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Colorectal Liver Metastases: (Neo)Adjuvant Chemotherapy and Prognostic Indicators

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

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ABSTRACT

The resection of colorectal liver metastases is the single most effective treatment modality. There is uncertainty regarding the role of chemotherapy when administered in addition to surgery. We hypothesized that chemotherapy was beneficial in those who underwent metastectomy. In addition, we hypothesized that an established clinical scoring system or immunohistochemical markers could be used to predict response to chemotherapy. We examined 103 patients who underwent potentially curative liver resections between 1988 and 2002. There was no clear demonstrable benefit from the indiscriminate addition of chemotherapy. Furthermore, thymidylate synthase and thymidine phosphorylase, individually or in combination, were not able to predict benefit attributable to chemotherapy. The Clinical Risk Score, however, was discriminatory in determining which patients were more likely to benefit from supplemental chemotherapy. We conclude that in addition to the resection of colorectal liver metastases, standard chemotherapy is most likely to benefit patients with low Clinical Risk scores.

ACKNOWLEDGEMENT

I would like to thank my supervisors, Drs. Linda Carroll, David Bigam and Charles Butts, for their unfaltering support and encouragement throughout this lengthy process. Each, in turn, selflessly gave of their time and expertise. Drs. Carroll and Butts at times may have rued the day they became involved in a project where their specialties were approached with a surgical mindset, but without their patience, assistance, and knowledge these ideas would never have reached fruition. Likewise Dr. Bigam may have had periods of regret in being the first surgeon to supervise a surgery resident through an epidemiology masters program, but his perseverance, support, and dedication allowed this project to happen. Although it is somewhat Machiavellian, I think that this resultant thesis demonstrates that their efforts were justified.

I appreciate the assistance of Doug Dover at the Alberta Cancer Board for his assistance in gathering data from the Alberta Cancer Registry.

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I would like to thank my supervisory committee: Drs. David Bigam, Linda Carroll, Charles Butts, and Stephen Newman, for their support, patience and expertise.

Finally, I gratefully acknowledge financial support from the Alberta Cancer Board Research Fellowship which, in conjunction with the Division of General Surgery, provided full salary support.

Most importantly, this work could not have been completed without the support, encouragement and patience extended to me by my wife, Sandra. These are but some of the many reasons why I dedicate this thesis to her.

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LIST OF ABBREVIATIONS

5-FU	5-fluorouacil
APC	adenomatous polyposis coli
CEA	carcinoembryonic antigen
CI	confidence interval
CRS	clinical risk score
CT	computed tomography
CTAP	computed tomography arterial portography
DCC	deleted in colon cancer
DFS	disease free survival
DPD	dihydropyrimidine dehydrogenase
HAI	hepatic arterial infusional
HR	hazard ratio
HRR	hazard risk ratio
IHC	immunohistochemical
IOUS	intra-operative ultrasound
MRI	magnetic resonance imaging
MSI	macrosatellite instability
OS	overall survival
PCR	Polymerase chain reaction
PD-EDF	platelet derived endothelial cell growth factor
PET	positron emission tomography
TP	thymidine phosphorylase
TS	thymidylate synthase
TTV	total tumor volume
VEGF	vascular endothelial growth factor

CHAPTER 1

Introduction

EPIDEMIOLOGY OF COLORECTAL CANCER

GEOGRAPHIC EPIDEMIOLOGY OF COLORECTAL CANCER

Worldwide, ten million new cancer* cases are diagnosed yearly. Of these, colorectal cancer is the fourth most common, accounting for approximately 783,000 or almost 8% of the new cancer cases reported in 1990[1-3]. These malignancies also account for significant mortality: 5.2 million deaths were attributable to cancer in 1990, with colorectal cancer being reported as the cause of death in between 400,000 and 500,000 people[2-4]. These cases are almost equally divided between genders with roughly 53% of cases in men and 47% in women[2-5].

Over the last 10 years, the global incidence rate of colorectal cancer has increased. In 2000, there were approximately 945,000 new cases of colorectal cancer diagnosed throughout the world. Refuting conventional belief that this disease is one of western culture; more than a third of these cases were in non-industrialized countries[2 4-6]. In the western world, colorectal cancer is the second most common malignancy as well as the second leading cause of cancer related death[3]. The majority (approximately 95%) of colorectal tumors diagnosed in the western world are sporadic in nature (i.e. not related to a genetic syndrome)[7].

In Europe, the incidence of colorectal carcinoma is 150,000 cases per year. Approximately 30,000 of these patients (20%) have metastatic disease at the time of first diagnosis. Each year 95,000 Europeans succumb to colorectal cancer.

In North America, approximately 1 in 20 people is afflicted with this disease[8]. The majority of these cases are in the United States, which has both the largest population as well as some of the highest incidence areas. In the U.S. colorectal cancer

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*all cancer statistics will exclude non-melanoma skin cancer

is the third leading cause of cancer-related mortality. Following only lung cancer and prostate cancer in men and lung cancer and breast cancer in women[9], it accounted for approximately 129,400 new diagnoses and 56,600 deaths (approximately 18%) in 1999. It is possible to separate colon cancer and rectal cancer for descriptive epidemiological purposes. Certain errors are inherent in this process however, making some of these epidemiological distinctions somewhat artificial. The main difficulty is related to the ICD-9 diagnostic coding system that is used to differentiate the two diseases. Inconsistent application of the ICD-9 codes in classifying the disease site is not uncommon. Often in the case of colon or rectal cancer the cause of death is reported as "cancer of the large intestine" which is then traditionally coded as ICD-9 153: colon cancer[10]. Also, the anatomical distinction as to what is labelled colon and what is labelled rectum is not always clear.

These concerns aside, when colon cancer is viewed as a distinct entity (i.e. selecting ICD-9 code 153); it is noted to be more prevalent in "developed" countries. The regions with the 10 highest age-standardized incidence rates of male colon cancer are located within the United States, Japan, Canada, and New Zealand. Although the orders and locations are somewhat altered, this translates to women as well[11]. The comparative incidence is lower in eastern Europe and lowest in Africa and south-east Asia[11].

When rectal cancer is viewed as a distinct entity (i.e. selecting ICD-9 code 154) the epidemiology of rectal cancer displays some subtle differences from that of colon cancer. There is a definite gender difference, with a 1.5-2.0 times greater incidence in males. The global distribution is somewhat altered as well with several European countries having incidence rates that rank within the top 10 globally. It is noteworthy

that European men living in Harare, Zimbabwe and Israeli women of both European and other descent have noticeably high incidence rates[2].

The geographic distribution of high and low incidence areas suggests that colorectal cancer is usually an acquired disease. Looking at some individual populations demonstrates the interaction of both acquired and hereditary components. This interaction is illustrated by Israeli Jews, in that stratification of this sub-population demonstrates the combination of environment and genetic predisposition involved in the development of colorectal cancer. Of this sub-population, Israeli Jews born in Europe have the highest incidence of colorectal cancer (22.5/100,000/year). Israeli Jews born in Israel have a lower incidence (18.1/100,000/year). Israeli Jews born in Africa or Asia have an even lower incidence rate (13.2/100,000/year). These three genetically related groups with differing incidence rates demonstrate the acquired component of this disease. Finally, Israeli Non-Jews, regardless of land of origin, have a much lower incidence rate (4.6/100,000/year), demonstrating that there is a distinct hereditary aspect to the development of colorectal cancer[12].

Epidemiology of Colorectal Cancer in Canada

The prominence of colorectal cancer in Canada mirrors that of the United States. In 2001, 134,100 new cases of cancer were diagnosed of which 68,600 were in men and the remaining 65,400 were found in women. Of these cancers, slightly more than 7% (17,174) were of colorectal origin, making it the fourth most common new cancer diagnosis behind lung cancer, breast cancer, and prostate cancer (Figure 1-1). When stratified by gender (Figures 1-2 and 1-3), the incidence of colorectal cancer is third in

both men and women. As is the case globally, the incidence in Canadian men (13.5%) is slightly greater than that in women (12.1%) [13].

Even though the number of new cases diagnosed each year is rising, the age-standardized incidence rate is actually decreasing and has been for over 15 years (Figuer 1-4). This discrepancy is largely due to the changing demographic in Canada combined with the fact that as a person increases in age, so does the likelihood of developing colorectal cancer (Figure 1-5). As the entire population ages, the number of new cases of colorectal cancer increases (Figures 1-6 and 1-7). This is also the case when looking at individual age cohorts, although within each age stratum the incidence is decreasing (Figure 1-8).

In Canada, cancer is a significant source of mortality, accounting for 894,000 potential years of life lost which is 29% of the annual total potential life years lost – more than any other cause. Among malignancies, colorectal cancer is the third leading cause of potential life years lost (84,000 years) following only breast cancer (95,000 years) and lung cancer (233,000 years)[13].

When expressed as individual cases, malignancies were responsible for 65,300 Canadian deaths in 2001. Colorectal cancer was the second leading cause of cancer-related death, responsible for approximately 10% of cancer-related mortality (Figure 1-9). Again, there was very little discrepancy between men (3,460 deaths = 10%) (Figure 1-10) and women (2,978 deaths = 9.7%) (Figure 1-11)[13]. Just as the age-standardized incidence is decreasing, so is age-standardized mortality (Figure 1-12). Yet, paralleling incidence, due to the ageing of the Canadian population as well as due to general population growth, the raw number of deaths continues to increase (Figures 1-13 and 1-14) even though age specific mortality is decreasing in all age brackets (Figure 1-15).

Fortunately, colorectal cancer treatment represents a minor success story in Canada. The age-standardized incidence and / or mortality rates of many malignancies have been increasing in Canada. Colorectal cancer is the one major malignancy where both the age-standardized incidence rate (Figures 1-16 and 1-17) and mortality rate (Figures 1-18 and 1-19) are decreasing irrespective of gender[13]. (Figure 1-20)

Epidemiology of Colorectal Cancer in Alberta

Within Canada, Alberta follows only British Columbia and Saskatchewan as having the lowest age-standardized incidence and mortality rates. (Figure 1-21) Provincial incidence rates for colorectal cancer were collected between 1995 and 1999. In men the incidence is reported to be 3323 per 100,000 over the past 4 years, while in women the incidence is reported to be 2552 per 100,000 over the past 4 years[13].

EPIDEMIOLOGY OF COLORECTAL CANCER - SURVIVAL

It is important to note that colorectal carcinoma is not a homogenous disease. This heterogeneity is emphasized when looking at mortality. Even though 5-year survival in patients with colorectal cancer is 40% overall[14], the five-year survival differs greatly when stratified by stage of disease. In patients who are diagnosed with lesions harboring stage 0 disease: either severe dysplasia or carcinoma-in-situ, 5-year survival after surgical treatment is approximately 100%. An 80-100% five-year survival can be expected in patients who have had a resection for a carcinoma classified as Stage I (Dukes A). As lesions become more extensive, survival decreases, leading to a 60%-85% 5-year survival in patients with a resected Stage II (Dukes B) lesion, and 30%-40% in those with Stage III disease (Dukes C). The diagnosis and treatment of stage

IV disease (Dukes D) has an abysmal prognosis with a 5-year survival of less than 5%. The poor prognosis of Stage IV disease is slightly misleading, since this stage can be differentiated into resectable and unresectable disease. Resectable Stage IV disease carries with it a 25-40% 5-year survival, while unresectable Stage IV disease has virtually no long-term survivors.

These disparate survival rates strongly reflect whether the disease in question is surgically removable, which in large part is determined by the presence or absence of distant disease (either hepatic or extra-hepatic). Even so, about two-thirds of patients presenting with colorectal cancer are able to undergo a potentially curative resection. Regrettably up to 50% of these patients will relapse and most will eventually succumb to their disease[15].

EPIDEMIOLOGY OF COLORECTAL CANCER – METASTATIC DISEASE

Approximately two-thirds of patients with colorectal cancer will be diagnosed with hepatic metastases at some point during the course of their disease[9]. Untreated, a patient with colorectal liver metastases has a median life expectancy of between 4 and 9 months, with as little as a 3% chance of being alive at 3 years[16]. Modest increases in survival have been demonstrated when metastatic disease was treated with chemotherapy without resection. Initially median survival was around 12 months, but this has been gradually increasing as newer agents are being used and new combinations of chemotherapeutic pharmaceuticals are being adopted. The introduction of irinotecan (CPT-11) and oxaliplatin are largely responsible for this increase.

In patients with colorectal liver metastases, resection has shown to be the single most beneficial treatment modality when measured in terms of increases in 5-year

survival. Studies have consistently reported 5-year survival between 25 and 40%, a dramatic improvement when compared to patients who did not undergo resection (whether they had chemotherapy or not)[17-21]. Unfortunately, due to both disease and patient related factors, only about 10% of patients will be eligible for a potentially curative resection.

Even with resection, there is an obvious need for improvements in treatment as 5-year survival is still well below 50%. The escalating survival seen in the current treatment of unresected metastatic disease, in combination with the results from adjuvant chemotherapy for node positive primary colorectal carcinoma have been extrapolated to resected colorectal liver metastases. Researchers and clinicians have started to use and investigate the benefits of adjuvant chemotherapy after liver resection. This idea was supported by the finding that the majority of patients who have had hepatic metastectomies recur; with the majority of recurrence isolated to or involving the remaining liver. Further support for the use of adjuvant chemotherapy came from pathological findings within resected liver specimens where as many as 35% of specimens contained clinically and radiologically (including intra-operative ultrasound) undetectable micrometastases.

Figure 1-1. Percentage Distribution of Estimated New Cases for Selected Cancer Sites (Both Sexes), Canada, 2001. (Source: http://www.ncic.cancer.ca)

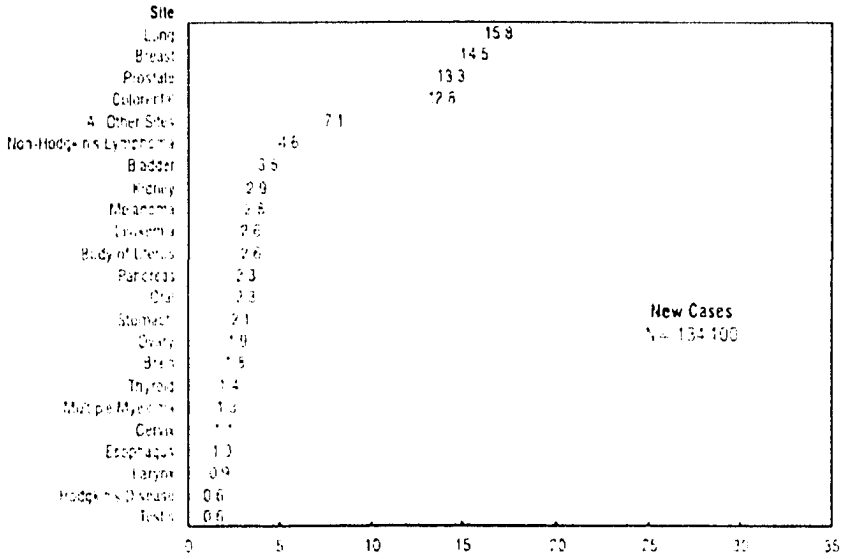


Figure 1-2. Percentage Distribution of Estimated New Cases for Selected Cancer Sites, Males, Canada, 2001. (Source: http://www.ncic.cancer.ca)

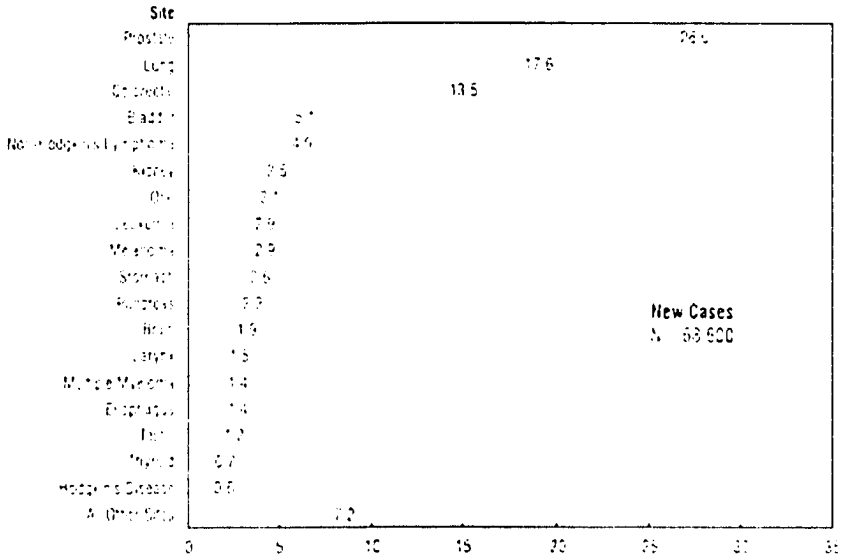


Figure 1-3. Percentage Distribution of Estimated New Cases for Selected Cancer Sites, Females, Canada, 2001. (Source: http://www.ncic.cancer.ca)

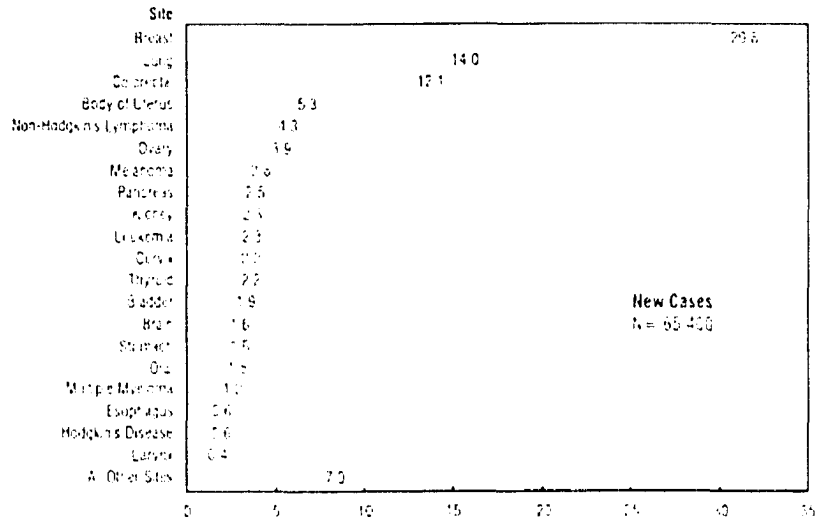
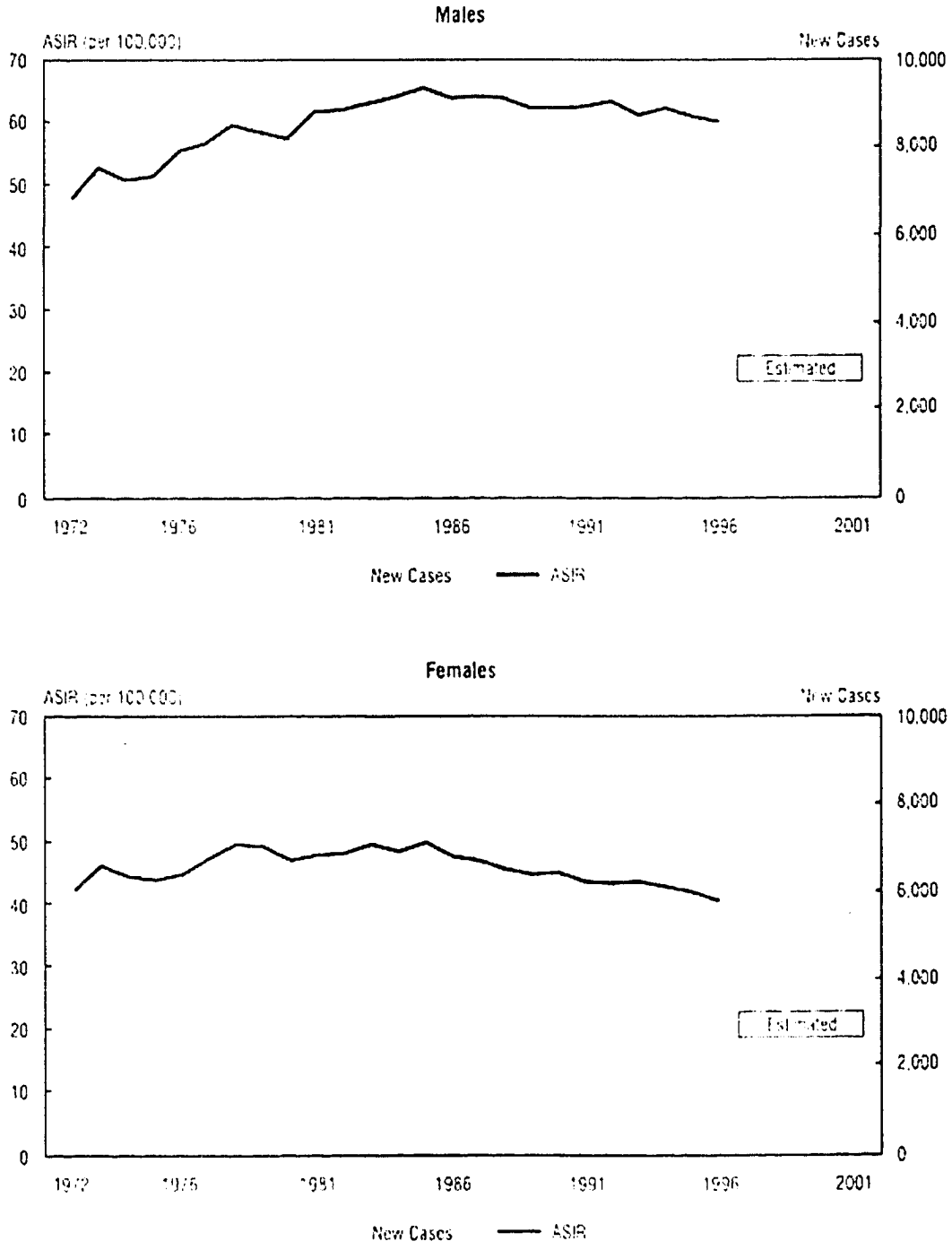


Figure 1-4. New Cases and Age-Standardized Incidence Rates (ASIR) for Colorectal Cancer, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)



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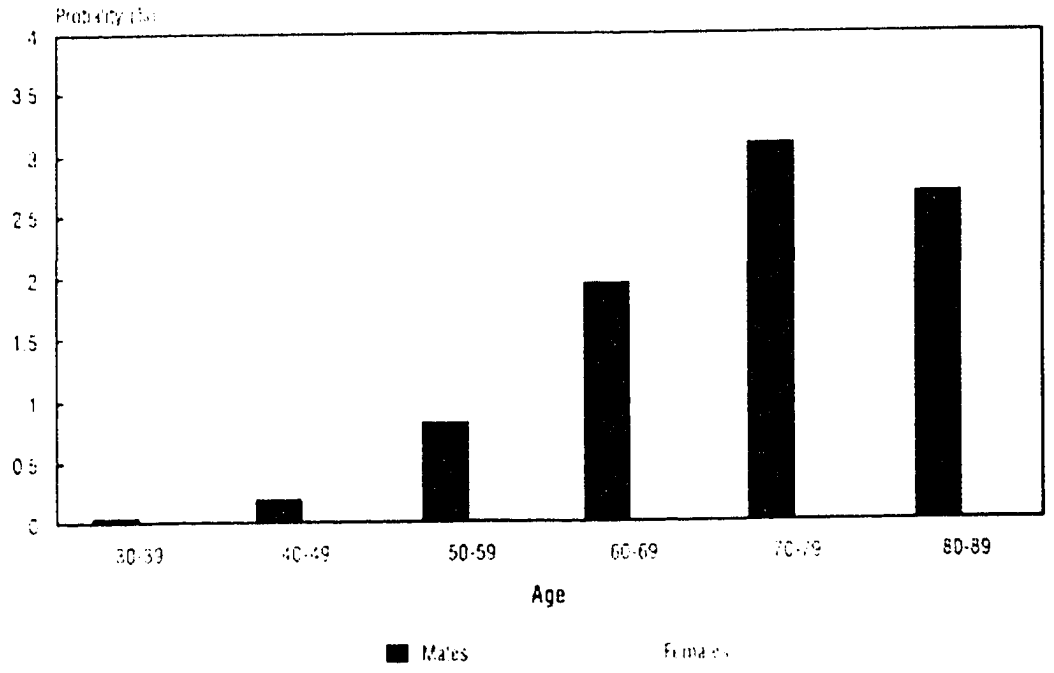

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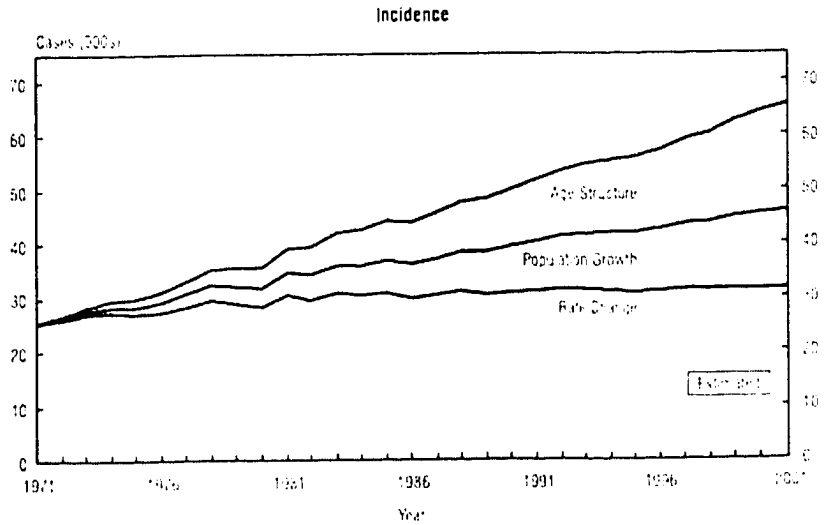
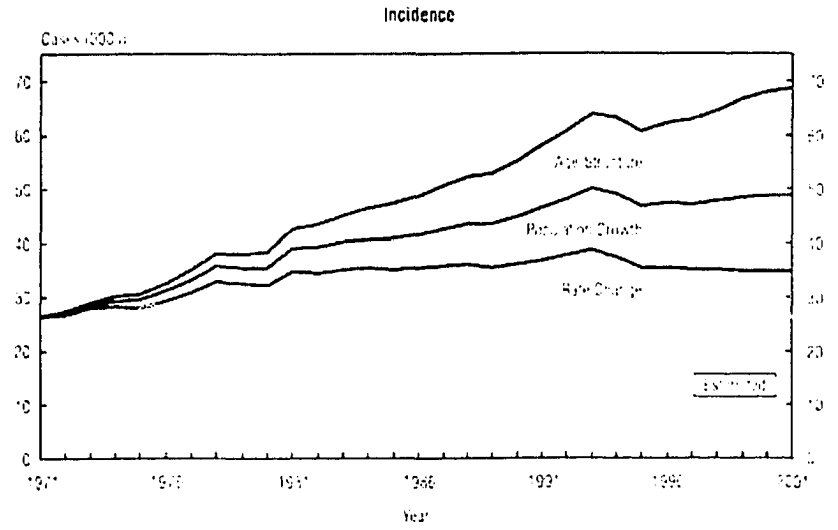


Figure 1-7. Trends in New Cases Attributed to Cancer Rate, Population Growth, and Population Age-Structure, All Cancers, All Ages, Males, Canada, 1971-2001. (Source: http://www.ncic.cancer.ca)



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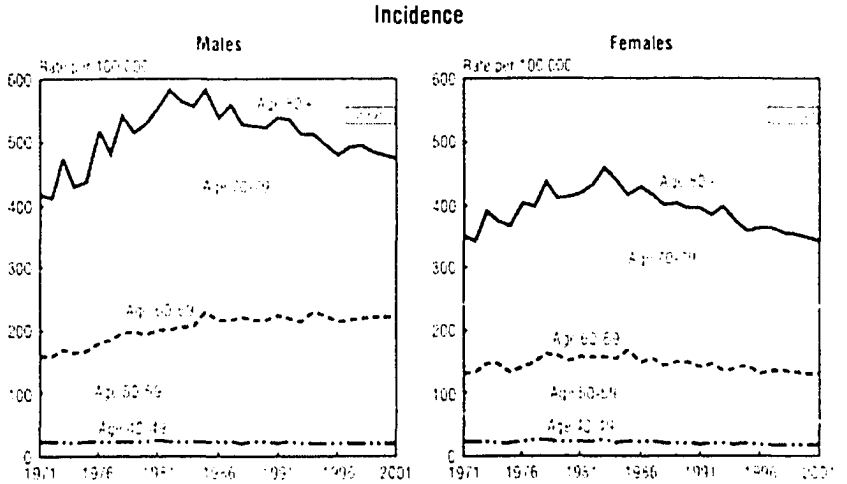

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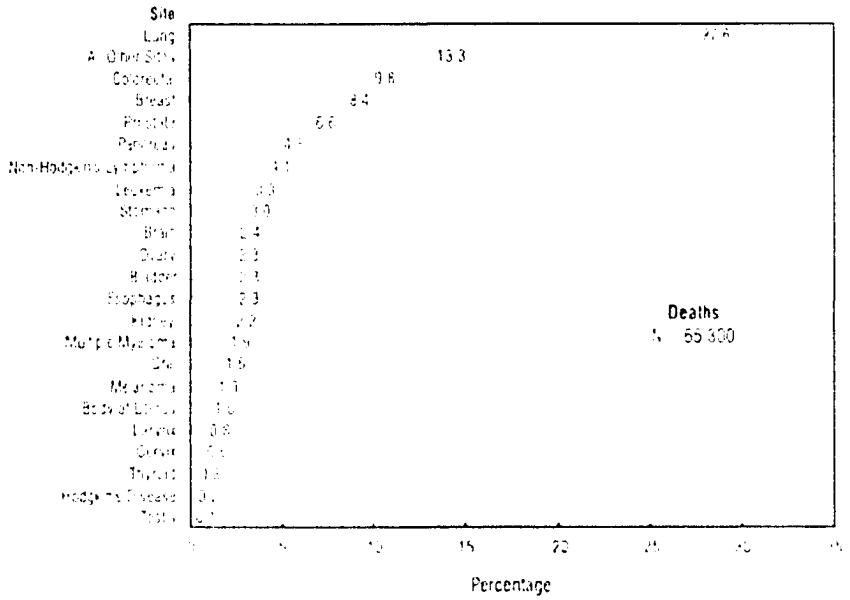


Figure 1-10. Percentage Distribution of Estimated Deaths for Selected Cancer Sites, Males, Canada, 2001. (Source: http://www.ncic.cancer.ca)

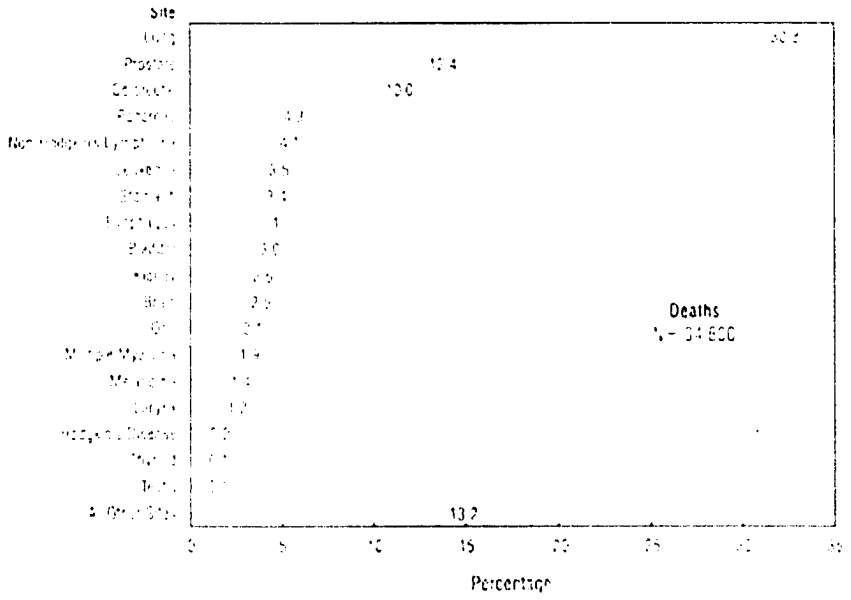


Figure 1-11. Percentage Distribution of Estimated Deaths for Selected Cancer Sites, Females, Canada, 2001. (Source: http://www.ncic.cancer.ca)

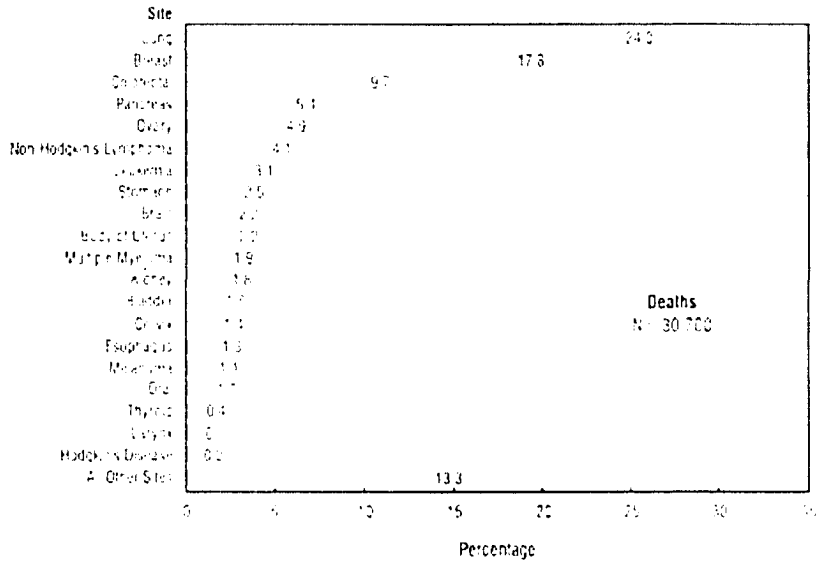


Figure 1-12. Deaths and Age-Standardized Mortality Rates (ASMR) for Colorectal Cancer, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)

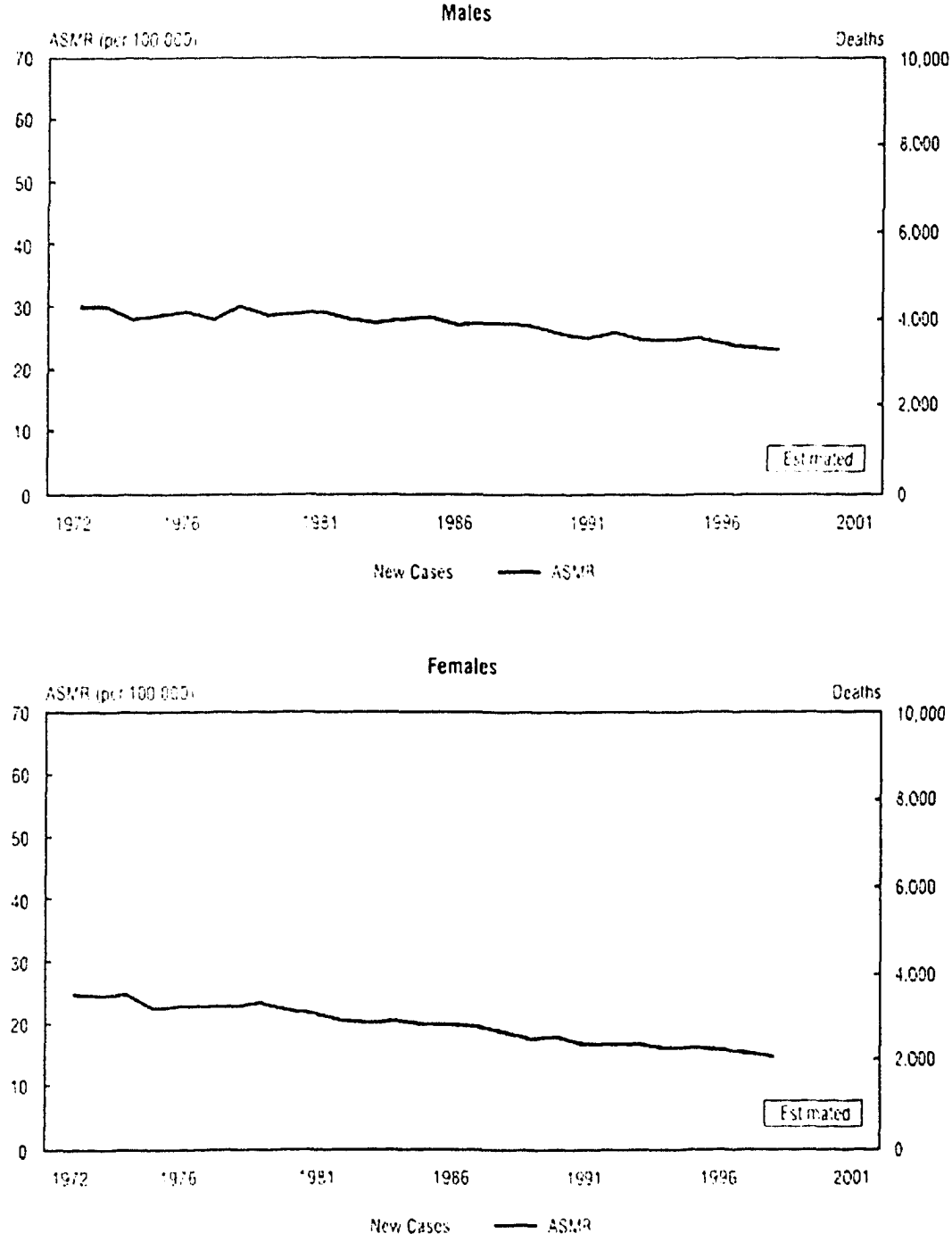


Figure 1-13. Trends in Deaths Attributed to Cancer Rate, Population Growth, and Population Age-Structure, All Cancers, All Ages, Females, Canada, 1971-2001. (Source: http://www.ncic.cancer.ca)

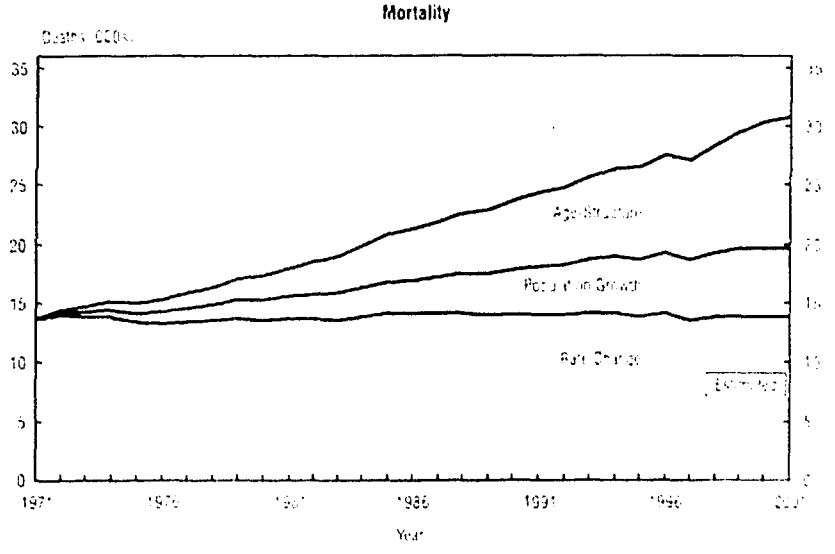


Figure 1-14. Trends in Deaths Attributed to Cancer Rate, Population Growth, and Population Age-Structure, All Cancers, All Ages, Males, Canada, 1971-2001. (Source: http://www.ncic.cancer.ca)

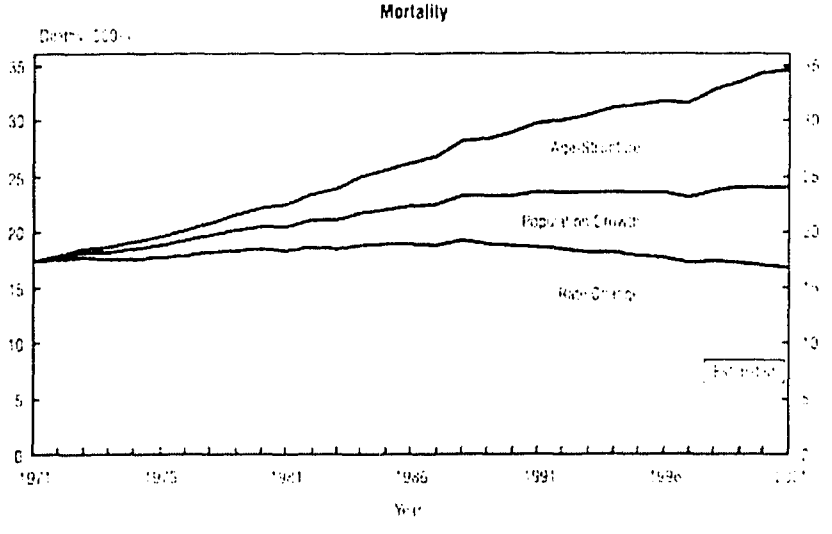


Figure 1-15. Age-Specific Mortality Rates, Colorectal Cancers, Canada, 1971-2001.

