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P53 and bcl-2 in colorectal cancer arising in patients under 40 years of age: Distribution and prognostic relevance

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ABSTRACT

Young people (⩽40 years of age) with colorectal cancer (CRC) represent a distinct subgroup with more aggressive disease behaviour compared to older patients. We evaluate whether p53 and bcl-2 could be useful in identifying young patients at higher risk of tumour progression. We reviewed 1340 CRC patients with 58 patients ⩽40 years (4.2%). They had more frequent moderately or poorly differentiated mucinous adenocarcinomas (26% versus 12.3%, $p = 0.03$); higher advanced stage at diagnosis; shorter 5-year overall survival (49.8% versus 71%; $p = 0.02$); more frequent p53 positive (89.8% versus 72.6%, $p < 0.05$) and bcl-2 negative (88.0% versus 66.2%, $p < 0.05$) tumours; no difference in DNA content or proliferation indexes. Moreover, p53+ and bcl-2– resulted in being independent predictors of survival with shorter survival for the p53+/bcl-2– patients. Combining p53 and bcl-2, we could identify young CRC patients at higher risk of progression, who probably require development of a more sophisticated therapeutic approach based on identification of predictive factors.

1. Introduction

The risk of colorectal cancer (CRC), the second major cause of cancer death in Europe and in the USA,[1] increases with age with a peak in the seventh–eighth decades of life. However, several reports described an uncommon population of CRC patients with age ⩽40 years,[2] a small, distinct subgroup ranging from 2% to 10%. Although in young age several premalignant conditions may predispose to CRC, in recent years an increasing number of young people affected by sporadic CRC has been observed.[3]

Young patients have a more aggressive disease with a worse prognosis than older patients or the population as a whole.[4,5] However, literature fails to indicate whether this is caused by a biologically more virulent cancer or simply by a delay in diagnosis due to the low suspicion for CRC in this

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age group.[6,7] On the other hand, some authors report a similar[8] or even better course of the disease compared to older people.[5,9]

Whereas no differences were found, in young and old patients, for the duration of symptoms up to diagnosis (ranging from less than 1 month to 24 months)[6] or for the tumor site (left colon and sigmoid-rectum were the most frequent sites),[6,10] a significant difference in the stage at diagnosis usually occurs. In fact, almost 2/3 of young patients present features of advanced disease at the time of diagnosis, with a greater incidence of Dukes C or D tumours than the older patients.[4,5,11]

Apart from conventional clinico-pathological parameters, in recent years the prognostic significance of a number of molecular markers has been reported.[12,13]

The objective of this study was to evaluate the possible differences in presentation and outcome of CRC in young versus old patients focusing on the prognostic implications of proteins involved in the control of proliferation and apoptosis (i.e. p53 and bcl-2). Moreover, we examined the influence of these biological parameters on survival of CRC patients aged ⩽40 years of age and attempted to identify the young patients at worse prognosis.

2. Materials and methods

2.1. Patients characteristics

The clinical records of 1340 patients with sporadic CRC diagnosed between 1977 and 2003 at the Regina Elena Cancer Institute, with a median follow up of 5 years, were examined. Forty years was selected as the cut-off point to define young patients being that CRC is a rare event before this age. The median age was 37 years (range 20–40 years) for young subjects and 66 years (range 41–99 years) for old patients.

Patients treated with chemotherapy and/or radiotherapy totalled 57.0% in the young group and 42.7% in the old group.

2.2. Flow cytometry

Flow cytometry analyses were performed on a single nuclei suspension obtained from frozen tumour biopsies, sampled from both cancer and healthy mucosa, as previously described.[14] Tumour ploidy was evaluated as DNA index and the percentage of the cells in the S-phase was estimated on the DNA content histograms by using a mathematical model.[15,16]

2.3. Immunohistochemistry

Immunoreactivity for p53 and bcl-2 were performed using the monoclonal antibodies (MAb) DO7 and 124 (Dako, Milan, Italy) respectively.[14] P53 was recorded as positive when tumour cell nuclei were stained, irrespective of the percentage of positive cells, whereas bcl-2 was recorded when a strong cytoplasmic immunoreaction (>30%) was evidenced. Tumour proliferative activity, assessed by Ki-67 (Mab MIB-1, Dako), was classified as high if the value was >25% (median value). The immunohistochemical (IHC) staining was performed using a streptavidin-biotin immunoperoxidase system (LSAB2 kit, Dako). The enzymatic activity was developed using 3-amino-9-ethylcarbazole as a chromogenic substrate. IHC results were evaluated independently and blindly by two investigators (MM, MD).

