Clinical and pathological predictors of survival for stage II and III colon cancer patients treated with or without chemotherapy: a population-based study

Submitted for the degree of Doctor of Philosophy at the University of Western Australia (2007)

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Abstract

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Aims

Using a population-based cohort of colorectal cancer (CRC), the major aims of this study were to:

1. Identify clinico-pathological markers that can be used to define a subset of stage II colon cancer patients with excellent prognosis and who therefore do not require referral for adjuvant chemotherapy

2. Investigate whether there is a survival benefit from the use of adjuvant chemotherapy in a population-based cohort of stage II colon cancer

3. Investigate stage III colon cancer patients for evidence of predictive markers for response to 5FU chemotherapy

4. Investigate CRC for age-related differences in clinico-pathological and molecular features

Hypotheses to be tested

1. A subset of good prognosis stage II colon cancers can be defined using routine pathological markers

2. Females colon cancer patients gain more survival advantage from 5FU chemotherapy than males

3. Tumours from young CRC patients have different molecular characteristics to those from older patients

4. The underlying molecular characteristics of tumour can impact upon the response to 5FU chemotherapy

Methods

The study cohort consisted of 5,971 cases diagnosed between 1993 and 2003 representing over 90% of the CRCs diagnosed in the state of Western Australia.

Results

The major findings of this translational research into colon cancer can be summarized as follows:

1. The morphological features of serosal and vascular invasion allow for prognostic stratification of stage II colon cancer into "good" and "poor" prognosis groups. Good prognosis patients can confidently be spared adjuvant chemotherapy, however greater effort should be directed towards ensuring that more poor prognosis patients are referred for chemotherapy.

2. Evidence was obtained for a survival advantage from the use of 5FU chemotherapy in stage II colon cancer patients, particularly women and those with poor prognosis features.

3. Completion of chemotherapy confers a survival advantage, whereas failure to complete regimens results in a survival disadvantage, when compared with patients treated by surgery alone. This was observed for both stage II and III colon cancers.

4. The factors associated with a survival advantage from the use of chemotherapy in stage III colon cancer were patient age >55years, female gender, perforation and lymphocytic response.

5. The age, site and sex distribution of CRCs revealed in this study provides further evidence for two major pathways of colorectal tumourigenesis. The CIN

pathway is predominant in distal tumours from young males, while the CIMP+ is predominant in proximal tumours from older females.

Conclusions

Population based studies are an important component of translational research. In part, they allow for auditing of disease management in a population. Guideline verification and analysis of the reasons for lack of adherence to guidelines is integral to improving the management of a disease. Laboratory-based research that is linked to clinical databases allows elucidation of the mechanisms of the disease and the response to treatments.

Publications arising from thesis

Morris M, Platell C, de Boer B, McCaul K and Iacopetta B: Population-based study of prognostic factors in stage II colonic cancer. *Br J Surg* 93: 866-871, 2006.

Morris M, Platell C, McCaul K, Millward M, van Hazel G, Bayliss E, Trotter J, Ransom D and Iacopetta B: Survival rates for stage II colon cancer patients treated with or without chemotherapy in a population-based setting. *Int J Colorectal Dis*. 2007 (in press)

Morris M, Platell C, Fritschi L and Iacopetta B: Failure to complete adjuvant chemotherapy is associated with adverse survival in stage III colon cancer patients. *Br J Cancer* 96:701-707, 2007.

Morris M, Platell C and Iacopetta B: A population-based study of age-related variation in clinicopathological features, molecular markers and outcome from colorectal cancer. *Anticancer Res*. 2007 (in press)

Stewart C, Morris M, de Boer B and Iacopetta B: Identification of serosal invasion and extramural venous invasion on review of Dukes' stage B colonic carcinomas and correlation with survival. *Histopathology* 2007 (in press)

Morris M, Iacopetta B, Platell C. Comparing survival outcomes for patients with colorectal cancer treated in public and private hospitals. *Med J Aust.* 186(6): 296-300, 2007.

Morris M and Platell C: Surgical volume influences survival in patients undergoing resections for stage II colon cancers. *ANZ J Surg*. 2007 (in press)

Watson N, Grieu F, Morris M, Schofield L, Goldblatt J, Harvey J, Stewart C and Iacopetta B: Heterogenous patterns of mismatch repair protein expression in colorectal tumours with microsatellite instability. *J Mol Diagnosis* (in press)

Presentations arising from thesis

Oral presentations

- Association of Coloproctology of Great Britain and Ireland (ACPGBI): Annual Meeting, Newcastle, United Kingdom, 2006.
- Royal Australasian College of Surgeons (RACS): Annual Scientific Conference, Sydney. 2006.
- Surgical Research Society of Australasia (SRS): Annual Scientific Meeting
    - Sydney, May 2006
    - Perth, May 2005
- West Australian Cancer Council Annual Conference: Perth, 2005.
- West Australian Cancer Education Meeting, Perth 2007 (WACOG)

Poster Presentations

- American Society of Clinical Oncology (ASCO) Annual Scientific Conference: Atlanta, Georgia, 2006; Chicago, Illinois, 2007.
- Royal Australasian College of Surgeons (RACS) Annual Scientific Meeting, Sydney, 2006.

Awards

Surgical Research Society of Australasia (SRS)

-Young Investigator of the Year 2006

- Travel Grant, 2005

Chapter 1 Introduction

Incidence of CRC

One million new cases of CRC (CRC) were diagnosed worldwide in 2002 (Parkin *et al*, 2005). CRC is a major health problem in Australia, with 12,600 new cases diagnosed annually and 4,700 deaths (AIHW, 2001). It is the most common cancer type reported to Australian cancer registries and was responsible for 13% of cancer deaths in Australia in 2001, ranking second only to lung cancer (AIHW, 2001). Premature death from CRC was responsible for 29,058 life-years lost before the age of 75 years, again ranking second only to lung cancer (AIHW, 2001).

The incidence of CRC varies by up to 25-fold worldwide (Kamangar *et al*, 2006). North America, Australia/New Zealand, Western Europe and Japan (particularly men) have the highest incidence rates (Parkin *et al*, 2005). CRC incidence tends to be low in Africa and Asia and intermediate parts of South America (Parkin *et al*, 2005). The geographical variation in incidence is more striking for colon than rectal cancer. In high risk populations, the ratio of colon to rectal cancer incidence is 2:1. The ratio has been noted to be higher in females of high risk populations. In low risk countries, the incidence of colon and rectal cancer is approximately the same (Parkin *et al*, 2005).

Aetiology of CRC

Geographic variations in the incidence of CRC are probably a result of different genetic, dietary/lifestyle and environmental factors. High per capita consumption of meat (Armstrong *et al*, 1975), animal fat (Prentice *et al*, 1990) and fibre (Trock *et al*, 1990) have been linked to these geographic variations in incidence. Epidemiologic studies find consistent evidence that physical inactivity, excess body weight, and a central

deposition of adiposity have a major influence on risk of colon cancer (Giovannucci *et al*, 2002). The risk of CRC is strongly dependent upon environmental factors, as demonstrated by studies of migrants that move from low incidence to high incidence populations and whereupon the incidence of CRC increases in the first generation (King *et al*, 1973; Kolonel *et al*, 1980). These studies strongly imply that dietary and environmental factors constitute a major component of risk. Modifiable dietary and lifestyle factors have been estimated to account for 70% of the attributable risk for CRC in Western populations (Giovannucci *et al*, 2002).

Hereditary conditions associated with increased risk of CRC include Familial Adenomatous Polyposis Coli (FAP) and Hereditary Non-Polyposis Coli (HNPCC), both of which are inherited as autosomal, dominant germline mutations (see below).

Clinicopathological Features of CRC

CRC is predominantly epithelial-derived, with 95% presenting as adenocarcinoma. Most CRCs are believed to develop from a benign precursor lesion referred to as adenoma. Gross tumour morphology varies between the polypoid, fungating masses commonly found in the proximal colon and the annular, encircling tumours often seen in the distal colon. Iron deficiency anaemia, disturbance of bowel habit such as bleeding, diarrhoea, constipation and obstruction and perforation can be signs at presentation and symptoms of CRC. CRC is often confined locally for a relatively long period of time before infiltrating through the bowel wall, peri-colic fat and adjacent structures as well as metastasising to regional lymph nodes and distant sites such as liver and lungs.

Molecular Pathogenesis of CRC

The Chromosomal Instability Pathway (CIN): Adenoma to carcinoma sequence

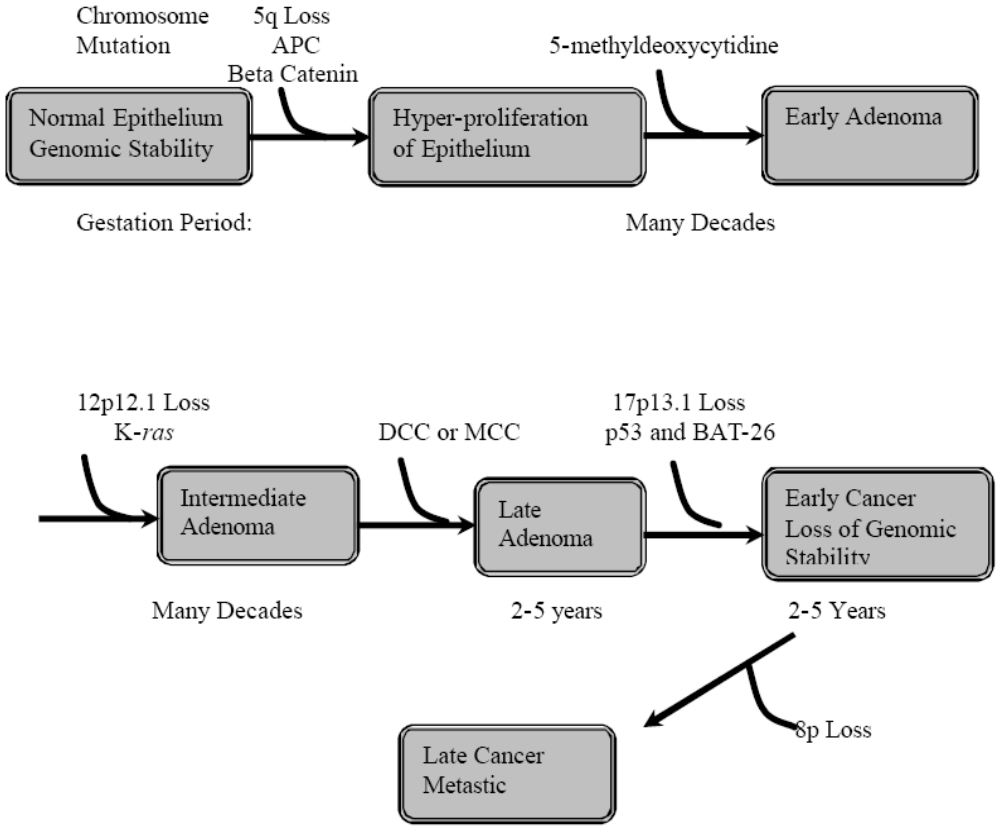
Tumourigenesis is a multi-step process in which genetic alterations accumulate and ultimately produce a neoplastic phenotype (Cho *et al*, 1992). Histological observations have led to the concept that most CRCs develop from normal mucosal epithelium through sequentially more aberrant degrees of adenomatous dysplasia (Muto *et al*, 1975).

Fearon and Vogelstein (1990) proposed a model of successive genetic changes that lead to CRC. Salient features of the model were that tumours arose as a result of mutational activation of oncogenes coupled with inactivation of tumour suppressor genes. Mutation in at least four or five genes were required to produce a malignant tumour and it was the total accumulation of these changes, rather than their chronological order, that was responsible for the tumour's biological properties (Cho *et al*, 1992).

The classic model of the adenoma to carcinoma sequence proposes that the vast majority of CRCs develop along the same linear sequence of genetic events and implicate the involvement of key "cancer" genes:

1. Initiation of adenoma: biallelic inactivation of the *APC* tumour suppressor gene (Kinzler *et al*, 1991; Powell *et al*, 1992)
2. Growth into a larger adenoma: mutation of the *Kras* oncogene
3. Transition from adenoma to carcinoma: mutation and loss of additional tumour suppressor genes (e.g. *DCC* and *p53*)

Figure 1. Pathway to invasive cancer involving activation of oncogenes and inactivation of tumour suppressor genes (from Fearon and Vogelstein, 1990).



The model represented by the adenoma to carcinoma sequence is referred to as the chromosomal instability (CIN) pathway because of the high frequency of aneuploidy and loss of heterozygosity (LOH). This classical model advocates a linear sequence of events for tumourigenesis. However it has been reported that simultaneous mutations of *APC*, *Kras* and *P53* were present in only 7% of CRC (Smith *et al*, 2002).

APC inactivation results in stabilization of the transcriptional activator β-catenin in the Wnt signalling pathway, leading to translocation to the nucleus and induction of expression of multiple oncogenes including c-*myc*. *APC* germline mutations result in the familial adenomatous polyposis (FAP) syndrome characterised by multiple adenomas within the gastrointestinal tract. Somatic *APC* mutations generally lead to a truncated APC protein (Miyoshi *et al*, 1992) and are detected in 60-75% of sporadic CRCs, most commonly those in the distal colon and rectum (Miyaki *et al*, 1994; Jass *et al*, 2002a). Mutations in the *Kras* oncogene lead to activation of the MAP kinase

signalling pathway. They can sometimes be detected in histologically normal mucosa and are found in approximately 60% of adenomas but only 40% of CRC (Jass *et al*, 2002a).

The P53 protein is important for the maintenance of DNA integrity. DNA damage results in P53-mediated arrest of the cell cycle in G1 phase, allowing repair to occur. If the DNA damage is too great, P53 can induce apoptosis. Loss of P53 function by mutation or deletion allows cells to accumulate mutations throughout the genome and results in karyotypic instability, impaired G1 cycle arrest and reduced apoptosis (Lane, 1992; Carder *et al*, 1993). Around 70% of CRCs show loss of the 17p chromosomal region containing the *P53* gene locus, with somatic mutation of the gene occurring in around 40-50% (Jass *et al*, 2002a).

Since the introduction of the Vogelstein and Fearon multistep model of CRC, further investigations of molecular pathways in this tumour type have elucidated other genetic and epigenetic events and alternative mechanisms have been proposed for the development of CRC.

The Methylator Phenotype Pathway (CIMP) in CRC: Hyperplastic polyp/serrated adenoma to carcinoma sequence

Methylation of CpG islands located in gene promoter regions appears to be the primary mediator of epigenetic inheritance in cancer cells (Jones *et al*, 1999). Hypermethylation of CpG islands in the promoter of tumour suppressor genes results in transcriptional inactivation of these genes. The term CpG island methylator phenotype (CIMP+) was first proposed in 1999 to describe a subgroup of CRCs with frequent and concurrent methylation of CpG islands (Toyota *et al*, 1999). Approximately 20-30% of CRCs display the CIMP+ phenotype and are characterised by distinctive clinical, pathological

and molecular profiles (Hawkins *et al*, 2002; Van Rijnsoever *et al*, 2002; Samowitz *et al*, 2005).

CIMP+ tumours occur frequently in the proximal colon of elderly females (Toyota *et al*, 1999; Hawkins *et al*, 2002; Van Rijnsoever *et al*, 2002). They are more likely to be poorly differentiated, mucinous adoenocarcinomas with frequent tumour infiltrating lymphocytes. Typical molecular features include frequent microsatellite instability (MSI+) and *BRAF* oncogene mutation, with low frequencies of *P53* mutation. It has been proposed that CIMP+ tumours arise from large hyperplastic polyps or serrated adenomas often found in the proximal colon (Jass *et al*, 2002b). These lesions show subtle histological differences to the hyperplastic polyps found in the distal colon and rectum and are often CIMP+ and *BRAF* mutant. Methylation of promoter CpG islands has been shown to occur early in colorectal carcinogenesis (Jass *et al*, 2002b; Minoo *et al*, 2006), however not all promoter CpG regions are affected. This has led to research aimed at defining a panel of CpG islands that can accurately identify CIMP+ tumours (Weisenberger *et al*, 2006).

Microsatellite instability in CRC occurs in both the CIN and CIMP pathways

Microsatellite instability (MSI) refers to a type of genetic instability associated with the lengthening or shortening of microsatellite DNA repeat sequences (Thibodeau *et al*, 1993). The underlying mechanism is a defect in the capacity to repair DNA mismatches that arise during cell replication. Approximately 20% of colon tumours and 1-2% of rectal tumours show MSI (Nilbert *et al*, 1999; Elsaleh *et al*, 2001). For sporadic CRC, the MSI almost always arises because of methylation-induced, bi-allelic silencing of the *MLH1* gene. For this reason, sporadic MSI tumours occur frequently in conjunction

with the CIMP+ phenotype and are also thought to arise from hyperplastic polyps or serrated adenomas (Hawkins *et al*, 2001).

The MSI phenotype is also found in the large majority of tumours arising in HNPCC (Lynch syndrome). The MSI tumours in this context arise because of germline mutations to one of the DNA mismatch repair genes, most commonly *MLH1*, *MSH2* or *MSH6*. The tumour develops when the second allele is inactivated by somatic mutation. In contrast to sporadic MSI tumours, those from HNPCC patients appear to develop along the CIN pathway and follow the adenoma to carcinoma sequence. Another discriminating feature is that *BRAF* mutations are common in sporadic MSI tumours but are never observed in MSI tumours from HNPCC cases (Deng *et al*, 2004).

Sporadic MSI tumours tend to be located proximal to the splenic flexure and are often poorly differentiated, mucinous, large and have tumour infiltrating lymphocytes (Wheeler *et al*, 2000). There is evidence that MSI tumours have better stage-specific prognosis and tend to be less metastatic, although this has not been a consistent finding (Popat *et al*, 2005).

Detection of CRC

Colonoscopy is currently the most accurate investigation for assessing the colon and rectum for cancer (Winawer *et al*, 2000). It has a sensitivity of 95% (Rex *et al*, 1997) and allows for biopsy and subsequent histological confirmation of the diagnosis. All patients with CRC should undergo colonoscopy as part of their preoperative assessment, unless there is a perforation or significant obstruction of the large bowel. The presence of synchronous pathologies in 5-10% of patients may alter the surgical approach (NH&MRC guidelines, 2005). Faecal Occult Blood testing (FOBT) is currently being evaluated in Australia as a population screening tool (AHTAC, 1997). Digital rectal

examination, flexible and rigid sigmoidoscopies as well as CT colonography are alternative methods for diagnosing CRC.

Surgical Resection of CRC

The objective of surgical treatment of CRC is to remove the primary tumour and any regional spread without causing further dissemination of the tumour, whilst at the same time preserving reasonable quality of life for the patient (NH&MRC guidelines, 2005). Approximately 30% of colon cancer patients in the population present as emergencies, with the majority of these presenting with obstruction (80%) and the remainder with perforation (Ohman, 1982; Mandava *et al*, 1996). Most perforations occur at the site of the cancer and lead to localised abscess or generalised peritonitis. Massive bleeding from CRC is an uncommon presentation (Repse *et al*, 1996).

Patients presenting as emergencies tend to be older, have other comorbidities and have a more advanced stage of cancer (Anderson *et al*, 1992; Runkel *et al*, 1991). The duration of hospitalisation tends to be longer and there is a higher incidence of permanent stoma (Anderson *et al*, 1992). Peri-operative morbidity and mortality are higher in patients presenting as emergency cases compared to those undergoing elective surgery (Scott *et al*, 1995). Operative mortality after surgery for perforated CRC is higher than for obstructed CRC (35% *vs.* 15%), especially if major sepsis is present (Kyllonen *et al*, 1987; Runkel *et al*, 1991).

The optimal technique for resection of colonic cancer has been the subject of considerable debate. Important elements include early isolation (+/- high ligation) of the lymphovascular pedicle before minimal manipulation of the tumour and wide excision. High ligation techniques failed to produce a substantial improvement in survival

(Sugarbaker *et al*, 1982) and the 'no touch isolation' technique also did not demonstrate a survival benefit (Garcia-Olmo *et al*, 1999).

Staging of CRC

Staging of CRC refers to classification of the tumour according to the extent of spread in a manner that has a clinically useful correlation with prognosis. Dukes' classification was the first well documented and tested staging system for CRC (Dukes, 1932; Dukes, 1951). The system is based on the extent of tumour spread and the presence or absence of lymph node metastases in the resected bowel specimen. There have been modifications to the Dukes classification, notably the Astler-Coller (1954) modification and the AJCC post-surgical pathological assessment with the TNM classification (AJCC, 2002).

Turnbull *et al* (1967) introduced the concept of clinico-pathological staging in which distant metastases found by the surgeon at the time of operation could determine the assigned stage. CRCs are usually staged after surgical exploration of the abdomen and pathologic examination of the surgical specimen. Recent developments have included the incorporation of pre-operative imaging (e.g. demonstrating liver metastases) into clinico-pathological stage (Davis *et al*, 1982; AJCC, 2002). The 6th Ed of the American Joint Committee on Cancer's Cancer (AJCC, 2002) Staging Manual has been used to stage all CRCs by the TNM classification as illustrated in Table 1.1. A comparison of staging systems is shown in Table 1.2.

Table 1. TNM staging system for CRC

T –spread of primary tumour
T0: no evidence of primary tumour **Tis:** in situ, non-invasive; intraepithelial. Invasive tumour confined to the mucosa and lamina propria. **T1:** invasive tumour within the confinements of submucosa **T2:** muscularis propria invaded **T3:** Invasion through muscularis propria or muscularis Non-peritonealised pericolic tissues invaded, subserosal tissue/subserosal fat invaded. Adherent to other organs or structures, but no microscopic tumour found in adhesions **T4:** Invasion of/through serosa (visceral peritoneum) Adherent to other organs or structures. Evidence of perforation.
N-regional lymph nodes
N0: no regional lymph node involvement **N1:** 1 to 3 clinically positive regional lymph nodes **N2:** 4-90 clinically positive regional lymph nodes
M-distant metastases
M0: No metastases **M1:** distant lymph nodes- common iliac, external iliac, para-aortic, retroperitoneal, superior and inferior mesenteric lymph nodes carcinomatosis. All other distant metastases e.g. liver, lung

Table 2. Staging systems used for CRC

Stage	**T**	**N**	**M**	**Dukes**	**Modified Astler-Coller**
0	Tis	N0	M0	-	
I	T1	N0	M0	A	A
	T2	N0	M0	A	B1
IIA	T3	N0	M0	B	B2
IIB	T4	N0	M0	B	B3
IIIA	T1-T2	N1	M0	C	C1
IIIB	T3-T4	N1	M0	C	C2/C3
IIIC	Any T	N2	M0	C	C1/C2/C3
IV	Any T	Any N	M1	-	D

Pathological evaluation should report histological grade of cancer, depth of penetration (T), number of lymph nodes evaluated, number of positive nodes (N) and the status of proximal, distal margins. It is recommended that the surgeon mark the area of the specimen with the deepest tumour penetration so that the pathologist can directly evaluate the radial margin (NCCN guidelines, 2007). There is no consensus as to the definition of what constitutes a positive margin of resection. Positive margin is defined as the presence of tumour within 1-2 mm of the transected margin and the presence of tumour cells within the diathermy of the transected margin (Volk *et al*, 1995; Ueno *et al*, 2004).

The surgeon is also encouraged to score the completeness of the resection. Pathological review of the margins categorises tumours as R0 for complete tumour resection with all margins negative, R1 for incomplete tumour resection with microscopic involvement of a margin and R2 for incomplete tumour resection with gross residual tumour not resected (NCCN guidelines, 2007). The AJCC and the American College of Pathologists recommend evaluation of a minimum of 12 lymph nodes to accurately identify stage II CRCs (Compton *et al*, 2000a; AJCC, 2002). The apical lymph node is the most proximal node within 1 cm of vessel ligation at the apex of the vascular pedicle and should be marked by the surgeon. Apical lymph node involvement is significantly associated with worse outcome (Sobin *et al*, 2001; Le Voyer *et al*, 2003). Modifications to the 6th ed of the AJCC Staging Manual include smooth metastatic nodules in the peri colic or peri rectal fat to be considered lymph node metastases and should be included in the N staging. Irregularly contoured metastatic nodules in the peritumoural fat are considered vascular invasion.

Prognostic and Predictive Markers for Colon Cancer

A *prognostic* marker provides information on patient outcome that can be used to guide therapeutic decisions (McLeod *et al*, 1999), whereas a *predictive* marker provides information as to the likely response to a treatment regimen. Identification of factors with strong prognostic significance would be of great benefit in guiding the use of adjuvant chemotherapy, particularly for early stage disease. Such markers are best evaluated in patient cohorts treated by surgery alone in order to avoid the possibility that adjuvant treatments differentially affect the survival of patient subgroups defined by prognostic features.

Carcinoembryonic antigen (CEA) levels can be used in the pre-operative work up to assist with staging and surgical treatment planning (Locker *et al*, 2006). Elevated pre-operative CEA (>5ng/mL) in the serum may correlate with a poorer prognosis (Locker *et al*, 2006), but detection of CEA staining in tumour samples does not provide useful prognostic information (McLeod *et al*, 1999). CEA is considered a valuable component of postoperative follow-up as it is the most frequent indicator of recurrence in asymptomatic patients and is the marker of choice for monitoring the response of metastatic disease to systemic therapy (Locker *et al*, 2006).

Pathological variables that are commonly reported and thought to have prognostic significance include tumour histological type (adenocarcinoma, mucinous adenocarcinoma, signet ring carcinoma, large cell undifferentiated), evidence of lymphovascular and perineural invasion, expanding or infiltrating margins and the presence of peritumoural and tumour infiltrating lymphocytes (NH&MRC guidelines, 2005). Angiogenesis has a vital role in tumour growth and metastasis. It can be assessed by microvessel counts in the tumour and/or by the analysis of angiogenesis promoting molecules such as VEGF and thymidine phosphorylase (Graziano *et al*, 2003). VEGF is

localised to tumour cells and VEGF positive tumours have a significantly poorer prognosis than VEGF negative tumours (Kang *et al*, 1997). Evaluation of microvessel density is technically challenging and there have been conflicting results as to its prognostic value (Lindmark *et al*, 1996; Boxer *et al*, 2005).

Numerous studies have been carried out on the potential prognostic significance of mutations in oncogenes and tumour suppressor genes. Several studies report that *Kras* mutation is an adverse prognostic indicator (Benhattar *et al*, 1993; Span *et al*, 1996), however this may depend on the specific type of mutation. *Kras* mutation is currently not recommended for use as a prognostic marker in CRC (Locker *et al*, 2006). The prognostic significance of other proto-oncogenes such as c-*erb* B2 and c-*myc* is unclear (McLeod *et al*, 1999). The prognostic significance of the *P53* tumour suppressor gene has been studied extensively in CRC (Russo *et al*, 2005; Royds *et al*, 2006). In contrast to breast cancer, mutation of this gene does not appear to have prognostic value for the outcome of CRC patients. The most recent guidelines conclude that with the current methods of assessment, *P53* status is a poor guide to both prognosis and prediction of response to chemotherapy (Locker *et al*, 2006).

