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Prognostic factors in patients with stage II colon cancer: Role of E-selectin gene polymorphisms

Dear Editor,

According to the most recent European Society for Medical Oncology (ESMO) guidelines, the use of adjuvant chemotherapy for patients with stage II (T3 or T4, N0, M0) colon cancer (CC) remains controversial [1]. The lack of robust data on the benefit of adjuvant chemotherapy for patients with stage II CC causes the need for further determination of prognostic and predictive factors for assessing adjuvant chemotherapy responses in these patients [1]. In this regard, we previously explored the potential involvement of the adhesion molecule E-selectin in metastasis [2].

E-selectin is expressed on endothelial cells and is involved in the adhesion and extravasation of leukocytes carrying the ligands sialyl-Lewis x or sialyl-Lewis a [3–5]. Carcinoma cells, especially those of breast or colorectal origin, may express these ligands. Indeed, E-selectin-mediated mechanisms have been implicated in the occurrence of distant metastases of gastrointestinal cancers [5]. In addition, the main biomarker of colorectal cancer (CRC), carcinoembryonic antigen (CEA), has been demonstrated to be an E-selectin ligand [6]. In our previous study, we found a significant correlation between the constitutional S128R polymorphism of the E-selectin gene and the risk of recurrence and death in patients with stage II/III CCs [2]. In the current study, we aimed to validate this finding in a larger validation cohort with a more homogeneous population, including patients enrolled in the phase III Multicenter International Study of Oxaliplatin/Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) trial [7,8].

MOSAIC is a phase III trial that randomly assigned 2246 patients who had undergone curative resection for stage II or stage III CC to receive 12 cycles of biweekly LVFU (LV5FU2) or FOLFOX4 treatment. Eligible patients were 18–75 years of age and had undergone complete resection of histologically proven stage II (T3 or 4, N0, M0) or stage III (any T, N1 or 2, M0) CC. Inclusion/exclusion criteria, the random assignment process, and treatment/dose schedules have been previously described [7,8]. All patients provided written informed consent before inclusion and the ethics committee at each participating center approved the study and follow-up protocol and the translational data research integration.

The E-selectin genotypes of the patients were determined using tissue specimens obtained during surgery. Genotypes were analyzed according to allelic discrimination using a TaqMan-based real-time polymerase chain reaction assay, as described previously [2]. Allelic frequencies were calculated by gene counting and the Hardy-Weinberg equilibrium was evaluated using a chi-squared test. For statistical analyses, we pooled all patients carrying the minor R allele (S/R and R/R genotype) and compared them to patients homozygous for the wild-type allele (S/S). Main baseline characteristics were compared between the two genotype groups using the Mann-Whitney U test for age and the chi-squared test for all other categorical variables. Overall survival and disease-free survival were estimated for each genotype group using the Kaplan–Meier method [9] and were compared between groups using the Cox proportional hazard model adjusted for the adjuvant treatment arm. The proportional hazard assumption was checked by plotting the Schoenfeld residuals against the rank of event time [10]. We further investigated the association between the genotype groups and adjuvant treatment arm by including the corresponding interaction term into the Cox proportional hazard model. Statistical testing was evaluated at the two-tailed α level of 0.05. Data were analyzed using the SAS software package, release 9.4 (SAS Institute, Cary, NC, USA).

Among the 899 patients that underwent complete resection of histologically proven stage II CC who were randomized in the MOSAIC trials, surgical samples from 316 patients were subject to assessment of the E-selectin S128R polymorphism. Of these patients, 162 received the FOLFOX4 regimen (oxaliplatin/fluorouracil/leucovorin) as adjuvant treatment for CC and the remaining 154 patients received the LV5FU2 regimen (fluorouracil/leucovorin). Genotype distribution of the E-selectin polymorphism deviated from Hardy–Weinberg equilibrium ($p = 0.033$); 257 patients had the S128S genotype, 52 had the S128R genotype, and seven had the R128R genotype, providing an allele frequency of 10.4% for the R variant. As shown in Table 1, there were no significant differences in the baseline characteristics between carriers and non-carriers of the E-selectin 128 R variant.