(Source: http://www.ncic.cancer.ca)

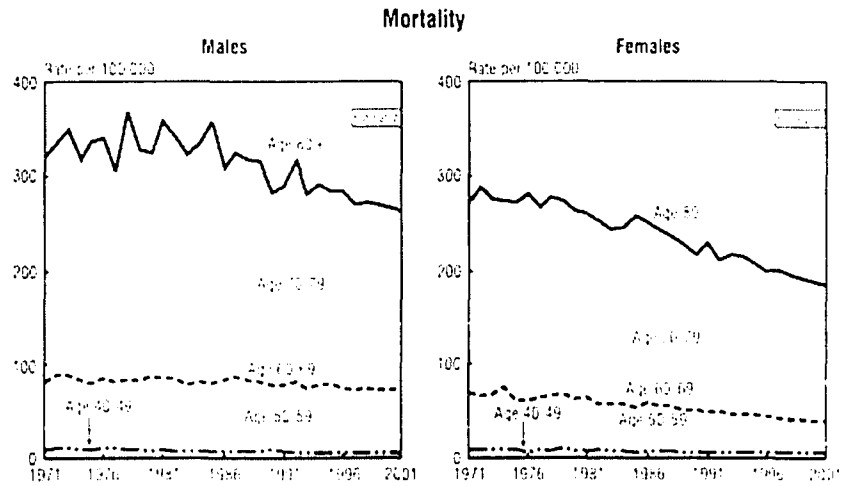


Figure 1-16. Age-Standardized Incidence Rates (ASIR) for Selected Cancer Sites, Females, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)

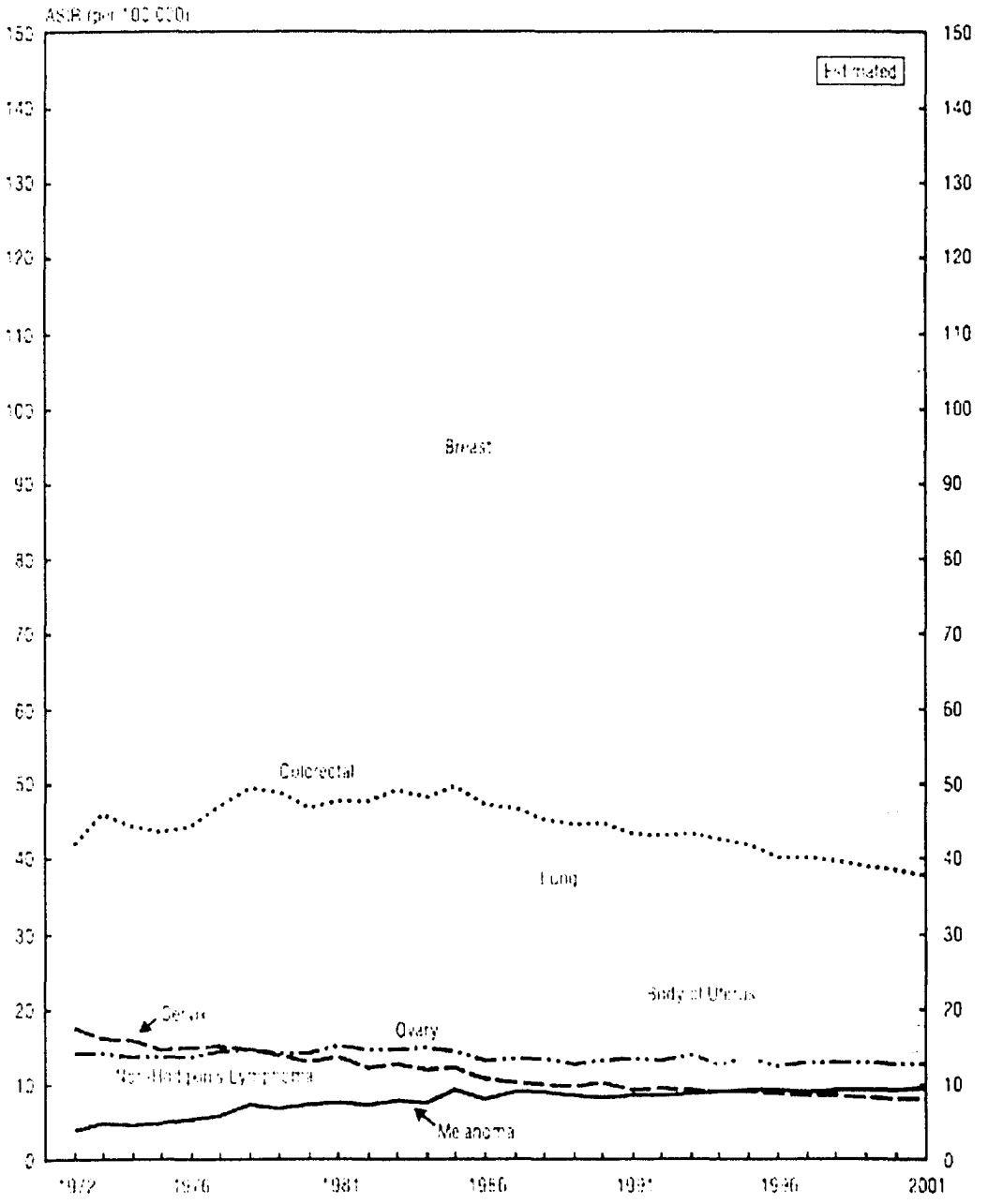


Figure 1-17. Age-Standardized Incidence Rates (ASIR) for Selected Cancer Sites, Males, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)

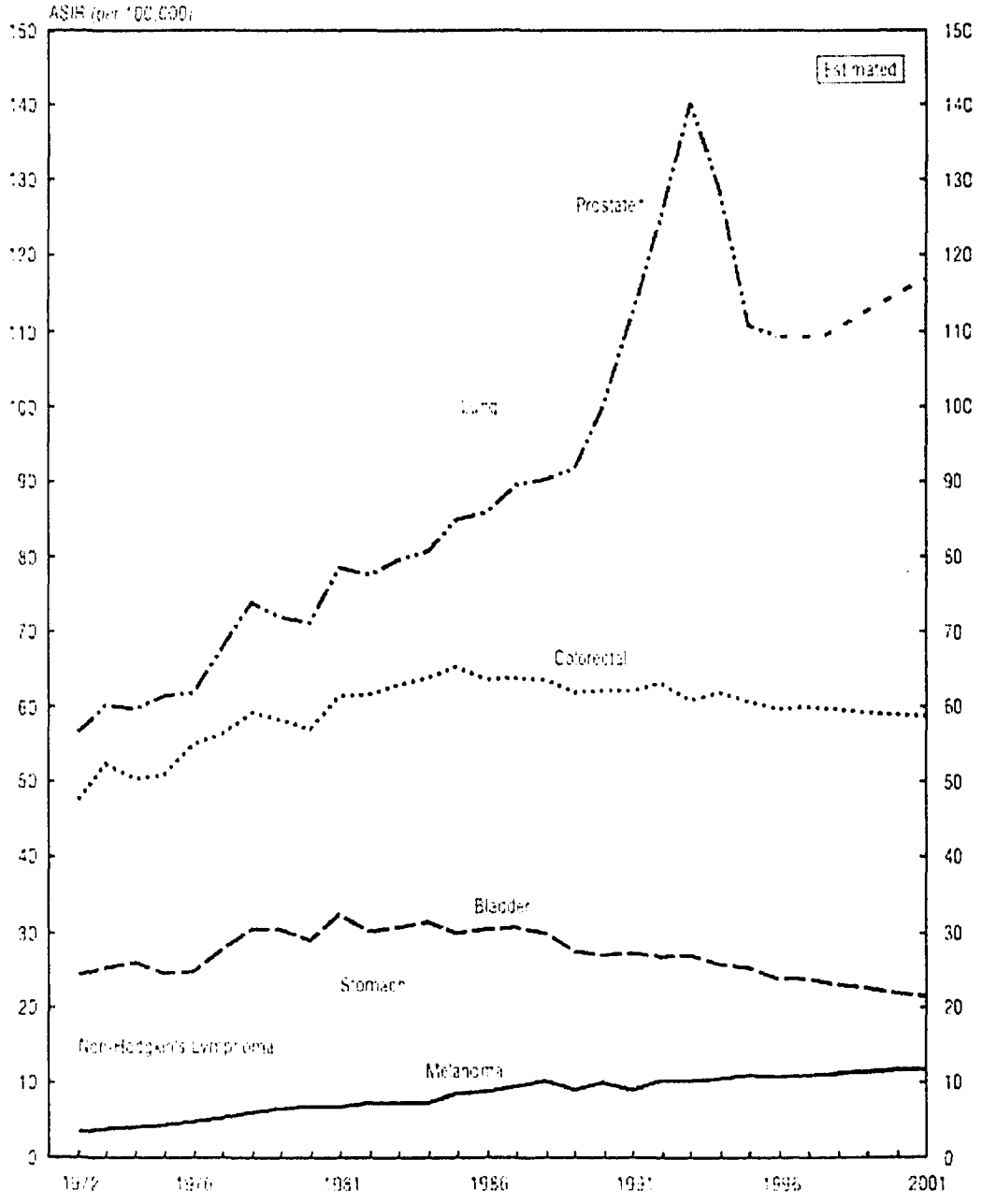


Figure 1-18. Age-Standardized Mortality Rates (ASMR) for Selected Cancer Sites, Females, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)

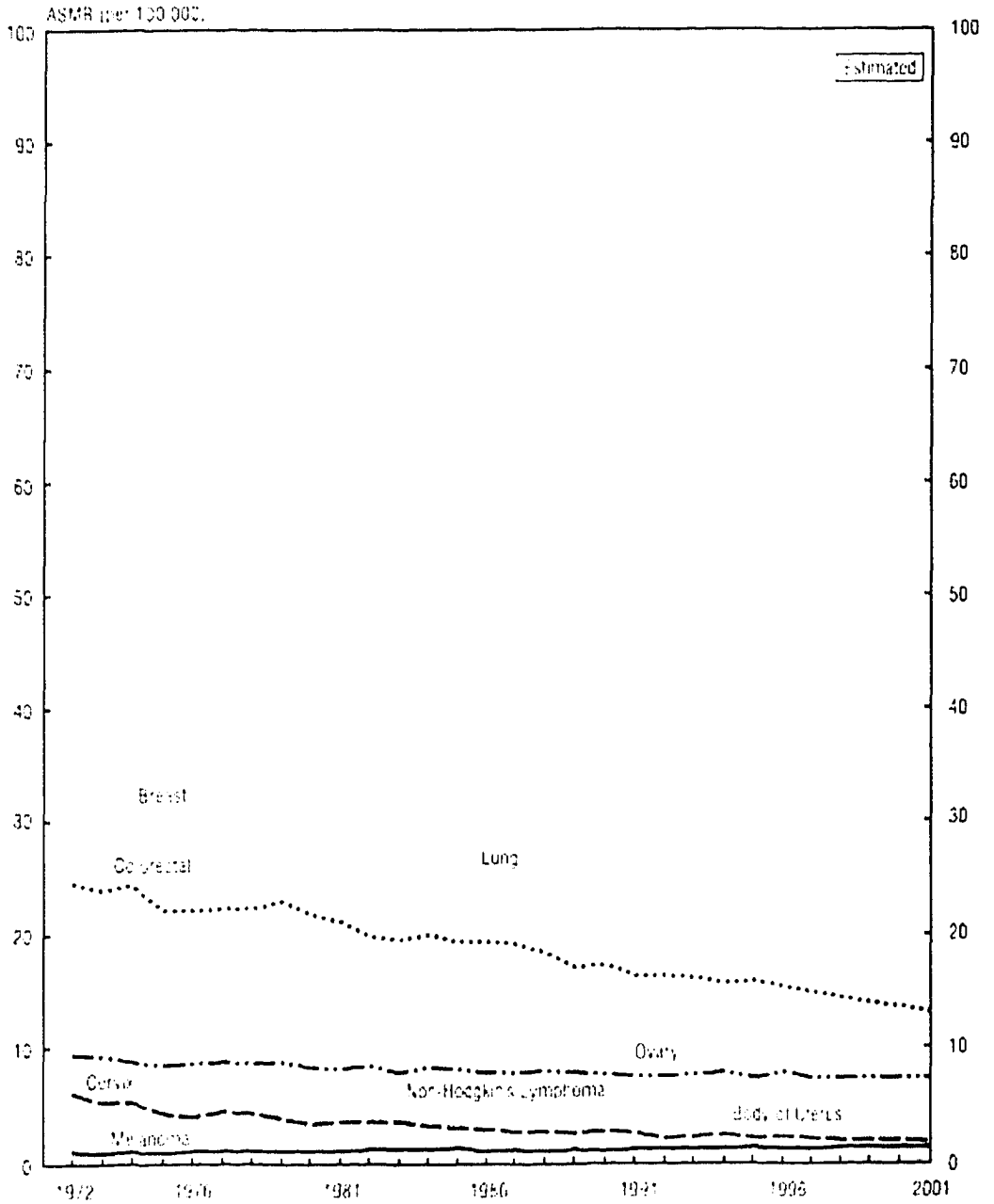


Figure 1-19. Age-Standardized Mortality Rates (ASMR) for Selected Cancer Sites, Males, Canada, 1972-2001. (Source: http://www.ncic.cancer.ca)

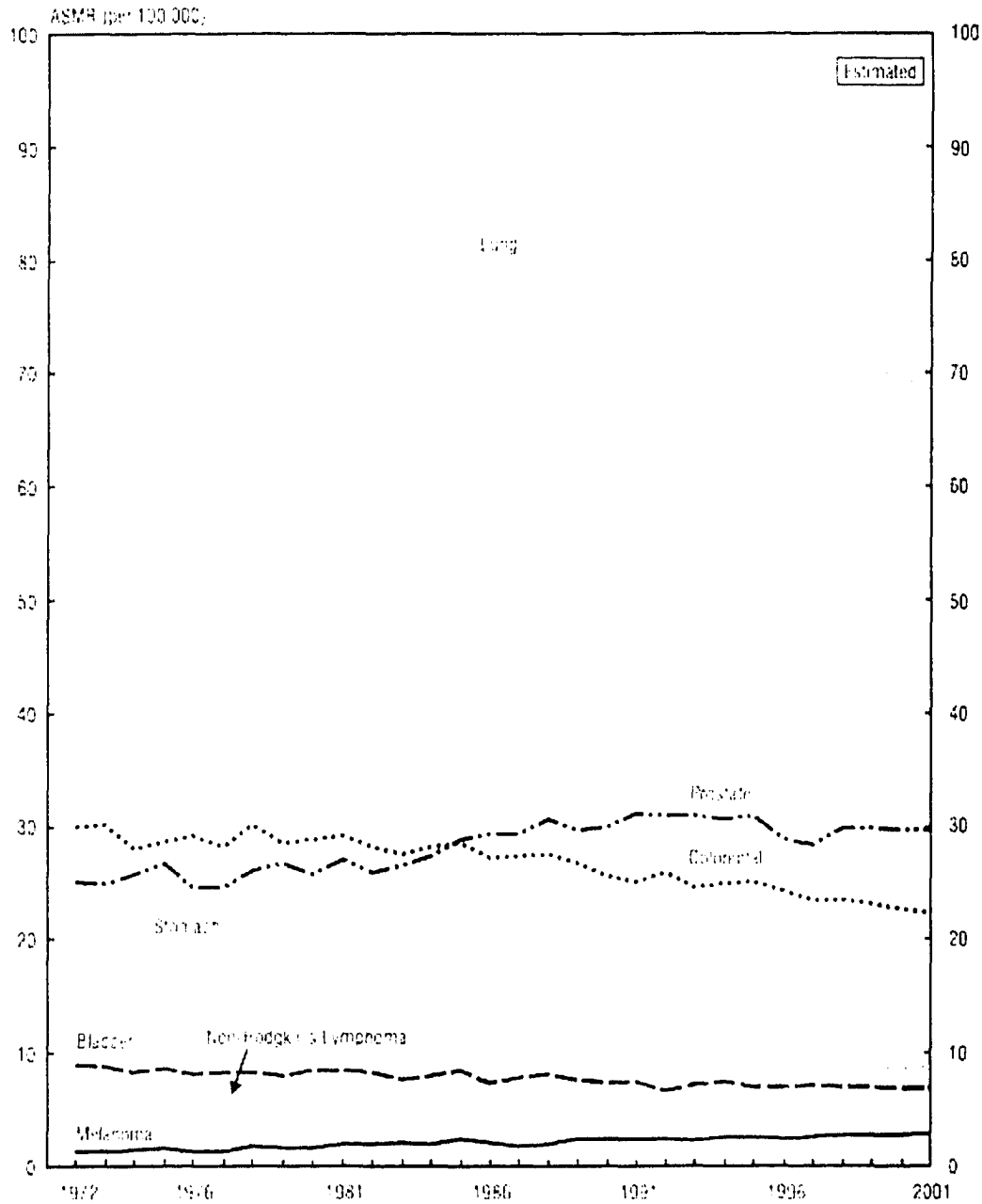


Figure 1-20. Average Annual Percent Change (AAPC) in Age-Standardized Incidence (1989-1996) and Mortality (1989-1997) Rates for Selected Cancer Sites, Canada. (Source: http://www.ncic.cancer.ca)

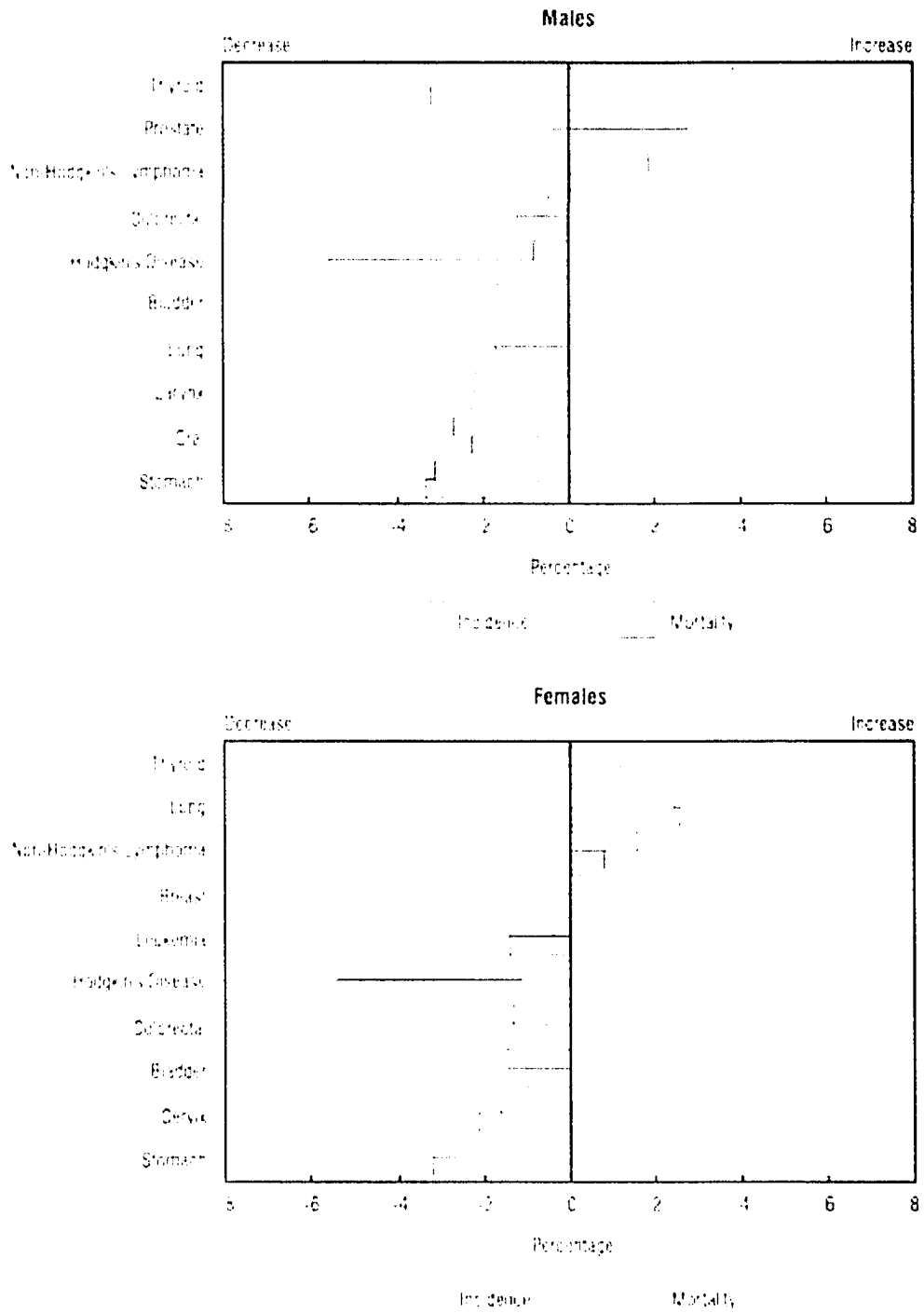
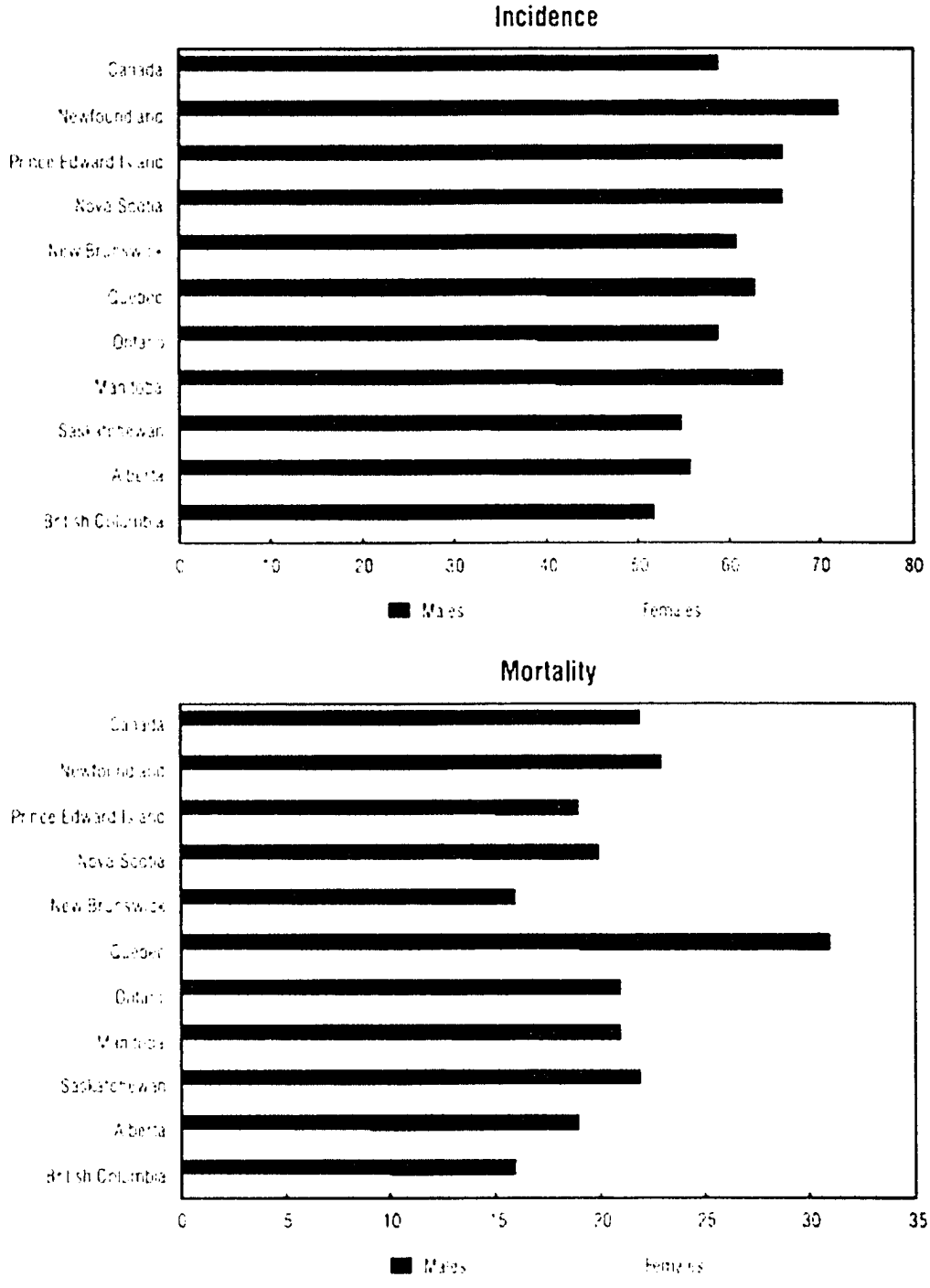


Figure 1-21. Estimated Age-Standardized Colorectal Cancer Rates per 100,000 by Province, Canada, 2001. (Source: http://www.ncic.cancer.ca)



PATHOLOGY

STAGING

The staging of patients with colorectal cancer serves several functions. It aids the clinician in determining what treatment is most appropriate and it aids in establishing a prognosis. In addition, staging allows for reasonable comparisons to be made between similar patients. As different prognoses are associated with different stages of disease, a comparison between treatment modalities is only useful if the patients have equal or similar prognosis. In this manner, disease stage can serve as a useful surrogate for prognosis in clinical trials.

The first widely adopted classification system for colorectal adenocarcinoma was developed by Cuthbert Dukes in 1930[22]. Updated versions of this system, such as the Astler-Coller modification[23], are still widely used. Unfortunately, this system is too simple to deal with many of the important anatomical and pathological factors encountered in colorectal tumors. This has led to the adaptation and widespread adoption of the TNM system to colorectal carcinoma. The TNM system is one used in the classification of most solid tumors. This classification system may be used based on clinical and / or pathology data. (Figure 1-22)

Three characteristics of the malignancy are involved: the extent of the primary Tumor, the involvement of regional lymph Nodes, and the presence of distant Metastases. The combination of these three characteristics correlates with prognosis. Unfortunately, no widely adopted system exists which specifically accounts for previous treatment (i.e. chemotherapy and / or radiotherapy) that the patient may have had. Most sites continue to use the TNM classification system with these patients, and may or may not note the presence of pre-existing treatment.

CLASSIFICATION

Classification of the Specimen

In patients with colorectal carcinoma, pathological examination of the biopsied or resected tumour specimen plays a crucial role in determining the correct diagnosis, the most appropriate treatment course, and the prognosis.

The grading of colorectal carcinoma is traditionally composed of three entities: Grades I through IIII. A Grade I lesion is histologically well-differentiated, and closely resembles normal epithelium including almost normal gland architecture. A Grade II lesion is histologically moderately differentiated. It is not well differentiated enough to be labelled a Grade I lesion, and it is not poorly differentiated enough to be labelled as Grade III. Finally, a Grade III lesion displays poor differentiation histologically, barely resembling normal epithelium. There is minimal gland formation, if any, and there may be mucous production. A fourth grade exists (Grade IV) for undifferentiated tumors, but by definition tumors classified as such are labelled as "undifferentiated carcinomas" and are usually not considered colorectal carcinomas. Approximately 75-80% of resected tumors are classified as grades I or II.

Classification of the resection

Resection is the cornerstone of the treatment of colorectal carcinoma. A formal classification scheme has been developed for the categorization of the extent of resection. This scheme has been well validated and is widely accepted.

A complete or R0 resection is the goal of any resection for primary colorectal carcinoma where the intent is curative. An R0 resection implies that there is no residual microscopic disease. If a patient has been completely resected macroscopically, but

there is residual microscopic disease, it is considered an R1 resection. An R2 resection, or a de-bulking procedure, implies that there is macroscopic residual disease. Hodgson et al demonstrated in 1986 that radical resection is superior to no or incomplete resection of colorectal liver metastases[24]. Almost all subsequent trials have confirmed this. In patients with colorectal liver metastases, there is no indication for an R2 resection. All efforts should also be made to avoid an R1 resection, as almost every study has demonstrated that an R1 resection is an independent prognosticator of a poor outcome. Many clinicians have suggested that if an R0 resection is not feasible, then a patient is not resectable.

Prognostic factors in patients with primary resected colorectal carcinoma

In patients who have undergone a successful R0 resection, several factors have proven to be of prognostic significance. These factors can be classified as treatment-related, tumour-related, and patient-related. The single treatment-related factor that has recently emerged as being significant is the skill of the surgeon[25-32]. Tumour-related factors include pathological TNM stage, histological grade, histological pattern at tumor margin, and the presence of venous invasion. Finally, patient related factors that significantly predict outcome include serum CEA level and the presence of co-morbidities.

Figure 1-22. Tumor Staging

	TNM[1]					Survival
	T	N	M	Dukes	M.A.C.*	(5-year)
0	Tis	N0	M0	-	-	100%
I						90-100%
	T1	N0	M0		A	
	T2	N0	M0		B1	
II				B		70-85%
IIa	T3	N0	M0		B2	
IIb	T4	N0	M0		B3	
III				C		30-45%
IIIa	T1-2	N1	M0		C1	
IIIb	T3-4	N1	M0		C1/C2	
IIIc	Any T	N2	M0		C1/C2/C3	
IV	Any T	Any N	M1	-	D	<5%

[1] J.C.C. 6th edition 2002
* Modified Astler-Coller Classifcation

TREATMENT OF RESECTABLE PRIMARY COLORECTAL CANCER

In 1833, Reybard performed the first colon resection and anastomosis[10]. Resection was then adopted as the treatment of choice for non-metastatic colorectal cancer.

The importance of completing an R0 resection is reflected in the disparate survival rates demonstrated by Durdey et al in patients with rectal cancer who underwent R0 resections and those who had unresected and pathologically documented local invasion (i.e. R1 disease)[33].

Durdey's study was completed in 1984, prior to the institution of modern chemotherapeutic and radiotherapuetic regimens which have somewhat tempered the poor prognosis after an R1 resection. When the two groups were matched for known prognostic factors including stage of disease, there was still a significant difference in 5-year survival in those that had an R0 resection (64.6% - 68.9% depending on whether the tumour was fixed by inflammation) and those that underwent an R1 resection (28.5%). Another strong example of the importance of completing an R0 resection was that patients with a Dukes B lesion who had an R1 resection had a 43.5% 5-year survival, while those with Dukes C disease who underwent an R0 resection had a 62.9% 5-year survival.[33]

Assuming an R0 resection has been completed, the prognosis of patients who have had their primary disease resected is influenced by a host of factors. Of these, stage at the time of resection is the most important prognostic factor[10].

Due to the sometime indolent nature of colorectal cancer progression, resection of primary colorectal tumors is performed on an emergent basis 15%-35% of the time. This occurs in patients presenting with colonic obstruction, perforation, and less-

commonly hemorrhage. Several series report a significantly worsened prognosis if the resection is performed on an emergent basis compared to those performed electively[34-36]

ADJUVANT THERAPY AFTER RESECTION OF COLORECTAL CARCINOMA

NSABP C-01 is an early study which demonstrated the benefit of adjuvant chemotherapy after resection of primary colorectal carcinoma. At the time, it established a survival advantage with the addition of vincristine, semustine, and 5-FU* chemotherapy after resection of Dukes B or C carcinoma. Interestingly, this study also demonstrated that the choice of agent was important as an arm treated with adjuvant BCG demonstrated no survival advantage over surgery alone[37].

One year after NSABP C-01 was published, a second randomized controlled trial demonstrated a survival advantage conferred by adjuvant chemotherapy. In this study the adjuvant treatment consisted of 5-FU and levamisole, an antihelminthic drug that demonstrated immunostimulatory properties via cell-mediated immunity. The survival benefit was only seen in patients who had a Dukes C carcinoma resected. An improvement in time to recurrence was demonstrated across both Dukes B and C carcinomas and was seen in both the group that received levamisole and, to a greater extent, the group that received levamisole in combination with 5-FU[38].

A second follow-up study demonstrated that in patients with nodal disease (Dukes C) there was a survival benefit from administering the combination of levamisole and 5-FU over both levamisole alone as well as when compared to no adjuvant therapy (both of which demonstrated basically equivalent survival)[39]. These results led to various governing bodies suggesting that the combination of 5-FU and levamisole should

* 5-FU or Fluorouracil is discussed elsewhere in this chapter

be adopted as adjuvant therapy in all patients with resected node-positive colorectal carcinoma[40 41].

Subsequent to these results, researchers started investigating compounds which might modulate the cytotoxic actions of 5-FU. Leucovorin (folinic acid) was one such biomodulator.

O'Connell et al initiated one of the earliest trials which included a biomodulated 5-FU arm. After resection of the primary in stage III and high-risk stage II patients, they demonstrated a significant improvement in time to relapse as well as overall survival with the addition of adjuvant 5-FU and leucovorin versus no adjuvant therapy[42]. This result led to numerous adjuvant chemotherapy trials, including NSABP C-04 which attempted to compare biomodulated 5-FU (5-FU + leucovorin) with 5-FU + levamisole. There was a slight improvement in the group treated with biomodulated 5-FU. No real difference in survival or recurrence was found when all drugs were combined (5-FU + leucovorin + levamisole), but there was an appreciable increase in chemotherapy related toxicities[43 44].

CARCINOEMBRYONIC ANTIGEN (CEA)

Gold and Freedman first described carcinoembryonic antigen, or CEA, in 1965. CEA is a tumor-associated antigen. Since its description, it has held a prominent role in the management of patients with a variety of cancers but colorectal cancer specifically. It has been used to monitor the quality of surgical resection, to monitor patients for recurrence, and even to judge the effectiveness of chemotherapy.

McCall et all prospectively evaluated the utility of serial CEA measurements in diagnosing recurrence in patients who have undergone curative resection of a primary colorectal carcinoma. They noted that even though CEA was the first indicator of recurrence in 58% of all patients, it was the first indicator of recurrence in 80% of patients that had developed liver metastases[45]. This property is present even if the patient had a "normal" CEA level prior to the resection of their primary lesion[46]. McCall and colleagues were unable to conclude, however, that this earlier detection afforded the patient any survival benefit[45].

Bakalakos et al demonstrated that preoperatively collected CEA is useful as both a prognostic marker, as well as in making the future diagnosis of metastatic disease in patients with resected non-metastatic colon cancer. In those patients that were scheduled to undergo a resection of their colorectal liver metatastases, a preoperative CEA $\geq$30ng/ml was associated with a significantly worse median survival (17 months versus 25 months) and a significantly lower chance of undergoing a curative (R0) resection. It was also noted that prognosis worsened with increased levels of CEA[47].

METASTATIC COLORECTAL CARCINOMA

In Europe, the incidence of colorectal carcinoma is 150,000 per year. Thirty-thousand (or 20%) of these patients have metastatic disease at the time of first diagnosis. Of the people who have no metastatic disease at the time of initial presentation, and then undergo a resection of their primary lesion (with curative intent - approximately 70-80% of patients) about 50% will recur. Broken down by stage, approximately 20 % of patients with stage II and 50% of patients with stage III disease will develop metastatic disease. A much smaller percentage develops local recurrence. Almost all those that recur will eventually succumb to the disease.

Gilbert et al demonstrated that recurrence after "curative" resection of primary disease was often multi-focal (73% in one series, 45% in another series)[48]. When recurrence proved to be isolated, the liver was the most common single site of recurrence, accounting for 40% of all recurrences. Lung, locoregional, and peritoneal recurrences accounted for approximately 15% each while retroperitoneal recurrences and lymph node recurrences were seen in 10% and 5% respectively. Others have demonstrated that between 38% and 70% of patients who develop metastatic disease have metastases confined to the liver[19,49].

The high number of patients who present with disseminated recurrent disease has led to numerous investigations into the most effective and appropriate way of following patients post-resection. Bruinvels et al concluded that intensive follow-up, including serial CEA measurements, could lead to a 9% improvement in survival mainly due to the early detection of resectable recurrence[50].

Mccall et al demonstrated the utility of CEA in following patients after resection of their primary colorectal disease. In these patients, CEA levels $\geq$ 5mg/l had a

sensitivity of 93%, a positive predictive value of 79%, and a negative predictive value of 83% for having recurrent disease. A median lead time of 6 months (8 months in those with hepatic recurrence) was afforded by following patients using serial CEA measurements when compared to what was standard follow-up at that time[45]. Currently combinations of serial serum CEA measurements, endoscopic surveillance and radiological imaging (i.e. CT scan) are used in follow-up.

THE DEVELOPMENT OF METASTATIC DISEASE

Hernandez et al used human colorectal cancer tissue lines and microarray technology to demonstrate the presence of 9 genetic differences between low stage colon cancer (Dukes B) and metastatic colon cancer[51]. The significance of this is not yet fully determined, but these genetic differences may represent the changes that permit the local cells to develop their metastatic ability.

Even the route of metastatic tumour spread is debated. Several theories have been developed to explain the development of metastatic disease. One such theory suggests that malignant cells disseminate sequentially via the portal system initially and then systemically. A different theory proposes that both the vascular system (including the portal system) and the lymphatic system are used during systemic dissemination. A third, more comprehensive theory advocates that there are three mechanisms of tumour dissemination. The first is local spread. Tumour may spread in a continuous or a discontinuous fashion or even be due to gross tumour spillage intra-operatively. Discontinuous spread is represented by satellite lesions. Intra-operative tumour spillage may lead to locoregional recurrence within the tumour bed or peritoneal metastases. Peritoneal metastases can also result from the serosal penetration or perforation of the

continuous spreading tumour. The second mechanism of tumour spread incorporates the lymphatic system. Lymphatic spread leads to regional lymph node metastases which may then lead to distant lymph node metastases which may progress via the thoracic duct or the caval system and present as lung metastases. Lung metastases may then give rise to disseminated spread. The third, and final mechanism incorporates venous spread. Lower rectal tumours may access the systemic circulation and develop into lung metastases. The remaining tumours access the portal circulation and may give rise to liver metastses. Both liver metastases and lung metastases may give rise to disseminated metastases[10].

It is possible that the presence of the primary lesion may somewhat inhibit the development of metastases[52]. This suggests that resection of the primary lesion may in fact encourage the proliferation of the remaining micrometastases. This is a possible explanation for the sometimes rapid development of significant metachronous disease after the resection of a primary lesion.

COLORECTAL LIVER METASTASES

It has been demonstrated that if untreated, patients diagnosed with synchronous liver metastases from colorectal carcinoma have a median survival of only 4.5 months[53]. The addition of chemotherapy (without resection) affords only a 6-12 month benefit in survival when compared to no-treatment.

Stangle et al prospectively followed 484 patients with colorectal liver metastases that received no treatment for their metastatic disease. For the entire cohort survival at 1, 2, 3, and 4 years were 31%, 7.9%, 2.6% and 0.9% respectively. They then attempted to elucidate prognostic factors using first univariate and then multivariate

analyses. Six factors emerged as being of prognostic significance. These factors were: the relative volume of liver replaced by tumor (%), the grade of malignancy of the primary tumor, the presence of extra-hepatic metastatic disease, the presence of positive mesenteric lymph nodes, the serum CEA level and the patients' age. Stratifying according to the presence or absence of these criteria created cohorts with widely disparate median survivals, ranging from 3.8 months to 21 months[54].

Rougier et al also analyzed numerous factors in an attempt to determine prognostic factors for patients with unresected colorectal liver metastases. Most predetermined factors proved to be insignificant. They then used the remaining factors to propose two grouping systems to stratify those patients with liver metastases. One classification scheme involved performance status and the number of liver segments involved. The second grouping system was based on a combination of performance status and a single biochemical marker (alkaline phosphatase). Based on having normal values in only these two factors, they were able to demonstrate a median survival of 11 months[55].

An early but influential trial by Wagner and colleagues directly compared patients who had their liver metastases resected with those that had potentially resectable disease but who were not resected. No patients in the unresected group survived 5 years while those that underwent a liver resection benefited from an approximately 25% 5-year survival[56].

Wilson et al and Wanebo et al were interested in the natural history of colorectal liver metastases and whether resection was beneficial. Each reported on the differential survival in similar patients that were or were not resected. Wilsons group noted a 28% 5-year survival in those that were resected, while the cohort with resectable disease that

was not resected had no 5-year survivors[57]. Wanebo compared patients with resectable solitary liver metastases and found that the resected cohort had a 25% 5-year survival while the unresected cohort also had no 5-year survivors (median survival was 36 months and 19 months respectively)[58].

