Original paper

Stromal CCL18 expression at the tumour invasion front and overall survival in node-positive gastric adenocarcinoma: a retrospective cohort study

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C-C motif chemokine ligand 18 (CCL18), mainly produced by M2-polarised tumour-associated macrophages, is implicated in immunosuppression, invasion, and metastasis, whereas *IgG4*-positive plasma cells may reflect humoral immunoregulation and immune evasion.
In this retrospective cohort study, stromal *CCL18* expression at the primary tumour invasion front was evaluated in relation to overall survival in 76 patients with node-positive gastric adenocarcinoma. Stromal *IgG4* was assessed secondarily, and concordance between primary tumours and matched lymph node metastases was examined. Immunohistochemical evaluation was restricted to the stromal compartment. *IgG4* expression was defined as ≥ 10 positive plasma cells per high-power field, and *CCL18* expression was dichotomised using study-specific median cut-offs.
Median overall survival was shorter in patients with high vs. low primary tumour stromal *CCL18* expression (9.3 vs. 23.2 months). In multivariable Cox regression adjusted for age, sex, histologic grade, and pathological stage, high primary tumour stromal *CCL18* expression was associated with shorter overall survival; this association persisted after adding stromal *IgG4* to the model. Primary tumour stromal *IgG4* showed no independent survival association. Concordance was higher for *IgG4* than for *CCL18*.
These findings support primary tumour stromal *CCL18* as a candidate tumour microenvironment marker associated with shorter overall survival in node-positive gastric adenocarcinoma.

Key words: immunoglobulin G4, adenocarcinoma, stomach neoplasms, lymphatic metastasis, chemokine CCL18.

Introduction

Gastric cancer is one of the leading causes of cancer-related mortality worldwide. According to GLOBOCAN 2022 data, gastric cancer ranks fifth in both new cancer cases and cancer-related deaths [1]. Gastric cancer is often diagnosed at an advanced stage, and its clinical course shows marked heterogeneity even among cases at similar stages. Therefore, there has been increasing interest in biomarkers that reflect the tumour microenvironment beyond traditional staging systems [2]. In particular, the tumour invasion front (TIF) has attracted prognostic interest as one of the regions where tumour stroma interactions and signals associated with metastatic behaviour are most prominently observed [2, 3]. C-C motif chemokine ligand 18 (*CCL18)* is a chemokine primarily secreted by M2-polarised tumour-associated macrophages (TAMs) and is asso-

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ciated with immunosuppression, tissue remodeling, invasion and metastasis [4]. In gastric cancer, tumour-associated macrophages, particularly M2-polarised populations, are increasingly recognised as key components of the tumour immune microenvironment, supporting the evaluation of TAM-derived mediators such as *CCL18* as candidate stromal prognostic markers [5]. The available data on *CCL18* in gastric cancer are not entirely consistent. The study by Leung *et al.* [6] identified *CCL18* as an independent prognostic marker and associated it with better survival whereas more recent data suggest that the *CCL18*/*CCR8* axis may be linked to recurrence and poor prognosis [7]. *IgG4*-positive plasma cells are considered indicators of tumour-associated humoral immune response and potential immune evasion. It has been shown that the *IgG4* response can weaken antitumour effector functions, and increased tissue *IgG4* levels in gastric cancer have been associated with tumour progression and poor prognosis [8, 9]. Therefore, *IgG4* was included in the study as a secondary biomarker complementing the microenvironment reflected by *CCL18* representing tumour-associated humoral immune response and potential immune evasion processes.

In addition, spatial profiling studies of patient-matched primary gastric cancers and locoregional metastases indicate that metastatic compartments may not simply mirror the primary tumour, supporting paired primary metastatic tissue assessment in biomarker studies [10]. However, studies evaluating *CCL18* expression at the TIF of the primary tumour in gastric adenocarcinoma in relation to overall survival assessing *IgG4* as a secondary biomarker within the same microenvironment and comparatively examining matched lymph node metastasis compartments are limited [6, 7, 9]. Therefore, in a retrospective cohort consisting of resection cases with lymph node metastasis, we aimed to test our primary hypothesis that high stromal *CCL18* expression at the TIF of the primary tumour is associated with shorter overall survival by scoring stromal *CCL18* and *IgG4* expression in the primary tumour TIF and matched lymph node metastasis.

Secondarily, we evaluated the clinicopathological and prognostic associations of *IgG4* infiltration. Concordance between the primary tumour and lymph node metastasis, marker combinations, and additional compartment-based comparisons were planned as exploratory analyses.

Material and methods

Case selection

A total of 167 cases who underwent total, distal or subtotal gastrectomy for gastric malignancy between January 2008 and September 2021 were screened. Thirty cases were excluded due to tumour types other than gastric adenocarcinoma, and 61 cases were excluded due to the absence of lymph node metastasis. Consequently, 76 resection cases with at least one metastatic lymph node and available formalin-fixed, paraffin-embedded tissue samples from both the primary tumour and the corresponding lymph node metastasis were included in the study.

Clinicopathological variables

Age, sex, date of pathological diagnosis, and clinical follow-up data were obtained from the electronic hospital database and pathology records. Age was categorised as < 65 years and ≥ 65 years.

Haematoxylin and eosin-stained slides from all cases were re-evaluated. All histopathological parameters, including diagnosis, histologic grade, tumour type, lymphovascular invasion, and perineural invasion, were assessed and determined by two pathologists through joint evaluation of all cases.

Tumour localisation was classified as proximal (cardia, fundus, and corpus), distal (antrum and pylorus), and diffuse. Lymphovascular invasion and perineural invasion were recorded as present or absent. Histologic grade was reassessed based on glandular differentiation. According to the Lauren classification, diffuse-type tumours were considered poorly differentiated for grading purposes. Tumour type was classified as intestinal, diffuse, and mixed according to the Lauren classification. Tumour staging was performed based on the pT, pN, and pM categories according to the 8th edition of the American Joint Committee on Cancer tumour-node-metastasis classification. In addition, the presence of desmoplasia in the primary tumour, desmoplasia in the metastatic lymph node, and intestinal metaplasia was recorded.

