

Infiltration of diametrically polarized macrophages predicts overall survival of patients with gastric cancer after surgical resection

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Abstract

Background Tumor-associated macrophages (TAMs), the most predominant tumor-infiltrating immune cells, are emerging prognostic factors and therapeutic targets for personalized therapy against malignant neoplasms. We aimed to evaluate the prognostic significance of diametrically polarized TAMs in gastric cancer and generate a predictive nomogram to refine a risk stratification system. **Methods** We evaluated polarized functional status of infiltrated TAMs by immunohistochemical staining of CD68, CD11c, and CD206 in 180 consecutive gastric cancer patients from Zhongshan Hospital, Shanghai, China. Prognostic values were assessed in these patients. We created a predictive nomogram by integrating polarized TAMs with the TNM staging system for overall survival of gastric cancer patients.

Results CD68⁺ TAMs display polarized programs comprising CD11c⁺ proinflammatory macrophages (M1) and CD206⁺ immunosuppressive macrophages (M2) that configure versatile infiltration files in gastric cancer. CD11c⁺ TAMs negatively correlated with lymph node metastasis ($p = 0.012$), whereas CD206⁺ TAMs correlated with the Lauren classification ($p = 0.031$). No prognostic difference was observed for overall survival for CD68 density (high vs low, $p = 0.1031$), whereas high versus low CD11c density ($p < 0.0001$) and low vs high CD206 density ($p = 0.0105$) indicate better overall survival. Multivariate Cox regression analysis identified CD11c and CD206 as independent prognostic factors ($p < 0.001$ and $p = 0.030$, respectively), which could be integrated with the TNM staging system to generate a predictive nomogram for patient outcomes.

Conclusion Infiltration of polarized TAMs, a novel identified independent prognostic factor, could be combined with the TNM stage to refine a risk stratification system and better stratify patients with different prognosis. Tipping TAMs to an antitumoral phenotype might be a promising therapeutic target for postoperative treatment.

Keywords Gastric cancer · Tumor-associated macrophages · Prognosis · Nomogram · Overall survival

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Introduction

Although the incidence of gastric cancer has declined for decades, it remains one of the commonest malignant cancers worldwide, and gastric cancer is the second-leading cause of cancer-associated death in both sexes worldwide [1]. Traditionally, the prediction of gastric cancer outcomes is based on tumor-cell-based risk stratification systems,

such as the TNM staging system, composed of tumor invasion depth, lymph node metastasis, and distant metastasis. However, these clinicopathological factors cannot provide complete prognostic information because they do not incorporate tumor-microenvironment information. Some patients with advanced-stage cancer can remain stable for years, whereas some early-stage patients progress rapidly [2]. Therefore, reliable biomarkers or stratification systems that can be used for more accurate prediction are urgently needed.

Compelling evidence has emerged in recent years that the tumor microenvironment plays a critical role in tumor progression [3]. Macrophages are one of the major components of the tumor microenvironment, are recruited by chemokines such as macrophage colony stimulating factor (M-CSF) and chemokine C–C motif ligand 2, and are produced mainly by tumor cells [4]. Macrophages are versatile cells that can express different functional programs in response to microenvironmental signals [5]. Bacterial stimuli, interferon- γ , and granulocyte-macrophage colony stimulating factor promote a proinflammatory M1-polarized phenotype, whereas M-CSF, interleukin-4, and interleukin-13 favor the generation of folate receptor β positive, interleukin-10-producing, immunosuppressive, M2-polarized macrophages [6]. Macrophages that infiltrate tumor tissues, also termed tumor-associated macrophages (TAMs), can be driven by tumor-derived and T-cell-derived cytokines to acquire such a polarized phenotype, and have a key role in subversion of adaptive immunity and in inflammatory circuits that promote tumor growth and progression [7]. Fully polarized M1 and M2 macrophages are the extremes of a continuum of functional states; in contrast to this binary M1/M2 definition, there are several other distinct populations that share features of both types [8]. Functions of these subpopulations in tumor progression need careful elucidation.

There have recently been extensive studies on the correlation between intratumoral macrophages and prognosis, but the results were heterogeneous because they did not discriminate the M1 and M2 macrophage subsets [9–14]. A handful of studies proved that the alternative activated M2 macrophages are the main infiltration files of TAMs in various tumors [15, 16]. The M2-polarized macrophages promote tumor progression and metastasis by activating circuits that regulate tumor growth, adaptive immunity, stroma formation, and angiogenesis, and can be used as prognostic indicators [7, 17, 18]. But the classically activated M1 cells also participate in the crucial process of tumor progression, and mainly provide an antitumor benefit by activating production of toxic intermediates and reactive oxygen intermediates. Characterization of the polarized-TAM-based immune status could be more useful for understanding the tumor-derived signals that guide the

polarization of macrophages, and could thus provide a molecular stratification strategy for predicting a more precise prognosis or even predicting the response of patients to specific therapies. According to this knowledge, we hypothesized that polarized-TAM-based immune status may correlate with the progression and prognosis of gastric cancer. Incorporating the immune status into a tumor-cell-centered stratification system may provide a more comprehensive understanding of tumor biology and more precise prognostic significance.