2.4. Statistical analyses

The Pearson's χ^2 test was used to assess the association between biological characteristics and age. Survival curves were calculated by the Kaplan–Meier method and differences between groups were compared by the log-rank test. Significance was defined at the $p < 0.05$ level. The relative risk and confidence limits were estimated for each variable using the Cox univariate model and adopting the most suitable prognostic category as a referent group. A multivariate Cox proportional hazard model was developed using stepwise regression (forward selection) with predictive variables that were significant in the univariate analyses. The enter limit and remove limit were $p = 0.10$ and $p = 0.15$ respectively. The SPPS (13.0) software was used for statistical analyses.

3. Results

3.1. Clinico-pathological characteristics and patient outcome

As described in Table 1, the population was composed of 1340 patients, of whom 58 (4.2%) were aged 40 or less, with no difference in sex distribution in both group (58.6% males versus 41.4% females in the young group and 57.4% versus 42.6% in the older group).

Table 1 – Clinical features of young (⩽40 years) and older patients

	Young patients (n = 58) Number of cases (%)	Old patients Number of cases (%)
Gender		
Male	40 (58.6)	735 (57.4)
Female	18 (41.4)	546 (42.6)
Site of tumour		
Right colon	10 (17.2)	298 (23.3)
Left colon	22 (37.9)	439 (34.3)
Rectum	26 (44.8)	543 (42.4)
Stage at diagnosis (Astler–Coller)		
A	8 (14.2)	160 (12.5)
B1/B2	2 (3.6)/15 (25.0)	127 (9.9)/447 (34.9)
C1/C2	6 (10.7)/9 (16.1)*	133 (10.3)/152 (11.8)
D	18 (30.4)*	263 (20.5)
Grading		
G3 (poorly differentiated)	14.0	14.7
Mucin	26.0**	12.3
Chemotherapy		
Yes	33 (57.0)	548 (42.7)
No	25 (43.0)	734 (57.3)

* $p < 0.05$.
** $p = 0.03$.

A similar involvement of the rectum (44.8% versus 42.4%), left (37.9% versus 34.3%) and right colon (17.2% versus 23.3%) was found between the young and old patients and no differences in tumour grading were observed in the two populations (16.3% G1, 67.4% G2 and 14.0% G3 versus 15.7% G1, 70.6% G2 and 14.7% G3). Only the percentage of mucinous carcinomas was significantly higher in young patients (26.0% versus 12.3%; $p = 0.03$).

Concerning the stage (Astler–Coller classification), we found in the young patients that 14.2% were stage A, 3.6% were stage B1, 25% were stage B2, 10.7% were stage C, 16.1% were stage C2 and 30.4% were stage D. In the older group, 12.5% were stage A, 9.9% were stage B1, 34.9% were stage B2, 10.3% were stage C1, 11.8% were stage C2 and 20.5% were stage D. Although the distribution trend for the stages was similar among the two groups, young patients more frequently have an advanced C/D Dukes tumour at diagnosis (57.1% versus 42.6%; $p < 0.05$). The overall 5-year survival rate, as shown in Fig. 1, was significantly lower in the young patients compared to the older ones (49.8% versus 71.5%; $p = 0.02$).

3.2. *Impact of biological parameters on survival in CRC young people*

As shown in Table 2, there was no significant difference between young and old patients in DNA content, S-phase and Ki-67. In contrast, the incidence of p53 positive (89.8% versus 72.6%) and bcl-2 negative tumours (88.0% versus 66.2%) was significantly higher in the young patient group compared to the older subjects.