Tissue area selection

For immunohistochemical evaluation, TIF was defined as the advancing edge of the tumour, irrespective of the presence of stromal reaction and/or tumour cell dissociation. In the primary tumour, non-tumoral mucosa located within 10 mm of the tumour periphery was considered the noncancerous area. A single inflammation-rich hotspot area was selected for evaluation in both the primary tumour and lymph node metastasis. In the final study design, the analyses were conducted in the stromal compartment.

Immunohistochemical analysis

After the paraffin blocks best representing the tumour were selected, 3-μm-thick sections were cut onto positively charged lysine-coated slides. Immunohistochemical staining was performed on a Ventana Benchmark XT automated staining system (Ventana Medical Systems, Roche, USA) using a validated immunoperoxidase method and DAB chromogen. *IgG4* (MRQ-44, 1 : 100 dilution) and *CCL18* (SC-374438, 1 : 100 dilu-

tion) antibodies were used. Tonsil tissue was used as the positive control.

Immunohistochemical evaluation

The slides were evaluated using an Olympus BX50 microscope. After an overall scan of the slides, a single inflammation-rich hotspot area showing the most intense staining was selected. *IgG4* expression was assessed by counting *IgG4*-positive plasma cells in the tumour stroma. C-C motif chemokine ligand 18 expression was recorded as the percentage of positively stained cells in the tumour stroma within the selected area. Evaluation was performed in a high-power field with an area of 0.238 mm² using a 10^x ocular and a 40^x objective. Evaluations were performed separately for the primary tumour and matched lymph node metastasis. Immunohistochemical evaluation was performed by two pathologists (Seda Keskin Gökmen and Emİne Kilinç Günay) blinded to clinicopathological outcomes and survival data. *IgG4* and *CCL18* scores were determined by consensus for both primary tumour and matched lymph node metastasis samples. Because the evaluation was consensus based, interobserver agreement was not calculated separately.

Marker definitions

IgG4 expression was evaluated as ≥ 10 *IgG4*-positive plasma cells per high-power field, whereas *CCL18* expression was assessed as the percentage of positively stained cells in the tumour stroma and classified as low or high according to study-specific median values. This approach is consistent with histopathological thresholds and median-based biomarker classifications reported in the literature [11–13]. Accordingly, values of ≥ 15% in the primary tumour and ≥ 10% in lymph node metastasis were considered high *CCL18* expression. These classifications were used to examine associations with clinicopathological variables, survival analyses, and concordance analyses between the primary tumour and metastatic focus.

Outcome measure

The primary outcome of the study was overall survival. Overall survival was defined as the time from the

Table IA. Univariable overall survival analysis according to clinicopathological variables and stromal marker expression

Clinicopathological variables	*N*	Deaths, *N*	Median OS [months]	*p*-value
Age				
< 65 years	28	21	18.5	0.002
≥ 65 years	48	44	5.7	
Sex				
Female	24	21	5.9	0.246
Male	52	44	13.8	
Tumour localisation				
Proximal	33	27	13.9	0.635
Distal	38	33	9.5	
Diffuse	5	5	9.1	
Lymphovascular invasion				
Present	54	48	9.3	0.198
Absent	22	17	15.8	
Perineural invasion				
Present	53	47	9.1	0.068
Absent	23	18	18.8	
Histologic grade				
Grade 1	12	10	11.2	0.016
Grade 2	25	19	16.8	
Grade 3	39	36	8.0	
Lauren type				
Intestinal	39	34	11.4	0.166
Diffuse	24	22	9.1	
Mixed	13	9	20.2	

Table IA. Cont.

Clinicopathological variables	*N*	Deaths, *N*	Median OS [months]	*P*-value
Pathological stage				
Stage II	12	8	19.4	0.020
Stage III	47	40	6.4	
Stage IV	16	16	7.6	
Pathological T category				
pT1	2	2	16.5	
pT2	3	1	—	0.016
pT3	40	33	15.6	
pT4	31	29	5.4	
Pathological N category				
pN1	21	17	19.4	0.103
pN2	24	19	15.8	
pN3	31	29	6.6	
Pathological M category				
pM0	60	49	13.8	0.015
pM1	16	16	7.6	
Stromal marker expression				
Primary tumour stromal IgG4				
High (≥ 10 cells/HPF)	20	14	16.5	0.088
Low (< 10 cells/HPF)	56	51	9.1	
Primary tumour stromal CCL18				
High (≥ 15%)	49	43	9.3	0.014
Low (< 15%)	27	22	23.2	
Lymph node metastasis stromal IgG4				
High (≥ 10 cells/HPF)	24	19	13.8	0.423
Low (< 10 cells/HPF)	52	46	9.1	
Lymph node metastasis stromal CCL18				
High (≥ 10%)	43	34	13.9	0.237
Low (< 10%)	33	31	8.0	

CCL18 – C-C motif chemokine ligand 18, HPF – high-power field, OS – overall survival

P-values were obtained using the log-rank test. Stage I was not included in the survival comparison because only one case was present in this category. Primary tumour stromal CCL18 was dichotomised using the study-specific median threshold of 15%, and lymph node metastasis stromal CCL18 using the median threshold of 10%.

date of pathological diagnosis to the date of death. Cases who were still alive were censored at the last available follow up. The univariable overall survival analysis is presented in Table IA.

Statistical analysis

The distribution of continuous variables was assessed using the Shapiro-Wilk test. Associations between categorical variables were examined using the Pearson χ^2 test, the likelihood ratio test, and exact tests when expected cell frequencies were low. The McNemar test was used for paired categorical comparisons such as primary tumour and lymph node metastasis, and concordance between primary and metastatic sites was evaluated using Cohen's κ test. For non-normally distributed numerical data the Kruskal-Wallis test was used for comparisons of more than two groups and the Mann-Whitney *U* test for pairwise comparisons, with Bonferroni correction applied for multiple comparisons. Overall survival was analysed using the Kaplan-Meier method and compared between categorical variables using the log-rank (Mantel-Cox) test.

