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Research Article

Prognostic and Predictive Value of the Tumor-Stroma Ratio in STAGE II Colon Cancer

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ABSTRACT

Background: Tumor-stroma ratio (TSR) is an independent prognosticator in colon cancer.

Objective: We set out to investigate the predictive power, as well as to validate the prognostic power of TSR in stage II colon cancer patients. Better identification of patients who could benefit from adjuvant chemotherapy remains an important issue in stage II disease.

Methods: TSR was microscopically determined on haematoxylin and eosin-stained primary tumor tissue slides of 212 patients who received either adjuvant chemotherapy or surveillance after curative resection in a prospective randomized clinical trial (ABCSCG-91).

Results: Stroma-high tumors were associated with significantly more cancer-related death ((CaDeath) HR 2.30, 95% CI 1.05–5.03; $p=0.037$) and significantly shorter distant recurrence-free survival (DRFS) HR 2.32, 95% CI 1.10–4.87; $p=0.027$) compared to stroma-low tumors. Backward multivariate Cox-regression analysis demonstrated TSR as an independent prognosticator for DRFS ($p=0.027$) and CaDeath ($p=0.031$). TSR did not validate as a predictive biomarker; CaDeath (HR 0.87, 95% CI 0.18–4.17; $p=0.87$), DRFS (HR 0.76, 95% CI 0.17–3.36; $p=0.71$) and OS (HR 0.96, 95% CI 0.29–3.21; $p=0.95$) for the type of chemotherapy given in ABCSCG-91.

Conclusions: TSR, an easily applicable and inexpensive observer-based method, is an independent predictor of poor prognosis in stage II colon cancer. Predictive value for adjuvant 5-FU/leucovorin could not be demonstrated.

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Introduction

The worldwide cancer incidence and mortality are rapidly growing, with an estimated 1.8 million new colorectal cancer cases and 881,000 deaths in 2018. Making colon cancer the third most common cancer [1]. This increase in incidence is partly due to countries undergoing major socioeconomic developments, influence of westernization (i.e. dietary patterns, alcohol consumption, smoking and obesity) and early detection and screening programs [2-4]. Although the screening programs generally lead to a temporary increase of newly detected colorectal tumors, which are mostly early-stage disease with favorable prognosis, they do have implications on subsequent treatment decisions. Currently, European guidelines recommend adjuvant systemic chemotherapy after primary tumor resection for stage III and “high-risk” stage II patients. Hereby, “high-risk” is defined by presence of at least one of the following features: vascular, lymphatic or perineural invasion, poorly differentiated tumors, tumor presentation with perforation or obstruction, pT4 stage and < 12 lymph nodes sampled [5, 6].

However, there is much debate on which high-risk stage II patients might benefit from adjuvant chemotherapy nowadays [7]. The issue is raised whether treatment decisions should be tailored to individual tumor characteristics in this group with localized disease since convincing evidence on adjuvant treatment benefit has not been proven in the general stage II disease group [8-10]. Risk assessment is presently performed by consideration of known tumor-related prognostic factors. Novel risk factors, such as MMR status, microRNA and BRAF mutational status, have proven to be prognostic, whereas the availability of predictors for chemotherapy derived benefits are scarce [11-14]. Promising results have been reported for markers like CDX2 and circulating tumor DNA. However, these are still far from routine clinical implementation [15, 16]. In the past years, our research group has repeatedly demonstrated that the amount of intratumoral stroma, referred to as the tumor-stroma ratio (TSR), is an independent prognosticator in colon cancer [17-21]. Most of these studies were performed in pooled stage I-III cohorts and reported subgroup analyses of stage II patients. We will assess if the TSR provides predictive information, which could serve as a marker for adjuvant therapy in this group and validate the prognostic power of the TSR in a group of exclusively stage II colon cancer patients.

Material and Methods

I Study Design

Between 1993-2003, the Austrian Breast and Colorectal Cancer Study Group (ABCSCG) included patients for various research purposes. As part of this initiative, a multicentre prospective randomized trial investigating the impact of adjuvant chemotherapy in histologically proven stage II colon cancer (defined as T3-4, N0, M0) was performed in 2007. Thirty-one hospitals were involved in this trial, whereby the protocol was approved by local ethical committees of the participating hospitals.