A small number of retrospective studies have found LOH at 18q is associated with a poor prognosis in stage II disease (Jernvall *et al*, 1999; Lanza *et al*, 1998, Martinez-Lopez *et al*, 1998). Similarly, determining the loss of the deleted in colon cancer (DCC) protein by immunohistochemistry found the expression of DCC was a prognostic factor for both stage II and stage III disease (Shibata *et al*, 1996). Current guidelines state that $18q^-$/DCC should not be used to determine prognosis, nor predict response to chemotherapy (Locker *et al*, 2006).

CIMP+ tumours have worse survival compared to CIMP– tumours in CRC treated by surgery alone (Van Rijnsoever *et al*, 2003; Ward *et al*, 2003). In comparison,

MSI+ tumours have better survival compared with MSI- patients treated by surgery alone (Popat *et al*, 2005; Sinicrope *et al*, 2006). At present neither CIMP+ nor MSI status are recommended as independent markers of prognosis (Locker *et al*, 2006). Patients with CIMP+ tumours demonstrated a survival advantage from 5FU adjuvant chemotherapy in a population based cohort of Stage III colon cancer (Van Rijnsoever *et al*, 2003). The predictive value of MSI is highly controversial, however, possibly because of the involvement of two different pathways (CIN and CIMP) with different responses to 5FU (Kim *et al*, 2007; Jover *et al*, 2006).

Thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) are enzymes involved in folate metabolism and as such have been postulated to have predictive value for response to 5FU chemotherapy (McCleod *et al*, 1999). None are presently recommended for use in the routine clinical setting (Locker *et al*, 2006). DNA microarray gene expression profiling allows measurement of mRNA expression levels for thousands of genes simultaneously in a single assay. It provides a strategy to search systematically for molecular markers of prognosis and for predictors of response to treatment. Research in this area is at an early stage and the results published to date require further validation in prospective trials before they can be recommended for routine use.

Adjuvant Chemotherapy for Colon Cancer

In 1989 the North Central Cancer Treatment Group (NCCTG) published a randomized trial comparing surgical resection alone with postoperative levamisole or 5-FU-levamisole (Laurie *et al*, 1989). An absolute survival benefit of approximately 12% (49% *vs.*. 37%) was seen in patients with stage III disease treated with 5-FU-levamisole. The Intergroup trial #0035 published in 1990 detected a significant survival advantage

for 5-FU plus levamisole compared with observation (Moertel *et al*, 1990). In 1990, the National Institutes of Health consensus statement recommended a one year combination of 5-FU plus levamisole as the standard of care for Dukes C colon cancer patients (NIH, 1990).

It has been found subsequently that levamisole alone does not confer survival benefit (Moertel *et al*, 1990). 5FU was then combined with the active form of the B complex vitamin folate, leucovorin. Multiple randomized trials that have enrolled over 4,000 patients demonstrate a relative reduction in mortality of between 22% and 33%, with 3-year overall survival rates increasing from 71-78% to 75-84% (Wolmark *et al*, 1993; IMPACT, 1995; O'Connell *et al*, 1993). Pooled analysis of randomized trials indicate that elderly patients (>70 years) derived equal benefit from adjuvant treatment as younger individuals and should not be excluded from these treatments based solely on age (Sargent *et al*, 2001).

5FU Regimenns

5-FU–based therapy has been administered in the past according to several schedules, including daily bolus for 5 days every 4 weeks for 6 months (Mayo Clinic regimenn) and weekly for 6 weeks with 2 weeks off (Roswell Park regimenn). These regimens can be administered on an outpatient basis and are considered to be equivalent to the International Multicentre Pooled Analysis of Colon Trials (IMPACT, 1995). There is no superiority in terms of patient survival with either of these regimens (Goyle *et al,* 2005; Saini *et al,* 2003). There is data to suggest increased toxicity with the 5-day schedule (Mayo Clinic) compared to the weekly regimenn (Roswell Park) without evidence of an improved therapeutic benefit (Tebbutt *et al*, 2000; Goyle *et al*, 2005). Subsequent studies examined the length of treatment and the use of combinations of levamisole,

leucovorin and interferon with 5FU-based chemotherapy. These have shown that treatment for 6-8 months with 5FU-leucovorin is equivalent to 12 months treatment, and that the addition of interferon increases toxic side effects without improving efficacy (Wolmark *et al*, 1998; Wolmark *et al*, 1999). Also, there is insufficient data to prove whether high-dose, intermediate-dose, or low-dose leucovorin is most advantageous as a modulator of 5FU.

Capecitabine is an oral fluoropyrimidine that undergoes a 3-step enzymatic conversion to 5FU with the last step occurring within the tumour cell. For patients with metastatic colon cancer, two studies have demonstrated the equivalence of capecitabine to 5FU/leucovorin (Van Cutsem *et al*, 2001; Hoff *et al*, 2001). For patients with stage III colon cancer in whom treatment with 5FU/leucovorin is planned, capecitabine is an equivalent alternative (Twelves *et al*, 2005). Capecitabine has been associated with a better toxicity profile except for more hand-foot syndrome (Twelves *et al*, 2005).

Mechanism of Action of 5FU

The active metabolite of 5FU, FdUMP, is a competitive inhibitor of the enzyme thymidylate synthase (TS) which catalyses the synthesis of thymidine nucleotide precursor using a methyl group provided by a folate cofactor (Pinedo *et al*, 1988; Parker *et al*, 1990). Leucovorin enhances the action of 5FU by binding to the enzyme thymidylate synthase (Peters *et al*, 1991). Dihydropyrimdine dehydrogenase (DPD) is the initial and rate limiting step in the catabolism of 5FU (Flemming *et al*, 1992). Low tumour levels of DPD have been associated with good sensitivity to 5FU (Lu *et al*, 1993). It is well documented that women suffer more toxicity than men (Milano *et al*, 1992; Chanksy *et al*, 1992; Zalcberg *et al*, 1998; Sloan *et al*, 2002). Studies have found that women have lower DPD activity and possibly as a consequence they suffer greater

toxicity from 5FU compared to males. Women have lower 5FU clearance rates compared to men and this is clinically relevant for both toxicity and response to 5FU (Milano *et al* 1992; Iacopetta *et al*, 2006). 5FU is misincorporated into DNA and RNA, with detrimental effects on the structure and function of these nucleic acids (Peters *et al*, 1991). It is widely believed that 5FU alters DNA metabolism, thereby causing strand breaks which in turn activates p53-dependent apoptosis.

Combination Therapies with FU

Irinotecan and oxaliplatin were developed and approved for the treatment of patients with advanced CRC. These drugs are now being tested in patients with local or recurrent disease. Irinotecan is a topoisomerase-I inhibitor with a 10% to 20% partial response rate in patients with metastatic colon cancer (Rougier *et al*, 1998; Cunningham *et al*, 1998). Phase III trials have demonstrated improved response rates and prolonged overall survival with irinotecan combined with 5FU-leucovorin when compared to 5-FU-leucovorin alone (Saltz *et al*, 2000; Douillard *et al*, 2000). Oxaliplatin is a platinum-based anti-neoplastic agent used in combination with an infusion of 5-FU/leucovorin (FOLFOX) in the adjuvant setting. The MOSAIC study compared the toxic effects and efficacy of FOLFOX4 with a 5FU/leucovorin regimenn administered for 6 months in 2,246 patients with resected stage II or stage III colon cancer (Andre *et al*, 2004). The preliminary results of the study demonstrated a significant improvement in disease-free survival at 3 years (78.2% *vs*.. 72.9%, $P = 0.0002$) in favour of FOLFOX4. Patients treated with FOLFOX4 experienced more frequent toxic effects consisting mainly of neutropenia and reversible peripheral sensorial neuropathy.

Current Australian Guidelines for the use of Adjuvant Chemotherapy in Stage II and III Colon Cancer

The Australian NH&MRC guidelines (1999) for the management colon cancer current at the commencement of this research thesis recommended that patients with resected node positive colon cancer (stage III) should be offered adjuvant chemotherapy. The preferred option was 5FU with low dose leucovorin for six months.

For early stage disease, the guidelines state: '*The value of adjuvant therapy in Dukes B (Stage II) colon cancer has not been demonstrated uniformly. Adjuvant therapy in this group is not recommended except for patients with 'poor prognosis' stage II disease, who after discussion, wish to have treatment by entry into appropriate clinical trial, which is recommended*.'

The potential value of adjuvant therapy for patients with stage II colon cancer remains controversial. An early randomized trial of postoperative 5FU plus levamisole compared to surgery alone in stage II disease showed no survival advantage for postoperative adjuvant chemotherapy (Moertel *et al*, 1995). National Surgical Adjuvant Breast and Bowel Project (NSABP) meta-analysis of 4 prior adjuvant NSABP studies demonstrated a 30% reduction in mortality with adjuvant chemotherapy. The reduction in risk of recurrence by adjuvant therapy in patients with stage II disease was of similar magnitude to the benefit seen in patients with stage III disease treated with adjuvant therapy, although an overall survival advantage was not established (Mamounas *et al*, 1999). A meta-analysis of 1,000 stage II patients whose experience was amalgamated from a series of trials indicated a 2% advantage in disease-free survival at 5 years when patients treated with 5-FU-leucovorin were compared to untreated controls (IMPACT

B2, 1999). The latest QUASAR study results, a randomised study of stage II colon cancer receiving adjuvant chemotherapy versus observation (n= 2092), demonstrate a small (1-5%) absolute survival benefit in stage II disease patients (Gray *et al*, 2004).

The American Society of Clinical Oncology (ASCO) currently does not recommend adjuvant chemotherapy for stage II colon cancer (Benson *et al*, 2004). The recommendation does however comment that there are populations of patients with stage II disease that could be considered for adjuvant chemotherapy, including patients with inadequately sampled nodes, T4 lesions, perforation or poorly differentiated histology.

Aims of the Current Study

Using a population-based cohort of CRC (n=5,971), the major aims of this study were to:

1) Identify clinico-pathological markers that can be used to define a subset of stage II colon cancer patients with excellent prognosis and who therefore do not require referral for adjuvant chemotherapy

2) Investigate whether there is a survival benefit from the use of adjuvant chemotherapy in a population-based cohort of stage II colon cancer

3) Investigate stage III colon cancer patients for evidence of predictive markers for response to 5FU chemotherapy

4) Investigate CRC for age-related differences in clinico-pathological and molecular features

Hypotheses to be tested

1) A subset of very good prognosis stage II colon cancers can be defined using routine pathological markers

2) Females colon cancer patients gain more survival advantage from 5FU chemotherapy than males

3) Tumours from young CRC patients have different molecular characteristics to those from older patients

4) The underlying molecular characteristics of tumour can impact upon the response to 5FU chemotherapy

Chapter 2 Methods

Study Population

Database construction

An ACCESS database was constructed comprising surgically resected CRC cases treated in the state of Western Australia during the years 1993-2003. Records were accessed from the Pathwest, Royal Perth Hospital, Fremantle Hospital and St John of God Pathology services. The pathology services at these hospitals also process surgical specimens from other minor district and country hospitals and consequently information was obtained for the total Western Australian Populations numbering 1.8-2 million inhabitants over the study period. Records identified by the appropriate SNOP codes (T6700, T6800, M8143) were retrieved for the aforementioned years for both biopsy and resection specimens. At this point, the data was cross checked with the Cancer Registry of Western Australia for the years 1993-2003. A further 575 patients were identified from the cancer registry as having had CRC during this time period, however only the pathology report from biopsy specimens was available or the pathology records were not registered with the 4 major pathology laboratories cited above. A total of 182 of these records were retrieved from the Western Diagnostic pathology service for stage II colon cancer aged <75 years. The remaining cases (n=393) are believed to originate from the Western Diagnostic and General Pathology Fremantle pathology services. The cohort described in this thesis therefore represents approximately 88% of all CRCs identified in the state of Western Australia during the period 1993-2003.

Classification of Pathology Reports

Resection specimen reports were read individually and classified according to the AJCC 6th Ed staging system. A total of 5,971 CRCs that underwent surgical resection were identified.

Exclusion Criteria for Analysis

Patients with colorectal tumours that were metachronous, had positive surgical margins, concomitant inflammatory bowel disease, familial adenomatous polyposis or previous malignancies were excluded from analysis (n=63).

Definition of Site

The anatomical site of the tumour in the large bowel was ascertained from the pathology report and was cross-checked with information from admission and procedure records. Colon cancers were classified as being proximal or distal to the splenic flexure. Rectal cancer was defined as being within 12cm of the anal verge. Site could not be ascertained for 649 cases, and these cases were excluded from subsequent analysis.

Stage II Colon Cancer Staging

Dukes' B CRC was identified in 2,272 patients during the study period. When classified according to the current AJCC guidelines (AJCC, 2002), a total of 361 patients were found to have Stage I disease and 36 were incorrectly staged in the initial pathology records (i.e. lymph node metastases were documented). A total of 1847 CRC patients fulfilled the criteria for analysis and 1468 of these were colon carcinomas.

Stage III Colon Cancer Staging

A total of 2,024 patients with CRC fulfilled the criteria for stage III CRC and 1,404 of these had colonic carcinoma.

Pathological Variables

Pathology records for Stage II and stage III colon cancers were individually reviewed for ascertainment of pathological variables such as differentiation, T stage, lymph nodes examined and involved, lymphatic and perineural invasion, obstruction, perforation, mucinous phenotype and lymphocytic response. Perforation was considered present if noted by the surgeon at operation (documented on pathology request form or in operative notes) or on histopathological review of the specimen. Current AJCC guidelines categorise perforation of a tumour as T4. Obstruction was considered present if clinical records documented patients presenting with obstruction or if noted by the pathologist to be an obstructing tumour with evidence of distension and thinning of the bowel wall proximal to the tumour. Lymphocytic response was considered present if there was documented evidence of lymphocytic response on the pathology report. The definition for tumour infiltrating lymphocytes (TILS) was not used (Ropponen *et al*, 1997) as early in the study time period it was not routine pathological reporting practice. A tumour was considered mucinous if documented by the pathologist as a mucinous adenocarcinoma. Percentage of a tumour demonstrating mucinous morphology was not used as the definition as this was seldom reported in the histopathology reports.

Adjuvant Chemotherapy

The WA data linkage system (Holman *et al*, 1999) was accessed to obtain morbidity records of procedure codes. Patients undergoing chemotherapy over the study period were identified by the codes 99.23, 90760-00, 90768-00, 13915-00, 13928-00, 13921-00, 13927-00, 13929-00, 13942-00, 34527-00 and 34528-00. Dates were obtained for the administration of chemotherapy. This data was then cross-checked with the cancer registries of the medical oncology departments of the three teaching hospitals and from the hospital coding database of the private hospital. Patients were considered to have received chemotherapy if one or more cycles were given. The date at which chemotherapy was given relative to the surgical date was used to distinguish adjuvant treatment from treatment for recurrence, with 6 months post surgery being the cut-off period. Individual records of patients who received adjuvant chemotherapy were reviewed for stage II and stage III disease and those receiving the Mayo regimen (Goyle *et al*, 2005) were identified.

Colonoscopy Procedures

Patients were identified as undergoing colonoscopy by procedure codes (45.23, 45.25, 45.27, 45.42, 32090-00, 32090-01, 32093-00). Partial colonoscopy (32084-00, 32084-01, 32087-00) and sigmoidoscopy (45.24, 48.23, 48.24, 32072-00, 32075-00, 32072-01, 32075-01, 32078-00, 32081-00) were also identified and the dates of all procedures recorded in the database.

Socio-Economic Indices

Each patient's post code address was obtained from the West Australian Cancer Registry database and this was linked to Socio-Economic Indexes for Areas

(advantage/disadvantage, economic resources and education/occupation) obtained from the 2001 Australian census (Australian Bureau of Statistics, 2001). These indices were then divided into quintiles of socio-economic status.

Survival Data

Mortality data was obtained from the Death Registry of the Health Department of Western Australia. Death reports were reviewed individually and classified as death due to colorectal cancer (C) or from other causes (O). Peri-operative mortality (P) was defined as death within 4 weeks of the surgical resection (C, n=2254; O, n=883; P, n=239). Follow up information concerning the date of death was updated throughout the project until 1/3/2006. This data was then cross-checked with the Cancer Registry of Western Australia's database. Discrepancies were identified for the cause of death in approximately 10% of patients. Death records were re-read and the discrepancy was found to be in the classification system used by the Cancer Registry for defining death from cancer, but not necessarily death from colorectal carcinoma. For example, patients with stage 1 colon cancer were identified as having died of cancer because they died of leukaemia.

Molecular Analysis

Molecular screening for the MSI phenotype and for somatic mutations in the *BRAF*, *KRAS* and *TP53* genes was performed in 1272, 845, 264 and 298 tumours, respectively, in patients aged $\leq$60 years. This represented 77%, 51%, 16% and 18% of cases in this age group, respectively. All 48 tumours available from the 52 patients aged $\leq$30 years were investigated for each of the 4 molecular markers. Because of limitations on

resources, only the numbers of samples indicated above were screened for molecular alterations in patients aged 31-60 years. These were chosen randomly.

DNA extraction from paraffin embedded tissue sections

One or two 20µm tumour sections were placed into 1.5ml Eppendorf tubes containing 200µl digestion buffer (50mM tris, 1mM EDTA, 0.5% Tween 20 and pH=8.5). Tubes were heated at 94^{o}C in a dri-bath for 20 minutes to melt the paraffin and centrifuged immediately at maximum speed (13,000 rpm) for 10 minutes. After two hours of refrigeration at 4^{o}C, the solidified paraffin was removed from the surface and the tumour tissue in digestion buffer was transferred to a new tube. Samples were incubated at 55^{o}C for three days following addition of 40µl of proteinase K solution (20mg/ml). The proteinase K was then activated by heating samples to 94^{o}C for 10 minutes. After 10 minutes of centrifugation at maximum speed, 60µl of the lowest, clear solution was transferred to a new tube and stored at 4^{o}C for use in PCR.

Fluorescent-single strand conformation polymorphism (F-SSCP) analysis

The Single Strand Conformation Polymorphism (SSCP) technique is based on the differential electrophoretic migration in non-denaturing acrylamide gels of single stranded DNA molecules having different primary sequences and therefore different secondary structures. In the current study, a fluorescent Gel-Scan 2000 system (Corbett Research, Sydney) was used to detect Hex-labelled fluorescent primers used in the amplification of Bat-26, KRAS, BRAF and TP53 genes. In summary, 3µl of amplified fluorescent-labelled PCR product was mixed with 9µl of deionized formamide loading buffer containing 0.05% w/v Bromophenol blue and 0.5M EDTA, and denatured by heating at 94^{o}C for 5 minutes. One µl of this mix was then loaded onto a non-denaturing

polyacrylamide gel (8% polyacrylamide/2% glycerol) and run on the Gel Scan 2000 real-time DNA fragment analyser according to manufacturer's instruction (Corbett Research, Sydney). Once loaded into the wells, samples were pulse loaded for 20 seconds at 1400V, the wells were then rinsed thoroughly and the gel was run for 120 minutes at 1400V in 0.8x TBE buffer at a constant temperature of 25^{o}C. ONE-D scan software (Scanalytics, Billerica, USA) was used to enhance contrast of the electrophoretogram and thus facilitate the reading of aberrant bands.

Molecular analysis

The MSI status of each tumour was determined by the fluorescent SCCP analysis of the BAT-26 mononucleotide repeat as described previously (Iacopetta *et al*, 2000a). Mutations in the BRAF (V600E), KRAS (codons 12 and 13) and TP53 (exons 5-8) genes were also determined by PCR-based, fluorescent single strand conformation polymorphism analysis as described earlier by our group (Li *et al*, 2006; Wang *et al*, 2003; Iacopetta *et al*, 2000b). Only those tumours found to be MSI+ and *BRAF* wildtype were subsequently investigated by IHC for the loss of MMR protein expression. Loss of mismatch repair gene expression was determined by immunohistochemistry as determined previously (Chai *et al*, 2004). Lymphocytes and normal colonic epithelium located adjacent to tumour cells served as internal controls for positive MMR protein expression. Cases were initially scored by a pathologist (JH) as positive for expression (MMR normal) if nuclear staining was present in any of the malignant cells.

Statistical Analysis

SPSS Version 12.0.1 was used for all statistical analyses (Chicago, IL). Chi-square analysis was used to compare the frequencies of clinico-pathological features and

molecular alterations. Survival analysis was conducted using both Kaplan-Meier analysis and Cox proportional hazards regression. Statistical significance was considered to be $P<0.05$. Survival was calculated in days from date of diagnosis to date of death from colon cancer. Survival times were censored at the date of death from other causes or at 1/3/2006, whichever came first.

Binomial and multinomial logistic regressions were performed regarding the likelihood of receiving chemotherapy and completing the course of chemotherapy. Cox proportional hazards regression was used to estimate the effect of chemotherapy in stage II and III colon cancer and adjust for other confounding factors. The potential confounding factors were then assessed using the "change-in-estimate" method (Rothman *et al*, 1998).

Ethics

Ethics approval for this project was obtained from the Human Research Ethics Committee at each hospital, the Confidentiality of Health Information Committee and the University of Western Australia.

Chapter 3

Population-based study of prognostic factors in stage II colon cancer

Morris M, Platell C, de Boer B and Iacopetta B: Population-based study of prognostic factors in stage II colonic cancer. Br J Surg 93: 866-871, 2006.

Abstract

Background: Guidelines for the use of adjuvant chemotherapy in stage II CRC state this treatment may be considered for patients whose tumours show features of poor prognosis. The aim of the current study was to evaluate the prognostic significance of commonly reported clinical and pathological features of this disease.

Methods: A population-based observational study encompassing all stage II colon cancer patients diagnosed in the state of Western Australia from 1993-2003 inclusive was performed. A total of 1306 patients treated by surgery alone were identified and had a median follow-up of 59 months (range 0-145).

Results: Multivariable analysis revealed the only independent prognostic factors for disease-specific survival were T4 stage (HR=1.75, 95%CI [1.32-2.32], $P<0.0001$) and vascular invasion (HR=1.63, 95%CI [1.15-2.30], $P<0.0001$). In the younger patient group (≤75 yrs) who are more likely to be considered for chemotherapy, the same two features showed independent prognostic significance but with higher HR values (1.96 and 2.73 respectively). T4 and/or the presence of vascular invasion identified a "poor" prognosis group comprising 26% of younger patients and having a 5-year survival rate of 71%. The remaining "good" prognosis group showed 84% survival at 5 years follow-up.

Conclusions: This study highlights the importance of accurate pathological assessment of T stage and vascular invasion for the prognostic stratification of stage II colon cancer. The results provide clarification of guidelines for the management of stage II disease in relation to recommendations for chemotherapy.

Introduction

Current clinical guidelines in the USA, UK and Australia recommend that stage II colon cancer patients with features of poor prognosis may be considered for adjuvant chemotherapy (NIH, 1990; ACPGBI, 2001; NH&MRC, 1999). These include perforation or obstruction, T4 level of invasion, poor differentiation, vascular invasion and inadequate nodal sampling (Petersen *et al*, 2002; Burdy *et al*, 2001). Petersen *et al* (2002) reported that peritoneal involvement (T4), vascular invasion (submucosal and extramural), positive surgical margins and perforation were independent factors for worse survival in a prospective study of 268 Dukes' B colon cancers. Burdy *et al* (2001) in a retrospective study of 108 stage II colon cancers found that male sex, bowel obstruction, T4 invasion and <14 nodes examined were independent prognostic factors for recurrence. The number of lymph nodes analysed is also a prognostic variable and current recommendations are that at least 10-15 nodes are assessed (Law *et al*, 2003; Swanson *et al*, 2003; Le Voyer *et al*, 2003). The ability to identify stage II colon cancer subgroups with a high risk of recurrence would clearly improve treatment strategies for stage II disease and perhaps also the subsequent outcomes.

Hypothesis-generating retrospective studies allow the evaluation of promising prognostic markers that also have sufficient prevalence to be clinically useful (Graziano *et al*, 2003). The aim of the present study was to evaluate the prognostic significance of commonly reported pathological features in a retrospective series of 1,306 stage II colon

cancers treated by surgery alone. Data was collected from all colon cancer patients diagnosed in the state of Western Australia from 1993-2003 inclusive. T4 stage and vascular invasion were found to be the most important prognostic indicators. This was seen in both the overall population and in younger patients (≤75 yrs) who are more likely to be considered for chemotherapy.

Methods

Pathology records from four major hospitals in the state of Western Australia were used to identify a total of 2,272 patients diagnosed with Dukes' B CRC during the period 1993-2003 inclusive. The pathology services at these hospitals also process surgical specimens from other minor district and country hospitals and consequently information was obtained for the total Western Australian population numbering 1.8-2 million inhabitants over the study period.

Tumour stage was classified according to the current AJCC guidelines (AJCC, 2002; Compton *et al*, 2004). A total of 361 patients were found to have Dukes' B1 or Stage I disease, 36 were incorrectly staged in the initial pathology records (i.e. lymph node metastases were identified). Exclusion criteria were metachronous colon cancer, positive surgical margins, concomitant inflammatory bowel disease, familial polyposis coli or previous malignancies (n=28). A total of 1847 CRC patients fulfilled the criteria and 1468 of these were colon carcinomas. Rectal carcinomas were defined as originating within 12 cm of the anal verge and were excluded from the analysis.

Information on each of the pathological variables listed in Table 3.1 was obtained from the histopathology reports. Current AJCC guidelines categorise perforation of a tumour as T4. Perforation was considered present if noted by the surgeon at operation or on histopathological review of the specimen. For the 1468 colon

cancers, histopathology data was not recorded for tumour size in 120 patients. All tumours were staged as T3 or T4 but physical measurement of depth of invasion was absent for 761 patients. In 214 patients, all nodes examined were negative, but the number of nodes evaluated was not documented. Anatomical site of the tumour was ascertained from the pathology reports and cross-checked with information from admission and procedure records. Colon cancers were classified as being proximal or distal to the splenic flexure.

Patients who received adjuvant chemotherapy (n=162) were excluded from the data set in order to avoid possible influences of adjuvant treatment on survival. These patients were identified by hospital coding records of chemotherapeutic drug administration and from individual hospital cancer registries. A total of 1306 colon carcinoma patients treated by surgical resection alone were thus identified.

Mortality data was obtained from the Death Registry of the Health Department of Western Australia. Death reports were individually reviewed and classified as death due to cancer or death from other causes. This enabled disease-specific survival (death due to metastatic colon cancer) and overall survival to be evaluated. For the overall cohort (n=1306), 220 (16.9%) patients died from unrelated causes and 247 (18.9%) from colon cancer. For patients aged $\leq$75 years (n=666), 59 (8.9%) died from unrelated causes and 131 (19.7%) from colon cancer. The median length of follow-up was 59 months (range 0-145), mean follow-up was 60 months and the follow-up study period ended in February 2005. Peri-operative death within 4 weeks of surgery was excluded from the disease-specific survival analysis (n=54). Ethics approval for this project was obtained from the Human Research Ethics Committee at each hospital, the Confidentiality of Health Information Committee and the University of WA.

