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ORIGINAL ARTICLE

Tumor infiltrative growth pattern correlates with the immune microenvironment and is an independent factor for lymph node metastasis and prognosis in stage T1 esophageal squamous cell carcinoma

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Abstract
In this retrospective study, we analyzed the association between the tumor infiltrative growth pattern (INF) and tumor immune environment and its predictive value for lymph node metastasis and overall survival (OS) in stage T1 esophageal squamous cell carcinoma (ESCC). In total, 593 patients with a diagnosis of stage T1 ESCC who underwent esophagectomy and regional lymphadenectomy between 2009 and 2018 were included. The INF type and elements of the tumor immune microenvironment, including tumor infiltrative lymphocytes (TILs) and tertiary lymphoid structures (TLSs), were microscopically evaluated within the tumor invasive margin with hematoxylin and eosin (HE)-stained slices. The infiltrative-type INF (INFc) was associated with low-grade TILs and the absence of TLSs, deep tumor invasion, poorly differentiated phenotype. Multivariate logistic regression identified INFc as one of the independent risk factors for lymph node metastasis. INFc and low-grade TILs were independent inferior predictive factors for OS. A novel histologic risk stratification model was classified as INFa/b and high-grade TILs, INFa/b and low-grade TILs, INFc and high-grade TILs, and INFc and low-grade TILs. The Kaplan-Meier curves showed that INFa/b and high-grade TILs were associated with the best prognosis, and INFc and low-grade TILs were associated with the worst prognosis, and there was significant difference between groups. In conclusion, INFc is an independent risk factor for lymph node metastasis and an independent inferior prognostic factor for stage T1 ESCC. Furthermore, INFc is associated with immunosuppression, and the combination of the INF and TILs is useful for the risk stratification of prognosis.

Keywords Esophageal squamous cell carcinoma · Tumor immune microenvironment · Infiltrative growth pattern · Tumor-infiltrating lymphocytes · Tertiary lymphoid structures · Overall survival

Introduction

Esophageal cancer is a kind of malignant tumor that seriously threatens human health, and its incidence and morbidity in China rank first in the world [1]. Esophageal squamous cell cancer (ESCC) accounts for 90% of all esophageal cancer cases in China.

In Japan, the infiltrative growth pattern (INF) has been routinely assessed as a pathologic characteristic of surgically resected specimens. Although the INF can be easily determined by hematoxylin and eosin (HE)-stained slices and used without specialized training, it has not gained widespread use in the clinic, and there are few reports on the predictive value of the INF regarding the outcome of ESCC [2–6].

Recently, a revolutionary concept shifted the focus from the tumor towards the microenvironment to determine the clinical disease course. Tumor-infiltrating lymphocytes (TILs), as a main component of the immune microenvironment, accumulate in many solid tumors, and their role in tumor progression remains controversial. Several reports have indicated that tumors with abundant TILs are associated

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with a better prognosis than tumors with scarce TILs in various cancers [7–10]. Tertiary lymphoid structures (TLSs), as the location where the generation of an efficient adaptive immune response against cancer occurs, reflect lymphoid neogenesis occurring in tumor peripheral tissues. Their presence is associated with a favorable prognosis in most solid malignant tumors [11].

In this study, we analyzed a series of 593 prospectively recruited stage T1 ESCC patients in China. The aim was to characterize the association between the INF type and the tumor immune microenvironment (TILs and TLSs), as well as to determine the predictive value of the INF type for lymph node metastasis and prognosis. In addition, we generated a novel grouping system to process the risk stratification of prognosis in T1 stage ESCC.

Materials and methods

Patient selection

This study enrolled 593 patients with a diagnosis of stage T1 ESCC who underwent esophagectomy and regional lymphadenectomy in our hospital between 2009 and 2018. The clinical and pathological data were obtained through a detailed retrospective review of the medical records. None of the patients had received any therapy before surgery. The study protocol was approved by the ethics committee of Shanxi Cancer Hospital, China (Reference number 201990).