For the original study, a total of 535 patients were included and randomized in two post-operative treatment groups; 1) 5-fluorouracil (5-FU) and leucovorin (LV), once weekly for 6 weeks in each 8-week cycle for a total of 7 chemotherapy cycles (=56 weeks of therapy); 2)

surveillance only. Follow-up was performed every 3 months during the first year, followed by every 6 months during year 2-5 and once yearly until year 10 after randomization. For more details with regard to the original study design, see Schippering *et al.* [22]. Patients from whom haematoxylin and eosin-stained (H&E) tumour tissue slides were available, were eligible for the currently described study. Since archival material was used in an anonymized manner, no additional informed consent was required.

II Histopathological Scoring of Tumour-Stroma Ratio

For all available H&E primary tumour tissue slides, the TSR was determined in a blinded manner by two investigators (GP, SV). Histopathological scoring was performed according to the method as described by Mesker *et al.*, whereby using a 2.5x or 5x objective, the area with the highest amount of stroma was microscopically selected. Next, using a 10x objective, image fields where neoplastic cells were present at all borders were scored. Scoring percentages were given per 10-fold (10%, 20% etc.) per image field. Subsequently, two groups were defined: stroma-low ($\leq 50\%$) and stroma-high ($>50\%$). This cut-off has previously proven to have a maximum discriminative power [18].

III Statistical Analysis

Statistical analyses were performed with SPSS software version 25. Interobserver variability for histopathological scoring was tested using Cohen's kappa coefficient (κ). The χ^2 test was used to compare statistical differences among categorical variables between the stroma-low and stroma-high group. For numerical variables, the unpaired t-test or Mann-Whitney U test was used, depending on the normality of the distribution. For time-to-event analyses, the Kaplan Meier method and log-rank test were used. Distant recurrence-free survival (DRFS) was defined as the interval between the date of randomization and date of last visit or date of distant-recurrence. Cancer-related death (CaDeath) was defined as the interval between the date of randomization and date of last visit or date of death caused by colon cancer. Overall survival (OS) was defined as the interval between randomization and date of last visit and/or death by any cause. Univariate and multivariate analyses were performed by using a Cox-regression model, whereby a backward selection model was applied for the multivariate analyses. Predictive analyses were performed using a Cox-proportional hazard model with interaction term between TSR and the treatment groups.

Results

I Baseline Characteristics

The original trial performed by ABCSCG included 535 patients. A total of 212 histological samples from this group were available for microscopic scoring in the current study. Upon scoring, baseline characteristics were added. Clinical data were not available from 34 patients, causing the final study population to consist of 174 patients with 88 patients (50.6%) in the 5-FU/LV group and 86 patients (49.4%) in the surveillance group, respectively. Baseline characteristics were evenly balanced between the two groups without significant differences. In total, 60 (34.5%) of the 174 patients died during a median follow-up period of 11.5 years. Table 1 provides a detailed description of all

characteristics. Furthermore, there were no significant differences between this subset and the original ABCSG study population (data not shown).

Table 1: Baseline characteristics.

	Stroma-low	Stroma-high	P-value
	N = 138 (%)	N = 36 (%)	
Age in years			
Median (range)	65.7 (35.4 - 78.0)	64.2 (37.5 - 77.2)	0.27
Gender			
Female	65 (47.1)	17 (47.2)	0.99
Male	73 (52.9)	19 (52.8)	
Therapy			
5-FU/LV	70 (50.7%)	18 (50.0%)	0.94
Surveillance	68 (49.3%)	18 (50.0%)	
T stage			
T3	118 (85.5)	26 (72.2)	0.06
T4	20 (14.5)	10 (27.8)	
Grade			
G1 and G2	113 (81.9)	28 (77.8)	0.58
G3 and G4	25 (18.1)	8 (22.2)	
Tumor location			
Coecum and right colon	35 (25.4)	7 (19.4)	0.67
Sigmoid and left colon	69 (50.0)	18 (50.0)	
Flexures and transverse colon	34 (24.6)	11 (30.6)	

TSR: Tumor-Stroma Ratio; 5-FU: 5-Fluorouracil; LV: leucovorin.