Prognostic nomograms are tools designed for prediction by combining all proven prognostic markers and quantify risk as precisely as possible instead of producing risk groups [19]. In this study, we investigated the prognostic significance of polarized TAMs in gastric cancer and studied its relation with clinical outcomes. On the basis of infiltration of polarized TAMs and the tumor-cell-centered TNM stratification system, we generated a powerful nomogram with high prognostic value in patients with gastric cancer. The results suggested the prognostic significance of macrophage infiltration in gastric cancer, and tipping the balance of polarized TAMs toward an antitumoral phenotype might be a promising target of postoperative adjuvant therapy for patients with gastric cancer.

Methods

Patients and specimens

One hundred eighty consecutive gastric cancer patients who received standard gastrectomy with lymph node resection from May 2002 to April 2006 were enrolled in our research with informed consent approved by the Clinical Research Ethics Committee of Zhongshan Hospital of Fudan University. None of these patients received any preoperative anticancer treatment. Baseline clinicopathological features of these patients including age, gender, tumor location, tumor size, tumor differentiation, Lauren classification, and TNM staging were collected retrospectively. Tumor stage and tumor differentiation grade were reclassified according to the seventh American Joint Committee on Cancer TNM classification by two independent gastroenterology pathologists. The clinicopathological features of the 180 gastric cancer patients are shown in Table 1. The median age of this cohort was 63 years (range 32–83 years), and 68.9 % of the cohort were male; 66.1 % had intestinal-type carcinoma, and the rest had diffuse-type carcinoma; 68.9 % of patients had lymph node metastasis, and six patients (3.3 %) had resectable distant metastases. Patients were observed until July 2013, with a median observation time of 59 months. Overall survival (OS) was defined as the interval between the dates of surgery and death or the last visit.

Table 1 Relations between CD68, CD11c, or CD206 expression and clinicopathological characteristics in patients with gastric cancer ($n = 180$)

Factors	Patients		CD68 expression			CD11c expression			CD206 expression		
	No.	%	Low ($n = 90$)	High ($n = 90$)	p	Low ($n = 90$)	High ($n = 90$)	p	Low ($n = 91$)	High ($n = 89$)	p
Mean age (years)			62.6	61.6	0.553	63.4	60.8	0.102	62.5	61.7	0.620
Gender					0.107			0.334			0.087
Female	56	31.1	23	33		25	31		23	33	
Male	124	68.9	67	57		65	59		68	56	
Localization					0.245			0.402			0.570
Proximal	30	16.7	15	15		12	18		16	14	
Middle	58	32.2	34	24		32	26		32	26	
Distal	92	51.1	41	51		46	46		43	49	
Tumor size ^a					0.881			0.655			0.762
<4 cm	93	51.7	47	46		48	45		46	47	
≥4 cm	87	48.3	43	44		42	45		45	42	
Differentiation					0.065			0.682			0.083
Good	6	3.3	5	1		2	4		5	1	
Moderate	67	37.2	38	29		33	34		38	29	
Poor	107	59.5	47	60		55	52		48	59	
Lauren classification					0.637			0.270			0.031
Intestinal type	119	66.1	61	58		56	63		67	52	
Diffuse type	61	33.9	29	32		34	27		24	37	
T classification					0.067			0.295			0.434
T1	32	17.8	18	14		13	19		17	15	
T2	15	8.3	9	6		5	10		8	7	
T3	11	6.1	9	2		6	5		8	3	
T4	122	67.8	54	68		66	56		58	64	
N classification					0.058			0.012			0.326
N0	56	31.1	35	21		24	32		33	23	
N1	43	23.9	18	25		15	28		21	22	
N2	28	15.6	16	12		19	9		15	13	
N3	53	29.4	21	32		32	21		22	31	
Distant metastasis					0.211			0.682			0.116
No	174	96.7	89	85		86	88		90	84	
Yes	6	3.3	1	5		4	2		1	5	
TNM stage					0.102			0.413			0.168
I	38	21.1	21	17		15	23		19	19	
II	35	19.5	22	13		17	18		22	13	
III	101	56.1	46	55		54	47		49	52	
IV	6	3.3	1	5		4	2		1	5	

T tumor depth, *N* lymph node, *TNM* tumor–node–metastasis

^a Split at median

Immunohistochemistry and evaluation

CD68 [20], CD11c [21–23], and the mannose receptor CD206 [24], which represented overall infiltrated, M1-polarized, and M2-polarized TAMs, respectively, were used to identify and quantify infiltration of polarized TAMs

in gastric cancer by immunohistochemical (IHC) staining. The tissue microarray and immunohistochemistry protocols were constructed as described previously [25, 26]. Primary antibodies were against CD68 (Dako, Glostrup, Denmark), CD11c (Abcam, Cambridge, MA, USA), and CD206 (Abcam, Cambridge, MA, USA). The density of positive

staining was measured with the use of a computerized image system composed of an Olympus CCD camera connected to a Nikon eclipse Ti-s microscope. The IHC staining sections were scanned at high magnification ($\times 200$) and captured by NIS-Elements F3.2 to identify the five independent microscopic fields with the densest infiltration of TAMs to ensure representativeness and homogeneity. Identical settings were used for each photograph. The density was counted by Image-Pro Plus version 6.0 (Media Cybernetics, Bethesda, MD, USA). The integrated optical density of all the positive staining in each photograph was measured, and its ratio to the total area of each photograph was calculated as the relative density. The results were expressed as the mean density for one computerized microscopic field. The evaluation of immunostaining was performed by two independent gastroenterology pathologists who were blinded to the patient outcomes and clinicopathological characteristics. Variations in the enumeration, within a range of 5 %, were reevaluated, and a consensus decision was made. For IHC density, the cutoff for the definition of high/low expression subgroups was the median value.