Evaluation of histopathological factors

All available HE-stained tumor slides were reviewed by two pathologists separately who were blinded to clinical data at the time of the histologic evaluation. Whenever there was uncertainty, a third experienced pathologist was consulted. The invasive depth of stage T1 ESCC was subclassified into 6 groups: (m1) intraepithelial carcinoma that did not break the basement membrane, (m2) lesions between m1 and m3, (m3) carcinoma that was extremely close to or infiltrating the lamina muscularis mucosa, and (sm1, sm2, and sm3) submucosal carcinomas equally dividing the depth of the submucosal layer.

Evaluation of the INF, TILs, and TLSs

According to the Japanese classification of esophageal cancer [12], the INF indicates the growth and infiltrative pattern of tumors and can be classified into one of the following three types with regard to the predominant pattern observed at the tumor margin: INFa (expansive type), expansive growth of tumor nests with a well-demarcated border from surrounding tissue; INFb (intermediate type), intermediate growth pattern between INFa and INFc; and INFc (infiltrative type), infiltrative growth of tumor nests with an ill-defined border from surrounding tissue.

TILs were assessed according to the criteria previously published by the International TILs Working Group 2014 for breast cancer [13]. In brief, TILs were defined as the mean percentage of stroma in the invasive carcinoma infiltrated by lymphocytes and plasma cells and are reported as the area occupied by mononuclear inflammatory cells over the total stromal area, not the number of stromal cells. TILs were scored as a continuous variable. In this study, we categorized patients into low-grade and high-grade groups based on the median value.

TLSs are ectopic lymph node-like structures characterized by lymphoid aggregation with high endothelial venules. Similar to secondary lymphoid organs, T and B lymphocytes in TLSs are present in separate areas and may contain a germinal center. We classified TLSs as present or absent according to TLS formation in the area adjacent to the tumor.

All these indexes were evaluated within the tumor invasive margin, which was defined as a region centered on the border separating the host tissue from tumor nests, with an extent of 1 mm.

Statistical analysis

The start of the follow-up period was defined as the date of surgery, and the end of the follow-up period was defined as either the date of death or the date of last censorship, whichever occurred first.

Interrelationships between the INF, TILs, TLSs, and clinicopathological characteristics were analyzed using the chi-square test. Univariate logistic regression and multivariate logistic regression analysis using a backward conditional method were utilized to identify the risk factors for and independent factors of lymph node metastases, respectively. To test the reproducibility of the evaluation method, kappa statistics were calculated for both intra- and interobserver agreement.

Overall survival (OS) was examined using the Kaplan-Meier survival analysis, and univariate Cox proportional hazards regression was used to calculate the hazard ratios and 95% confidence intervals. Variables found to be statistically significant ($p < 0.05$) on the univariate analysis were entered into the multivariate analysis.

For the logistic and Cox regression analyses, a two-tier system for the INF was used along with the presence or absence of INFc to facilitate risk stratification. For all analyses, a two-sided p value of < 0.05 was considered statistically significant. Analyses were conducted using the STATA software program (version 14.0; Stata Corp., College Station, TX).

Results

INF: association with TILs, TLSs, and clinicopathologic features

Of 593 patients, the INFa type was present in 179 patients, the INFb type was present in 218 patients, and the INFc type was present in 196 patients. The relationship between the INF type and TILs, TLSs, and clinicopathologic features of patients with T1 ESCC is shown in Table 1. INFc was associated with low-grade TILs ($p < 0.001$) and the absence of TLSs ($p < 0.001$), deep tumor invasion ($p < 0.001$), poorly differentiated phenotype ($p < 0.001$), and a strong possibility of lymphovascular invasion ($p < 0.001$) and lymph node metastasis ($p < 0.001$). All these statistically significant parameters gradually increased or decreased along the order of INFa-INFb-INFc.

INF: an independent risk factor for lymph node metastases

The univariate logistic regression analysis showed that the following variables were significantly associated with lymph node metastases: male sex, tumor size ≥ 20 mm, deep invasion, poorly differentiated phenotype, lymphovascular invasion, INFc, low-grade TILs, and the absence of TLSs. Multivariate backward stepwise logistic regression identified tumor size ≥ 20 mm ($p = 0.022$), deep invasion ($p = 0.009$ and $p = 0.000$), lymphovascular invasion ($p = 0.001$), and INFc ($p = 0.045$) as independent risk factors for lymph node metastasis (Table 2).