For multivariable survival analysis, a parsimonious Cox proportional hazards model was constructed based on the primary study hypothesis and established clinicopathological prognostic factors. Primary tumour stromal *CCL18* expression was included in the model as the main exposure variable, and age, sex, histologic grade, and pathological stage were included as adjustment variables. In the multivariable analysis, histologic grade was entered as grade 1–2 and grade 3, and pathological stage as stage

I–II and stage III–IV in a dichotomised form. To avoid overadjustment and collinearity, pathological stage and its components pT, pN, and pM were not included in the same model. In the secondary model, primary tumour stromal *IgG4* expression was additionally included to assess whether the prognostic effect of *CCL18* was independent of IgG4. The proportional hazards assumption was visually assessed using log-minus-log survival plots. The results of the multivariable analysis are presented in Table IB. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA).

Reporting guideline

The reporting of this retrospective prognostic marker study was guided by the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK), where applicable [14].

Results

The clinicopathological characteristics of the 76 patients are summarised in Table II. Forty-eight patients (63.2%) were aged ≥ 65 years and 52 (68.4%) were male. Distal tumours were observed in 38 patients (50.0%), lymphovascular invasion in 54 (71.1%), perineural invasion in 53 (69.7%), grade 3 tumours in 39 (51.3%) and intestinal type tumours in 39 (51.3%). Most patients had pT3–pT4 disease [71/76 (93.4%)] and stage III–IV disease [63/76 (82.9%)].

Representative stromal *CCL18* and *IgG4* staining patterns are shown in Figures 1 and 2. In the primary tumour, stromal *IgG4* expression was high in 20 cases and stromal *CCL18* expression was high in 49 cases. *IgG4* expression was associated with pT and pN categories ($p = 0.034$ and $p = 0.024$, respectively). C-C motif chemokine ligand 18 expression was associated with histologic grade ($p = 0.019$), with grade 3 tumours occurring more frequently in the high-expression group [31/49 (63.3%)] than in the low-expression group [8/27 (29.6%)]. No other significant associations were identified (Table III).

In matched lymph node metastases, stromal *IgG4* and *CCL18* expression was high in 24 and 43 cases. *IgG4* expression was associated with histologic grade and Lauren type ($p = 0.002$ and $p = 0.012$), whereas *CCL18* expression was associated with sex ($p = 0.023$). No other significant associations were observed.

Primary tumour lymph node concordance was higher for *IgG4* than for *CCL18*. *IgG4* concordance was observed in 58/76 cases (76.3%; $\kappa = 0.426$, $p < 0.001$), whereas *CCL18* concordance was observed in 44/76 cases (57.9%; $\kappa = 0.125$, $p = 0.271$). McNemar tests showed no significant differences between paired sites for *IgG4* ($p = 0.481$) or *CCL18* ($p = 0.377$) (Table IV).

During follow-up, 65 deaths occurred. In univariable analysis, age, histologic grade, pathological stage, pT category, pM category, and primary tumour stromal *CCL18* expression were associated with overall survival (Table IA). Stage I was excluded from the stage comparison because only one patient was present in this category. Median overall survival was shorter in patients with high than in those with low primary tumour stromal *CCL18* expression (9.3 vs. 23.2 months, respectively, $p = 0.014$). Primary tumour stromal *IgG4* expression was not significantly associated with survival ($p = 0.088$), and neither lymph node marker was associated with survival (IgG4, $p = 0.423$; *CCL18*, $p = 0.237$).

Table IB. Multivariable Cox regression analyses for overall survival in patients with node-positive gastric adenocarcinoma

Parameters	Category/ comparison	Model 1 Adjusted HR	95% CI	*p*-value	Model 2 Adjusted HR	95% CI	*p*-value
Demographic factors							
Age	≥ 65 vs. < 65	3.27	1.84–5.81	< 0.001	3.13	1.74–5.64	< 0.001
Sex	Female vs. male	1.51	0.86–2.62	0.149	1.43	0.81–2.52	0.220
Tumour-related covariates							
Histologic grade	Grade 3 vs. grade 1–2	2.05	1.10–3.81	0.024	1.90	0.98–3.65	0.056
Pathological stage	Stage III–IV vs. stage I–II	2.16	1.02–4.56	0.044	2.16	1.01–4.58	0.046
Main marker							
Primary tumour stromal CCL18	High vs. low	2.09	1.06–4.09	0.032	2.29	1.11–4.75	0.025
Secondary marker							
Primary tumour stromal IgG4	High vs. low	—	—	—	0.78	0.40–1.55	0.485

CCL18 – C-C motif chemokine ligand 18
Reference categories were age < 65 years, male sex, histologic grade 1–2, pathological stage I–II, low primary tumour stromal CCL18 expression, and low primary tumour stromal IgG4 expression.

Table II. Clinicopathological characteristics of the study cohort ($N = 76$)

Characteristic	*n* (%)
Age [years]	
< 65	28 (36.8)
≥ 65	48 (63.2)
Sex	
Female	24 (31.6)
Male	52 (68.4)
Tumour localisation	
Proximal	33 (43.4)
Distal	38 (50.0)
Diffuse	5 (6.6)
Lymphovascular invasion	
Present	54 (71.1)
Absent	22 (28.9)
Perineural invasion	
Present	53 (69.7)
Absent	23 (30.3)
Histologic grade	
Grade 1	12 (15.8)
Grade 2	25 (32.9)
Grade 3	39 (51.3)
Lauren type	
Intestinal	39 (51.3)
Diffuse	24 (31.6)
Mixed	13 (17.1)
Primary tumour desmoplasia	
Present	72 (94.7)
Absent	4 (5.3)
Desmoplasia in lymph node metastasis	
Present	45 (59.2)
Absent	31 (40.8)
Intestinal metaplasia	
Present	28 (36.8)
Absent	48 (63.2)
Pathological T category	
pT1	2 (2.6)
pT2	3 (3.9)
pT3	40 (52.6)
pT4	31 (40.8)
Pathological N category	
pN1	21 (27.6)
pN2	24 (31.6)
pN3	31 (40.8)
Pathological M category	
pM0	60 (78.9)
pM1	16 (21.1)
Pathological stage	
Stage I	1 (1.3)
Stage II	12 (15.8)
Stage III	47 (61.8)
Stage IV	16 (21.1)