Nordlinger et al abstracted data from 1568 patients to demonstrate a 2-year survival of 64% and a 5-year survival of 28% in patients who had colorectal liver metastases resected. The disease eventually recurred in approximately 67% of these patients[59].

In 1997 Fong and colleagues describe 456 consecutive metastectomies in patients with colorectal liver metastases. They found a 38% 5-year survival and a median survival of 46 months. They then stated that no trials comparing resection with no resection should be attempted[19].

Two years later, Sheele and Altendorf-Hofmann reviewed the available literature and reported that 5-year survival ranged from 21% to 48% in patients who had colorectal liver metastases resected. They also suggested that "adjuvant chemotherapy or radiotherapy after R0-resection is unlikely to improve [on these] results"[60].
The only treatment modality that potentially grants long-term survival or even cure to patients with colorectal liver metastases is surgical resection. This fact makes early detection crucial, as early liver metastases may be resectable and thereby confer a substantial survival benefit.

Due to the substantial benefit from surgery relative to other treatment modalities, most research has historically focused on increasing the percentage of patients who are candidates for liver resection. Unfortunately, only 10-20% of patients that develop liver metastases have disease that is amenable to surgery.

There are technical factors that have improved patient outcome after resection of colorectal liver metastases. One of the most significant changes has been the increase in R0 resections.

Advances in chemotherapy have improved median survival dramatically for patients with colorectal liver metastases who do not undergo surgery. But, due to the immense gap between survival after resection alone and after chemotherapy alone, even an improvement in median survival up to 18 months is still far lower than after complete resection[61-63]. Even so, probably the largest advance in chemotherapy can be attributable to multi-drug regimens. The first to show benefit was the addition of irinotecan (CPT-11*) to 5-FU containing regimens in patients with unresected metastatic disease. Several investigators demonstrated the benefits of adding irinotecan to the standard treatment with 5-FU and leucovorin[Δ62-64]. This addition led to decreases in risk of death of up to 20% with concomitant increases in survival [62-64]. Following this, the next major potential chemotherapeutic advance involves the addition of oxaliplatin.[65].

DIAGNOSTIC IMAGING FOR THE DETECTION OF METASTATIC DISEASE

The "gold-standard" in imaging colorectal liver metastases is intra-operative ultrasound (IOUS). It is able to detect lesions that are missed by both pre-operative imaging as well as intra-operative inspection and palpation[66-68]. Some studies suggest that intra-operative inspection and palpation of the liver in the absence of IOUS will miss at least 10-17% of liver metastases[69].

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* Irinotecan is discussed elsewhere in this chapter

Δ Elsewhere the significant toxicity associated with some of these regimens is discussed.

Diagnostic imaging plays a large role in the detection and treatment of colorectal liver metastases. Currently helical computed tomography specifically plays a large role in the detection of new liver metastases, the description of liver metastases, as well as the detection of extra-hepatic disease. The description of the metastases allows a patient to be evaluated as to whether these lesions are surgically resectable – done by evaluating the size and distribution of the lesions.

It should be noted that the reported detection abilities of computed tomography may be inflated due to often being compared to inadequate standards of reference or "gold standards". Several other biases have also been responsible for exaggerating the capabilities. A significant yet commonly present bias is verification bias i.e. only the part of the liver with a suspected metastatic disease is examined using a reference standard, thereby ignoring possible lesions in the remaining hepatic parenchyma. This was exemplified by the numerous studies that either failed to use intra-operative ultrasound, or used it only on areas of liver with suspected metastases[70].

Helical Computed Tomography (CT) and CT Arterial Portography (CTAP)

Valls et al prospectively compared helical computed tomography (CT) with CTAP. Even though both methods demonstrated similar sensitivities (76% for helical CT versus 74% for CT portography), there were significant differences seen in both positive predicted values (90% versus 69%) and area under the respective receiver operating characteristics curves (0.96 versus 0.86)[71]. These characteristics have led to the adoption of helical CT as the preferred pre-operative diagnostic imaging modality in most patients[72]. These same characteristics have led to the diminished use of CT portography in the pre-operative work-up of patients with known or suspected colorectal

liver metastases. CTAP also was shown to have a high false positive rate. This is especially troubling in the patient population with potentially resectable colorectal liver metastases as it may incorrectly label someone as unresectable.

Valls et al also prospectively evaluated the diagnostic ability of helical CT compared to a "gold standard" combination of intra-operative ultrasound, intra-operative palpation, and post-resection histopathological examination in patients with colorectal liver metastases (94% of patients underwent a potentially curative resection). Helical CT demonstrated a good sensitivity (85%) and an impressive positive predictive value (96%). An acceptable false positive rate of 3.9% was also documented. Unfortunately, there was a high false-negative rate associated with this modality. Fourteen percent of lesions detectable by the "gold-standard" combination were not detected.

Biphasic computed tomography has become the standard pre-operative method for determining the presence and extent of liver metastases due to its relatively high sensitivity and specificity[69]. There are also efforts to use the CT images in more intricate ways. Glombitza and colleagues have developed a modeling process that may allow accurate pre-operative assessment of the resectability of lesions based on their location in relation to major vascular structures. This same method is purported to be able to accurately predict post-operative liver failure based on the volume functioning hepatic parenchyma spared after the proposed resection[73 74]

Positron Emission Tomography (PET)

^{18}F-Fluorodeoxyglucose positron emission tomography (18F-FDG PET), like CT and MRI, has excellent sensitivity in terms of detecting intra-hepatic disease that is greater than one centimeter in size, but a much poorer sensitivity when examining

lesions less than 1 centimeter[69]. PET has started to show promise as being able to detect extrahepatic disease, and has been shown to alter treatment in patients[75]. It has been shown to have similar sensitivity to CTAP but, largely due to the number of false positives with CTAP, PET has a greater accuracy (93% versus 76%)[75].

PET's greatest contribution may lie in its ability to detect extra-hepatic disease. As many as 10-30% of patients eligible for resection of colorectal liver metastases have been shown to have undetected extra-hepatic disease that, if known, would obviate the need for laparotomy[75]. (Figure 1-23)

Magnetic Resonance Imaging (MRI)

Both CT and MRI have high sensitivities (94% and 99% respectively) for the detection of lesions at least one centimeter in size. When examining smaller lesions, CT and MRI have much reduced detection rates (52% and 47% respectively) for these lesions that are detectable surgically or by intra-operative ultrasound[69]. Both CT and MRI are handicapped by the limited contrast between liver metastases and normal hepatic parenchyma. This may result in an effective 10-fold reduction in resolution. This reduced resolution drastically impairs our ability to detect lesions that are one or two millimeters or smaller[69].

Colorectal liver metastases usually appear as hypo-intense lesions on T1 weighted images and moderately hyper-intense lesions on T2 weighted images. (Figure 1-24)

MRI, like CT, demonstrates some utility in identifying metastatic nodes by size criteria. This is done with an accuracy of approximately 64%[76]. Metastases may also be distinguished by an increased signal during the arterial phase and a decreased signal

during the portal phase of the scan. This discrepancy is due to the metastases receiving most of their blood supply from the arterial system (in contrast to normal hepatic parenchyma which receives most of its blood supply from the portal system)[77]. Finally, a super-paramagnetic agent such as ferumoxides can be used to increase sensitivity and specificity as this contrast is preferentially taken up in kuppfer cells (which are not present in colorectal metastases). This has led to substantial promise to be associated with MRI as contrast enhanced magnetic resonance imaging may prove to be the most accurate pre-operative imaging method, but is as of yet not universally accepted or used.

Intra-operative ultrasonography

Intra-operative ultrasonography (IOUS) has become standard-of-care in terms of the patient with potentially resectable hepatic colorectal metastases. Paul et al demonstrated that IOUS changed the staging and therefore management in 11% of their patients. There are however, both false-positives and false-negatives[78]. Valls et al reported a series of parameters regarding the use of IOUS. In a review of 290 colorectal liver metastases, IOUS displayed a sensitivity of 99.3% and a positive predictive value of 98.2%[72]. Zacherl et al noted the recent advances in diagnostic imaging and re-evaluated the role of IOUS as the "gold standard" imaging technique. They noted that along with the other imaging modalities, IOUS had improved as well, and remained the modality of choice. In this cohort of patients, it was more sensitive than MRI and helical CT (95.2% versus 84.9% and 82.5%) and was able to be the sole detector of findings that changed management in 22.8% of cases[79].

Future Trends

There is a suggestion that smaller metastases may be better detected using the "Hepatic Perfusion Index": a measure of the ratio of arterial inflow and portal inflow[80 81]. Future studies will, hopefully incorporate this or other new techniques in an effort to identify metastases at earlier and smaller stages of development.

Laparoscopy is also beginning to play a role as patients can now be evaluated with this technique which includes the use of laparoscopic ultrasound, to determine resectability. This may spare a significant number of patients the morbidity of a laparotomy if they are in fact unresectable.

SELECTION CRITERIA FOR LIVER RESECTION

There are three generally accepted criteria for selecting a candidate for resection of colorectal liver metastases. The patient must be a reasonable operative candidate for a major laparotomy and liver resection. There should be no extrahepatic disease. Finally, disease is of such a distribution that the surgeon has the ability to completely resect all hepatic disease with preservation of adequate liver parenchyma (~20-25%).

DETERMINATION OF UNRESECTABILITY

The utility of liver resection in patients with hepatic metastatic disease is well known. It is the single most beneficial treatment available. Due to the potentially significant survival advantage resection confers; an important step in the treatment of patients with colorectal liver metastases involves the determination of whether a specific patient is a candidate for liver resection.

Arguably, one of the most significant recent advances in the treatment of colorectal liver metastases has been the improvement in peri-operative care allowing patients historically deemed unresectable to become re-classified as resectable.

A number of contra-indications to resection still do exist, however. Surgery is clearly contraindicated if there is inadequate control of the primary lesion or in most cases in the presence of extra-hepatic metastases. For this reason, it is important that the patient has been evaluated for both recurrent primary disease, as well as extra-hepatic disease prior to undergoing hepatic resection. The presence of pulmonary metastases can be deduced via chest X-ray (CT is only indicated if abnormalities are found on x-ray). Bone and brain imaging for metastases is only warranted if clinically indicated.

It has long been known that few, if any, patients demonstrate 5-year survival in the presence of unresectable extra-hepatic disease including nodal disease in the hepatoduodenal ligament. Reported 5-year survival in these patients ranges from 0% to 20%[59 82 83].

There are two significant exceptions, however. Recurrence of the primary tumor, if resectable, does not preclude resection of hepatic metastases. Also, the presence of resectable lung metastases may not necessarily preclude liver resection for metastases. A 5-year survival of up to 25% has been reported in patients who have had resection of synchronously presenting lung and liver metastases[84].

Certain traits of the colorectal liver metastases themselves may contra-indicate resection. A patient can be classified as unresectable due to several reasons inherent to the liver metastases themselves: 1) the lesions are situated in anatomical positions so as to make their resection technically impossible; 2) the disease present would require a

resection which would render remaining liver parenchyma insufficient for functioning (and lead to post-operative liver failure).

Lesions in "Unresectable" anatomical locations

Unfortunately, even a small lesion in an awkward anatomical position (i.e. at the bifurcation of the portal vein) may make the lesion unresectable. Alternatively, inadequacy of the surgical margin attainable may be one reason that a lesion (or lesions) may be deemed technically unresectable. Adding to this criteria is the debate over what is classified as an acceptable surgical margin. Historically, a 1 centimeter or greater margin was considered the standard acceptable margin[85]. Recent studies have supported this ideal, showing a slight improvement in survival with margins greater than or equal to one centimeter[19 86]. Others researchers have not been able to replicate this conclusion and have, in fact, demonstrated the equality, in terms of survival, of patients resected with margins less than 4mm and greater than 4 mm [60 87-89].

What is generally agreed upon is the importance of the resection being an R0 resection (i.e. no microscopic tumor detectable at the surgical margin). The survival advantage conferred by liver resection is dramatically decreased in patients who have microscopic or macroscopic disease remaining.

There are techniques becoming available that may make these people who are classified as having unresectable disease due to the location of the lesions be reclassified as resectable. Ablative techniques (laser or microwave thermal ablation, cryoablation, radiofrequency ablation, ethanol injection, and hepatic artery ligation) may allow patients who were previously regarded as having unresectable disease due to anatomical or technical considerations to undergo their maximal attainable resection

with subsequent ablation of the involved tissues at the surgical margin. This may convert what would otherwise be an R1 resection to an R0 resection[90 91].

Cryotherapy, consists of alternating freeze / thaw cycles of the intrahepatic lesion via inserted cryoprobes. The tissue freezes causing cellular death. Radiofrequency ablation is performed via an electrode inserted in to the metastasis. Heat from the radiofrequency energy is transmitted to the malignant tissues inducing a coagulative necrosis. Both these techniques are rapidly gaining in popularity and are being widely used although there is currently little evidence supporting their utility. Currently, there is no proven role for these techniques in patients with metastases that are amenable to complete resection. It is important to note, however, that some studies are starting to suggest that there may be a role for these techniques in such lesions in the future[90]. Curley et al recently reported remarkable results using a combination of laparotomy, radiofrequency ablation, and the Pringle maneuver[92].

Number and Distribution of Liver Metastases

Many different opinions exist as to whether the pre-operatively determined number of metastases has prognostic significance. Historically, the number of metastases present within the liver was regarded as one of the most important prognostic factors. This attitude was borne of research which concluded that survival in patients with a single intra-hepatic metastasis was significantly greater than in those with 3 or more lesions, with no long-term survivors having had 4 or more lesions resected[18 85]. Since then numerous researchers have concluded that such a relationship is not clear and if such a relationship does exist it is definitely less drastic than previously thought[20 59 93]. Even more recently Fong et al endorsed the prognostic

significance of having greater than one hepatic lesion and found it to be an independent predictor of poor outcome in his review of 1001 patients and as such was incorporated into his clinical risk score[94].

Patient Factors

Significant co-morbidity (especially cardiopulmonary) makes a number of patients unresectable due to the unacceptable risk of peri-operative morbidity and mortality. This is largely due to the general operative risk associated with an extensive resection. . A patient with underlying liver dysfunction may also be classified as unresectable if the dysfunction would classify them as a Child class B or C. As liver resection decreases hepatic reserve (in some instances as much as 75%) it is imperative that there is little if any pre-operative liver dysfunction.

Even though cirrhosis and fibrosis are rarely an issue in patients with colorectal liver metastases, focus must still be placed on other causes and markers of liver dysfunction. Previous experience with chemotherapy, especially if it is prolonged, may induce hepatic changes (steatosis, portal / periportal fibrosis, or microvascular changes like peliosis or sinusoidal congestion). Usually hepatic function can be examined clinically and via serum markers. Occasionally, supplemental testing is indicated such as indocyanin green or bromosulfophtalein retention tests, or even a needle biopsy.

TIMING OF SURGERY

Due to more comprehensive and sophisticated pre-operative work-up, an increased number of patients are known to have metastatic disease to the liver prior to their initial colorectal resection. This has led to a debate over whether timing of surgery

is an important factor. Patients with synchronous metastatic disease may have their primary and their metastases resected during the same operation; alternatively, the metastases may be resected subsequently requiring a second operation; patients with newly diagnosed metachronous metastases may undergo resection near the time of diagnosis or may have serial investigations to determine whether they have additional metastases that become detectable and may change or contra-indicate a resection. In the first instance, i.e. patients with newly diagnosed primary colorectal carcinoma and synchronous liver metastases, several authors have reported similar morbidity and mortality between staged resections versus a synchronous resection[95]. Authors have also reported that survival remains largely unaffected when comparing lesions with equivalent pre-operative prognosis[95-99]. Contrary to previously held views, new information suggests that these equivalent results are not necessarily limited to minor resections, and that more extensive resections may be performed synchronously without affecting peri-operative morbidity and mortality[95 99-101].

SURGERY FOR COLORECTAL LIVER METASTASES

The first resection performed for metastatic disease in the liver was a right lobectomy executed over 50 years ago by Lortart-Jacob in 1952. Since then the mortality associated with liver resections for metastatic colorectal disease has steadily diminished. The mortality associated with liver resection for colorectal metastases has consistently been reported at being less than 5%[20 59 82 83 94].

The dramatic improvement in 5-year survival due to resection of colorectal liver metastases is well established. Large series have reported 5-year survival between 25 and 48% with median survival ranging between 30 and 45 months[19-21 60 82 86 94 102-107].

These results have led prominent researchers to conclude that randomized trials of resectable patients involving a no-surgery arm are not ethical.

Liver transplantation has been attempted, but without success due to the high rate of recurrence.

Types of resection

Kokudo et al analyzed a series of 174 liver resections in patients with colorectal liver metastases. They noted that there was no survival advantage in the cohort that had undergone anatomical resections versus those that underwent non-anatomical resections. There was a trend towards more intra-hepatic recurrence in those that had non-anatomical resections. They noted however, that about 90% of those recurrences were amenable to re-resection[108]..

As recently as June 2002 Choti et al published results from over 200 liver resections performed over a span of 15 years. They noted 5% of their resections were R1 resections. They also suggest a significant improvement in outcomes over the last 6 years (1993-1999) versus the first 8 years (1984-1992). They noted that this trend is due to several factors including better patient selection, improved preoperative imaging techniques, the increased use of IOUS, the introduction of adjuvant chemotherapy, and the increased rate of repeat resection for intra-hepatic re-recurrence[109].

Minagawa et al retrospectively reviewed 235 hepatic resections at a single institution. They noted that even though they identified several factors that were associated with poor prognosis, the survival benefit realized in patients who were radically (i.e. R0) resected outweighed any decrease in survival due to these prognostic variables.[110]

Liver resection has also been shown to be cost-effective when viewed in terms of life-years gained and when compared to non-surgical options[111].

Unfortunately, even in light of these positive results, up to 70% of patients will have a recurrence after resection of colorectal liver metastases.[112].

COMPLICATIONS OF LIVER RESECTION (MORBIDITY AND MORTALITY)

Hepatic resection for metastatic colorectal disease has repeatedly been shown to be safe and effective[17 18 56 82 94 113-124]. It is not however, a benign procedure. Significant morbidity and mortality can be associated with liver resection. Reported mortality from more modern studies ranges from 0 to 5% [19 94 119 125 126]. There is an approximately 20-40%[19 20 60 94 104 127] morbidity rate post-resection. Both morbidity and mortality are caused by factors that can be categorized as procedure related or patient related. Patient related factors can be separated into liver-related and general co-morbidity related.

Intra-operative hemorrhage is the one of the most significant procedure-related events and can occur immediately intra-operatively or present in a delayed fashion during the post-operative period. Hemorrhage is consistently reported but relatively infrequent (1-2%)[17 19 85].

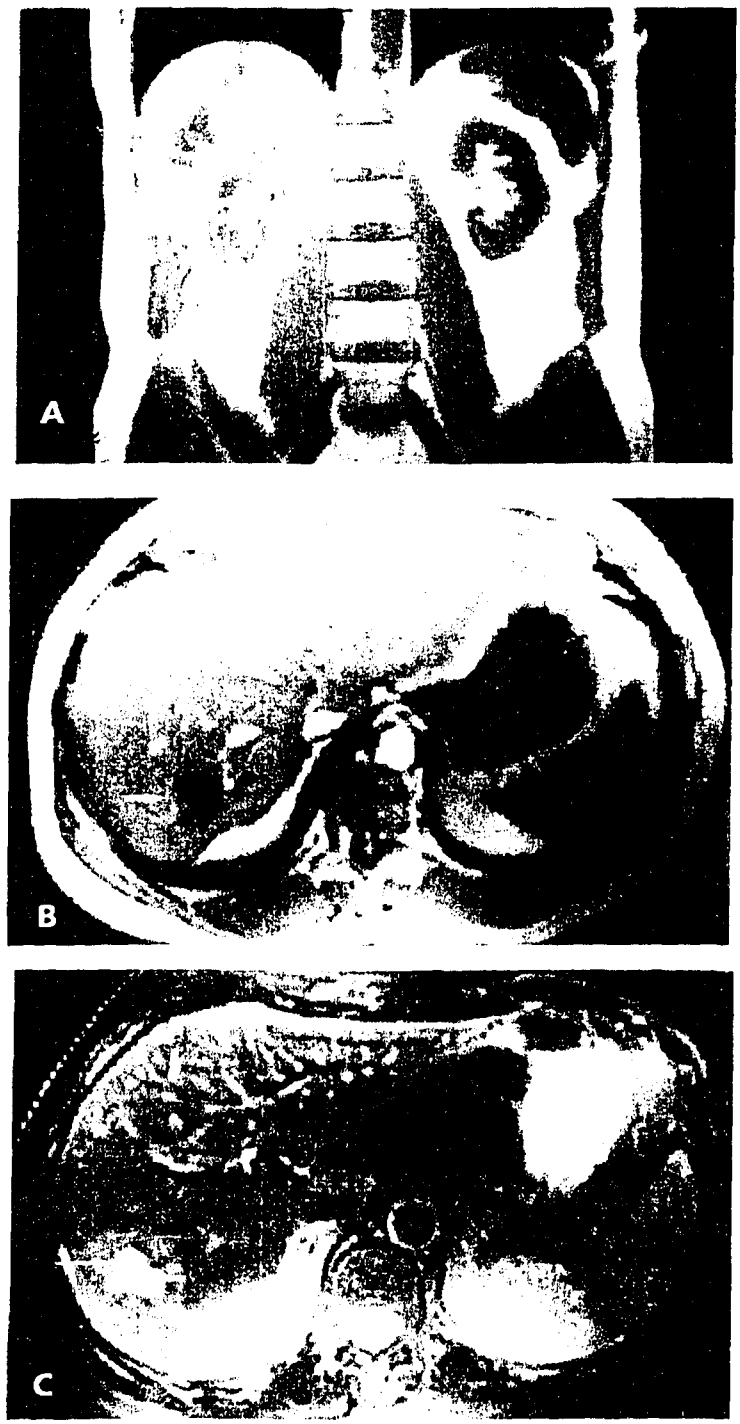
Liver failure is one of the most significant post-operative complications and is reported to occur in 1-4% of cases[17 19 85]. Even though liver failure is largely dependant on the quantity of functional parenchyma remaining after resection, it is also greatly influenced by the presence of any clinical or sub-clinical liver dysfunction present pre-operatively. Due to this interaction, liver function is carefully evaluated prior to surgery through a combination of clinical indicators and biochemical markers. Liver dysfunction

is a relatively common cause of a patient with anatomically resectable liver metastases being relegated to palliative chemotherapy.

Other potential complications include bilomas, biliary fistulas, abscesses, wound infections, sepsis, deep venous thromboses, pulmonary emboli, pleural effusions, and pneumonias.

Figure 1-23. (A) Coronal PET demonstrating 2 liver metastases (only one was seen on CT) (B) (same patient) Sagittal PET demonstrating local pelvic recurrence

Figure 1-24. T1 weighted images of the liver demonstrating typical hypo-intense hepatic metastases: (A) Coronal (B) Axial (C)(Same patient) Axial T2 weighted image demonstrating the lesion has a high signal centre and an ill-defined margin



DETERMINING PROGNOSIS

Recent advances in peri-operative care have allowed for a greater percentage of patients to be eligible for potentially curative liver resections. Even with this improved care, liver resection is still associated with significant morbidity and mortality. Also, between 60 and 80% of patients resected will develop early recurrent disease, with many reporting recurrences within a year after surgery. These recurrences are mostly confined to the liver (50-67% of cases). In a further 20-30% of patients, recurrence is both intra and extra-hepatic. Another 10-30% of patients present with only extra-hepatic recurrence. Given this, it is imperative to know which patients are most likely to benefit from this invasive but potentially beneficial treatment modality. Conversely, it is also important to know who should be offered alternative treatments and who should be offered adjuvant therapy. For these reasons, prognostic and predictive factors are considered.

Prognostic factors provide prospective information regarding patient outcome and thereby aid in determining what the likely disease course will be. Predictive factors provide information regarding likely response to a treatment option and thereby allow clinicians and patients to make better treatment decisions[128].

The search for reliable prognostic factors is not isolated to colorectal carcinoma. McGuire published eight criteria needed for a study which purports to evaluate a prognostic factor. These restated criteria include[129]:

1. The new factor should be rooted in biological plausibility
2. Predetermine whether the investigation is a pilot study or definitive study
3. Perform sample size calculations prior to patient or sample accrual
4. Identify the likely selection biases involved

5. Use validated methods of measuring the factor in question (or validate any newly developed methods)
6. Include optimal representation of the factor in the analyses
7. Perform univariate as well as multivariate analyses including standard and known prognostic factors
8. Validate the tests using both internal and external data sets

In a following communication, a note was made of the increased likelihood of committing a type I error due to the measuring of multiple factors and multiple cut-points within individual factors. It was recommended that this potential error be decreased by using independent validation sets and / or other statistical methods[130]. August et all analyzed a series of 33 patients who underwent liver resection between 1980 and 1983. Of note, 21 patients received intraperitoneal 5-FU while the remainder received no adjuvant treatment. They reported a 53% 4-year survival and a median survival of 38 months. They reported two factors predictive of survival: The number of metastases (with 4 or more lesions having a worse prognosis) and the distribution of the metastases (unilobar versus bilobar disease with bilobar disease having a worse prognosis). They also found that lack of an R0 resection led to a decreased time to recurrence [131].

In 1992 a series of 280 consecutive hepatic resections performed at the Mayo clinic were analyzed. Several negative prognostic factors were revealed. Extra-hepatic disease and extra-hepatic lymph node involvement were both associated with a poor prognosis in terms of overall survival. Today, these factors would lead to the conclusion that a patient has unresectable disease. They also noted that metastases with a satellite configuration and metastases initially detected via abnormal liver enzymes were

associated with a poor prognosis. The only positive prognostic factor found was if a patient presenting with Dukes B disease was discovered to have synchronous metastases[83]. Gayowski et al confirmed the negative prognostic impact of extra-hepatic disease and positive margins, noted similar poor prognostic factors as seen by August (4 or more metastases, bilobar distribution), as well as adding 2 new factors (aged 60 years or greater, and 24 months or less between resections for primary and metastatic disease)[86].

Shirabe et al noted in a series of 31 patients who underwent liver resection for colorectal metastases that a poor prognosis was associated with several factors that had not been prominently described before. Tumour size greater than 4cm, 6 months or less between primary and secondary resections, 4 or more tumours detected pre or intra-operatively, bilobar distribution, and resection margin less than 10 mm. Cox proportional hazard modeling retained only tumour size and margin less than 10 mm as prognostic for poor outcome in a multivariate model[132].

It is important to note that several of the studies that looked at surgical margins included positive margin in the less than 10mm category[132]. Knowing that residual microscopic disease markedly worsens prognosis, the inclusion of positive margins in the less than 10mm category tends to bias results in favor of the $\geq$10mm group. In current practice, a positive margin is not acceptable when attempting to perform a curative resection.

Taylor et al from the University of Toronto analyzed 123 consecutive liver resections. They concluded that only the number of hepatic lesions could be used as a prognostic marker, with a solitary lesion imparting a superior prognosis compared to multiple lesions or to a solitary lesion amid satellite lesions[133].

Nagashima et al published results of an analysis of 65 hepatic resections for colorectal liver metastases. They performed both univariate and multivariate analyses on 59 of these patients and determined that in addition to microscopically positive margins and the presence of extrahepatic metastases, two further histological criteria were poor prognosticators. Both lymphatic invasion seen in the primary tumor, as well as the presence of an infiltrative growth pattern seen in the resected liver metastases were found to predict a poor prognosis. An important confounder in this study pertains to the chemotherapy received by each patient. 41% of patients received intra-arterial adjuvant chemotherapy, and 7% received portal venous chemotherapy. The authors do not provide details regarding how many also (or only) received systemic chemotherapy. There was an attempt to investigate if either adjuvant intra-arterial or portal venous chemotherapy was of prognostic significance, but without knowing whether these patients also received systemic chemotherapy, or whether the other patients received systemic chemotherapy, we can not be sure of the meaning of these results[134].

Yamamoto et al reviewed 96 patients who had at least one liver resection to determine factors associated with overall survival. After separating histologically positive margins from margins that were less than 1 centimeter or greater than or equal to 1 centimeter, they found that the extent of the surgical margin remained a significant prognostic indicator. A histologically negative margin less than 1 centimeter was still associated with a poorer prognosis. They also noted that having 4 or more metastases was associated with a poorer prognosis when compared to having 1 or 2 - 3 metastases. There were also three new pathological factors presented as prognostic indicators. Portal vein involvement, hepatic vein involvement, and the absence of a psuedocapsule

were all found to have negative prognostic implications.[135]. Weber et al later supported the prognostic significance of the absence of a tumour psuedocapsule[136].

A variety of novel factors have been elucidated recently. Koike's group noted that higher numbers of entrapped liver cells within tumors were associated with a worse prognosis than when fewer cells were entrapped[137]. Weber et al noted that a higher proliferative index (evaluated using Ki-67 labelling) was associated with a worse prognosis. An increased proliferative index was also detected in patients with 4 or more lesions[136]. Contrary to some previous studies, Weber et al noted synchronous metastases were associated with a worse prognosis than metachronous disease[136]. Ercolani et al looked at 245 consecutive patients who underwent potentially curative R0 resections for colorectal liver metastases. The percentage of hepatic parenchyma involved by tumor was found to significantly affect survival. Less than 25% liver involvement was associated with a better overall survival than if either greater than 25% of liver was involved, or greater than 50% of liver was involved. Percentage involvement was then translated into a concept known as Total Tumor Volume (TTV: calculated by adding the calculated volume of each liver lesion present). It was then shown that a higher TTV was associated with a worse overall survival. Even when comparing patients with multiple metastases with those who had a single lesion, TTV was the factor that was related to prognosis, overriding the number of lesions present. They also concluded that, if an R0 resection was feasible, the number of metastases should not be used as a contraindication to resection as a 20% 5-year survival was still feasible[138].

SCORING SYSTEMS: COMBINING PROGNOSTIC FACTORS

The cohort of patients that are eligible for resection of their colorectal liver metastases are not a homogenous group. Both survival and early recurrences seem to be heterogeneously allocated. This distribution has led to a significant effort being directed towards finding a scoring system that can be used in patients that are otherwise candidates for resection. This system would then be used to identify patients that have the least benefit from surgery: Patients in whom the morbidity, mortality, and recovery associated with the operation render it a poor treatment option when compared to the potential benefit they may receive. Ideally, a system would also be able to stratify patients based on their likelihood of recurrence and early recurrence in particular. This stratification may then be used in the construction of clinical trials. Those patients at higher risk for recurrence may then be followed differently and/or be offered different adjuvant chemotherapy. This may allow clinicians to spare the more toxic therapeutic regimens for the patients that are likely to benefit the most, leaving the rest of the patient population with a less toxic chemotherapy (if any at all).

Nordlinger – 1996

In 1996 Nordlinger et al proposed such a scoring system[59]. They used univariate and multivariate analyses to evaluate the importance of identifiable factors on both 2 and 5-year survival in 1568 patients with resected colorectal liver metastases. Using their results, they then developed a scoring system that allowed the division of the population into 3 risk groups each with distinct 2-year survivals. The factors incorporated into their scoring system included: age > 60, size of largest metastasis $\geq$ 5cm (or CEA level), stage of the primary tumor, disease free interval less than 2 years, number of liver nodules, and resection margin[59].

Iwatsuki – 1999

In 1999 Iwatsuki et al proposed a scoring system for metastatic hepatic tumors. Their initial analysis included patients with positive margins of resection and / or extra-hepatic nodal involvement. They noted that the patients who had positive margins and / or extra-hepatic nodal disease had uniformly poor survival. This led the authors to re-analyze their data after the exclusion of patients who were positive for one or both of these factors. The 243 remaining patients revealed that (1) tumor number of three or more, (2) tumor size greater than 8 cm, (3) time to hepatic recurrence of 30 months or less, and (4) bilobar tumors were independent indicators of poor prognosis. They then proposed a scoring system based on these factors. Patients would be categorized into one of 5 groups according to increasing score (no factors present=0, and 1 point is awarded for each factor, to a maximum of all factors present=4) As the number of factors present increased, overall survival decreased. The number of factors could then be adjusted using coefficients determined via Cox regression to provide an actual risk score which proved to be good a predictor of survival[139].(**Error! Reference source not found.**)

Fong – 1999

Also in 1999, Fong et al investigated 1001 consecutive resections performed between 1985 and 1998. He noted seven clinical factors were predictive of poor longer-term survival. Of these factors, two were found to associate with such a poor prognosis that they in fact should be considered contra-indications to surgery. These two factors; positive margin and extra-hepatic disease, mirrored results from previous studies. The

remaining five factors (node-positive primary, disease-free interval from primary to metastases <12 months, number of hepatic tumors >1, largest hepatic tumor >5 cm, and carcinoembryonic antigen level >200 ng/ml) were used to generate a score out of five since the presence of each factor is given a single point. This score proved to consistently predict outcome. It was found that even though the calculated actuarial survival was 14%, no patients with a score of 5 were actual long term survivors[94].

The addition of adjuvant chemotherapy was not reported in this study, but can be assumed to be almost universal. There is no indication of when CEA levels were taken in relation to the resection of the metastases (i.e. it is possible that some of the CEA levels were taken when the primary lesion was still in-situ). Even in light of these shortcomings, the size of this study alone makes it a significant contribution to the demand for a scoring system that may be used to prognosticate survival.

A summary of the factors in each of the above mentioned scoring systems is presented (Figure 1-25), and the associated survival based on these scores is presented (Figure 1-26).

Mala et al recently published a validation of Fong's Clinical Risk Score (CRS). 113 patients who had previously undergone hepatic metastectomies were stratified using a modification of the Clinical Risk Score. A change was made in the classification of CEA status with patients being classified according to the presence of CEA levels greater than 100ng/ml. Once a score was applied, patients were classified into two categories: 0-2 and 3-5 (although no patients were scored a five). A significant survival advantage was found in the lower scoring group[140].

The CRS has also been used to evaluate a patients likelihood of harboring unresectable disease when pre-operative clinical and radiological work-up suggests

resectability. Jarnagin et al found that in patients with a CRS of $\leq$ 2 disease was deemed unresectable at laparotomy in only 2% of patients. For patients with a CRS of $\geq$3 however, 42% of patients were found to have unresectable disease at laparotomy[141]. If patients at high risk were examined laparoscopically first, a significant percentage of unresectable patients would be identified without the added morbidity and mortality associated with a laparotomy.

All reviewed studies did demonstrate a survival benefit inpatients who underwent resection regardless of score when compared with reported survival in patients who are not resectable.

All 3 of the major scoring systems presented are designed to predict survival (disease free and / or overall). These systems may also be applicable in the prediction of the utility of adjuvant chemotherapy in general and possibly even the type of chemotherapy. Further studies are needed before any inferences can be made regarding these properties.

The minimal contribution of these scoring systems lies in their ability to stratify patients. Future clinical trials should show that patient groups are comparable based on standard demographic, surgical, and pathological factors as well as with regards to prognostic score.

Many of the studies have short comings with respect to their overall design. Several of the studies do not delineate whether patient's received adjuvant chemotherapy. Two often quoted negative prognostic markers are an R1 or R2 resection and extra-hepatic disease. Both these markers are contraindications to curative surgery and therefore should not really be considered prognostic markers. Except for very select cases (i.e. solitary lung metastases) in which it has been

demonstrated that survival is not drastically altered, there is no indication for performing a liver resection in someone with extra-hepatic metastases. Likewise, there is no indication for performing palliative liver resections, therefore only R0 resection should be attempted.

MISCELLANEOUS PROGNOSTIC MARKERS

Many proposed prognostic markers have shown some initial promise, but most have been refuted by subsequent studies. The size of the primary tumour was such an example[83 133 142]. There are others whose relevance is still under investigation. Memon and Beckingham[143] classified potential prognostic indicators into 4 categories: patient factors, primary tumor features, liver metastases features, and surgical factors.

Patient Factors

The most prominent examples of patient factors are: 1)age which was only endorsed by two studies[59 82], and 2) gender which has been widely refuted (except for 2 studies which suggest a favorable prognosis with opposite genders).

Primary Tumor Features

The stage of the primary tumour and the site of the primary tumour are the most prominent potential factors in this group. 12 studies endorse the primary tumour as a prognostic factor, while 8 studies refute it. Only 2 studies endorse the use of the site of the primary tumour and they disagree on the indicator. One study suggests that right sided tumors are associated with a worse prognosis[144], while the other study suggests

that rectal tumors are associated with a worse prognosis[59].

Liver Metastases Features

The number of metastases present has been shown to be significant in numerous studies, but the cut-off has varied (i.e. 1 versus >1[94]; 1-3 versus ≥4[59], 1-2 versus >2 [139]), and several studies have suggested that the presence of satellite lesions, as a separate entity from number of metastases, is a negative prognostic marker [20 83]. Tumor size is often regarded as helpful in prognosticating, but again, there is disagreement as to which cut-off appropriately classifies poor prognostic markers (i.e. >5[59 94] or >8cm[139]). The timing of the diagnosis of metastatic disease, in relation to the detection of the primary tumour, has been found to be non-significant but alternatively, a poor prognosis has been attributed to metastases presenting synchronously, less than 1 year from diagnosis, and less than two years from diagnosis. Bilobar disease has often been quoted as a poor prognostic variable. Bolton et al demonstrated that if complete resection was possible, a significant survival benefit is gained from resecting bilobar disease. They did note that, as expected, surgically related mortality was increased when compared to single lobe or lesser resections. But even when this mortality is included in the analyses, a significant survival benefit is seen[145].

Surgical Factors

A microscopically positive resection margin has almost universally been found to be a negative prognostic indicator, as has extra-hepatic disease (including lymph node involvement) except in rare circumstances. So much so that both entities should be considered contra-indications to resection. In a thorough systematic review, Rodgers

and McCall concluded that when there is lymph node involvement, the prognosis is poor regardless of whether there was a lymph node dissection or not[146]

There is some controversy as to what constitutes an adequate margin. Originally, a margin of $\geq$ 1 cm was advocated[85 142 147]. More recently this has been questioned. Kokudo et al attempted to definitively answer this question. They looked at 194 patients retrospectively and prospectively enrolled 58 patients undergoing 62 resections. In addition to the standard microscopic assessment of surgical margins, they evaluated margins using polymerase chain reaction with primers for K-ras and p53. They noted that the best results were found in those that had a margin $\geq$ 1 cm as was classically advocated. They also noted, however, that with any negative margin, there was no significant difference in overall survival, but there was a strong trend favouring a margin $\geq$ 1cm in terms of disease free survival. Margins < 2mm had a cut-end recurrence rate of 20%, but when the margin was greater than 2mm, the rate of cut-end recurrence reduced to 5.6-7.5% for margins between 2 and 4mm), and to 0% for margins greater than 1 cm. They concluded that a $\geq$ 1cm margin should be the goal of a curative resection, but the inability to get such a wide margin should not preclude a patient from undergoing hepatic resection , as long as there is a negative margin[148]. This study can be criticized for using genetic analysis in determining whether a margin is positive or not, as this is not what is routinely practiced. In defense of this practice, a recent study indicates that a margin found positive by non-standard means (in this case immunohistochemistry) also indicates a significantly worsened prognosis[149].

Figure 1-25. *The components of three different scoring systems. For each system the factor associated with increased risk is shown.*

Factor	Nordlinger (1996)	Iwatsuki (1999)	Fong (1999)
Tumor Number	> 4	> 2	> 1
Size of Largest Tumor	≥ 5 cm	> 8 cm	> 5 cm
"Disease Free Interval" (Interval between resection of primarn and diagnosis of metastases	< 2 years	Not Used	< 12 months
Interval Between Resection of Primary and Resection of Metastases	Not Used	≤ 30 months	Not Used
Tumor Distribution	Not Used	Bilobar	Not Used
Primary Tumor	1. Invades into serosa 2. Lymphatic spread present	Not Used	Node Positive
CEA Level	Not Used	Not Used	> 200 ng/ml
Age of Patient	> 60 years	Not Used	Not Used
Resection of Margin	< 1 cm	Not Used	Not Used
Score Range	0-7	0-4 (0-1.4361)	0-5

Figure 1-26. Prognostic system scores and resultant survival

Scoring System	Points	1-year survival	2-year survival	3-year survival	5-year survival
Nordlinger (1996)	0-2		79%		
	3-4		60%		
	5-7		43%		
Fong (1999)	0	93%		72%	60%
	1	91%		66%	44%
	2	89%		60%	40%
	3	86%		42%	20%
	4	70%		38%	25%
	5	71%		27%	14%

EXTRA-HEPATIC METASTASES

For the majority of patients, extra-hepatic recurrence (aside from local recurrence of the primary lesion) signifies that a patient has unresectable disease. There is one uncommon but notable exception: the presence of lung metastases does not necessarily preclude the patient from potentially curative surgery. Ten to 20% of patients with resected colorectal carcinoma will develop pulmonary recurrences. Of these about 2-4% are amenable to resection[150 151]. Lung resection for colorectal metastases has been shown to be a low mortality and low morbidity procedure which significantly prolongs survival. Murata et al presented a series of 30 patients who had both hepatic and pulmonary colorectal metastases resected. Mortality was reported to be 0% and morbidity was a very acceptable 10% (3 instances in 30 patients). A 5-year survival of 43.8% was realized[152]. The goal surrounding resection of lung metastases parallels that surrounding hepatic resection for liver metastases. The aim should be for an R0 resection.