INF and TILs: independent predictive factors for OS

The median follow-up period was 42 months (range, 1–112 months). During the follow-up, 61 patients died. The overall 1-, 3-, and 5-year survival rates were 97.0%, 92.1%, and 90.1%, respectively.

The univariate Cox analysis revealed that INFc, low-grade TILs, the absence of TLSs, tumor size ≥20 mm, deep invasion, poorly differentiated phenotype, lymphovascular invasion, and lymph node metastases were significantly associated with poor OS. Furthermore, INFc ($p = 0.015$) and low-grade TILs ($p = 0.002$) were also independent inferior predictive factors on the multivariate analysis (Table 3) (Fig. 1a–c).

INF and TILs: a novel histologic risk stratification model

Based on the independent prognostic factors INF and TILs, a novel histologic risk stratification model for OS was generated as follows: INFa/b and low-grade TILs ($n = 107$, 18%), INFa/b and high-grade TILs ($n = 287$, 48%), INFc and low-grade TILs ($n = 89$, 15%), and INFc and high-grade TILs ($n = 110$, 19%) (Fig. 2). The Kaplan-Meier curves showed that patients with INFa/b and high-grade TILs had the best prognosis, and patients with INFc and low-grade TILs had the worst prognosis. The other two groups demonstrated similar prognoses. The log-rank test showed that the difference between groups was significant ($p < 0.001$) (Fig. 1d).

Intra- and interobserver variation

To test the reproducibility of the evaluation method, two observers independently evaluated the INF, TILs, and TLSs on 40 randomly selected patients from this study. After 1 month, one observer performed another evaluation to assess the intraobserver variation. The kappa values were 0.73, 0.64, and 0.70 for the intraobserver assessment and 0.41, 0.50, and 0.55 for the interobserver assessment of the INF, TILs, and TLSs, respectively, which showed moderate to good agreement and validated the reproducibility of the evaluation method.

Discussion

The interdependent interaction between the tumor and its microenvironment is a crucial topic in cancer research, and this interaction orchestrates the fate of tumor progression. In this study, we focused on the tumor INF type and local immune microenvironment (TILs and TLSs) in stage T1 ESCC. In some studies, according to their location, TILs and TLSs are classified as intratumoral and stromal TILs and intratumoral and peritumoral TLSs. In this study, we chose the invasive margin to evaluate these parameters. Because the invasive margin is reported to constitute a critical interface between pro- and anti-tumor factors, tumor-immune cell interactions at the invasive margin are a biologic driver of tumor aggressiveness [14].

We demonstrated that INFc was more closely associated with the immunosuppressive microenvironment (low-grade TILs and the absence of TLSs) and tumor aggressiveness (deep invasion, poor differentiation) than INFa and INFb. Some reports have shown that INFc is significantly associated with a poorly differentiated phenotype and deep invasion in gastric and pharyngeal cancers [3, 6], compatible with our results. However, there is very little published literature describing the association between the INF type and the tumor immune microenvironment. A previous report indicated that some small tumor nests at the invasive front were inversely associated with CD8+ TILs [15]. These small tumor nests were described as cancer-initiating cells or tumor budding (a cluster of fewer than five cancer cells) in several tumor types [16, 17]. Ito et al. found that tumor budding was strongly correlated with INFc, and the concordance rate was approximately 80% [2]. In our opinion, the evaluation of the INF is based on the invasive form of tumor nests in whole tissue at low magnification, while tumor budding

Table 1 The relationship between INF and clinicopathologic features of patients with T1 ESCC