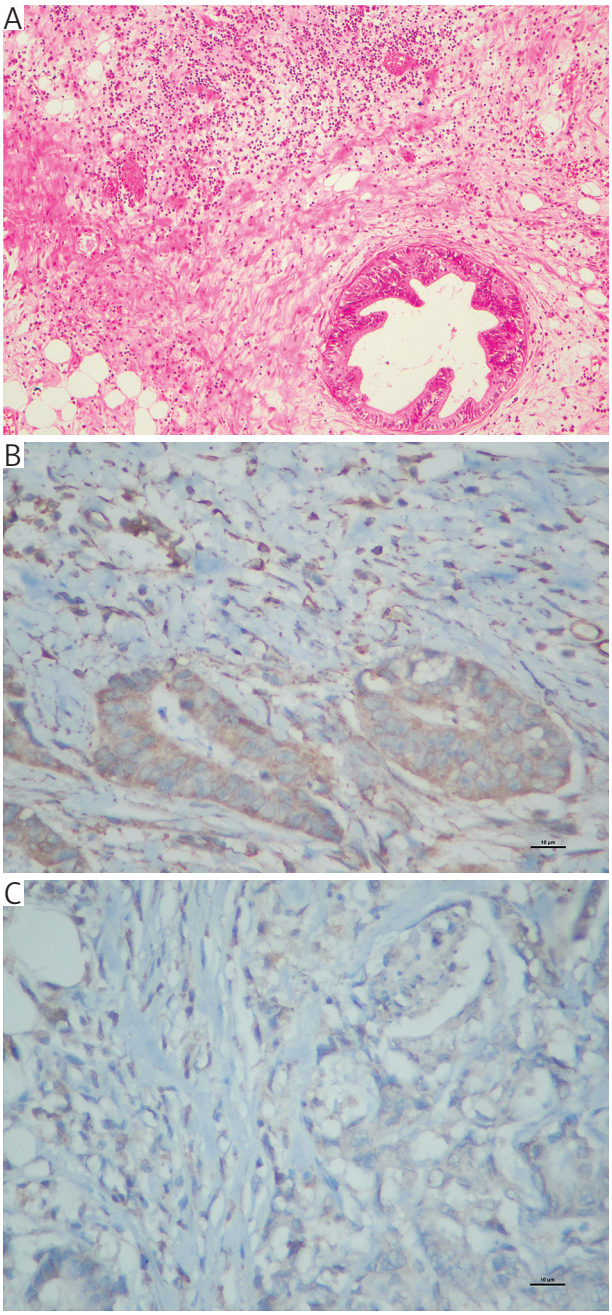

Figure 1. Histopathological appearance and stromal C-C motif chemokine ligand 18 (CCL18) expression at the primary tumor invasion front. **A**) Representative hematoxylin and eosin-stained section showing gastric adenocarcinoma at the tumor invasion front within desmoplastic and inflammation-rich stroma (original magnification 100×). **B**) High stromal CCL18 expression, with strong cytoplasmic immunoreactivity in approximately 60% of stromal cells (original magnification 400×). **C**) Low stromal CCL18 expression, with weak immunoreactivity in approximately 5% of stromal cells and focal epithelial staining (original magnification 400×)

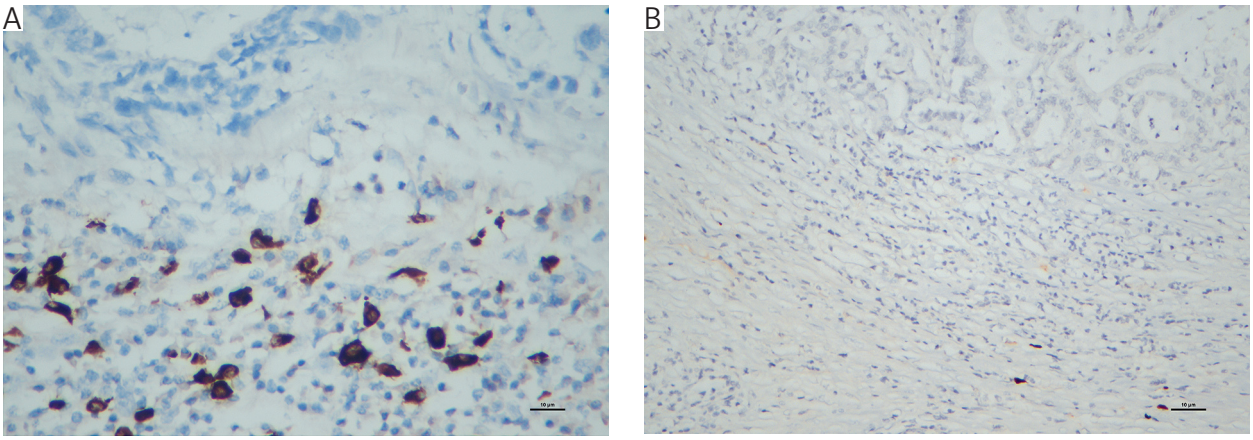

Figure 2. Representative stromal IgG4 expression in the primary tumor. **A**) High stromal IgG4 expression, with ≥ 10 IgG4-positive plasma cells per high-power field in the tumor stroma (original magnification 400×). **B**) Low stromal IgG4 expression, with < 10 IgG4-positive plasma cells per high-power field in the tumor stroma (original magnification 400×)

Table III. Associations of stromal IgG4 and C-C motif chemokine ligand 18 expression in the primary tumour with clinicopathological parameters