As the liver is the most common site of recurrence after hepatic resection, the lung is the most common site of recurrence following pulmonary resection. And, again like liver, repeat resections may be offered in ideal situations.

PERI-OPERATIVE CHEMOTHERAPY

Even though we expect randomized controlled trials to help differentiate useful treatment from ineffective treatment, the ideas for the treatment combinations come from a combination of basic science research and smaller, often retrospective, studies. These studies often use "tumor response" to determine if a treatment has any efficacy. Tumor response is also often used in the smaller prospective (pilot) studies. It is important to not rely too heavily on tumor response over survival data as there is evidence that tumor response alone may not predict overall survival. Buyse et al completed a systematic review demonstrating that, even though it was a useful surrogate for survival, tumour response alone does not explain the variation in survival seen in individual studies. They suggest that individual studies should augment tumour response with other biomarkers[153].

ADJUVANT THERAPY AFTER RESECTION OF COLORECTAL LIVER METASTASES

The most common hypothesis regarding recurrence of colorectal carcinoma after R0 resection of the primary presumes that micrometastases remain after surgery. This has led to the use of adjuvant therapy in the treatment of colorectal carcinoma. Similar hypothese exist in the setting of colorectal liver metastases. It has been shown that even though survival is improved by liver resection, recurrence is still common. It is postulated that these recurrences arise from undetectable liver metastases present at the time of resection as well as isolated tumour nests. Weitz et al demonstrated the presence of tumor cells both within the vasculature as well as within the bone marrow of patients undergoing hepatic resection[154]. These remaining metastases may then be spurred on by mitosis promoting growth factors released by the regenerating liver[155 156].

The previously mentioned benefits of adjuvant chemotherapy in node-positive primary colorectal has led to the use of adjuvant chemotherapy after the resection of colorectal liver metastases. Unfortunately, due to disparate results from numerous studies, many modalities of administration and many different combinations of cytotoxic pharmaceuticals or immunologic agents are used. Studies have looked at intra-arterial, intraperitoneal, portal venous, and systemic chemotherapy. Confusing the issue even further, most of these modes of administration have studies supporting them, studies demonstrating them to be ineffective, and even studies showing them to be detrimental. Much of the data used to support adjuvant treatment of resected colorectal metastases, especially the data used to support adjuvant systemic chemotherapy, is extrapolated from research on unresectable hepatic metastases. There is surprisingly little prospective research in the population of patients that have had colorectal liver metastases resected. It seems as if the addition of systemic adjuvant chemotherapy has been taken for granted, and all the research focuses on the utility of adding regional chemotherapy. Unfortunately, the role of adjuvant systemic chemotherapy has never been scientifically established.

SYSTEMIC ADJUVANT CHEMOTHERAPY

Little evidence is available that supports the use of adjuvant systemic chemotherapy, and little evidence exists that refutes its use. However, due to the success of adjuvant chemotherapy in the setting of node positive primary colorectal cancer, clinicians have extrapolated the results so that adjuvant systemic chemotherapy has essentially become standard in chemotherapy naïve patients with resected colorectal liver metastases[157].

Recently, chemotherapeutic combinations have proved to be effective in metastatic disease that was considered unresectable[8 62-64]. Both Douillard et al and Saltz et al demonstrated improved survival and disease free survival after administration of 5-FU in combination with irinotecan [62 63]. Unfortunately, the method of administration proved to be an important factor and one of these early studies was associated with significant toxicity, causing some trials in progress to be halted. Fortunately, these adverse events have not led to the abandonment of combination therapy but have led increased monitoring of drug administration.

A retrospective study looked at 235 patients who had undergone 256 liver resections. 55% of these patients received adjuvant chemotherapy, usually 5-FU and leucovorin. The addition of adjuvant chemotherapy significantly improved 5-year survival[112]. In their conclusion regarding the role of adjuvant systemic chemotherapy after the resection of colorectal liver metastases, the authors note that "prospective studies are necessary to define its exact role"[112].

Many authors recognize the limitations in knowledge due to the lack of prospective trials evaluating the efficacy of the addition of systemic chemotherapy as adjuvant treatment after resection of colorectal liver metastases. Unfortunately, this did not translate into better patient accrual for a well designed randomized trial that would answer this question. The ENG trial was a cooperative between the EORTC, NCIC CTG, and GIVIO. In 5 years only 129 patients were enrolled. This ambitious trial was thus forced to close prematurely. Analysis of the patients that were enrolled demonstrated a trend towards a survival benefit attributable to the addition of 5-FU and leucovorin chemotherapy, but this trial lacked the power to demonstrate significance[158].

HEPATIC ARTERIAL INFUSIONAL (HAI) ADJUVANT CHEMOTHERAPY

It is hypothesized that the portal vein carries metastatic cells from the colorectal primary allowing implantation in the liver. These cells initially support themselves via diffusion from adjacent blood supply – mainly the portal venous system[159 160]. Once a critical mass is reached, tumour masses gain the ability to induce angiogenesis, and with the growth of new vasculature, the cells within the tumour masses begin to get their nourishment from the hepatic arterial supply. This has led to research involving chemotherapy infused via the hepatic artery. As the tumour masses are too small to be detected radiologically, they are hypothesized to be the ones left behind after resection that may eventually give rise to hepatic recurrences. These same tumour masses quickly become large enough to develop their own arterial based blood supply. It is believed that targeting these cells via their unique blood supply is possible via hepatic arterial infusion and that this may be effective in increasing survival and reducing intra-hepatic tumour recurrence[157]. Another justification for regional chemotherapy is based on the step-wise model of metastatic progression which hypothesizes that the liver becomes involved with metastases initially (via the portal vein) and then eventually, the metastases spread beyond the liver to the other organs (i.e. lung, brain, etc.)
Most of the studies that report an impact on over-all survival by using HAI chemotherapy alone, or in combination with systemic chemotherapy are retrospective in nature. Ambiur et al reviewed 78 patients who received HAI and compared them to 30 who had received portal vein infusional chemotherapy and 66 who received no regional adjuvant chemotherapy. A significant benefit in terms of overall survival was noted in patients treated with adjuvant HAI chemotherapy[161].

A systematic review published in 1996 suggested that in patients with unresected colorectal liver metastases, regional chemotherapy in the form of FUDR administered via the hepatic artery confers a small (10%) but significant benefit in 1-year survival when compared to systemic chemotherapy (5-FU and FUDR). The survival advantage at 2-years is less (6%) and no longer significant[162].

One of the first trials to prospectively examine HAI in patients who have had their colorectal liver metastases resected was from Kemeny et al in 1986. In this ambitious but small study, she found a non-significant trend that suggested HAI may improve survival[163].

Curley et al prospectively analyzed a series of 20 consecutive patients who received adjuvant 5-FU infused via the hepatic artery following resection of their colorectal liver metastases. 18 patients were analyzed. Of these 18, only 10 completed the 6 month course of chemotherapy. 8 patients had to discontinue chemotherapy; four due to toxicity and four due to catheter related complications. Five of the patients that completed the course required dose reduction due to toxicity. This pilot study demonstrated a significant increase in median survival in those patients that did not develop intra-hepatic recurrence of their disease.[164]

Historically, a major morbidity from this method of infusion was related to the catheterization of the hepatic artery with an arterial pump. In some patients this led to catheter failure due to clotting, hepatic artery thrombosis, infection, as well as duodenal ulcers. This procedure has since been abandoned in favour of a different type of surgically implantable device. The newer devices have lowered the instances of infection and thrombosis while maintaining long-term patency[165-167].

The majority of trials that used hepatic arterial infusional therapy chose floxuridine (FUDR or 5-fluor-2`deoxyuridine) as their cytotoxic compound that was to be infused into the hepatic artery. FUDR was chosen as it has significant first pass extraction (approximately 94-99%)which is much greater than that of 5-FU[168]. FUDR is often given in conjunction with dexamethasone as the combination has been shown to be less hepatotoxic as well as potentially improving response rate and most importantly, survival[169]. This represents an up to 100 fold increase in hepatic drug exposure in HAI FUDR when compared to systemic exposure. FUDR has the advantage of an up to 400 fold increase in hepatic exposure when compared to systemic exposure. This property should result in minimizing systemic toxicity thereby allowing improved patient quality of life, as well as the use of higher doses, and favouring maximal exposure of the liver metastases to the chemotherapuetics.

One of the drawbacks of HAI is that it fails to address concerns regarding extra-hepatic recurrences. About 2/3 of patients will develop extra-hepatic recurrence (either solely or in combination with intra-hepatic recurrences). This concern has led to the addition of systemic chemotherapy to regimens using regional (HAI) chemotherapy. There is, of course, a cost associated with the addition of HAI to a therapeutic regimen. It has been estimated by Durand-Zaleski et al using data from single institutions in Palo Alto, California and Paris, France that (in 1996 US dollars) the addition of HAI adds approximately $20,000 to the costs associated with treatment. This then translates into an cost-effectiveness of between $72,000 and $74,000 per life-year[170]. By most criteria, this cost-effectiveness ratio would be considered too expensive.

One of the earlier groups that added adjuvant chemotherapy after liver resecion was Rudroff et al. Patients who were resected between 1984 and 1985 were randomized to

receive HAI chemotherapy with mitomycin C and 5-FU versus no adjuvant therapy. They reported no difference in long-term disease-free status or long-term survival[171].

Lygidakis et al presented a study where they randomly allocated twenty patients to treatment after hepatic metastectomy. Half the patients received no adjuvant therapy, and the other 20 patients received locoregional chemotherapy in combination with locoregional immunotherapy. This small study found significant differences in mean survival and disease-free survival all in favor of the adjuvant chemotherapy group[172].

Lorenz et al started accrual of patients from 26 different hospitals into a protocol comparing HAI 5-FU and folinic acid as adjuvant treatment after liver resection for colorectal liver metastases. At the first interim analysis, interpreting from their confidence intervals, they noted that at most there may be a small (15%) overall survival benefit due to the addition of HAI chemotherapy, but it was just as likely that there was a significant increase in the risk of death. This was taken in combination with the fact that 44 patients (63%) (out 73 for which they had chemotherapy related data) demonstrated grade 3 or 4 toxicity. As they had demonstrated no improvement in survival from the addition of HAI 5-FU and leucovorin and that there may be a significant risk of harm, patient accrual for this protocol was thus terminated[173]. Lorenz et al did not demonstrate any advantage in survival by using HAI + systemic 5-FU after resection of colorectal liver metastases when compared to resection alone[173].

In 1999 Nancy Kemeny et al reported on their randomized trial in which they accrued 156 patients who were randomly assigned to receive systemic chemotherapy post-resection of colorectal liver metastases or to receive systemic chemotherapy in addition to regional HAI chemotherapy. The investigators started with 514 eligible

patients of which 111 were, appropriately, not resected or had no tumor. 17 eligible patients, however, had "miscellaneous" reasons for not being included. Of the remaining patients, 105 declined, 28 were ineligible due to exclusion criteria that were changed part way through this study, and 65 were excluded for vague reasons including "surgeons decision", "not appropriate for the protocol", and "miscellaneous". 11 had "poor arterial blood supply to the liver". 13 were also listed as "living too far away". This discrepancy between patients eligible and patients included, and the reasons given for exclusion, bring up a host of concerns about this study. There is a very significant likelihood of the presence of selection bias. There is a likelihood of a treatment bias as the protocol changed part way through the study, even though the patients who had previous experience with chemotherapy appeared evenly distributed. Of the 156 patients who underwent hepatic resection, 21 (14%) did not have an R0 resection, and 1 even had positive portal nodes. This is a greater than usual amount of non-radical resections.

Nonetheless, this study did show significant differences in 2-year actuarial survival, with the combined chemotherapy group displaying 86% 2-year survival while the monotherapy group demonstrated 72% 2-year survival. Median survival was not significantly different between the two groups. These results are promising, but should be regarded with caution in light of the above mentioned concerns. There was, however, no significant difference in 5-year survival.

These observations indicate that long-term survival results are crucial in evaluating treatment regimens. The magnitude of the treatment associated toxicity is demonstrated in part by the fact that only 26% of patients in the combined therapy arm received at least 50% of their intended chemotherapy dose. 29 patients needed

hospitalization for management of toxicity- related concerns versus 18 in the monotherapy arm – a significant difference[174]. Also of note, the significant results are from subgroup analyses that may not have been prospectively defined. The kaplan-meier curves were analysed using the normal approximation test which is not the test normally performed in this analysis. A skeptic may believe that this test was used because the log-rank test and the wilcoxon test were non-significant (p=0.21 and p=0.11 respectively) When this study was designed, a one-tailed test was used during sample size calculations. After data was gathered, the analyses was performed using a two-tailed test which, due to lack of power, failed to give a significant result. (if a two-tailed test was used during the sample size calculations, a recruitment of 196 patients would have been necessary[175]).

M. Kemeny et al completed an ambitious and important study on the efficacy of HAI[176]. They accrued 109 patients over 7 years. The patients were randomized to receive either hepatic artery infusional therapy with FUDR to be followed by systemic 5-FU or exclusively resection of their colorectal liver metastases. Unfortunately, there are a number of difficulties that are encountered when interpreting this work. Of the 109 patients randomized pre-operatively, only 80 ended up in their assigned arm. Of those, 35 were assigned to the experimental arm, of which only 30 were able to be assessed. Of the 30 patients in the experimental arm, 10 patients (33%) did not receive their allotted treatment (4 (13%) received 75% of intended dose, 4 (13%) received half of their intended dose, (1) 3% received 25% of their intended dose, and 1 (3%) received no HAI therapy at all). According to the design, all 30 of these patients were also supposed to receive systemic chemotherapy, but only 13 received the full course. Seven discontinued due to recurrent disease and 9 had dose reductions due to toxicity, all of

which was within protocol. Of greater concern are the 3 who did not receive dose escalation after completing HAI therapy (with no reason given). Due to the large discrepancy between those randomized and those that actually received their allotted treatment, analysis by intention to treat was judged to be futile. To remedy this situation they analyzed their results by treatment actually received. This is also problematic as the high number of patients not receiving their allocated treatment likely has introduced significant (selection) bias into these results[176]. The authors concluded that there was no significant difference in overall and 4-year survival. They did find significant differences in 4-year disease free survival and 4-year liver-specific disease – free survival. From this they concluded that the combination of HAI followed by systemic chemotherapy was "the current best regional treatment."[176], citing a previous study by N. Kemeny as support. Brief mention was made of the study by Lorenz which did not demonstrate any benefit from using HAI after resection of colorectal liver metastases. Of note is that this study was never designed to detect differences in overall survival.

Tono et al randomized 19 patients into two groups: one group received HAI chemotheraspy post hepatic metastectomy, the other group did not while both grouped received systemic 5-FU administered orally. The control group received oral 5-FU for two years starting approximately 4 weeks after resection, while the experimental group only started receiving oral 5-FU after the HAI courses were complete. All patients in the experimental group completed their course of HAI chemotherapy. After more than 5-years of follow-up, they noted significant differences in disease free survival, but not in overall survival. Also of interest was the fact that toxicity was minor with only one patient experiencing toxicity (grade I nausea and anorexia)[177].

Of note is that in all three of the major trials, a relatively large number of patients did not receive the HAI therapy as they were assigned. This may be due to numerous factors and raises the spectre of bias due to both patient and centre selection, but regardless of the cause, it demonstrates a difficulty in generalization as these highly specialized centers running a fairly rigid protocol were unable to administer this treatment to a significant number of patients. A final concern is the lack of long-term survival benefit realized.

Depending on the chemotherapeutics used, HAI may be limited by the relative lack of extrahepatic drug exposure. This is important in light of the fact that about 2/3 of patients will have extra-hepatic recurrence. Also of note is the fact that in the randomized trials comparing systemic chemotherapy versus HAI chemotherapy and / or combination chemotherapy, the systemic chemotherapy chosen is not the most active or efficacious. 5-FU and leucovorin plus irinotecan is considerably more active than 5-FU and leucovorin alone[62-64] and the addition of oxaliplatin may improve on this even further. Until a trial looks at the most active systemic chemotherapy with and without additional HAI, there is no scientific rational for the use of HAI chemotherapy.

NEOADJUVANT THERAPY

There are three possible roles for neoadjuvant therapy: 1) To "downstage" unresectable disease in patients into resectable disease 2) For people who already are resectable to make resection technically simpler 3) To increase survival benefits over surgery alone (possibly to a similar or greater degree than post operative adjuvant chemotherapy)

Once again, the results seen in primary colorectal cancer are seen as indicators that this type of therapy may be beneficial in those with metastatic disease. There are numerous studies which show that down staging of some initially unresectable tumors to a stage where they are resectable is not only possible, but also significantly increases five-year survival versus someone who did not receive treatment and was therefore unresectable[178 179]. As many as a third of tumors are able to be downstaged in this manner.

Shankar et al demonstrated that treatment with neoadjuvant therapy may downstage a patient enough so as to allow liver resection. Three different modalities were used (systemic chemotherapy, HAI chemotherapy, and chemotherapy plus interstitial laser therapy. All of the patients demonstrated a response to treatment, and survival was comparable to those reported in patients who were initially resectable. This is a small study, and conclusions must be interpreted with caution[180].

Adam et al treated 701 patients with unresectable colorectal liver metastases with neoadjuvant chemotherapy usually consisting of chronomodulated 5-FU, leucovorin and oxaliplatin. They noted that 13.5% of the patients were eventually resected. Those that were resected appeared to have similar 5-year survival (35%) to what has been widely reported for patients who undergo curative resection without neoadjuvant chemotherapy[178]. Giachetti et al published similar results[181].

The benefits of therapy come with substantial costs. One such cost is the added toxicity of the neoadjuvant chemotherapy. As many as 68% of patients experienced grade III or IV toxicity as stated by Alberts et al in an interim report from the North Central Cancer Treatment Group neoadjuvant trial[65]. A second cost is purely financial,

cost estimates have been as high as 42000 (EUROS) per patient downstaged into resectable[182].

Without demonstrated evidence in patients who initially have resectable colorectal liver metastases,many authors, including Nordlinger and Rougier, have suggested that "surgical resection without prior administration of chemotherapy should remain the treatment of choice for most patients"[183].

RECURRENCE

As many as 2/3 of patients will recur after potentially curative liver resections. About 30% of patients that recur after liver resection for colorectal metastases will have recurrence isolated to the remaining liver[184-186]. Resection of these isolated hepatic "re"-recurrences is increasingly reported[184 185 187-196]. Petrowsky et al examined 126 re-resections and noted morbidity and mortality comparable to initial hepatic resections (morbidity 28% and mortality 1.6%). They also noted 1,3, and 5 year survival rates of 86%, 51% and 34% respectively[197]. When looking at prognostic factors, they found them to be similar to those in initial hepatic metastectomies. Number of recurrent tumors and size of the largest recurrent tumor were the only significant factors on multivariate analysis[197].

Yamaguchi et al examined the relationship between the pathologically determined "mode of infiltrative growth" (INF) and the likelihood of intra-hepatic recurrence in patients who had less than 4 lesions, each less than 6 cm in size. They noted 2 distinct growth patterns: INF α – expansive growth with a sharp boundary with adjacent tissue; and INF γ – invasive growth without a boundary with adjacent tissue. They also noted the existence of an intermediary pattern named INF β. These classifications were associated with distinct 5-year disease-free survival. Patients with tumors that displayed INF α or INF β type growth had an estimated median survival of 40.7 +/- 3.2 months with a 64% 5-year disease-free survival rate. Those with lesions that displayed INF γ type growth had a significantly worse prognosis with an estimated median survival of 12.5 +/- 2.3 months and a 5-year disease free survival rate of 14%[198]. The authors suggest that there was a difference in surgical resection margins between the two groups, in that those with INF γ were less likely to have resection

margins $\geq$ 1cm. Extent of surgical resection margin was not a significant independent prognostic marker in these patients however.

Yamaguchi et al then tried to extrapolate these prognostic factors to computed tomography. They noted that the outline of nodules displaying different types of growth (i.e. INF α, INF β, INFγ) were fairly distinct (81% of nodules with INF γ growth had irregular contours while 78% of nodules with INF α or INF β growth had regular contours). Two factors were significant on univariate analysis: the ratio of length to width of the nodule $\geq$1.5 versus < 1.5 (0% and 33% 5-year disease-free survival rate);and irregular contour versus regular contour (0% and 40% 5-year disease-free survival rate). Multi-variate analysis demonstrated that contour was the only independent prognostic factor[199].

PREDICTIVE / PROGNOSTIC BIOLOGICAL MARKERS

Knowing that no single treatment can boast a 100% response rate, the treatment of a patient with colorectal liver metastases involves the making of several important treatment decisions. Will this patient benefit from resection of his intra-hepatic metastatic disease? Should this patient have adjuvant chemotherapy after his resection (or even pre-operative neoadjuvant)? If chemotherapy is indicated, what combination of chemotherapeutic drugs would be optimal? The goal of the oncological team, is to make decisions that maximize survival and minimize the exposure to toxicity. Currently these clinical decisions are based, mostly, on a combination of generalized results from trials, what the team and patient feel are acceptable and probable outcomes. It would be extremely beneficial if these decisions were made with the aid of patient specific information. For instance when, in hopes of increasing survival, deciding whether a patient should be exposed to adjuvant chemotherapy and its associated toxicities it would be valuable to be able to predict what the most likely benefits and harms of the available alternatives are in this particular patient.

THYMIDYLATE SYNTHASE (TS)

Thymidylate Synthase (TS, N^5, N^{10}-methylenetetrahydrofolate, dUMP *C*-methyltransferase, EC 2.1.1.45) catalyzes the irreversible conversion of 2-deoxyrudulate (dUMP or 2`deoxyuridine-5`-monophosphate) to thymidylate (dTMP or 2`-deoxythymidine-5`-monophosphate). This is the cell's only de novo source of thymidylate. As the thymidylate is then used in the construction of DNA, halting de novo synthesis of thymidylate arrests DNA synthesis. The most commonly used cytotoxics in the treatment of colorectal cancer are fluorinated pyrimidines such as

floxuridine and fluorouracil. These fluorinated pyrimidines are metabolized to fdUMP which, using a folate cofactor, then binds TS and blocks the dUMP binding site, thereby halting dTMP synthesis and consequently arresting DNA synthesis. Increased TS levels (resulting from gene amplification) have been shown to lead to cellular resistance to fluoropyrimidines[200-202].

TS has long been of interest to those who work with colorectal cancer as it is the target of fluoropyrimidine based chemotherapy. This role has led to the wealth of knowledge that exists regarding the genetic coding, make-up, and function of this enzyme. An even more important role may be emerging for TS as new chemotherapeutic agents are found to be efficacious in the treatment of colorectal cancer[203]. These new drugs have activity at different sites within the tumor cell and are therefore not reliant on TS levels for function or resistance. This potentially gives the classification of TS levels a role in determining which patient receives which therapeutic regimen. Patients who are found to have high levels of TS (which has been shown to be associated with poor response to fluoropyrimidine based therapy) may be selected to receive non-fluoropyrimidine based treatment.

There are several ways to quantify TS within tissue. Polymerase Chain Reaction (PCR) can accurately quantify the amount that the gene for TS is expressed. The inference is that over or under expression of the gene specific for TS correlates directly with the over or under expression of TS itself. Alternatively, immunohistochemical (IHC) techniques may be used to demonstrate actual TS levels within the tissue of interest. Research has shown that the amount of TS genetic expression as measured by PCR and the amount of TS enzyme expression as determined by IHC are closely associated[203-206].

As can be inferred from the use of PCR in the detection of TS genetic expression, the genetic sequence which codes for TS is known, as is its locale (18q). Studying the gene has revealed the existence of a polymorphism at the 5`-terminal, consisting of either a double or a triple repeat. The different sequences have been linked to different gene expression frequencies[207].

The initial work on TS demonstrated its role in resectable primary colorectal cancer. Later its role in unresectable metastatic colorectal cancer was elucidated. Finally, TS's role in patients with resected intra-hepatic colorectal metastases is being brought to light.

Immunohistochemical techniques for the determination of TS levels within resected colorectal cancer specimens have been developed. Edler showed that TS levels quantified using immunohistochemical techniques applied to tissue gathered from paraffin blocks accurately reflects the enzymes activity in the tumour[208].

Trials have attempted to elucidate the effect of TS levels on both response to chemotherapy and on overall survival. One of the earliest such trials in colorectal cancer was Johnston et al who found immunohistochemically detected thymidylate synthase levels to be an independent prognostic factor of overall and disease-free survival in patients with resected Dukes B and Dukes C rectal cancer. It was noted that 5-year survival was 50% greater in the group of patients that were low TS expressers. Although TS expression was correlated with Dukes stage at the time of resection (high TS expression was correlated with higher stage disease) TS expression remained an important prognostic indicator after patients were stratified by stage. The authors agreed with the theory that this relation was due to the interaction between TS and the adjuvant fluoropyrimidine based chemotherapy. This relationship was found even

though the patients in this study were on a chemotherapeutic regimen that consisted of Lomustine (methylCCNU), 5-FU, and vincristine[209]. In retrospect, these results hinted at the relative importance of fluoropyrimidines and the relative lack of importance of methylCCNU and vincristine in treating colorectal cancer in an adjuvant setting. Leichman et al followed up on the results presented by Johnston.

Leichmans' study involved patients who had metastatic colorectal cancer and who may have been exposed to 5-FU based chemotherapy in the past. These patients also differed from Johnstons cohort in that they were concurrently being treated with a different regimen consisting of biomodulated fluororymidines (i.e. 5-FU and leucovorin). Finally, PCR based TS quantification (relating levels to beta actin - a "house keeping" gene) was used. Results demonstrated, once again, that there was an association between high TS expression and resistance to fluoropyrimidine based therapy – no patient with high TS levels responded to chemotherapy. Of interest was that in the group of patients that had previously been treated with 5-FU based therapy, intra-tumoral TS expression was elevated[203].

Conversely, Sanguedolce et al found that patients with decreased TS levels had an increased risk of death[210].

Early trials using patients with metastatic disease enrolled both patients with disseminated unresectable disease, as well as resected non-metastatic disease. It appears that TS levels may predict whether a patient is likely to respond to fluoropyrimidine based chemotherapy in patients with metastatic disease that was not resected. High TS levels have reliably predicted non-response, while low TS levels have suggested response[203]. Less convincing are results that suggest that, in patients with resected non-metastatic disease who had high TS expression, adjuvant chemotherapy

improved overall and disease-free survival. In low TS expressers, the addition of adjuvant chemotherapy made no difference in overall or disease-free survival[209]. TS levels continued to be independent predictors of survival (with high TS expressers doing worse). Unfortunately, this last study, along with several others in this field, suffers from several notable drawbacks. Firstly, most patients received more than one type of chemotherapy. Because there are now several different compounds that have shown to have some benefit in patients with colorectal cancer, if a patient progresses while receiving 5-FU, they are often put on a different chemotherapy protocol. Also, many of these studies use immunohistochemistry to quantify TS levels within tissue. When performed properly, immunohistochemistry is fairly reliable, however, it is a difficult procedure wrought with sources of error. Few labs have shown reliable and valid results using consistent monoclonal antibodies or have shown good interobserver reliability. Aschele et al were able to show that responsiveness to systemic infusional fluoropyrimidine chemotherapy in patients with metastatic colorectal cancer was related to TS expression as determined using immunohistochemistry. Low TS expression was the single greatest predictor of response to the chemotherapy. Not surprisingly, they also found that disease free and overall survival were significantly greater in patients with lower TS expression [211]. Cascinu et al demonstrated similar response results in patients with disseminated colorectal cancer who received bolus fluoropyrimidine chemotherapy[212].

Davies et al investigated 36 patients with resected colorectal liver metastases. He attempted to correlate response to intra-hepatic fluoropyrimidine chemotherapy and TS levels. 56% of patients who displayed low levels of intra-tumoral TS responded to fluoropyrimidines compared to only 15% of patients who displayed high levels of intra-

tumoral TS. This discrepancy was significant. Other findings of interest were related to the fact that neither relation was absolute, i.e. not all high TS expressers were treatment resistant and not all low expressers demonstrated a treatment response. This led to the hypothesis that TS was not the sole mechanism by which patients responded or became resistant to fluoropyrimidine based chemotherapy[213].

Gorlick et al noted the decreased responsiveness to fluoropyrimidines seen in pulmonary metastases. They postulated that a mechanism similar to the treatment resistance seen in some liver metastases may be involved. In support of their hypothesis they found consistently elevated TS RNA levels as well as elevated TS protein levels in colorectal lung metastases when comparisons were made to colorectal liver metastases[214].

The mechanism responsible for disparate TS levels may have been elucidated recently. Lacopetta et all noted that polymorphisms at the TS gene promoter enhancer region correlated with survival in patients with resected Dukes C colorectal primaries who were treated with 5-fluorouracil based chemotherapy. They noted that triple repeat homozygotes (3R/3R) demonstrated no significant benefit from chemotherapy, while double repeat homozygotes (2R/2R) or heterozygotes (2R/3R) demonstrated a significant benefit from the addition of fluoropyrimidine based chemotherapy[215]. This relationship paralleled the relationship between TS expression and survival seen previously. This parallel was likely due to the fact that triple repeat homozygotes had been shown to have 100-300% greater expression of TS than double repeat homozygotes or heterozygotyes[216].

These results were confirmed by Pullarkat et al who noted that the presence of the triple repeat correlated with TS expression, as well as predicting response to

fluoropyrimidine chemotherapy. They demonstrated that among patients with metastatic colorectal cancer, double tandem repeat homozygotes (2R/2R) had lower TS mRNA levels than those homozygous for triple tandem repeats (3R/3R). Heterozygotes (2R/3R) were also noted to express higher results than the tandem repeat homozygotes. This group was also able to confirm response rates. A 50% response rate was seen in 2R/2R homozygotes while heterozygotes exhibited a response rate of 15% and 3R/3R homozygotes had response rates of only 9% to systemic 5-FU infusion. Additionally, a relation between toxicity and TS was tested. Somewhat surprisingly 2R/2R homozygotes were more likely to suffer from grade 3 toxicities than the other 2 groups, while 3R/3R homozygotes where more likely to suffer milder grade 1 toxicities[217].

Bathe et al noted that regardless of the addition of surgical treatment, TS levels played a role in the response to treatment and the resulting survival in patients with colorectal liver metastases. It was noted that no patients with high TS levels responded to fluoropyrimidine therapy. Median overall and disease-free survival were shorter in those with high TS expression regardless of whether or not a resection was performed[218].

Recently, Edler et al demonstrated that high TS expression in primary tumors could be used to prognosticate (negatively when compared to low TS expressors) locoregional recurrence, distant metastases, disease-free survival and overall survival [219].

THYMIDINE PHOSPHORYLASE (TP)

TP is identical to platelet-derived endothelial cell growth factor (PD-EDF). It is an enzyme that inhabits part of the anabolic mechanism of the cell where it catalyzes the phosphorylation of thymidine to thymine. Unlike the reaction catalyzed by TS, this is a

reversible process. This reaction, and therefore this enzyme, is used during the promotion of angiogenesis. It also performs a role during cell growth where it is involved in cell motility or chemotaxis.

Knowing that TP levels are generally higher in malignant cells than in normal tissue, it may be used as a key "selector" when using cytotoxic pharmaceuticals whose mechanism involve this enzyme and / or its related pathways. Capecitabine* is such a compound. This fluoropyrimidine prodrug uses TP during its final transformation into a cytotoxic chemical. Theoretically, this allows targeted killing in areas where TP is elevated, namely malignant cells. Unfortunately, even though increased levels of TP allow for selective activation of chemotherapeutic agents, increased tissue levels are associated with a worse prognosis[220].

Metzger et al, using 38 resected colorectal specimens, demonstrated that TP expression level was associated with responsiveness to 5-FU. Specifically, high TP expression was associated with non-response to infusional 5-FU chemotherapy in patients with metastatic or locally recurrent colorectal cancer[221].

DIHYDROPYRIMIDINE DEHYDROGENASE (DPD)

DPD is the rate-limiting enzyme in the catabolism of 5-FU. Authors have observed that severe toxicity to fluoropyrimidine based therapies seems to correlate with decreased levels of DPD[222 223]. DPD exists in numerous human cell lines, but within a single host different tissues express different levels of this enzyme. Therefore, DPD must be measured in the tissue of interest, and can not be extrapolated from surrogate sites (i.e. peripheral blood). As DPD is involved in inactivating 5-FU, high levels were

* Capecitabine will be discussed later in this chapter

predicted to correlate with resistance to fluoropyrimidine treatment. This has been borne out, as Salonga et al demonstrated that high levels of DPD, like high levels of TS, predicted non-response to fluoropyrimidine based chemotherapy[224]. The potentially important role of DPD is supported by research from Japan. Nita et al demonstrated in human colorectal cancer lines that high levels of DPD expression was associated with resistance to 5-FU and that the tumour cell lines that were sensitive to 5-FU had undetectable DPD levels. They, however, noted no correlation between sensitivity to 5-FU and TS expression[225]. DPD and its activities gain increased prominence when viewed in the context of eniluracil, a potent inhibitor of DPD that may be used in combination with 5-FU based chemotherapy[226].

The fact that low levels of DPD expression are associated with improved survival in patients treated with fluoropyrimidines is countered by the observation that DPD also plays a role in the governing of 5-FU mediated toxicity. Low levels of DPD have been associated with increased chances of developing significant toxicity[227].

p53

p53 is a tumor suppressor gene and thereby plays a major role in the regulation of cell-cycle activity. Loss of this gene removes a cell-cycle regulator and increases the chance a cell may undergo unchecked proliferation. Through this mechanism, disruptions of this gene and / or its function have been shown to be involved in the development of a host of human carcinomas. p53 has long been known to also play a significant role in the development of colorectal carcinoma[228]. Mutations of this gene seem to be strongly associated with a poor prognosis in non-metastatic colorectal carcinoma[229]. It likely also plays a role in the modulation of specific chemotherapeutic

effects on the cancer cells, but the exact mechanism and magnitude of this interaction is poorly understood. This mutation or overexpression of p53 has been estimated to occur in 51%-74% of colorectal cancer cases[230-233].

In 1998 Lenz et al published work which attempted to relate p53 status and thymidylate synthase status. Both had previously and repeatedly been shown to have prognostic efficacy in terms of survival as well as response to chemotherapy for colorectal cancer. They demonstrated, using RT-PCR, that at a genetic level for both entities (and at a protein level for p53 only) the two are possibly linked. They suggested that mutated p53 was associated with higher TS expression. Patients with both mutated p53 and high TS expression had a tendency to be poor or non-responders when treated with 5-FU chemotherapy, and their overall survival was also lower. To a similar extent, the opposite association was noted in patients who had non-mutated or "wild type" p53 gene expression and protein as well as low TS levels[234]. If this relationship is confirmed, it makes TS an even more significant prognostic protein, as TS expression may then be used to infer p53 type.

Yang et al noted that mutated p53, in the absence of chemotherapy, was associated with a greater cumulative survival after potentially curative liver resection than was wild type p53. No known reason exists for this finding, but several possible explanations were offered. One of which suggests that altered response in the p53 mutated cells to the surge of growth factors expressed as the liver regenerates, a theory that had been suggested in previous studies[235].

ADENOMATOUS POLYPOSIS COLI (APC)

Adenomatous polyposis coli is a tumor suppressor gene located on chromosome 5q21. It is involved in cell migration adhesion, and proliferation. A mutation distal to the 5` end is found in patients with familial adenomatous polyposis, a hereditary condition which predisposes a person to colorectal cancer, and is found in 52%-60% of colorectal cancers[236-238]. Patients with this mutation develop numerous polyps at a fairly early age. These polyps, even though individually of low malignant potential, virtually guarantee the patient will develop colon cancer.

DELETED IN COLON CANCER (DCC)

Deleted in Colon Cancer was thought to be a tumor suppressor gene found at chromosomal location 18q. A loss of heterozygosity at this location was identified as one of the potentially important steps in the malignant transformation from adenoma to carcinoma[228]. Kato et al attempted to use loss-of-heterozygosity of the DCC gene in the primary tumour to predict metastatic potential, and thereby the likelihood of developing liver metastases. No significant result was found. However they reported on a trend suggesting that in patients who were metastases free at the time of initial resection, the presence of LOH at the DCC locus increased the risk of developing subsequent liver metastases[239]. So far, none of the major chemotherapeutic agents used in the treatment of colorectal carcinoma are known to have any interaction with this gene or its (lack of) products

More recent results illustrate one of the problems associated with the use of genetic markers. It appears that DCC may not be the tumor suppressor gene that was involved in the aberrations at locus18q. It may be that the gene named DCC is actually involved with neuronal axons and not with the development of colorectal cancer. The

lack of in-depth knowledge regarding the genetic code and its interpretation led researchers into believing they were observing effects related to DCC when in fact the observations were due to 18q loss[240]. 18q allelic loss is present in approximately 73% of colorectal cancers[241 242].

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND MICROSATELITE INSTABILITY (MSI)

Some of the more recent prognostic markers of interest include vascular endothelial growth factor (VEGF) and microsatelite instability (MSI). VEGF plays an important role in angiogenesis, a crucial aspect of tumor growth. As targeted therapies are being developed, investigators are exploring a variety of potential targets. Angiogenesis is a logical and likely important target, thereby increasing the likely role of VEGF.

Microsatelites are segments of DNA consisting of 1-5 base pairs (or tandem repeats) that are repeated many times. MSI is a mutation noted in the minority of colorectal cancer patients who have hereditary disease. It has been noted that MSI might have utility in predicting the efficacy of 5-FU based chemotherapy[243]. This is even more evident when the MSIs are divided by stability into high-level MSIs and low-level MSIs. Research into this area is just beginning, but it already appears to be a promising avenue of research.

Future research will likely continue to focus on expected response to specific therapies, as well as the genetic determinants of survival. Such research has already offered some promising results in patients with resected primary colorectal tumors that

have been treated with fluoropyrimidine based therapy. In addition, genetic markers have been identified that suggested improved outcome[244].

USING PREDICTIVE / PROGNOSTIC MARKERS IN COMBINATION

Much like the clinical scoring systems under development, it is hypothesized that combinations of tumor markers may be used to more accurately predict response to chemotherapy and prognosticate survival.

Lenz et al attempted to build on their results from their previous studies that demonstrated the utility of TS expression in predicting response to fluoropyrimidine based chemotherapy, as well as in helping with prognosis. They also noted the emergence of p53 as a possible prognostic and predictive marker. They postulated that the classification of patients with disseminated colorectal cancer by p53 levels and by TS levels may allow for more accurate prediction and prognostication. To their surprise, TS remained an important marker, but the importance of p53 was vastly overshadowed by the TS. In fact, p53 was no longer an independent predictor of response or survival when TS was included in the analyses. They did note an association between wild type p53 and lower levels of TS expression[234].

Saw et al studied 63 patients with resected colorectal liver metastases. They investigated the prognostic significance of three markers (DCC, p53, and TS) using both immunohistochemistry and RT-PCR. No significant prognostic benefit was found for any of the three markers. These results are opposite of what had been shown previously by several research groups. There are numerous difficulties with accepting the results of this study. First, no mention is made of adjuvant chemotherapy. It is therefore unclear if all, none or only some of the patients received it. Any of these scenarios would alter

the interpretability of the results. Second, the authors acknowledge that they may not have used an optimal method of immunohistochemistry. Immunohistochemistry needs to be performed in a lab that has experience and whose process and results have been validated. Also, the antibodies used should be consistent between studies. Finally, only a very small number of metastases and an even smaller number of primary tumors were used[245].

Van Triest et al looked at both TS and TP individually in 32 patients with curative primary colorectal resections. They noted that both markers were significant prognostic indicators. Unfortunately, the small group of patients involved were not homogenously treated. Several different chemotherapy protocols were running concurrently at the two different hospitals. No mention of stratification based on chemotherapy received was noted in the analysis, possibly an important omission since each marker has previously been shown to potentially have significant interactions with certain chemotherapeutic regiments [245].