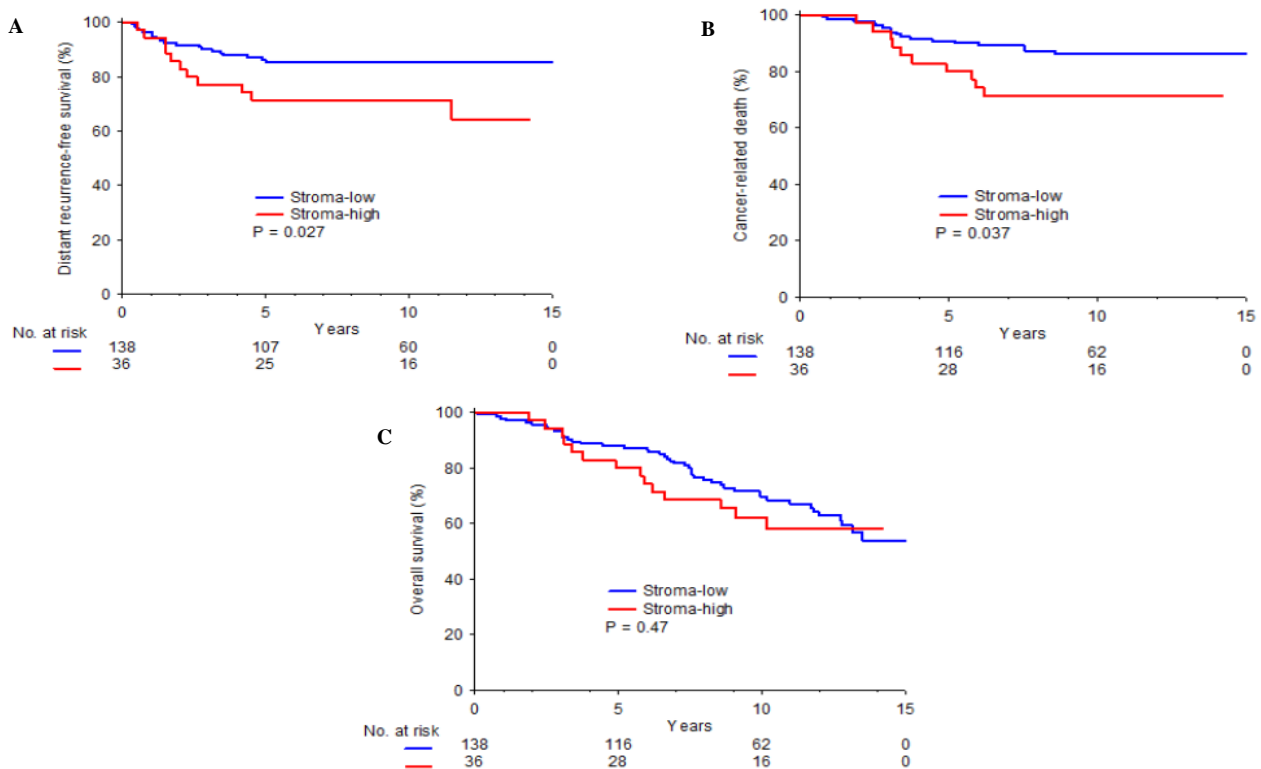


Figure 1: Kaplan-Meier curves for survival endpoints according to TSR category:(A) Distant recurrence-free survival, (B) Cancer-related death, (C) Overall survival. Blue line = stroma-low; Red line = stroma-high.

Table 2: Univariate and multivariate analyses.

