Improving diagnostic, prognostic and predictive biomarkers in colorectal cancer: the role of proteomics and stromatogenesis

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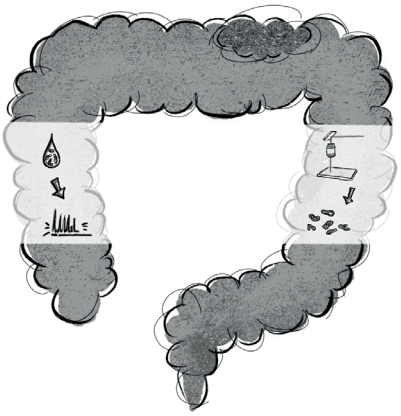

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The value of additional bevacizumab in patients with high-risk stroma-high colon cancer. A study within the QUASAR2 trial, an open-label randomized phase 3 trial.

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ABSTRACT

Introduction: Patients with a high stroma percentage within the primary tumor have a poor prognosis. In this study we investigate whether anti-angiogenic therapy might improve survival of patients with a stroma-high profile with potentially increased angiogenesis.

Materials and Methods: Tissue samples of the primary tumor of 965 colon cancer patients participating in the QUASAR2 trial were analyzed for tumor-stroma ratio (TSR). Stroma-high (>50%) and stroma-low (≤50%) groups were evaluated with respect to survival.

Results: Disease free survival (DFS) was significantly lower in the stroma-high group (HR 1.53, 95%CI 1.19-1.95, p=0.001). No difference in DFS was seen with respect to treatment with capecitabine alone (CAP) or capecitabine with bevacizumab (CAPBEV) (Stroma-high HR 1.00, 95%CI 0.69-1.46, p=0.996; stroma-low HR 1.02, 95%CI 0.75-1.41, p=0.883). A significant difference in survival was seen comparing groups with or without vascular invasion (DFS p<0.001). A correlation between vascular invasion and stroma-high was seen (χ2-test p=0.043).

Discussion and Conclusions: The TSR confirmed to be a strong prognosticator for disease-free survival in a selected high-risk patient population. No benefit was found in response to treatment with bevacizumab when stratified for TSR. TSR showed to have an additional prognostic value in patients with vascular invasion present in the primary tumor.

INTRODUCTION

The tumor-stroma ratio (TSR) in colon cancer (CC) patients has previously been reported by our group and others as a strong independent prognostic parameter. Patients with CC and a high stroma percentage within the primary tumor have a poor prognosis (1-5).

The knowledge of interactions between cancer cells and their tumor microenvironment (TME) and its associated stromal cells is of increasing importance. There is an interaction between non-malignant cells of the microenvironment and malignant cells with growth factors and chemokines that stimulate cancer cell growth, migration and invasion (6). The tumor-stroma itself has been shown to play an important role in tumor formation and progression (7). The tumor-stroma environment contains multiple different cells including (cancer-associated) fibroblasts, angiogenic vascular cells and infiltrating immune cells (8). One of the hallmarks of tumor progression is angiogenesis which the tumor stroma facilitates. When changes occur in the TME, stroma can modulate cancer development and progression (9). Although some aspects of stroma are understood quite well, in particular the contribution of tumor angiogenesis and remodeled extracellular matrix (ECM), it becomes more evident that stromal cells play a much larger role in tumor growth and progression than previously thought (6).

The prognostic value of the TSR has been previously shown, but examining the TSR and its use in therapy selection is a promising new approach. Personalized therapy based on the characteristics of the individual tumor could improve survival and decrease adverse effects induced by unnecessary therapy. The stromal environment contributes to tumor angiogenesis, which supplies the oxygen and nutrients needed for tumor growth and progression (10). Anti-angiogenic therapy, for example with bevacizumab, a monoclonal antibody against vascular-endothelial growth factor, can therefore play an important role in treating patients with increased angiogenesis. Therapy targeting the TME could make a difference in survival, especially for the stroma-high group. This patient group shows a worse survival compared to stroma-low patients and recent literature indicates the resistance of stroma-high patients to current standard chemotherapy regimens (11).

In this present study we investigate the additional value of bevacizumab to standard chemotherapy for stroma-high patients. Furthermore, the relation between the tumor-stroma ratio and the presence of vascular invasion is analyzed.

MATERIAL AND METHODS

Patients

Tissue samples of patients with colon cancer were obtained from the study population of the Quick and Simple and Reliable trial (QUASAR2)(9), a phase III randomized trial of adjuvant capecitabine (CAP) ± bevacizumab (BEV) after complete surgical resection of high-risk stage II and stage III colorectal cancer. Inclusion criteria were histologically proven stage II (stage T4, lymphatic invasion, vascular invasion, peritoneal involvement, poor differentiation, obstruction and perforation of the primary tumor during the pre-operative period and T3 as long as they also have one of the previous listed poor prognostic features) and stage III (any T, N+, M0)(12). QUASAR2 samples were recruited in 123 UK and 61 non-UK participating hospitals. For detailed trial design see http://www.oncology.ox.ac.uk/trial/quasar-2.