Statistical analysis

Analysis was performed with SPSS 19.0 (SPSS, Chicago, IL, USA) and R (<http://www.r-project.org/>). Pearson's χ^2 test or Fisher's exact test was used to compare qualitative variables, and quantitative variables were analyzed by the *t* test or Pearson's correlation test. Kaplan–Meier analysis was used to determine the survival. The log-rank test was used to compare patient survival between subgroups. The stepwise Cox regression model was used to perform multivariate analysis. Numbers at risk were calculated for the beginning of each time period. A nomogram was created with R using the “rms” package. A calibration plot was generated to examine the performance characteristics of the nomogram. The bootstrap-corrected concordance index and the Akaike information criterion were used to compare the prognostic accuracy. All statistical analyses were two-sided, and $p < 0.05$ was considered statistically significant. Results are reported according to Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines [27].

Results

IHC findings

The positive staining of CD68, CD11c, and CD206 seen in the cytoplasm and/or membrane of macrophages and confined to the accessory cells surrounding the tumor cells in a diffuse manner confirmed the specificity of these markers

Fig. 1 Versatile polarization status of intratumoral tumor-associated macrophages (TAMs) in gastric cancer patients. Consecutive sections were used for immunohistochemical study: **a** CD68 high, CD11c low, CD206 high; **b** CD68 high, CD11c high, CD206 low; **c** CD68 high, CD11c high, CD206 high; **d** CD68 low, CD11c low, CD206 low. The scatter plots for immunohistochemistry scores of CD68, CD11c and CD206 in different TNM stages are also shown (**e**). Positive macrophages were stained *brown* ($\times 200$ magnification)

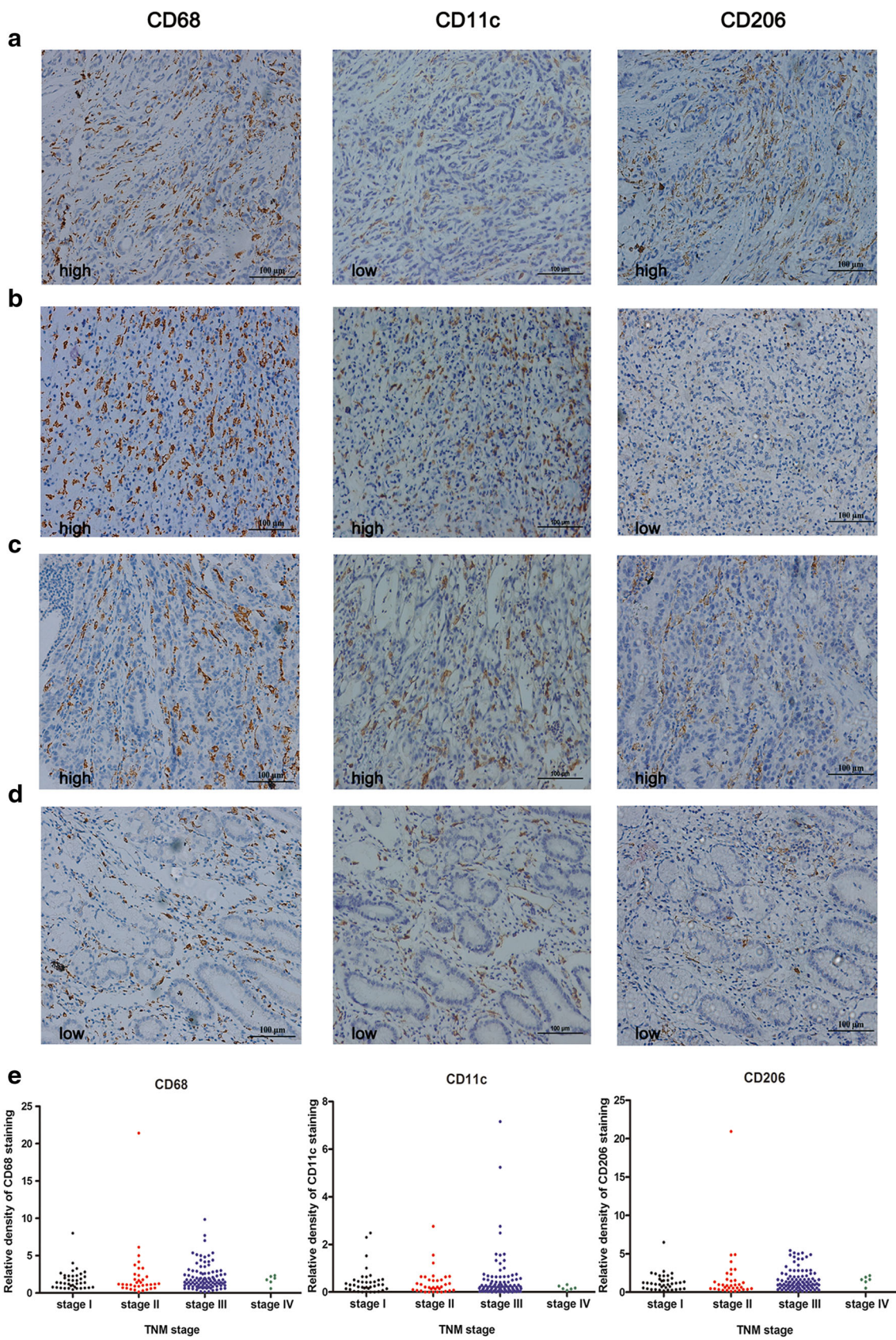
in indicating TAMs (Fig. 1, panels a–d). CD11c⁺ and CD206⁺ macrophages existing within the area where CD68⁺ macrophages were located confirmed that the CD11c⁺ and CD206⁺ cells are subsets of CD68⁺ macrophages (Fig. 1, panels a–d). We evaluated the density of positive staining in sequential slides per specimen, and found that the infiltration files of polarized TAMs varied largely. The relative density of IHC staining was 2.03 ± 2.11 (median, 1.45; range, 0.15–21.4), 0.44 ± 0.79 (median, 0.21; range, 0–7.15), and 1.58 ± 1.93 (median, 1.10; range, 0.12–20.9) for CD68, CD11c, and CD206, respectively. Some patients had a higher M2 infiltration (Fig. 1, panel a), some had a higher M1 infiltration (Fig. 1, panel b), and some had a high infiltration or a low infiltration of both M1 and M2 (Fig. 1, panels c and d, respectively). The IHC density of CD68, CD11c, and CD206, exhibits no significant difference among samples of different TNM stage (Fig. 1, panel e).

