A Population-based Study of Age-related Variation in Clinicopathological Features, Molecular Markers and Outcome from Colorectal Cancer

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Abstract. *Background: To investigate age-related differences in clinicopathological features, molecular alterations and patient survival in a large, population-based series of CRC. Patients and 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: Patients aged ≤30, ≤40, ≤50 and ≤60 years comprised 0.9%, 3.1%, 10.6% and 27.8% of all cases, respectively. The proportion of rectal cancers and tumors with poor differentiation was higher in ≤30-year-old patients and decreased progressively with age. The incidence of tumors 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 K-ras mutations were less common (p=0.0001) when comparing the same age groups. Conclusion: These results provide evidence for major age-related differences in the clinical and molecular features of CRC.*

Colorectal carcinomas (CRC) are thought to develop from adenomas following the accumulation of mutations to oncogenes and tumor suppressor genes (1). This process 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 (2). Frequent methylation of gene promoter regions characterises this second pathway and the resulting tumors are referred to as CpG island methylator phenotype, or CIMP (3). 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 tumors arise more frequently in the proximal colon of older patients (4), whereas CIN tumors are more common in the distal colon and rectum (5). In addition to anatomical site, other major influences on the profile of genetic and epigenetic changes present within CRC are gender and age (6). Population-based data reveals that patients with caecal tumors have the highest mean age at diagnosis and contain the highest proportion of females (7). Both the mean age and the percentage of female patients then decline progressively as the tumor site becomes more distal. These epidemiological observations can be explained by predominance of the CIMP pathway in older females with proximal tumors and of the CIN pathway in younger males with distal tumors.

Tumors 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 (8, 9). 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 old) who comprise approximately 3% of total cases, but represent a considerably higher proportion in terms of years of life lost to CRC.

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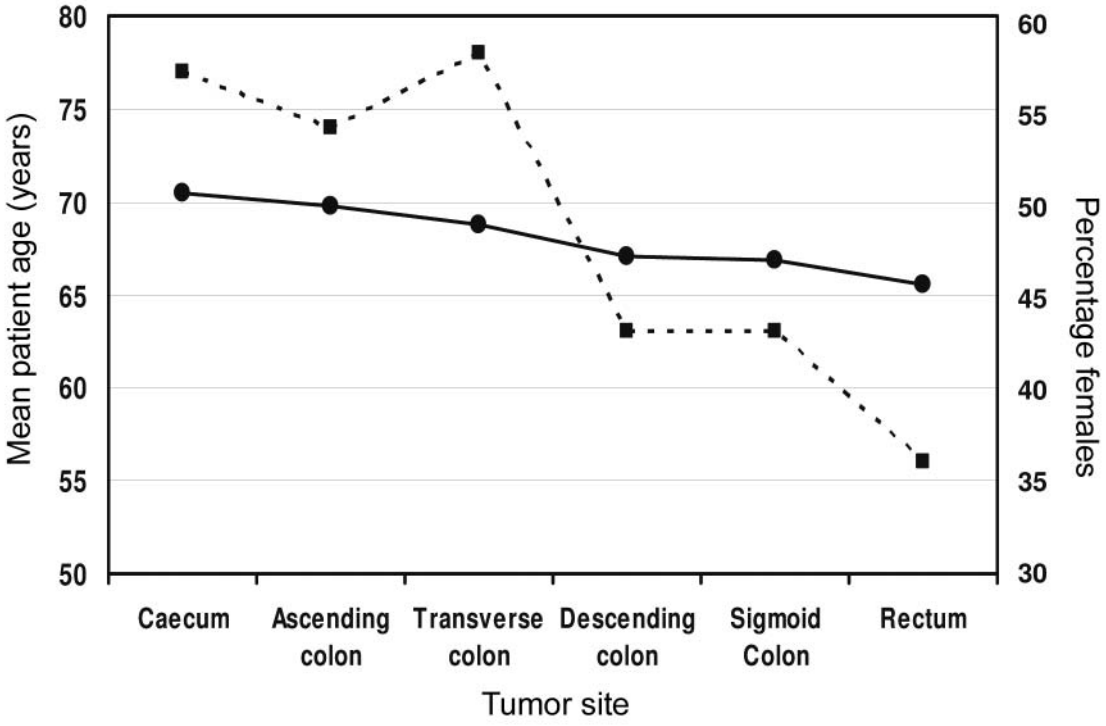