Statistical Analysis

Survival analysis was conducted using both Kaplan-Meier survival analysis and Cox proportional hazards regression. Survival was calculated from the date of diagnosis to date of death from colon cancer. Survival times were censored at the date of death from other causes or at 28 February 2005, whichever came first. Multivariable Cox proportional hazards regression was conducted to identify significant prognostic factors. All variables were initially modelled and variables of little prognostic significance were identified and removed via backward elimination conducted manually. The variables of tumour size, depth of invasion, and number of nodes examined were not included in this analysis because of missing data, but separate analyses indicated these did not have strong prognostic significance.

Results

Details of the pathological features assessed in this study population are shown in Table 3.1. Data is shown for both the overall (n=1306) and younger (n=666, ≤75 yrs) patient groups. The distribution of tumours in the colon was caecum (19%), ascending colon (23%), transverse colon (8%), descending colon (14%) and sigmoid colon (36%). Figure 3.1 illustrates the number of nodes examined for all patients. The mean number of nodes analysed (n=12) is consistent with recommendations in the literature (Petersen *et al*, 2002; Swanson *et al*, 2003).

The prognostic significance of each clinical and pathological feature for disease-specific survival is shown in Table 3.2 for 1306 stage II colon cancer patients, as well as the 666 patients aged ≤75 yrs. Age, sex, site, number of nodes examined, tumour size, depth of invasion, histological grade, lymphatic invasion, perforation, mucinous differentiation and lymphocytic response showed no prognostic significance in either of

the two patient groups. There was no survival difference between patients with ≤12 nodes examined (n=660) compared to those with >12 nodes examined (n=431; *P*=0.291). T4, vascular invasion, perineural invasion and obstruction were significant prognostic indicators for the overall group. The same features showed prognostic significance in the younger patients, although obstruction failed to reach significance, possibly reflecting the lower frequency of obstruction in younger patients (Table 3.1). Higher HR's were observed in the younger patient group relative to overall patients for both T4 (2.03 *vs.* 1.83) and vascular invasion (2.82 *vs.* 1.76).

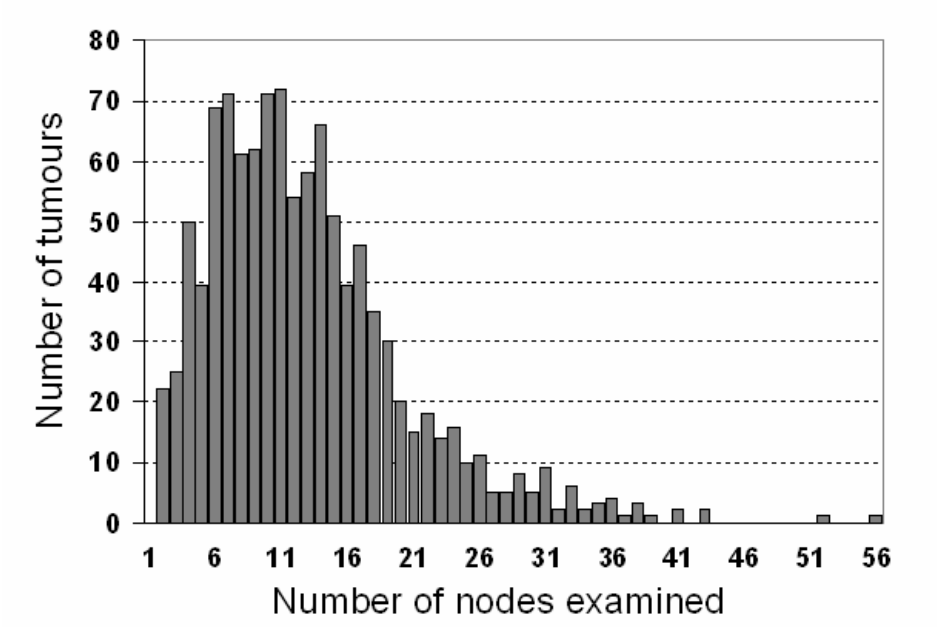


Figure 3.1. Number of nodes examined for the 1306 stage II colon cancers investigated in this study.

Table 3.1. Clinical and pathological features of stage II colon cancer patients treated by surgery alone.

Feature	**Overall**	**≤75 yrs**
Total	1306	666
Age (yrs)		
Mean (S.D.)	73.2 (11.4)	64.9 (9.3)
Median (range)	75 (18-97)	68 (18-75)
Number of nodes examined		
Mean (S.D.)	11.8 (7.5)	12.2 (7.6)
Median (range)	10 (1-55)	11 (1-55)
Tumour size (mm)		
Mean (S.D.)	48 (20.2)	48 (19.3)
Median (range)	45 (3-140)	45 (3-140)
Depth of invasion (mm)		
Mean (S.D.)	17.4 (12.9)	17.0 (12.3)
Median (range)	14 (1-122)	14 (1-100)
Sex		
Male	639 (48.9)	369 (55.4)
Female	667 (51.1)	297 (44.6)
Subsite		
Proximal colon	652 (49.9)	282 (42.3)
Distal colon	654 (50.1)	384 (57.7)
T stage		
T3	1045 (80.0)	547 (82.1)
T4	261 (20.0)	119 (17.9)
Histological grade		
Well/moderate differentiation	1160 (88.9)	585 (90.1)
Poor differentiation	145 (11.1)	66 (9.9)
Vascular invasion		
present	153 (11.7)	77 (11.6)
absent	1153 (88.3)	589 (88.4)
Lymphatic invasion		
present	142 (10.9)	79 (11.9)
absent	1164 (89.1)	587 (88.1)
Perineural invasion		
present	64 (4.9)	40 (6.0)
absent	1242 (95.1)	626 (94.0)
Perforation		
present	115 (8.8)	69 (10.4)
absent	1191 (91.2)	597 (89.6)
Obstruction		
present	230 (17.6)	93 (14.0)
absent	1076 (82.4)	573 (86.0)
Mucinous differentiation		
present	318 (24.3)	139 (20.9)
absent	988 (75.7)	527 (79.1)
Lymphocytic response		
present	208 (15.9)	91 (13.7)
absent	1098 (84.1)	575 (86.3)

Table 3.2. Prognostic significance of clinico-pathological features in stage II colon cancer patients treated by surgery alone.

Feature	Overall group			Patients ≤75 yrs		
	HR	**95% CI**	***P***	**HR**	**95%CI**	***P***
T stage						
T3	1.00			1.00		
T4	1.83	1.38-2.42	<0.0001	2.03	1.37-3.02	<0.0001
Vascular invasion						
Absent	1.00			1.00		
Present	1.76	1.25-2.48	0.002	2.82	1.82-4.35	<0.0001
Perineural invasion						
Absent	1.00			1.00		
Present	1.95	1.21-3.15	0.013	2.33	1.31-4.14	0.009
Obstruction						
Absent	1.00			1.00		
Present	1.45	1.07-1.96	0.020	1.51	0.96-2.37	0.090
Grade						
Well/moderate	1.00			1.00		
Poor	1.35	0.94-1.95	0.122	1.00	0.81-2.40	0.245
Mucinous						
Absent	1.00			1.00		
Present	0.81	0.59-1.10	0.172	0.68	0.42-1.11	0.113
Perforation						
Absent	1.00			1.00		
Present	1.21	0.79-1.87	0.387	1.55	0.92-2.62	0.119
Lymphatic invasion						
Absent	1.00			1.00		
Present	1.18	0.80-1.75	0.418	1.00	0.77-2.19	0.309
Lymphocytic response						
Absent	1.00			1.00		
Present	0.95	0.66-1.39	0.810	0.84	0.52-1.66	0.827
Sex						
Female	1.00			1.00		
Male	0.90	0.70-1.16	0.418	1.02	0.72-1.45	0.903
Subsite						
Proximal colon	1.00			1.00		
Distal colon	1.08	0.85-1.39	0.527	1.12	0.79-1.61	0.525
Nodes examined						
<6	1.00			1.00		
6-10	0.79	0.54-1.15		0.98	0.58-1.67	
11-15	0.64	0.42-0.98		0.79	0.44-1.41	
>15	0.79	0.53-1.18	0.242	1.04	0.61-1.78	0.786
Tumour size (mm)						

<35	1.00					
35-44	0.87	0.60-1.29		0.77	0.45-1.33	
45-59	0.92	0.63-1.33		0.84	0.52-1.34	
>59	0.91	0.63-1.32	0.922	0.86	0.54-1.37	0.820
Tumour depth (mm)						
<10	1.00					
10-14	0.86	0.46-1.61		0.86	0.41-1.84	
15-19	0.77	0.36-1.70		0.38	0.12-1.17	
>19	1.35	0.77-2.36	0.255	0.80	0.38-1.66	0.319
Age (years)						
<55	1.00			1.00		
55-59	0.60	0.27-1.33		0.61	0.27-1.37	
60-64	0.68	0.33-1.42		0.75	0.36-1.58	
65-69	0.79	0.42-1.49		0.80	0.42-1.54	
70-74	1.32	0.78-2.25		1.35	0.79-2.30	0.230
75-79	1.12	0.66-1.92				
80-84	1.02	0.59-1.76				
>84	1.34	0.76-2.36	0.068			

In multivariable analysis, the only independent prognostic features identified in the overall group were T4 (HR=1.75; 95%CI [1.32-2.32], $P<0.0001$) and vascular invasion (HR=1.63; 95%CI [1.15-2.30], $P<0.0001$). The same two features were also the only independent prognostic factors found in the younger age group (T4: HR=1.96; 95%CI [1.32-2.90], $P<0.001$; vascular invasion: HR=2.73; 95%CI [1.76-4.21], $P<0.0001$).

T stage and vascular invasion (VI+) were therefore used to define four prognostic groups (T3/VI-, T3/VI+, T4/VI- and T4/VI+). Survival curves for these subgroups in the overall and younger patient cohorts are shown in Figures 3.2A and 3.2B, respectively. The presence of vascular invasion was associated with worse prognosis in the younger age group. The good prognosis group was defined as T3/VI- and comprised 72% and 74% of the overall and younger patient categories, respectively. The poor prognosis group was defined as T3/VI+, T4/VI- or T4/VI+ and comprised 28% and 26% of the overall and younger patient cohorts, respectively. Five-year

survival rates in the overall patient cohort were 84% and 74% for good and poor prognosis groups ($P<0.0001$), respectively, while for the younger patient cohort they were 84% and 71% ($P<0.0001$).

Discussion

Clinical guidelines for the management of stage II colon cancer state that the standard of care is surgical resection alone (NIH, 1990; ACPGBI, 2001; NH&MRC, 1999). However, these guidelines are qualified by the statement that adjuvant chemotherapy may be considered in cases where there are pathological features of poor prognosis such as T4 stage, perforation or obstruction, poor differentiation and vascular invasion. Although there has been considerable effort directed towards the evaluation of prognostic significance for novel molecular-based prognostic markers (Graziano *et al*, 2003), surprisingly little attention has been paid to accurate evaluation of the conventional pathological prognostic markers. The aim of the current work was to investigate the commonly reported pathological variables in a large, population-based series of stage II colon cancers. More than 1300 patients diagnosed over an 11-year period and with a 5-year median follow-up time were examined. In order to facilitate the interpretation of survival data, only patients treated by surgery alone were included in the study. Chemotherapy is recommended for consideration in young stage II colon cancer patients with pathological features of poor prognosis and hence special attention was given here to the prognostic significance of markers in patients aged $\leq$75 years.

T4 and vascular invasion were the only independent clinico-pathological markers of poor prognosis found in this study, both in the overall and younger patient cohorts. Petersen *et al* (2002) also identified these factors in a series of 268 stage II

Fig 3.2A

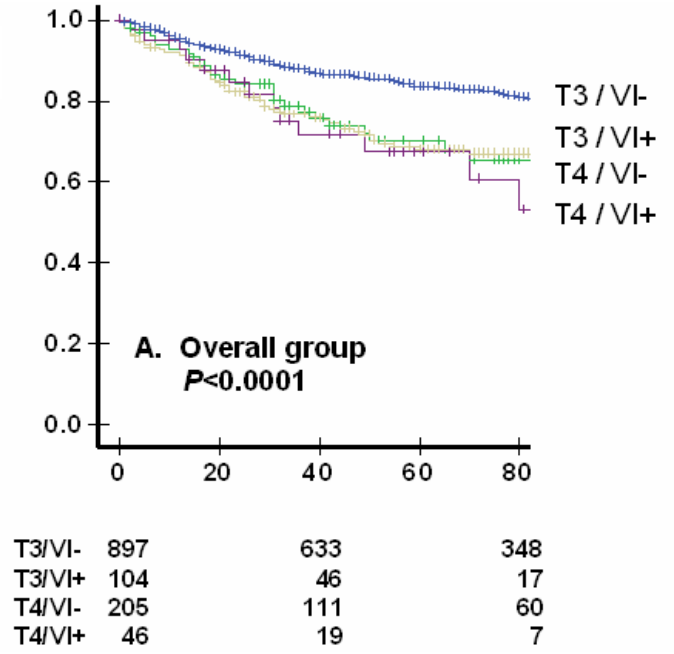


Fig 3.2B

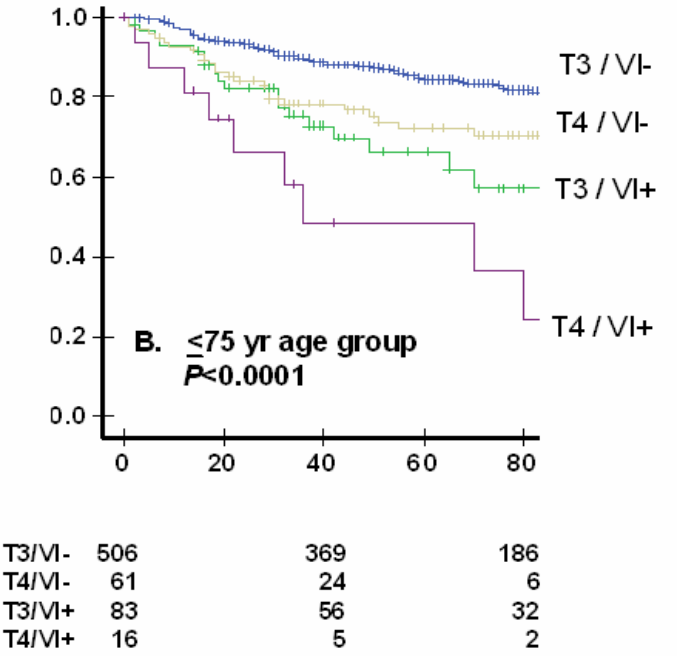


Figure 3.2. Kaplan-Meier survival analysis for disease-specific survival of stage II colon cancer patients treated by surgery alone. A, overall patient cohort; B, patients aged ≤75 years. Good prognosis patients were defined as T3 stage and without vascular invasion (T3/VI-), while poor prognosis patients were defined as T3/VI+, T4/VI- or T4/VI+. The *P* value compares survival between good and poor prognosis groups.

colon cancers, together with positive surgical margins and perforation. Burdy *et al* (2001) identified T4 in a study of 108 colon cancers and also reported independent significance for male gender, bowel obstruction and the number of nodes examined. None of the clinico-pathological factors other than T4 and vascular invasion reached significance in the current study involving a large (n=1306), population-based cohort, although obstruction was of borderline significance (HR=1.34; 95%CI [0.99-1.82], *P*=0.061) in multivariable analysis. Mulcahy *et al* (1997) found a trend for prognostic significance of vascular invasion in rectal but not colon cancers. Several previous groups have suggested the number of sampled lymph nodes is an important prognostic factor for colon cancer (Burdy *et al*, 2001; Le Voyer *et al*, 2003; Jestin *et al*, 2005; Caplin *et al*, 1998), with recommendations that at least 10-15 nodes be evaluated. The mean number of nodes examined in this cohort (n=12) was therefore within the recommended sample size for accurate staging. It is worth noting that although 25% of patients in this study had 6 or fewer nodes examined, these did not have significantly worse survival (Table 3.2).

Using the two independent prognostic markers identified in this study we were able to define good and poor prognosis groups with 5-year survival rates of 84% and 71%, respectively, for the younger patient cohort (Figure 3.2B). Histological review of tumour sections is currently in progress to determine whether more accurate pathological assessment of T stage and vascular invasion might have resulted in an even better survival rate for good prognosis patients. Any false negative assessment of these markers would have an adverse impact on the survival of the T3/VI- group. The use of conventional pathological markers to identify stage II colon cancer patients with $\geq$90% 5-year survival would clearly be advantageous in terms of indications for chemotherapy.

The relatively low 5-year survival rate (71%) observed for the poor prognosis group defined by T stage and vascular invasion (T3/VI+, T4/VI- or T4/VI+) warrants their strong consideration for chemotherapy, particularly for young and healthy patients. Data from the Western Australian population indicates that over the past decade, only 23% of stage II colon cancer patients aged $\leq$75 years and with the features of poor prognosis defined above were treated with chemotherapy (Chapter 4, Morris *et al*, 2007). This is of particular concern in light of recent evidence for a survival benefit from chemotherapy in stage II disease (Gray *et al*, 2004).

In conclusion, this population-based study highlights the importance of accurately evaluating T stage and vascular invasion for the prognostic assessment of stage II colon cancer. Identification of these markers as independent prognostic factors provides clarification of the guidelines for the management of this disease in relation to recommendations for chemotherapy.

Chapter 4

Survival rates for stage II colon cancer patients treated with or without chemotherapy in a population-based setting

Morris M, Platell C, McCaul K, Millward M, van Hazel G, Bayliss E, Trotter J, Ransom D and Iacopetta B: Survival rates for stage II colon cancer patients treated with or without chemotherapy in a population-based setting. Int J Colorectal Dis. 2007 (in press)

Abstract

Background: There is considerable uncertainty as to whether adjuvant 5-fluorouracil-based chemotherapy provides survival benefit for colon cancer patients with stage II disease. Consequently, the current rates of chemotherapy use for this disease are low, despite 5-year survival rates of only 70-80%. The aim of the present study was to compare the survival rate of stage II colon cancer patients treated by surgery alone with that of patients also treated by chemotherapy.

Methods: A population-based, observational study was conducted on the survival of stage II colon cancer patients (n=812) diagnosed in Western Australia from 1993-2003. The study was restricted to patients aged ≤75 years, of whom 18% (n=142) were treated with chemotherapy. Only 0.9% of patients older than 75 years received chemotherapy.

Results: Patients who received chemotherapy were significantly younger (mean age 60 years) than those treated by surgery alone (65 years, $P<0.001$) and their tumours were more often positive for vascular invasion ($P=0.007$). Multivariate analysis that included all prognostic factors revealed adjuvant chemotherapy was associated with improved survival (HR=0.62, 95%CI [0.39-0.98], $P=0.043$), with females gaining more benefit

(HR=0.48, 95%CI [0.20-1.22], *P*=0.09) than males (HR=0.94, 95%CI [0.54-1.64], *P*=0.8).

Conclusions: In view of the apparent survival benefit from chemotherapy for stage II colon cancer, the present study raises concerns about the current low rates of adjuvant treatment for this disease in the community, particularly for female patients.

Introduction

Between 30-40% of colon carcinomas are diagnosed as AJCC stage II disease (T3/4N0M0) at resection (Jessup *et al*, 1996; Jemal *et al*, 2004). Five-year survival rates for these patients vary between 60-80% (Zaniboni *et al*, 2004) and although up to 40% will develop disease recurrence during their lifetime, the role of adjuvant chemotherapy in this setting is presently uncertain (Macdonald, 1999). Randomised controlled trials conducted in the 1980's demonstrated that 5-fluorouracil (5FU)-based therapy resulted in a 10% absolute improvement in 5-year survival for stage III CRC patients (Moertel *et al*, 1990). As a result of these trials the National Institutes of Health recommended in 1990 the routine administration of FU-based adjuvant chemotherapy for medically fit patients with completely resected stage III colorectal carcinoma (NIH, 1990). It did not however recommend any specific adjuvant therapy for stage II patients outside of clinical trials. Subsequent randomised controlled trials and meta-analyses relevant to stage II disease have unfortunately been underpowered to determine whether such patients derive an overall survival benefit from adjuvant chemotherapy (Moertel *et al*, 1995; IMPACT B2, 1999). The International Multi-center Pooled Analysis of B2 Colon Cancer Trials compared 6 months of 5FU/leucovorin chemotherapy against surgery alone in 1,016 stage II colon cancer patients from five different trials (IMPACT B2, 1999). The absolute reduction in risk of death in patients receiving chemotherapy was

only 2% and this was not statistically significant. On the basis of these results, the routine use of adjuvant chemotherapy was not recommended for stage II colon cancer (IMPACT B2, 1999; Marsoni, 2001). More recently Gill *et al* (2004) presented a pooled dataset of 3,302 patients with stage II colon cancer from seven randomised trials comparing 5FU/leucovorin or 5FU/levamisole versus surgery alone (Gill *et al*, 2004). A disease-free survival benefit was seen for patients receiving chemotherapy but this did not translate into significant overall survival benefit.

Other studies however have found evidence of survival benefit from 5FU treatment in stage II colon cancer patients. The NSABP group carried out a pooled analysis of outcome data from four of their trials (C-01 to C-04) comprising over 1,500 stage II patients in total. A reduction in mortality of 30% was observed with adjuvant therapy (Mamounas *et al*, 1999). Significantly, subgroup analysis revealed this benefit also occurred in the poor prognosis groups of T4 tumour stage and patients with obstruction or perforation (Mamounas *et al*, 1999; Mamounas, 2000). Another recent meta-analysis on adjuvant therapy for stage II colon cancer reported data for 4,187 patients (Figueredo *et al*, 2004). The reduction in mortality for treated patients was approximately 13%, however the result just failed to reach significance (HR=0.87, 95%CI [0.75-1.01], P=0.07).

The standard of care for stage II colon cancer therefore remains surgical resection alone. USA, UK and Australian national guidelines state that adjuvant chemotherapy may be considered for patients who have “poor prognosis” markers or in the setting of clinical trials (NIH, 1990; ACPGBI, 2001; NH&MRC, 1999). Estimates of chemotherapy use in stage II colon cancer are 15% in Australia (Clinical Governance Unit, 2002) and approximately 30% in the USA (Schrag *et al*, 2002; Potosky *et al*, 2002) but there is widespread variation depending upon clinician bias, patient age and

geographical location. The aim of the present study was to evaluate survival rates for stage II colon cancer patients treated with or without chemotherapy in a population-based setting. The study was restricted to cases aged ≤75 years at diagnosis because adjuvant chemotherapy for early stage disease concerns almost exclusively the younger patient group. Approximately one fifth of these patients received adjuvant chemotherapy, allowing investigation of the predictive significance of various clinical and pathological features for survival benefit from 5FU chemotherapy.

Methods

Patient and tumour information

Pathology records from four major hospitals in the state of Western Australia were used to identify a total of 2,272 patients diagnosed with stage II CRC during the period 1993-2003 inclusive. The pathology services at these hospitals also process surgical specimens from district, country and minor private hospitals and consequently information was obtained for the total Western Australian population numbering 1.8-2 million inhabitants over the study period. Tumour stage was classified according to current AJCC guidelines (AJCC, 2002; Compton *et al*, 2004) Information on the anatomical site of the tumour and on each of the pathological variables listed in Table 4.1 was obtained from histopathology reports and cross checked with information from admission and procedure records. Colon cancers were classified as being proximal or distal to the splenic flexure.

Exclusion criteria were age >75 years at diagnosis, metachronous colon cancer, concomitant inflammatory bowel disease, familial polyposis coli or previous malignancies and positive surgical margins. The age limit was chosen arbitrarily in an attempt to eliminate co-morbidities from the data set and because adjuvant

chemotherapy was given to only 6 of the 656 (0.9%) stage II colon cancer patients aged >75 years in this population. A total of 1,085 CRC cases fulfilled the criteria and 812 of these were colon carcinomas. Rectal carcinomas were defined as originating within 12cm of the anal verge and were excluded from the current analysis. For the 812 colon cancers, data was not recorded for tumour size in 43 cases, number of nodes evaluated in 100 cases and quantitative assessment of the depth of invasion in 430 cases.

Information on treatment with adjuvant 5FU-based chemotherapy was obtained from the cancer registries of the medical oncology departments of the three teaching hospitals and from the hospital coding database at the private hospital. Patients were considered to have received chemotherapy if one or more cycles were given. The date at which chemotherapy was given relative to the surgical date was used to distinguish adjuvant treatment from treatment for recurrence, with 6 months post-surgery being the cut-off period. 5-Fluorouracil-based regimens were used in the chemotherapy of all patients. Of the 812 colon cancer patients, 146 (18.0%) received chemotherapy.

Disease-specific mortality data was obtained from the Death Registry of the Health Department of Western Australia. Of the 666 colon cancer patients treated by surgery alone, 131 (19.7%) died from their disease and 59 (8.9%) from other causes. Of the 146 patients who received chemotherapy, 24 (16.4%) died of their disease and 7 (4.8%) from other causes. Median length of follow-up was 61 months (range 0-145 months), mean follow-up was 60 months and the follow-up study period for survival ended in February 2005. Peri-operative deaths within 4 weeks of surgery were excluded from the analysis. Ethics approval for this project was obtained from the Human Research Ethics Committee at each hospital, the Confidentiality of Health Information Committee and the University of Western Australia.

Statistical analysis

Survival analysis was conducted using both Kaplan-Meier survival analysis and Cox proportional hazards regression. Statistical significance was deemed if $P<0.05$. Survival was calculated in days from date of diagnosis to date of death from colon cancer. Survival times were censored at the date of death from other causes or at 28 February 2005, whichever came first. Initial analyses suggested that patient sex, tumour site, and prognostic significance were modifying the effect of chemotherapy. Cox proportional hazards regression was used to estimate the effect of chemotherapy and also to identify and adjust for other confounding factors. For example, a Cox model was initially fitted for the effect of sex, the effect of chemotherapy and an interaction between these two factors thus enabling the effect of chemotherapy to be estimated in males and in females. The potential confounding by other factors was then assessed using the "change-in-estimate" method (Rothman *et al*, 1998). A factor was considered to be a confounder if, after adjustment for that factor, the effects of chemotherapy in either males or females changed by more than 15%. Similar models were developed to estimate the effects of chemotherapy by tumour site and by prognostic group.