Correlations between TAM polarization status and clinicopathological features

Correlations between IHC variables and clinicopathological features are summarized in Table 1. CD11c staining was negatively correlated with the involvement of lymph nodes ($p = 0.012$). CD206 staining was correlated with the Lauren classification ($p = 0.031$). No association between IHC variables and other clinicopathological factors was observed.

Prognostic value of TAM polarization status

Using median values obtained from the IHC density as a cutoff, we performed Kaplan–Meier analysis. As shown in Fig. 2, low CD11c expression or high CD206 expression was associated with reduced survival (Fig. 2b, c; $p < 0.0001$ and $p = 0.0105$, respectively), whereas CD68 staining in tumor has no significant relation with OS (Fig. 2a; $p = 0.1031$). To investigate further the effect of polarized TAMs in stratifying patients with different TNM stages, we considered TNM stages I and II as early-stage tumor, and TNM stages III and IV as advanced-stage tumor. CD11c was positively correlated with OS and CD206 had no significant relation with OS in patients with early-stage tumor (Fig. 3a, b; $p = 0.0187$ and $p = 0.6093$,



respectively). Moreover, CD11c was positively correlated with OS and CD206 was negatively correlated with OS in patients with advanced-stage tumor (Fig. 3c, d; $p = 0.0007$ and $p = 0.0126$, respectively). These results suggested that polarized TAMs might provide additional prognostic information in different tumor stages.

Univariate and multivariate survival analysis

The clinical factors statistically significantly associated with reduced OS (Table 2) were tumor size ($p = 0.002$), T classification ($p < 0.001$), N classification ($p < 0.001$), distant metastasis ($p < 0.001$), TNM stage ($p < 0.001$), CD11c expression ($p < 0.001$), and CD206 expression ($p = 0.012$). Variables demonstrating a significant effect on OS were included in the multivariate analysis. Moreover, to avoid confounding factors, TNM stage was excluded in the multivariate analysis. T classification ($p = 0.010$), N classification ($p = 0.008$), distant metastasis ($p = 0.007$), CD11c expression ($p < 0.001$), and CD206 expression ($p = 0.030$) were identified as independent prognostic factors for OS after adjustment of covariates (Table 2).

Predictive nomogram model for overall survival of gastric cancer patients

To provide a quantitative method to better stratify patients with different prognosis, we constructed a nomogram that integrated the proven prognostic factor consisting of tumor invasion depth, lymph node involvement, distant metastasis, and polarized macrophage infiltration (Fig. 4a). In this nomogram, a higher total number of points indicates poorer prognosis. The calibration plot showed that the nomogram performs well compared with the ideal prediction model (Fig. 4b). The bootstrap-corrected concordance index for OS was 0.7426 in the nomogram model compared with 0.6872 in the TNM stage model, and the Akaike information criterion was reduced to 816.7575 compared with 832.4341 in the TNM model. A wide range of predicted survival could be identified in each TNM stage (Fig. 4c). Furthermore, the range of predicted survival was wider for earlier stages. All these results indicated that incorporation of polarized TAMs into the TNM staging system could further stratify patients with different prognosis.