Parameters	Primary tumour stromal IgG4 ≥ 10		*p*-value*	Primary tumour stromal CCL18		*p*-value*
	High, *n* (%)	Low, *n* (%)		High, *n* (%)	Low, *n* (%)	
Age						
< 65	11 (55.0)	17 (30.4)	0.050	19 (38.8)	9 (33.3)	0.638
≥ 65	9 (45.0)	39 (69.6)		30 (61.2)	18 (66.7)	
Sex						
Female	3 (15.0)	21 (37.5)	0.092	14 (28.6)	10 (37.0)	0.447
Male	17 (85.0)	35 (62.5)		35 (71.4)	17 (63.0)	
Tumour localisation						
Proximal	10 (50.0)	23 (41.1)	0.358	24 (49.0)	9 (33.3)	0.233
Distal	10 (50.0)	28 (50.0)		21 (42.9)	17 (63.0)	
Diffuse	0 (0.0)	5 (8.9)		4 (8.2)	1 (3.7)	
Lymphovascular invasion						
Present	12 (60.0)	42 (75.0)	0.204	36 (73.5)	18 (66.7)	0.531
Absent	8 (40.0)	14 (25.0)		13 (26.5)	9 (33.3)	
Perineural invasion						
Present	12 (60.0)	41 (73.2)	0.269	37 (75.5)	16 (59.3)	0.140
Absent	8 (40.0)	15 (26.8)		12 (24.5)	11 (40.7)	
Histologic grade						
Grade 1	4 (20.0)	8 (14.3)	0.232	6 (12.2)	6 (22.2)	0.019
Grade 2	9 (45.0)	16 (28.6)		12 (24.5)	13 (48.1)	
Grade 3	7 (35.0)	32 (57.1)		31 (63.3)	8 (29.6)	
Lauren type						
Intestinal	13 (65.0)	26 (46.4)	0.331	21 (42.9)	18 (66.7)	0.073
Diffuse	4 (20.0)	20 (35.7)		19 (38.8)	4 (14.8)	
Mixed	3 (15.0)	10 (17.9)		9 (18.4)	5 (18.5)	
Pathological stage						
Stage II	6 (30.0)	6 (10.7)	0.069	7 (14.3)	5 (18.5)	0.839
Stage III	11 (55.0)	36 (64.3)		31 (63.3)	16 (59.3)	
Stage IV	2 (10.0)	14 (25.0)		11 (22.4)	5 (18.5)	

Table III. Cont.

Parameters	Primary tumour stromal IgG4 ≥ 10		*p*-value*	Primary tumour stromal CCL18		*p*-value*
	High, *n* (%)	Low, *n* (%)		High, *n* (%)	Low, *n* (%)	
Pathological T category						
pT1	2 (10.0)	0 (0.0)	0.034	1 (2.0)	1 (3.7)	0.379
pT2	2 (10.0)	1 (1.8)		1 (2.0)	2 (7.4)	
pT3	9 (45.0)	31 (55.4)		24 (49.0)	16 (59.3)	
pT4	7 (35.0)	24 (42.9)		23 (46.9)	8 (29.6)	
Pathological N category						
pN1	8 (40.0)	13 (23.2)	0.024	12 (24.5)	9 (33.3)	0.576
pN2	9 (45.0)	15 (26.8)		15 (30.6)	9 (33.3)	
pN3	3 (15.0)	28 (50.0)		22 (44.9)	9 (33.3)	
Pathological M category						
pM0	18 (90.0)	42 (75.0)	0.211	38 (77.6)	22 (81.5)	0.688
pM1	2 (10.0)	14 (25.0)		11 (22.4)	5 (18.5)	

CCL18 – C-C motif chemokine ligand 18
** P-values were obtained using Pearson χ^2, likelihood ratio, or exact tests, as appropriate.*

Table IV. Concordance of stromal *IgG4* and C-C motif chemokine ligand 18 expression between the primary tumour and matched lymph node metastasis

Panel A. Stromal *IgG4*

Primary tumour status	Lymph node metastasis, high	Lymph node metastasis, low	Total
Primary tumour, high	13	7	20
Primary tumour, low	11	45	56
Total	24	52	76

Concordance, *n*/*N* (%)	58/76 (76.3)
Discordance, *n*/*N* (%)	18/76 (23.7)
Cohen's κ (*p*-value)	0.426 ($p < 0.001$)
McNemar (*p*-value)	0.481

Panel B. Stromal C-C motif chemokine ligand 18

Primary tumour status	Lymph node metastasis, high	Lymph node metastasis, low	Total
Primary tumour, high	30	19	49
Primary tumour, low	13	14	27
Total	43	33	76

Concordance, *n*/*N* (%)	44/76 (57.9)
Discordance, *n*/*N* (%)	32/76 (42.1)
Cohen's κ (*p*-value)	0.125 ($p = 0.271$)
McNemar (*p*-value)	0.377

Panel A presents stromal IgG4 concordance and Panel B presents stromal C-C motif chemokine ligand 18 concordance between paired primary tumour and lymph node metastasis specimens.

High IgG4 expression was defined as ≥ 10 positive plasma cells per high-power field. High C-C motif chemokine ligand 18 (CCL18) expression was defined using study-specific median-based cutoffs (primary tumour stromal CCL18 ≥ 15%; lymph node metastasis stromal CCL18 ≥ 10%).

In Model 1 high primary tumour stromal *CCL18* expression remained independently associated with shorter overall survival (adjusted HR = 2.09, 95% CI: 1.06–4.09, $p = 0.032$). Age ≥ 65 years, grade 3 histology, and stage III–IV disease were also significant. After adding primary tumour stromal *IgG4* expression in model 2, *CCL18* remained significant (adjusted HR = 2.29, 95% CI: 1.11–4.75, $p = 0.025$) whereas *IgG4* was not (adjusted HR = 0.78, 95% CI: 0.40–1.55, $p = 0.485$). Age and pathological stage remained significant, while histologic grade did not (Figure 3, Table IB).

In exploratory analyses, primary tumour *IgG4-CCL18* groups were associated with histologic grade and Lauren type ($p = 0.033$ and $p = 0.029$) whereas lymph node groups were associated with sex and pN category ($p = 0.025$ and $p = 0.049$) (Table V).

Discussion

Principal findings

In this retrospective immunohistochemical study, stromal *CCL18* expression at the TIF of the primary tumour was associated with overall survival in patients with node-positive gastric adenocarcinoma. Cases with high primary tumour stromal *CCL18* expression had shorter median overall survival, and this association persisted in the multivariable Cox model adjusted for age, sex, histologic grade, and pathological stage. Adding primary tumour stromal *IgG4* expression to the model did not alter this association. In contrast, primary tumour stromal *IgG4* expression showed no independent association with overall survival (Table I).