Results

Clinical and pathological information for the 812 stage II colon cancer patients aged $\leq$75 yrs investigated in this study is shown in Table 4.1. Only 18% of patients received adjuvant chemotherapy and these were on average 5 years younger than those treated by surgery alone. A significantly higher proportion of cases with vascular invasion received adjuvant chemotherapy. No significant differences in the use of chemotherapy were apparent for any of the other clinical or pathological features evaluated, with the exception of marginally less frequent use in patients with mucinous tumours. The use of

adjuvant chemotherapy during the study period peaked at 25-30% of patient in the late 1990's but then decreased to less than 15% in 2002 and 2003 (Figure 4.1).

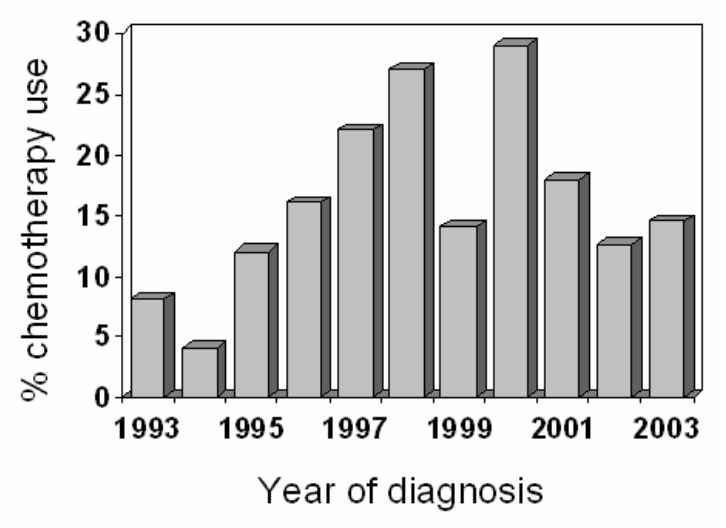


Figure 4.1. Adjuvant chemotherapy use in stage II colon cancer patients aged ≤75 years diagnosed in Western Australia from 1993-2003.

The age distribution of patients who received adjuvant chemotherapy is shown in Figure 4.2. Approximately 25% of patients aged ≤65 yrs received chemotherapy compared to only 10% of those aged between 66-75 yrs.

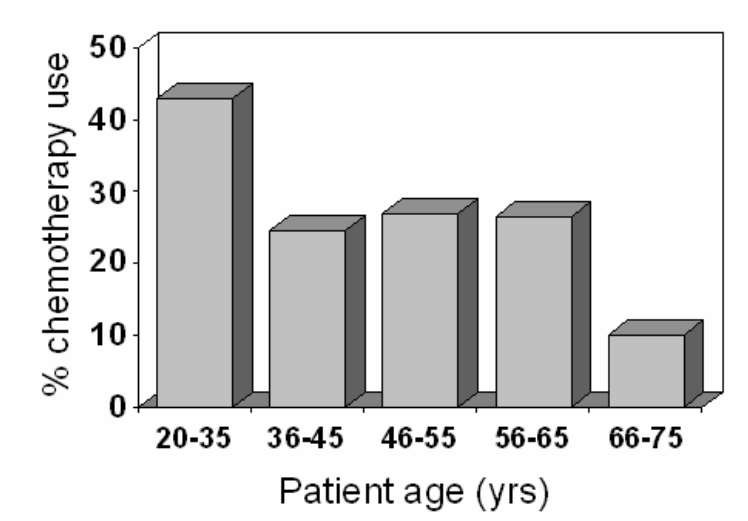


Figure 4.2. Adjuvant chemotherapy use in stage II colon cancer patients from different age groups in the Western Australian population.

Although this population-based series was not randomized for adjuvant treatment, the 146 patients who received chemotherapy were relatively well matched to those treated by surgery alone (Table 4.1). In the overall patient group, adjuvant chemotherapy was associated with a relative survival benefit of approximately 35% and this was of borderline significance (HR=0.64, 95%CI [0.40-1.01], *P*=0.055).

The predictive values for each clinical and pathological feature were estimated by comparing survival rates for patients treated with or without adjuvant chemotherapy (Table 4.2). These should be regarded as exploratory because of the relatively small sample size in many of the subgroups, particularly for the number of patients receiving adjuvant chemotherapy. Features associated with trends for survival benefit from adjuvant chemotherapy in univariate analysis were female gender, proximal site, vascular invasion and larger tumour size.

Table 4.1. Pathological features of stage II colon cancer patients (≤75 yrs) treated by surgery alone or with surgery and adjuvant chemotherapy.

Feature (n)	**Surgery (%)**	**Chemotherapy (%)**	***P***
Total (812)	666 (82)	146 (18)	
Age (yrs)			
Mean	64.9	59.8	<0.001
Median (range)	68 (18-75)	62 (30-75)	
Sex			
Male (454)	369 (81)	85 (19)	0.54
Female (358)	297 (83)	61 (17)	
Number of nodes examined (712)			
Mean	12.2	13.0	0.31
Median (range)	11 (1-55)	12 (1-56)	

Tumour size (mm) (769)			
Mean	48	50	0.23
Median (range)	45 (3-140)	49 (15-120)	
Depth of invasion (mm) (382)			
Mean	17.0	17.3	0.56
Median (range)	14 (1-100)	14 (5-100)	
Subsite			
Proximal colon (344)	282 (82)	62 (18)	0.98
Distal colon (468)	384 (82)	84 (18)	
T stage			
T3 stage (659)	547 (83)	112 (17)	0.13
T4 stage (153)	119 (78)	34 (22)	
Histological grade			
Well/mod. differentiation (714)	585 (82)	129 (18)	0.44
Poor differentiation (77)	66 (86)	11 (14)	
Vascular invasion			
present (106)	77 (73)	29 (27)	0.007
absent (706)	589 (83)	117 (17)	
Lymphatic invasion			
present (96)	79 (82)	17 (18)	0.97
absent (716)	587 (82)	129 (18)	
Lymphovascular invasion			
present (159)	126 (79)	33 (21)	0.31
absent (653)	540 (83)	113 (17)	
Perineural invasion			
present (47)	40 (85)	7 (15)	0.58
absent (765)	626 (82)	139 (18)	
Perforation			
present (86)	69 (80)	17 (20)	0.65
absent (726)	597 (82)	129 (18)	
Obstruction			
present (115)	93 (81)	22 (19)	0.73
absent (697)	573 (82)	124 (18)	
Mucinous differentiation			
present (159)	139 (87)	20 (13)	0.05
absent (653)	527 (81)	126 (19)	
Lymphocytic response			
present (108)	91 (84)	17 (16)	0.51
absent (704)	575 (82)	129 (18)	

In a multivariate model that included all prognostic factors, the use of adjuvant chemotherapy was associated with significantly improved survival (HR=0.62, 95%CI [0.39-0.98], *P*=0.043). Multivariate analysis adjusted for age and vascular invasion confirmed the gender difference in response, with females showing a trend for more survival benefit than males (Table 4.3). We also examined survival benefit from

adjuvant chemotherapy within good and poor prognosis groups defined by T stage and vascular invasion (Chapter 3; Morris *et al*, 2006)). Good prognosis patients were T3 and lacked vascular invasion, while poor prognosis patients were T4 and/or showed vascular invasion. Although not statistically significant, poor prognosis patients appeared to gain more benefit from chemotherapy than good prognosis patients (Table 4.3).

Figure 4.3 shows Kaplan-Meier analysis for disease-specific survival of good and poor prognosis patients treated with and without adjuvant chemotherapy. In good prognosis patients the 5-year survival rate improved with chemotherapy from 85% to 95% (P=0.064, log-rank test) in females but showed no change in males (84% *vs.* 82%). For the poor prognosis patients, survival rates for both females (65% *vs.* 79%) and males (72% *vs.* 78%) improved with the use of chemotherapy, however this did not reach significance in either group (P=0.22 and P=0.26, respectively).

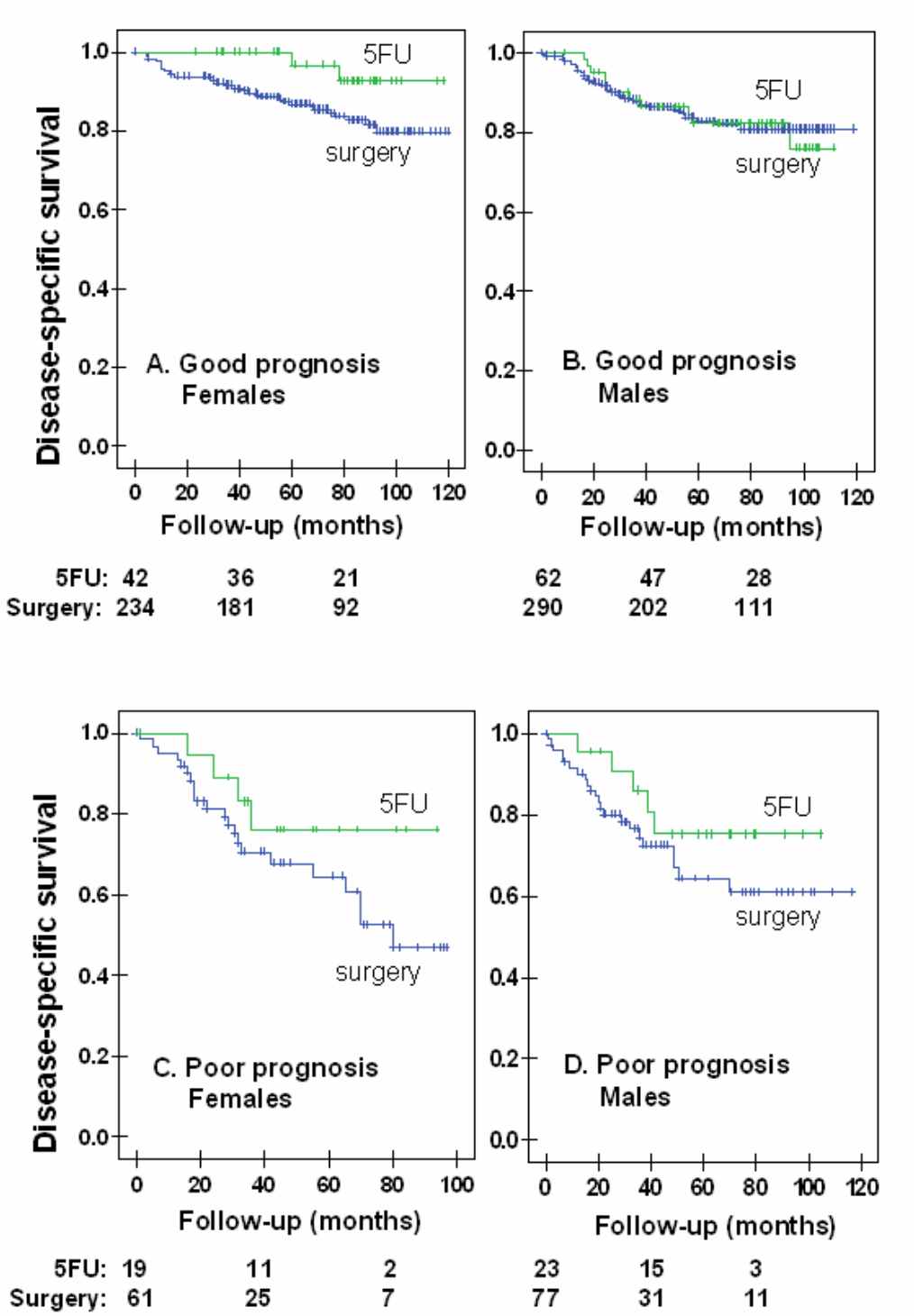


Figure 4.3. Evidence for survival benefit from chemotherapy in female (A, C) and male (B, D) patients with good and poor prognosis stage II colon cancer.

Table 4.2. Predictive significance of clinical and pathological features for overall survival benefit from 5FU in stage II colon cancer patients aged ≤75 yrs.

Feature (n1,n2) [a]	**HR**	**95% CI**	***P***
Total (666,146)	0.64	0.40-1.01	0.055
Age groups (years)			
<55 (94,36)	0.55	0.18-1.63	0.28
55-59 (74,17)	1.47	0.40-5.42	0.57
60-64 (87,40)	0.80	0.28-2.28	0.68
65-69 (129,33)	1.08	0.43-2.69	0.87
70-75 (282,20)	0.71	0.26-1.95	0.51
Sex			
Male (369,85)	0.77	0.44-1.34	0.36
Female (297,61)	0.47	0.20-1.09	0.08
Nodes examined			
<6 (106,23)	1.18	0.47-2.93	0.73
6-10 (183,30)	1.19	0.53-2.67	0.68
11-15 (142,36)	0.70	0.24-2.03	0.51
16+ (155,37)	0.63	0.24-1.64	0.35
Tumour size (mm)			
<35 (143,25)	0.84	0.32-2.14	0.71
35-44 (155,28)	2.00	0.97-4.12	0.06
45-59 (165,38)	0.38	0.12-1.26	0.11
60+ (174,41)	0.47	0.17-1.34	0.16
Depth of Invasion (mm)			
<10 (66,16)	0.58	0.13-2.58	0.47
10-14 (87,27)	0.55	0.16-1.92	0.35
15-19 (51,15)	2.77	0.62-12.42	0.18
20+ (100,20)	0.57	0.13-2.47	0.45
Subsite			
Proximal colon (282,62)	0.50	0.22-1.10	0.09
Distal colon (377,82)	0.74	0.42-1.31	0.30
T stage			
T3 (547,112)	0.62	0.34-1.10	0.10
T4 (119,34)	0.54	0.25-1.17	0.12
Histological grade			
Well/moderate diff. (585,128)	0.74	0.46-1.19	0.22
Poor differentiation (65,11)	0.03	0.00-10.79	0.25
Vascular invasion			
Present (78,29)	0.42	0.17-1.04	0.06
Absent (588,117)	0.65	0.38-1.12	0.12
Lymphatic invasion			
Present (78,17)	0.47	0.11-2.05	0.32
Absent (588,129)	0.66	0.41-1.08	0.10
Perineural invasion			
Present (40,7)	0.67	0.15-2.99	0.60
Absent (627,139)	0.65	0.4-1.06	0.08
Perforation			
Present (69,17)	0.58	0.17-1.99	0.38
Absent (597,129)	0.64	0.39-1.05	0.08
Obstruction			

Present (93,22)	0.48	0.14-1.59	0.23
Absent (573,124)	0.67	0.41-1.11	0.12
Mucinous histology			
Present (139,20)	0.27	0.04-1.99	0.20
Absent (572,126)	0.68	0.42-1.10	0.11
Lymphocytic response			
Present (83,13)	0.31	0.04-2.38	0.26
Absent (583,133)	0.64	0.40-1.05	0.08

[a] n1 = surgery alone, n2 = surgery and chemotherapy

Table 4.3. Effect of adjuvant chemotherapy on survival of stage II colon cancer patients according to sex, tumour site and prognostic group. Multivariate Cox regression analysis adjusted for age and vascular invasion.

	Feature	**Hazard Ratio**	**95% CI**	***P***
	Total	0.62	0.39 - 0.98	0.043
Model 1	Female	0.48	0.20 - 1.22	0.09
	Male	0.94	0.54 - 1.64	0.80
Model 2	Proximal	0.62	0.28 - 1.40	0.25
	Distal	0.78	0.47 - 1.46	0.50
Model 3	Good prognosis	0.82	0.45 - 1.49	0.52
	Poor prognosis	0.60	0.29 - 1.26	0.18

Discussion

Western Australia has an isolated and relatively non-migratory population of approximately 2 million people. Centralized health records make this state an excellent site for population-based research (Holman *et al*, 1999). The current retrospective, observational study has evaluated survival rates for the state's entire stage II colon carcinoma population over an 11year period, starting from the time at which 5FU-based

adjuvant chemotherapy was introduced for this cancer type. In terms of research design, the results of randomised controlled trials are considered to provide the highest level of evidence whereas observational studies are viewed as having less validity because they reportedly over-estimate treatment effects (MacMahon *et al*, 2001; Collins *et al*, 2001). This viewpoint has recently been challenged by workers who argue that results from observational studies may be less prone to heterogeneity because they more broadly represent the population at risk (Concato *et al*, 2000; Benson *et al*, 2000). In contrast, individual randomised controlled trials may comprise distinct groups of patients as a result of specific inclusion and exclusion criteria and the experimental treatment protocol may not be representative of general clinical practice.

The present study was restricted to patients aged ≤75 years at diagnosis in order to minimize the influence of co-morbidities and because only 0.9% of stage II colon cancer patients aged >75 years received adjuvant chemotherapy. This finding demonstrates that clinicians are very cautious in their use of adjuvant treatments for older patients. Changes to this practice may occur following the recent publication of evidence for survival benefit from chemotherapy in elderly colon cancer patients (Sargent *et al*, 2001; Gill *et al*, 2004). As expected, the major determining factor in the present patient cohort for the use of adjuvant chemotherapy in stage II colon cancer was young age (Figure 4.2). In accordance with recommendations on the use of adjuvant chemotherapy for stage II disease, patients having the poor prognosis feature of vascular invasion were also significantly more likely to receive chemotherapy (Table 4.1). Interestingly however, several features of poor prognosis identified in current guidelines including T4 stage, larger tumour size, poor histological grade, perforation and obstruction did not influence the likelihood of adjuvant treatment in this community-based cohort. We conclude from our results that patient age and vascular invasion were

the only factors that significantly influenced the use of adjuvant chemotherapy for stage II colon carcinoma in the Western Australian population.

Fewer than 20% of patients aged ≤75 years were treated with chemotherapy over the study period. Adjuvant chemotherapy use in Western Australia peaked in 2000 at almost 30% and has since decreased to less than 15% in recent years (Figure 1). A similar frequency of use was reported by an Australia-wide pattern of care study into this disease (Clinical Governance Unit, 2002). In light of evidence presented here (Tables 4.2 and 4.3 and Figure 4.3) and elsewhere (Mamounas *et al*, 1999; Mamounas *et al*, 2000; Figueredo *et al*, 2004; Gray *et al*, 2004) for a survival benefit from 5FU in stage II colon cancer, this low rate is of concern. Furthermore, only 23% of the poor prognosis patients defined as being T4 stage and/or having vascular invasion were treated with adjuvant chemotherapy, highlighting the lack of strict adherence to guidelines even in relatively young patients.

Multivariate analysis revealed an interesting gender difference. Similar to previous results by our group for a population-based series of stage III CRC patients (Elsaleh *et al*, 2000), females with stage II colon cancer showed a trend for survival benefit from chemotherapy (Table 4.3), particularly for the good prognosis females (Figures 4.3A). It is well established that females suffer more toxicity from 5FU treatment than males (Sloan *et al*, 2002; Patel *et al*, 2004; Chansky *et al*, 2005), probably due to gender differences in the metabolism of this drug (Milano *et al*, 1992; Milano *et al*, 1994). Gender differences in response were not observed in a recent pooled analysis of stage II and III colon cancers from randomized trials (Gill *et al*, 2004). This discrepancy may reflect differences in patient profiles between controlled trials and observational studies, as alluded to earlier. Trial patients are more likely to have poor prognosis stage II disease and we also found less evidence for gender

differences in benefit from adjuvant chemotherapy in this group (Figures 4.3C and 4.3D). Interestingly, in the present study the 5-year survival rate for females with good prognosis tumours treated by surgery alone was 85% and increased to 95% with chemotherapy (Figure 4.3A). We hypothesize that increasing the 5FU treatment dose for males in order to reach the same level of toxicity as that observed for females may result in a similar level of survival benefit between the two sexes.

Histopathology data obtained from public and private pathology services in the state of Western Australia has recently allowed us to identify a subgroup of stage II colon cancer patients with poor prognosis (5-year survival rate of 71%, Chapter 3; Morris *et al*, 2006). These comprised 26% of all cases and were defined as being T4 and/or presenting with vascular invasion. Current guidelines recommend these patients be considered for adjuvant chemotherapy, however whether they derive survival benefit from this treatment has yet to be conclusively established. Results from the present study suggest that chemotherapy is a reasonable option for poor prognosis patients. Although not reaching statistical significance, evidence for a survival benefit was observed for both sexes in this population-based study (Table 4.3 and Figures 4.3C and 4.3D). Recent discussion has centred on whether the use of combination regimenns including oxaliplatin with 5FU may allow high risk stage II colon cancer patients to derive increased benefits over standard adjuvant 5FU regimens (Grothey *et al*, 2005).

Mature results are eagerly awaited for the QUASAR trial which randomised 2,092 patients with stage II colon cancer to adjuvant treatment with a 5FU-based regimen or to surgery alone. Conclusions published so far in abstract form indicate that chemotherapy produces a small absolute survival benefit of 1-5% for stage II patients and that for high-risk, younger patients this may be sufficient to outweigh the

inconvenience, toxicity and cost of treatment (Gray *et al*, 2004). The authors highlight the need for longer follow-up and for clarification of the benefits to older patients.

In summary, results from the current observational study suggest that women with stage II colon cancer are likely to gain survival benefit from 5FU-based adjuvant chemotherapy, although this requires confirmation in further studies. Both men and women with bad prognosis tumours also appear to benefit from 5FU chemotherapy and these could be considered for entry into trials of novel treatments.

Chapter 5

Failure to complete adjuvant chemotherapy is associated with adverse survival in stage III colon cancer patients

Morris M, Platell C, Fritschi L, Iacopetta B: Failure to complete adjuvant chemotherapy is associated with adverse survival in stage III colon cancer patients. Br J Cancer 96: 701-707, 2007.

Abstract

Background: Two recent North American studies have shown that completion of 5-fluorouracil-based adjuvant chemotherapy is a major prognostic factor for the survival of elderly stage III colon cancer patients. The aim of the present study was to confirm this finding in a population-based series from Australia.

Methods: The study cohort comprised 851 stage III colon cancer patients treated by surgery alone and 461 who initiated the Mayo chemotherapy regimen.

Results: One-third of patients who initiated chemotherapy failed to complete more than 3 cycles of treatment. Independent predictors for failure to complete were treatment in district or rural hospitals, low socio-economic index and treatment by a low volume surgeon. Patients who failed to complete chemotherapy showed worse cancer-specific survival compared not only to those who completed treatment (HR=2.24; 95%CI [1.66-3.03], $P<0.001$) but also to those treated by surgery alone (HR=1.37; 95% CI [1.09-1.72], $P=0.008$).

Conclusions: The current and previous studies demonstrate the importance of completing adjuvant 5-fluorouracil based chemotherapy for colon cancer. Further prospective studies are required to better identify the physiological and socioeconomic

factors responsible for failure to complete chemotherapy so that appropriate improvements in health service delivery can be made.

Introduction

Randomised controlled trials conducted in the 1980's demonstrated that 5-fluorouracil (5FU)-based chemotherapy resulted in a 10% absolute improvement in 5-year survival for stage III CRC (CRC) patients (Moertel *et al*, 1990). As a result of these trials the National Institutes of Health recommended in 1990 the routine administration of FU-based adjuvant chemotherapy for medically fit patients with completely resected stage III CRC (NIH, 1990). In the early 1990's, adjuvant chemotherapy with 5FU was used in combination with levamisole or leucovorin and regimens varied from 6 to 12 months in length. By 1995, the standard of care in many countries, including Australia, had become the Mayo regimen of intravenous 5FU/leucovorin for 6 months.

Randomised controlled clinical trials generally analyse the benefits of treatment in patient cohorts with few comorbidities. Participants in the earlier randomised clinical trials for CRC were highly selected and most patients were aged <70 years. These do not accurately represent all patients who may ultimately become candidates for treatment in the general population. Nevertheless, several reports have recently documented a similar degree of survival benefit from 5FU in older patient groups from a population-based setting (Sundararajan *et al*, 2002; Neugut *et al*, 2006; Iwashyna *et al*, 2002; Dobie *et al*, 2006; Jessup *et al*, 2005). These results support earlier evidence from randomised control trials and clearly establish benefit from 5FU-based adjuvant chemotherapy in stage III colon cancer.

Two recently published studies using the SEER database examined early termination of adjuvant chemotherapy regimens in the elderly population in relation to

survival (Neugut *et al*, 2006; Dobie *et al*, 2006). These papers reported that patients who failed to complete 5FU-based chemotherapy showed significantly worse survival compared to those who completed the treatment. Confirmation of the findings with respect to completion of treatment has important implications for the delivery of effective healthcare to patients with colon cancer. This paper examines the effect on survival of failure to complete adjuvant chemotherapy in a population-based cohort that includes patients of all ages and who were treated exclusively with the Mayo regimen.

Methods

Study Population

Pathology records from the four major hospitals in Western Australia were used to identify patients diagnosed with CRC during the period 1994-2001 inclusive. The pathology services at these hospitals also process specimens from minor district and country hospitals. This patient list was cross-checked with the Cancer Registry of Western Australia. Approximately 90% of all CRC patients who underwent surgical resection were identified for the population of Western Australia, comprising 1.8-2 million people over the study period. Tumour stage was classified according to the current American Joint Committee on Cancer (AJCC) guidelines (AJCC, 2002). A total of 2,024 patients with CRC fulfilled the criteria for stage III CRC and 1,404 of these had colonic carcinoma. All cases showed clear margins (R0 resections). Rectal carcinomas were defined as originating within 12 cm of the anal verge and these were excluded from the analysis. Information on pathological variables was obtained from the histopathology reports. Perforation was considered to be present if noted by the surgeon at operation or on histopathological review of the specimen. Clinical records were used to classify tumours presenting with obstruction. Anatomical site of the

tumour was cross-checked with information from admission and procedure records. Colonic cancers were subclassified as being proximal or distal to, and including, the splenic flexure.

Surgical case load was defined as low (≤10), medium (11-50) and high (>50) for stage II and III colon cancer resections over the 8-year study period. Hospitals were classified as teaching (university affiliated), private (fee for service), district (non-teaching and non-private institutions located in the metropolitan region of Perth) or rural (non-metropolitan). Each patient's post code address was obtained from the West Australian Cancer Registry database and this was linked to Socio-Economic Indexes for Areas (advantaged/disadvantaged and economic resources) obtained from the 2001 Australian census (Australian Bureau of Statistics, 2001). Patients with an advantaged/disadvantaged score of 1 were the most deprived quintile in socioeconomic terms, while a score of 5 corresponds to the most advantaged group. Patients with a score of 1 for economic resources had the least financial resources, while those with a score of 5 had the most. Ethics approval for the project was obtained from individual hospital Human Research Ethics Committees, the University of Western Australia, and the Confidentiality of Health Information Committee.

Adjuvant chemotherapy

Procedure codes from the morbidity database of the Data Linkage Unit, Health Department of Western Australia were used to identify patients who began chemotherapy within 120 days of resection. The adjuvant chemotherapy regimens used in Western Australia varied during the study period. Cases were individually reviewed using hospital records and only those patients (n=461) who received the Mayo regimen were included in the study. A total of 92 patients who received the Roswell or other

regimens were excluded. Patients who received chemotherapy for a recurrence were also documented (n=150). Less than 15 doses administered was defined as ≤3 cycles (n=156) and 16-30 doses as 4-6 cycles (n=305). Therefore, the study cohort (n=1,312) comprised 851 patients treated by surgery alone and 461 who initiated the Mayo chemotherapy regimen.

