

ORIGINAL ARTICLE

Nuclear maspin expression as a predictive marker for fluorouracil treatment response in colon cancer

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ABSTRACT

Background. Maspin is a member of the serpin family of protease inhibitors whose function in colorectal cancer is not fully understood. The objective of this study was to determine whether level of maspin expression could have prognostic or predictive value in colorectal cancer.

Material and methods. Maspin expression was assessed using immunohistochemistry on tissue microarrays obtained from 380 patients with stage II and III colorectal cancer randomized to adjuvant chemotherapy with fluorouracil and levamisole (5-FU/Lev) or to surgery only (control), with scores (0–300) based on presence (0–100) and intensity (0–3) of maspin expression. Associations with disease-free survival (DFS), cancer-specific survival (CSS) and prognostic factors were determined.

Results. Maspin expression was predominantly nuclear and present in tumor tissue in 99% of the cases. No associations with clinicopathological factors were identified. In colon cancer patients receiving adjuvant chemotherapy, maspin expression level was significantly associated with CSS [HR 1.43 per 50 points increase in maspin score ($p = 0.021$)] in multivariate analyses, and a significant interaction between treatment status and maspin expression ($p = 0.045$) was found. Kaplan-Meier plots from colon cancer patients showed a significant treatment benefit in patients with low maspin expression, but not for individuals with medium/high expression. Level of maspin expression was not significantly related to clinical outcome in rectal cancer or in any of the control groups.

Conclusions. In patients with colon cancer a low nuclear maspin expression was an independent predictor of benefit from adjuvant chemotherapy with 5-FU/Lev. A prognostic value of maspin expression was not found in this material.

Colorectal cancer (CRC) is currently the third most common cancer and cause of cancer related deaths in the Western world [1], also representing a major health problem worldwide with approximately 1.2 million new cases in 2008 [2].

After surgical resection of colon cancer, patients are presently selected for chemotherapy based on histopathological evaluation only. Benefit of adjuvant chemotherapy for patients with stage III colon cancer is well established, but administration to high-risk patients with stage II disease remains controversial [3,4]. Fluorouracil and levamisole (5-FU/Lev) was the first chemotherapy-regimen to significantly increase survival in stage III disease [5,6]. Levamisole was later replaced by calciumfolinate

[7]. The MOSAIC and NSABP C07 trials showed an increased benefit of adding oxaliplatin to 5-FU and calciumfolinate, and the FOLFOX or FLOX regimens are now the standard adjuvant chemotherapy for colon cancer stage III [3,4]. Patients with rectal cancer generally receive preoperative chemoradiotherapy for down-staging of locally advanced tumors, but in Europe adjuvant chemotherapy is not administered routinely in all countries [8].

A better identification of high-risk patients benefiting from adjuvant chemotherapy could not only improve survival, but also spare many patients from needless toxicity and economic loss. Thus, identifying molecular markers with predictive value is highly warranted. Maspin (serpin B5) is a promising marker

and has in addition been proposed as a potential therapeutic target [9]. Maspin is a member of the serine superfamily of protease inhibitors (serpins). It differs from other clade B serpins by not being able to go through the 'stressed to relaxed'-transition to inhibit a target protease, hence, the biological functions of maspin are not yet fully known [9].

Maspin was first identified in mammary epithelium and breast cancer cell lines in 1994 [10]. It has later been described in several types of cancer and cell lines including primary tumors from prostate, ovary, bladder, lung, head and neck, stomach and colon [11–17]. Maspin generally exerts tumor suppressing activities *in vitro* and *in vivo*, inhibiting tumor formation, cancer cell migration, invasion, metastasis and angiogenesis [18]. Interestingly, maspin knockout is lethal at an early embryonic stage [18,19].

Although the anti-tumor effects of maspin are well established by experimental data, results regarding its clinical significance in cancer development are conflicting [18]. The vast majority of studies on maspin expression have been performed on breast and ovarian cancer where maspin expression is correlated with positive prognostic factors, and the tumor-suppressing abilities of maspin seem to be dependent on nuclear presence of maspin [19]. Recent studies imply that maspin may exert different functions depending on tumor type [18]. In most studies on colorectal cancer, maspin expression is associated with adverse prognostic factors indicating an oncogenic function in this tumor group [17,20–23].

In the current study maspin expression was examined retrospectively in 380 patients with colorectal cancer stage II and III randomized to surgery only or adjuvant 5-FU and levamisole chemotherapy after surgery. We hypothesize that maspin expression measured in the primary tumor at the time of diagnosis may help identifying cases benefiting from adjuvant chemotherapy.

Material and methods

The REMARK guidelines were carefully considered when reporting this study [24].