	N=	Distant recurrence-free survival			Cancer-related death			Overall survival					
		Univariate			Multivariate			Univariate			Multivariate		
		HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value	HR (95% CI)	P- value
Age (mean)	174	0.98 (0.953 - 1.017)	0.338			0.99 (0.960 - 1.029)	0.721			1.04 (1.011 - 1.069)	0.006	1.04 (1.015-1.070)	0.002
Gender													
Male	92	0.65 (0.314 - 1.333)	0.238			0.49 (0.224 - 1.069)	0.073			1.02 (0.612 - 1.693)	0.947		
Female	82												
Grade													
G1 and G2	141	0.49 (0.147 - 1.603)	0.236			0.54 (0.163 - 1.793)	0.314			0.79 (0.388 - 1.604)	0.512		
G3 and G4	33												
T stage													
T3	144	0.73 (0.255 - 2.096)	0.561			1.10 (0.417 - 2.907)	0.846			1.32 (0.716 - 2.448)	0.37		
T4	30												
Tumor location													
Caecum and right colon	42	Reference				Reference				Reference			
Sigmoid and left colon	87	0.60 (0.246 - 1.474)	0.267			0.34 (0.136-0.827)	0.018	0.32 (0.131-0.796)	0.014	0.38 (0.205-0.691)	0.002	0.36 (0.196-0.661)	0.001
Flexures and transverse colon	45	1.08 (0.425 - 2.730)	0.875			0.62 (0.246-1.583)	0.321	0.56 (0.220-1.1435)	0.228	0.72 (0.382-1.371)	0.321	0.78 (0.412-1.482)	0.45
Therapy													
Surveillance	86	1.06 (0.518 - 2.175)	0.871			1.01 (0.476 - 2.153)	0.976			0.89 (0.536 - 1.476)	0.651		
5-FU / LV	88												
TSR													
Stroma-low	138	2.32 (1.102 - 4.872)	0.027	2.32 (1.102 - 4.872)	0.027	2.30 (1.054 - 5.027)	0.037	2.38 (1.082-5.220)	0.031	1.25 (0.685 - 2.271)	0.47		
Stroma-high	36												

TSR: Tumor-Stroma Ratio.

II Tumor-Stroma Ratio

Two hundred and twelve H&E tissue slides were scored for TSR. Four samples (1.9%) were excluded due to the absence of the material or an insufficient amount of invasive tumor tissue for scoring. Finally, 163 (76.9%) were scored as stroma-low tumors and 45 (21.1%) as stroma-high tumors. The interobserver agreement showed a good level of agreement for TSR scoring ($\kappa = 0.81$) Noteworthy, as previously mentioned, clinical data was not available from 34 patients. Hence, the final study population comprised 174 patients.

III Prognostic Value of TSR

In total, 138 (79.3%) primary tumors were scored as stroma-low and 36 (20.7%) as stroma-high. Patients with stroma-high tumors experienced significantly more CaDeath (HR 2.30 (95% CI 1.05–5.03; $p = 0.037$)) and had a shorter DRFS (HR 2.32 (95% CI 1.10–4.87; $p = 0.027$)) compared to patients with stroma-low tumors. A survival difference was ruled out for OS (HR 1.25, 95% CI 0.69–2.27; $p = 0.470$) (Figure 1). After 10 years of follow-up, 10 (27.8%) patients with stroma-high tumors died of a cancer-related cause versus 17 (12.3%) patients with stroma-low tumors, whereas for distant recurrence, this was 11 (30.6%)

patients and 19 (13.8%) patients, respectively. Multivariate analyses validated the TSR as an independent prognosticator for DRFS (HR 2.32 (95% CI 1.10–4.87; $p = 0.027$)), as well as for CaDeath (HR 2.38 (95% CI 1.08–5.22; $p = 0.031$)), but not for OS. Additionally, the backward model revealed that tumor location also retained its prognostic power with respect to CaDeath and OS (Table 2).

IV Predictive Value of TSR

Within the 5-FU/LV group, 70 (79.9%) patients had a stroma-low tumor and 18 (20.5%) a stroma-high tumor. For the surveillance group, this was 68 (79.1%) for the former and 18 (20.9%) for the latter category (Table 1). The time-to-event analysis demonstrated no significant differences in survival between stroma-high and stroma-low patients amongst the two treatment arms (Figure 2). Predictive analysis ruled out an interaction between TSR and therapy with respect to CaDeath (HR 0.87 (95% CI 0.18–4.17; $p = 0.87$)), DRFS (HR 0.76 (95% CI 0.17–3.36; $p = 0.71$)) and OS (HR 0.96 (95% CI 0.29–3.21; $p = 0.95$)). Unfortunately, due to the limited amount of patients who met the ASCO guideline criteria of “high-risk” stage II disease ($n = 48$), we were unable to perform a formal predictive analysis in this group.