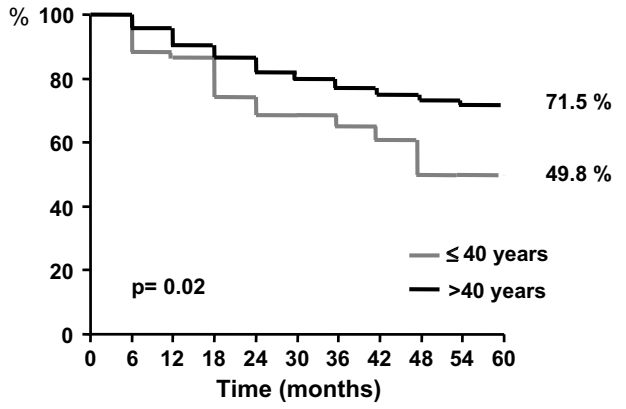

Fig. 1 – Overall 5-year survival of young (⩽40 years) and older patients.

Table 2 – Correlation between biological characteristics and age in young (⩽40 years) and older patients

	Young patients	Old patients	P*
Aneuploidy	47.6% ($n = 42$)	73.0% ($n = 502$)	n.s.**
S phase>18.8%	47.4% ($n = 42$)	61.0% ($n = 420$)	n.s.
p53 +	89.8% ($n = 56$)	72.6% ($n = 638$)	0.05
bcl-2 –	88.0% ($n = 56$)	66.2% ($n = 634$)	0.049
Ki-67>30%	80.0% ($n = 48$)	80.1% ($n = 632$)	n.s.

* χ^2 test.
** n.s.: not significant.

The univariate analysis (Table 3) identified advanced stage ($p < 0.0001$), lack of bcl-2 expression ($p < 0.0001$), p53 nuclear accumulation ($p = 0.001$), male sex ($p = 0.02$), younger age ($p = 0.025$), aneuploidy ($p = 0.069$) and high tumour grade ($p = 0.11$) as significant unfavourable prognostic factors, so these variables were included in the Cox proportional risk model. S-phase did not emerge as a prognostic factor (data not shown). In multivariate analysis, stage ($p < 0.0001$), bcl-2 downregulation ($p = 0.015$), male sex ($p = 0.046$) and p53 positivity ($p = 0.09$) were confirmed as independent prognostic factors for survival.

As shown in Fig. 2, the overall 5-year survival was worse in young and old patients for p53+ and bcl-2– tumours. When the two parameters were combined, the overall 5-year survival of the subgroup with a p53+/bcl-2– tumour phenotype was lower than that observed in the subgroup with an opposing pattern. In young patients this difference was markedly higher (90.5% in p53–/bcl-2+ versus 35.2% in p53+/bcl-2– patients, $p < 0.001$) compared to the older patients (92% in p53–/bcl-2+ versus 61.8% in p53+/bcl-2– patients, $p < 0.025$) (Fig. 3).

4. Discussion

This study showed a distinct clinico-pathological and biological profile of CRC in young patients compared to older subjects. Young patients usually presented more advanced stage at diagnosis, mucin production, p53 alteration and lack

Table 3 – Univariate and multivariate Cox proportional hazard analysis of prognostic factors in young (⩽40 years) people

Univariate			Multivariate		
Factor	HR (95% CI)	p	Factor	HR (95% CI)	p
Stage	4.61 (3.54–5.99)	<0.0001	Stage	4.61 (2.76–7.65)	<0.0001
bcl-2 –	3.02 (1.89–4.83)	<0.0001	bcl-2 –	3.02 (1.15–3.52)	0.015
p53 +	2.48 (1.44–2.27)	0.001	p53 +	2.48 (1.01–2.33)	0.046
Sex	1.34 (1.05–1.72)	0.02	Sex	1.34 (0.91–3.38)	0.09
Age	1.76 (1.07–2.87)	0.025			
Ploidy	1.51 (0.97–2.37)	0.069			
Grading	1.32 (0.94–1.85)	0.11			

HR = hazard ratio; CI = confidence interval.