Salonga et al demonstrated, in tumour specimens collected from patients with metastatic or recurrent colorectal cancer, that low expression of TS, DPD and TP was significantly associated with improved survival in patients receiving systemic 5-FU chemotherapy. The survival advantage was noted when the group of low expressers was compared with those that were high expressers in one or more of the markers (i.e. all other patients)[224].

Ikeguchi et al partially contradicted these results when they published their analyses of 189 tumour specimens from patients ranging from Dukes A to D (metastases were confined to the liver and not resected). About 120 of these patients received adjuvant fluoropyrimidine based chemotherapy after resection of the primary lesion.

Patients with Dukes A, B, and C disease received systemic chemotherapy, while the 20 with synchronous metastatic disease received hepatic arterial infusional (HAI) chemotherapy. No survival differences were found based on DPD, TP, or DPD + TP combinations in patients who received adjuvant chemotherapy, in those who did not receive adjuvant chemotherapy, and in those with metastatic disease[246]. These results raise some doubt as to the reliability of the earlier results that demonstrated prognostic and predictive abilities associated with TP and DPD.

CYTOTOXIC COMPOUNDS

5-FU has a greater than forty year association with treatment for colorectal carcinoma. It has been extensively used and investigated. Recently Irinotecan, only the second drug with significant demonstrable cytotoxicity in colorectal carcinoma has entered in to the regular armamentarium of clinicians that treat this disease. Even more recently oxaliplatin has started to show promise in treatment of patients with metastatic colorectal carcinoma. It is likely that oxaliplatin is the first of many new compounds that will be tried in the treatment of colorectal cancer. As scientific knowledge (rapidly) increases regarding the molecular biology and genetics of this disease, many more potential targets become elucidated.

FLUOROPYRIMIDINES

Fluoropyrimidines, and 5-FU specifically, are cytotoxic agents used in the treatment of numerous solid tumors. 5-FU (**Error! Reference source not found.**) represents one of the earliest chemotherapeutics designed to target a specific function within tumour cells.

5-fluourouracil or 5-FU has been widely studied due to its use in the treatment of numerous solid tumours. Until recently, it was also the only known effective pharmaceutical in the definitive treatment, adjuvant treatment, and palliation of patients with colorectal carcinoma[39 247]. There are several dosing regimens used, each with their own advantages and disadvantages. Bolus administration provides an approximately 10% response rate. A systematic review in 1998 reviewed some of the regimens and concluded that prolonged infusion afforded the least hematological toxicity at the

expense of increased hand-foot syndrome while providing a small survival advantage when compared to bolus administration[248].

Hepatic arterial infusion may use 5-FU or may use FUDR. FUDR is 5-fluoro-2-deoxyuridine. It has high hepatic clearance and first-pass metabolism (up to 99%) and a short plasma half-life. During HAI, hepatic exposure to the active metabolites may reach up to 400x that of systemic exposure (**Error! Reference source not found.**).

Oral Fluoropyrimidines

5-fluorouracil is unpredictably absorbed via the gastrointestinal tract, therefore it can not reliably be used as an oral (or p.o.) agent. Capecitabine is predictably absorbed via the gastrointestinal tract. It is a prodrug and gradually gets converted to 5-FU via 5`-deoxy-5-fluorouridine (5`-DUFR) by thymidine phosphorylase through an enzymatic pathway found in the liver and in certain tumours (**Error! Reference source not found.**). This makes capecitabine a tumour selective fluoropyrimidine carbamate. Its selectivity is demonstrated by it being found in higher concentrations in tumours than in the surrounding tissues (i.e. 1.17x greater in liver metastases than in normal parenchyma).

A second oral formulation exists. It is a fixed 1:4 combination of tegafur, a prodrug converted to 5-FU by the hepatic microsomal system, with uracil which is an inhibitor of dihydropyrimidine dehydrogenase (DPD)[249]. DPD inhibition allows the 5-FU to reach elevated concentrations.

Both drugs demonstrate similar efficacy to bolus 5-FU and leucovorin in the treatment of advanced colorectal carcinoma with less toxicity[250-253]. The benefits of

these oral formulations, including the ease of administration, may become less significant as treatment becomes increasingly poly-pharmaceutical.

Biomodulation

Fluoropyrimidines are often administered in conjunction with a biomodulator – leucovorin. Leucovorin is a reduced folate that enhances 5-FU activity by enhancing the binding of FdUMP (fluorodeoxyuridine monophasphate, the active intracellular metabolite of 5-FU) and thymidylate synthase. The addition of leucovorin to 5-FU treatment has been shown to increase tumor response by as much as 100%, but no consistent survival advantages have been reported[254, 255].

FdUMP forms a ternary covalent complex with thymidine synthase as well as the folate cofactor 5,10-methylene tetrahydrofolate [255]. The formation of this complex is directly dependent on the concentration of 5,10-methylene tetrahydrofolate, a reduced folate. The addition of leucovorin expands the intracellular reduced folate pool including 5,10-methylene tetrahydrofolate. This augmented complex formation is then responsible for the increase in the anti-tumor activity of 5-FU.

Numerous other biomodulators have been tested, including methotrexate, trimethrexate, interferon, dipyridamole, and N-phosphonacetyl-L-aspartic acid. Most of these demonstrated little if any positive effects.

Toxicities

As fluoropyrimidines exert the majority of effects on rapidly dividing tissues, this is reflected in the toxicity profile. Mode of administration also plays a large role in determining potential toxicity associated with fluoropyrimidine therapy. The more

common, and often severe, toxicities include: myelosuppresion, mucositis, diarrhea, hand-foot syndrome, and ataxia.

IRINOTECAN

Camptothecan is a derivative from the Chinese tree *Camptotheca acuminata.* Irinotecan (CPT-11 or 7-ethyl-10-[4-(1-piperidino)-1piperidine]carbonyloxy-camptothecin) is a semisynthetic camptothecin derivative acting as a topoisomerase I inhibitor. Topoisomerase I is a nuclear enzyme which participates in DNA replication, DNA transcription, DNA recombination, and DNA repair. This participation consists of relaxing the supercoiled DNA double helix. These actions are due to the irinotecan metabolite SN-38 which interferes with DNA replication and cell division. It is S-phase specific, during which it causes single stranded breaks in DNA which results in arrest of cell replication. In colorectal cancer, irinotecan has demonstrated efficacy as first line therapy alone or in combination with 5-FU as well as having demonstrated efficacy as second line therapy after the failure of 5-FU based therapy[256].

Irinotecan has a mechanism of action that is dissimilar to that of 5-FU. This in combination with its efficacy alone or after the failure of 5-FU based therapy led to the research in to combination chemotherapy. Adding irinotecan to a bolus 5-FU and folinic acid regimen demonstrated superior response rates and overall survival when compared to bolus 5-FU and folinic acid alone (Mayo clinic regimen) in patients with disseminated colorectal metastases. This result was replicated using an infusional administration of 5-FU and folinic acid in combination with irinotecan (de Gramont regimen) compared with infusional 5-FU and folinic acid alone. Results were convincing enough to recommend

this regimen as first line therapy for both locally advanced and metastatic colorectal cancer.

Toxicities

Having a unique mechanism, irinotecan also has its own side effect profile. The most frequently reported toxicities include both hematologic and non-hematologic toxicities. Hematologic toxicities include neutropenia which in one trial was present at a grade III or IV level in 66% of patients[8]. In this same trial, non-hematologic toxicities included delayed onset diarrhea (63%), acute cholinergic syndrome (57%), alopecia (77%), fatigue (72%), stomatitis (49%), vomiting (43%), anorexia (34%), hand and foot syndrome (12%). Non-hematoligic grade III and IV toxicities were less common and were mainly diarrhea (16%), nausea (11%) and vomiting (9%). Hepatic toxicity was minimal[8].

Toxicity of Combination Therapy with 5-FU and Irinotecan

In early 2001, three randomized and controlled studies were simultaneously testing the value of combination chemotherapy in an adjuvant setting. Intergroup N9741 was investigating irinotecan and bolus 5-FU + leucovorin as adjuvant therapy in patients with recurrent and / or metastatic colorectal cancer. Concurrently CALGB (Cancer and Leukemia Group B) C89803 was testing a similar regimen in a similar group of patients. Within 60 days of Intergroup N9741 enrolment, an unexpectedly high number of deaths occurred. These deaths all occurred in the arm receiving irinotecan and bolus 5-FU and leucovorin. The deaths were not disease related, and in fact were mostly due to gastrointestinal toxicities (diarrhea, nausea, vomiting, febrile neutropeina,

electrolyte disturbances) and / or thrombo-embolic events MI's, CVA's, PE's). Early analysis of C89803 was performed, and these same disturbing events were noted within this trial. There was a 300% increase in treatment related deaths[257]. Previous trials using similar cohorts of patients had reported treatment related mortality ranging between 0 and 0.8%. A second CALGB trial, PETACC-3, was using 5-FU and leucovorin in combination with irinotecan, but without the treatment related mortality. In this trial the 5-FU was administered as a 2-weekly infusion. This has led to the arrest of trials and / or arms of trials that used bolus 5-FU and leucovorin in combination with irinotecan. Combination trials are now using infusional 5-FU or modified administration of 5-FU.

OXALIPLATIN

Oxaliplatin is a third generation platinum derivative with a 1,2-diaminocyclohexan (DACH) carrier ligand (**Error! Reference source not found.**). It is related to cisplatin and carboplatin, but with noticeably less renal or bone marrow toxicity, likely due to this ligand. Action is via its active metabolite DACH-platin causing DNA crosslinking and thereby inducing apoptosis.

It has been shown to have a good response rate, but randomized trials are underway to identify its role in the treatment of primary and secondary colorectal carcinoma.

Toxicities

Hematological toxicities are commonly reported during oxaliplatin therapy, as are nausea, vomiting, diarrhea, mucositis, and early-onset cold induced dysaesthesia.

Oxaliplatin does have a unique peripheral neuropathy which can be dose limiting[249] with cumulative peripheral sensory neuropathy causing dose reduction in 10-15%. The paraesthesias can become persistent after several cycles.

ONGOING STUDIES

Numerous studies are underway examining what is the most beneficial regimen of administering these three compounds, both individually and in any number of combinations.

OTHER DRUGS

Raltitrexed is a thymidylate synthase inhibitor that works via a different mechanism than 5-FU. It functions by rapidly polyglutamylating folates whereby which it is retained within cells. One study has demonstrated it to be inferior to standard treatment with 5-FU and folinic acid[258], but the remainder of investigations to date have shown equivalent results to this well used combination. Raltitrexed is associated with serious toxicities including GI, hematological and asthenic toxicities. Up to 6% of patients may succumb to treatment related mortality. Fatalities are often due to a combination of gastrointestinal toxicity (diarrhea) and hematological toxicity (neutropenia) in the setting of nephrotxicity (~50% of the drug is excreted unchanged in the urine).

FUTURE TRENDS

One of the most troubling problems within this clinical arena, is the rapid adoption of new treatment regimens. The adoption of new regimens has started to become a large hindrance in accruing for new trials as patients seem to be demanding treatment, even if there is little evidence to support it. A good example of this is the NCIC trial which attempted to explore the advantages and disadvantages of adjuvant chemotherapy after resection of colorectal liver metastases. This well designed trial was closed prior to reaching sufficient sample size. The reason for closing this trial was poor accrual, largely due to patients and clinicians not accepting what was perceived as a "no treatment" arm. It was also handicapped by the limited numbers of patients that were treatment naïve. The lack of patient accrual is not necessarily limited to chemotherapy trials[158]. Other large, well designed, and organized trials have also had difficulty accruing enough patients to study a variety of surgical and radiotherapuetic topics. As survival increases and recurrences decrease, the sample sizes necessary to demonstrate significant differences start to increase. This is then augmented in trials with greater than 2 comparative treatments.

The goal of research in the treatment of colorectal cancer with cytotoxic chemotherapy is not only to increase overall and disease free survival, but also to minimize toxicities in general and decrease exposure to unnecessary and ineffectual chemotherapy and their toxicities specifically.

Some very small studies have looked at all three of enzymes in combination, but no firm conclusions were able to be drawn from them[224].

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CHAPTER 2

Introduction to Project

Surgery, when possible, continues to be the most efficacious and important treatment modality for those with colorectal liver metastases. The role of chemotherapy, specifically whether it should be added at all and if so what type, remains somewhat enigmatic. Even though it is currently widely used, it is still important to determine whether supplemental chemotherapy is beneficial in this population. It is also essential to determine whether supplemental chemotherapy is equally important in all patients, and if not, are there methods, clinical and/ or biochemical, whereby one may select those who are most likely to benefit from the addition of chemotherapy despite its related toxicities. These questions are escalating in importance as chemotherapeutic options are becoming increasingly complex and varied.

This thesis is composed of three separate but interrelated studies. All three involve the same study population: patients who have undergone resection of colorectal liver metastases by either of two hepatobiliary surgeons from the University of Alberta (DLB, NMK) between 1988 and June 2002. We retrospectively examine outcomes in these patients and attempt to determine whether we can attribute improved outcomes to the addition of supplemental chemotherapy and whether we can use a scoring system or immunohistochemically detected tumour markers to determine prognosis and/or who, if anyone, should receive supplemental chemotherapy.

The first paper entitled "*The Role of Peri-Operative Chemotherapy in Patients with Resectable Colorectal Liver Metastases*" divides this entire cohort of patients into two groups: those who received chemotherapy given as "adjuvant" or "neoadjuvant" treatment in relation to their hepatic metastectomies, and those who did not. Survival analysis was used to determine whether any benefits were realized due to the addition of chemotherapy to the patient's treatment.

The second paper entitled "*Predicting the Benefit of Supplemental Chemotherapy in Patients with Colorectal Liver Metastases using the Clinical Risk Score*" takes this entire cohort of patients and applies the prognostic scoring system known as the Clinical Risk Score [1] to each individual patient. Once the score has been applied, those who are determined to have low scores will be compared to those who are determined to have high scores. Initially, differences in overall and disease-free survival were elucidated in an effort to replicate and thereby add to the validation of this scoring instrument. Once the scoring system was shown to function as intended, we investigated whether there was a differential effect due to the addition of supplemental chemotherapy depending upon whether a patient was classified as high-score or a low-score.

The third and final paper entitled "*Thymidylate Synthase and Thymidine Phosphorylase Expression in Resected Colorectal Liver Metastases Do Not Predict Overall Survival or Response to Chemotherapy*" involves the greater than 95% of this cohort for whom preserved tissue samples were available. In collaboration with Dr. Maroun's research group in Ottawa, thymidylate synthase (TS) and thymidine phosphorylase (TP) levels in the resected tumour specimens were determined using immunohistochemistry. These enzyme levels, both individually and in combination, were scored. Patients were categorized according to the scores representative of the presence or absence of TS, TP and a combination of the two. Overall and disease-free survivals were then compared between the two strata in each of the three categories. Finally, we determined whether these scores allow for the selection of patients that would most likely benefit from supplemental chemotherapy.

We believe that each individual paper investigates important questions regarding this patient population. Specifically whether chemotherapy, in addition to resection, is

beneficial and whether it is equally beneficial in all patients; whether the Clinical Risk Score[1] can be used to differentiate those who will maximally benefit from supplemental chemotherapy and; whether biochemically determined levels of specific proteins are similarly useful. More importantly, as a collective, these papers may serve to help clinicians in their approach to these unique patients. The results may be used to help determine which, if any, additional chemotherapeutic regimen would be optimal for a particular patient. Most importantly, this thesis may serve as a springboard for future hypotheses and research involving this growing patient population. It describes the heterogeneity of this patient population in terms of response to chemotherapy and thereby suggests the importance of using a validated clinical scoring system to describe and stratify these patients when enrolled in trials such as those involving supplemental chemotherapy.

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CHAPTER 3

Materials and Methods

The records of two hepatobiliary surgeons from the University of Alberta (DLB, NMK) were examined. All patients referred for liver lesions or resections were identified. In addition all liver resections performed by either surgeon between 1988 and June 2002 were identified. From each of the two lists, all patients who had liver resection performed for hepatic colorectal metastases were identified. This single cohort of patients was used to conduct all three studies

Data was gathered from the office charts of the involved surgeons as well as from the hospital charts and Cross Cancer Institute charts of the patients identified previously. Mortality of in-province patients was cross-referenced using the Alberta Cancer Registry. Several patients were either only initially treated at the Cross Cancer Institute or never received treatment there. Efforts were made to contact their primary care physicians and/or their local cancer board (i.e. Tom Baker Cancer Centre – Calgary, British Columbia Cancer Agency – Vancouver and Kelowna, Alan Blair Cancer Centre - Saskatchewan etc).

Demographic, disease-specific, surgery-specific, pathologic and chemotherapeutic data were extracted from the various patient records. Data were recorded using novel forms made expressly for this project (Figure 3-1). All data were then double entered in a SPSS database made solely for these projects.

Immunohistochemical analysis was performed on paraffin embedded specimens retrieved from the University Of Alberta Department Of Pathology. These specimens were transported to Dr. Maroun's laboratory facilities in Ottawa. The actual immunohistochemical staining was performed under the direct supervision of Dr. Moyana. Each paraffin embedded specimen was used to make three microscopic slides,

two of which were used to perform individual TS and TP staining and were subsequently used in this project. Dr. Moyana also interpreted each individual slide.

Figure 3-1. Data Collection Form

Patient Name:		**DOB** (d/m/yr):	
Male (1) / Female (2)		**Hospital #:**	

Description if not standard:	

WWX #:		**Path #:**	

Date of Original Surgery (d/m/yr):

ORIGINAL SURGERY				
Site of Primary:	Right Colon (1)	Transverse Colon (2)	Left Colon (3)	Sigmoid Colon (4)
	Rectosigmoid (5)	Rectum (6)	Anus (7)	Unknown (99)
Resection Performed:	Right Hemicolectomy (1)	Transverse Colectomy (2)	Left Hemicolectomy (3)	Sigmoid Colectomy (4)
	Low Anterior Resection (5)	APR (6)	Sub-total Colectomy (7)	Total Colectomy (8)
	Other (9) :	Unknown (0)		

PATHOLOGY OF PRIMARY

Stage:	0 (0) Tis, N0, M0
	I (1) T1-2, N0, M0
	II (2) T3-4, N0, M0
	III (3) Any T, N1-2, M0
	IV (4) Any T, Any N, M1
	Not stated (99)

Duke's Classification	A = not penetrating submucosa (1)		C1 = to but not through serosa + regional nodes positive (4)
	B1 = to but not through serosa (2)		C2 = through serosa, involves adjacent organs + regional nodes positive (5)
	B2 = through serosa, involves adjacent organs (3)		D = distant mets (6)
	Not Specified (99)		

T – Primary Tumor	**N – Regional Lymph Nodes**	**M – Distant Metastases**
Tx = can not be assessed (0)	Nx = can not be assessed (0)	Mx = can not be assessed (0)
T0 = no evidence of primary tumor (1)	N0 = no regional node metastases (1)	M0 = no distant metastases (1)
Tis = in situ (2)	N1 = 1-3 regional nodes involved (2)	M1 = distant metastases (2)
T1 = invades submucosa (3)	N2 = ≥ 4 regional nodes involved (3)	Not Specified (9)
T2 = invades muscularis propria (4)	Not Specified (9)	
T3 = invades into subserosa / into non-peritonealized pericolic or perirectal tissue (5)		
T4 = (6)		
Not Specified (9)		

Chemotherapy:	None (0)	5-FU (1)	Other (2)	Unknown (3)	Not Specified (9)
Radiation:	None (0)	Yes (1)	Unknown (9)		

Metastases:	Present at initial resection (0)	<1 year (1)	1-5 years (2)	> 5 years (3)	Not Spec (9)

PRE-OPERATIVE EVALUATION:

Number of hepatic metastases:		Unknown (99)		N/A (0)			
Size of largest metastasis: (cm)		Unknown (99)		N/A (0)			
Modalities involved:	U/S (1)	CT (2)	U/S + CT (3)	MRI (4)	U/S + CT + MRI (5)	Other (6)	Not Spec (9)

Extra-hepatic disease:	N/A (0)	None (1)	Lung (2)
	Other (3)		Not Spec (9)

Bilirubin (μmol/L):		Unknown (0)
Albumin (g/L):		Unknown (0)
INR:		Unknown (09)

CEA:		Unknown (0)

Operative Data:

Date of OR(d/m/yr):	**Surgeon:**	Dr. Bigam (1) / Dr. Kneteman (2)

OR start time (24hr):		**OR end time** (24hr):		**Resectable**	N (0)	Y (1)

Procedure:	Biopsy (0)	Non-anatomic / Wedge resection (1)	Left Lateral Segmentectomy (2)	Right Lobectomy (3)
	Left Lobectomy (middle vein stays) (4)	Extended Right Lobectomy (middle vein goes) (5)	Extended Left Lobectomy (middle vein goes) (6)	Other (9)

Vascular reconstruction	No (0)	Yes (1)	Not Spec (9)

Vascular Control:	None (0)	Pringle (1)	Total Vascular Exclusion (2)	Unknown (9)

Cross-Clamp Time:(min)		Unknown (999)	None (0)

Intra-operative Transfusions: (units pRBC)	None (0)		Not Spec (99)

Extra-Hepatic Disease:	None (0)	Peritoneal deposits (1)	Extrahepatic nodes (2)
	Diaphragm (3)	Other (9) describe:	

PATHOLOGY

Mass of Resected Liver (g)	N/A (0)		Unknown (9999)
Number Of Metastases	N/A (0)		
Sattelite Lessions	No (0)	Yes (1)	
Largest Metastasis (mm)	N/A (0)		
Closest Surgical Margin (mm)	N/A (999)	Not Spec (555)	

Grade:	1 (1)
	2 (2)
	3 (3)
	Not Stated (9)

TS:	Low (1)	High (2)	Unknown (9)	
Polyomies:		Not Enrolled - declined (0)	Not Enrolled - other (1)	Enrolled (2)

POST-OPERATIVE

ICU Admission	No (0)	Yes (1)
Days in ICU	N/A (0)	

Date of Discharge / Death (d/m/yr):	Not Spec (11.11.11)		**Disposition:**	Dead (0)	Alive (1)

Complications: List All	None (0)	Bile Leak (1) Bleeding (2) Liver Failure (3) Wound Infection (4) Other (9) – List:

LONG-TERM FOLLOW-UP

Chemotherapy:	None (0)	5-FU (1)	5-FU / CPT-11 (2)	CPT-11 (3)	Other (4)	5-FU + CIS (6)	5-FU + LEV (7)	? (9)

Date of Recurrence: d/m/yr		None at last Fu (11.11.11)	N/A (11.11.22)	Not specified (11.11.33)

Date of Last Follow-up (d/m/yr)		Not Spec (11.11.11)

Date of Death: d/m/yr		N/A (11.11.11)

Status at Follow-up:	Alive w/o Disease (0)
	Alive with Disease – primary site (1)
	Alive with Disease – Liver (2)
	Alive with Disease – lung (3)
	Alive with Disease – other (4)
	Death due to disease (5)
	Death due to other (6)
	Alive – not resected (7)
	Dead – not resected (8)
	Not Specified (9)

CHAPTER 4

The Role of Peri-operative Chemotherapy in Patients with Resectable Colorectal Liver Metastases

A version of this chapter was presented at the at the American Hepato-Pancreato-Biliary Association Meeting - 4th Americas Congress, Miami, USA, February 27 - March 2, 2003.

INTRODUCTION

Globally, as many as 400,000 to 500, 000 deaths per year are attributable to colorectal cancer. The incidence of this disease is also alarmingly high: nearly 1,000,000 people worldwide were diagnosed with colorectal cancer in 2000, making it the fourth most common new cancer diagnosis1-5. Up to 50% will present with, or develop metastatic disease at the time of diagnosis or will develop metastases during the course of their illness.

Untreated, metastatic disease confers a grim prognosis. Median survival is reported to be between 4 and 9 months, with only a 3% chance of surviving three years[6]. The addition of chemotherapy has led to only modest increases in survival. Until recently chemotherapy, in the absence of metastectomy, resulted in a median survival of around 12 months in patients with colorectal liver metastases. This has been gradually increasing as newer agents like irinotecan (CPT-11) and oxaliplatin are introduced and new combinations of chemotherapeutic pharmaceuticals are being adopted[7 8].

Resection of colorectal liver metastases has consistently been shown to be the treatment modality that provides the greatest survival benefit, and the only significant chance for long-term survival, offering a 5-year survival of between 25% and 40%. These results can be achieved with relatively low morbidity and mortality rates in the range of 20-40% and 0-5% respectively[9-12].

Unfortunately, most patients with resected colorectal liver metastases eventually succumb to their malignancy. Clinicians observed that the majority of patients who have hepatic metastectomies experience recurrence of their disease; with the majority of recurrences isolated to or involving the remaining liver. Pathologists also noted that

within resected liver specimens, as many as 35% contained clinically and radiologically undetectable micrometastases.

The addition of adjuvant chemotherapy after resection of colorectal liver metastases was a result of extrapolating the utility of the addition of adjuvant chemotherapy in node positive primary colorectal carcinoma. However, despite the frequent practice of administering chemotherapy after resection of colorectal liver metastases, there is little prospective evidence to support a survival benefit[13].

PATIENTS AND METHODS

Ethical approval for this study was obtained from the Health Research Ethics Board of the University of Alberta and the corresponding panel from the Alberta Cancer Board, Cross Cancer Institute. Administrative approval for this study was received from the Capital Health Authority.

All surgeries were performed by either of two hepatobiliary surgeons (DLB and NMK) at the University of Alberta Hospital. Patients diagnosed with liver metastases were identified. All patients who underwent resection for metastatic colorectal cancer were included. Between February 1988 and June 2002, 113 patients underwent 131 liver resections for the treatment of histologically confirmed colorectal liver metastases. Three of these patients had 2-stage resections in an effort to render them disease-free. Fourteen patients underwent second resections for recurrent disease after the initial metastectomies with a single patient undergoing a subsequent third resection. All 131 resections in 113 patients were used to calculate the surgical morbidity and mortality. Eight patients were excluded from the subsequent analysis for the following reasons: local ablative therapy prior to or during the first resection (n=3); in-hospital post-operative mortality (n=2); failure to reach a disease-free state (n=2, both due to unresected pulmonary metastases); death from other causes within two months of the liver resection (n=1). Two more patients were lost to follow-up shortly after liver resection. Thus, the analysis is based on the 103 remaining patients.

Demographic, disease-specific, surgery-specific, and follow-up data were obtained using a structured data abstraction form. Data was retrieved from physician notes, operative notes, as well as pathology, radiology, and laboratory reports found at the University of Alberta Hospital, regional cancer centres within Canada, and physician's

offices. Additional survival information was obtained using the Alberta Provincial Cancer Registry.

All chemotherapy administered was systemic. Patients were allocated to groups depending upon whether or not they received chemotherapy. Those that received chemotherapy were categorized as having received only adjuvant chemotherapy (adjuvant chemotherapy only group), or having received neoadjuvant chemotherapy with or without subsequent adjuvant chemotherapy (neoadjuvant chemotherapy group). Comparisons were then made between the adjuvant chemotherapy only group and all other patients as well as between the peri-operative chemotherapy group (consisting of both the adjuvant and neoadjuvant chemotherapy groups) and all remaining patients. Survival within each group was determined using the date of surgery and the date of the most recent follow-up or death. Disease-free survival was determined using the date of surgery and the date of diagnosis of disease recurrence. For patients that underwent more than one resection, these intervals were calculated using the date that the patient was first rendered disease free until either the most recent follow-up or death for survival, or the first recurrence for disease-free survival.

Survival curves were generated using the Kaplan-Meier method. We report median survival times (in years) and their 95% confidence intervals. The log-rank test was used to test for survival differences between groups. Univariate analyses using the Cox proportional hazards model were used where appropriate. Frequencies were compared using the Fischer's exact or χ^2 tests. Our a priori level of statistical significance was set at $p<0.05$. All data were analyzed using SPSS Statistical Software (version 11.0, Chicago IL)

RESULTS

The study sample consists of 103 patients who have undergone 119 liver resections (Table 4-1).

MORBIDITY AND MORTALITY

All 113 patients who underwent 131 liver resections were included in the determination of morbidity and mortality (Table 4-2). There were two (1.5%) in-hospital deaths following partial hepatectomy: one was secondary to hepatic failure, while the second followed a stroke in an elderly patient with a history of symptomatic cerebrovascular disease. Thirty-one (24%) patients experienced 39 (30%) distinct morbidities listed in Table 4-2. Of the five patients who experienced bile leaks, two required surgical intervention, two were treated via ERCP, and one was self-limited.

SURVIVAL

Overall Survival (Figure 4-1)

The proportion of the 103 patients surviving at one year was 93% [95% CI: 87.7 – 97.9]. This fraction dropped to 61% [95% CI: 49.5 – 71.6] at three years, and to 37% [95% CI: 23.9 – 50.0] at 5 years. Median survival was 4.35 years [95% CI: 3.24-5.47].

Disease-Free Survival (Figure 4-2)

Median disease-free survival of the entire study sample was 1.52 years [95% CI: 1.00 – 2.05]. Disease-free survival at one year was 57.9% [95% CI: 48.2 – 67.7],

30.5% [95% CI: 20.3 – 40.7] at three years, and 25.7% [95% CI: 15.2 – 36.3] at 5-years.

ADJUVANT CHEMOTHERAPY

Forty-two patients received adjuvant chemotherapy. Of these, three received neoadjuvant therapy as well and were excluded from this group leaving 39 patients. Almost all patients received 5-fluorouracil (5-FU) based treatment. Thirty-three patients were given 5-FU + folinic acid. Three patients were treated with 5-FU and CPT-11 (1) or 5-FU and levamisole (2). Two further patients received CPT-11 monotherapy, while in one patient the type of chemotherapy administered could not be determined. Patients were classified according to whether they received adjuvant chemotherapy only and were compared versus all those that did not (64 patients). Demographic, primary tumor, and metastases factors for these two cohorts were comparable (Table 4-3).

Survival (Figure 4-3)

Median survival for the patients that received adjuvant chemotherapy only was 4.79 years [95% CI: 3.74 – 5.83] compared to 3.52 years [95% CI: 1.66-5.38] for those who did not receive solely adjuvant therapy. Differences in overall survival at 1-year, 3-years, or 5-years did not reach statistical significance.

Disease-Free Survival (Figure 4-4)

Median disease-free survival in patients who were treated with adjuvant chemotherapy after resection of colorectal liver metastases was 1.91 years [95% CI: 1.40 – 2.41], compared to 0.94 years [95% CI: 0.57 – 1.31] for those not treated. This

is reflected in a significant improvement in 1-year disease-free survival (log-rank p=0.02). At two years this difference was no longer statistically significance but a trend remained (log-rank p=0.13).

PERI-OPERATIVE CHEMOTHERAPY

Fifty-five patients received peri-operative chemotherapy. Of these, 39 received adjuvant chemotherapy only, as described above. Sixteen patients were administered neoadjuvant chemotherapy, all of which was 5-FU based. Eight patients were given 5-FU + folinic acid / leucovorin. Five patients were treated with 5-FU and oxaliplatin (1), 5-FU and CPT-11 (2) or 5-FU and levamisole (2). Three of these 16 patients were given both adjuvant and neo-adjuvant chemotherapy. As can be seen in tables 3-5, the groups were comparable among demographic, primary tumor, and metastases factors.

Survival (Figure 4-5)

Median survival for the patients that received peri-operative chemotherapy was 4.79 years [95% CI: 3.84 – 5.74] while those that did not receive peri-operative chemotherapy had a median survival of 3.43 years [95% CI: 1.31 - 5.55]. There was no significant difference in either overall survival or survival at 1-year, 3-years, or 5-years marks.

Disease-Free Survival (Figure 6)

Median disease-free survival in patients who were treated with peri-operative chemotherapy after the resection of colorectal liver metastases is 1.69 years [95% CI: 1.23 – 2.15]. In those that did not receive peri-operative chemotherapy, median

survival is 0.94 years [95% CI: 0.54 – 1.34]. Similar to what was seen when analyzing adjuvant chemotherapy alone, the addition of peri-operative chemotherapy after the resection of colorectal liver metastases demonstrates a trend towards improving disease-free survival at 1-year post-resection (log-rank p=0.11). This trend was no longer apparent subsequently as three-year, five-year, and overall disease-free survival are not significantly different in patients.

Table 4-1. Description of study sample (n=103).

Female gender, n (%)	34	(33%)
Median age in years, (range)	63	(32.7 – 84.5)
Initial diagnosis of liver metastases, n (%)		
Synchronous	50	(49%)
Metachronous	53	(51%)
Number of Lesions, n (%)		
1	55	(53%)
2-4	40	(39%)
>4	7	(8%)
Unknown	1	(1%)
Operations for hepatic resection[\]	119	
Procedures performed[◇]	148	
Wedge / non-anatomic resection	72	
Left lateral lobectomy	9	
Right lobectomy	39	
Left lobectomy	9	
Extended right lobectomy	16	
Extended left lobectomy	3	

[\] 15 analyzed patients underwent 2 resections, 1 patient underwent a third resection

[◇] More than one procedure may be performed during one operation(i.e. left lateral lobectomy + wedge resection of segment VII = 2 procedures)

Table 4-2. Morbidity and mortality. Percentages displayed are calculated using the total number of resections performed for colorectal liver metastases (N=131).

Mortalities	2	(1.5%)
Morbidities		
All	39	(30%)
Wound infection / seroma	7	(18%)
Bile leak	5	(13%)
Deep venous thrombosis / pulmonary embolus / superficial thrombophlebitis	4	(10%)
Pneumonia	3	(8%)
Intra-abdominal abscess	2	(5%)
Small bowel obstruction	2	(5%)
Pleural effusion	2	(5%)
Prolonged ileus	2	(5%)
Supra-ventricular tachycardia / premature ventricular contractions	2	(3%)
Hypercarbic respiratory failure	1	(3%)
Transient liver failure	1	(3%)
Bleeding varices	1	(3%)
Pneumothorax due to central vein cannulation	1	(3%)
Dehiscence	1	(3%)
Stroke	1	(3%)
Delirium	1	(3%)
Pyelonephritis	1	(3%)
Lymphatic fistula	1	(3%)
Renal dysfunction	1	(3%)

Figure 4-1. Survival following partial hepatectomy for colorectal metastases in years following liver resection – including 95% confidence intervals.

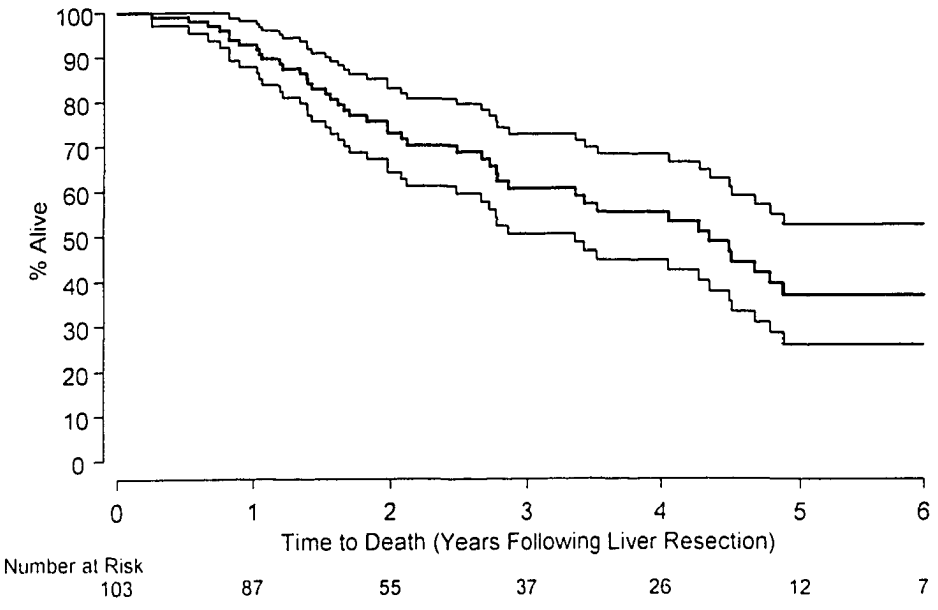


Figure 4-2. Disease-free survival following partial hepatectomy for colorectal metastases in years following liver resection – including 95% confidence intervals

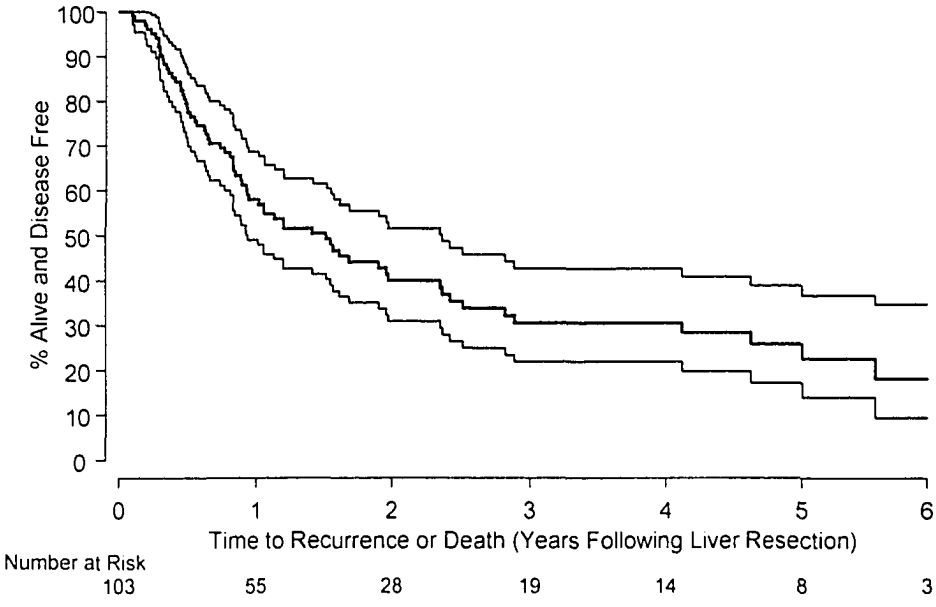


Table 4-3. Demographics and tumor characteristics.

	Peri-operative Chemotherapy					
	Neoadjuvant Chemotherapy N=16		Adjuvant Chemotherapy Only N=39		No peri-operative Chemotherapy N=48	
Female gender, n (%)	5	(31%)	11	(28%)	18	(38%)
Median age in years (range)	65.2	(49.8-75.7)	59.7	(40.0-75.8)	67	(32.47-84.5)
Location of primary						
Right colon, n (%)	6	(38%)	12	(31%)	17	(35%)
Transverse colon, n (%)	1	(6%)	0	(0%)	0	(0%)
Left Colon, n (%)	2	(13%)	0	(0%)	2	(4%)
Rectosigmoid, n (%)	5	(31%)	22	(56%)	13	(27%)
Rectum, n (%)	2	(13%)	5	(13%)	15	(31%)
Missing, n (%)	0	(0%)	0	(0%)	1	(2%)
T stage primary						
T1, n (%)	0	(0%)	1	(3%)	0	(0%)
T2, n (%)	1	(6%)	8	(21%)	5	(10%)
T3, n (%)	11	(69%)	26	(67%)	32	(67%)
T4, n (%)	4	(25%)	2	(5%)	8	(17%)
Missing, n (%)	0	(0%)	2	(5%)	3	(6%)
N stage primary						
N0, n (%)	5	(31%)	18	(46%)	19	(40%)
N1, n (%)	9	(56%)	14	(36%)	17	(35%)
N2, n (%)	2	(13%)	7	(18%)	10	(21%)
Missing, n (%)	0	(0%)	0	(0%)	2	(4%)

	Peri-operative Chemotherapy					
	Neoadjuvant Chemotherapy N=16		Adjuvant Chemotherapy Only N=39		No peri-operative Chemotherapy N=48	
Time to Metastases						
Synchronous, n (%)	15	(94%)	24	(62%)	13	(27%)
<1 year, n (%)	1	(9%)	4	(10%)	12	(25%)
1-5 years, n (%)	0	(0%)	10	(26%)	20	(42%)
>5 years, n (%)	0	(0%)	1	(3%)	3	(6%)
Number of Metastases						
1, n (%)	6	(38%)	21	(54%)	28	(58%)
>1, n (%)	10	(63%)	17	(44%)	18	(38%)
Missing, n (%)	0	(0%)	1	(3%)	2	(4%)
Size of Largest Metastasis						
<5cm, n (%)	12	(75%)	22	(56%)	27	(56%)
≥5cm, n (%)	4	(25%)	16	(41%)	20	(42%)
Missing, n (%)	0	(0%)	1	(3%)	1	(2%)
CEA						
<5, n (%)	7	(44%)	12	(31%)	8	(17%)
≥5, n (%)	6	(38%)	18	(46%)	30	(63%)
Missing, n (%)	3	(19%)	9	(23%)	10	(21%)
Surgical Margin						
+ Microscopically, n (%)	1	(6%)	1	(3%)	4	(8%)
<1cm, n (%)	6	(38%)	15	(38%)	13	(27%)
≥1cm, n (%)	8	(50%)	20	(52%)	28	(58%)
Missing, n (%)	1	(6%)	3	(8%)	3	(6%)

Figure 4-3. Survival following partial hepatectomy for colorectal metastases in years following liver resection – patients who received adjuvant chemotherapy versus those that received no adjuvant chemotherapy. No significant difference is noted.