Discussion

This study proved the hypothesis that the infiltration of diametrically polarized TAMs influences OS of patients with surgically resected gastric cancer. In this study, we

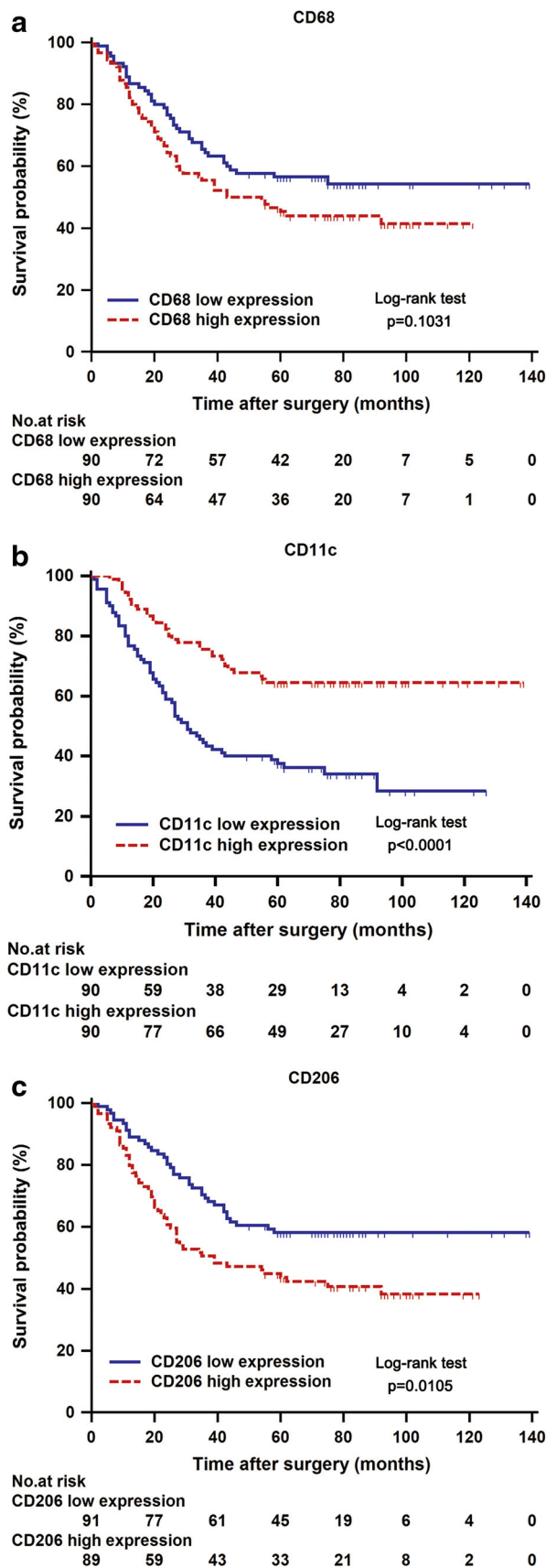


Fig. 2 Kaplan–Meier survival curves for gastric cancer patients with diametrically polarized TAMs: **a** CD68 (high vs low, $p = 0.1031$); **b** CD11c (high vs low, $p < 0.0001$); **c** CD206 (high vs low, $p = 0.0105$). The p values were calculated by the log-rank test

observed that TAMs indicated by CD68 positive staining irrespective of functional status have no significant correlation with OS, whereas single use of CD11c or CD206 has a significant positive and negative correlation with OS, respectively. Cox regression analysis confirmed that the polarized TAMs emerged as an independent prognostic factor. Incorporating the polarized TAM system into the established TNM system generates a nomogram that can

quantify the prognostic risk more precisely. However, the incorporation of macrophage infiltration into current prognostic models requires independent and more data to validate it.

There is growing appreciation that inflammation in the tumor microenvironment is the root of many malignancies [28]. Activated macrophages are central to tumor-associated inflammation in combination with other immune cells, which has been considered as proof that the host interacts with the growing tumor [29]. But the precise infiltration files of these immune cells and their functional status are still obscure. In this study, we focused on TAMs, and found that the diametrically polarized functional status of