The secondary findings of the study showed that stromal immune markers were not preserved to the same extent between the primary tumour and matched

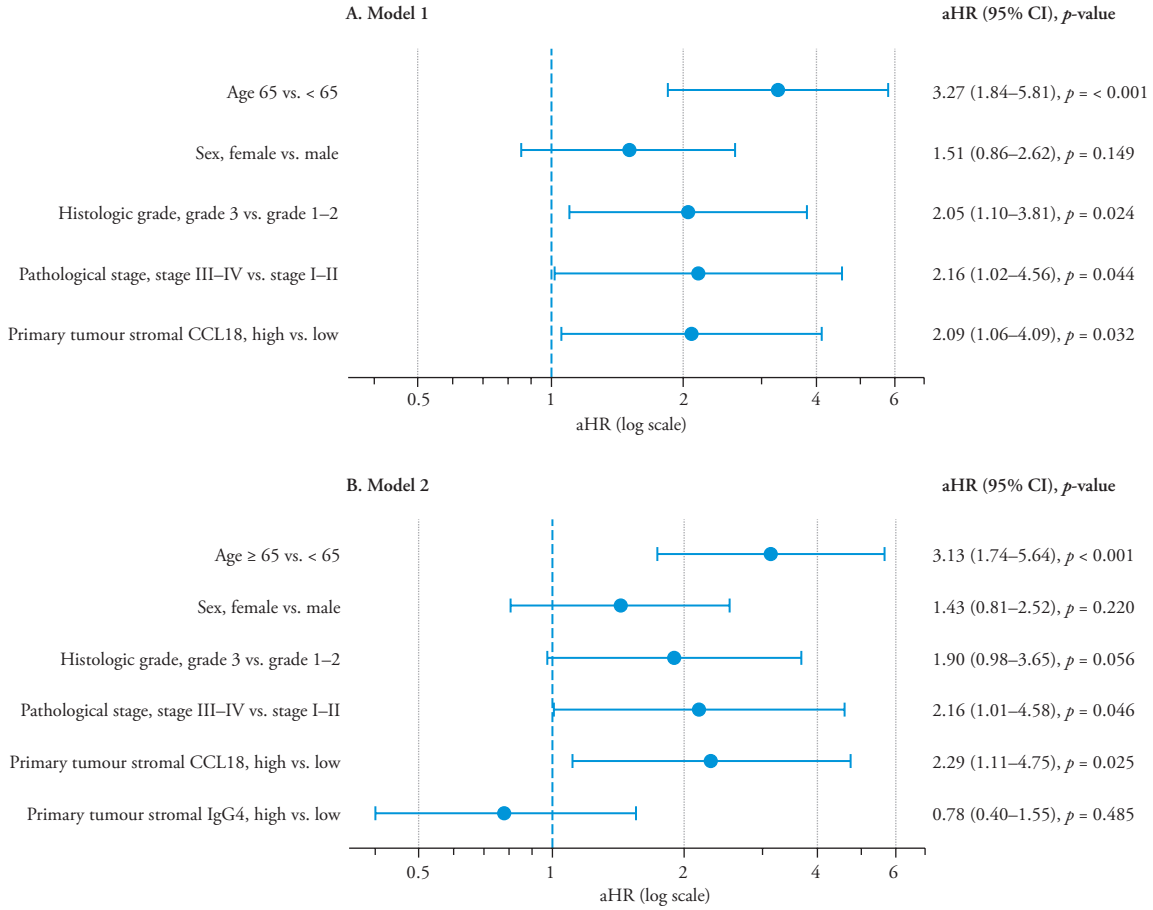

Figure 3. Multivariable Cox regression analyses for overall survival

aHR – adjusted hazard ratio, CCL18 – C-C motif chemokine ligand 18
Reference categories: age < 65 years, male sex, histologic grade 1–2, pathological stage I–II, low primary tumour stromal CCL18 expression, and low primary tumour stromal IgG4 expression.

Table V. Exploratory associations of *IgG4-CCL18* combination groups with clinicopathological characteristics in the primary tumour and matched lymph node metastasis

Panel A. Primary tumour

Characteristic	High IgG4 + high CCL18, *n*	High IgG4 + low CCL18, *n*	Low IgG4 + high CCL18, *n*	Low IgG4 + Low CCL18, *n*	*p*-value
Age					
< 65	6	4	11	7	0.858
≥ 65	9	5	17	16	
Sex					
Female	4	5	5	9	0.131
Male	11	4	23	14	
Tumour localisation					
Proximal	7	3	11	11	0.814
Distal	7	6	14	11	
Diffuse	1	0	3	1	
Lymphovascular invasion					
Present	9	5	21	18	0.451
Absent	6	4	7	5	
Perineural invasion					
Present	11	6	20	16	0.986
Absent	4	3	8	7	

Panel A. Cont.

Characteristic	High IgG4 + high CCL18, *N*	High IgG4 + low CCL18, *N*	Low IgG4 + high CCL18, *N*	Low IgG4 + Low CCL18, *N*	*P*-value
Histologic grade					
1	6	2	3	1	0.033
2	6	4	8	7	
3	3	3	17	15	
Lauren type					
Intestinal	11	6	9	13	0.029
Diffuse	1	1	15	6	
Mixed	3	2	4	4	
Pathological stage					
II	4	1	4	2	0.169
III	6	8	17	16	
IV	5	0	6	5	
Pathological T category					
pT1	0	0	1	1	0.451
pT2	2	0	0	1	
pT3	8	5	17	9	
pT4	5	4	10	12	
Pathological N category					
pN1	6	0	9	5	0.124
pN2	4	6	7	7	
pN3	5	3	12	11	
Pathological M category					
pM0	10	9	22	18	0.140
pM1	5	0	6	5	

CCL18 – C-C motif chemokine ligand 18
P-values were calculated using exact tests, as appropriate.