Figure 1. *The mean age of patients at diagnosis (circles) and the percentage of female patients (squares) are shown for tumors arising at each anatomical subsite in the large bowel.*

Patients and 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=5,971). 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. Tumor stage was classified according to current AJCC guidelines (10). 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 tumor 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 from 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 (11). Mutations in the *BRAF* (V600E), K-*ras* (codons 12 and 13) and *TP53* (exons 5-8) genes were also determined by PCR-based, fluorescent single strand conformation polymorphism analysis as described elsewhere by our group (12-14). Molecular screening for the MSI phenotype and for somatic mutations in the *BRAF*, K-*ras* and *TP53* genes was performed in 1272, 845, 264 and 298 tumors, respectively, in patients aged ≤60 years. This represented 77%, 51%, 16% and 18% of cases in this age group, respectively. All 48 tumors available from the 52 patients aged ≤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 clinicopathological 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, USA).

Table I. *Clinicopathological features of colorectal cancer by age group.*

Feature	Total	≤30 years	31-40 years	41-50 years	51-60 years	61-75 years	>75 years	*P*
Total	5,971	52 (0.9%)	132 (2.2%)	445 (7.5%)	1,028 (17.2%)	2,616 (43.8%)	1,698 (28.4%)	
Gender								
Male	3,227 (54%)	32 (62%)	60 (46%)	228 (51%)	605 (59%)	1,505 (58%)	797 (47%)	
Female	2,744 (46%)	20 (38%)	72 (54%)	217 (49%)	423 (41%)	1,111 (42%)	901 (53%)	<0.001
Tumor site								
Proximal colon	1,738 (33%)	16 (31%)	32 (27%)	86 (22%)	245 (26%)	722 (31%)	637 (42%)	
Distal colon	2,251 (42%)	14 (27%)	45 (38%)	186 (47%)	414 (45%)	1,026 (44%)	566 (38%)	
Rectal	1,333 (25%)	22 (42%)	41 (35%)	126 (31%)	264 (29%)	583 (25%)	297 (20%)	<0.001
Histological differentiation								
Well, moderate	5,112 (86%)	36 (69%)	108 (82%)	365 (82%)	884 (86%)	2,276 (87%)	1,443 (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	2,166 (36%)	17 (33%)	41 (31%)	110 (25%)	311 (30%)	926 (35%)	761 (45%)	<0.001
III	2,109 (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	

Results

The mean age and percentage of females in patient groups classified according to the anatomical site of the primary tumor are shown in Figure 1. The mean age decreased progressively from patients with caecal tumors (70.5 years) through to patients with rectal tumors (65.6 years). The percentage of female patients also decreased progressively from proximal to distal tumor sites, with the exception of a small rise from ascending to transverse colonic tumors. Similar observations have been reported in another large, population-based study (7) 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.

Clinicopathological features. Patients aged ≤30, ≤40, ≤50 and ≤60 years comprised 0.9%, 3.1%, 10.6% and 27.8% of cases, respectively (Table I). The proportion of males amongst patients aged ≤30 years was 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 old). As suggested by the results shown in Figure 1, the proportion of rectal tumors was highest in young patients and declined progressively with increasing patient age (Table I). The frequency of poorly differentiated tumors also showed a progressive decrease with age. Young patients showed a significantly higher incidence of late stage (node-positive) disease than older patients ($p<0.001$).

Table II. *Cancer-specific survival according to patient age.*

Age (yrs)	n	HR[1]	95% CI	*P*	5-year 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- to 75-year-old patients as the reference group.

Table III. *Molecular features of tumors from young colorectal cancer patients.*

Feature	Patient age (% of tumors with molecular alteration)				*P*
	≤30 years	31-40 years	41-50 years	51-60 years	
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]K-*ras* codon 12 and 13 mutations; [4]*TP53* exon 5-8 mutations.