Patients

In order to confirm the results from Laurie's and Moertel's studies [5,6], the Norwegian Gastrointestinal Cancer Group launched a prospective randomized multicenter study assessing the effects of adjuvant chemotherapy with 5-FU/Lev in patients undergoing radical surgery for colorectal cancer. The study population, inclusion and exclusion criteria have been described previously [25]. In summary, 425 patients aged 18–75 years with colorectal cancer

stage II and III were included from January 1993 to October 1996 after going through radical surgical resection of a histopathologically confirmed local primary adenocarcinoma of the colon or rectum with the regional lymph nodes. Patients were randomly assigned to either adjuvant chemotherapy with 5-FU/Lev or surgery alone. None of the patients with rectal carcinomas had preoperative radiotherapy. The regional ethics committee, The Norwegian Medicines Agency and the Norwegian Data Inspectorate all approved the study. Before randomization all patients signed a written, informed consent.

Chemotherapy and follow-up

Administration of 5-FU/Lev chemotherapy strictly followed Moertel's regimen [5,25]. Follow-up included consultation every six months for at least five years with chest x-ray, abdominal ultrasound, blood count and CEA, and relevant endoscopic examination including colonoscopy every three years. The recording of data ended March 2002. Median follow-up by reverse Kaplan Meier method [24] was 7.6 years.

Tissue microarray and immunohistochemistry

Tissue microarrays (TMAs) with cores of 1.0 mm were made from archival formalin fixed and paraffin-embedded tissue. From each patient, 1–3 samples were taken from the primary tumor in addition to cores from normal colon or rectal mucosa. The immunohistochemical procedure was performed according to previous studies [17,20,21]. After deparaffinization and rehydration, sections of 3–5 μm were subjected to heat-induced epitope retrieval with a pressure cooker in 10 mM citrate buffer (pH 7.2). Sections were incubated with primary antibody (1:50 dilution, anti-human maspin, P/N 554292, BD Pharmingen™, BD Biosciences, San Jose, CA, USA) for 60 minutes. Staining was performed using a Ventana Discovery® system and a DABMap-kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). Slides were counterstained with hematoxylin (P/N 790-2208, Ventana), dehydrated and mounted. Negative control with no primary antibody (diluent only) was included.

Evaluation of maspin staining intensity

Cytoplasmic and nuclear staining intensity were evaluated separately and semi-quantitatively by two of the authors, who were both blinded from the patients' clinical data. The final analyses were restricted to nuclear maspin expression in primary tumors. The staining intensity was evaluated as: 0: no staining; 1:

weak; 2: moderate; and 3: strong staining, and was recorded from primary tumor tissue and normal colorectal mucosa. The fraction of maspin-positive tumor cells was then assessed as 0–100%. The product of staining intensity and fraction of positive cells was used to calculate the total staining index, a continuous score ranging from 0–300. In cases with heterogeneous staining intensity slight qualitative adjustments were made per case based on a total evaluation of the specific slide.

Statistics

Disease-free survival (DFS) and cancer-specific survival (CSS) were used to assess the prognostic and predictive value of maspin. CSS was defined as time from randomization to death from colorectal cancer or treatment complications, and DFS as time from randomization to the first occurrence of either locoregional or distant recurrence with patients dying before cancer recurrence being censored at the time of death. Overall survival (OS) was defined as time from randomization until death from any cause (Supplementary Tables III–IV and Figure 1, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>).

Student's t-test was utilized to detect possible associations between clinicopathological variables and levels of maspin expression. The normal distribution of maspin expression was assessed graphically. In absence of an established cut-off point maspin expression was in all analyses except the Kaplan-Meier plots treated as a continuous variable. Agreement between the evaluations of maspin staining intensity was assessed by intraclass correlation (absolute agreement).

Cox regression analyses were used to assess DFS, CSS and OS in univariate and multivariate analyses. The analyses were performed in the whole group (colorectal cancer), and for colon and rectal cancer separately. In addition, univariate analyses of the maspin variable were performed separately for patients receiving adjuvant chemotherapy and patients treated with surgery only, to assess the impact of maspin expression in each treatment group. The prognostic value of maspin expression was evaluated based on the results in the control group (surgery only) as recommended by Barratt et al. [26].

In the multivariate analyses, DFS, CSS and OS were modeled by randomization group and maspin expression, adjusting for possible confounding effects of other variables, as detailed below. The multivariate analyses were performed in a hierarchical manner, with models without and subsequently including a randomization group by maspin interaction.

The maspin variable was centered on three different values to facilitate comparisons between the randomization groups at specific values of the maspin score. The centering values included low, middle and high values based on histograms of maspin expression and were 60, 130, and 180, respectively. Hazard ratios (HRs) were determined per 50 point increase in total maspin score. Maspin expression and the most important clinicopathological variables (stage, tumor differentiation, randomization group, sex and age) were added regardless of statistical significance in univariate analyses. The proliferation marker Ki-67 was also added, as a previous study in our group has identified its predictive value in this patient material [27]. The assumption of proportional hazards was tested as recommended by Therneau and Grambsch [28].