After a median follow-up of 10.4 yr (interquartile range, 9.3–11.4 yr), there were 95 events of disease-free survival and 77 deaths. As shown in Fig. 1, there were no differences in overall survival (hazard ratio [HR] = 0.71; 95% confidence interval [CI], 0.39–1.30; $p = 0.27$) or disease-free survival (HR = 0.63; 95% CI, 0.35–1.11; $p = 0.11$) between carriers and non-carriers of the E-selectin 128 R variant. When the analysis was stratified according to the allocated adjuvant treatment, there was no evidence of heterogeneity between the LV5FU2 and FOLFOX4 regimen groups (p for interaction = 0.36 for both overall and disease-free survival). For overall survival, the HR for the 128 R variant carriers was 0.90 (95% CI, 0.43–1.85) in the LV5FU2-treated patients and 0.46 (0.14–1.50) in the FOLFOX4-treated patients. For disease-free survival, the HR for the 128 R variant carriers was 0.79 (0.40–1.54) in the LV5FU2-treated patients and 0.40 (0.12–1.30) in the FOLFOX4-treated patients.

Table 1
Main baseline characteristics, overall and according to E-selectin 128 R variant status.

		E-selectin S128R		
Characteristics	Overall (n = 316)	S/S (n = 257)	S/R + R/R (n = 59)	*p*
Age, years; median (IQR)	61 (53 to 67)	61 (53 to 67)	62 (54 to 67)	0.42
Men	166 (52.5)	132 (51.4)	34 (57.6)	0.38
Location				
Right side	114 (39.9)	93 (40.3)	21 (38.2)	0.96
Left side	127 (44.4)	102 (44.1)	25 (45.4)	
Both sides	45 (15.7)	36 (15.6)	9 (16.4)	
Treatment arm				
LV5FU2	154 (48.7)	122 (47.5)	32 (54.2)	0.35
FOLFOX4	162 (51.3)	135 (52.5)	27 (45.8)	
Differentiation grade				
Well	80 (26.2)	69 (27.9)	11 (19.0)	0.33
Moderate	196 (64.3)	154 (62.4)	42 (72.4)	
Poor	29 (9.5)	24 (9.7)	5 (8.6)	
Obstruction	51 (16.1)	41 (16.0)	10 (17.0)	0.85
Perforation	30 (9.5)	26 (10.1)	4 (6.8)	0.40
pTNM stage				
pT3N0M0	250 (79.1)	201 (78.2)	49 (83.1)	0.41
pT4N0M0	66 (20.9)	56 (21.8)	10 (17.0)	
Number of patients with ≥10 lymph nodes removed	210 (67.1)	167 (65.5)	43 (74.1)	0.21
MMR status				
pMMR	274 (86.7)	222 (86.4)	52 (88.1)	0.72
dMMR	42 (13.3)	35 (13.6)	7 (11.9)	
BRAF mutation status				
Wild type	284 (90.2)	232 (90.3)	52 (89.7)	0.89
Mutant	31 (9.8)	25 (9.7)	6 (10.3)	

Values are number (percentage) unless otherwise indicated.
Abbreviations: IQRinterquartile range; FOLFOX, fluorouracil, leucovorin, and oxaliplatin; LV5FU2, leucovorin and fluorouracil; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Overall, the current results indicated that in the MOSAIC trial cohort the S128R variant of the E-selectin gene was not significantly associated with shorter disease-free survival or overall survival. Therefore, E-selectin polymorphisms do not appear to be predictive factors of metastatic relapse in CC. One explanation for the contradictory results from earlier work may be the less homogeneous retrospective cohort in our previous study, including patients from several centers with tumor resections performed between 1987 and 2007 [2].

Other studies have assessed E-Selectin gene polymorphisms for their association to prognosis significance. One study demonstrated that presurgical median baseline levels of serum soluble E-selectin are higher in patients with CRC (43 ng/mL) compared to that in controls (36 ng/mL) or in patients with benign colonic diseases (31 ng/mL, $p < 0.001$) [11]. The serum levels of E-selectin were found to be significantly associated with CEA mRNA positivity by RT-PCR ($p < 0.05$) [9]. Another more recent study assessed a potential relationship of P-selectin, L-selectin, and E-selectin in CRC patients in relation to tumor advancement according to TNM classification and tumor location. The mean levels of all selectins are significantly higher in CRC patients compared to that in healthy controls. The highest levels of serum soluble P-selectin are observed in patients with CRC metastases to the liver (stage IV) and are significantly higher compared to that in patients without metastases (stage I/II) or with lymph node metastases (stage III; $p = 0.02$) [12].