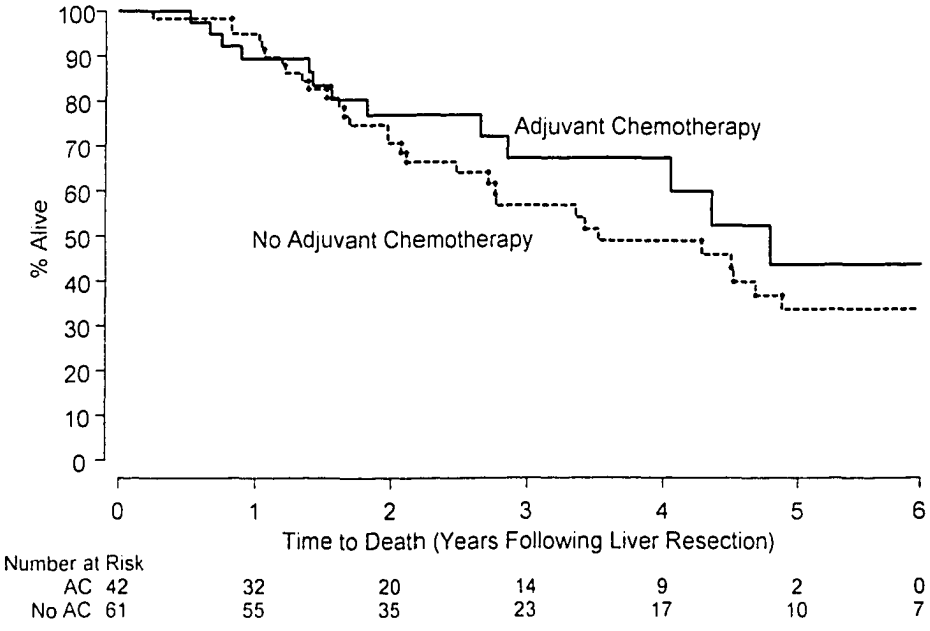


Figure 4-4. Disease-free survival following partial hepatectomy for colorectal metastases in years following liver resection – patients who received adjuvant chemotherapy versus those that received no adjuvant chemotherapy. There is a statistically significant improvement in disease-free survival at 1-year with the addition of adjuvant chemotherapy (p=0.02). This trend remains at the 2-year mark (p=0.09), but then disappears beyond the second year.

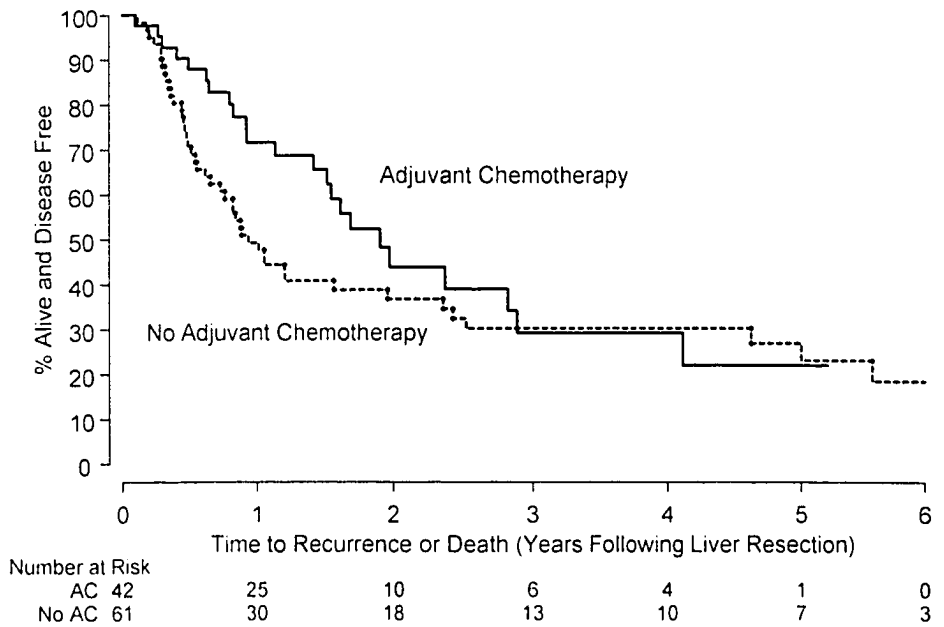


Figure 4-5. Survival following partial hepatectomy for colorectal metastases in years following liver resection – patients who received peri-operative (neoadjuvant and/or adjuvant) chemotherapy versus those that received no chemotherapy. No significant difference is noted.

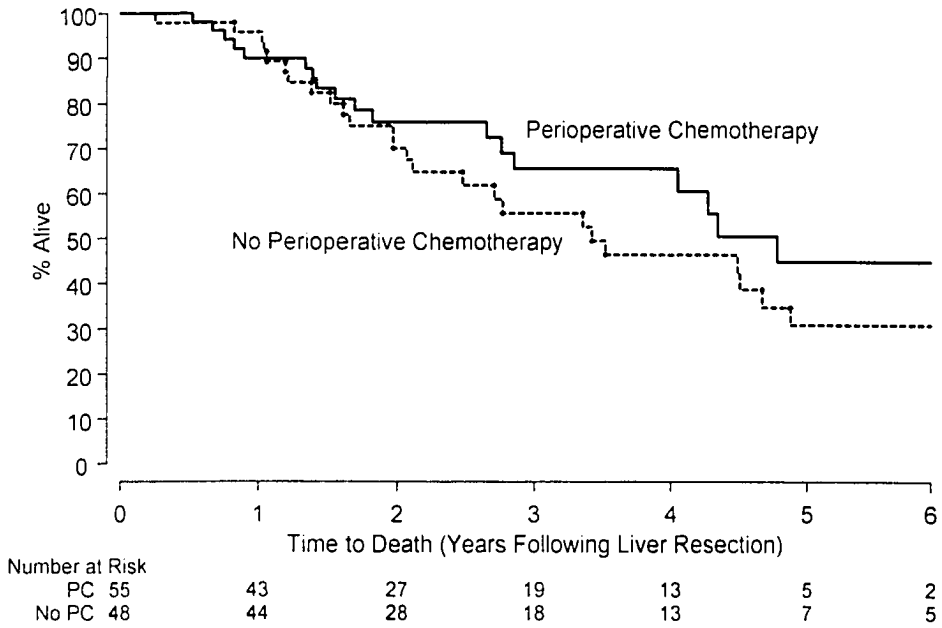
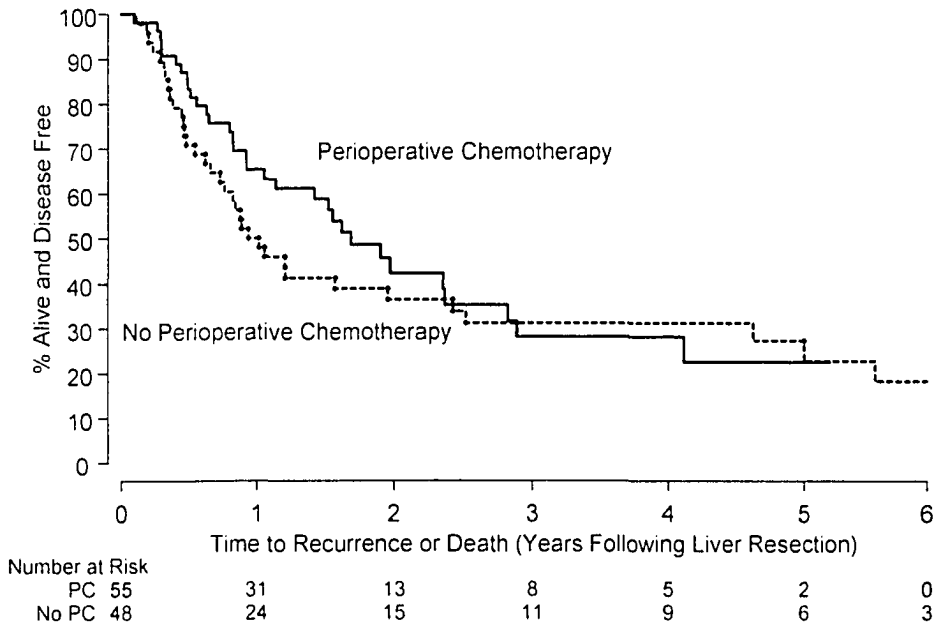


Figure 4-6. Disease-Free Survival Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection – patients who received peri-operative (neoadjuvant and/or adjuvant) chemotherapy versus those that received no chemotherapy. There is a trend towards a chemotherapy-associated improved disease-free survival at 1-year (log-rank p=0.11), which is lost beyond the first year.



DISCUSSION

Surgery is the single most effective treatment for patients with colorectal liver metastases[14-18]. Unfortunately, the resultant survival remains below 50% at five years. Recently efforts towards improving this figure have focused on the addition of chemotherapeutic agents. The potential benefits from the addition of chemotherapy logically derive from several observations. First, the majority of post-metastectomy patients die from recurrence of their malignancy which can be intra-hepatic, extra-hepatic or both. Secondly, chemotherapy has been proven to prolong survival in patients with resected node positive colorectal carcinoma.

Research in this area currently focuses on the addition of adjuvant intra-arterial chemotherapy to adjuvant systemic chemotherapy after partial hepatectomy for colorectal metastases[19-23]. However, the role of adjuvant systemic chemotherapy, which is widely regarded as "standard"[24], has never been convincingly demonstrated. Randomized and controlled trials were attempted by several cooperatives including the EORTC, NCIC CTG, and GIVIO. Regrettably, these trials failed to accrue sufficient patients. Although there was insufficient power, a trend towards improved overall survival with the addition of adjuvant systemic chemotherapy (consisting of 5-FU and folinic acid) was demonstrated[25].

Retrospective studies have now become the most likely avenue for describing the effects of adjuvant chemotherapy in patients who have undergone resection of colorectal liver metastases. Figueras et al (2001) described an improvement in survival attributable to adjuvant systemic chemotherapy. They concluded, however, that "prospective studies are necessary to define its exact role"[26]. Considering the wide-

spread adoption of adjuvant chemotherapy in this setting, such prospective trials are unlikely to be done.

Given the current absence of prospective studies, we have attempted to further describe the role of adjuvant chemotherapy in the treatment of colorectal liver metastases. In 103 patients who have undergone one or more partial hepatectomies for colorectal liver metastases we noted that both overall and disease-free survival compared favorably with that reported previously.

When post-metastectomy patients were stratified by chemotherapy, differences in median disease-free and overall survival favored those who received adjuvant chemotherapy only when compared to all other patients. However, while potentially clinically significant, these differences failed to reach statistical significance likely due small numbers involved.

A further analysis was performed, this time combining patients who received either adjuvant chemotherapy or neo-adjuvant chemotherapy, or both (the "peri-operative chemotherapy" group). These patients were then compared to the remaining subjects, none of which had received any peri-operative chemotherapy. Paralleling the results from the adjuvant chemotherapy only analysis, while an advantage was noted in terms of both survival and disease-free survival in those that received peri-operative chemotherapy, statistical significance was not reached.

When interpreting these results, two factors must be noted. First, this analysis lacked the power necessary to detect a statistically significant improvement in survival. A cohort roughly 4 times this size, with similar ratios of treatment and non-treatment patients, would be needed to achieve a power of at least 80%. It must be noted, however, that this study is sufficiently powered to detect differences in disease-free

survival. Secondly, the timing of the resections and subsequently the recurrences must be noted. In the "no peri-operative chemotherapy" group, 19 of the 25 recurrences (61%) occurred in 1999 or earlier and therefore prior to the publication of the more effective palliative chemotherapy combinations that have led to increased survival[7 27]. This is in contrast to the peri-operative chemotherapy group, in which 17 of 30 recurrences (57%) occurred after the publication of these improved palliative chemotherapy protocols.

The multinational cooperative study mentioned previously used similar chemotherapeutic agents and demonstrated similar trends[25]. It, like this study, was handicapped by the limited number of patients available for analysis. Due to the acceptance of the role of systemic chemotherapy in the treatment of resected colorectal liver metastases, it is unlikely that such an endeavor will be undertaken again. With the emergence of newer chemotherapeutic agents being used in the treatment of this disease; future research will likely concentrate on determining the optimal systemic chemotherapeutic regime rather than on whether systemic chemotherapy has a role in resected metastatic colorectal cancer.

CONCLUSION

Metastectomy established a median survival of 4.35 years [95% CI: 3.24 – 5.47] and median disease-free survival of 1.52 years [95% CI: 1.00 – 2.05] in 103 patients with colorectal liver metastases. The role of adjuvant and neo-adjuvant chemotherapy in these patients who have undergone partial hepatectomies for colorectal metastases remains unclear. Both survival and disease-free survival demonstrated potentially clinically important improvements due to the addition of adjuvant chemotherapy only or any peri-operative chemotherapy. Yet, aside from a statistically significant improvement in 1-year disease-free survival (and a trend at 2-years) in those who received adjuvant chemotherapy only after resection of their liver metastases, there was no statistically significant improvement in survival or disease-free survival experienced by patients who have received any chemotherapy. Although the power for detecting a difference in disease-free survival exceeded 80%, this analysis lacked the power necessary to detect a statistically significant difference in survival.

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CHAPTER 5

Predicting the Benefit of Supplemental Chemotherapy in Patients with Colorectal Liver Metastases using the Clinical Risk Score

A version of this chapter was presented at the Perspectives in Colorectal Cancer Meeting in Barcelona, Spain, June 26-June 28, 2003 and has been submitted for publication in the Journal of Clinical Oncology.

INTRODUCTION

Each year, there are up to 500,000 deaths and twice that number of new diagnoses attributable to colorectal cancer worldwide.[1-6] The majority of these patients will have metastatic disease either at diagnosis or during the course of their disease.[7]

In those with metastatic disease confined to the liver, surgery remains the gold standard treatment modality; conferring a 5-year survival of between 25% and 40%[8-12] in suitable patients who undergo resection. Improvement upon these results is being sought through the addition of supplemental systemic and / or regional chemotherapy.

Until recently, it was difficult to consistently determine the likelihood of long-term survival in individual patients with resectable colorectal liver metastases based on the biologic parameters of their disease. Many attempts have been made at developing systems that allow prediction of post-operative survival. For a system to be clinically relevant, however, it must use readily available pre-operative data. Only a few of these systems are so constructed.[9,10,13] Fong et al (1999) used a cohort of 1001 patients to develop the Clinical Risk Score (CRS).[9] The CRS uses five accessible pre-operative markers (nodal status of the primary tumour, disease-free interval until the development of metastatic disease, size of the largest metastasis, number of metastases, and serum carcinoembryonic antigen (CEA) level) to classify patients with a score from 0 to 5. Furthermore, patients can be aggregated and classified as either "low-score" (CRS 0-2) or "high-score" (CRS 3-5), with the "low-score" group displaying improved survival. These results have been replicated by other authors, and this system is starting to become widely used.[14]

The stated benefits of the CRS are multiple: it can aid in the selection of appropriate surgical candidates[9]; it can be used to determine which patients necessitate

further pre-operative work-up[15]; it can be used to stratify patients within the context of a clinical trial; and it can also be used to direct subset analyses.[16]

The usefulness of the CRS may also be extrapolated to the investigation of the benefits of supplemental systemic chemotherapy when added to the resection of colorectal liver metastases. There is little evidence supporting the routine use of supplemental chemotherapy in the setting of resected colorectal liver metastases. Unfortunately, a large multi-national randomized trial initiated to define the role of systemic chemotherapy failed to accrue enough patients and was prematurely terminated.[17]

As supplemental systemic chemotherapy is routinely used in the "control arm" of randomized trials, it is unlikely that there will be another opportunity for a prospective, randomized study.[16,18,19] It is possible, however, to determine whether all patients derive equal benefit from this therapy.

PATIENTS AND METHODS

Ethical approval for this study was obtained from the Health Research Ethics Board of the University of Alberta and the corresponding panel from the Alberta Cancer Board, Cross Cancer Institute. Administrative approval for this study was received from the Capital Health Authority.

All surgeries were performed by either of two hepatobiliary surgeons (DLB and NMK) at the University of Alberta. Patients diagnosed with liver metastases were identified. All patients who underwent resection for metastatic colorectal cancer were included. Between February 1988 and June 2002, 113 patients underwent 131 liver resections for the treatment of histologically confirmed colorectal liver metastases. Three of these patients had 2-stage resections in an effort to render them disease-free. Fourteen patients underwent second resections for recurrent disease after the initial metastectomies with a single patient undergoing a subsequent third resection. All 131 resections in 113 patients were used to calculate the surgical morbidity and mortality. Eight patients were excluded from the subsequent analysis for the following reasons: local ablative therapy prior to or during the first resection (n=3); in-hospital post-operative mortality (n=2); failure to reach a disease-free state (n=2, both due to unresected pulmonary metastases); death from other causes within two months of the liver resection (n=1). Two more patients were lost to follow-up shortly after liver resection. Thus, the analysis is based on the 103 remaining patients who had undergone 119 resections.

Demographic, disease-specific, surgery-specific, and follow-up data were obtained using a structured data abstraction form. Data was retrieved from physician notes, operative notes, as well as pathology, radiology, and laboratory reports found at

the University of Alberta Hospital, regional cancer centres within Canada, and physician's offices. Additional survival information was obtained using the Alberta Provincial Cancer Registry.

The clinical risk score was calculated for each of the 103 included patients. Patients were then allocated to either the low-score (CRS 0-2) or high-score (CRS 3-5) group based on their CRS. Patients were determined as having supplemental chemotherapy if in relation to the hepatic metastectomies they had received either neoadjuvant chemotherapy or adjuvant chemotherapy or both. All chemotherapy administered was systemic. Those who received supplemental chemotherapy were then compared to those that did not.

Survival was calculated using the date of surgery and the date of the most recent follow-up or death. Disease-free survival was determined using the date of surgery and the date of diagnosis of disease recurrence. For patients who underwent more than one resection, these intervals were calculated using the date that the patient was first rendered disease free until either the most recent follow-up or death for survival, or the first recurrence for disease-free survival.

STATISTICS

Survival curves were generated using the Kaplan-Meier method. We report median survival times (in years) and their 95% confidence intervals. The log-rank test was used to test for survival differences between groups. Univariate analyses using the Cox proportional hazards model were used where appropriate. Our a priori level of statistical significance was set at $p<0.05$. All data were analyzed using SPSS Statistical Software (version 11.5, Chicago IL).

RESULTS

The study sample consists of 103 patients who have undergone 119 liver resections (Table 6-1). Fifty-five patients received supplemental chemotherapy. All of these, except for two, were administered 5-FU based chemotherapy.

Thirty-nine patients received adjuvant chemotherapy only. Thirty-three patients were given 5-FU + folinic acid. Three patients were treated with 5-FU and CPT-11 (1) or 5-FU and levamisole (2). Two further patients received CPT-11 monotherapy, while in one patient the type of chemotherapy administered could not be determined.

Sixteen patients were administered neoadjuvant chemotherapy, all of which was 5-FU based. Eight patients were given 5-FU + folinic acid / leucovorin. Five patients were treated with 5-FU and oxaliplatin (1), 5-FU and CPT-11 (2) or 5-FU and levamisole (2). Three of these 16 patients were given both adjuvant and neo-adjuvant chemotherapy.

No patients received intra-arterial, intra-portal, or other regional chemotherapy.

MORBIDITY AND MORTALITY

All resections for colorectal liver metastases (131 resections in 113 patients) were used in calculating the surgical morbidity and mortality. There were two (1.5%) in-hospital deaths following partial hepatectomy: one was secondary to hepatic failure, while the second followed a stroke in an elderly patient with a history of symptomatic cerebrovascular disease. Thirty-one (24%) patients experienced 39 (30%) distinct morbidities listed in Table 6-2. Of the five patients who experienced bile leaks, two required surgical intervention, two were treated via ERCP, and one was self-limited.

Overall Survival (Figure 6-1A)

The proportion of the 103 patients surviving at one year was 93% [95% CI: 87.7 – 97.9]. This fraction dropped to 61% [95% CI: 49.5 – 71.6] at three years, and to 37% [95% CI: 23.9 – 50.0] at 5-years. Median survival was 4.35 years [95% CI: 3.24-5.47].

Disease-Free Survival (Figure 6-1B)

Median disease-free survival of the entire study sample was 1.52 years [95% CI: 1.00 – 2.05]. Disease-free survival at one year was 57.9% [95% CI: 48.2 – 67.7], 30.5% [95% CI: 20.3 – 40.7] at three years, and 25.7% [95% CI: 15.2 – 36.3] at 5-years.

CLINICAL RISK SCORE

Data regarding the individual components of the CRS were collected and tabulated. Pre-operative Serum CEA levels were unavailable for 22 (21.4%) patients. As per the scoring system, positive components were awarded a score of one, while negative or missing components were awarded a score of zero (Table 6-3). Individual patient scores were then calculated and were used to stratify patients. Sixty-three patients were stratified to the low-CRS (CRS 0-2) group, while 40 patients were stratified to the high-CRS (CRS 3-5) group (Table 6-4).

Low-CRS versus High-CRS (Figure 6-2)

Median overall survival in the low-CRS group was 4.51 years [95% CI: 4.02 – 5.00]. This was significantly different from the overall survival of patients in the high-

score group: 2.66 years [95% CI: 1.38 – 3.93] (p=0.02). Similarly, there was a significant difference noted in disease-free survival between patients in the low-score group (1.91 years [95% CI: 0.7 – 3.11]) and patients in the high-score group (0.82 years [95% CI: 0.26 – 1.38]) (p=0.03)

Low-CRS and Supplemental Chemotherapy (Figure 6-3)

Examining the low-CRS group in isolation reveals a significant advantage in terms of both survival and disease-free survival due to the addition of supplemental systemic chemotherapy. Overall 4-year survival is significantly improved by the addition of supplemental chemotherapy Hazard Ratio (HR) = 0.26 [95% CI: 0.074 – 0.899] . Similarly, the addition of supplemental chemotherapy benefits 1-year disease-free survival in the low-CRS group HR = 0.37 [95% CI: 0.143 – 0.937]]. This benefit persists as a trend up for up to 4 more years.

High-CRS and Supplemental Chemotherapy (Figure 6-4)

Examining the high-CRS group in isolation reveals no advantage in terms of survival (HR = 0.84 [95% CI: 0.33 – 2.2]) or disease-free survival (HR = 0.97 [95% CI: 0.45 – 2.09]) attributable to the addition of supplemental systemic chemotherapy.

Table 5-1. Description of study sample (n=103).

Female gender, n (%)	34	(33%)
Median age in years, (range)	63	(32.7 – 84.5)
Operations for hepatic resection[\]	119	
Procedures performed[◇]	148	
Wedge / non-anatomic resection	72	
Left lateral lobectomy	9	
Right lobectomy	39	
Left lobectomy	9	
Extended right lobectomy	16	
Extended left lobectomy	3	

[\] 15 analyzed patients underwent 2 resections, 1 patient underwent a third resection

[◇] More than one procedure may be performed during one operation(i.e. left lateral lobectomy + wedge resection of segment VII = 2 procedures)

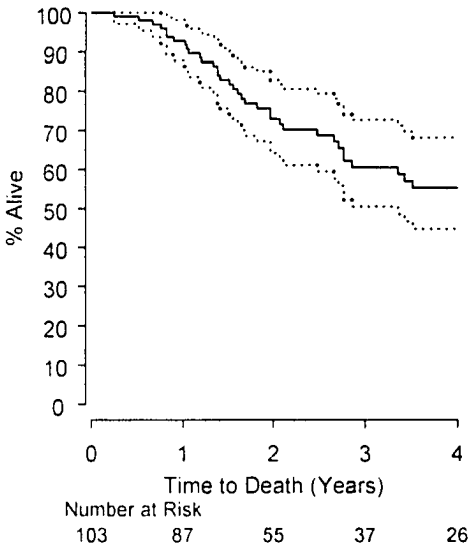
Table 5-2. Morbidity and mortality. Percentages displayed are calculated using the total number of resections performed for colorectal liver metastases (N=131).

Mortalities	2	(1.5%)
Morbidities		
All	39	(30%)
Wound infection / seroma	7	(18%)
Bile leak	5	(13%)
Deep venous thrombosis / pulmonary embolus / superficial thrombophlebitis	4	(10%)
Pneumonia	3	(8%)
Intra-abdominal abscess	2	(5%)
Small bowel obstruction	2	(5%)
Pleural effusion	2	(5%)
Prolonged ileus	2	(5%)
Supra-ventricular tachycardia / premature ventricular contractions	2	(3%)
Hypercarbic respiratory failure	1	(3%)
Transient liver failure	1	(3%)
Bleeding varices	1	(3%)
Pneumothorax due to central vein cannulation	1	(3%)
Dehiscence	1	(3%)
Stroke	1	(3%)
Delirium	1	(3%)
Pyelonephritis	1	(3%)
Lymphatic fistula	1	(3%)
Renal dysfunction	1	(3%)

Figure 5-1.

(A) Survival Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection (Entire Study Sample: n=103)

(B) Disease-Free Survival (Broken Line) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection (Entire Study Sample: n=103)



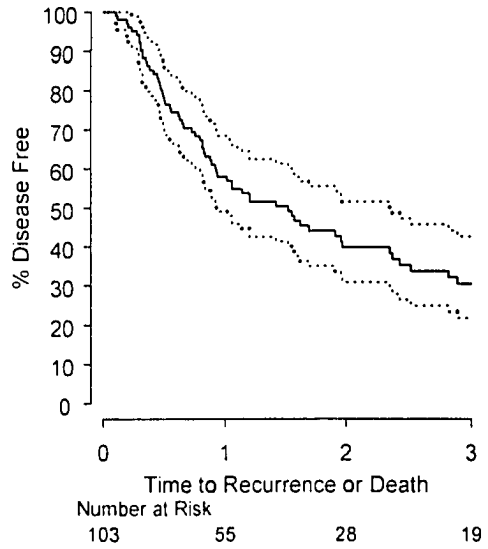


Table 5-3. Components of the clinical risk score: cccurrence in 103 patients [n (%)]

	No (CRS = 0)	Yes (CRS = 1)	Missing (CRS=0)
Node positive primary	42 (40.8%)	59 (57.3%)	2 (1.9%)
Time to metastasis < 1 Year	34 (33%)	69 (67%)	0 (0%)
>1 metastasis	56 (54.4%)	47 (45.6%)	0 (0%)
Largest metastasis > 5cm	70 (68.0%)	31 (30.1%)	2 (1.9%)
CEA > 200ng / ml	74 (71.8%)	7 (6.8%)	22 (21.4%)

Table 5-4. Study sample clinical risk scores.

Score	n	
0	9 (8.7%)	
1	25 (24.3%)	Low-CRS n=63 (61%)
2	29 (28.2%)	
3	30 (29.1%)	
4	10 (9.7%)	High-CRS n=40 (39%)
5	0 (0%)	

Figure 5-2.

(A) Survival Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Clinical Risk Score (Low-CRS (CRS = 0-2) and High-CRS (CRS = 3-5)). Patients in the Low-CRS (solid line) group demonstrated significantly longer survival (p =0.03) and disease-free survival (p=0.02) than those in the High-CRS group (broken line).

(B) Disease-Free Survival Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Clinical Risk Score (Low-CRS (CRS = 0-2) and High-CRS (CRS = 3-5)). Patients in the Low-CRS group (solid line) demonstrated significantly longer survival (p =0.03) and disease-free survival (p=0.02) than those in the High-CRS group (broken line).

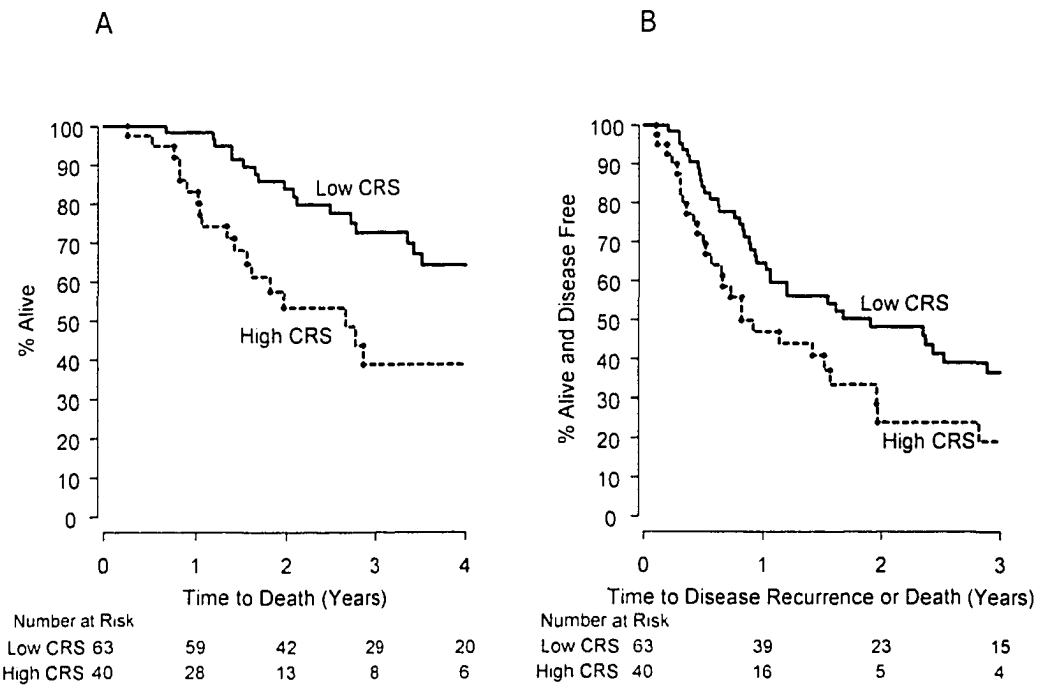


Figure 5-3.

(A) Survival in Low Clinical Risk Score Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of Supplemental Systemic Chemotherapy (No Supplemental Chemotherapy versus Supplemental Chemotherapy). The addition of supplemental systemic chemotherapy significantly improved 4-year survival (p=0.02).

(B) Disease-Free Survival in Low Clinical Risk Score Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of Supplemental Systemic Chemotherapy (No Supplemental Chemotherapy versus Supplemental Chemotherapy). The addition of supplemental systemic chemotherapy significantly improved 1-year disease-free survival (p=0.03). This difference maintained as a strong trend beyond 4-years.

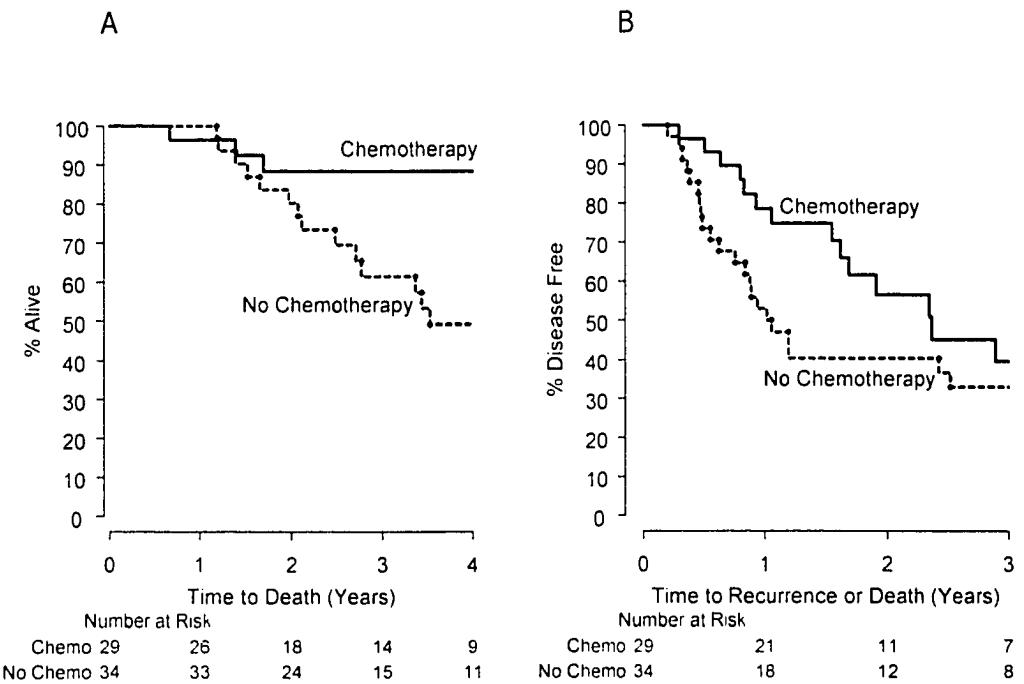
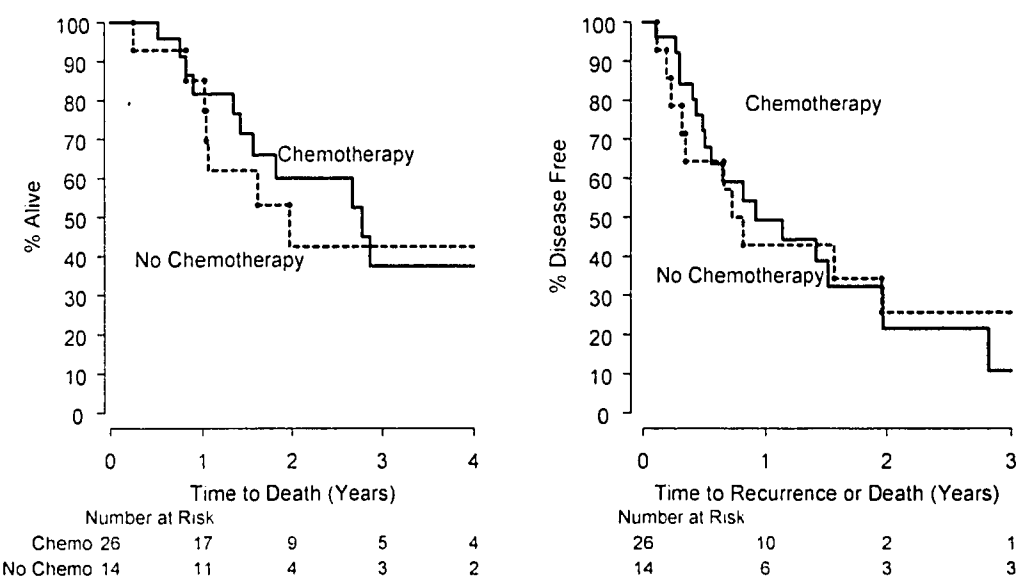


Figure 5-4.

(A) Survival in High Clinical Risk Score Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of Supplemental Systemic Chemotherapy (No Supplemental Chemotherapy versus Supplemental Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group (p=0.71).

(B) Disease-Free Survival in High Clinical Risk Score Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of Supplemental Systemic Chemotherapy (No Supplemental Chemotherapy versus Supplemental Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group (p=0.94).



DISCUSSION

Surgery remains the single most effective treatment for patients with colorectal liver metastases.[8,20-22] Metastectomy is, however, associated with significant morbidity (10% - 30%) and mortality (0 -5%) rates.[23,24] One of the roles of the oncology team is to evaluate the potential survival benefit achievable through surgery against the potential for harm inherent in the procedure. To adequately accomplish this, several prognostic scoring systems have been developed.[9,10,13]

The Clinical Risk Score is the most widely applicable of these systems. It is also derived from the largest sample, 1001 patients. Analysis of numerous factors led to the elucidation of five independent components; the presence or absence of which determine the final score. The five factors are: time to metastases < 12 months, node-positive primary, largest metastasis > 5cm, > 1 metastasis, and serum CEA > 200 ng / ml.[9] These are all readily available, pre-operative factors

Using 103 patients who had undergone hepatectomy for metastatic colorectal carcinoma, an attempt was made to reproduce the prognostic capability of the CRS demonstrated previously by both the creators of the CRS as well as an independent party.[9,14] Of the 103 patients, those classified by a low-CRS (CRS = 0-2) demonstrated significantly lengthened overall survival (4.51 years [95% CI: 4.02 – 5.00]) compared to patients with high-CRS (CRS = 3-5) (2.66 years [95% CI: 1.38 – 3.93]). Similar significant differences were noted when comparing disease-free survival between the two groups: 1.91 years [95% CI: 0.7 – 3.11] in the low-CRS group versus 0.82 years [95% CI: 0.26 – 1.38] in the high-CRS group.

After confirmation of the prognostic ability of the CRS, a possible role for the CRS in the administration of supplemental systemic chemotherapy was explored. The use of

supplemental systemic chemotherapy has become ubiquitous, and is considered the control-arm in many studies.[16,18] Unfortunately, little adequately controlled data has been published describing its role. We investigated whether the CRS was able to predict who would benefit from the addition of supplemental systemic chemotherapy.

Among patients with a low CRS, there was a significant benefit in 4-year overall survival and in 1-year disease-free survival derived from the addition of supplemental systemic chemotherapy. Analysis of the high-CRS group failed to demonstrate such a benefit. In patients with a high CRS stratified by the receipt of supplemental chemotherapy, overall and disease-free survival were almost identical.

These results suggest that the CRS may have added utility in selecting supplemental chemotherapy for patients who have resectable colorectal liver metastases. In our sample, patients with low CRS demonstrated a benefit from the addition of 5-FU based supplemental systemic chemotherapy. High-CRS patients however, failed to derive any appreciable benefit from the same addition. Supplemental systemic, primarily 5-FU based chemotherapy was ineffective in prolonging either survival or disease-free survival in this group. Future research will be needed to determine whether alternative supplemental chemotherapy is more effective in this group, or whether supplemental chemotherapy should be avoided altogether.

The CRS is able to separate patients with colorectal liver metastases by prognosis in terms of disease-free and overall survival as well as by their likelihood of benefit from supplemental chemotherapy. This suggests that the CRS can and should be used as a stratification variable in trials that involve this specific population.

A potential shortfall of this analysis concerns the large amount of missing data. Approximately 21% of patients in this study did not have useable pre-operative serum

CEA levels. In individual patients, missing CRS components were assigned a score of 0 (or status equivalent to the absence of the marker) as was done in the original description of the CRS (personal communication Y. Fong, 2003). A sensitivity analysis was performed. During this second analysis, all missing CRS components were assigned a value of 1 (or status equivalent to the presence of the marker). Of the 24 patients who were missing a value for a CRS component (two of which were missing values for two components), only seven patients changed groups when missing components were treated as being present. Logically, all 7 patients moved from the low-CRS group to the high-CRS group. Analysis of the new groups demonstrated that the relationship between CRS and the effectiveness of supplemental systemic chemotherapy was maintained.

In patients with resectable colorectal liver metastases, the Clinical Risk Score is an easily applied prognostic system that uses readily available pre-operative data. There are a wide range of potential uses for such a validated prognostic system. The CRS should continue to be used to predict survival, to stratify patients within the context of a clinical trial, and to determine which patients need enhanced pre-operative work-up.[9,14,15,25] The CRS may also have a role in determining which supplemental chemotherapy is most appropriate for a given patient. Specifically, low-CRS patients should be started on traditional supplemental systemic chemotherapy. The supplemental treatment of high-CRS patients however, needs to be re-evaluated as these patients did not appear to derive any benefit from the mainly 5-FU based supplemental systemic chemotherapy.

CONCLUSION

The effectiveness of the CRS in predicting survival was replicated in our patient group. Patients stratified to the low-CRS (CRS = 0-2) group demonstrated a prolonged overall survival when compared to those in the high-CRS (CRS 3-5) group: median survival of 4.51 years versus 2.66 years (p=0.02). A similar advantage was found when comparing disease-free survival between low-CRS and high-CRS groups: median disease-free survival of 1.91 years versus 0.82 years (p=0.03).

In addition, we propose that the CRS may have additional function in choosing supplemental systemic chemotherapy. Patients with low-CRS demonstrated significantly increased 4-year survival and 1-year disease-free survival attributable to the addition of systemic chemotherapy, which was almost exclusively 5-FU based. Patients with high-CRS did not demonstrate a similar benefit from the addition of supplemental chemotherapy. This indicates that the CRS may be used to determine which patients will receive the most benefit from traditional supplemental chemotherapy, and which patients should avoid such treatment or be placed on alternative regiments.