To illustrate differences in survival between groups with varying maspin expression, the maspin score was grouped for use in Kaplan-Meier plots with log rank test. The scores were initially divided into three equal groups according to nuclear Maspin expression; low, medium and high. Preliminary analyses revealed a difference between low and medium/high expression, but not between the latter two groups (not presented). The material was therefore dichotomized at the cut-off point between the low (<97.5) and medium or high (≥ 97.5) maspin expression groups in the presented plots.

All p-values were two-sided and considered statistically significant if $p < 0.05$. As the analysis of biomarker data was explorative, no adjustments were made for multiple comparisons.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp. Released 2011) and R (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline clinical characteristics and maspin staining

The original randomized study included 425 patients with colorectal cancer from which tissue microarrays were constructed successfully in 409. Thirteen of these patients had stage I disease and were excluded from the final analyses. Of the remaining 396 cases, 16 could not be evaluated, either as a result of technical issues or lack of tumor cells in tissue cores included in the TMA, leaving 380 cases successfully stained for maspin and available for analyses. Their clinical characteristics are presented in Table I. A total of 1377 cores (2–5 cores from each patient) were examined.

The intraclass correlation between the two scorers' mean nuclear score was 0.618. Samples

Table I. Patient characteristics and maspin expression for patients included in the analyses.

Covariate	Colorectal			Colon			Rectum		
	Surgery alone n = 190	Adjuvant (5-FU/LEV) n = 190	Total n = 380	Surgery alone n = 132	Adjuvant (5-FU/LEV) n = 137	Total n = 269	Surgery alone n = 58	Adjuvant (5-FU/LEV) n = 53	Total n = 111
Age at inclusion									
Mean (SD)	62 (9)	60 (10)	61 (9)	63 (9)	61 (10)	62 (9)	62 (9)	59 (10)	60 (10)
Gender									
Male	98 (52%)	99 (52%)	197 (52%)	63 (48%)	64 (47%)	127 (47%)	35 (60%)	35 (66%)	70 (63%)
Female	92 (48%)	91 (48%)	183 (48%)	69 (52%)	73 (53%)	142 (53%)	23 (40%)	18 (34%)	41 (37%)
Stage (TNM)									
Stage II	115 (61%)	110 (58%)	225 (59%)	83 (63%)	86 (63%)	169 (63%)	32 (55%)	24 (45%)	56 (50%)
Stage III	75 (39%)	80 (42%)	155 (41%)	49 (37%)	51 (37%)	100 (37%)	26 (45%)	29 (55%)	55 (50%)
Differentiation ^a									
High/moderate	158 (83%)	155 (82%)	313 (82%)	103 (78%)	109 (80%)	212 (79%)	55 (95%)	46 (87%)	101 (91%)
Low	30 (16%)	30 (16%)	60 (16%)	27 (21%)	24 (18%)	51 (19%)	3 (5%)	6 (11%)	9 (8%)
Ki67 ^b									
< 40%	78 (38%)	89 (43%)	167 (39%)	58 (44%)	62 (45%)	62 (45%)	18 (31%)	26 (49%)	44 (40%)
≥ 40%	92 (45%)	78 (38%)	170 (40%)	57 (43%)	56 (41%)	56 (41%)	32 (55%)	21 (40%)	53 (48%)
Histological type ^c									
Adenocarcinoma	170 (90%)	170 (90%)	340 (90%)	114 (86%)	120 (88%)	234 (87%)	56 (96%)	50 (94%)	106 (96%)
Mucinous carcinoma	11 (6%)	14 (7%)	25 (7%)	10 (8%)	13 (10%)	23 (9%)	1 (2%)	1 (2%)	2 (2%)
Signet ring cell carcinoma	4 (2%)	1 (0.5%)	5 (1%)	3 (2%)	1 (1%)	4 (2%)	1 (2%)	0	1 (1%)
Localization									
Proximal	69 (36%)	77 (40%)	146 (39%)	69 (52%)	77 (56%)	146 (54%)			
Distal	63 (33%)	60 (32%)	123 (32%)	63 (48%)	60 (44%)	123 (46%)			
Rectum	58 (31%)	53 (28%)	111 (29%)						
Maspin expression (0–300)									
Mean (SD)	118 (57)	130 (60)	124 (58)	121 (55)	136 (62)	128 (59)	112 (60)	114 (52)	113 (56)
Low	70 (37%)	65 (34%)	135 (36%)	47 (36%)	43 (31%)	90 (33%)	23 (40%)	22 (42%)	45 (40%)
Medium or High	120 (63%)	125 (66%)	245 (64%)	85 (64%)	94 (69%)	179 (67%)	35 (60%)	31 (58%)	66 (60%)

^aData regarding differentiation were missing from 7 patients; ^bdata regarding Ki67-score were missing from 88 patients; ^cdata regarding histological type were missing from 10 patients.

from normal colorectal mucosa were available in 282 cases. In the normal mucosa, both nuclear and cytoplasmic staining were infrequent, and present in only 22 (8%) and 5 (2%) of the cases, respectively. In contrast, the staining in tumor tissue was present in almost all cases and was predominantly nuclear (376 patients, 99%), while cytoplasmic staining was weak and recorded in 23% of the patients only (Figure 1). Initial univariate Cox analyses indicated no significant association between cytoplasmic maspin staining and clinical outcome (data not shown).