The majority of the E-selectin polymorphisms related studies were performed nearly ten years ago, which increases the chances of conflicting results or negative results that were never published. Therefore, the search for prognostic and predictive factors for relapse should continue, particularly for patients with stage II CCs, so as to better refine the indications for adjuvant chemotherapy. This is particularly relevant as the survival benefit of adjuvant chemotherapy remains only moderate (2–5% absolute value based on the risk of recurrence with only fluoropyrimidine).

Currently, the most promising candidates for predictive factors of CC relapse are circulating tumor DNA [13], Immunoscore [14], and conventional tumor markers such as CEA determined postoperatively [15]. The CEA cut-off of 2.35 ng/ml for patients with stage II CC has already been validated in the MOSAIC cohort. Patients with a CEA level greater than 2.35 ng/ml are most at risk for recurrence or death [16]. The Immunoscore, which is based on the calculation of CD3+ and CD8+ T cell densities in tumors and the invasion margins, was validated in a large cohort of patients with stage II CC (n = 1434). The results showed that the risk of recurrence at five years is significantly different between patients with high and low Immunoscores ($p < 0.0001$), as well as the risk of death based on multivariate analysis using Cox's model ($p < 0.0001$). Immunoscore demonstrates the highest relative contribution to the risk of cancer recurrence compared to all other conventional clinical-histological criteria, including TNM classification. Another promising marker is circulating tumor DNA (ctDNA); however, there is currently little evidence for ctDNA as a marker for stage II CC. Two randomized trials of adjuvant chemotherapy guided by ctDNA are underway (DYNAMIC; NCT03737539 and PRODIGE70-CIRCULATE; EudraCT no. 2019-000935-15).

In conclusion, E-selectin polymorphisms were not predictive factors of metastatic relapse in stage II CC in a large homogeneous cohort of patients. Therefore, determination of E-selectin status does not appear to be a feasible marker moving forward. The search for effective prognostic markers of CC relapse after surgery should continue. This is particularly relevant for patients with stage II CCs since the benefits of adjuvant chemotherapy remain unclear and are controversial.