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CHAPTER 6

Thymidylate Synthase and Thymidine Phosphorylase Expression in Resected Colorectal Liver Metastases Do Not Predict Overall Survival or Response to Chemotherapy

A version of this chapter was presented at the American Society of Clinical Oncology Meeting in New Orleans, USA June 4-8, 2004 and has been submitted for publication in the Journal of Clinical Oncology.

INTRODUCTION

Colorectal cancer affects millions of people worldwide, with almost one million new diagnoses and half a million deaths per year[1-3]. Although solitary primary tumors are usually treatable, as many as one-third of patients diagnosed with colorectal cancer present with synchronous metastatic disease in the liver, and up to one-third more will eventually develop hepatic metastatic disease. Resection of liver metastases has proven to be the only potentially curative treatment; with 5-year survival of 25-40%[4-7]. Several clinically based prognostic scoring systems have recently been developed, some of which are becoming widely adopted[5,6,8]. More recently, application of these systems has progressed beyond simple prognostication to the selection of patients who would most benefit from liver resection and these systems may eventually be used to stratify patients in clinical trials.

The discovery and application of biochemical prognostic factors has lagged behind that of the clinical prognostic factors. Numerous groups have attempted to elucidate enzymatic and genetic factors associated with prognosis and treatment response. The latter is especially important given that there is a relatively low (20-30%) response rate associated with the most common regimen; 5-fluorouracil (5-FU) and leucovorin[9,10]. Unfortunately, conclusions regarding the predictive value of various biochemical tests have largely been inconsistent.

Thymidylate synthase (TS) inhibition is the proposed mechanism of action for fluoropyrimidines, such as 5-FU, which are the cornerstones of the chemotherapy used in this disease. As such, TS levels have been proposed to be a prognostic factor in patients with metastatic colorectal carcinoma[11]. Thymidine Phosphorylase (TP) catalyzes

the activation of 5-FU and has also been investigated for its potential prognostic and predictive properties[12,13].

In the setting of resected colorectal liver metastases, we investigated whether levels of TS and TP may be of prognostic significance. These enzymes may also be of discriminatory significance in determining which patient may benefit from a specific type of chemotherapy.

PATIENTS AND METHODS

Ethical approval for this study was obtained from the Health Research Ethics Board of the University of Alberta and the corresponding panel from the Alberta Cancer Board - Cross Cancer Institute. Administrative approval for this study was received from the Capital Health Authority.

PATIENTS

The records of two hepatobilliary surgeons (DLB and NMK) from the University of Alberta were examined for patients who underwent potentially curative liver resections for colorectal metastases. We identified 113 patients who received potentially curative hepatic resections for histologically confirmed colorectal liver metastases between February 1988 and June 2002. There were two in-hospital post-operative mortalities and one mortality within 2 months of liver resection. Three patients had local ablative therapy prior to or during their first resection. Two patients were never considered disease-free due to the presence of unresected pulmonary metastases, and two more patients were lost to follow-up shortly after liver resection. Of the 103 remaining patients, we were able to acquire pathologic specimens from initial resections that were suitable for immunohistochemical analysis for 99 patients.

Fifty-three patients received pre and / or post-operative systemic chemotherapy. Forty-five patients received modulated 5-FU (41 leucovorin, 4 levamisole), four patients received irinotecan and bolus 5-FU / LV, 2 received single agent irinotecan. One patient received an experimental vaccine based treatment, while in a final patient the type of chemotherapy administered could not be determined.

Demographic, disease-specific, surgery-specific, and follow-up data were obtained using a structured data abstraction form. Data were retrieved from physician notes, operative notes, as well as pathology, radiology, and laboratory reports filed at the University of Alberta Hospital, regional cancer centres within Canada, and physician's offices. Additional survival information was obtained using the Alberta Provincial Cancer Registry.

Overall survival (OS) was calculated from the date of liver resection to the date of death or last follow-up. Disease-free survival (DFS) was measured from the date of resection to the date of first recurrence or death if no recurrence was noted prior to death or date of last follow-up. In patients who underwent subsequent resections for recurrent disease, OS and DFS were calculated starting at the time of the first resection. In the three patients who underwent staged resections, OS and DFS were calculated starting at the time when the patients were first rendered disease-free; i.e. after the second stage of the resection.

TISSUE SAMPLES

Tissue blocks from the above-described surgically resected specimens were retrieved from the archives of designated pathology departments. The specimens had been fixed in 10% neutral buffered formalin, and routinely processed in paraffin. A section was cut from each block, and stained with hematoxylin and eosin to re-affirm its representative nature (Figure 6-1A). Following this, the tissue blocks were submitted for immunohistochemistry.

IMMUNOHISTOCHEMISTRY FOR TS

Sections of 3-micron thickness were cut from the tissue blocks. They were deparaffinized and rehydrated using standard methods. For antigen retrieval, the slides were heated for 5 minutes in a pressure cooker using 10 mM citrate buffer at pH 6.0. The endogenous peroxidase was inactivated with 0.3% H_2O_2 in absolute methanol. The slides were blocked with 20% normal goat serum. The sections were incubated overnight at 4°C with rabbit polyclonal antibody hTS7.4 which was obtained from Roche Diagnostics (Laval, Quebec, Canada), and used as outlined in their catalogue in a 1:100 dilution. The sections were then incubated with Dakocytomation Envision+ rabbit reagent K4002 (Dakocytomation Inc., Mississauga, Ontario, Canada) for 30 minutes . Dakocytomation diaminodenzidine substrate K3467 was used for color development. Sections were counterstained using hematoxylin. Positive controls included human liver tissue. Negative controls included the omission of the primary antibody. Both positive and negative controls were run in parallel.

IMMUNOHISTOCHEMISTRY FOR TP

As for TS, 3-micron thick sections were cut, deparaffinized, rehydrated, and heated in a pressure cooker however, the buffer used was 1 mM EDTA at pH 8.0. Endogenous peroxidase was inactivated, and the slides were blocked in a similar fashion. The sections were incubated overnight at 4°C with mouse monoclonal antibody 1C6-203 which was also obtained from Roche Diagnostics, Laval, Québec, and used as outlined in their catalogue in a 1:100 dilution. The sections were then incubated with Dakocytomation Envision+ mouse reagent K4000 (Dakocytomation Inc., Mississauga, Ontario, Canada), and Dakocytomation diaminodenzidine substrate K3467 was used for

color development. Sections were counterstained using hematoxylin. Positive (tonsillar tissue) and negative controls were run as for TS.

SCORING SYSTEM

The immunohistochemistry slides for TS (Figure 6-1B) and TP (Figure 6-1C) were evaluated by a pathologist experienced in these immunohistochemistry methods (TM). The evaluator was blinded to all operative, clinical, and pathological data. Individual slides were assigned two scores based on the intensity and the pattern of staining. Intensity was evaluated on a 4-point scale (0 = no staining, 1 = mild-, 2 = moderate-, and 3 = strong positivity respectively) while the staining pattern was assessed using a 6-points scale (0 = no staining, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% of tumor cells stained respectively). These two scores were then multiplied to obtain a composite score between 0 and 15. Scores equal to or greater than six were considered "positive"[14]. A combination TS/TP score was defined as positive if TS and / or TP were positive and negative if both TS and TP were negative.

STATISTICAL ANALYSIS

Survival curves were generated using the Kaplan-Meier method[15]. We report median survival times (in years) and their 95% confidence intervals. The log-rank test was used to test for survival differences between groups[16]. Univariate analyses using the Cox proportional hazards model or the Kaplan-Meier method were used where appropriate[17]. Multivariate analysis was planned if either of the enzymes were found to be significant prognosticators or predictors and the second enzyme would then be forced into the model. If neither enzyme were found to be a significant prognosticator

or predictor, the combination score would be calculated and analyzed using a univariate model. Our a priori level of statistical significance was set at <0.05 using two-sided p-values. All data were analyzed using SPSS Statistical Software (version 11.5, Chicago IL)

Figure 6-1A. A photomicrograph showing liver tissue with metastatic adenocarcinoma. The tumor cells in upper right of field are separated from normal liver (lower left of field by a lymphocytic infiltrate at the tumor/host interface zone (Hematoxylin and eosin, original magnification x100)

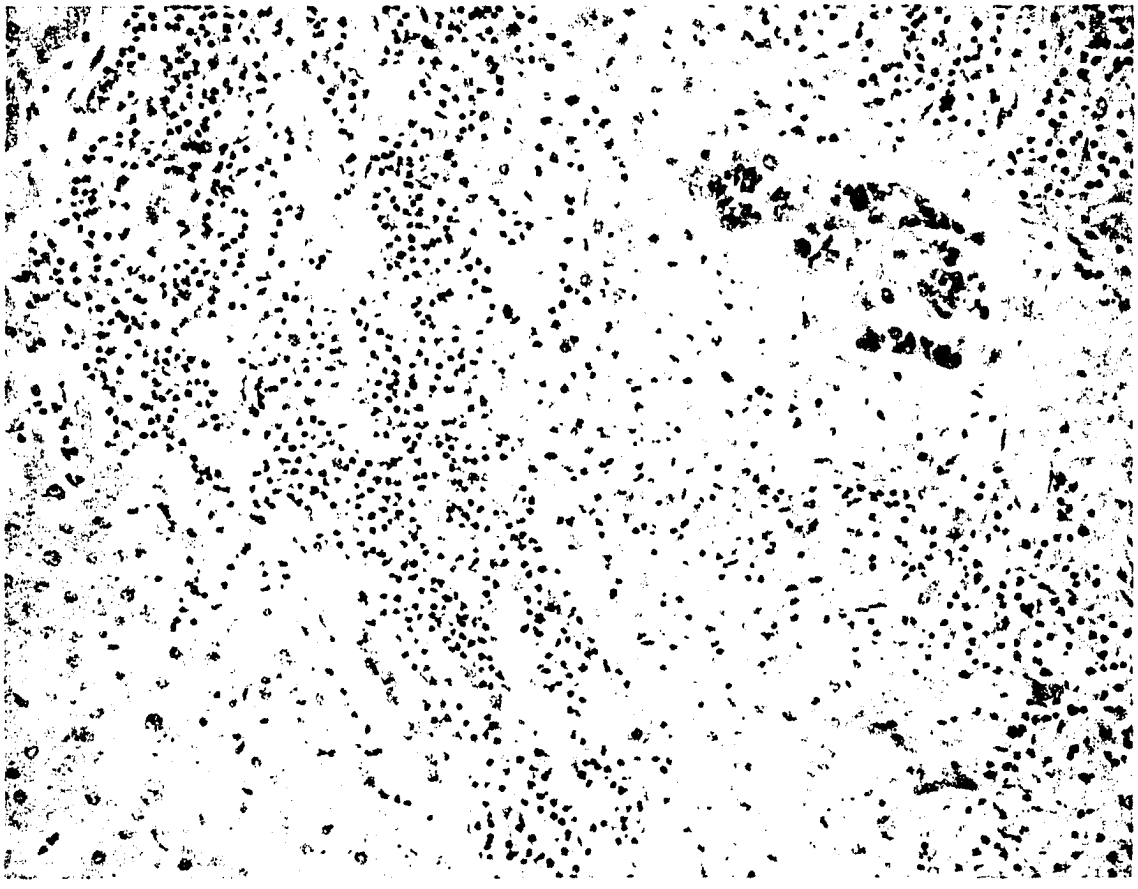
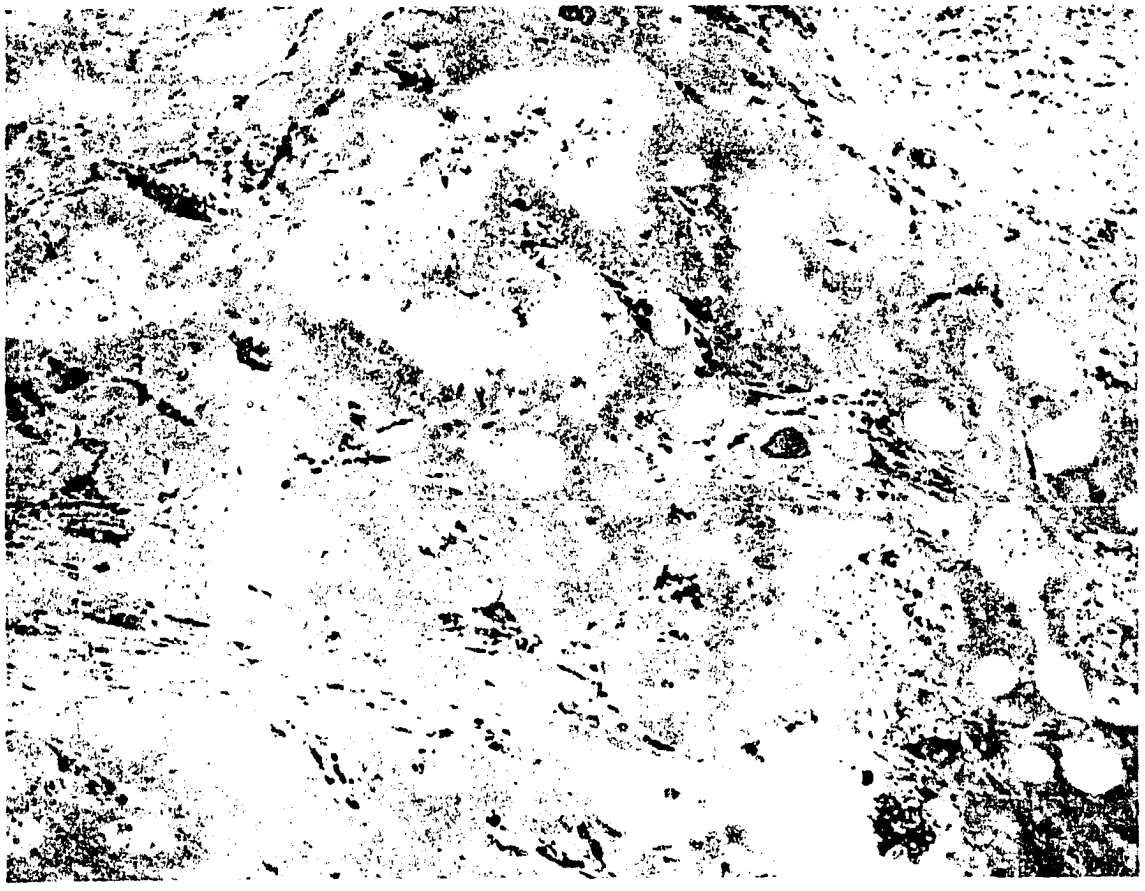


Figure 6-1B. Photomicrograph depicting strong positivity for TS in most of the tumor cells (Immunohistochemistry, original magnification x100)

Figure 6-1C. Photomicrograph illustrating a moderate degree of positivity for TP in the tumor cells while the stromal cells are strongly positive (Immunohistochemistry, original magnification x100)



RESULTS

Median overall survival of the entire sample of 99 patients included in the study was 4.28 years [95% CI: 3.26, 5.30]. Median disease-free survival was 1.52 years [95% CI: 0.76, 2.28].

THYMIDYLATE SYNTHASE

Fifty-two tumors were classified as being TS negative and 47 were classified as TS positive. The TS positive and TS negative groups were evenly distributed in terms of gender, age, (neo)adjuvant chemotherapy administration, and Clinical Risk Score (CRS)[5] [Table 6-1].

Median OS for the TS positive group was 4.51 years [95% CI: 2.82 – 6.20], this did not differ significantly from the median OS for the TS negative group: 4.05 years [95% CI: 2.44 – 5.67] (Log-rank test: p=0.44) (Figure 6-2A). There was a trend towards longer DFS for TS positive patients. Median DFS of the TS positive group was 2.37 years [95%CI: 0.92-3.82] while in the TS negative group median DFS was 1.14 years [95% CI: 0.37 – 1.90] (log-rank test: p=0.10) (Figure 6-2B).

Predictive Utility of TS staining

The TS group was also analyzed to determine whether it demonstrated predictive properties in regards to the differential effects of (neo)adjuvant chemotherapy. The TS positive group was divided into those that received (neo)adjuvant chemotherapy (n = 26) and those that did not (n = 21). The TS negative group was similarly divided into those that received (neo)adjuvant chemotherapy (n = 27) and those that did not (n = 25) [Table 6-2].

Supplemental chemotherapy did not improve OS or DFS in patients regardless of whether they were determined to be TS negative or TS positive, although trends toward improved OS and DFS were demonstrated in patients who were determined to be TS positive and who had received supplemental chemotherapy [Table 6-3].

THYMIDINE PHOSPHORYLASE

Eighty-nine tumors were classified as being TP negative and 10 were classified as TP positive. Once again, gender, age, (neo)adjuvant chemotherapy administration, and CRS were fairly evenly distributed [Table 6-1].

Median OS for the TP positive group was 4.35 years [95% CI: 0.89 – 7.81] which was not significantly different from that of the TP negative group of 4.05 years [95% CI: 2.78 – 5.33] (log-rank: p=0.66) (Figure 6-3A). A trend towards improved DFS for TP negative patients was noted. Median DFS in the TP negative group was 1.52 years [95% CI: 0.71 – 2.34] while in the TP positive group, DFS was 0.50 [95% CI: 0 – 2.23] (log-rank test: p = 0.24) (Figure 6-3B).

THYMIDYLATE SYNTHASE AND THYMIDINE PHOSPHORYLASE

Tumors were segregated based upon both their TS and TP staining. Tumors staining negative for both enzymes (TS/TP negative; n=49) were compared to those that stained positive for either or both enzyme (TS/TP positive; n=50). The two groups were evenly matched when looking at gender, age, (neo)adjuvant chemotherapy administration, and CRS [Table 6-1].

No significant difference was noted in median OS of the TS/TP negative group (4.05 years [95% CI: 2.49 – 5.62]) when compared with the median OS of the TS/TP positive group (4.51 [95% CI: 2.75 – 6.28] log-rank test: p = 0.47) (Figure 6-4A). Median DFS was again similar in the two groups; however a trend towards improved DFS was noted in the TS/TP positive group. Median DFS for the TS/TP negative group was 1.20 years [95% CI: 0.49 – 1.92] while in the TS/TP positive group median DFS was 1.96 years [95% CI: 0.56 – 3.36] (log-rank test: p = 0.17) (Figure 6-4B).

Predictive Utility of TS/TP staining

The TS/TP groups were analyzed to determine whether they demonstrated predictive properties in regards to the differential effects of (neo)adjuvant chemotherapy. Twenty-nine patients in the TS/TP positive group received (neo)adjuvant chemotherapy and 21 did not. The TS/TP negative group was similarly divided into those that received (neo)adjuvant chemotherapy (n = 24) and those that did not (n = 25) [Table 6-3].

Like the results of TS and TP individually, this combined analysis failed to demonstrate any difference in OS or DFS. Any suggestions of a trend towards improved survival due to the addition of supplemental chemotherapy in the TS positive group, was lost when the two markers were combined. [Table 6-4] (Figures 6-5 and 6-6).

Table 6-1. Patient Characteristics

	TS		TP		TS/TP	
	Negative	Positive	Negative	Positive	Negative	Positive
Gender, n (%)						
Male	36 (69%)	31 (66%)	60 (67%)	7 (70%)	34 (69%)	33 (66%)
Female	16 (34%)	16 (34%)	29 (33%)	3 (30%)	15 (31%)	17 (34%)
Age, years						
Median	64.6	60.5	63.0	63.6	64.6	60.4
Range	35.1-84.5	41.0-83.7	35.1-84.5	49.6-76.4	35.1-84.5	41.0-83.7
(Neo)adjuvant chemotherapy received, n (%)						
No	25 (48%)	21 (60%)	42 (47%)	4 (40%)	25 (51%)	21 (42%)
Yes	27 (52%)	26 (55%)	47 (53%)	6 (60%)	24 (49%)	29 (58%)
CRS, n (%)						
Low	31 (60%)	28 (60%)	51 (57%)	8 (80%)	29 (59%)	30 (60%)
High	21 (40%)	19 (40%)	38 (43%)	2 (20%)	20 (41%)	20 (40%)

The CRS is based on five factors: node positive primary, time to metastasis < 1 year, > 1 metastasis, largest metastasis > 5cm, CEA > 200 ng/ml. A Low score is determined by the presence of 0-2 factors, and high by the presence of 3-5 factors.

Figure 6-2A. Overall Survival (OS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Thymidylate Synthase (TS) classification (TS negative and TS positive). There was no statistically significant difference between TS negative (solid line) and TS positive (broken line) p=0.44.

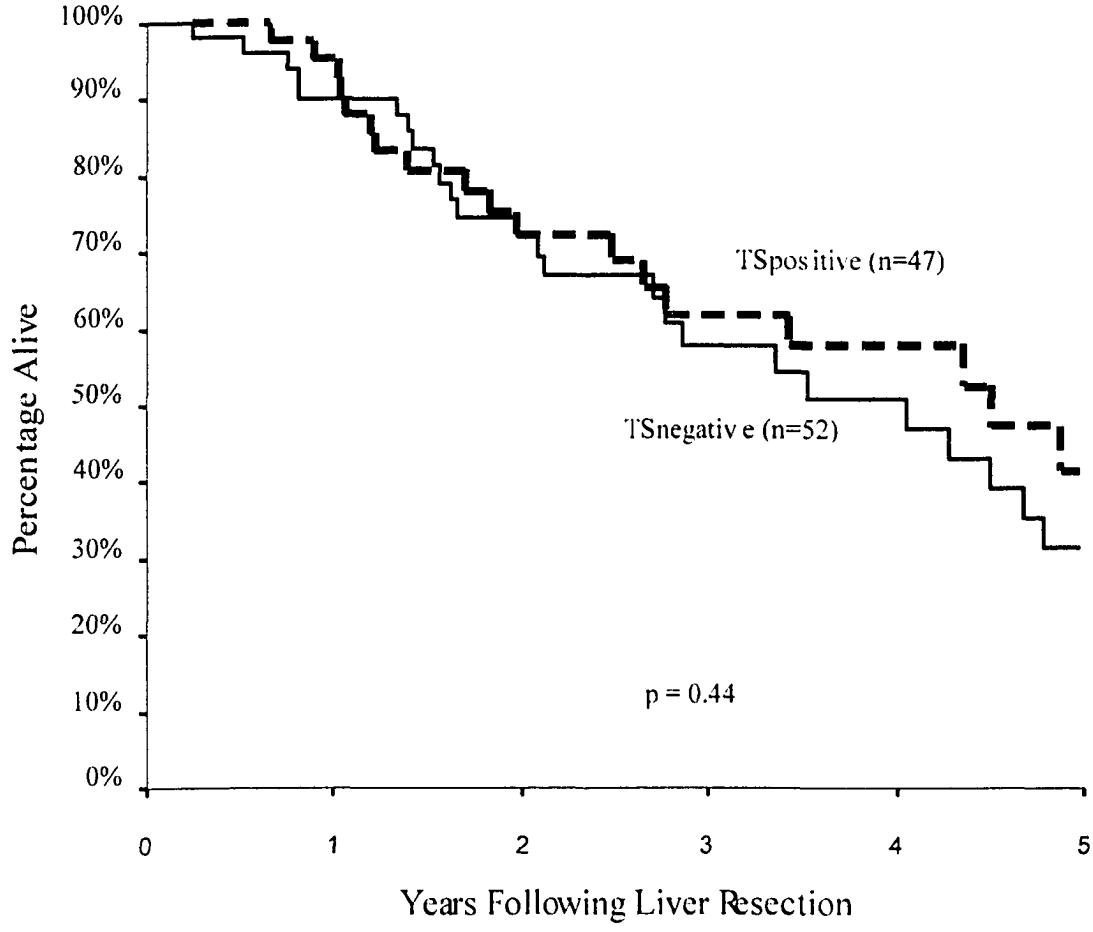


Figure 6-2B. Disease-Free Survival (DFS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Thymidylate Synthase (TS) classification (TS negative and TS positive). There was no statistically significant difference between TS negative (solid line) and TS positive (broken line) p=0.10.

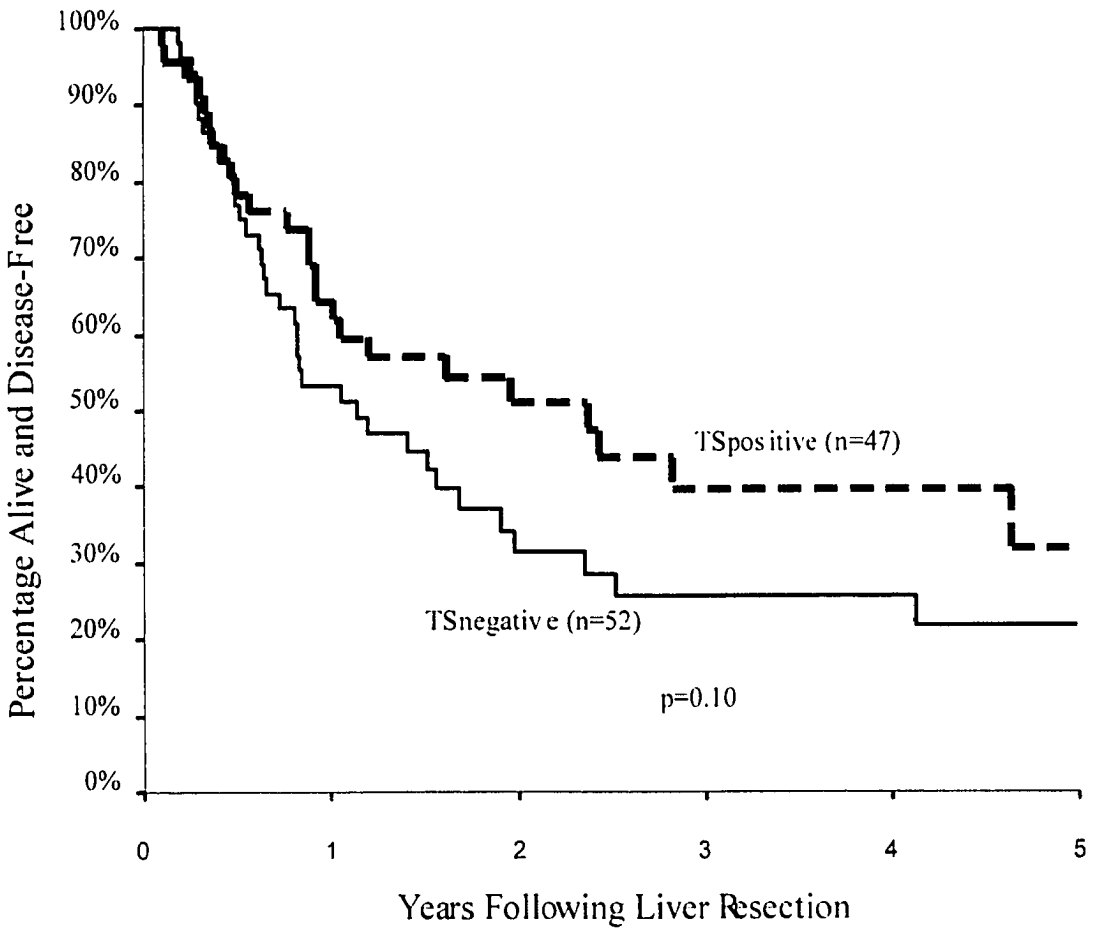


Table 6-2. Patient Characteristics by TS and (Neo)adjuvant chemotherapy.

	TS Negative		TS Positive	
Neo(adjuvant) chemotherapy	Yes	No	Yes	No
Gender, n (%)				
Male	19 (70%)	17 (68%)	19 (73%)	12 (57%)
Female	8 (30%)	8 (32%)	8 (27%)	9 (43%)
Age, years				
Median	61.2	66.8	57.6	68.8
Range	40.0-74.3	35.1-84.5	41.0-75.8	41.7-83.7
CRS, n (%)				
Low	14 (52%)	17 (68%)	13 (50%)	15 (71%)
High	13 (48%)	8 (32%)	13 (50%)	6 (29%)

Table 6-3. Patient Characteristics by TS/TP and (Neo)adjuvant chemotherapy.

	TS/TP Negative		TS/TP Positive	
Neo(adjuvant) chemotherapy	Yes	No	Yes	No
Gender, n (%)				
Male	17 (71%)	17 (68%)	21 (72%)	12 (57%)
Female	7 (29%)	8 (32%)	8 (28%)	9 (43%)
Age, years				
Median	61.6	66.8	57.8	68.8
Range	40.0-74.3	35.1-84.5	41.0-75.8	41.7-83.7
CRS, n (%)				
Low	12 (50%)	17 (68%)	15 (52%)	15 (71%)
High	12 (50%)	8 (32%)	14 (48%)	6 (29%)

Figure 6-3A. Overall Survival (OS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Thymidine Phosphoralase (TP) classification (TP negative and TP positive). There was no statistically significant difference between TP negative (solid line) and TP positive (broken line) p=0.66.

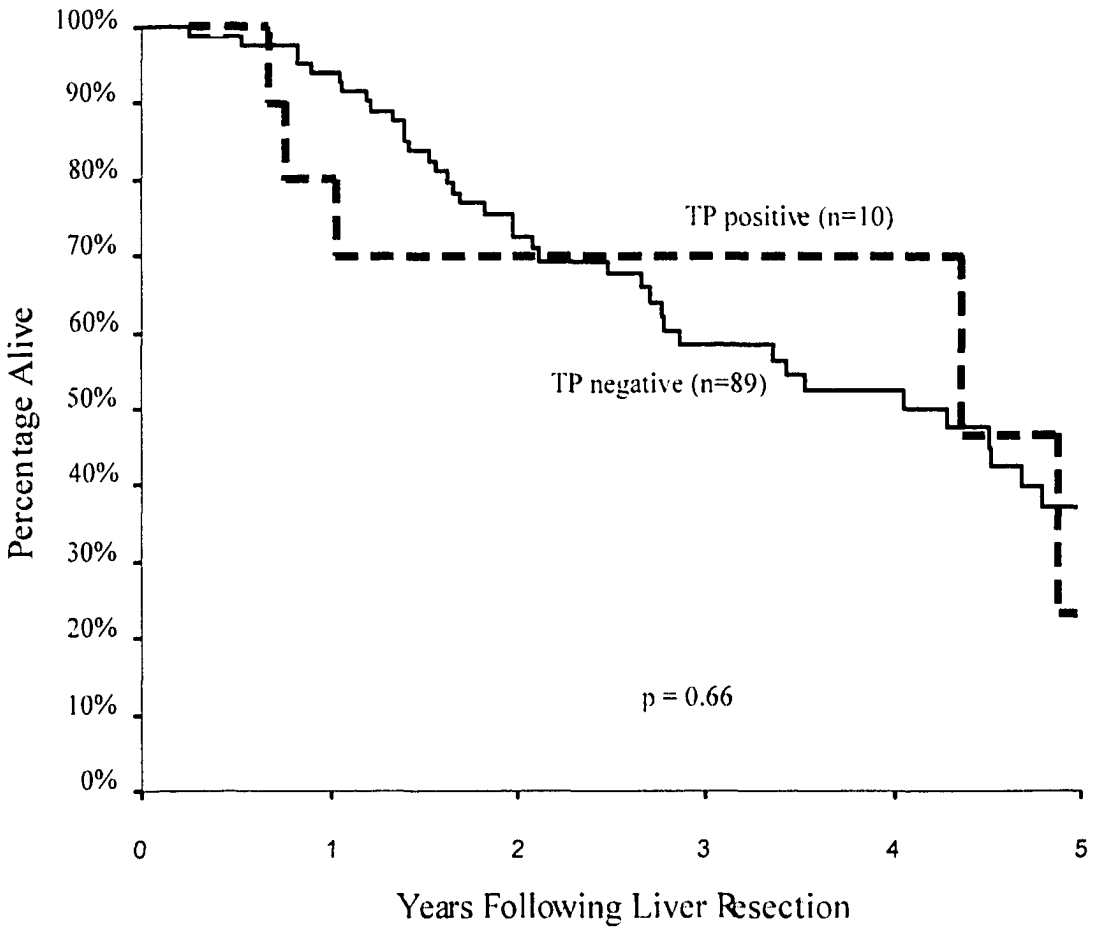


Figure 6-3B. Disease-Free Survival (DFS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by Thymidine Phosphorylase (TP) classification (TP negative and TP positive). There was no statistically significant difference between TP negative (solid line) and TP positive (broken line) p=0.24.

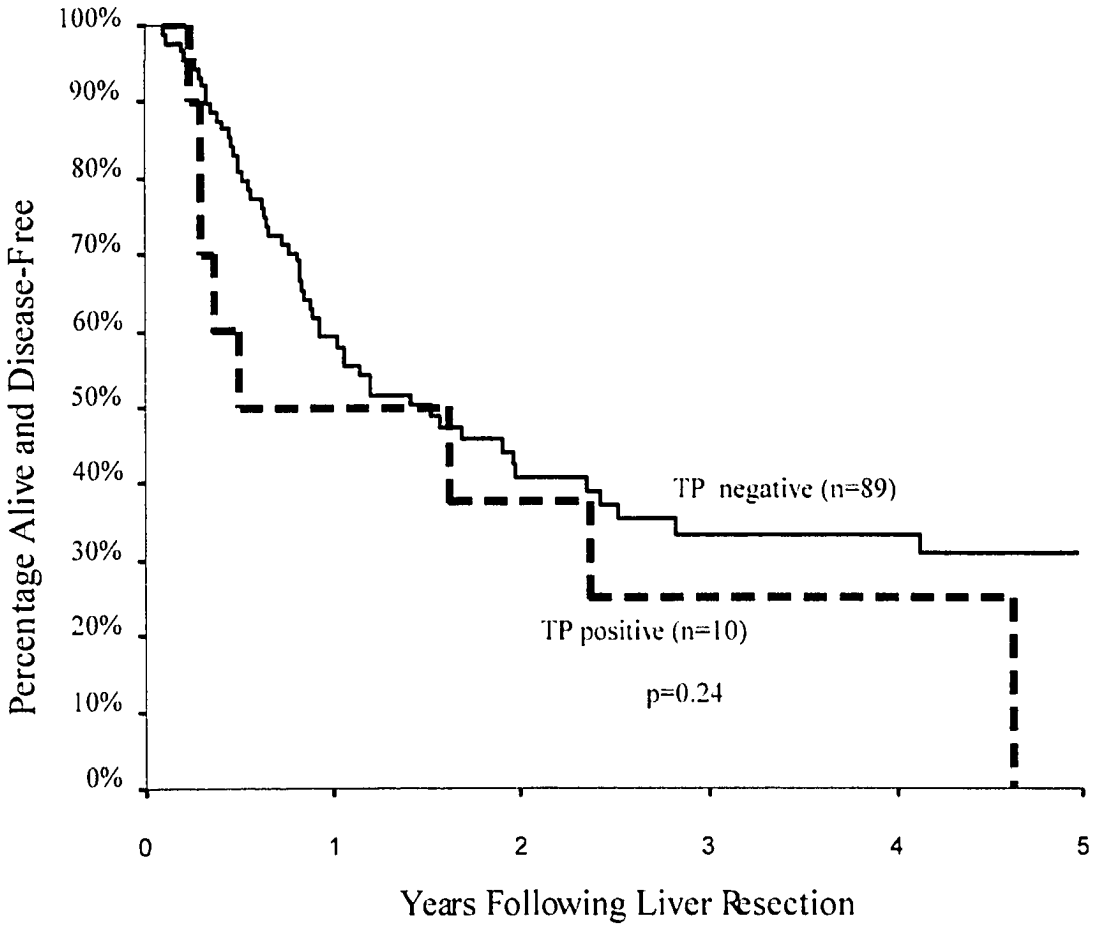


Figure 6-4A. Overall Survival (OS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by TS/TP combination (TS/TP negative and TS/TP positive). There was no statistically significant difference between TS/TP negative (solid line) and TS/TP positive (broken line) p=0.47.

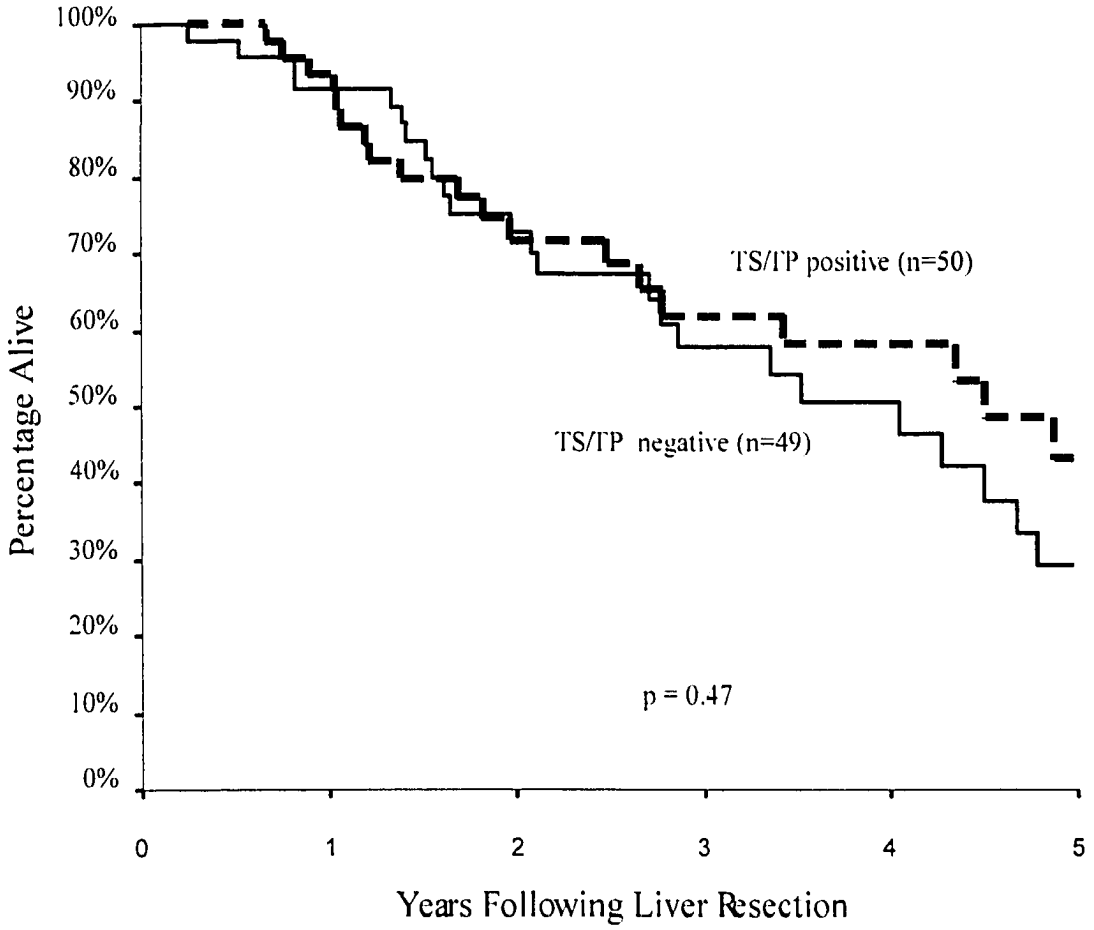


Figure 6-4B. Disease-Free Survival (DFS) Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by TS/TP classification (TS/TP negative and TS/TP positive). There was no statistically significant difference between TS/TP negative (solid line) and TS/TP positive (broken line) p=0.17.