A small but statistically significant difference in nuclear maspin expression score was found in colon cancer patients compared to rectal cancer patients (128 vs. 113, $p = 0.017$) (Table II). Despite randomization, the nuclear maspin score was also slightly higher among colon cancer patients who received adjuvant therapy compared to surgery only (136 vs. 121, $p = 0.039$). No significant associations between nuclear maspin expression and other clinicopathological variables were found.

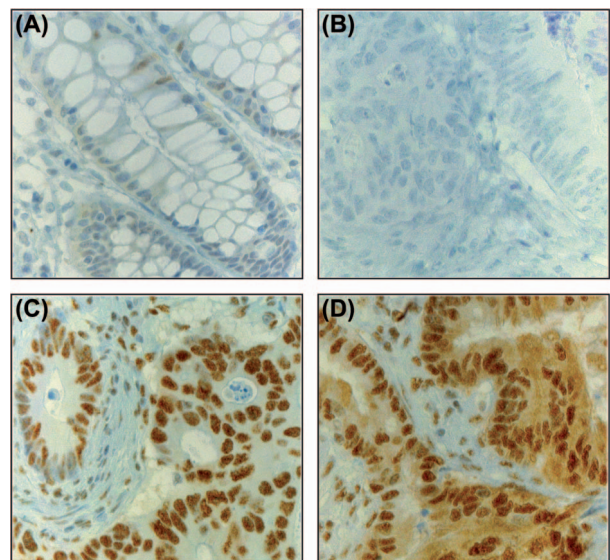


Figure 1. Immunohistochemistry for maspin. (A) Normal colon. (B) Colon tumor, negatively stained. (C) Colon tumor, strong nuclear staining. (D) Colon tumor, strong nuclear and intermediate cytoplasmic staining.

Table II. Nuclear maspin expression score according to patient and tumor characteristics.

	Colorectal tumors			Colon tumors			Rectal tumors		
	N ^a	Mean (SD)	p ^b	N ^a	Mean (SD)	p ^b	N ^a	Mean (SD)	p ^b
Sex									
Male	197	122 (56)	0.470	127	127 (56)	0.698	70	112 (55)	0.922
Female	183	126 (61)		142	130 (62)		41	113 (57)	
Age									
≤ 65	221	124 (58)	0.954	150	130 (59)	0.744	71	112 (54)	0.755
> 65	159	124 (59)		119	127 (59)		40	115 (60)	
Randomization group									
Adjuvant therapy	190	130 (60)	0.055	137	136 (62)	0.039	53	114 (52)	0.846
Surgery alone	190	118 (57)		132	121 (55)		58	112 (60)	
Stage									
Stage II	225	121 (63)	0.207	169	127 (64)	0.519	56	103 (57)	0.057
Stage III	155	128 (51)		100	132 (50)		55	123 (53)	
Differentiation									
High/moderate	313	123 (58)	0.608	212	130 (58)	0.608	101	110 (56)	0.097
Low	60	127 (59)		51	125 (61)		9	142 (50)	
Ki67									
< 40%	164	126 (57)	0.849	120	127 (60)	0.264	44	126 (49)	0.052
≥ 40%	166	125 (63)		113	135 (60)		53	103 (62)	
Localization									
Proximal colon				146	127 (62)	0.627			
Distal colon				123	130 (56)				
Colon	269	128 (59)	0.017						
Rectum	111	113 (56)							

^aNumber of patients; ^bStudent's t-test.

Relation between nuclear maspin expression and outcome

In patients treated with surgery only, no significant relations between maspin expression score and DFS or CSS were found in univariate analyses in either colorectal, colon or rectal cancer patients (Table III and Supplementary Table I, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>). These findings do not support a prognostic value of maspin expression in this material. However, for individuals who received adjuvant chemotherapy, maspin expression was significantly

associated with DFS and CSS in univariate analyses, with a higher hazard of death with increasing maspin score in colon cancer patients (HR for DFS 1.30 and for CSS 1.48 per 50 points increase in maspin expression score, $p = 0.040$ and $p = 0.004$, respectively), but not in rectal cancer patients alone (Table III and Supplementary Table I, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>). This significant association in cases receiving adjuvant chemotherapy indicates that a low maspin expression level predicts response to chemotherapy with 5-FU/Lev in patients with colon cancer.