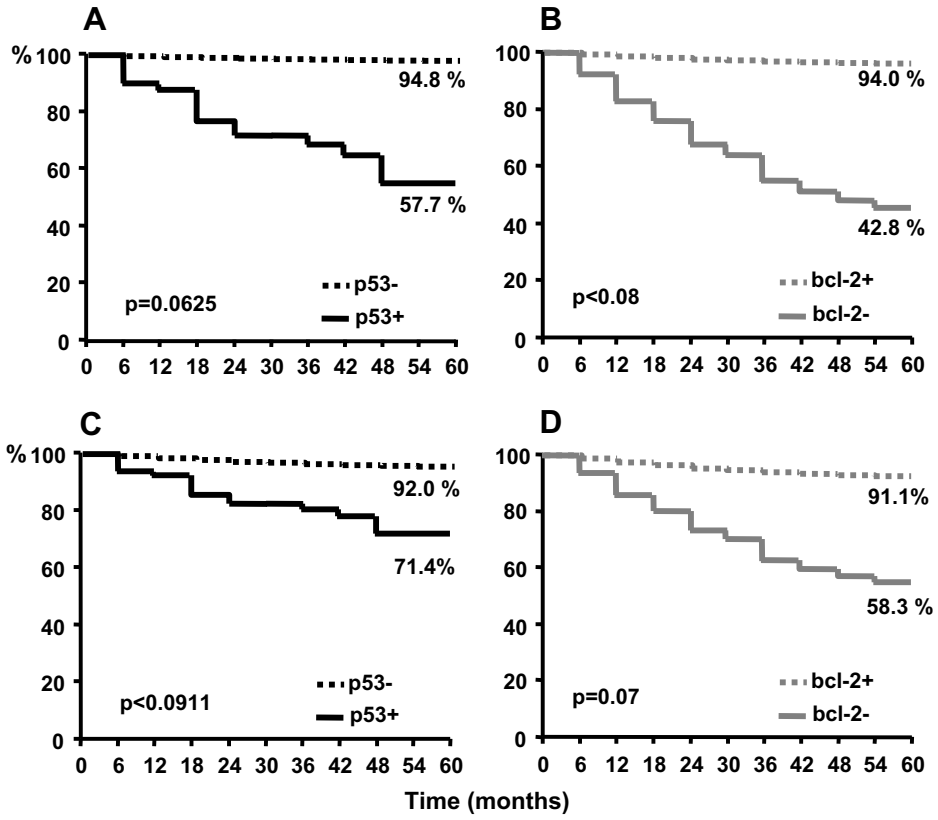

Fig. 2 – Overall 5-year survival of young (⩽40 years) (panel A and B) and older patients (panel C and D) with respect to p53 and bcl-2 status.

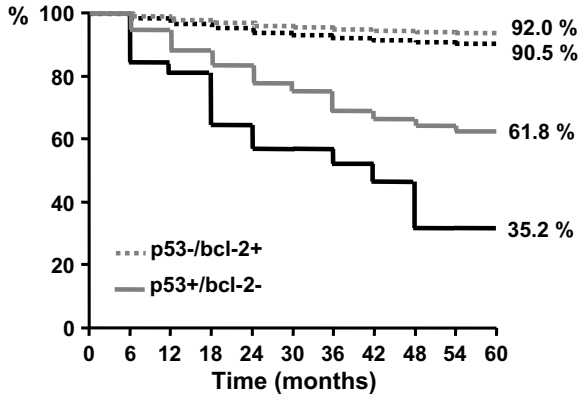

Fig. 3 – Overall 5-year survival on the basis of p53/bcl-2 pattern of young patients (black line) versus old patients (grey line).

of bcl-2 expression, and most of these parameters were prognostically relevant. Young and old patients had a similar trend regarding sex distribution and site of primary tumour while stage at diagnosis was found to be more advanced in young patients. For some authors, this increased rate of advanced tumours may be due to a delayed diagnosis,[6,17] whereas others believe it is due to a different and probably more aggressive behaviour of CRC in young patients.[5,10]

In agreement with several authors who consider mucinous adenocarcinomas to be associated with a poorer prognosis,[18,19] we found that young patients have a higher significant incidence (26%) of mucinous tumours compared to older patients. As confirmed by Cascinu and colleagues,[20] no differences in histologic type, grading, Ki-67 or S-phase proliferation index were found. Young patients have a significantly higher frequency of tumours with p53 nuclear accumulation and lack of bcl-2 than their older counterparts. We have previously reported that p53 positive/bcl-2 negative CRC patients, independently of age, have a more adverse outcome. Nevertheless, in the same study we observed a higher incidence of this tumour phenotype among B2 stage younger patients.[14,21] This initial observation made it possible for us to focus on the prognostic impact of p53 and bcl-2 in young CRC patients. To our knowledge, this is one of the few studies in which p53 and bcl-2 have been concomitantly analysed taking into account the age of patients.