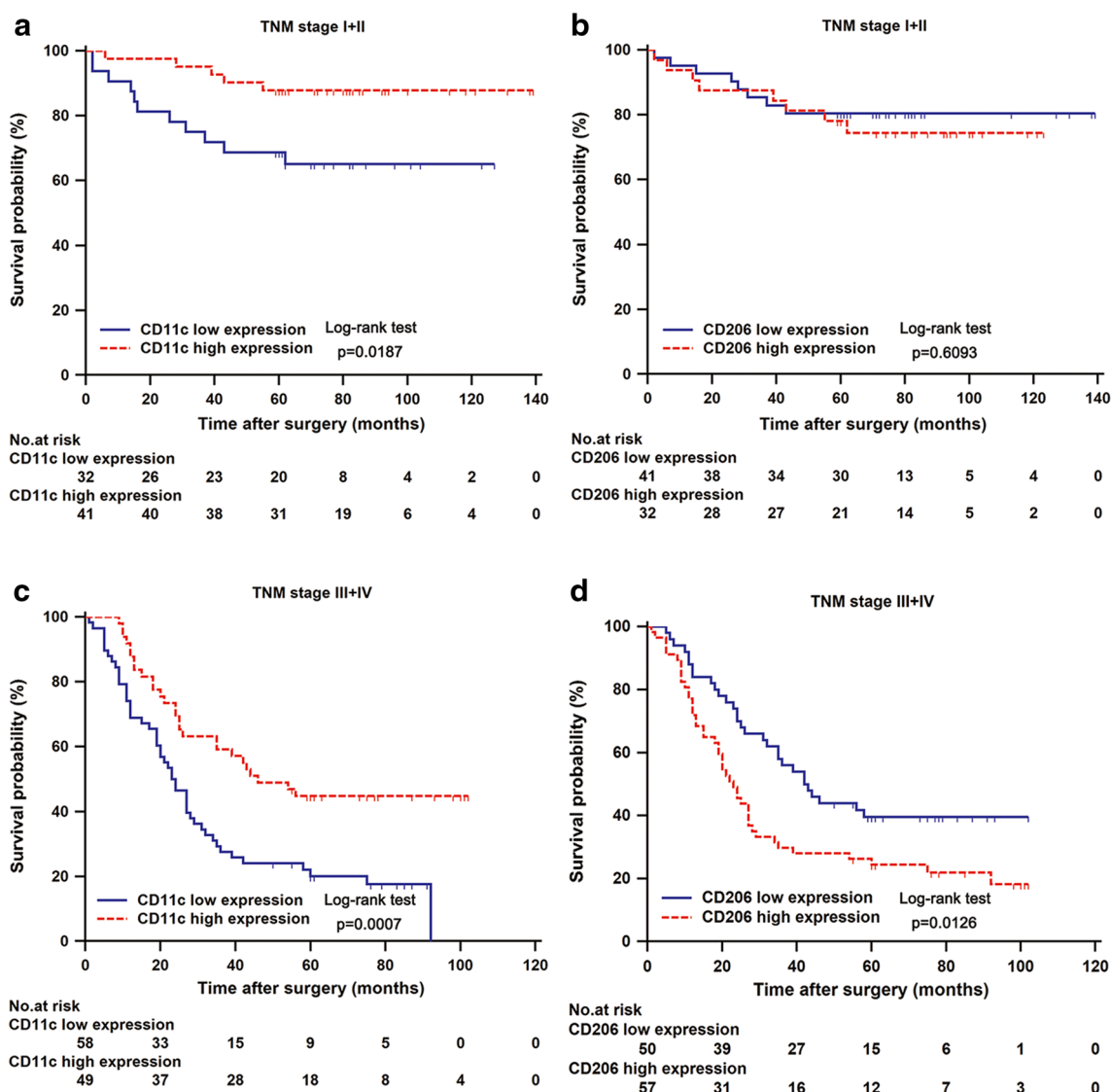


Fig. 3 Kaplan–Meier analysis for overall survival of patients with gastric cancer according to infiltration of polarized TAMs in different tumor stages: **a** patients with early-stage tumor (TNM stages I and II) according to CD11c expression (high vs low, $p = 0.0187$); **b** patients with early-stage tumor (TNM stages I and II) according to CD206

expression (high vs low, $p = 0.6093$); **c** patients with advanced-stage tumor (TNM stages III and IV) according to CD11c expression (high vs low, $p = 0.0007$); **d** patients with advanced-stage tumor (TNM stages III and IV) according to CD206 expression (high vs low, $p = 0.0126$). The p values were calculated by the log-rank test

Table 2 Univariate and multivariate Cox regression analyses for overall survival of patients with gastric cancer ($n = 180$)

Factors	Univariate		Multivariate	
	Hazard ratio (95 % CI)	<i>p</i>	Hazard ratio (95 % CI)	<i>p</i>
Age	1.014 (0.995–1.034)	0.154		
Gender		0.570		
Female	1 (reference)			
Male	0.878 (0.559–1.377)			
Localization		0.127		
Middle vs proximal	0.594 (0.320–1.101)	0.098		
Distal vs proximal	0.957 (0.558–1.642)	0.875		
Differentiation		0.308		
Moderate vs good	3.382 (0.463–24.731)	0.230		
Poor vs good	4.015 (0.555–29.035)	0.169		
Lauren classification		0.208		
Intestinal	1 (reference)			
Diffuse	1.314 (0.859–2.010)			
Tumor size ^a		0.002		0.096
<4 cm	1 (reference)		1 (reference)	
≥4 cm	1.969 (1.290–3.006)		1.454 (0.936–2.260)	
T classification		<0.001		0.010
T1	1 (reference)		1 (reference)	
T2 + T3 + T4	14.478 (3.559–58.889)		6.863 (1.585–29.712)	
N classification		<0.001		0.008
N0	1 (reference)		1 (reference)	
N1 + N2 + N3	5.368 (2.775–10.383)		2.570 (1.286–5.135)	
Distant metastasis		<0.001		0.007
No	1 (reference)		1 (reference)	
Yes	5.746 (2.424–13.617)		3.353 (1.401–8.023)	
TNM stage		<0.001		
II vs I	3.939 (1.268–12.236)	0.018		
III vs I	10.504 (3.820–28.885)	<0.001		
IV vs I	39.645 (10.806–145.452)	<0.001		
CD68 expression		0.107		
Low	1 (reference)			
High	1.407 (0.929–2.129)			
CD11c expression		<0.001		<0.001
Low	1 (reference)		1 (reference)	
High	0.399 (0.259–0.615)		0.422 (0.272–0.654)	
CD206 expression		0.012		0.030
Low	1 (reference)		1 (reference)	
High	1.709 (1.125–2.595)		1.610 (1.047–2.477)	