Survival data

Mortality data were obtained from the Death Registry of the Health Department of Western Australia. Death reports were reviewed individually and classified as death due to colon cancer or from other causes. The peri-operative mortality rate (4.8%) was defined as death within 30 days of surgery. At the end of the study period, 155 (11.8%) patients died from unrelated causes and 657 (50.1%) from recurrence of colonic cancer. Of the 461 patients who initiated adjuvant chemotherapy, 3 patients died as a result of chemotherapy treatment (0.65%). Sepsis and pancytopaenia were responsible for 2 deaths and 1 patient died of gastrointestinal haemorrhage secondary to a duodenal ulceration. One other patient died from a cerebrovascular accident whilst on chemotherapy 3 months post resection. Survival time was calculated from the date of diagnosis to date of death from cancer or 1/3/2006, whichever came first. This enabled cancer-specific and overall survival to be evaluated. The mean length of follow-up was 52 months and the median was 36 months (range 0-147 months).

Statistical analysis

Chi square analysis was used to identify factors influencing the rates of chemotherapy initiation and completion. A multiple logistic regression model in which each demographic, pathological and clinical variable listed in Table 5.1 was adjusted for all

others was used to estimate odds ratios and 95% confidence intervals for independent predictors of chemotherapy initiation or completion. Survival analysis was conducted using both unadjusted Kaplan-Meier analysis and Cox proportional hazards regression. The log-rank test was used to determine the significance for Kaplan Meier analysis. A Cox proportional hazards regression model was developed for survival in which each variable was adjusted for all others. Statistical significance was deemed if $P<0.05$.

Results

In this population-based cohort, just over one-third of stage III colon cancer patients initiated chemotherapy using the Mayo regimen (Tables 5.1 and 5.2). From 1997 to 2001 the rate remained steady at approximately 40% of cases. This is probably reflective of stabilization of surgical referral and oncological practice following the initial period of 5FU chemotherapy implementation. As expected, chemotherapy use declined with increasing age. Patients treated in private hospitals and those whose tumours were detected by colonoscopy or sigmoidoscopy were more likely to initiate chemotherapy. These same three factors were found in multivariate analysis to be independent predictors for the initiation of chemotherapy (Figure 5.1). None of the pathological variables was associated with the commencement of chemotherapy.

Table 5.1. Initiation of adjuvant chemotherapy for stage III colon cancer patients according to demographic factors (n=1312).

Characteristic	Percentage of total cases	Rate of chemotherapy initiation, %

Total	100.0	35.1
Year of diagnosis		
1994	11.2	16.3 [a]
1995	11.7	32.5
1996	13.7	33.9
1997	16.1	39.6
1998	13.7	40.7
1999	13.5	47.0
2000	10.1	39.4
2001	9.9	40.0
Age		
≤ 55 yrs	16.8	52.5 [b]
56-65 yrs	21.8	52.1
66-75 yrs	32.4	31.1
≥ 76 yrs	29.0	16.8
Sex		
Male	51.6	37.0
Female	48.4	33.4
Advantage/Disadvantage		
1	22.0	33.6
2	23.3	36.5
3	18.8	32.9
4	18.5	35.6
5	17.4	39.6
Economic Resources		
1	22.2	32.5
2	23.1	34.0
3	17.6	32.6
4	19.1	40.2
5	18.0	39.3
Hospital		
Teaching	46.0	31.8 [b]
District	11.2	21.1
Rural	14.3	33.3
Private	28.5	47.1

[a] $P<0.025$; [b] $P<0.0001$

Table 5.2. Initiation of adjuvant chemotherapy for stage III colon cancer patients according to pathological and clinical factors (n=1312).

Characteristic	Percentage of total cases	Rate of chemotherapy initiation, %

Total	100.0	35.1
Pathology		
Site		
Proximal	53.5	33.0
Distal	46.5	37.5
Grade		
Well/moderate	76.1	34.8
Poor	23.9	36.1
T stage		
T1/T2	5.0	41.6
T3	71.0	35.8
T4	24.0	32.1
N Status		
N1	64.9	34.2
N2	35.1	36.9
Vascular invasion		
Absent	69.7	35.2
Present	30.3	35.0
Perineural invasion		
Absent	87.7	34.3
Present	12.3	41.0
Perforation		
Absent	91.8	35.1
Present	8.2	35.5
Mucinous		
Absent	73.7	35.1
Present	26.3	35.4
Lymphocytic Response		
Absent	85.7	34.4
Present	14.3	39.4
Clinical		
Obstruction		
Absent	86.2	35.4
Present	13.8	33.7
Colonoscopy or		
Yes	56.9	40.2 [a]
No	43.1	28.5
Surgical Case Load		
Low	13.8	32.0 [b]
Medium	47.4	31.2
High	38.8	41.1

[a] $P<0.0001$; [b] $P<0.002$

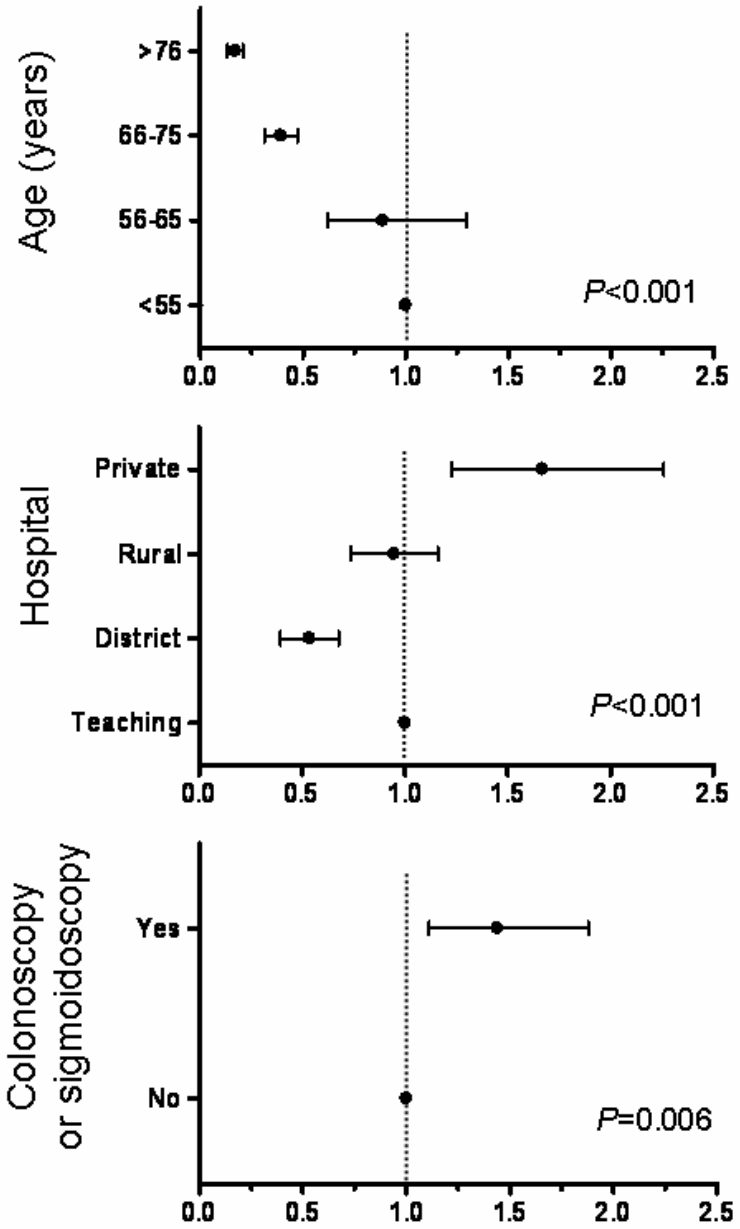


Figure 5.1. Predictors for the initiation of 5FU adjuvant chemotherapy in stage III colon cancer patients adjusted in multivariate analysis.

The survival of patients who initiated chemotherapy is shown in Table 5.3 according to the number of cycles received. Compared to patients treated by surgery alone, those who received only 1 cycle of chemotherapy showed significantly worse survival. A trend for worse survival was also observed for patients treated with 2 or 3

cycles. In contrast, patients treated with 4, 5 or 6 cycles showed better survival than those treated by surgery alone. Based on these results and for the purposes of this study, patients who received 4-6 cycles were classified as having completed chemotherapy, whereas those treated with 1-3 cycles were deemed not to have completed this treatment. The former group was estimated to have a 30% survival advantage and the latter group a 40% survival disadvantage compared to patients treated by surgery alone (Table 5.3 and Figure 5.2).

Table 5.3. Mortality hazard ratios according to number of completed cycles of adjuvant 5FU chemotherapy, multivariate adjusted.

Chemotherapy	Cancer-specific survival			Overall survival		
(n)	HR	95% CI	*P*	HR	95% CI	*P*
None (851)	1.00			1.00		
1-3 cycles (156)	1.37	1.09-1.72	0.008	1.09	0.88-1.35	NS
4-6 cycles (305)	0.67	0.54-0.83	<0.001	0.55	0.45-0.67	<0.001
1 cycle (68)	1.72	1.24-2.38	<0.001	1.26	0.93-1.71	NS
2 cycles (40)	1.19	0.79-1.80	NS	0.92	0.63-1.36	NS
3 cycles (48)	1.17	0.79-1.74	NS	1.04	0.73-1.48	NS
4 cycles (41)	0.74	0.46-1.20	NS	0.61	0.39-0.97	0.035
5 cycles (105)	0.77	0.56-1.07	NS	0.59	0.43-0.81	0.001
6 cycles (159)	0.53	0.39-0.72	<0.001	0.43	0.32-0.58	<0.001

Two-thirds of patients who initiated chemotherapy completed 4-6 cycles of treatment (Tables 5.4 and 5.5). Factors associated with higher rates of completion were N1 nodal status, high surgeon case load, treatment in teaching and private hospitals and high socioeconomic indices. Multivariate analysis revealed that independent predictors for completion of chemotherapy were the type of treatment hospital, high

socioeconomic index and high surgical volume (Table 5.6). Females showed a trend for less likelihood of completion (*P*=0.08).

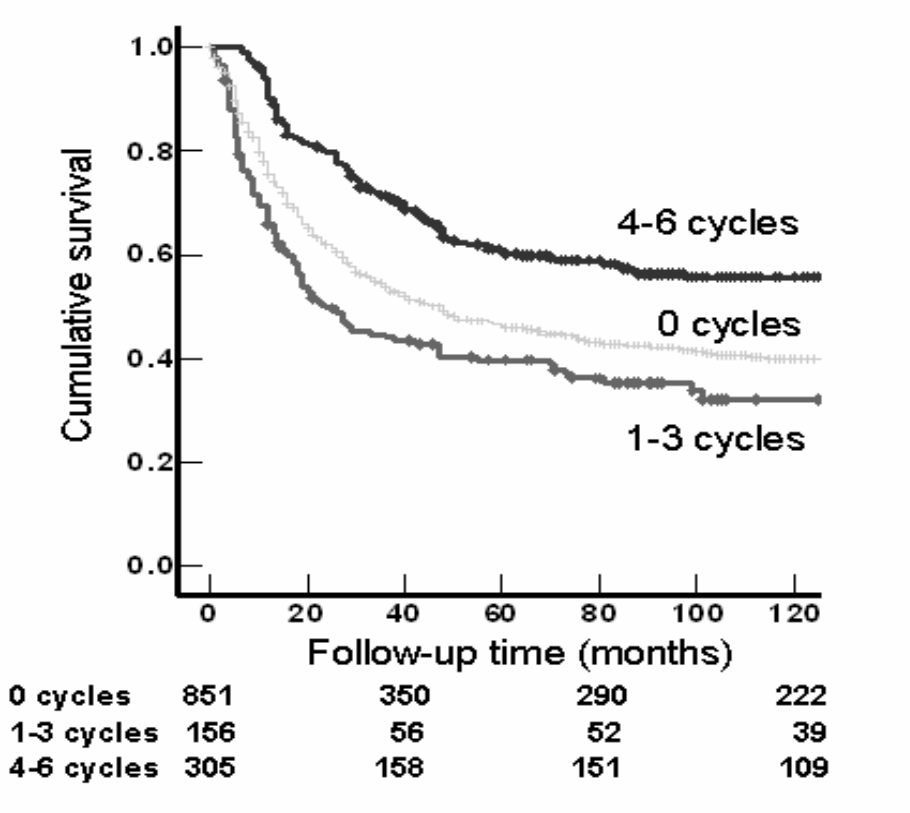


Figure 5.2. Kaplan-Meier survival analysis for stage III colon cancer patients treated with surgery alone (0 cycles, light grey), 1-3 cycles (incomplete chemotherapy, dark grey) or 4-6 cycles (complete chemotherapy, black) of 5FU adjuvant chemotherapy using the Mayo regimen. Log-rank test: *P*=0.021 for incomplete chemotherapy *vs.* surgery alone; *P*<0.0001 for complete chemotherapy *vs.* surgery alone; *P*<0.0001 for complete chemotherapy *vs.* incomplete chemotherapy.

Table 5.4. Completion of adjuvant chemotherapy for stage III colon cancer patients according to demographic factors (n=305).

Characteristic	Percentage of total cases	Rate of completion, %
Total	100.0	66.3
Year of diagnosis		
1994	3.3	41.7
1995	9.2	68.0
1996	12.5	67.7
1997	20.0	66.3
1998	14.4	63.8
1999	15.4	69.3
2000	11.8	66.7
2001	13.4	72.0
Age		
≤ 55 yrs	25.2	66.4
56- 65 yrs	34.4	70.5
66-75 yrs	26.9	62.1
≥ 76 yrs	13.4	64.1
Sex		
Male	53.1	68.9
Female	46.9	63.3
Advantage/Disadvantage		
1	16.4	52.6 [a]
2	23.6	66.1
3	18.0	65.8
4	20.0	69.0
5	22.0	76.1
Economic Resources		
1	13.8	45.2 [b]
2	24.6	74.3
3	16.1	58.9
4	24.3	75.5
5	21.3	72.2
Hospital		
Teaching	43.6	69.3 [b]
District	4.9	48.4
Rural	9.2	45.2
Private	42.3	73.3

[a] $P<0.02$; [b] $P<0.001$

Table 5.5. Completion of adjuvant chemotherapy for stage III colon cancer patients according to pathological and clinical factors (n=305).

Characteristic	Percentage of total cases	Rate of completion, %
Total	100.0	66.3
Pathology		
Site		
Proximal	47.2	62.1
Distal	52.8	70.3
Grade		
Well/moderate	78.0	68.4
Poor	22.0	59.3
T stage		
T1/T2	6.6	74.1
T3	71.1	65.2
T4	22.3	67.3
Nodal Status		
N1	67.2	70.4 [a]
N2	32.8	58.8
Vascular Invasion		
Absent	70.2	66.5
Present	29.8	65.5
Perineural Invasion		
Absent	85.6	66.1
Present	14.4	66.7
Perforation		
Absent	90.8	65.5
Present	9.2	73.7
Mucinous		
Absent	73.4	66.1
Present	26.6	66.4
Lymphocytic Response		
Absent	82.0	64.6
Present	18.0	66.2
Clinical		
Obstruction		
Absent	87.5	66.8
Present	12.5	62.3
Colonoscopy or sigmoidoscopy		
Yes	69.2	70.3 [b]
No	30.8	58.4
Surgical Case Load		
Low	11.5	60.3 [c]
Medium	35.1	55.2
High	53.4	78.0

[a] $P<0.02$; [b] $P<0.01$; [c] $P<0.001$

Table 5.6. Predictors for completion of adjuvant 5FU chemotherapy, multivariate adjusted.

	Odds Ratio	95% CI	*P*
Age of diagnosis			
≤ 55 yrs	1.00		
56-65 yrs	0.98	0.55-1.76	NS
66-75 yrs	0.77	0.43-1.38	NS
≥ 76 yrs	0.72	0.34-1.53	NS
Sex			
Male	1.00		
Female	0.68	0.44-1.05	0.08
Site			
Proximal	1.00		
Distal	1.37	0.84-2.24	NS
Surgical Case Load			
Low	1.00		0.007
Medium	0.88	0.45-1.72	NS
High	2.06	0.99-4.25	0.05
Advantage/Disadvantage			
1	1.00		
2	1.83	0.96-3.50	NS
3	1.45	0.74-2.85	NS
4	1.62	0.81-3.23	NS
5	2.18	1.07-4.44	0.032
Economic Resources			
1	1.00		
2	3.55	1.79-7.04	<0.001
3	1.61	0.80-3.23	NS
4	3.20	1.58-6.48	0.001
5	2.54	1.26-5.14	0.009
Hospital			
Teaching	1.00		
Private	0.91	0.52-1.58	NS
District	0.50	0.21-1.18	NS
Rural	0.40	0.20-0.79	0.009

Discussion

Two recent studies using SEER data reported that stage III colon cancer patients who failed to complete 5FU chemotherapy showed worse survival compared to those who completed the regimenn (Neugut *et al*, 2006; Dobie *et al*, 2006). The present investigation of an Australian population-based cohort of stage III colon cancer confirms the findings of the US groups. Moreover, the current study also found that patients who initiated but did not complete chemotherapy (1-3 cycles received) showed significantly worse survival than patients treated by surgery alone (Table 5.3).

There were several differences in study design between the current investigation and the US reports. Patients of all ages were included here, whereas only >65 year old patients were investigated previously. Despite the younger cohort, only 35% of patients initiated chemotherapy (Tables 5.1 and 5.2) compared to 54% and 55% for the SEER-derived cohorts (Neugut *et al*, 2006; Dobie *et al*, 2006). The completion rate for patients who initiated chemotherapy in the present investigation (66%, Tables 5.4 and 5.5) was comparable to the US report (64%) from the same study period (Neugut *et al*, 2006). Both were slightly lower than the second North American report (78%) which investigated an earlier study period, included both 12 and 6 month regimens and presented 3-year cancer-specific mortality data (Dobie *et al*, 2006). Although the present study had a smaller sample size, individual patient records were reviewed for pathology, chemotherapy regimen and cause of death.

In spite of these minor differences in study design, all 3 investigations have observed a significant survival advantage associated with the completion of chemotherapy. The survival advantage was estimated at 33% (Table 5.3) and 21% (Dobie *et al*, 2006) compared to patients treated by surgery alone. When patients who

did not complete chemotherapy were used as the reference group, the survival advantage was even greater at 47% (Neugut *et al*, 2006) and 55% in the current investigation (HR=0.45; 95%CI [0.33-0.60], *P*=0.005). Importantly, the same pattern of survival advantage from the completion of chemotherapy was also observed in this study in a population-based cohort of stage II colon cancer patients. Using patients treated by surgery alone as the reference group (n=1,362), patients who completed chemotherapy (n=142) showed a significant survival advantage (HR=0.63; 95%CI [0.41-0.96], *P*=0.03) whereas those who did not complete (n=49) showed worse survival (HR=1.27; 95% CI [0.71-2.29], *P*=0.42).

The predictors for initiation of chemotherapy were found in multivariate analysis to be younger patient age, treatment in a private hospital and preoperative colonoscopy or sigmoidoscopy (Figure 5.1). The first two factors are likely to reflect patients with lower comorbidities and higher socioeconomic status, respectively, while the third factor may represent non-emergency presentation. Interestingly, none of the reported pathological variables was predictive for the initiation of chemotherapy in stage III colon cancer. District hospitals were defined here as non-teaching and non-private institutions located in the metropolitan region of Perth. Patients treated in these hospitals showed a 2-fold lower initiation rate for chemotherapy compared to teaching institutions, suggesting deficiencies in access to oncology services.

Patients with greater levels of psycho-social support and financial resources would be expected to show higher rates of chemotherapy completion. This is supported by the observation that patients with a high socioeconomic index or who were treated in teaching or private hospitals showed higher rates of completion (Table 5.6). Previous US studies have shown that married status is also predictive for the completion of chemotherapy (Neugut *et al*, 2006; Dobie *et al*, 2006). It is of concern that patients

treated in district and rural hospitals showed approximately half the rate of chemotherapy completion compared to patients from teaching and private hospitals (Table 5.6). There is clearly a need to identify the underlying reasons leading to low initiation and completion rates for the 25% of colon cancer patients treated in these centres so that equality of health provision can be improved.

There are several current and commonly used 5FU regimens ranging from protracted, continuous infusional 5FU delivery to bolus schedules. Protracted infusional regimens were developed in order to maximise 5FU dose intensity. They have been found to be as effective as the bolus regimenns and less toxic, with less impact on quality of life (Goyle *et al,* 2005; Saini *et al,* 2003; Andre *et al*, 2003). The Mayo and Roswell Park regimens are commonly used in the USA and have different safety profiles. The Mayo regimen demonstrates more haematological but less gastrointestinal toxicity compared with the Roswell Park regimen. In the United Kingdom and Europe the Lokich, LV5FU2 (de Gramont) or QUASAR-type regimenns are commonly used and have advantages in terms of toxicity when compared with the Mayo regimen, but comparable survival rates (Goyle *et al,* 2005).

The other major factor likely to result in failure to complete chemotherapy is toxicity. It is well documented that bolus schedules of 5FU cause more severe leucopenia and stomatitis in elderly patients and particularly in females (Tebbutt *et al*, 2000; Chanksy *et al*, 1992; Milano *et al*, 1992; Zalcberg *et al*, 1998; Popescu *et al*, 1999; Sloan *et al*, 2002; Meta-analysis Group in Cancer, 1998). In agreement with these findings, the current study found that females were less likely to complete adjuvant chemotherapy when adjusted for other variables (Table 5.6) and we postulate this is due to an increased incidence of toxicity. The retrospective nature of this population-based study meant that information on treatment-related toxicity was not available. The skill

and experience of the administrators of chemotherapy in the management of toxicities and the capacity to provide psycho-social support to patients may impact on the likelihood of a patient to complete their chemotherapy regimen.

Many of the toxicities that lead to termination of 5FU chemotherapy culminate around the time of the third cycle (Tebbutt *et al*, 2000). Unfortunately, steady-state plasma 5FU levels do not correlate with toxicity (Jodrell *et al*, 2001) and hence pharmacokinetic monitoring is not used to identify patients who are at increased risk of toxicity (Tebbutt *et al*, 2000). In randomised controlled trials, dose reductions are common after the first two cycles and 15-30% of patients fail to complete chemotherapy (Wolmark *et al*, 1993; O'Connell *et al*, 1997; Poplin *et al*, 2005). A relationship between systemic exposure and treatment efficacy has not been demonstrated and can only be ascertained by conducting prospective randomised studies that compare a targeted dose adjustment to a fixed dose (Milano *et al*, 1994).

A novel and unexpected observation from this study was that stage III colon cancer patients who failed to complete chemotherapy showed significantly worse cancer-specific survival compared to patients treated by surgery alone (Table 5.3 and Figure 5.2). Dobie *et al* (2006) did not find a significant difference in survival between these two patient groups using 3-year cancer mortality data and Neugut *et al* (2006) did not report this comparison. It is unlikely that 5FU toxicity accounts for the increased mortality observed here as recent trials have reported a chemotherapy-related death rate of only 0.5% (Andre *et al*, 2004). The chemotherapy related death rate in this population based cohort of stage III colon cancer was 0.65% (3/461). The 60 day mortality for patients that initiated adjuvant chemotherapy was 0.4%. This is comparable to published benchmark data for the safety of adjuvant chemotherapy in colon cancer (Katopodis *et al*, 2004).

One possible explanation is that failure to complete chemotherapy is indicative of high toxicity and this may in turn be associated with a more aggressive tumour phenotype or impairment of the host immune response. The CpG island methylator phenotype has worse prognosis (Van Rijnsoever *et al*, 2003; Ward *et al*, 2003) and is linked to the folate pathway (Kawakami *et al*, 2003). This latter pathway has been implicated in toxicity to 5FU (Pinedo *et al*, 1988).

The results of the current study have relevance for the introduction of new therapies for colon cancer. Oral fluoropyrimidines (UFT and capecitabine) mimic protracted venous infusional 5FU. Benefits of these agents include convenience, elimination of risks from indwelling central venous catheters and a different toxic profile. They are of comparable efficacy to the Mayo regimen but with less toxicity (Twelves *et al*, 2005; Douillard *et al*, 2002; Carmichael *et al*, 2002). Toxicity profiles of FOLFOX and FOLFIRI (Allegra *et al* 2005; O'Connell *et al,* 2004; Andre *et al,* 2004) may reduce the completion of these treatments, but fewer cycles of these regimens may be as efficacious as the completed Mayo regimen. A recently published safety analysis of the XELOX NO16968 study (Schmoll *et al,* 2007) found more frequent treatment discontinuations with XELOX compared to the FU/LV Mayo and Roswell Park regimens, however a comparable number of patients completed 12 weeks of therapy (88% and 82%, respectively).

In conclusion, this study confirms two recent reports (Neugut *et al*, 2006; Dobie *et al*, 2006) that stage III colon cancer patients who fail to complete 5FU adjuvant chemotherapy show worse survival than patients who completed this treatment. In addition, the current study found that patients who do not complete chemotherapy may in fact have worse survival than patients treated by surgery alone. These results have implications for the delivery of oncology services. Further research is needed to identify

factors that could increase both initiation and completion rates of 5FU chemotherapy from colon cancer.

Chapter 6

Pathological predictors of survival benefit from 5FU-based adjuvant chemotherapy in stage III colon cancer: does the immune response play a role?

Morris M, Platell C, Fritschi L, McCaul K, Iacopetta B (manuscript in preparation)

Abstract

Background: There is currently insufficient evidence to recommend any molecular marker to guide clinical practice in the use of 5FU-based adjuvant chemotherapy in colon cancer. The aim of this study was to investigate routinely reported clinico-pathological variables as possible predictive markers of survival benefit from 5FU.

Methods: The study cohort comprised 851 stage III colon cancer patients treated by surgery alone and 461 who initiated the Mayo 5FU chemotherapy regimen.

Results: Propensity score analysis revealed that perforation and lymphocytic response were independent predictors of survival benefit from 5FU chemotherapy in stage III colon cancer patients who received at least 4 cycles of this treatment. The survival benefit associated with 5FU was estimated at approximately 40% for patients presenting with perforation (HR=0.61, 95%CI [0.30-1.24]) or with a tumour lymphocytic response (HR=0.61, 95%CI [0.36-1.02]).

Conclusions: The pathological features of perforation and lymphocytic response may reflect an activated immune system that acts synergistically with 5FU chemotherapy, resulting in better patient outcomes.

Introduction

Randomised controlled trials conducted in the 1980's demonstrated that 5FU-based chemotherapy resulted in a 10% absolute improvement in 5-year survival for stage III colon cancer patients (Moertel *et al*, 1990). As a result of these trials the National Institutes of Health recommended in 1990 the routine administration of FU-based adjuvant chemotherapy for medically fit patients with completely resected stage III CRC. A role for immunotherapy, either alone or in combination with chemotherapy, has yet to be established for the treatment of colon cancer.