Panel B. Matched lymph node metastasis

Characteristic	High IgG4 + high CCL18, *N*	High IgG4 + low CCL18, *N*	Low IgG4 + high CCL18, *N*	Low IgG4 + low CCL18, *N*	*P*-value
Age					
< 65	8	3	3	14	0.258
≥ 65	7	2	9	30	
Sex					
Female	2	1	8	13	0.025
Male	13	4	4	31	
Tumour localisation					
Proximal	9	1	6	17	0.384
Distal	6	4	5	23	
Diffuse	0	0	1	4	
Lymphovascular invasion					
Present	9	3	11	31	0.237
Absent	6	2	1	13	
Perineural invasion					
Present	10	2	9	32	0.512
Absent	5	3	3	12	

Panel B. Cont.

Characteristic	High IgG4 + high CCL18, *N*	High IgG4 + low CCL18, *N*	Low IgG4 + high CCL18, *N*	Low IgG4 + low CCL18, *N*	*P*-value
Histologic grade					
1	2	2	1	7	0.349
2	7	2	2	14	
3	6	1	9	23	
Lauren type					
Intestinal	9	4	5	21	0.614
Diffuse	3	1	5	15	
Mixed	3	0	2	8	
Pathological stage					
II	4	2	0	6	0.100
III	10	1	9	27	
IV	1	1	3	11	
Pathological T category					
pT1	1	1	0	0	0.142
pT2	1	1	0	1	
pT3	7	2	4	27	
pT4	6	1	8	16	
Pathological N category					
pN1	5	3	1	12	0.049
pN2	8	1	3	12	
pN3	2	1	8	20	
Pathological M category					
pM0	14	4	9	33	0.408
pM1	1	1	3	11	

CCL18 – C-C motif chemokine ligand 18
P-values were calculated using exact tests, as appropriate.

lymph node metastasis. Concordance between the primary tumour and lymph node metastasis was more pronounced for *IgG4* expression, whereas concordance was more limited for *CCL18* expression. This finding suggests that *CCL18* may emerge in different stromal contexts in the primary tumour and metastatic lymph node microenvironments (Table IV).

Overall, these findings support that primary tumour stromal *CCL18* expression may represent a survival-associated microenvironmental marker in node-positive gastric adenocarcinoma. However, in this study, *IgG4* should be considered not as a main prognostic marker but as a secondary marker complementing the *CCL18*-related stromal immune context (Table V).

Primary tumour stromal C-C motif chemokine ligand 18 in the context of the gastric cancer microenvironment

The primary TIF is a dynamic microenvironmental area in which tumour cells directly interact with stromal and immune cells. Therefore, stromal biomarkers evaluated in this region may reflect not only tumour burden but also tumour stroma interactions. Interest in the prognostic relevance of the invasion front in gastric cancer has increased in recent years. In particular, the SARIFA approach has shown that stromal response features at the invasion front may be associated with clinical outcomes in gastric and oesophagogastric cancers. Although these studies did not directly evaluate *CCL18*, they support the broader concept that histological and stromal features at the invasion front may carry prognostic information in gastric and oesophagogastric adenocarcinoma [3, 15].

In this study, *CCL18* was evaluated not as the expression in tumour cells but as the percentage of positively stained cells in the primary tumour stroma. This distinction is important because *CCL18* has been described as a chemokine mainly associated with M2-like TAMs and other immune stromal cells, and TAMs are increasingly recognised as central regulators of invasion, immune suppression, angiogenesis, and metastatic progression in gastric cancer. In different tumour models, *CCL18* has been shown to be associated with immunosuppression, invasion, migration, epithelial-mesenchymal transition, and metastatic behaviour in the tumour microenvironment. Therefore, the asso-

ciation between high primary tumour stromal *CCL18* expression and shorter overall survival is consistent with the view that stromal immune organisation, particularly macrophage polarisation, may be linked to the clinical course in gastric cancer [16–19].

The literature on *CCL18* in gastric cancer is not entirely consistent. In the expression profiling study by Leung *et al.* [6], *CCL18* level was reported as an independent prognostic factor for overall survival and disease-free survival. However, the direction, measurement approach, and biological interpretation of that study do not fully overlap with more recent studies. More recent data suggest that the *CCL18*/*CCR8* axis may be associated with a more adverse tumour biology in gastric cancer. Yi *et al.* [7] reported that *CCL18* expression was increased in gastric cancer tissue compared with adjacent normal tissue and was associated with tumour recurrence, whereas CCR8 expression was associated with poor overall survival. At the experimental level, *CCL18* has been shown to increase invasion and migration in gastric cancer cells and to be associated with increased MMP-3 and Slug, decreased E-cadherin, and ERK1/2/NF-κB activation [17].

Methodological differences may partly explain the inconsistent findings across *CCL18* studies. Antibody selection and interpretation strategy can influence immunohistochemical classification [20]. Specimen type and sampled site can affect biomarker concordance [21]. Histomorphological correlation is important when interpreting immunophenotypic findings in heterogeneous tumours [22]. Previous studies evaluated *CCL18* in different tissue compartments and applied different cut-off values. In the present study, *CCL18* was scored specifically in stromal cells at the primary TIF and dichotomised using a study-specific median cut-off.

Compartmental differences between the primary tumour and lymph node metastasis

The concordance analysis between the primary tumour and matched lymph node metastasis showed that stromal immune markers were not preserved to the same extent in the metastatic compartment. A more pronounced concordance was observed between the primary tumour and lymph node metastasis for *IgG4* expression, whereas concordance was more limited for *CCL18* expression. This finding suggests that *CCL18* may emerge in different stromal contexts at the primary TIF and in the metastatic lymph node microenvironment (Table IV). Spatial profiling studies of patient-matched primary gastric cancers and locoregional metastases have similarly shown that metastatic compartments may display distinct biological features rather than simply reproducing the primary tumour architecture [10].