Table III. Univariate Cox regression analysis for cancer-specific survival.

Covariate	Colorectal		Colon		Rectum	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex (male vs. female)	1.12 (0.78–1.60)	0.544	0.99 (0.63–1.55)	0.957	1.28 (0.68–2.42)	0.451
Age (continuous)	0.99 (0.98–1.01)	0.510	1.01 (0.98–1.03)	0.562	0.97 (0.94–0.99)	0.042
Stage (III vs. II)	4.75 (3.20–7.03)	<0.001	4.30 (2.69–6.88)	<0.001	5.72 (2.74–12.0)	<0.001
Tumor differentiation (low vs. medium/high)	1.62 (1.04–2.52)	0.032	1.74 (1.04–2.89)	0.034	1.65 (0.65–4.18)	0.295
Ki67 [low (< 40%) vs. high (≥ 40%)]	1.89 (1.28–2.80)	0.001	2.25 (1.37–3.70)	0.001	1.36 (0.70–2.65)	0.360
Tumor localization (rectum vs. colon) ^a	1.34 (0.94–1.99)	0.099				
Tumor localization (distal vs. proximal colon) ^a			1.18 (0.76–1.85)	0.462		
Randomization group ^b	1.07 (0.75–1.53)	0.714	0.98 (0.62–1.53)	0.913	1.31 (0.72–2.39)	0.376
Maspin expression pr 50						
Total	1.13 (0.97–1.31)	0.130	1.14 (0.94–1.38)	0.187	1.17 (0.90–1.52)	0.254
In patients receiving adjuvant chemotherapy	1.33 (1.08–1.64)	0.008	1.48 (1.13–1.94)	0.004	1.21 (0.82–1.78)	0.339
In patients treated with surgery only	0.92 (0.73–1.15)	0.460	0.82 (0.62–1.10)	0.185	1.12 (0.77–1.62)	0.551

^aVariable not included in multivariate analyses; ^badjuvant chemotherapy versus surgery only.

Kaplan-Meier curves illustrate the significant difference in both DFS and CSS for patients receiving adjuvant chemotherapy compared to treatment with surgery only in colon cancer cases with low (5-year survival 86% vs. 61% for DFS and 95% vs. 69% for CSS) but not medium/high maspin expression scores (Figure 2), suggesting that an increased maspin expression limits the effect of adjuvant 5-FU/Lev in colon cancer.

The effect of maspin expression levels in patients treated with chemotherapy compared to surgery was most prominent for the colon cancer stage III subgroup (5-year survival for patients with low maspin expression: 82% vs. 35% for DFS and 91% vs. 41% for CSS) (Figure 3).

The multivariate Cox regression analyses that did not include the randomization group by maspin expression interaction, showed no significant asso-

ciation between randomization group or maspin expression with DFS or CSS in any tumor location (colorectal, colon or rectum) (Table IV and Supplementary Table II, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>). In the subsequent analyses with randomization group by maspin interaction included in the models, this interaction was not significant for DFS in any location (Supplementary Table II, available online at <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>). However, for CSS, there was a significant interaction between randomization group and maspin expression for colon cancer ($p = 0.045$), supporting that the effect of adjuvant chemotherapy varies with the level of maspin expression. For colon cancer, maspin was significantly associated with CSS in chemotherapy-treated patients with a HR for cancer-related death of 1.43