The ability to individually tailor a patient's treatment for cancer is a major goal for oncology clinicians and researchers. Prognostic markers provide information on patient outcome that can be used to guide therapeutic decisions, whereas predictive markers provide information as to the likely response to treatment regimens (McLeod, 1998). Molecular-based markers hold promise as clinically useful prognostic and predictive factors in CRC, but these hopes have so far failed to materialize. Thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) are enzymes involved in folate metabolism and as such have been postulated to have predictive value for response to 5FU chemotherapy (Ackland *et al*, 2003). No molecular marker is presently recommended for use in routine clinical practice (Locker *et al*, 2006). The major limitations associated with molecular prognostic or predictive markers are the reproducibility and cost of assays, the requirement with many assays for frozen tissues, and the difficulty in quantifying protein expression levels detected by immunohistochemistry.

The current study investigated clinico-pathological features for predictive information on the survival benefit from 5FU-based adjuvant chemotherapy in a large, retrospective series of stage III colon cancer. These features have the advantage of being

routinely assessed as part of normal clinical practice, thus requiring no additional analysis of tumour tissues.

Methods

Study population, adjuvant chemotherapy and survival data

Information on the study cohort, 5FU chemotherapy and patient survival was obtained as described in the methods section of chapter 5 (pages70-72).

Statistical analysis

Chi square analysis was used to compare clinico-pathological features of patients treated by surgery alone or chemotherapy (both incomplete and complete as defined in Chapter 5). Survival analysis was conducted using Kaplan-Meier analysis and Cox proportional hazards regression. The log-rank test was used to determine significance for Kaplan Meier analysis. A Cox proportional hazards regression model was developed for survival in which each variable was adjusted for all others. During the first 6 months of patient follow-up there were 150 deaths due to cancer, with 29 of these occurring in patients treated with 5FU chemotherapy. Consequently, incomplete chemotherapy could not be treated as a baseline covariate in the proportional hazard regressions and was instead modelled as a time-dependent covariate. The proportional hazards assumption was evaluated by testing for non-zero slope in a linear regression of the scaled Schoenfeld residuals over the log of time (Grambsch *et al*, 1995). The prognostic and predictive significance of each clinico-pathological variable was examined by multivariate analysis within patient groups treated by surgery alone, incomplete chemotherapy or complete chemotherapy. Statistical significance was deemed as $P<0.05$.

Propensity scores were derived from a logistic regression model that predicted the probability of starting chemotherapy using as covariates the following baseline variables: age group, sex, site of tumour (proximal or distal), nodal status, vascular invasion, differentiation, obstruction, and mucin. Propensity score analysis (Adamina *et al*, 2006) was used to examine for independent effects of clinico-pathological variables and use of chemotherapy on the risk of death from cancer.

Results

Clinical and pathological information for the 1,312 stage III colon cancer patients investigated in this study were presented in Chapter 5. Although this population-based series was not randomized for adjuvant treatment, the 461 patients who initiated chemotherapy were relatively well matched, except for age, to patients treated by surgery alone (Table 6.1).

Prognostic significance

The prognostic significance of each clinical and pathological feature for disease-specific survival was determined by multivariable analysis in patients treated by surgery alone (n=851). Older patient age and the pathological features of proximal tumour location, poor differentiation, T4 or N2 status, vascular invasion and perforation were significant factors for poor prognosis in stage III colon cancer (Table 6.2). Gender, lymphocytic response, obstruction and mucinous histology did not show prognostic significance.

Clinical and pathological variables were also evaluated for prognostic significance within 5FU adjuvant treatment groups (Table 6.2). For patients who completed adjuvant chemotherapy (>3 cycles), multivariate adjusted analysis revealed

Table 6.1. Clinico-pathological features of stage III colon cancer in patients treated by surgery alone, incomplete (1-3 cycles) and complete (4-7 cycles) chemotherapy.

	Cycles of Chemotherapy				
	0 cycles	**1-3 cycles**	**4-7 cycles**	**Total**	***P* value**
Total	851(64.9)	156 (11.9)	305 (23.2)	1312	
Age of diagnosis					
≤ 55	105 (47.5)	39 (17.7)	77 (34.8)	221 (16.8)	<0.001*
56-65	137 (47.9)	44 (15.4)	105 (36.7)	286 (21.8)	
66-75	293 (68.9)	50 (11.8)	82 (19.3)	425 (32.4)	
≥ 76	316 (83.1)	23 (6.1)	41 (10.8)	380 (29.0)	
Sex					
Male	400 (63.0)	73 (11.5)	162 (25.5)	635 (48.4)	NS
Female	451 (66.6)	83 (12.3)	143 (21.1)	677 (51.6)	
Site					
Proximal	470 (66.9)	88 (12.5)	144 (20.6)	702 (53.5)	0.04*
Distal	381 (62.4)	68 (11.2)	161 (26.4)	610 (46.5)	
Histological Grade					
Well/moderate	651 (65.2)	110 (11.0)	238 (23.8)	999 (76.1)	NS
Poor	200 (63.9)	46 (14.7)	67 (21.4)	313 (23.9)	
T stage					
T1/T2	38 (58.5)	7 (10.8)	20 (30.7)	65 (5.0)	NS
T3	599 (64.3)	116 (12.4)	217 (23.3)	932 (71.0)	
T4	214 (67.9)	33 (10.5)	68 (21.6)	315 (24.0)	
Nodes examined					
Mean (S.D.)	11.1 (7.8)	11.8 (7.9)	12.6 (7.9)	11.5 (7.9)	
Median (range)	10.0 (1-53)	10.0 (1-43)	11.0 (1-51)	10.0 (1-53)	
N status					
N1	560 (65.8)	86 (10.1)	205 (24.1)	851 (64.9)	0.02*
N2	291 (63.1)	70 (15.2)	100 (21.7)	461 (35.1)	
Vascular Invasion					
Present	258 (65.0)	48 (12.1)	91 (22.9)	397 (30.3)	NS
Absent	593 (64.8)	108 (11.8)	214 (23.4)	915 (69.7)	
Perineural Invasion					
Present	95 (59.0)	22 (13.7)	44 (27.3)	161 (12.3)	NS
Absent	756 (65.7)	134 (11.6)	261 (22.7)	1151 (87.7)	
Perforation					
Present	69 (64.5)	10 (9.3)	28 (26.2)	107 (8.2)	NS
Absent	782 (64.9)	146 (12.1)	277 (23.0)	1205 (91.8)	
Obstruction					
Present	120 (66.3)	23 (12.7)	38 (21.0)	181 (13.8)	NS
Absent	731 (64.6)	133 (11.8)	267 (23.6)	1131 (86.2)	
Mucinous					
Present	223 (64.6)	41 (11.9)	81 (23.5)	345 (26.3)	NS
Absent	628 (64.9)	115 (11.9)	224 (23.2)	967 (73.7)	
Lymphocytic response					
Present	114 (60.6)	19 (10.1)	55 (29.2)	188 (14.3)	NS
Absent	737 (65.6)	137 (12.2)	250 (22.2)	1124 (85.7)	

Table 6.2. Prognostic significance of clinico-pathological variables in treatment subgroups of stage III colon cancer.

Total (1312)	**Surgery alone (n=851)**			**1-3 cycles chemotherapy (n=156)**			**4-7 cycles chemotherapy (n=305)**		
	HR	95%	*P*	HR	95%	*P*	HR	95%	*P*
Age at diagnosis (yrs)									
≤ 55	1.00			1.00			1.00		
56-65	1.29	0.87-1.90	NS	1.12	0.67-1.88	NS	1.07	0.63-2.42	NS
66-75	1.62	1.15-2.28	0.005*	1.27	0.77-2.10	NS	0.92	0.59-3.92	NS
≥ 76	1.84	1.31-2.57	<0.001*	1.43	0.66-3.15	NS	2.01	0.97-4.20	NS
Sex									
Male	1.00			1.00			1.00		
Female	0.95	0.79-1.13	NS	0.91	0.61-1.35	NS	0.78	0.55-1.11	NS
Site									
Proximal	1.00			1.00			1.00		
Distal	0.81	0.68-0.97	0.02*	0.90	0.61-1.35	NS	0.73	0.51-1.03	NS
Histological Grade									
Well/moderate	1.00			1.00			1.00		
Poor	1.43	1.17-1.75	<0.001*	1.15	0.75-1.78	NS	1.42	0.95-2.12	NS
T stage									
T1/T2	1.00			1.00			1.00		
T3	1.33	0.83-2.15	NS	1.30	0.41-4.11	NS	1.56	0.68-3.56	
T4	2.23	1.31-3.47	0.002*	2.10	0.64-6.99	NS	1.42	0.59-3.45	NS
N status									
N1	1.00			1.00			1.00		
N2	1.59	1.33-1.91	<0.001*	1.59	1.07-2.36	0.02*	1.63	1.13-2.34	0.01*
Vascular Invasion									
Absent	1.00			1.00			1.00		
Present	1.42	1.18-1.72	<0.001*	1.43	0.95-2.16	NS	1.25	0.86-1.82	NS
Perforation									
Absent	1.00			1.00			1.00		
Present	1.58	1.18-2.11	0.002*	0.92	0.40-2.09	NS	0.62	0.30-1.27	NS
Mucinous									
Absent	1.00			1.00			1.00		
Present	1.07	0.88-1.30	NS	0.88	0.56-1.40	NS	1.16	0.79-1.71	NS
Lymphocytic response									
Absent	1.00			1.00			1.00		
Present	0.95	0.73-1.24	NS	0.84	0.46-1.55	NS	0.58	0.34-0.98	0.04*

that N2 status was associated with poor survival, whereas the presence of lymphocytic response within the tumour was associated with significantly improved survival in this patient subgroup. None of the other clinico-pathological markers showed prognostic significance in adjuvant treated patient groups.

Predictive significance

Individual clinico-pathological variables were examined for association with response to 5FU by comparing the survival of patients treated by surgery alone to those treated with >3 cycles of chemotherapy. The factors of older age, female gender, perforation, mucinous histology and lymphocytic response were all associated with a greater survival benefit from complete chemotherapy (Table 6.3 and Figure 6.1).

Table 6.3. Predictive significance of clinico-pathological variables for survival benefits from 5FU chemotherapy in stage III colon cancer patients. Univariate analysis.

Feature (n1,n2)	**Surgery alone**	**Complete chemotherapy**	**95% CI**	***P***
Age				
<55y (105,77)	1.00	0.84	0.48-1.45	NS
≥55y (746,228)	1.00	0.61	0.48-0.79	<0.001
Sex				
Male (400,162)	1.00	0.72	0.53-0.97	0.03
Female (451,143)	1.00	0.57	0.41-0.80	0.001
Perforation				
Present (69,28)	1.00	0.20	0.08-0.52	0.001
Absent (782,277)	1.00	0.73	0.58-0.91	0.006
Mucinous				
Present (223,181)	1.00	0.58	0.38-0.87	0.008
Absent (628,224)	1.00	0.74	0.57-0.97	0.03
Lymphocytic response				
Present (114,55)	1.00	0.26	0.13-0.52	<0.001
Absent (737,250)	1.00	0.72	0.57-0.91	0.006

n1=surgery alone, n2=complete chemotherapy

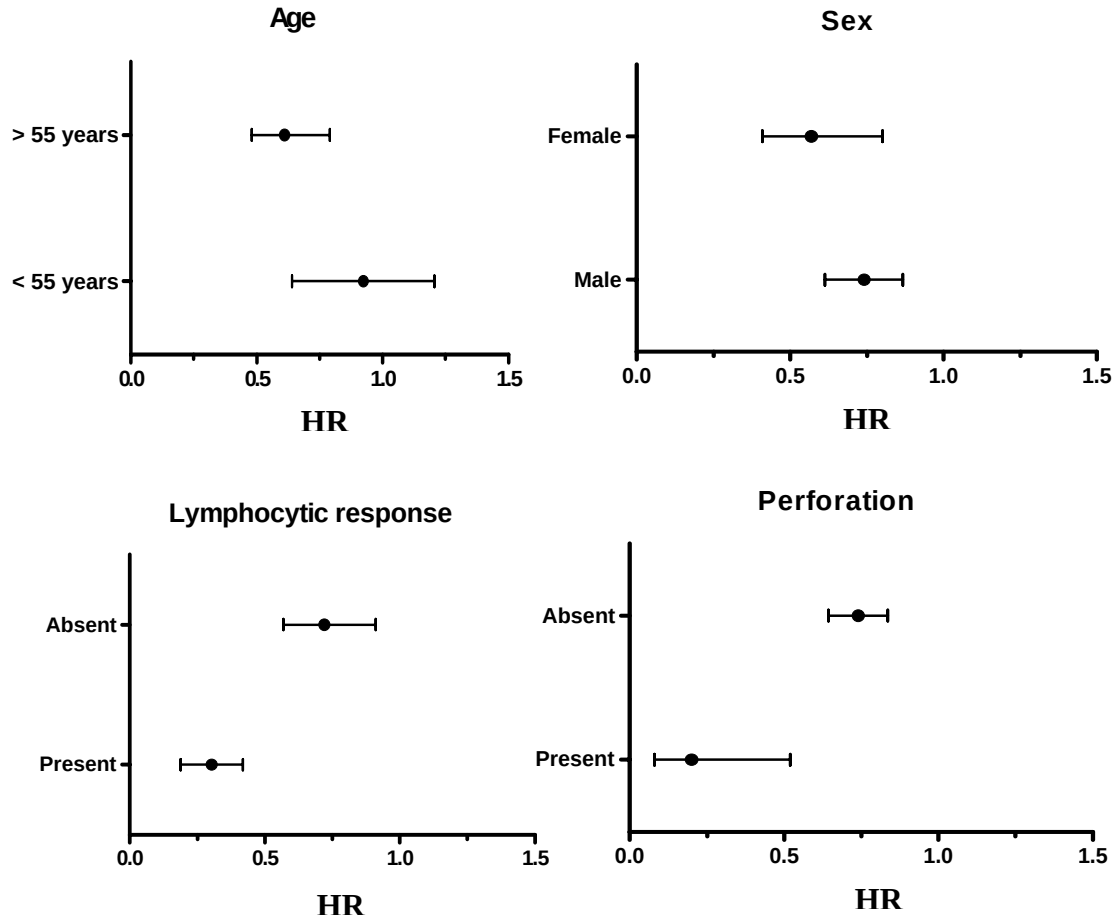


Figure 6.1. Survival benefits from complete 5FU chemotherapy in clinico-pathological subgroups of stage III colon cancer. Greater survival benefits can be seen for older and female patients, as well as those with perforation or with a tumour lymphocytic reaction.

Propensity score analysis was used to model the risk of death from colon cancer in conjunction with chemotherapy. By this assessment, the only clinico-pathological variables that showed independent predictive value for survival benefit from 5FU chemotherapy were perforation and lymphocytic response (Table 6.4). Perforation increased the risk of death by 65% in patients treated by surgery alone. However for patients who completed chemotherapy, perforation was associated with an almost 40% reduction in the risk of death. Lymphocytic response was not associated with a survival difference in patients treated by surgery alone. However the risk of death was again

reduced by almost 40% for patients whose tumours showed evidence of lymphocytic response and who completed chemotherapy.

Table 6.4. Propensity score analysis showing independent predictive values of perforation and lymphocytic response for survival benefit from 5FU chemotherapy in stage III colon cancer.

	Surgery alone	Incomplete Chemotherapy	Complete Chemotherapy
Perforation			
Absent (HR)	1.00	1.59	1.04
95% CI		1.24-2.05	0.82-1.33
Present (HR)	1.65	1.53	0.61
95% CI	1.23-2.22	0.67-3.47	0.30-1.24
Lymphocytic Response			
Absent (HR)	1.00	1.52	1.04
95% CI		1.18-1.98	0.81-1.33
Present (HR)	0.98	1.35	0.61
95% CI	0.75-1.27	0.75-2.42	0.36-1.02

Kaplan Meier survival curves for patient subgroups classified according to perforation or lymphocytic response and 5FU chemotherapy are shown in Figure 6.2. Treatment with at least 4 cycles of chemotherapy is associated with a survival benefit for both subgroups.

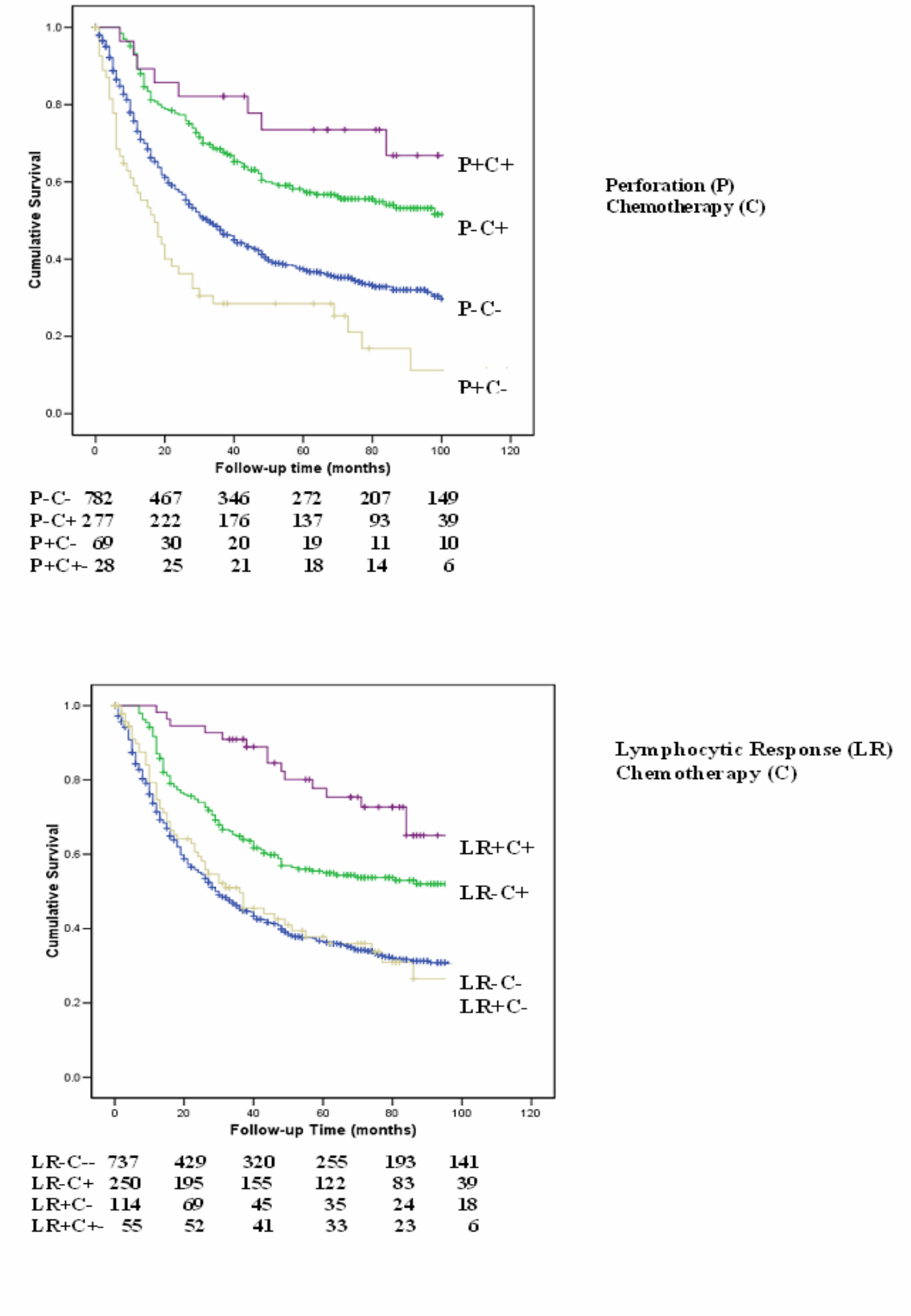


Figure 6.2. Survival of stage II colon cancer patients according to perforation (P), lymphocytic response (LR) and treatment with 4 or more cycles of chemotherapy (C).

Discussion

The ability to identify colon cancer patients who will gain benefit from 5FU adjuvant chemotherapy regimens has so far proven to be difficult. An extensive search that includes the introduction of micro-array technology has so far failed to find a robust molecular predictive marker for response to adjuvant 5FU-based chemotherapy. Although many factors have been investigated, including for example TS expression, p53 mutation and MSI status, none have been validated in prospective clinical studies. ASCO guidelines currently state there is insufficient evidence to recommend any marker to guide routine clinical practice in the use of 5FU (Locker *et al*, 2006)

The major limitation of the present study is that patients were not randomised to receive adjuvant FU chemotherapy. Consequently, unknown confounding factors could account for the differences in survival. Observational studies are viewed as having less validity because they reportedly over-estimate treatment effects (MacMahon *et al*, 2001; Collins *et al*, 2001). However, recent literature argues that population based observational studies, such as the current study, may be less prone to heterogeneity because they more broadly represent the population at risk (Concato *et al*, 2000; Benson *et al* 2000). In contrast, individual randomised controlled trials may comprise distinct groups of patients due to specific inclusion and exclusion criteria. Furthermore, the experimental treatment protocol may not be representative of general clinical practice. Propensity score analysis (PSA) can be used to reduce the biases that are inherent in all observational studies (Adamina *et al*, 2006; Yue, 2007; Rubin, 2007). It does this through matching, stratification and regression adjustment.

It was not unexpected that clinico-pathological features of poor prognosis in stage III colon cancer patients treated by surgery alone included older age, poor histological

grade, T4 status, N2 nodal status and evidence of vascular invasion or perforation (Table 6.2). Tumour infiltrating lymphocytes in melanoma, ovarian and CRCs have been shown to inhibit tumour growth and are associated with improved prognoses (Clemente *et al*, 1996; Zhang *et al*, 2003; Naito *et al*, 1998). In the present study, no prognostic significance was observed for lymphocytic response in stage III (Table 6.2) or stage II colon cancers (Chapter 3). These results are contrary to a recent report that the type, density and location of immune cells in CRC had prognostic value superior to and independent of the UICC-TNM classification (Galon *et al*, 2006). The chemotherapy status of the patients, however, was not reported. As shown in Figure 6.2, this treatment can influence the survival of colon cancer patients. The retrospective nature of the current study did not allow verification of the accuracy of pathology reporting for lymphocytic response, nor was the lymphocytic response identified by subsets that may have proven important for prognosis.

The present study evaluated the commonly reported clinical and pathological markers in stage III colon cancer for prediction of survival benefit from 5FU. Because patients were not randomized to adjuvant treatment, this investigation should be considered as hypothesis generating. Features associated with greater survival benefit from 5FU included older age, female gender and mucinous histology (Table 6.3). These factors lost significance, however, when propensity score analysis was performed (Table 6.3). Only perforation and lymphocytic response were independent predictors of response to a complete course of adjuvant 5FU chemotherapy (Table 6.4).

Tumour infiltrating lymphocytes may reflect recognition of the tumour by the immune system, or that the tumour is permissive to the infiltration of anti-tumour effectors. The cancer immuno-editing hypothesis recently put forward by Dunn *et al* (2002) is a 3 stage process of elimination, equilibrium and escape in which the immune

system protects the host against tumour development. However, it can also promote tumour growth by selecting for tumour escape variants with reduced immunogenicity (Khong *et al*, 2002). In the early 1990s a number of observations demonstrated that most antigens expressed by tumour cells were not necessarily neo-antigens present uniquely in cancer cells, but rather tissue-differentiation antigens that were also expressed in normal cells (Boon *et al*, 2006; Rosenberg, 1999). Complex mechanisms are involved in establishing T cell tolerance against self tumour antigens. This phenomenon is termed 'tumour anergy' (Rabinovich *et al*, 2006). The primary tumour plays a causative role in initially establishing a tolerogenic milieu in the tumour-draining lymph node (TDLN). Once established, the TDLN behaves like a continuously active 'factory' for generating and maintaining acquired peripheral tolerance toward tumour antigens (Munn *et al*, 2006). Immune tolerance must be broken for the immune system to effectively recognize and eliminate tumours. In the majority of colon cancer cases the TDLN is removed with surgical resection of the tumour.

Conventional cytotoxic chemotherapy can be a potent activator of anti-tumour immune responses (Lake *et al*, 2005; Nowak *et al* 2006). The effect of chemotherapy is multifactorial in that it:

(1) creates a wave of dying tumour cells that enter the antigen-presentation pathway

(2) it creates a milieu during the recovery phase (particularly from lymphopaenia) in which the immune system is receptive to breaking tolerance

(3) it transiently reduces the number and functional activity of T regulatory cells.

As seen in Figure 6.2, patients whose tumours show evidence of lymphocytic response and received adjuvant chemotherapy showed improved survival for the first 7-8 years following surgery, however they eventually succumbed to metastatic disease.

An unexpected finding of this study is that tumour perforation resulted in a survival advantage to patients who received adjuvant chemotherapy (Tables 6.3 and 6.4, Figures 6.1 and 6.2). Tumour perforation results in an acute inflammatory response, often characterized by the systemic inflammatory response syndrome (SIRS) and culminating in septic shock. Perforation in patients treated by surgery alone was associated with a survival disadvantage of 65% (Table 6.4). Faecal contents and gram negative sepsis elicit a strong innate immune response. Stimulation of Toll-like receptors by microbial products leads to activation of signalling pathways that result in induction of antimicrobial genes and inflammatory cytokines (Janeway *et al*, 2006; Beutler *et al*, 2006). In addition, stimulation of Toll-like receptors triggers dendritic cell maturation and results in the induction of co stimulatory molecules and increased antigen-presenting capacity. Microbial recognition by Toll-like receptors helps to direct adaptive immune responses. If the patient survives perforation and completes adjuvant chemotherapy, a survival advantage of 39% is achieved compared to patients without tumour perforation and treated by surgery alone (Table 6.4). The survival advantage is even greater when compared to patients with perforation who did not receive adjuvant chemotherapy (Table 6.4).

Most chemotherapies kill target cells by apoptosis and until recently this mode of cell death was regarded as either non-stimulatory or able to produce immune tolerance. *In vitro* work suggests that 5FU may improve tumour antigen uptake by monocyte-derived dendritic cells, increase the efficiency of cross presentation, and may also induce the production of heat shock protein (Rovere *et al*, 1999; Matzinger *et al*, 1994; Ohtsuboa *et al* 2000). Cells exposed to 5FU *in vitro* may also upregulate Fas expression and ICAM-1, leading to enhanced cytotoxic lymphocyte (CTL) mediated lysis. It is now clear that innate immunity can be triggered by apoptosis. Restifo (2000) has postulated

that apoptosis following viral infections or ligation of the death receptor Fas is intrinsically coupled to the production of inflammatory signals that can trigger powerful immune responses. There is increasing evidence that, under the right circumstances, tumour cell death induced by chemotherapy can set the stage for an effective anti-tumour immune response.