Patient survival. The incidence of perioperative mortality was 4% for the overall cohort and increased progressively with age (Table I). For each tumor stage, young patients (≤60 years) generally showed better cancer-specific survival compared to older patients (Table II). 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 K-*ras* oncogenes and *TP53* tumor suppressor gene are recognized as major genetic alterations in the development and progression of CRC. These molecular features were investigated in young patients (Table III). The frequencies of MSI and *TP53* mutation were highest in ≤40-year-old patients and decreased with advancing age. In contrast, the frequency of K-*ras* 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 colorectal cancer (HNPCC), with two known to have germline mutations in *MLH1* and two in *MSH2*. Known familial cancer syndromes therefore accounted for 7/52 (13%) of these very early onset CRC. The single familial cancer genetic service in the state of Western Australia had received referrals for 13 out of the 52 (25%) patients. Of the 9 MSI+ cases detected in ≤30-year-old patients (Table III), 4 were the HNPCC cases described above and 5 are yet to undergo germline testing.

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

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 clinicopathological 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 (7, 15), we observed a slight predominance of females amongst elderly CRC patients (>75 years old; Table I). 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 I). A progressive decrease in the proportion of poorly differentiated tumors with advancing age was also observed and has been noted previously by others (16-18). The current results also concur with previous reports that young CRC patients present more often with late stage disease (16-19). However, in agreement with other population-based studies (19-21), late stage presentation did not translate into worse survival for young patients (Table II). Trends for better survival of young patients were in fact observed, reaching significance in 41- to 60-year-old patients with stage III disease. These results argue against the existence of an aggressive tumor phenotype in very young patients. The initial reports of worse prognosis for young patients were mostly based upon highly selected series (22, 23).

The large size of this population-based cohort allowed examination of molecular features in the low frequency group of patients diagnosed at a young age (≤60 years old). Tumors 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 K-*ras* oncogenes. This pathway is likely to involve the adenoma to carcinoma sequence proposed in the Vogelstein model (1). The MSI phenotype was originally proposed as an alternate pathway, however more recent data suggests it can occur in tumors with either CIN or CIMP features (24).

The frequency of MSI+ tumors in the current study was approximately 3-fold higher in very young patients (≤40 years old) compared to those aged 41-60 years (18.3% *vs.* 6.6%). Two other population-based studies of Caucasians found similar frequencies of 17% (25) and 19.7% (26) in patients aged <50 years and <45 years, respectively. Interestingly, two population-based studies of Asians reported higher MSI+ frequencies of 29.4% (16) and 31.3% (27). The MSI phenotype in tumors from young CRC patients is a hallmark of HNPCC. Of the 9 patients aged ≤30 years with MSI+ tumors, 4 (44%) were known to have germline mutations in either the *MLH1* or *MSH2* mismatch repair genes. The remaining 5 cases have yet to be tested for germline 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 germline mutations estimated to be missed using this information alone (16, 28). In the present study, only 25% of ≤30 and 12% of 31 to 40-year-old patients were referred to the single familial cancer program that serves the state of Western Australia. Routine MSI screening for young patients (≤60 years old) might therefore be a useful triage allowing positive patients to be recommended for further genetic counselling and germline mutation testing (28). The finding of a germline 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- to 60-year-old patients (Table III). This might in part be explained by the higher proportion of rectal cancers in the very young CRC population (Table I). Previous studies have shown a higher frequency of *TP53* mutation in rectal compared to colon cancers (29). In contrast to MSI+ and *TP53* mutation, the incidence of K-*ras* mutation in very young patients was significantly lower than in 41 to 60-year-old patients. This finding is similar to another recent report (30), although the frequency of K-*ras* mutation (6%) in that population-based study was unusually low compared to other studies. No significant differences in the frequency of *BRAF* mutation were found within the ≤60 years age group. *BRAF* mutations are known to occur in a majority of MSI+ tumors from older patients but have never been observed in MSI+ tumors from HNPCC patients (31). They are also very rare in MSI+ tumors from young patients (32). 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– tumors in young patients.

Conclusion

The current population-based study found clear evidence for age-related differences in site distribution, histological grade and stage of presentation. This adds to recent data showing ethnic differences in the presentation of CRC (33). Despite evidence for a more aggressive tumor phenotype, the survival of young patients was not worse than 61- to 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 tumor molecular characteristics should assist with the development of novel treatment strategies in the future.

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Conflict of Interest

All authors state that there is no conflict of interest.

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