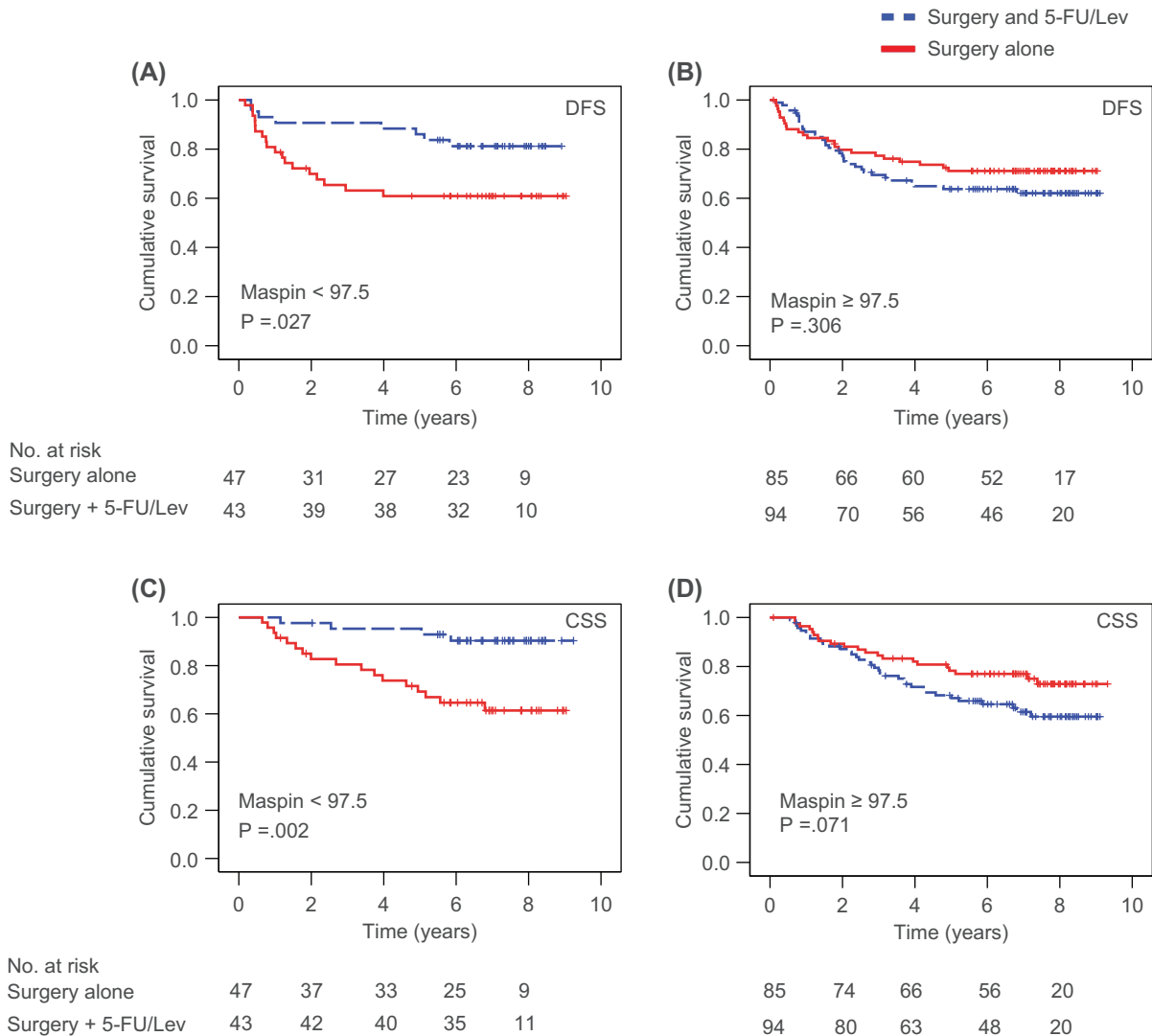


Figure 2. Survival of colon cancer patients (stage II+III) according to nuclear maspin expression and treatment. Survival is presented as DFS (A and B) and CSS (C and D) for low (A and C) versus high (B and D) expression of maspin. CSS, cancer-specific survival; DFS, disease-free survival.