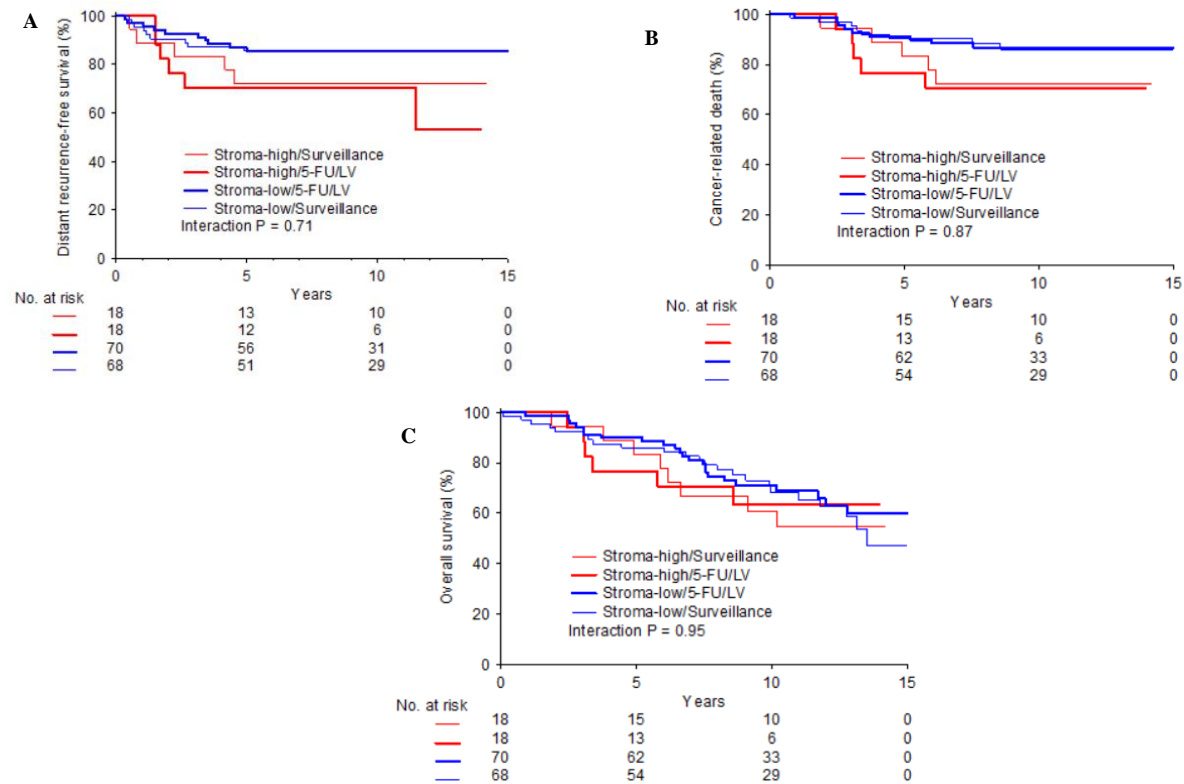


Figure 2: Kaplan-Meier curves for survival endpoints according to TSR category, stratified by treatment arms: Distant recurrence-free survival, (B) Cancer-related death, (C) Overall survival; Blue bold line = stroma-low / 5-FU/LV; Blue fine line = stroma-high/ surveillance; Red bold line = stroma-high / 5-FU/LV; Red fine line = stroma-high / surveillance.

Discussion

The indication of adjuvant chemotherapy for stage II colon cancer patients remains a topic of discussion, since the evidence on therapeutic

benefit in this population is inconsistent, despite the availability of pathologically based high-risk disease stratifiers [6-10, 23-26]. The TSR has previously validated as an independent prognosticator in several stages of colon cancer. This led us to further explore the prognostic and

predictive qualities of this biomarker in a population of exclusively stage II colon cancer. Our analysis demonstrated that tumors with a high amount of stroma were independently associated with more CaDeath and a shorter DRFS in stage II colon cancer. This was not the case for OS. The latter result was somewhat surprising, since the majority of the TSR studies in colon cancer also reported the TSR as an independent prognosticator for this survival endpoint, either in pooled cohorts or subgroup analyses [17-20, 27-31]. Nonetheless, demonstrating the prognostic power of the TSR with respect to CaDeath in a cohort of solely stage II disease is a valuable result, since this generally represents a group of individuals diagnosed via early detection screening programs and subsequently treated with local surgery. Using the CaDeath provides us with a more precise indicator of therapeutic impact on survival and subsequently gives a better illustration of the discriminating power of a new biomarker.