The survival advantage from 5FU adjuvant chemotherapy in stage III colon cancer is only observed when a complete course (>3 cycles) is administered (Neugut *et al*, 2006; Dobie *et al* 2006; Chapter 5). A survival advantage was not observed here for patients with perforation or lymphocytic tumour response if they did not receive a full course of adjuvant chemotherapy. The reasons for this are not yet clear, but probably relate to the effective therapeutic dose.

It can be hypothesised that administration of adjuvant chemotherapy to patients with perforation or evidence of a tumour lymphocytic response results in a synergistic effect that activates the immune system. This enables suppression of micrometastatic tumour cell growth for longer periods than in patients lacking these pathological features and hence improved overall survival. Future work must firstly involve validation of these observations in an independent clinical data set. Closer examination of the immune system in patients with perforation or lymphocytic reaction is also warranted before, during and after the administration of adjuvant chemotherapy. Little is known concerning the immune modulating role of 5FU-based chemotherapy. An animal model could also be established to investigate the effects of perforation on markers of the immune response and following treatment with 5FU. Ultimately, such studies may lead to more effective combinations of chemotherapy and immunotherapy for the treatment of colon cancer.

Chapter 7

A population-based study of age-related variation in clinico-pathological features, molecular markers and outcome from CRC

Morris M, Platell C, Iacopetta B: A population-based study of age-related variation in clinicopathological features, molecular markers and outcome from colorectal cancer. Anticancer Res, 2007 (in press)

Abstract

Background: To investigate age-related differences in clinico-pathological features, molecular alterations and patient survival in a large, population-based series of CRC.

Methods: The study cohort consisted of 5,971 cases diagnosed between 1993 and 2003 and representing over 90% of the CRCs diagnosed in the state of Western Australia.

Results: Patients aged ≤30, ≤40, ≤50 and ≤60 years comprised 0.9, 3.1, 10.6 and 27.8% of cases, respectively. The proportion of rectal cancers and tumours with poor differentiation was highest in ≤30 year old patients and decreased progressively with age. The incidence of tumours with microsatellite instability was significantly higher in patients aged ≤40 years (18.3%) compared to those aged 41-60 years (6.6%; $P<0.0001$). *TP53* mutations were also more frequent ($P=0.002$), however *KRAS* mutations were less common ($P=0.0001$) when comparing the same age groups.

Conclusions: These results provide evidence for major age-related differences in the clinical and molecular features of CRC.

Introduction

CRC carcinomas (CRC) are thought to develop from adenomas following the accumulation of mutations to oncogenes and tumour suppressor genes (Fearon *et al*, 1990). This has been referred to as the chromosomal instability (CIN) pathway because of the high frequency of aneuploidy. An alternate pathway to CRC has also been proposed and involves serrated adenomas and hyperplastic polyps as the precursor lesion (Jass *et al*, 2002b). Frequent methylation of gene promoter regions characterises this second pathway and the resulting tumours are referred to as CpG island methylator phenotype, or CIMP (Toyota *et al*, 1999). While there are undoubtedly other pathways for CRC leading to additional molecular phenotypes, the CIN and CIMP groups have so far received the most attention. A third phenotype characterised by microsatellite instability (MSI) can arise within either the CIN or CIMP phenotypes depending on the mechanism for inactivation of the DNA mismatch repair system.

CIMP tumours arise more frequently in the proximal colon of older patients (Van Rijnsoever *et al*, 2002), whereas CIN tumours are more common in the distal colon and rectum (Delattre *et al*, 1989). In addition to anatomical site, other major influences on the profile of genetic and epigenetic changes present within CRC are gender and age (Breivik *et al*, 1997). Population-based data reveals that patients with caecal tumours have the highest mean age at diagnosis and contain the highest proportion of females (Gonzalez *et al*, 2001). Both the mean age and the percentage of female patients then decline progressively as the tumour site becomes more distal. These epidemiological observations can be explained by predominance of the CIMP pathway in older females with proximal tumours and of the CIN pathway in younger males with distal tumours.

Tumours arising via the CIMP, CIN and possibly also other pathways that are influenced by age, site and sex are likely to show important differences in clinical

behaviour, including prognosis and response to chemotherapy (Iacopetta *et al*, 2002; Gervaz *et al*, 2004). Although a relatively large number of studies have compared CRC between younger and older patients, these have often been limited by a small sample size and by the biased selection of younger patients with a positive family history. This could account for the publication of contradictory results, particularly in relation to the prognosis of younger patients.

The aim of the present study was to investigate age-related differences in clinicopathological features, molecular alterations and patient survival using a well defined, population-based cohort of 5,971 CRC cases with long follow-up. Particular attention was paid to very young patients (≤40 years) who comprise approximately 3% of total cases, but represent a considerably higher proportion in terms of years of life lost from CRC.

Methods

Patient information

Pathology records from the four major hospitals in the state of Western Australia were used to identify all CRC cases diagnosed over the period 1993-2003 inclusive (n=5971). The pathology services at these hospitals also process surgical specimens from minor district and country hospitals and consequently information was obtained from the total Western Australian population numbering 1.8-2 million inhabitants during the study period. Tumour stage was classified according to current AJCC guidelines (AJCC,2002). Information on the histological grade of differentiation was obtained from the pathology report and was classified as well/moderate or poor. The anatomical site of tumour origin in the large bowel was also ascertained from the pathology report and was cross-checked with information from admission and procedure records. Colon cancers were classified as

being proximal or distal to the splenic flexure. Rectal cancer was defined as being within 12 cm of the anal verge. Site could not be ascertained for 649 cases.

Mortality data was obtained from the Death Registry of the Health Department of Western Australia. Death reports were individually reviewed and were classified as death due to cancer or death from other non-related causes. Peri-operative death within 4 weeks of surgery was excluded from the cancer specific survival analysis (n=239). Ethics approval for the project was obtained from the Human Research Ethics Committee at each hospital, the Confidentiality of Health Information Committee and the Human Research Ethics Committee of the University of Western Australia.

Molecular analyses

Microsatellite instability (MSI) was determined by screening for deletions within the BAT-26 mononucleotide repeat (Iacopetta *et al*, 2000a). Mutations in the *BRAF* (V600E), *KRAS* (codons 12 and 13) and *TP53* (exons 5-8) genes were also determined by PCR-based, fluorescent single strand conformation polymorphism analysis as described earlier by our group (Wang *et al*, 2003; Iacopetta *et al*, 2000b; Li *et al*, 2006). Molecular screening for the MSI phenotype and for somatic mutations in the *BRAF*, *KRAS* and *TP53* genes was performed in 1272, 845, 264 and 298 tumours, respectively, in patients aged $\leq$60 years. This represented 77%, 51%, 16% and 18% of cases in this age group, respectively. All 48 tumours available from the 52 patients aged $\leq$30 years were investigated for each of the 4 molecular markers. Because of limitations on resources, only the numbers of samples indicated above were screened for molecular alterations in patients aged 31-60 years. These cases were selected at random.

Statistical analysis

Chi-square analysis was used to compare the frequencies of clinico-pathological features and molecular alterations between different age groups, with *P*-values of <0.05 considered to be significant. Survival times were calculated from the date of diagnosis to the date of death from CRC recurrence. Patients who died from non-cancer related causes were censored at the time of death. Survival estimates were made using Cox regression analysis. SPSS Version 12.0.1 was used for all statistical analyses (Chicago, IL).

Results

The mean age and percentage of females in patient groups classified according to the anatomical site of the primary tumour is shown in Figure 7.1 The mean age decreased progressively from patients with caecal tumours (70.5 years) through to patients with rectal tumours (65.6 years). The percentage of female patients also decreased progressively from proximal to distal tumour sites, with the exception of a small rise from ascending to transverse colonic tumours. Similar observations have been reported in another large, population-based study (Gonzalez *et al*, 2001) and suggest the existence of multiple, age-dependent pathways for CRC development. This was investigated here in more detail by evaluating several major clinicopathological and molecular features in relation to patient age. For the purposes of this study, young patients were defined as ≤60 years and very young as ≤40 years at diagnosis.

Clinicopathological features

Patients aged ≤30, ≤40, ≤50 and ≤60 years comprised 0.9, 3.1, 10.6 and 27.8% of cases, respectively (Table 7.1). The proportion of males amongst patients aged ≤30 years was

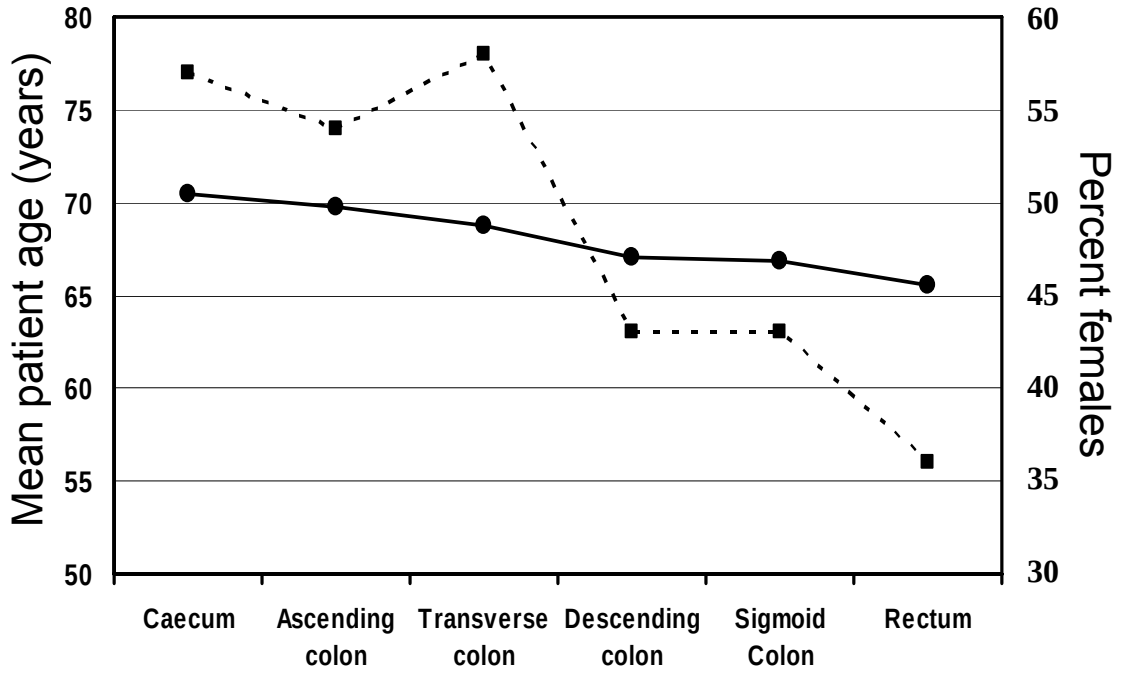


Figure 7.1. The mean age of patients at diagnosis (circles) and the percentage of female patients (squares) are shown for tumours arising at each anatomical subsite in the large bowel.

similar to that of the general CRC population (60:40), whereas between the ages of 31-50 years there was an even gender distribution. A slight predominance of females was found in elderly patients (>75 years). As suggested by the results shown in Figure 7.1, the proportion of rectal tumours was highest in young patients and declined progressively with increasing patient age (Table 7.1). The frequency of poorly differentiated tumours also showed a progressive decrease with age. Young patients showed a significantly higher incidence of late stage (node positive) disease than older patients (P<0.001).

Patient survival

The incidence of peri-operative mortality was 4% for the overall cohort and increased progressively with age (Table 7.1). For each tumour stage, young patients (≤60 years)

generally showed better cancer-specific survival compared to older patients (Table 7.2). This observation was independent of chemotherapy (results not shown).

Molecular features of young patients aged ≤60 years

The MSI phenotype and mutations in the *BRAF* and *KRAS* oncogenes and *TP53* tumour suppressor gene are recognized as major genetic alterations in the development and progression of CRC. These molecular features were investigated in young patients (Table 7.3). The frequencies of MSI and *TP53* mutation were highest in ≤40 year old patients and decreased with advancing age. In contrast, the frequency of *KRAS* mutation was lowest in the very young patients and increased with age. These results demonstrate that significant differences in molecular phenotype occur within the young patient cohort.

CRC in patients aged ≤30 years

Of the 52 patients aged ≤30 years at the time of CRC diagnosis, one was known to have Familial Adenomatous Polyposis syndrome, one had Turcot's syndrome and one had Li-Fraumeni syndrome. Four patients had hereditary non-polyposis CRC (HNPCC), with 2 known to have germ-line mutations in *MLH1* and 2 in *MSH2*. Known familial cancer syndromes therefore accounted for 7/52 (13%) of these very early onset CRCs. The single familial cancer genetic service in the state of Western Australia had received referrals for only 13 of the 52 (25%) patients. Of the 9 MSI+ cases detected in ≤30 year old patients (Table 7.3), 4 were the HNPCC cases described above and 5 are yet to undergo germ-line testing.

Brain neoplasms (3 glioblastoma multiformes, 2 astrocytomas and 1 glioma) had previously or subsequently been diagnosed in 4 patients, including one with Turcot's syndrome, one with Li-Fraumeni and one with HNPCC.

Table 7.1. Clinico-pathological features of CRC by age group

Feature	Total	≤ 30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	61-75yrs	> 75 yrs	*P*
Total	5971	52 (0.9%)	132 (2.2%)	445 (7.5%)	1028 (17.2%)	2616 (43.8%)	1698 (28.4%)	
Sex								
Male	3227 (54%)	32 (62%)	60 (46%)	228 (51%)	605 (59%)	1505 (58%)	797 (47%)	
Female	2744 (46%)	20 (38%)	72 (54%)	217 (49%)	423 (41%)	1111 (42%)	901 (53%)	<0.001
Tumour site								
Proximal colon	1738 (33%)	16 (31%)	32 (27%)	86 (22%)	245 (26%)	722 (31%)	637 (42%)	
Distal colon	2251 (42%)	14 (27%)	45 (38%)	186 (47%)	414 (45%)	1026 (44%)	566 (38%)	
Rectal	1333 (25%)	22 (42%)	41 (35%)	126 (31%)	264 (29%)	583 (25%)	297 (20%)	<0.001
Histological differentiation								
Well-moderate	5112 (86%)	36 (69%)	108 (82%)	365 (82%)	884 (86%)	2276 (87%)	1443 (85%)	
Poor	859 (14%)	16 (31%)	24 (18%)	80 (18%)	144 (14%)	340 (13%)	255 (15%)	<0.001
Stage								
I	907 (15%)	2 (4%)	19 (14%)	62 (14%)	189 (18%)	425 (16%)	210 (12%)	<0.001
II	2166 (36%)	17 (33%)	41 (31%)	110 (25%)	311 (30%)	926 (35%)	761 (45%)	<0.001
III	2109 (35%)	19 (36%)	57 (43%)	190 (43%)	377 (37%)	925 (35%)	541 (32%)	<0.001
IV	789 (13%)	14 (27%)	15 (11%)	83 (19%)	151 (15%)	340 (13%)	186 (11%)	<0.001
Mortality								
Perioperative	239 (4%)	0 (0)	0 (0)	4 (1)	21 (2)	78 (3)	136 (8)	
Follow-up (months)								
Mean	60	47	74	72	68	63	46	
S.D.	49.47	42.75	51.25	51.10	51.11	49.95	43.89	
Range	0-205	0-175	2-190	0-196	0-203	0-205	0-202	

Table 7.2. Cancer-specific survival according to patient age.

Age (yrs)	n	HR [1]	95% CI	*P*	5-year cancer-specific survival (%)
Stage I					
≤ 40	21	1.55	0.48-5.06	0.46	85.7
41-60	251	0.87	0.49-1.54	0.64	92.0
61-75	425	1.00	-	-	90.1
≥ 76	210	2.35	1.47-3.75	0.001*	82.9
Stage II					
≤ 40	48	0.67	0.344-1.31	0.24	83.3
41-60	321	0.84	0.66-1.07	0.16	77.7
61-75	926	1.00	-	-	76.7
≥ 76	761	1.37	1.13-1.66	0.001*	74.6
Stage III					
≤ 40	76	0.79	0.57-1.11	0.18	52.6
41-60	567	0.83	0.72-0.96	0.01*	49.0
61-75	925	1.00	-	-	46.1
≥ 76	541	1.26	1.09-1.45	0.002*	48.1
Stage IV					
≤ 40	29	0.89	0.57-1.38	0.60	15.4
41-60	234	0.84	0.68-1.03	0.09	22.4
61-75	340	1.00	-	-	16.6
≥ 76	186	1.09	0.87-1.36	0.44	30.1

[1] Patient survival for each age group is compared to 61-75 year old patients as the reference group.

Table 7.3. Molecular features of tumours from young CRC patients.

	Patient age (% of tumours with molecular alteration)				
Feature	≤ 30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	*P*
MSI [1]	9/48 (18.7)	17/94 (18.1)	28/338 (8.3)	47/792 (5.9)	<0.001
BRAF mutation [2]	5/48 (10.4)	5/60 (8.3)	12/224 (5.3)	37/513 (7.7)	NS
KRAS mutation [3]	11/48 (23.0)	5/18 (27.8)	32/57 (56.1)	68/141 (48.2)	<0.01
TP53 mutation [4]	25/48 (52.1)	7/15 (46.6)	19/56 (34.0)	51/179 (28.5)	0.02

[1] MSI was determined using the BAT-26 mononucleotide repeat; [2] *BRAF* V600E mutation; [3] *KRAS* codon 12 and 13 mutations; [4] *TP53* exon 5-8 mutations

Discussion

Western Australia has an isolated and relatively non-migratory population of approximately 2 million people. Centralized health records make this state an excellent site for population-based research. Population studies are not subject to selection bias and are therefore the only way to carry out proper assessment of the clinico-pathological characteristics of a disease. The present study on 5,971 CRC from a predominantly Caucasian population is the largest to date that assesses pathological and molecular features, as well as survival. It has allowed us to make several important observations, particularly in the young patient group.

Similar to other population-based studies (Gonzalez*et al*, 2001; Matanoski *et al*, 2006), we observed a slight predominance of females amongst elderly CRC patients (>75 years; Table 7.1). As far as we are aware, the current study is the first to show a progressive decrease in the proportion of rectal cancers with increasing patient age (Table 7.1). A progressive decrease in the proportion of poorly differentiated tumours with advancing age was also observed and has been noted previously by others (Liang *et al*, 2003; O'Connell *et al*, 2004; Lin *et al*, 2005). The current results also concur with previous reports that young CRC patients present more often with late stage disease (Mitry *et al*, 2001; Liang *et al*, 2003; O'Connell *et al*, 2004; Lin *et al*, 2005). However, in agreement with other population-based studies (Mitry *et al*, 2001; Chung *et al*, 1998; Kam *et al*, 2004), late stage presentation did not translate into worse survival for young patients (Table 7.2). Trends for better survival of young patients were in fact observed, reaching significance in 41-60 year old patients with stage III disease. These results argue against the existence of an aggressive tumour phenotype in very young patients. The initial reports of worse prognosis for young patients were mostly based upon highly selected series (Heys *et al*, 1994; Lee *et al*, 1994).

The large size of this population-based cohort allowed examination of molecular features in the less frequent patients diagnosed at a young age (≤60 years). Tumours from young patients are generally believed to have a hereditary component and also appear to follow the CIN rather than CIMP pathway. The CIN pathway involves frequent loss of heterozygosity and mutations to the *APC*, *TP53* and *KRAS* oncogenes. This pathway is likely to involve the adenoma to carcinoma sequence proposed in the Vogelstein model (Fearon *et al*, 1990). The MSI phenotype was originally proposed as an alternate pathway, however more recent data suggests it can occur in tumours with either CIN or CIMP features (Kambara *et al*, 2004).

The frequency of MSI+ tumours in the current study was approximately 3-fold higher in very young patients (≤40 years) compared to those aged 41-60 years (18.3% *vs.* 6.6%). Two other population-based studies of Caucasians found similar frequencies of 17% (Gryfe *et al*, 2000) and 19.7% (Losi *et al*, 2005) in patients aged <50 years and <45 years, respectively. Interestingly, two population-based studies of Asians reported higher MSI+ frequencies of 29.4% (Liang *et al*, 2003) and 31.3%(Suh *et al*, 2002). The MSI phenotype in tumours from young CRC patients is a hallmark of HNPCC. Of the 9 patients aged ≤30 years with MSI+ tumours, 4 (44%) were known to have germ-line mutations in either the *MLH1* or *MSH2* mismatch repair genes. The remaining 5 cases have yet to be tested for germ-line mutations and hence the incidence of HNPCC cannot yet be inferred from the MSI results. The importance of family history information for identifying HNPCC has recently been challenged, with up to 50% of germ-line mutations estimated to be missed using this information alone (Liang *et al*, 2003; Southey *et al*, 2005). In the present study, only 25% of ≤30 and 12% of 31-40 year old patients were referred to the single familial cancer program that services the state of Western Australia. Routine MSI screening for young patients (≤60 years) might therefore be a useful triage

allowing positive patients to be recommended for further genetic counselling and germ-line mutation testing (Southey *et al*, 2005). The finding of a germ-line mutation has implications for surveillance of the patient and any affected family members.

Similar to MSI+, the frequency of *TP53* mutation was also significantly higher in ≤40 year old compared to 41-60 year old patients (Table 7.3). This might in part be explained by the higher proportion of rectal cancers in the very young CRC population (Table 7.1). Previous studies have shown a higher frequency of *TP53* mutation in rectal compared to colon cancers (Russo *et al*, 2005). In contrast to MSI+ and *TP53* mutation, the incidence of *KRAS* mutation in very young patients was significantly lower than in 41-60 year old patients. This finding is similar to another recent report (Alsop *et al* 2006), although the frequency of *KRAS* mutation (6%) in that population-based study was unusually low compared to other studies. *BRAF* mutations occur in the majority of MSI+ tumours from older patients but have never been observed in MSI+ tumours from HNPCC patients (Deng *et al*, 2004). They are also very rare in MSI+ tumours from young patients (Samowitz *et al*, 2005). In the current study, *BRAF* mutation was only seen in association with the MSI+ phenotype in patients aged 54 years or older. Nevertheless, *BRAF* mutations were present in 7% of MSI- tumours in young patients.

In summary, the current population-based study found clear evidence for age-related differences in site distribution, histological grade and stage of presentation. Despite evidence for a more aggressive tumour phenotype, the survival of young patients was not worse than 61-75 year olds. In the younger cohort of patients, striking age-related differences were observed in the frequency of several important molecular alterations, suggesting these may be important in CRC etiology. Increased knowledge of age-related differences in tumour molecular characteristics should assist with the development of novel treatment strategies in the future.

Chapter 8 Discussion

General Discussion

It is the personal choice of any patient as to whether they wish to receive chemotherapy for treatment of their colon cancer. Clinicians have a responsibility to ascertain how much information a patient would like to know regarding their prognosis. Explanation of the potential side effects of therapy compared to the potential benefits is equally important in allowing the patient autonomy in making an informed choice about their treatment options. Clinical judgement is a necessary and important contributor to the decision making process. Assessment of the health status of the patient in terms of other physical co-morbidities, psychological well being and social support is essential before the implementation of a course of adjuvant chemotherapy. The availability of support services may play a vital role in the successful completion of a course of adjuvant chemotherapy.

Guidelines for the adjuvant treatment of stage II colon cancer

At the commencement of this thesis in 2004, the evidence from randomised controlled trials was conflicting as to whether stage II colon cancer patients derived a survival benefit from adjuvant chemotherapy. Investigators from the National Surgical Adjuvant Breast and Bowel Project have indicated that the reduction in risk of recurrence by adjuvant therapy in patients with stage II disease is of similar magnitude to that observed for stage III, although an overall survival advantage has not been firmly established (Mamounas *et al*, 1999). Randomised controlled trials have been underpowered to determine whether stage II patients derive an overall survival benefit from adjuvant

chemotherapy. The IMPACT B2 investigators found the absolute reduction in risk of death for stage II colon cancer patients receiving adjuvant chemotherapy was only 2% and was not statistically significant (IMPACT B2, 1999). Mature results from the QUASAR randomised controlled trial are awaited, but preliminary results indicate an absolute survival benefit of 1-5% for stage II patients (Gray *et al*, 2004).

International guidelines current at the commencement of this work qualified their assertions of no proven survival benefit from adjuvant chemotherapy in stage II disease by statements such as: "*If the tumours had features of poor prognosis then adjuvant chemotherapy could be considered, or could be used in the setting of clinical trials*". However the definition of what actually constitutes a 'poor prognosis' stage II tumour was neither clear nor uniform. Factors such as having less than 12 lymph nodes examined, poor histological differentiation, aneuploidy, high S phase analysis, deletion of chromosome 18q, tumour perforation, bowel obstruction, invasion of surrounding structures and evidence of positive margins have all been suggested in the classification of a 'poor prognosis' stage II colon cancer.

The American Society for Clinical Oncology (ASCO) currently does not recommend adjuvant chemotherapy for stage II colon cancer (Benson *et al*, 2003). The most recent Australian NH&MRC guidelines (2006) have been revised and now state:

'There is a small but statistically significant benefit from the use of adjuvant chemotherapy in Stage II colon cancer. A decision regarding treatment should be made following discussion of the relative merits and side effects of chemotherapy. High risk sub-groups are more likely to benefit from adjuvant chemotherapy.'

In the majority of current guidelines, adjuvant chemotherapy for stage II colon cancer is only considered for high risk subgroups and commonly involves the 5FU/ leucovorin regimens. New agents such as oral 5FU (capecitabine and UFT) and

combinations of 5FU and oxaliplatin (FOLFOX) are being considered for use in stage II disease, although firm evidence from randomised controlled trials for their efficacy is still lacking. Only the National Comprehensive Cancer Network of the US advocates in its 2006 guidelines the consideration of adjuvant chemotherapy for stage II colon cancer even in the absence of high risk features (NCCN, 2007).

The 5-year survival rate for stage II colon cancer is approximately 75%, although reported estimates range from 60-85%. Survival rates for 'poor prognosis' stage II colon cancer are comparable to those of stage IIIA colon cancer. Therefore it seems reasonable that if 'poor prognosis' stage II tumours could be accurately identified, adjuvant chemotherapy should be offered to these patients with the aim of improving their survival.

Prognostic stratification of stage II colon cancer (Chapter 3)

Molecular-based markers hold considerable promise as clinically useful prognostic factors in CRC, but these hopes have so far failed to materialize. Candidate molecular prognostic markers for this disease were discussed in detail in the Introduction (Chapter 1). The major limitations associated with molecular markers are the reproducibility and cost of assays, the requirement with some assays for frozen tissues, and the difficulty in quantifying protein expression levels detected by immunohistochemistry. The current study investigated the routinely evaluated histopathological features for their ability to provide independent prognostic information in stage II colon cancer (Chapter 3).