Histopathological scoring

Tissue samples consisting of 5 µm Haematoxylin and Eosin (H&E) stained formalin-fixed paraffin-embedded sections from the most invasive part of the primary tumor were used for TSR scoring using conventional microscopy. TSR was defined as the percentage intra-tumor stroma tissue relative to the neoplastic cell component. The protocol for TSR scoring has been described previously (1, 4). In short, the area with the highest amount of stroma on each slide was selected using a 2.5x or 5x objective. Using a 10x objective areas where tumor cells are present at all borders of the image field were scored. Scoring percentages were given per tenfold (10, 20, 30% etc.) per image-field. When mucinous tissue was present within a field that matched our scoring criteria, the mucinous tissue was visually excluded for the scoring. Two investigators (GvP,AH) estimated the stromal percentage in a blinded manner. In case of discrepancy slides were reviewed to reach consensus. In case no consensus could be reached a third observer (V. Smit) was decisive. For statistical analysis stromal ratio groups were divided into stroma-high (>50%) and stroma-low (≤50%) (Figure 1).

Statistical analysis

Statistical analysis was performed using SPSS software version 23.0. Disease-free survival (DFS) was defined as the time from randomization until confirmation of relapse or death of any cause. If no recurrence occurred DFS was calculated as the time period until the date of last follow-up. Inter-observer variability was analyzed using the Cohen's kappa coefficient. Analysis of the survival curves was performed using Kaplan-Meier Survival Analysis and differences in survival distributions were tested using Log Rank Statistics. The Cox proportional hazard model was used to determine the Hazard Ratio (HR) of explanatory variables for DFS.

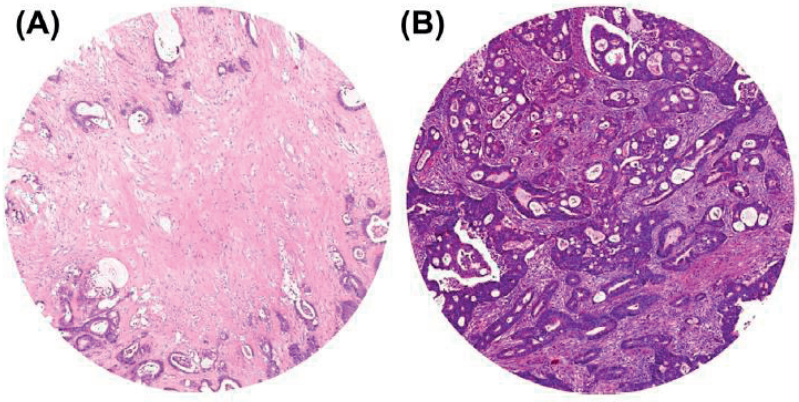

Figure 1. Examples of stroma-high (a) and stroma-low (b) haematoxylin and eosin (H&E) stained paraffin sections at the most invasive part of primary colon cancers (200x magnification).

RESULTS

Patients

In the QUASAR2 trial a total of 1389 patients with colorectal cancer were recruited between 2005 and 2010. A total of 1069 histological samples were obtained from the participating clinics. After scoring all samples for TSR, additional patient information was collected. Rectal cancer patients were excluded from the analysis (N=76) due to the fact that most of them received pre-operative radiotherapy (RT) with known effect of stromal formation. Of 15 samples it was not possible to score a proper TSR due to inferior histological quality. Another 13 patients were excluded due to tumor location or additional pathology information (N=3 small bowel or appendix, N=7 double tumor, N=2 pM1, N=1 stage I). As shown in table 1 the final TSR study cohort comprised of 965 patients (356 stage II, 609 stage III). The study population consisted of 548 men and 417 women, with a mean age of 63.8 years (SD 9.8) years. Within this group 459 patients received CAP and 506 received CAPBEV. Vascular invasion was present in 357 patients (37.0%), in 568 patients there was no vascular invasion (58.9%) and of 40 patients this data was missing (4.1%).

Tumor-stroma ratio

Of in total 965 patients, 323 (33.5%) patients were classified with a stroma-high tumor and 642 (66.5%) with a stroma-low tumor. Cohen's Kappa coefficient revealed a good agreement in classification (k=0.73, 87% concordance). From 357 patients with vascular invasion 135 (37.8%) were stroma-high and 222 (62.2%) were stroma-low. Within the group without vascular invasion (N=568) the division stroma-high versus stroma-low was 178 (31.3%) versus 390 (68.7%), respectively.