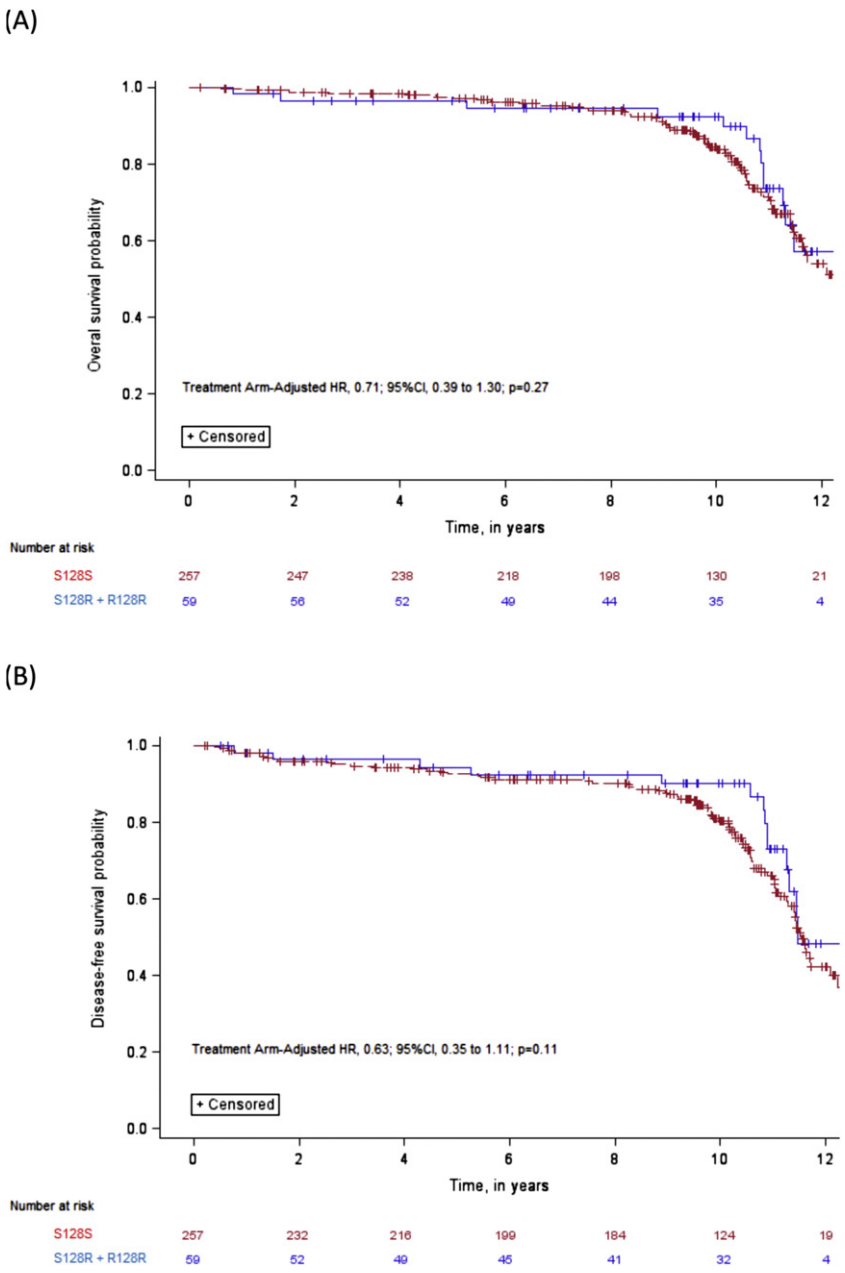

Fig. 1. Kaplan–Meier estimates of overall survival (A) and disease-free survival (B) in patients carrying the E-selectin 128 variant (n = 59) and in patients with the wild-type S128S genotype.

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Conflicts of interest

The authors declare no conflict of interest.

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High hepatitis B virus seroprevalence in pregnant women coming from middle and high-endemicity countries: An unresolved issue

Sir,

In a recent issue of Digestive and Liver Disease, Lembo et al. [1] presented an interesting survey on hepatitis B and hepatitis C prevalence in pregnancy in Southern Italy. Regarding hepatitis B virus (HBV), analyzing a cohort of 7,558 pregnant women, a prevalence of 0.5% was reported. Hepatitis B surface Antigen (HBsAg) seroprevalence was significantly higher among the 569 foreigners compared to the 5,559 Italian women (3% vs. 0.2%, $p < 0.001$), but still lower than was described in previous studies [2,3].

Since in Italy a significant cluster of HBV carriers is represented by people coming from high endemicity countries, we conducted an epidemiological study in non-Italian pregnant women (with the exception of those coming from Western Europe and Northern America) consecutively hospitalized for the delivery between May 2012 and January 2014 in the Obstetric Department of Policlinico Umberto I in Rome. We focus on the local unpublished data of a multicentric national survey promoted by the Italian Society of Infectious and Tropical Diseases [3]. The main goal was to estimate the overall HBsAg seroprevalence in pregnant migrant women, stratified by area of origin, whereas secondary aims were to control the coverage rate of mandatory HBV screening in pregnancy, to verify the rate of retention in follow-up in non-Italian women and of appropriate screening of their household contacts. Data on HBsAg serological status were collected using the clinical records, whereas data on socio-demographic characteristics by a questionnaire. HBsAg-positive patients were enrolled for follow-up and screening for HBV was offered to all their household contacts.

A total of 714 pregnant women coming from 62 different countries were prospectively evaluated. Of them, 682 (95.11%) correctly attended the HBV prenatal screening. The analyzed population was mainly represented by Eastern European women, followed by Asian, Central-South American, Sub-Saharan African and North African women (43.6%, 33.7%, 9.5%, 7.0% and 6.2%, respectively). Overall HBV seroprevalence was 5.13% (35/682). HBsAg-positive patients came from four macro-areas and only eleven countries, namely Albania, Bangladesh, Burkina Faso, China, Egypt, Philippines, Ghana, Kenya, Moldova, Romania and Tunisia. HBsAg seroprevalence was 6.67% (4/60), 6.64% (14/211), 5.62% (15/267) and 3.57% (2/56) in women coming from Sub-Saharan Africa, Asia, Eastern Europe and Northern Africa, respectively. Focusing on the nationality, the most relevant seroprevalence was reported in Chinese (10/42, 23.81%), Albanian (3/20, 15%) and Romanian women (10/132, 7.58%) (Table 1).

46.7% (14/30) of patients who accepted to fulfill the survey discovered HBV infection during pregnancy. Of the remaining 53.3% (16/30), only 12.5% was currently retained in care. Examining data