patient population has a high prevalence of CRLM, which may affect the results. Future studies are required in other patient populations, such as among general patients with colorectal cancer, in order to determine the diagnostic accuracy of gadofosveset-enhanced MRI for CRLM in a more general setting.

7.4.4 LGH with hepatobiliary specific contrast agents

Many centres are moving towards using hepatobiliary specific contrast agents, such as gadoxetate, for preoperative staging of CRLM, due to increased sensitivity of these agents for detection of lesions (56). Future studies are required in order to determine whether

LGH of CRLM with hepatobiliary-specific contrast agents is also associated with survival. Our group recently published a study, which provided preliminary evidence that this may be the case (191). Gadoxetate is classified as a hepatobiliary-specific contrast agent, but has a dual extracellular and hepatobiliary-specific component. It is possible that the extracellular component accounts for the LGH seen in this study and that the mechanism by which LGH occurs is the same as the mechanism of LGH with extracellular contrast agents presented in this thesis. Additional studies are required in order to validate this preliminary finding and to better understand the mechanism and pathophysiology by which LGH with hepatobiliary-specific contrast agents occurs.

7.5.5 Beyond LGH

Based on the results of this thesis, LGH in CRLM with extracellular contrast agents has potential as a possible imaging biomarker of prognosis. However, other imaging features on MRI may also represent possible imaging biomarkers. Future studies (such as radiomics studies) may help to elucidate some of these other features. This could eventually lead to an “imaging signature” similar to molecular signatures that could help predict prognosis and drive treatment decisions. The advantage of MRI biomarkers over molecular or histopathologic biomarkers is that MRI is noninvasive, is already routinely performed as part of the diagnostic and staging workup of CRLM, and can be performed at multiple time points (e.g. pre- and post-treatment).

7.5 THESIS IN THE CONTEXT OF THE BIOMARKER DEVELOPMENT FRAMEWORK

The work presented in this thesis involves a part of the development of late gadolinium hyperintensity as a semi-quantitative imaging biomarker of prognosis.

In Chapter 3, we established the presence of late gadolinium hyperintensity on MRI with extracellular contrast agents. We also identified the semi-quantitative technique of measuring CNR. Based on the framework for imaging biomarker development established by O'Connor et al and presented in section 1.8 of this thesis, this would fall under the discovery domain.

In Chapter 4, we established the unmet clinical need for late gadolinium hyperintensity on MRI with extracellular contrast agents as a biomarker of prognosis of CRLM. Based on the framework for imaging biomarker development, this would fall under the discovery domain. We also started preliminary work in the validation domain in a single institution. We performed preliminary technical validation by establishing target tumour enhancement as a measurement of late gadolinium hyperintensity, determining cutoff thresholds for target tumour enhancement and determining inter-rater reliability of target tumour enhancement. We also performed preliminary biological and clinical validation by determining the relationship of target tumour enhancement with tumour fibrosis and overall survival.

In Chapter 5, we established the unmet clinical need for late gadolinium hyperintensity on MRI with intravascular contrast agents as a diagnostic biomarker for excluding CRLM. Based on the framework for imaging biomarker development, this would fall under the discovery domain. We then established the sensitivity and specificity and diagnostic accuracy of this imaging biomarker in our population. Based on the framework for imaging biomarker development, this would fall under the biological and clinical validation component of the validation domain.

Therefore, the work presented in this thesis involves the very early stages of imaging biomarker development (the discovery domain and the early stages of the validation domain). As such, there is significant future work to be performed in the late validation and qualification stages.

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