This interpretation is consistent with studies reporting that primary and metastatic lesions in gastric cancer may differ in terms of the immune microenvironment. Son *et al.* [23] compared CD4+ and CD8+ T-cell density, PD-L1 expression, mismatch repair status, and immune-related gene expression in matched primary and metastatic gastric cancer samples and showed that metastatic lesions have distinct tumour immune microenvironment features compared with primary tumours. Single cell analyses of gastric cancer have also demonstrated heterogeneity in tumour-cell states and immune microenvironmental composition between primary tumours and metastatic lymph nodes, further supporting compartment-specific interpretation of stromal markers [24]. Similarly, in a systematic meta-analysis including different tumour types, PD-L1, PD-1, PD-L2, and TIL levels were reported to show marked discordance between primary lesions and matched metastases, whereas tumour mutational burden and microsatellite instability status were more stable [25]. These data support that metastatic sites are not merely passive extensions of the primary tumour but may harbour reshaped microenvironments in terms of immune and stromal features.

In our study, stromal *CCL18* expression in lymph node metastasis showed no significant association with overall survival. In contrast, primary tumour stromal *CCL18* expression was associated with overall survival in both univariable analysis and multivariable Cox models. This distinction suggests that the survival association related to *CCL18* became more evident at the primary TIF than in metastatic lymph nodes in this cohort (Table I).

Stromal IgG4 and exploratory IgG4-CCL18 combination analyses

IgG4 is a complementary marker that may reflect humoral immune responses and immunoregulatory processes in the tumour microenvironment. *IgG4* antibodies are characterised by low complement activation and weak effector functions; therefore, in some tumour contexts, they may represent a response that limits antitumour immunity. Karagiannis *et al.* [8] showed that *IgG4* can suppress IgG1-mediated tumouricidal functions in melanoma and that the *IgG4* response may be associated with tumour-induced Th2-skewed inflammation [8]. In gastric cancer, Miyatani *et al.* [9] associated a high number of *IgG4*-positive cells with tumour progression and poorer prognosis.

However, in this study, primary tumour stromal *IgG4* expression showed no independent association with overall survival. In the univariable analysis, primary tumour stromal *IgG4* did not reach statistical significance, and adding *IgG4* to the multivariable model did not alter the association between primary tumour stromal *CCL18* and overall survival (Table I). This difference may be related to the inclusion of only node-positive cases in our study, the limited sample size, the assessment of *IgG4* based on stromal hot spot cell counts, and the lack of evaluation of the IgG4/IgG ratio.

The significance of *IgG4* in cancer may vary depending on tumour type and microenvironmental context. In some studies, IgG4-positive cells or the IgG4/IgG ratio have been associated with adverse clinical outcomes, whereas in other tumours *IgG4* expression may show different or more complex prognostic patterns. For example, in intrahepatic cholangiocarcinoma, *IgG4* expression and the IgG4/IgG ratio at the invasion front have been associated with poor survival, whereas in tongue squamous cell carcinoma the prognostic significance of *IgG4* may differ [26, 27]. Therefore, it is not appropriate to generalise *IgG4* as a uniformly favourable or unfavourable prognostic marker on its own.

IgG4 and C-C motif chemokine ligand 18 combination analyses were performed to explore additional patterns of the stromal immune microenvironment. In the primary tumour, combination groups were associated with histologic grade and Lauren type, whereas in lymph node metastasis they were associated with sex and pN category (Table V). However, because of the small subgroup sizes, these findings should be considered exploratory

Limitations

A strength of this study is the joint evaluation of primary tumour and matched lymph node metastasis samples in a well-defined cohort of node-positive gastric adenocarcinoma. This enabled assessment not only of the distribution of stromal *CCL18* and *IgG4* expression in the primary tumour but also their counterparts in metastatic lymph nodes. In addition, *CCL18* and *IgG4* assessments were restricted to the stromal compartment, and survival analysis was conducted in line with the predefined primary hypothesis focusing on primary tumour stromal *CCL18* expression. Adjustment for key clinicopathological variables such as age, sex, histologic grade, and pathological stage in the multivariable Cox model also strengthens the interpretation of the findings.

However, this study has several limitations. The retrospective single-centre design is subject to selection bias and incomplete records. The sample size is small, and the cohort consists only of resection cases with lymph node metastasis; therefore, the results cannot be directly generalised to patients with node-negative disease, early-stage disease, unresectable tumours, or those managed with different treatment protocols. The C-C motif chemokine ligand 18 thresholds are based on study-specific median values and should not be interpreted as validated clinical cut-offs. Evaluation based on a single hotspot area may not fully capture intratumoural heterogeneity. In addition, the phenotype of *CCL18*-positive stromal cells was not validated with additional immune markers, no independent validation cohort was used, and *IgG4-CCL18* combination analyses were based on small subgroups. For these reasons, the findings should be considered supportive for further studies rather than confirmatory. Furthermore, the lack of inclusion of treatment details, recurrence data, disease-specific survival, and molecular subtypes constitutes additional limitations in terms of residual confounding and outcome specificity.

Conclusions

In conclusion, this study suggests that high stromal *CCL18* expression at the TIF of the primary tumour is associated with shorter overall survival in node-positive gastric adenocarcinoma. This association persisted after adjustment for age, sex, histologic grade, pathological stage, and stromal *IgG4* expression. In contrast, stromal *IgG4* showed no independent association with overall survival in this cohort and should be considered a complementary stromal immune marker rather than a primary survival determinant.

These findings support further evaluation of primary tumour stromal *CCL18* as a candidate tumour microenvironment marker associated with survival in gastric adenocarcinoma. However, because the *CCL18* thresholds are based on study-specific median values, the lack of an independent validation cohort, and the single-centre retrospective design, the current results should not be interpreted as evidence for a biomarker ready for clinical application. Larger independent and preferably multicentre cohorts with standardised immunohistochemical scoring, digital pathology, or multiplex immune marker panels are required for validation.

Disclosures

1. Institutional review board statement: This retrospective observational study was approved by the Kahramanmaraş Sütçü İmam University Medical Research Ethics Committee (decision no. 2022/02-04, dated 11 January 2022). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and internationally accepted standards for research involving human participants. Patient data and archival tissue materials were evaluated retrospectively, and confidentiality of patient information was maintained throughout the study.
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