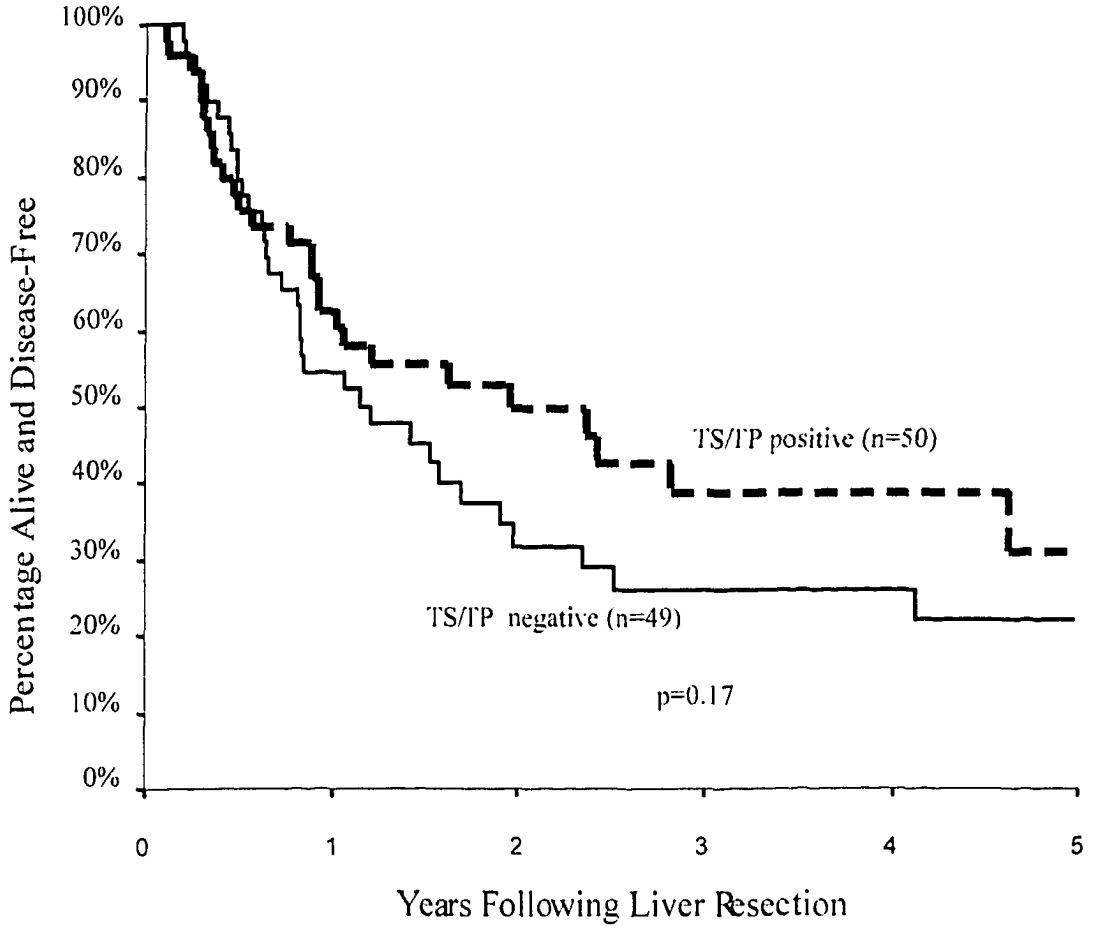


Table 6-4. TS staining and effect of chemotherapy

	Neo(adjuvant chemotherapy)				
	No		Yes		
	Median, years	(95% CI)	Median, years	(95% CI)	Log-rank test p-value
TS Positive	(n=21)		(n=26)		
OS	3.43	(0.97 – 5.89)	5.95	(3.60 – 8.30)	0.49
DFS	1.20	(0.00 – 2.72)	2.82	(1.06 – 4.58)	0.28
TS Negative	(n=25)		(n=27)		
OS	3.36	(1.43 – 5.29)	4.28	(2.38 – 6.18)	0.73
DFS	0.84	(0.30 – 1.38)	1.42	(0.42 – 2.42)	0.91

Figure 6-5A. Overall Survival (OS) in TS/TP positive Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of (Neo)adjuvant Chemotherapy (No Chemotherapy versus (Neo)adjuvant Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group (p=0.67).

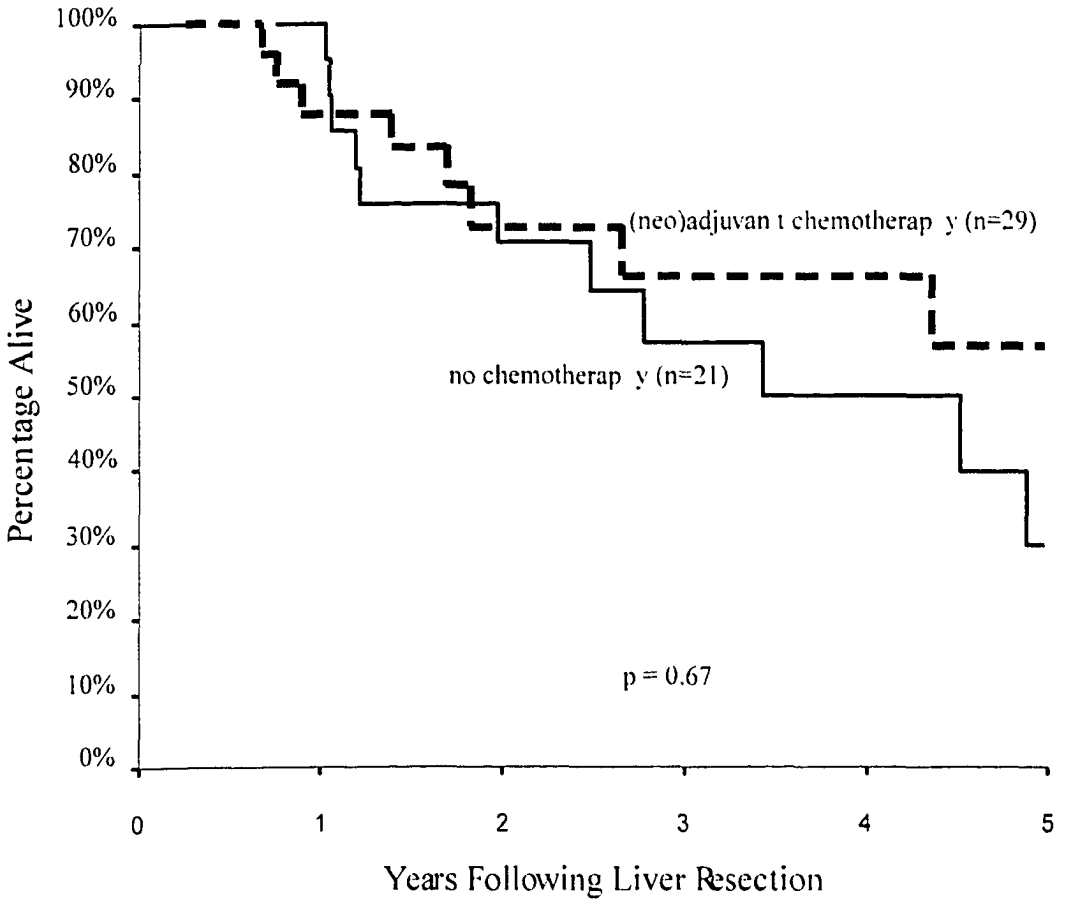


Figure 6-5B. Disease-Free Survival in TS/TP positive Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of (Neo)adjuvant Chemotherapy (No Chemotherapy versus (Neo)adjuvant Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group (p=0.40).

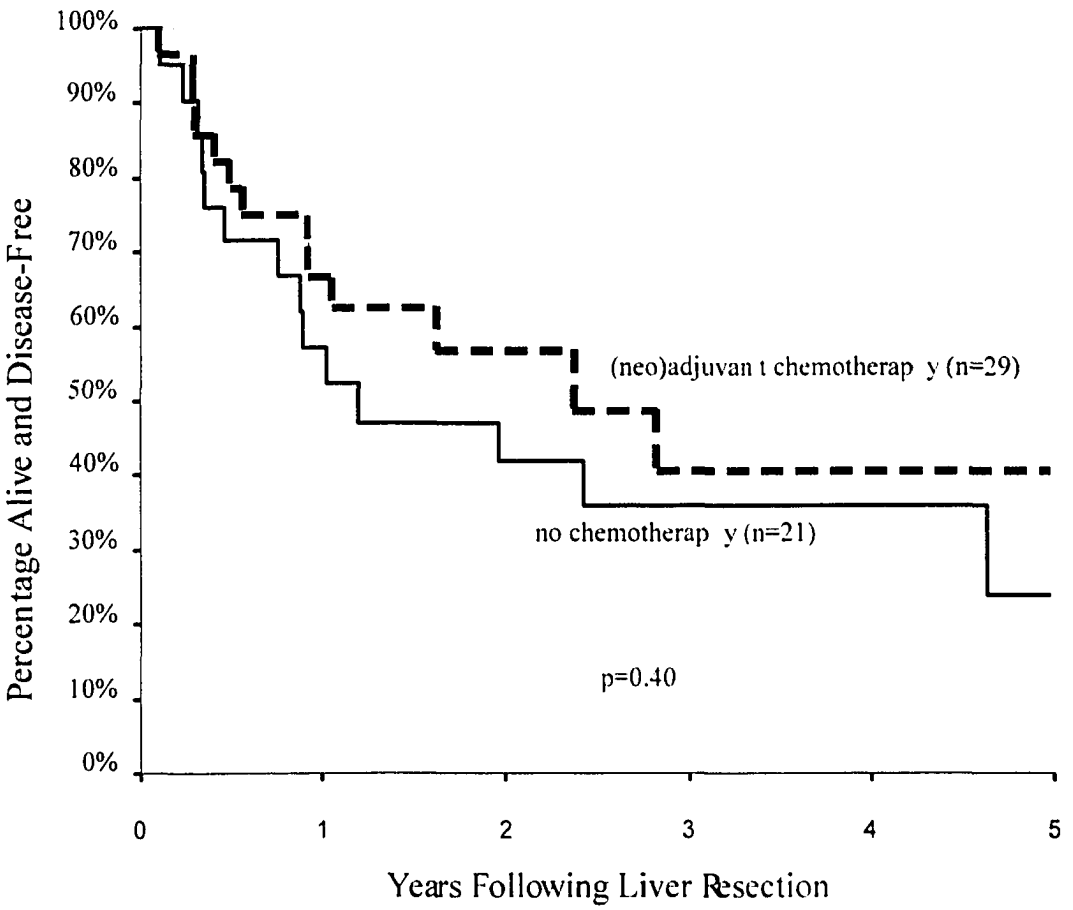


Figure 6-6A. Overall Survival (OS) in TS/TP negative Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of (Neo)adjuvant Chemotherapy (No Chemotherapy versus (Neo)adjuvant Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group (p=0.73).

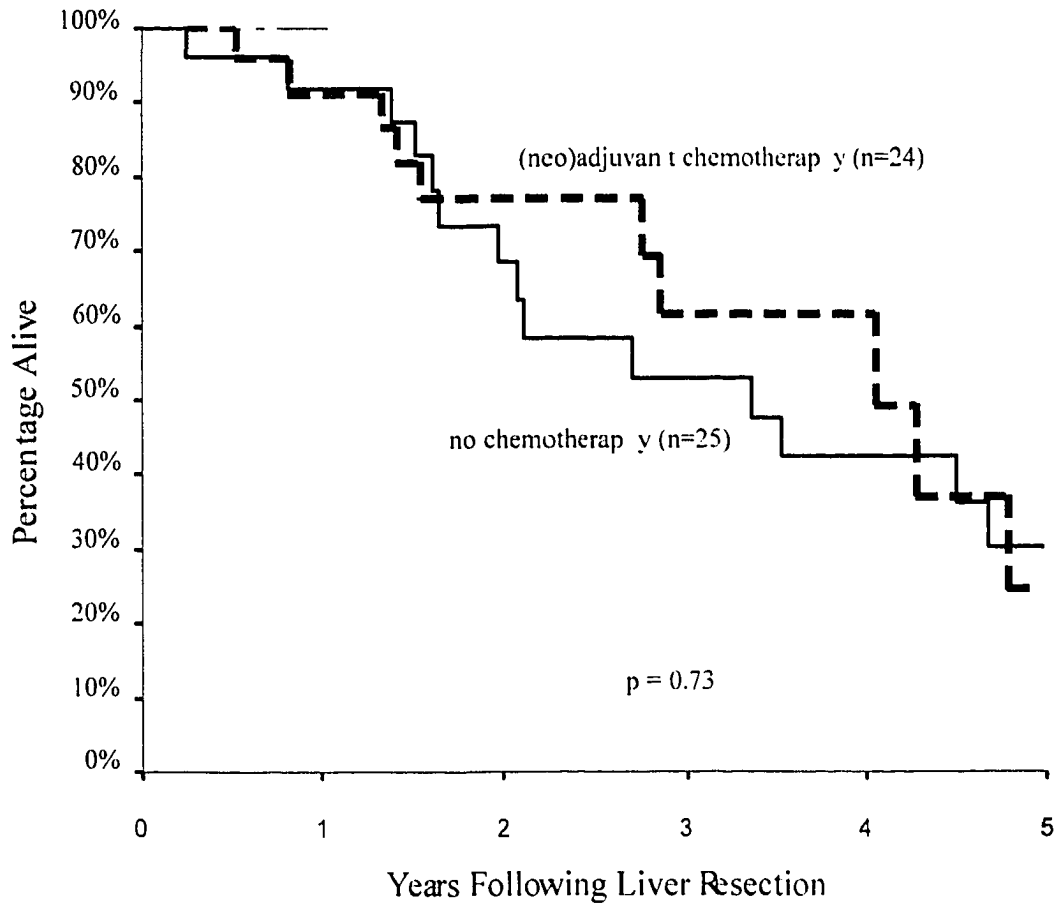
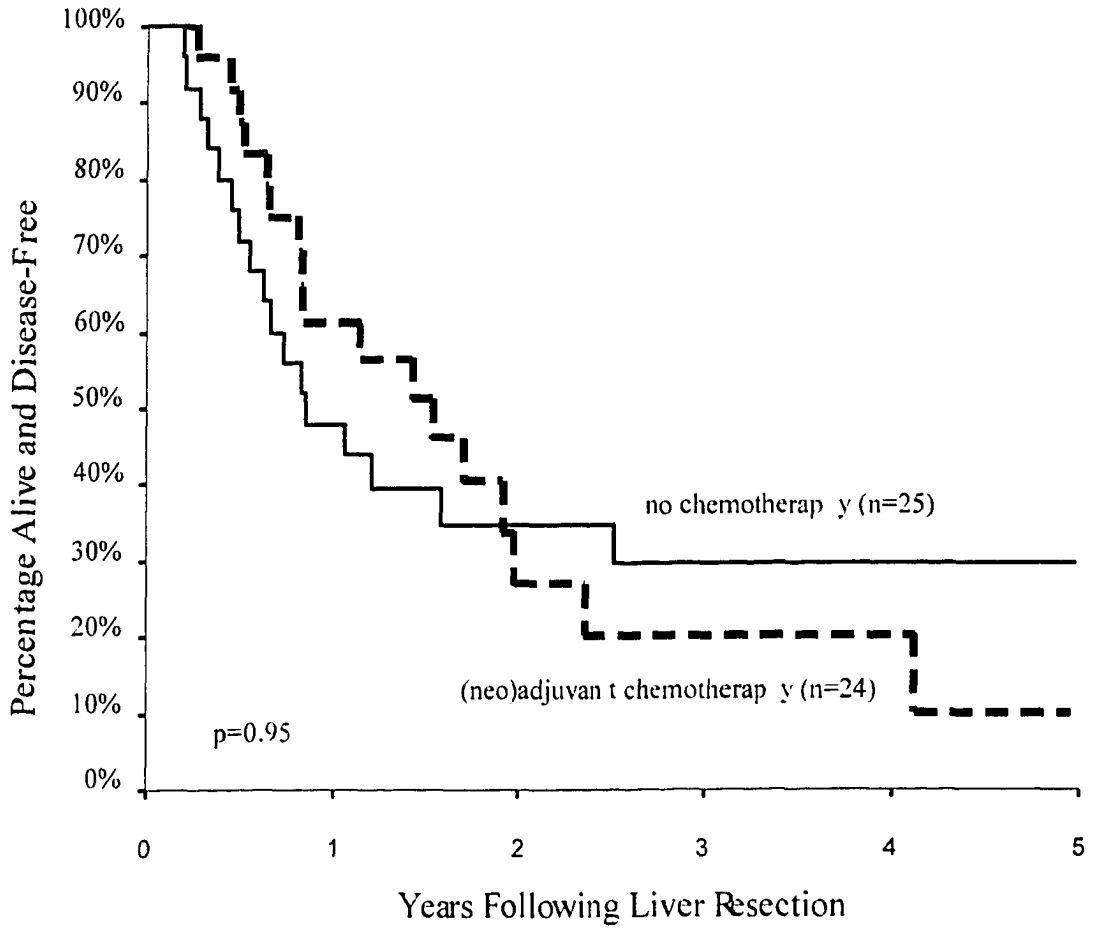


Figure 6-6B. Disease-Free Survival in TS/TP negative Patients Following Partial Hepatectomy for Colorectal Metastases in Years Following Liver Resection After Stratification by the Administration of (Neo)adjuvant Chemotherapy (No Chemotherapy versus (Neo)adjuvant Chemotherapy). There was no noticeable benefit from the addition of supplemental chemotherapy in this group p=0.95).



DISCUSSION

TS is the enzyme that catalyzes the irreversible conversion of 2`deoxyuridine-5`-monophosphate (dUMP) to thymidylate (dTMP or 2`-deoxythymidine-5`-monophosphate). This pathway represents the sole *de novo* source of thymidylate. As thymidylate is used in the synthesis of DNA, halting *de novo* synthesis of thymidylate leads to the arrest of DNA synthesis. *In vitro* studies have confirmed that fluouropyrimidine resistance is associated with increased TS activity.[18,19] TS levels have been measured in both primary colorectal cancer as well as in metastatic disease, with numerous studies reporting relationships between both TS and survival as well as TS and response to chemotherapy.

TS was reported to be a significant prognostic marker in patients who have undergone resection of primary colorectal cancer. Specifically, elevated TS levels were associated with worse outcomes[20]. In addition, Edler et al (2002) noted that TS was an independent prognostic indicator, and that TS levels were correlated with response to fluoropyrimidine chemotherapy. Notably, high TS levels were associated with worse outcomes overall but may predict the potential for benefit from chemotherapy, while low TS levels were indicative of both decreased overall and disease-free survival in the setting of adjuvant chemotherapy[21]. Several others developed similar conclusions[22-25].

TS levels in the metastases can not be inferred from those measured in the primary tumor and therefore must be specifically measured[26-28]. Johnston et al noted the TS levels in the primary tumor of 219 patients with unresectable metastatic disease and commented on the lack of a correlation between TS levels and overall survival, summarizing that "primary colorectal tissue can not be used as a reliable surrogate for tissue from metastatic disease sites to predict response to 5–FU-based regimens"[29].

One of the explanations for the lack of correlation between primary and metastatic TS expression may be the influence of subsequent chemotherapy after resection of the primary lesion, but before the presentation of the metastases[30].

Several authors have recently concluded that TS expression in metastases is a significant prognostic indicator[26] Corsi et al demonstrated an inverse relationship between survival and TS staining in resected liver metastases[26]. A similar result was presented by Bathe et al, employing RT-PCR to examine TS gene-expression and determining that higher TS levels are associated with both treatment effectiveness as well as a poorer outcome[31]. Leichman at all replicated these findings, also using PCR technology[32]. Though not statistically significant, trends were demonstrated when examining disease-free survival.

Gonen et al demonstrated that TS remained a significant prognostic factor even when analyzed using multivariate techniques incorporating other well-known prognostic factors. It is possible that these results are somewhat magnified, however, as this study was performed using tissue collected from patients enrolled in a randomized-clinical trial comparing two different post-resection chemotherapy regimens. The authors state that this may lessen the impact of the clinical prognostic factors and subsequently the strength of the impact of TS[33]. Predating this study, Aschele et al (1999) concluded that TS was a strong enough predictor in recurrent, advanced, and metastatic disease to allow selection of patients who should and should not be treated with 5-FU based adjuvant chemotherapy[34]. Most recently this has been generalized to resected colorectal liver metastases with some concluding that TS levels are also predictive of response to chemotherapy[26,33,34].

Others have determined that there is no important relationship between TS levels in metastases and prognosis. Saw et al (2002) could not demonstrate any relationship between IHC determinations of TS, TP, or DCC in colorectal metastases and survival[14]. They also were unable to demonstrate this correlation using IHC results from the primary tumors[14].

Thymidine Phosphorylase (TP) is the enzyme which catalyzes the conversion of thymidine to thymine. TP expression has generally been found to be increased in tumors compared to neighboring benign tissue. This has therapeutic advantages, as TP is involved in the "activation" of traditional fluoropyrimidines and related compounds to cytotoxic agents[35-37]. This would suggest that elevated TP expression might be beneficial in the right chemotherapeutic setting. Capecitabine, a pro-drug, uses TP during its final transformation into a cytotoxic chemical theoretically allowing targeted killing of malignant cells that may contain elevated levels of TP. Unfortunately, it appears that the increased angiogenesis associated with elevated TP expression is correlated with a worse prognosis[38]. This is likely as PD-EDF has been postulated to be involved in cell motility as well as angiogenesis, crucial steps in the growth and spread of malignancy[39].

Metzger et al, using 38 resected colorectal specimens, demonstrated that TP expression level was also associated with responsiveness to 5-FU. Specifically, high TP expression was associated with non-response to infusional 5-FU chemotherapy in patients with metastatic or locally recurrent colorectal cancer[13]. This prognostic value has recently been questioned by Ikeguchi et al (2002) as they failed to demonstrate a relationship between TP and response to chemotherapy in both resected primary

colorectal cancer as well as in advanced disease[40]. The addition of DPD did not increase the effectiveness of TP as a prognostic marker[40].

Several methods to determine TS or TP presence exist, including PCR which allows the quantification of genetic expression[41]. Assays including FdUMP binding assay and TS catalytic assays as well as TP activity assays have also been used[42]. Globally however, immunohistochemistry has been one of the more common methods used to detect either enzyme. Immunohistochemistry allows the relatively inexpensive analysis of archived specimens. Fortunately, immunohistochemical TS expression has been shown to adequately reflect TS activity[43]. Correlation between TS mRNA expression and immunohistochemical determination of enzyme expression have also been demonstrated[44]. Thereby, IHC can confidently be used to identify TS presence in archived specimens.

Our group set out to clarify the roles of TS and TP individually and in combination in determining the prognosis of patients who have undergone potentially curative resections of colorectal liver metastases.

The archived specimens from 99 patients who underwent potentially curative resection of colorectal liver metastases were used. IHC was used to determine the extent of TS and TP in each patient's lesions. A simple computational model was then used to classify patients in to TS (or TP) positive or negative categories. Comparisons in OS and DFS were then made between the dichotomous categories of each enzyme. We noted no significant differences between positive or negative categorization of TS or TP or combination and both OS and DFS.. This suggests that individually, neither enzyme is of prognostic value. The same lack of prognostic value was found when we categorized

patients as TS/TP positive or TS/TP negative based on the combined expression of both enzymes.

We also attempted to elucidate the role of elevated levels of TS alone or in combination with TP in selecting patients for chemotherapy. Our results failed to show any correlation between TS / TP in combination and benefit from systemic 5-FU based chemotherapy. When investigating TS individually, even though no statistically significant difference was noted, there is a suggestion that systemic 5-FU based chemotherapy may differentially benefit patients with elevated TS expression in their metastases (Median OS 5.95 years in those with high TS expression who received chemotherapy versus 3.43 years in those with high TS expression who did not receive chemotherapy). We can not conclude that enzyme levels determined using IHC are predictive of improved survival in response to chemotherapy and therefore can not yet be used in the stratification or selection of patients.

It is noteworthy that these results support some of the previously mentioned works, but are contradictory to others. The observed discrepancies may be at least partially explained by the heterogeneity in the methods of IHC. More importantly, this heterogeneity may be directly explained by the lack of consistency in the methods used in the scoring of the results[33]. Some groups did not use an *a priori* established scoring system and instead chose the extreme level in their group of interest as a cut-off[12]. We attempted to accommodate this potential short-coming by performing sensitivity analyses using several of the other scoring methods[11,14,21,24,26,33,45]. We noted that the method chosen of scoring the IHC did not change our results. Our results did not differwhen analyzed according to the alternative scoring systems.

Also, many of the studies, including ours, use immunohistochemistry to quantify TS and or TP levels within tissue. When performed properly, IHC is fairly reliable, however, it is a difficult procedure wrought with multiple potential sources of error. Even though few labs have shown reliable and valid results using consistent monoclonal antibodies, the majority of the cited works took place in labs with tremendous experience using these techniques.

A further explanation of these discrepancies may be found by extrapolating from results found by Fukunari et al (2003). They described the existence of genetic heterogeneity within individual primary colorectal tumors[46]. It may well be that metastases also harbor within-tumor differences.

Finally, this is a predictive analysis using retrospectively collected tissue, and thereby is vulnerable to the potentially low power associated with it. Unfortunately, short of a large prospective multi-institution study, there was little choice in the methodologies used. Even so, it is unlikely that a larger sample would have altered the results significantly.

We conclude that TS and TP, either individually or in combination, are not predictive of survival or response to chemotherapy. However, as more chemotherapeutic options become available, the determination of which regimens will work best for an individual patient becomes increasingly vital. Future research can use microarrays to direct research into which proteins should be studied with immunohistochemistry. This may then lead to the discovery of protein markers which will aid in prognostication as well as in the selection of supplemental chemotherapy for patients with resectable colorectal liver metastases.

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CHAPTER 7

Supplemental Analyses

The examination into the role of chemotherapy in patients who undergo potentially curative liver resection for colorectal metastases was a multi-step process. The addition of chemotherapy was first studied individually. These analyses demonstrated that the addition of chemotherapy did not confer a survival benefit to patients experiencing hepatectomies. In fact, the only demonstrable benefit in favor of supplemental chemotherapy was in 1-year disease-free survival. When more closely examining the Kaplan-Meier graphs, there were however, noticeable trends suggesting that there may still be an effect of chemotherapy, if not in the entire group, possibly in a subgroup.

After first replicating the utility of the Clinical Risk Score in determining prognosis, it was demonstrated that this score could be used to identify patients who may have benefited (expressed in terms of improved survival and / or disease-free survival) from the addition of chemotherapy. From this analysis it was concluded that patients with lesser disease, as identified by a CRS between zero and two, had significant survival benefits due to supplemental chemotherapy. Conversely, those identified as having more severe disease (CRS between three and five) did not demonstrate improvements in survival attributable to chemotherapy.

Finally, using a scoring system based on the immunohistochemically determined presence of TS and TP, we were unable to demonstrate a significant predicting or prognosticating effect of either enzyme individually or in combination.

In an effort to summarize this process the individual components; expressed as three variables, were evaluated for inclusion in a multivariate model of overall survival prognosis. The initial results regarding supplemental chemotherapy, the clinical risk score, and the two enzymatic markers were reviewed. Based on the results of the main

analyses (summarized in chapter 6), it was determined that including the enzymatic markers in a multivariate model would be both problematic and unlikely to be important. The lack of trends toward discrepant survival between any of the enzyme based categorizations suggests that their inclusion would not likely be informative. In addition, even though this is a large cohort of patients in terms of the published international literature, it is still relatively small and including terms that are very likely to be non-significant would be at the expense of power to detect the effects of the variables that are more likely to be important.

The remaining two variables; supplemental chemotherapy (presence versus absence) and clinical risk score (low versus high) were considered using both univariate and multivariate proportional hazards models. Using supplemental chemotherapy to construct a univariate main-effects model (Table 7-1) resulted in a risk ratio of 0.837 [95% CI: 0.465 - 1.507] (p=0.55). This supports the previously expressed results that the receipt of supplemental chemotherapy can not be used to differentiate between groups with different prognosis in terms of overall survival. When clinical risk score (high versus low) was similarly used to construct a model (Table 7-1), the results were also as expected from the previous analyses. A patient clinically determined to be in the high score group (CRS 3-5) was at twice the risk of death when compared to a patient determined to be in the low score group (CRS 0-2): HR 1.91 [95% CI: 1.048 – 3.481] (p=0.035).

These two variables were then forced into a main effects model using the multivariate proportional hazards model (Table 7-1). When the chemotherapy variable was added to a model already containing CRS as a variable it did not become a significant determinant of overall survival as demonstrated by a hazard ratio of 0.698

[95% CI: 0.379 – 1.285] (p=0.25). Interestingly, censoring the data at four years (Table 7-2) resulted in a strong trend towards including chemotherapy (HR 0.525 [95% CI: 0.262 – 1.051] (p=0.069)). It is possible that the reason for the appearance of this trend at 4-years while not present in overall survival is multi-factorial. Firstly, chemotherapy is given to alter the natural history of the disease. This history suggests that most deaths will occur in the first 4-5 years. Therefore the mortality avoided should be during this time frame. In addition, as one looks at points further in time from the initial resection, it is done at the expense of the power to determine a difference. This is reinforced when realizing that the number of subjects at risk steadily drops when moving away from time t=0.

When originally analyzing CRS as a variable, the Kaplan-Meier method was used to determine the effect of chemotherapy in the low-score and high-score groups individually. The results were somewhat surprising. There was a significant demonstrable benefit attributable to receiving chemotherapy in the low-score group. This benefit was non-existent in the high-score group. This discrepancy between the two CRS groups in regards to the benefit of chemotherapy suggests that there is an interaction between the CRS variable and the chemotherapy variable. Therefore, a model was created including both CRS and chemotherapy as well as an interaction term (Table 7-3). Unexpectedly, this term was non-significant (HR = 1.235 [95% CI: 0.351 – 4.342] (p=0.74)), thereby suggesting no interaction. To further explore these findings a multivariate proportional hazards model was fit after creating a set of contrast variables that would allow comparison of each of the four individual situations (low CRS / no chemotherapy, low CRS / chemotherapy, high CRS / no chemotherapy, high CRS / no chemotherapy) (Table 7-4). The low CRS / chemotherapy group was chosen as the

standard as this group had previously been demonstrated to have an enhanced survival when compared to the other three groups. Using this method, computation of the interaction term after the individual hazard ratios were determined does not allow one to determine significance, nor a confidence interval. It did, however; reinforce the results of the multivariate Cox regression that included the interaction term as the resultant terms were similar.

It is important to reconcile these differences: namely that the initial analyses illustrate a significant interaction between the CRS and chemotherapy terms, while the multivariate proportional hazards models explicitly did not. This important difference can be reconciled by examining the use of the proportional hazards models. Although this is inherently a robust test, there are certain assumptions that must be met to allow implementation of the test as well as confidence in the resultant hazard ratios. One of the three primary assumptions states that the relative risk between the hazard rate of two subjects should be constant over time. This is not the case in this cohort. In fact, when graphically depicted, there are multiple points where the survival curves created using Cox regression analysis intersect (Figures 7-1 and 7-2). Alternatively stated, there appears to be an interaction between time and the covariates. This is enough to invalidate the conclusion derived from the proportional hazards models that there is no interaction effect. Thus the conclusions reached using the simpler, yet more appropriate, Kaplan-Meier methods that there is an interaction between CRS and Chemotherapy are retained. Specifically, supplemental chemotherapy has no significant effect on survival in patients assigned to the high-score category, while those in the low-score category receive benefit from the addition of chemotherapy as measured by an improved survival.

Table 7-1. Association between supplementary chemotherapy and clinical risk score, and overall survival. Unadjusted and adjusted hazard rate ratios and 95% Confidence Intervals

Factor	Unadjusted HRR* (95% CI)	p-value	Adjusted HRR† (95% CI)	p-value
Supplemental Chemotherapy				
No	1.00		1.00	
Yes	0.84 (0.47 – 1.51)	0.55	0.70 (0.38 – 1.29)	0.25
Clinical Risk Score				
Low risk	1.00		1.00	
High risk	1.91 (1.05 – 3.48)	0.04	2.11 (1.13 – 3.94)	0.02

*HRR refers to hazard rate ratios. CI refers to confidence intervals.
† Hazard rate ratios are adjusted for the other factor in the model.

Table 7-2. Association between supplementary chemotherapy and clinical risk score, and 4-year survival. Unadjusted and adjusted hazard rate ratios and 95% Confidence Intervals

Factor	Unadjusted HRR (95% CI)	p-value	Adjusted HRR* (95% CI)	p-value
Supplemental Chemotherapy				
No	1.00		1.00	
Yes	0.66 (0.33 – 1.30)	0.23	0.53 (0.26 – 1.05)	0.07
Clinical Risk Score				
Low risk	1.00		1.00	
High risk	2.56 (1.31 – 4.98)	0.006	2.96 (1.49 – 5.87)	0.002

*Hazard rate ratios are adjusted for the other factor in the model.

Table 7-3. Association between supplementary chemotherapy, clinical risk score, and the possibility of interaction and overall survival or 4-year survival. Adjusted hazard rate ratios and 95% Confidence Intervals

	Overall Survival		4-Year Survival	
Factor	Adjusted HRR* (95% CI)	p-value	Adjusted HRR* (95% CI)	p-value
Supplemental Chemotherapy				
No	1.00		1.00	
Yes	0.64 (0.28 – 1.46)	0.29	0.28 (0.08 – 0.97)	0.04
Clinical Risk Score				
Low risk	1.00		1.00	
High risk	1.90 (0.784 – 4.62)	0.16	2.01 (0.81 – 5.00)	0.14
Interaction Term	1.24 (0.35 – 4.34)	0.74	2.91 (0.61 – 13.96)	0.18

*Hazard rate ratios are adjusted for the other factor in the model.

Table 7-4. Multivariate main effects model using combination variables and overall survival or 4-year survival. Adjusted hazard rate ratios and 95% Confidence Intervals

Factor		Overall Survival		4-Year Survival	
		Adjusted HRR* (95% CI)	p-value	Adjusted HRR* (95% CI)	p-value
Clinical Risk Score	Supplemental Chemotherapy				
Low risk	Yes	1.00		1.00	
Low risk	No	1.57 (0.68 – 3.61)	0.29	3.60 (1.04 – 12.54)	0.044
High risk	Yes	2.35 (0.96 – 5.78)	0.062	5.84 (1.63 – 20.96)	0.007
High risk	No	2.99 (1.08 – 8.31)	0.035	7.23 (1.86 – 28.04)	0.004

*Hazard rate ratios are adjusted for the other factor in the model.

Figure 7-1. Overall Survival of High-CRS Group: First 2 years - Illustrating the Non-Proportionality of the Survival Curves

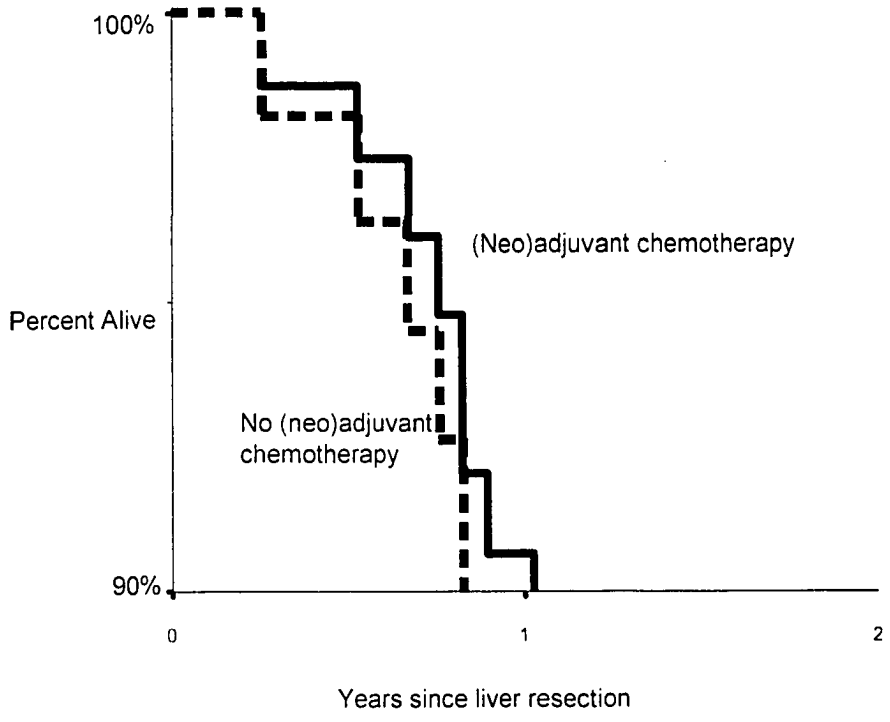
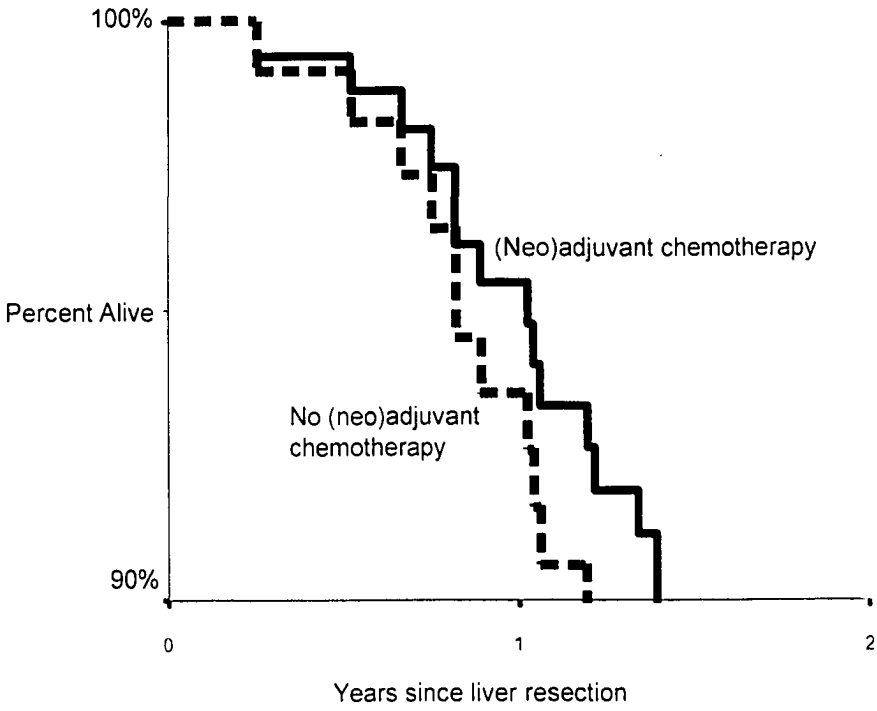


Figure 7-2: Overall Survival of Low-CRS Group: First 2 years - Illustrating the Non-Proportionality of the Survival Curves.



CHAPTER 8

Conclusion

Surgery is currently the only potentially curative option in patients with resectable colorectal liver metastases. Even though it has mostly become standard-of-care in North America, the addition of supplemental chemotherapy has yet to be experimentally justified as there are no randomized and controlled trials that conclusively support its role.

Although this thesis does not demonstrate that there is a statistically significant increase in overall and disease-free survival in patients which is attributable to the addition of supplemental chemotherapy, it does illustrate a trend towards increased survival when comparing all those that received such chemotherapy against those that did not. These results add to the growing body of literature that supports the use of supplemental chemotherapy in this population.

We believed that a prognostic scoring system such as the Clinical Risk Score may allow the selection of patients that are most likely to benefit from supplemental chemotherapy. In testing this hypothesis, we first demonstrated the generalizeability of the CRS by reproducing its results in our novel population. These findings will also add to the validity of this scoring system. Our next step involved determining whether this system had any utility in identifying patients who are most likely to benefit from supplemental chemotherapy. We demonstrated that those identified as having low Clinical Risk Scores achieved prolonged overall and disease-free survival attributable to the addition of supplemental chemotherapy. Somewhat surprisingly, we also established that those with high Clinical Risk Scores did not significantly benefit from the addition of supplemental chemotherapy.

Finally we aimed to elucidate a possible biologic basis for the differences in response to chemotherapy. The extensive literature pertaining to primary colorectal

disease and the emerging literature regarding colorectal liver metastases suggested that thymidylate synthase (TS), an enzyme involved in the de novo synthesis of pyrimidines; and to a lesser extent thymidine phosphorylase (TP), an enzyme involved in the activation of the most common chemotherapeutic used in this patient population; may both be clinically applicable most likely to allow the prediction of benefit from supplemental chemotherapy. Our results contradict the current literature and suggest that neither enzyme individually or in combination can predict overall or disease-free survival nor benefit from supplemental chemotherapy. Admittedly, even though they appeared to be among the most promising, the markers chosen are but two of many possible, and there may still be a single (or combination of two or more) biological marker(s) that predicts response to chemotherapy. Moreover, these results should be confirmed by correlating enzyme levels with genomic expression.

It is likely that future research into biological markers will be aided by employing DNA or RNA microarrays to provide stronger pre-trial indicators of which genes or proteins to pursue.

The results of this thesis may be used in several ways. We encourage the continued use of chemotherapy in addition to resection of colorectal liver metastases. We recommend however that the approach towards administration should be changed somewhat. Firstly, all patients should be stratified pre-operatively as either high-score or low-score according to the Clinical Risk Score. Secondly, there is evidence that those that are low-score should continue to receive standard supplemental 5-fu based chemotherapy. Those that are high-score however may not benefit from this standard chemotherapy, but could still possibly benefit from different chemotherapeutic regimens.

The possible benefits from these regimens should be investigated further or preferably in the setting of a clinical trial.

Our results should also change the construction of clinical trials regarding this population of patients. As we have demonstrated that not only do high and low score patients have different prognoses, they also tend to respond to chemotherapy differently; we feel that it is important that future trials should include a pre-randomization stratification of patients based on their clinical risk score.