Survival analysis

In the stroma-high population the five year survival rate for DFS was 65% versus 75% within the stroma-low population. As expected, the DFS within the stroma-high group was significantly lower compared to the stroma-low group (HR=1.53 (95% CI: 1.19 – 1.95, p=0.001))(Figure 2). In multivariate analysis, after adjusting for age, sex, stage, lymphatic invasion and vascular invasion, the TSR was an independent prognostic factor (HR=1.52 (95%CI: 1.18 – 1.96, p=0.001)).

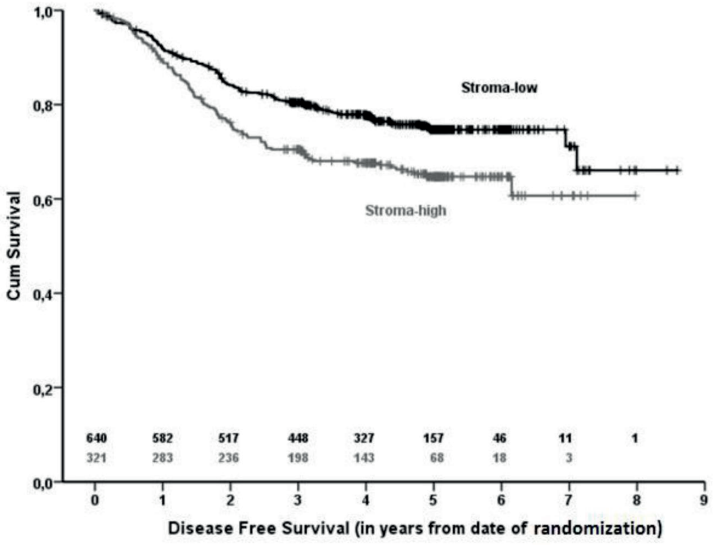

Figure 2. Kaplan-Meier disease free survival curve of the total patient group stratified for the tumor-stroma ratio.

Because of our hypothesis that stroma-high patients might benefit from bevacizumab due to its potential anti-angiogenic effect, we compared the results of therapy for this group of patients. No significant difference for stroma-high patients who received CAPBEV compared to those who were treated with CAP was observed (Stroma-high HR 1.00, 95%CI 0.69-1.46, p=0.996; stroma-low HR 1.02, 95%CI 0.75-1.41, p=0.883) (Figure 3).

Vascular invasion is a prognostic parameter for patients with colorectal cancer. To evaluate a possible interaction between vascular invasion and the TSR, survival times were compared. The DFS between patients with or without vascular invasion showed a significant difference (HR 1.64, 95%CI 1.28-2.10, p<0.001) with a shorter disease-free survival for patients with vascular invasion. Within this group with vascular invasion TSR could further subdivide for patients with worse survival (HR 1.44, 95%CI 1.01-2.06,p=0.041)(Figure 4). A correlation between the presence of a high amount of stroma and vascular invasion was observed (χ2-test p=0.043).

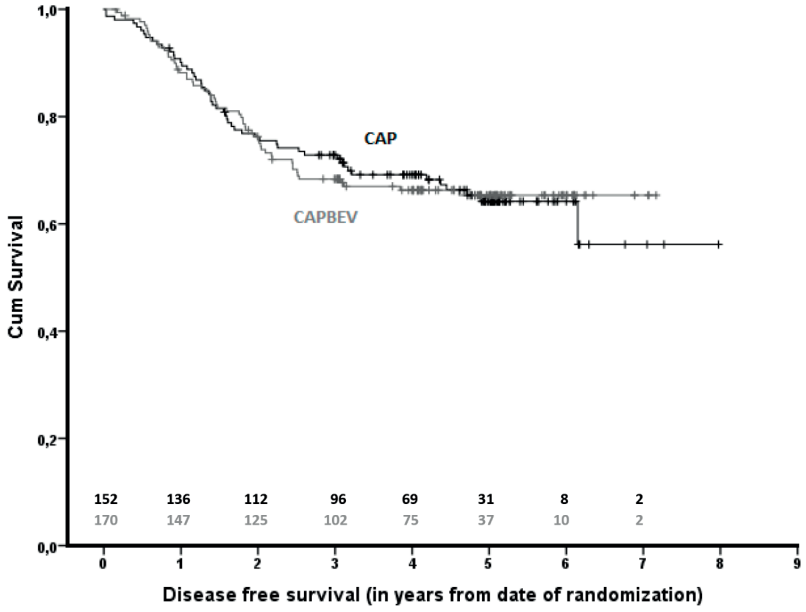

Figure 3. Kaplan-Meier disease free survival curve for the stroma-high patient group stratified for treatment.