With respect to our hypothesis, that tumors with more stroma should be considered as more aggressive based on an increased metastatic potential and tumor burden due to an activated tumor microenvironment with consequent enhanced growth factor and cytokine production, it could be suggested that the amount of intratumoral stroma, expressed as the TSR, might be considered as a potential additional risk stratifier [32]. Upon predictive analysis, we presumed that patients with high amounts of intratumoral stroma would have a different response to therapy, at least for the survival endpoints DRFS and CaDeath. However, a predictive value was ruled out for all endpoints. This negative finding was more or less in agreement with results from our previous study in a cohort of stage II and III colon cancer, wherein we could only demonstrate a trend for adjuvant therapeutic survival benefit though this was in relation to chemotherapy and targeted therapy [28]. Based on the reports from Mezheyeuski *et al.*, the suggestion is raised that observer-dependent scoring methods simply might not possess a predictive power with regard to response to adjuvant chemotherapy. In this particular study, the authors investigated the prognostic and predictive capability of multifractal analyses (i.e. a computer-assisted method which quantitatively evaluates the morphological composition of the tumor-stroma interface) versus the histomorphological parameters tumor budding, tumor grade and tumor border configuration. Herein, they demonstrated that like all histomorphological parameters, multifractional analyses validated as an independent prognostic marker in stage II colon cancer. However, only the multifractional analyses contained a predictive capacity and was subsequently able to identify patients who experienced significant improvement of CSS after receiving adjuvant 5-FU [33]. Nevertheless, we feel that we should not abandon the predictive potential of observer-based parameters right away since these methods are generally more cost-effective as well as easier to integrate into current clinical workflows.

Apart from the retrospective design of our study, the chemotherapy regimen of only 5FU/LV, which was administered could be indicated as a second limitation. This regimen is currently considered outdated, ever since it was demonstrated that addition of oxaliplatin to 5-FU/LV significantly increased the disease-free survival in stage II colon cancer [9, 34]. Therefore, we cannot fully extrapolate the results to the current clinical situation. Thirdly, in the current study population, approximately 20% of the tumors were classified as stroma-high, whereas in previous studies, the amount of stroma-high tumors usually ranged between 25-

35% [19, 28, 35]. Lastly, we must acknowledge we were unable to perform analyses in the subgroup of “high-risk” patients, a clinically relevant group in need of additional predictive biomarkers.

In conclusion, our study validated an easily applicable and inexpensive method, such as the TSR, as an independent predictor of poor prognosis in stage II colon cancer. This provides the perspective of the implementation of this parameter as an additional prognostic clinical risk stratifier for colon cancer. Prospective studies to validate this are currently pending (NTR7270) [36]. Predictive value of the TSR for adjuvant 5-FU/leucovorin could not be demonstrated.

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Conflicts of Interest

Dr. Greil reports honoraria, travel funding, and research funding from AbbVie, Amgen, Astra Zeneca, Bristol-Myers Squibb, Celgene, Merck, Merck Sharp & Dohme, Novartis, Takeda, Sandoz, and Roche. Dr. Gnant reports personal fees and non-financial support from Amgen, grants, personal fees and non-financial support from AstraZeneca, personal fees and non-financial support from Celgene, personal fees and non-financial support from Eli Lilly, grants, personal fees and non-financial support from Novartis, personal fees from NanoString Technology, grants and personal fees from Roche, other from Accellsoir, grants and non-financial support from Pfizer, non-financial support from Ipsen. Dr. Filipits reports personal fees and research funding from Astra Zeneca, and personal fees from Bayer, Biomedica, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme, Myriad Genetics Inc., Pfizer, and Roche. No other disclosures are reported.

Ethics Approval

The original study protocol was approved by local ethical review committees. For the current study, archival material was used in an anonymized manner. Therefore, no additional informed consent was required.

Contributions

All authors actively contributed to the paper and take responsibility and accountability for the accuracy and integrity of the work. Conception and design: MF, MG, HG, WM, RT Collection and assembly of data: MF Data analysis and interpretation: GP, SV, MF, SZ, MG Manuscript writing: MF, SZ, MG, HG, WM, RT.

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