Although p53 alteration is widely considered to be a negative prognostic factor in CRC,[14,21,22] its use in the clinical setting remains controversial. Lan and colleagues[23] found that accumulation of p53 protein could have a favourable, but not independent, prognostic value in CRC patients. In a series of Taiwanese patients, Liang and colleagues [24] demonstrated that although young patients usually present a more advanced disease at diagnosis, with a higher incidence of mucinous tumours, they have a better prognosis associated to microsatellite instability (MSI-H) and p53 wild type. In the ASCO 2006 recommendations,[25] p53 status is a poor guide when defining prognosis and response to therapy in CRC pa-

tients. In the adjuvant setting, some retrospective analyses demonstrated a non-significant trend towards a worse survival in p53-mutated patients, indicating that a p53 routine test is not powerful enough to identify patients who should receive adjuvant therapy. However, Westra and colleagues[26] demonstrated that in patients with stage III colon cancer submitted to adjuvant treatment, the presence of a p53 mutation should be considered as a better predictor for a short disease free survival than MSI status.

Concerning bcl-2 in CRC, few studies have investigated its role in predicting the clinical course of this disease. It was reported that bcl-2 expression associated to a better prognosis,[27] whereas other authors have stated that the lack of bcl-2 is an adverse prognostic factor,[14] recently confirmed by Han and colleagues[28] who evaluated the p53 and bcl-2 status in CRC patients. The expression of the bcl-2 family member correlates with early clinical stage and lack of lymphovascular invasion and lymph node involvement, indicating it as a favourable marker. In contrast, p53 overexpression correlates with less differentiated status and perineural invasion, suggesting it as an adverse prognostic marker. The question is why the lack of bcl-2, that should be associated with an aggressive phenotype due to its capability of blocking apoptosis, appears, on the contrary, to be predictive of a poorer clinical course in CRC. Probably, it is not possible to ascertain if bcl-2 is directly involved in the determination of the cancer phenotype. One explanation might be the presence of an anti-proliferative-like domain in bcl-2 which would lower the rate of tumour proliferation, or perhaps downstream apoptotic pathways could be still functional. Moreover, the effect of bcl-2 may be widely variable, depending on the cellular context.[29]

In our series of CRC patients under 40 years of age, IHC positivity of p53 and lack of bcl-2 could be considered predictive of a worse outcome. It is likely that this tumour profile may contribute to the disadvantage of young patients conferring a more aggressive behaviour to the tumour and interfering with response to chemotherapy. In fact, in stage D colorectal cancer, Liang and colleagues[30] reported that p53 status was associated with poor chemosensitivity to 5-FU-based therapy. Similarly, Bunz and colleagues[31] showed that the presence of p53 mutations seems to be predictive of decreased cancer cell sensitivity to chemotherapy, particularly to 5-fluorouracil.

In conclusion, although stage at diagnosis remains the most powerful and relevant predictor of prognosis in CRC, independently of age, our analyses indicate that p53 nuclear accumulation or bcl-2 downregulation may be clinically relevant to better define the prognosis of young CRC patients. Our data suggested that the p53 positivity associated to bcl-2 loss may permit a more accurate identification of a subset of young patients with worse prognosis. These findings have been indirectly confirmed by Watson and colleagues[32] who found that CRC patients with the opposite pattern of p53–/bcl-2+ tumours, independent of age, have a more favourable prognosis.

Our results might have a clinical application for diagnosis and treatment of this malignancy in young patients. The tendency to have a more advanced tumour stage at diagnosis with a potentially faster tumour progression should alert physicians and it is imperative to investigate promptly to detect the tumour at an early stage, thereby offering a better prospect of cure. Therefore, the clinical application of biological factors could be useful in selecting young patients who could benefit from novel active regimens. The possibility to identify therapeutic targets in p53+/bcl-2– patients could open up new possibilities in the treatment of these patients by using polichemotherapy or target therapies or both. In a retrospective microarray study restricted to tumour-node-metastasis stage II CRC patients who did not undergo adjuvant chemotherapy, Resnick and colleagues[33] interestingly found that p53 and EGFR overexpression are associated to a decreased survival of patients. Moreover, these molecular parameters associated to lymphovascular invasion are independent predictors of disease recurrence.

However, the mechanisms underlying the clinical and biological features of CRC in young patients need further investigation in order to define their impact on the potential modifications of current clinical approaches.

Conflict of interest statement

None declared.

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