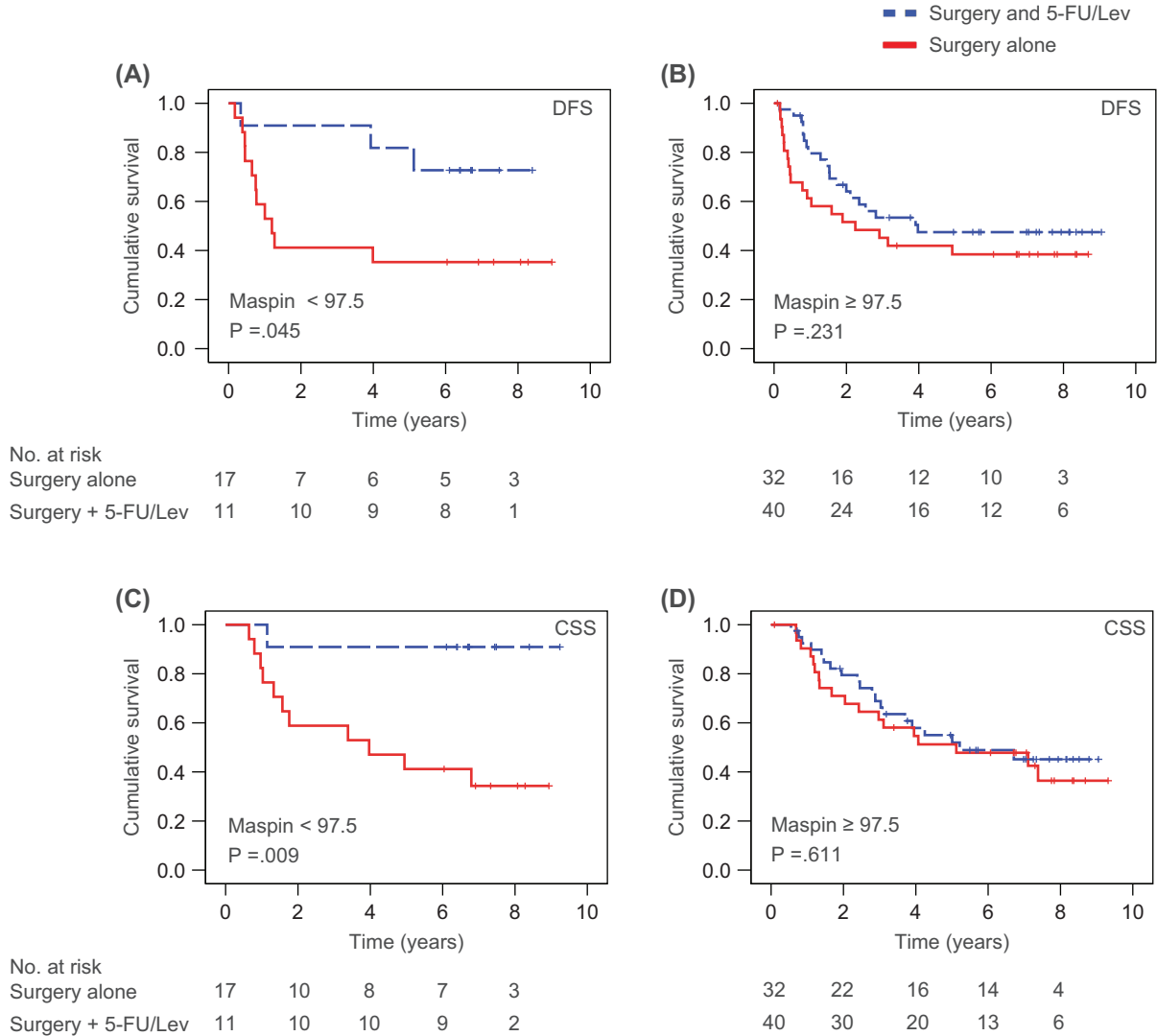


Figure 3. Survival of colon cancer patients stage III according to nuclear maspin expression and treatment. Survival is presented as DFS (A and B) and CSS (C and D) for low (A and C) versus high (B and D) expression of maspin. CSS, cancer-specific survival; DFS, disease-free survival.

for each 50 points increase in maspin score ($p = 0.021$), compared to insignificant results in patients treated with surgery only (HR 0.89, $p = 0.523$). These results imply that maspin is a predictive factor and that a low maspin expression level predicts response to chemotherapy with 5-FU/Lev in colon cancer, also when adjusted for other relevant clinicopathological variables and Ki-67. The analyses do not support that maspin is a prognostic factor as there were no significant associations between maspin expression and survival in patients treated with surgery alone.

Neither nuclear maspin expression nor treatment significantly affected survival (DFS or CSS) in patients with rectal cancer, suggesting that the possible predictive value of nuclear maspin expression is restricted to colon cancer only.

As expected, stage (III vs. II) was highly associated with DFS and CSS in both colon and rectal cancer with respective HRs of 3.97 and 4.56 for colon cancer, and 4.87 and 6.84 for rectal cancer (all $p < 0.001$). The results from OS analyses correlated with DFS and are included as Supplementary Tables III-IV, available online at: <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>. Low (vs. high) level of Ki-67 was associated with DFS (HR 2.12, $p = 0.003$) and CSS (HR 2.21, $p = 0.002$) in colon cancer, but not in rectal cancer.

Discussion

Our results indicate a possible role for nuclear maspin expression as an independent predictive marker of response to chemotherapy with 5-FU/Lev in patients

Table IV. Multivariate Cox regression analysis for cancer-specific survival.