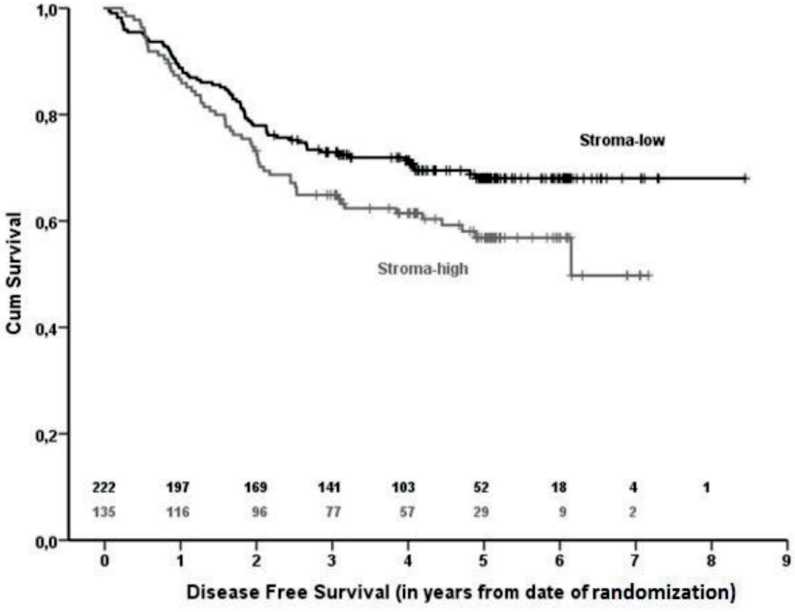

Figure 4. Subgroup analysis within patients positive for the presence of vascular invasion; Kaplan-Meier disease free survival curve stratified for tumor-stroma ratio.

DISCUSSION

Although our study population consisted of only high-risk patients, the TSR proved again to be a strong independent prognostic factor for CC patients. In addition, a worse survival for patients with vascular invasion was confirmed, which is known to be significantly related to reduced disease free and overall survival (13-15). The relation between patients with a stroma-high tumor and vascular invasion found in this study has not been described earlier. This correlation confirms the hypothesis of the important role angiogenesis plays in the stromal environment and the choice for therapy. Targeting the TME can make a difference in survival, especially for the subgroup of stroma-high patients.

The original study (12) did not show a benefit for the addition of bevacizumab for the total study population. In our study, analyzing subgroups of patients based on the pattern of stromal formation within the primary tumor, additional treatment with bevacizumab as anti-angiogenic therapy also did not improve the survival of stroma-high patients. Bevacizumab is a humanized monoclonal antibody which binds to VEGF, thereby prohibiting binding to VEGFR-1 and VEGFR-2. Carmeliet et al. described the complex role of inhibition of angiogenesis. Inhibition of a single target, for instance anti-VEGF therapy, could lead to upregulation of additional angiogenic factors (like PDGF). Combined treatment of anti-angionic agents could increase efficacy and may give the tumor(-microenvironment) less chance to escape from treatment (16). Multiple studies are further investigating the role of the TME and its stromal cells. Their relationship is fundamental for understanding tumor progression and therapeutic resistance. It has been recognized that the tumor-stroma influences drug uptake and sensitivity by different mechanisms. The tumor-stroma is for example involved in buffering the acidic tumor micro-milieu. During rapid tumor growth the TME becomes hypoxic. This induces the immigration of vessels into the tumor and also forces tumor cells to use alternate metabolic pathways creating an acidic microenvironment (7, 17). Moreover, the physical properties and composition of the TME can limit drug-uptake through a dysfunctional vasculature and increased interstitial fluid pressure (6, 7). The organization of the stromal matrix formation might also be an important factor for prediction of therapy response. Efficient organization of this matrix might increase the effective path of molecules towards the target cells (18). This might influence drug diffusion and treatment efficacy. A recent study confirms this hypothesis by describing tumors (of breast cancer patients) with stroma consisting of organized collagen showing a higher benefit from neo-adjuvant chemotherapy compared to tumors with disorganized stroma (19).

In this study, analyzing a pre-selected high-risk patient population with colon cancer, the TSR confirmed to be a strong prognosticator for disease-free survival. Furthermore, it proved to have an additional prognostic value in patients with vascular invasion present in the primary tumor. No benefit was found for the stroma-high group in response to treatment with bevacizumab. Further knowledge of the stromal composition might lead to new targeted treatment regimens focusing on patients with stroma-high and thus more aggressive tumors.

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