CI confidence interval, *T* tumor depth, *N* lymph node, *TNM* tumor–node metastasis

^a Split at median

macrophages configured a complex immune status in predicting prognosis of gastric cancer patients.

Macrophages derived from the CD34⁺ bone marrow progenitors develop into monocytes, and extravasate into various tissues, where they differentiate into resident macrophages [15]. The monocyte–macrophage lineage is diverse and plastic, attributed to a complex transcriptome [30]. To facilitate immune function and maintain tissue

homeostasis, macrophages adopt a wide range of activation states that can be classified within the classic activated M1 and alternative activated M2 models of macrophage polarization by interaction with the tumor-microenvironment milieu [31]. Accumulating evidence indicates that TAMs play an important role in tumor initiation, progression, and metastasis [32]. However, the polarized TAMs have diametrically different functions in these processes.

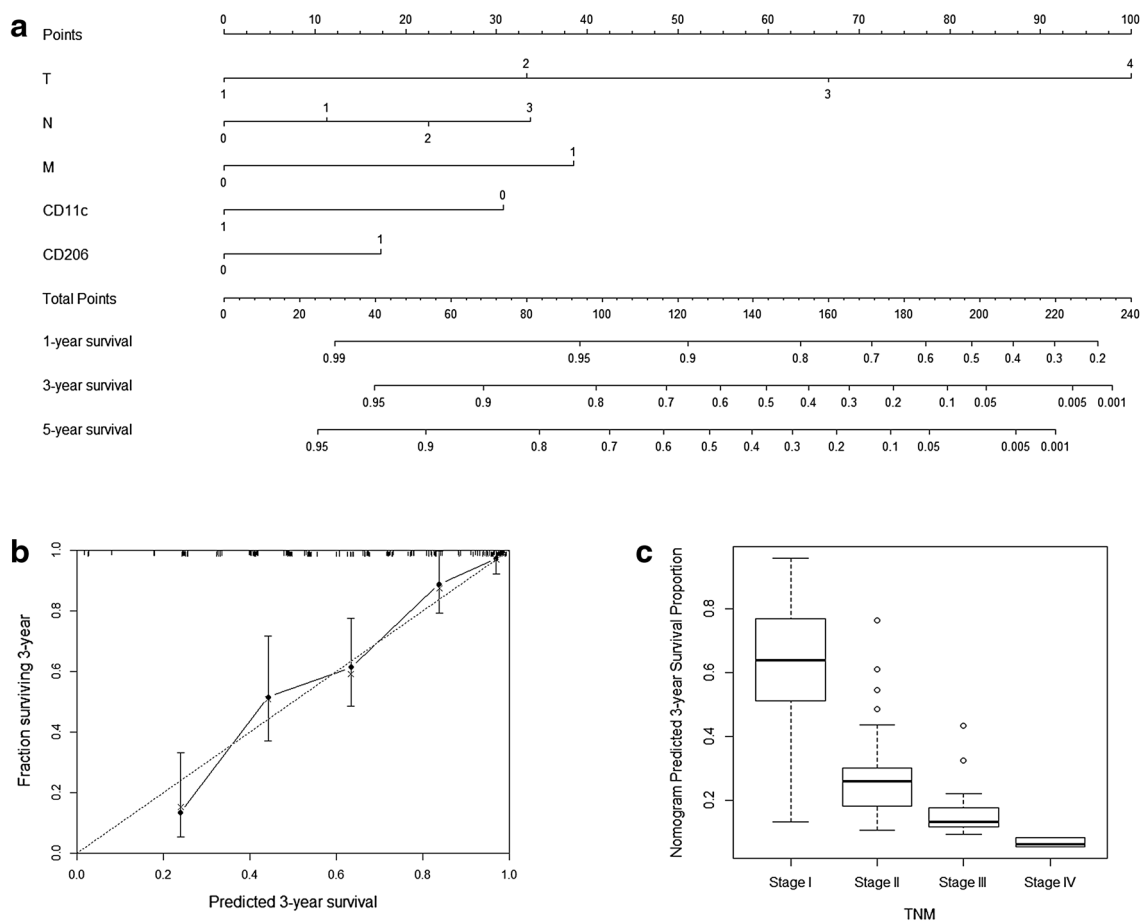


Fig. 4 Prognostic nomogram to predict overall survival in patients with gastric cancer. **a** Nomogram generation for predicting outcomes integrated with polarized TAMs (CD11c and CD206; 0 represents low expression, 1 represents high expression), tumor invasion depth (*T*; 1 represents T1, 2 represents T2, 3 represents T3, 4 represents T4), lymph node involvement (*N*; 0 represents N0, 1 represents N1, 2 represents N2, 3 represents N3), and distant metastasis (*M*;