Covariate	Colorectal		Colon		Rectum	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex (male vs. female)	1.14 (0.77–1.69)	0.506	0.98 (0.60–1.60)	0.944	1.62 (0.81–3.27)	0.176
Age (continuous)	0.99 (0.97–1.01)	0.370	1.00 (0.97–1.03)	0.978	0.97 (0.93–1.00)	0.055
Stage (III vs. II)	5.00 (3.24–7.73)	<0.001	4.56 (2.73–7.63)	<0.001	6.84 (2.85–16.4)	<0.001
Tumor differentiation (low vs. medium/high)	1.52 (0.93–2.50)	0.098	1.50 (0.84–2.66)	0.169	2.12 (0.66–6.83)	0.209
Ki67 [low (<40%) vs. high (≥40%)]	1.77 (1.19–2.64)	0.005	2.21 (1.32–3.68)	0.002	1.16 (0.57–2.37)	0.677
Interaction Randomization group by maspin expression		0.101		0.045		0.571
Randomization group ^a						
for maspin expression score 60	0.59 (0.30–1.15)	0.122	0.49 (0.21–1.15)	0.101	0.95 (0.31–2.92)	0.935
for maspin expression score 130	0.90 (0.60–1.36)	0.632	0.94 (0.56–1.59)	0.827	0.74 (0.35–1.56)	0.431
for maspin expression score 180	1.23 (0.72–2.11)	0.448	1.51 (0.77–2.96)	0.232	0.62 (0.23–1.66)	0.343
Maspin expression pr 50						
In patients receiving adjuvant chemotherapy	1.31 (1.01–1.69)	0.039	1.43 (1.06–1.94)	0.021	0.99 (0.62–1.57)	0.949
In patients treated with surgery only	0.96 (0.74–1.25)	0.762	0.89 (0.63–1.26)	0.523	1.18 (0.78–1.79)	0.442
Total ^b	1.13 (0.94–1.36)	0.188	1.17 (0.93–1.48)	0.169	1.09 (0.80–1.49)	0.592
Randomization group ^{a,b}	1.09 (0.73–1.62)	0.682	1.02 (0.62–1.66)	0.944	1.34 (0.63–2.82)	0.445

All information is based on a model including the Randomization group by maspin interaction, unless otherwise stated.

^aAdjuvant chemotherapy versus surgery only; ^bwithout Randomization group by maspin interaction in model.

with stage II and III colon cancer (analyzed jointly), but not for patients with rectal cancer. A prognostic value of maspin was not found in this material and no significant associations with other prognostic variables were identified.

Reports on the clinical role of maspin in colorectal cancer are limited in number and present inconsistent results. In the majority of reports maspin expression seems to be related to factors indicating an adverse outcome like high grade tumor budding [21,22], advanced stage [22,23], depth of invasion [22], high CEA-level [23], and high tumor grade [17,20,21,23,29,30]. Recent studies support that high maspin expression is an adverse prognostic factor in CRC [17,23,30]. In contrast, two studies support tumor suppressing abilities of maspin in colon cancer, reporting a high expression of maspin in normal colon, lower maspin expression in carcinomas [31,32] and loss of maspin correlating with poor prognosis [32]. Others have found no prognostic value of maspin [29,33]. One other study has assessed the predictive value of maspin in colon cancer. Dietmaier et al. studied 172 colon cancer patients in a non-randomized study including patients with stage III disease, where 96 received 5-FU based chemotherapy [17]. In contrast to our results, they report a benefit from 5-FU based chemotherapy in cases with *high* nuclear maspin expression. The methodological approach in these two studies correlates in regard to immunohistochemistry, but patient selection criteria differ. In Dietmaier's study treatment was assigned by physicians' choice, not randomized, and the study had a long recruitment period (1993–2001) leaving data regarding predic-

tive values probably more prone to recruitment bias. In most of the previously published studies maspin expression was treated as a dichotomized variable and not analyzed as a continuous variable [20,21, 23,30–32]. This may contribute to information loss and makes comparison between the studies more difficult in the absence of an established cut-off point of maspin expression levels.

Our study is, to the best of our knowledge, the first report on maspin protein function in colorectal cancer to present data from a randomized trial. This material offers a unique opportunity to assess predictive values, since comparing adjuvant chemotherapy in stage III colon cancer to surgery only is no longer considered ethically appropriate. In this study the adjuvant chemotherapy consisted of fluorouracil and levamisole. Since oxaliplatin is added in the current FOLFOX/FLOX regimen the possibility that our results may not be applicable to this treatment must be considered. However, it is well documented that fluorouracil is the main contributor in FOLFOX/FLOX [3,4], and for this reason there are, to our knowledge, no indications that results from this study are not relevant to the adjuvant chemotherapy of today. In this material a *low* expression of maspin predicted response to adjuvant chemotherapy with 5-FU/Lev. We propose that an anti-apoptotic impact of maspin expression in colon epithelial cells limits the effects of 5-FU treatment, either as a feature in wild type maspin [34] or as a result of a maspin polymorphism [35]. Preventing apoptosis may decrease a tumor's vulnerability to chemotherapy-induced cell death and therefore result in a decreased effect of treatment with 5-FU/Lev. Findings in this study cohere with other reports from our group per-

formed on the same patient material, both proposing a higher sensitivity to chemotherapy in tumors with high replication rates [27,36].

Maspin is a multifaceted protein whose function is not completely understood. It was initially characterized as a tumor suppressor, but reports regarding its effect in colorectal cancer indicate an oncogenic function and a relation to adverse prognostic factors. In this study low maspin protein expression was associated with treatment benefit of adjuvant chemotherapy in patients with colon cancer. Further studies are needed to confirm a possible role of maspin as a predictor of adjuvant chemotherapy response in colon cancer.

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Supplementary material available online

Supplementary Figure 1 and Table I-IV available online at: <http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.952386>.