Retrospective studies allow the evaluation of promising prognostic markers that are sufficiently prevalent to be clinically useful. The current population-based, retrospective study evaluated the commonly reported pathological variables for their

prognostic significance in patients treated by surgery alone. The major finding of this study was that careful pathological assessment of T stage and vascular invasion allowed the prognostic stratification of stage II colon cancer (Chapter 3). This research has provided clarification of the guidelines in terms of defining "poor prognosis" stage II colon cancer patients who should be considered for adjuvant chemotherapy.

"Poor prognosis" stage II colon cancers were defined here as being T4 stage (tumour invasion of free serosal surface) and/or showing vascular invasion (VI+). The hazard ratio for worse survival associated with T4 was 1.96 (95%CI: 1.32-2.90, $P<0.001$) and for VI+ it was 2.73 (95%CI: 1.76-4.21, $P<0.0001$) compared to T3 and VI-, respectively. T4/VI+ tumours comprised 26% of all stage II colon cancers in this population-based cohort and showed a 5-year disease-specific survival rate of just 71%. In comparison, the remaining "good" prognosis patients (T3/VI-) comprised 74% of cases and showed a 5-year survival rate of 84%.

Other workers have also reported T stage and vascular invasion to be independent prognostic markers in stage II colon cancer (Newland *et al*, 1995; Shepherd *et al*, 1997; Khankhanian *et al*, 1997; Mulcahy *et al*, 1997; Burdy *et al*, 2001; Petersen *et al*, 2002; Merkel *et al*, 2001; Lennon *et al*, 2003; Ludeman *et al*, 2005). The definition of peritoneal involvement (T4, serosal invasion) varies amongst different reporting guidelines. Its diagnosis is dependent upon appropriate tumour sampling and the diligence with which it is investigated. Consequently, the reported frequency of peritoneal involvement in stage II colon cancer ranges from 12-43% (Newland *et al*, 1995; Shepherd *et al*, 1997).

Following pathological assessment of 268 stage II colon cancers, Petersen *et al* (2002) found that peritoneal involvement, venous spread (both submucosal and extramural), spread to involve the surgical margin and perforation through the tumour

were all independent prognostic factors in multivariable analysis. Only 31% of patients in their study showed none of the above "poor" prognostic features, while 42% showed evidence of peritoneal involvement and 43% showed venous invasion. In comparison, the current population-based cohort found that T4 stage was reported in 20% of stage II colon cancers and venous invasion in only 11%. Consequently, a relatively higher proportion (74%) of tumours was classified having neither of these "poor" prognostic features.

The "good prognosis" stage II colon cancer patients in the current study showed a 5-year survival rate of 84%. A pilot study was performed to ascertain the frequency of false negative reporting for T4 and vascular invasion (VI) within this "good prognosis" group (Stewart *et al*, 2007). The hypothesis for the study was that following histopathological review, "good prognosis" patients who subsequently died of disease recurrence would show a higher frequency of false negative reporting for T4 and VI compared to patients who survived. The tumour cohort selected for blinded pathological review (n=82) was enriched for patients who had subsequently died of cancer recurrence (n=35, 43%). Following review, 26 cases (32%) were reclassified as being T4, VI+ or both. Importantly, 18/35 (51%) of the patients who died of cancer were upstaged compared to only 8/47 (17%) of those who survived (P=0.0004). These results indicate that a more rigorous assessment of T stage and VI will lead to improved stratification of stage II colon cancer into "good" and "poor" prognosis groups.

Proforma-based reporting of CRC by Western Australian pathology services was introduced midway through the study period (cf 1998). As described by other workers, introduction of the proforma has resulted in an increase in the reported frequencies of certain pathological variables (Beattie *et al*, 2003; Rigby *et al*, 1999). In this study, the frequency of T4 stage increased from 18% to 22% (P=0.03) and that of vascular invasion

from 9% to 16% ($P<0.0001$). Consequently, fewer stage II colon cancers were classified as "good prognosis" following the introduction of proforma (67% *vs.* 76%, $P<0.0002$). Importantly, the 5-year survival rate for "good prognosis" patients (T3/VI-) increased from 77% pre-proforma to 88% post-proforma ($P<0.0008$). This suggests the quality of reporting became more accurate in terms of prognostic stratification following the introduction of proforma. When the major target group for chemotherapy is analysed (patients aged $\leq$ 75 yrs), the introduction of proforma was associated with an increase in the 5-year survival of "good prognosis" patients from 78% to 91% ($P=0.0003$).

The goal of future work is to define a robust prognostic model for stage II colon cancer that identifies "good prognosis" patients having >90% 5-year survival and who therefore do not require referral for adjuvant chemotherapy. The introduction of proforma reporting in Western Australia appears to have led to more accurate reporting of T stage and vascular invasion, thus allowing stage II colon cancers diagnosed in younger patients ($\leq$ 75 yrs) to be stratified into a "good prognosis" group with a 5-year survival rate of >90%. This group accounts for about two thirds of stage II colon cancers and could reasonably be treated by surgery alone. In contrast, "poor prognosis" patients showed a 5-year survival of just 74% and should be strongly considered for adjuvant chemotherapy.

Further study is required to determine whether the use of elastin staining will allow even greater accuracy in the evaluation of T4 and VI. The addition of routine elastin staining to the assessment of colon cancer specimens is technically feasible and cost effective (Abdulkader *et al*, 2006). Moreover a recent study by Shinto *et al* (2004) suggests that tumour relationship to the elastic lamina provides prognostic information in T3 carcinomas. There is a danger however that elastin staining may lead to an increase in

false positive reporting for T4 and VI and therefore a decrease in the prognostic significance of these markers.

A consensus conference on prognostic factors held by the American Joint Committee on Cancer (Compton C *et al*, 2000) noted that host lymphoid response was a potentially promising prognostic factor that needed further evaluation and confirmation before inclusion in routine pathological reports. In the current study cohort, the lack of a uniform, standardised approach for the reporting of lymphocytic response was likely to have prevented accurate evaluation of its prognostic significance. Nevertheless, lymphocytic response appears to have no prognostic significance in stage II and III colon cancer patients treated by surgery alone (Chapters 3 and 6). This is in contradistinction to recent reports (Galon *et al*, 2006; Galon *et al*, 2007; Ohtani *et al*, 2007) that demonstrate prognostic significance for TILs. The retrospective nature of the current study with collection of data on pathological variables from earlier reports did not allow the identification of lymphocyte subsets that may have proven important for prognosis. Verification of the accuracy of pathology reporting for lymphocytic response was not performed. Quantitative analysis of lymphocytic response is required to address the question of the possible prognostic significance of TILs in this cohort.

Adjuvant chemotherapy in stage II colon cancer (Chapter 4)

The ability to identify ‘poor’ prognosis stage II colon cancers that should be referred for adjuvant chemotherapy leads to the question of whether such patients will receive a survival benefit from this treatment. The current study attempted to answer this by comparing the survival of patients treated with chemotherapy to those treated by surgery

alone. Statistical modelling was performed to adjust for the non-randomized nature of the adjuvant treatment.

Over the course of the study period (1993-2003), the use of adjuvant chemotherapy for stage II colon cancer peaked at 25-30% of ≤75 yr old patients in the late 1990s but then decreased to <15% in 2002 and 2003 (Chapter 4). Following multivariate adjustment, a significant survival advantage was seen for patients who received adjuvant chemotherapy (HR=0.62, 95%CI [0.39-0.98], *P*=0.04).). It is of some concern that only 23% of patients aged ≤ 75 years and with "poor prognosis" tumours (T4 stage and VI+) received chemotherapy during the study period. This most likely reflects a lack of awareness by surgeons of the adverse prognosis associated with T4 and VI+, together with continued scepticism surrounding the benefits of chemotherapy for stage II colon cancer patients. Whilst failing to reach statistical significance, there was evidence that patients with "poor prognosis" tumours gained a survival advantage from adjuvant chemotherapy (HR=0.60, 95%CI [0.29-1.26], *P*=0.18).

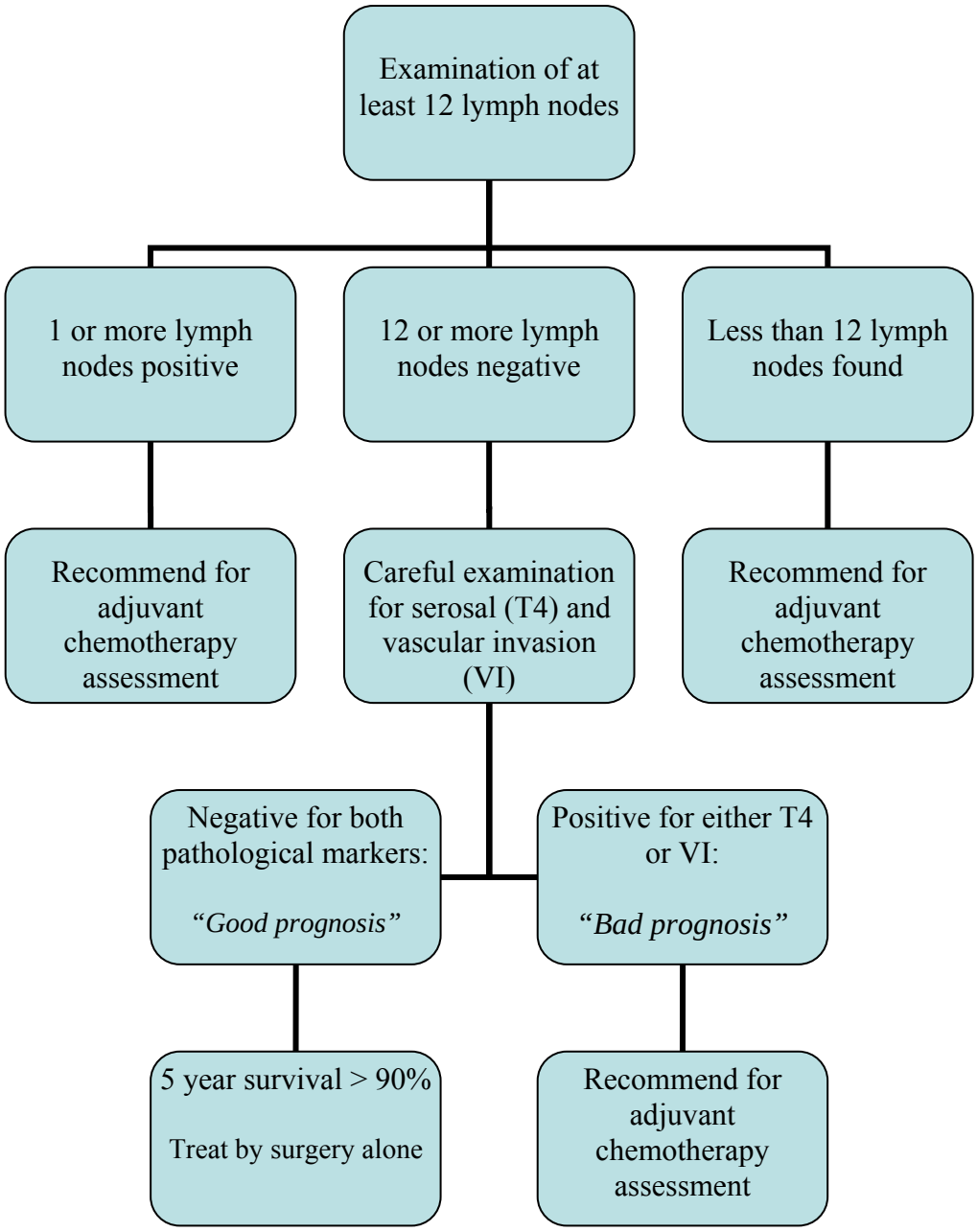
It is important to acknowledge the retrospective, population-based nature of this study and the fact that patients were not randomised to receive adjuvant chemotherapy. The above result should therefore be interpreted with caution because unidentified confounders may have biased the result for benefit from chemotherapy. Propensity score analysis (PSA) can be used to reduce these biases that are inherent in all observational studies (Adamina *et al*, 2006; Yue, 2007; Rubin, 2007). The propensity score estimate reduces bias through matching, stratification and regression adjustment, or via a combination of all three. PSA was used here for the survival analysis of stage II colon cancer patients. The results showed that survival benefits from adjuvant chemotherapy were comparable to those obtained by multiple variable regression (results not shown).

Clarification of the Australian NH&MRC guidelines (NH&MRC, 2005) is necessary to highlight that T4 stage and VI in stage II colon cancer are associated with poor prognosis and that such patients should therefore be recommended for adjuvant chemotherapy. Education of surgeons and oncologists concerning the prognostic significance of T4 and VI is vital in order to improve the targeting of adjuvant chemotherapy to patients who are most in need. A diagnostic and clinical management pathway for colon cancer is proposed in Figure 8.1. Randomised clinical trials will be necessary to determine whether "poor prognosis" stage II colon cancer patients obtain survival benefits from chemotherapy. Prospective studies will also be required to confirm that stage II patients treated by surgery alone and classified as "good prognosis" by virtue of T3 and VI- tumour status have 5-year survival rates in excess of 90%. Improved targeting of 5FU-based chemotherapy for stage II colon cancer will help achieve more cost-effective healthcare, as well as preventing side-effects and risks associated with adjuvant treatment for patients who could have been effectively treated by surgery alone.

Completion of adjuvant chemotherapy and impact upon survival (Chapter 5)

One of the major findings of this study was that failure to complete chemotherapy was associated with adverse survival in both stage II and III colon cancer patients (Chapter 5). This result confirms two recent North American studies that used survival information from the SEER database (Neugut *et al*, 2006; Dobie *et al*, 2006). In the present population-based study, approximately one third of stage III colon cancer patients who initiated chemotherapy failed to complete this treatment. The novel observation made here was that patients who failed to complete adjuvant chemotherapy ($\leq$3 cycles) showed worse survival compared not only to patients who completed adjuvant chemotherapy

Figure 8.1. Flow diagram illustrating diagnostic and clinical pathways for the management of colon cancer.



(HR=2.24, 95%CI [1.66-3.03], $P<0.001$) but also to those treated by surgery alone (HR=1.37, 95%CI [1.09-1.72], $P=0.008$). Similar observations were made for stage II colon cancers, although with a smaller sample size.

The retrospective nature of this study and the absence of recorded toxicity data complicate the interpretation of these results. The worse survival of patients who failed to complete chemotherapy is clearly not due to mortality from 5FU-related toxicities, since this rate is generally estimated to be approximately 0.5%. Many of the patients who withdrew from treatment are likely to have experienced debilitating side effects. Their poor survival could be explained by a link between increased toxicity to 5FU and a more aggressive tumour phenotype or impaired host immune response. Prospective studies that correlate tumour properties and immune status with toxicity to 5FU-based treatments and the reasons for non-completion of treatment are required to address these issues.

Psychosocial support is vital for patients who commence chemotherapy (Chantler *et al*, 2005; Duric *et al*, 2007). Colon cancer is especially common in elderly patients and these are known to gain similar levels of survival benefit from adjuvant chemotherapy as younger patients (Sargent *et al*, 2001; Gill *et al*, 2004). However, elderly patients often have pre-existing co-morbidities, have less access to services such as transportation and are frequently isolated socially. Together, these factors are likely to have an adverse impact upon the ability of such patients to complete their chemotherapy regimen.

Failure to complete adjuvant chemotherapy regimens has major implications for the introduction of new anti-neoplastic drugs for use in combination with 5FU-based chemotherapy. Combination therapies such as FOLFOX (5FU/oxaliplatin) have worse toxicity profiles than 5FU alone (Table 8.1), leading potentially to higher non-completion rates. Protocols that address the management of toxicity complications with 5FU-based therapies are needed and dose adjustments and/or selective drug withdrawal may be

required to allow the completion of 5FU regimens. Capecitabine has a better toxicity profile than 5FU/leucovorin (Twelves *et al*, 2005) and may therefore offer a solution for the delivery of more effective adjuvant therapy in colon cancer.

Table 8.1. Frequency of toxicities from 5FU-based chemotherapy in CRC.

Toxicity type	5FU [1]	Capecitabine [1]	FOLFOX [2]	XELOX [3]
	Frequency of toxicity: all grades/Grades 3&4 (%)			
Thromboctyopenia	0/0	0/0	75/2	20/5
Neutropenia	60/25	35/5	80/40	20/5
Diarrhoea	65/15	50/10	55/10	65/15
Mucositis	60/15	20/5	40/5	20/0
Nausea/Vomiting	50/5	35/5	75/10	70/15
Neurological	0/0	0/0	90/15	85/15
Hand/foot syndrome	10/0	60/15	10/0	40/5

[1] Twelves C, *et al* (2005)

[2] Andre T, *et al*. (2004)

[3] Cassidy J, *et al*. (2004)

Health service provision

A striking finding in this study was that patients treated in district hospitals were less likely to initiate chemotherapy and that both district and rural patients were less likely to complete chemotherapy (Chapter 5). This study also found that patients treated in private hospitals were more likely to initiate chemotherapy and those with higher financial resources were more likely to complete chemotherapy. The reasons underlying the failure to complete chemotherapy in approximately one third of patients who begin this treatment require further investigation in prospective studies. This should involve

documentation of the toxicities experienced with different regimens and the reasons for not initiating treatment and/or withdrawing before completion. A prospective study would allow areas of need in health service provision to be identified and strategies to improve these services investigated and trialled. Health care services need to ensure that colon cancer patients who are socio-economically disadvantaged, lack psychosocial support or are treated in smaller centres are not disadvantaged in terms of access to medical care and the opportunity to complete 5FU chemotherapy.

Adjuvant 5FU chemotherapy in stage III colon cancer and pathological predictors of survival benefit (Chapter 6)

The rate of initiation of adjuvant chemotherapy (predominantly the Mayo regimen) for stage III colon cancer patients (<75 years of age) was 57% during the study period 1993-2003. The survival benefit from chemotherapy for patients who initiated chemotherapy was estimated at 16% (HR=0.84, 95%CI [0.71-0.98], $P<0.05$) following adjustment for possible confounders (Chapter 6). Since there are currently no validated molecular predictive markers, an attempt was made here to identify possible pathological predictors of survival benefit from 5FU-based chemotherapy.

In both stages II and stage III colon cancer, female gender was associated with a trend for greater survival benefit from 5FU-based chemotherapy than males (Chapters 4 and 6). It is well established that females suffer more toxicity from 5FU treatment than males, probably due to gender differences in the metabolism of this drug (Milano *et al*, 1992; Zalcberg *et al*, 1998; Sloan *et al*, 2002; Patel *et al*, 2004; Chansky *et al*, 2005). Conversely, it is possible that patients who suffer more toxicity also gain more therapeutic benefit. Although toxicity data was not available for this population-based

cohort, the lower chemotherapy completion rate observed here for females compared to males (24% *vs.* 31%, $P<0.02$*) is in accordance with previous studies (Chanksy *et al*, 1992; Milano *et al*, 1992; Zalcberg *et al*, 1998; Meta-analysis Group in Cancer, 1998; Sloan *et al*, 2002). It can be hypothesized that increasing the 5FU treatment dose in males to reach the same level of toxicity as that observed for females would result in a similar survival benefit between the two sexes. Pooled analyses of stage II and III colon cancers from randomized trials did not find evidence for gender difference in benefit from 5FU chemotherapy (Gill *et al*, 2004). This may reflect differences in patient profiles between controlled trials and observational studies, as alluded to earlier.

Patients aged >55 years showed a trend for greater survival benefit from adjuvant chemotherapy than patients aged ≤55 years (Table 6.3). This may be an indication that different age-related pathways of CRC tumourigenesis (Chapter 7) are associated with a different response to 5FU chemotherapy, as discussed below.

Exploratory subgroup analysis was performed to compare the survival of pathologically defined subgroups of patients treated with or without chemotherapy in stage III colon cancer. Patients whose tumours demonstrated a lymphocytic response or showed evidence of perforation appeared to gain the most survival advantage from adjuvant chemotherapy (Chapter 6). It can be hypothesised these two pathological variables act synergistically with 5FU-based chemotherapy to overcome the body's immunological tolerance to the tumour, thereby activating tumour clearance by enabling immune system recognition. The associations between perforation and lymphocytic response with apparent benefit from 5FU chemotherapy warrant closer examination of immune system activation and of a possible synergistic relationship with adjuvant chemotherapy.

Age-related variation in pathological and molecular features of CRC (Chapter 7)

The large, population-based nature of this study cohort allowed the investigation of CRC incidence and survival in relation to patient age and gender, as well as to various clinico-pathological features including tumour subsite (Chapter 7). As summarized in Figure 8.2, distal tumours occurred predominantly in younger males and their incidence decreased with advancing age in both sexes. In contrast, the number of proximal tumours increased progressively with age. It can be hypothesised that age, site and gender differences reflect the existence of two major pathways of CRC tumourigenesis. The chromosomal instability pathway (CIN) is commonly found in distal tumours from young males, whereas the methylator phenotype pathway (CIMP) is characteristically seen in elderly females with proximal tumours (Iacopetta, 2006).

Clinical database construction and tissue banking

There is a critical need to collate data in an attempt to address clinical questions and to provide guidance to clinicians and patients alike in the management of cancer. The research conducted in this thesis highlights the importance of accurate and routine collection of data for colon cancer patients at a population level, specifically for:

- Clinicopathological features including nodal status, serosal invasion, vascular invasion, lymphocytic reaction and perforation.
- Adjuvant treatment, including regimenn, dose, number of cycles and toxicity.

In addition to the above clinicopathological data, considerable value-adding can be achieved by the collection, with patient-consent, of tumour tissues. This allows

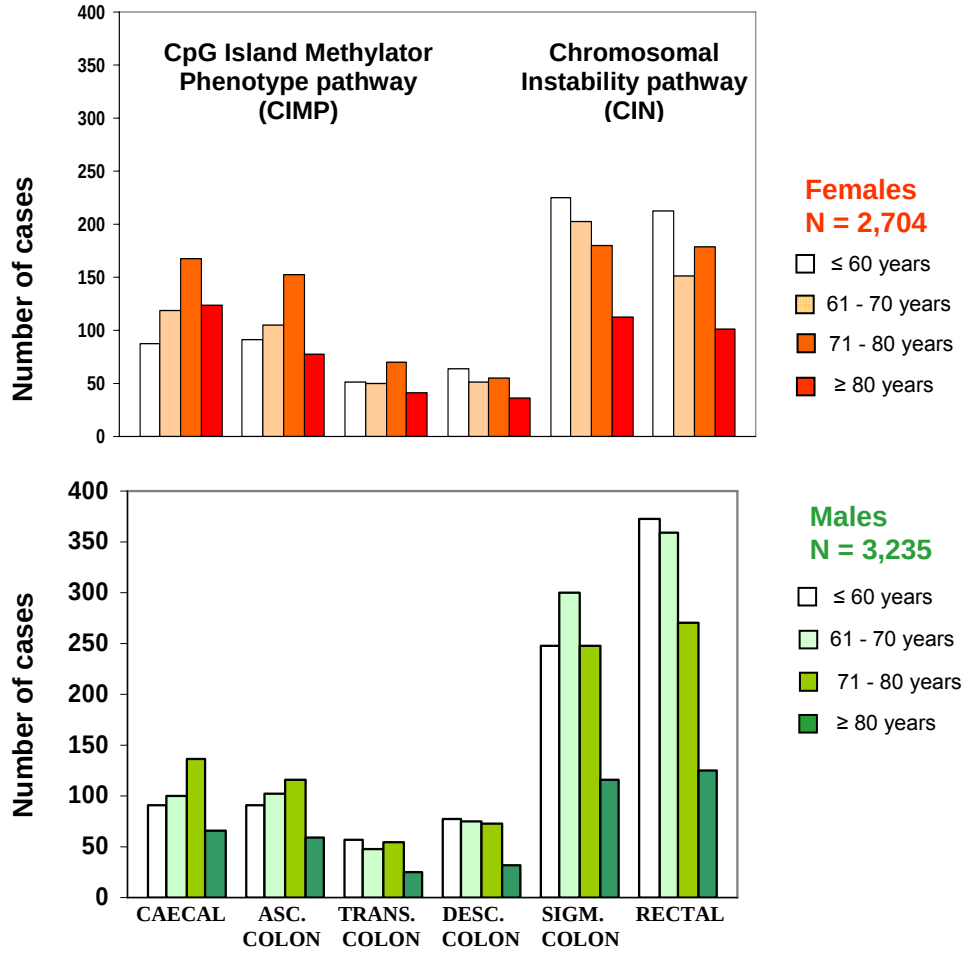


Figure 8.2 Incidence of CRC in Western Australia distribution according to age, sex and site. All stages.

morphological and molecular features to be correlated with toxicity to treatment and patient survival. A comprehensive approach to understanding the factors that influence patient outcomes in the management of colon cancer is clearly necessary. This involves not only the collection of clinical data concerning all aspects of cancer management, but also tissue samples allowing the investigation of molecular mechanisms for toxicity and response.

Conclusions

Population-based studies are an important component of translational research. In part, they allow for auditing of disease management in a population. Guideline verification and analysis of the reasons for lack of adherence to guidelines is integral to improving the management of a disease. Laboratory-based research linked to clinical databases should allow mechanisms of disease to be investigated in greater detail, together with prediction of response to current treatments and the development of novel therapeutic agents. The major findings of this translational research into colon cancer can be summarized:

1. The histopathological features of serosal and vascular invasion allow prognostic stratification of stage II colon cancer into “good” and “poor” prognosis groups. Good prognosis patients can confidently be spared adjuvant chemotherapy. Greater effort should be directed towards ensuring that a much higher proportion of poor prognosis patients are referred for chemotherapy than is currently the case.

2. Evidence was obtained for a survival advantage from the use of 5FU chemotherapy in stage II colon cancer, particularly for women and patients with tumour features of poor prognosis.

3. Completion of 5FU chemotherapy confers a survival advantage, whereas failure to complete chemotherapy results in a survival disadvantage, when compared to

patients treated by surgery alone. This was observed for both stage II and III colon cancer patients.

4. Perforation and tumour lymphocytic response were associated with a survival advantage from the use of chemotherapy in stage III colon cancer.

5. The age, site and sex distribution of CRCs revealed in this study provides further evidence for two major pathways of CRC tumourigenesis. The CIN pathway is predominant in distal tumours from young males, while the CIMP+ is predominant in proximal tumours from older females.

Future work

The results from the present study suggest the following additional investigations are warranted:

1. Prospective evaluation of whether elastin staining and careful assessment of serosal and vascular invasion enable the identification of a "good prognosis" subgroup of stage II colon cancer patients with >90% 5-year survival.

2. Prospective study to determine the reasons for non-completion of 5FU chemotherapy amongst colon cancer patients.

3. Correlation of toxicity to 5FU with host and tumour factors such as methyl group metabolism.

4. Prospective studies to further evaluate perforation, tumour lymphocytic response and host immune system activation as candidate predictive markers of benefit from 5FU chemotherapy.

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