0 represents absence, 1 represents presence). **b** Calibration curve for nomogram-predicted and observed 3-year outcomes. **c** The box plot represents the distribution of nomogram-predicted 3-year survival according to seventh American Joint Committee on Cancer TNM classification. A wide range of predicted survival can be identified in each TNM stage

The M1-skewed macrophages are able to release large amounts of proinflammatory cytokines, reactive nitrogen intermediates, and reactive oxygen intermediates, have higher expression of major histocompatibility complex class II and costimulatory molecules, and have efficient antigen presentation to favor their tumoricidal activity, whereas the M2-skewed macrophages produce molecules that promote tumor cell growth directly, enhance angiogenesis, tune inflammatory responses and adaptive immunity, and catalyze structural and substantial changes of the extracellular matrix compartment to facilitate tumor metastasis [33]. The functional status of TAMs in tumor is not immutable, and there is a dynamic switch. The local cytokine milieu in the tumor microenvironment is important to the phenotype switch of those infiltrated TAMs. With tumor progression, cytokines derived from tumor cells and their surrounding microenvironment, such as

M-CSF, educate macrophages to perform trophic functions and adopt an immunosuppressive M2-skewed macrophage phenotype [32, 34]. Moreover, variations on the typical M1/M2 phenotype have also been noted depending on the tumor type and organ involved. Once resident in tissues, macrophages acquire a distinct, tissue-specific phenotype in response to different microenvironment signals present within individual tumors [35–37]. Thus, the infiltration files and prognostic significance of TAMs in gastric cancer may differ from those of other tumors and need further investigation.

Studies attempting to elucidate the prognostic significance of TAMs have generated heterogeneous results, most likely owing to not discriminating the M1/M2 subsets [38]. Many studies have identified the important role of TAMs in gastric cancer. Wu et al. [39] found that TAMs could promote angiogenesis and lymphangiogenesis of gastric

cancer via elevated levels of vascular endothelial growth factor and vascular endothelial growth factor C. Okita et al. [40] proved the prognostic significance of antigen-presenting cells in patients with gastric cancer. Ishigami et al. [41] found a negative correlation between TAMs and prognosis in gastric cancer, and Ohno et al. [14, 41] revealed that the aggregation of TAMs within the tumor nest had a beneficial effect. These studies have proved the prognostic significance of TAMs in gastric cancer; however, the diametrically opposed functional status of TAMs was beyond these investigations. A recent study in renal cell carcinoma tried to prove the prognostic significance of M1/M2 phenotypes using combined analysis of CD11c and CD206 [42]. This may be better to help elucidate the prognostic significance of diametrically polarized infiltrated macrophages. In the present study, we investigated the prognostic significance of the M1/M2 phenotype in patients with gastric cancer and found that the M1/M2 phenotype may better stratify patients with different prognosis compared with overall infiltrated TAMs. Moreover, diametrically polarized infiltrated macrophages can be incorporated with TNM staging parameters to generate a predictive nomogram for OS with better accuracy. These results may help clinicians to better stratify patients who need more aggressive adjuvant therapy or who need more intensive follow-up.

In our effort to clarify the infiltration of polarized TAMs in gastric cancer progression, we found that patients with low CD11c expression developed lymph node metastasis more easily, whereas CD206 expression correlated with the Lauren classification (Table 1). These results partially revealed the effects of polarized TAMs mediated the tumor cell biological phenotype. Although there are many biological factors of innate tumor that determine tumor biological behaviors, more and more evidence has suggested that the tumor microenvironment also plays critical roles in determining tumor biology [32, 43]. In this study, we have proved that the infiltration of polarized TAMs (the most abundant immune cells in the microenvironment) in gastric cancer is correlated with lymph node metastasis and can be used to stratify patients with different prognosis, even patients with disease of the same stage. Moreover, adding the prognostic information derived from polarized TAMs to the TNM staging system could provide additional prognostic information that might be used in better distinguishing patient outcomes and making corresponding follow-ups.

Along with the predictive significance of myeloid immune cell infiltration, accumulating evidence indicates that the success of anticancer therapies, including conventional cytotoxic compounds, radiotherapy, and targeted agents, depends (at least partially) on the activation of anticancer immune responses [44]. The phenotype of

polarized M1/M2 macrophages can, to some extent, be reversed in vitro and in vivo [45, 46]. Thus, restoration of the complex immune system to the antitumor state by blocking the transition alone or in combination with chemotherapy may open a new avenue for treatment of patients with gastric cancer.

In conclusion, our study focused on the prognostic significance of polarized-TAM-based immune status in gastric cancer. Combining the polarized TAMs with the proven TNM stage can generate a nomogram that may become a useful prognostic tool in prognosis, follow-up, and intervention counseling for patients with gastric cancer.

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Conflict of interest The authors made no disclosures.

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