Variables	INFa $N = 179$ (30.19%)	INFb $N = 218$ (36.76%)	INFc $N = 196$ (33.05%)	p value
Patients characteristics				
Age(years)				0.624
< 65	123 (69)	143 (66)	137 (70)	
≥ 65	56 (31)	75 (34)	59 (30)	
Gender				0.05
Male	88 (49)	121 (56)	121 (62)	
Female	91 (51)	97 (44)	75 (38)	
Tumor characteristics				
Tumor size (mm)				0.696
< 20	68 (38)	77 (35)	77 (39)	
≥ 20	111 (62)	141 (65)	119 (61)	
Depth of tumor invasion				0.000
M1 + M2	128 (70)	56 (26)	6 (3)	
M3 + Sm1	44 (25)	99 (45)	93 (47)	
Sm2 + Sm3	7 (5)	63 (29)	97 (50)	
Differentiation				0.000
Well-moderately	174 (97)	198 (91)	141 (72)	
Poorly	5 (3)	20 (9)	55 (28)	
Lymphovascular invasion				0.000
Absence	177 (99)	207 (95)	174 (89)	
Presence	2 (1)	11 (5)	22 (11)	
Lymph node metastasis				0.000
Absent	175 (98)	192 (88)	154 (79)	
Present	4 (2)	26 (12)	42 (21)	
Immune microenvironment characteristics				
TILs				0.000
Low-grade	40 (22)	67 (31)	89 (45)	
High-grade	139 (78)	151 (69)	107 (55)	
TLSs				0.000
Absence	61 (34)	89 (41)	124 (63)	
Presence	118 (67)	129 (59)	72 (37)	

INFa, expansive type growth pattern; *INFb*, intermedia type growth pattern; *INFc*, infiltrative-type growth pattern; *TILs*, tumor-infiltrating lymphocytes; *TLSs*, tertiary lymphoid structures

is based on the cellular level at high magnification. In addition, "small tumor nests" and "tumor budding" were included in the concept of an infiltrative-type growth pattern (INFc), which refers to tumor nests at the tumor margin with an ill-defined border from surrounding tissue. van Wyk et al. described an inverse association between tumor budding and the intensity of peritumoral inflammation in colorectal cancer, which is in accordance with our results regarding the relationship between the INF and immune reaction [18]. Viktor et al. revealed that these small tumor nests frequently lost major histocompatibility complex-I expression when its expression was maintained in differentiated tumor cells in colorectal cancer; consequently, these small tumor nests may be able to reduce immunogenicity and evade the antitumoral host immune response and then undergo epithelial mesenchymal transition, invasion, and metastasis [19]. This explains why patients with the INFc type in our study always exhibited deep invasion and strong lymph node metastasis ability. We also found that the shallower the infiltration was, the richer the TILs and TLSs, and this result was also confirmed in a previous study on colorectal cancer [20]. At an early disease stage, an active immune reaction could be a major determinant for controlling tumor evolution, as with tumor progression, immune cells gradually decreased. Therefore, we focused on stage T1 ESCC to explore the immune microenvironment in our study. Further work is required to elucidate the molecular basis of the interaction between the INF and the

Table 2 Univariate and multivariate logistics analysis of risk factors for lymph node metastases

Variables	Univariate analysis			Multivariate analysis (backward)		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age (< 65/≥ 65)	0.99	0.59–1.69	0.985			
Gender (female/male)	1.82	1.08–3.07	0.025			
Size (< 20 mm/≥ 20 mm)	2.29	1.28–4.10	0.005	2.06	1.11–3.83	0.022
Depth	6.26	2.16–18.18	0.001	4.35	1.45–13.12	0.009
(m1+m2/m3+sm1/sm2+sm3)	14.65	5.11–41.95	0.000	7.96	2.60–24.38	0.000
Differentiation (well-moderately/poorly)	2.70	1.50–4.87	0.001			
Lymphovascular invasion (absence/presence)	5.75	2.77–11.91	0.000	3.65	1.69–7.86	0.001
INF (a+b/c)	3.34	2.01–5.53	0.000	1.77	1.01–3.09	0.045
TILs (low-grade/high-grade)	0.50	0.31–0.83	0.007			
TLSs (absence/presence)	0.50	0.30–0.83	0.008			

INFa+b, expansive and intermedia type growth pattern; *INFc*, infiltrative-type growth pattern; *TILs*, tumor-infiltrating lymphocytes; *TLSs*, tertiary lymphoid structures; *OR*, odds ratio; *CI*, confidence interval

immune microenvironment, which can help us design more efficient immunotherapeutic strategies.

According to the univariate logistic analysis, low-grade TILs and the absence of TLSs were associated with lymph node metastasis. We believe that a weak immune reaction reflects a defect of the host response to tumor challenge, leading to an increased likelihood of lymph node metastases. In the multivariate analysis, INFc was identified as an independent risk factor for lymph node metastasis, which was also validated in early pharyngeal cancers. The evaluation of the INF type needs to be performed in the tumor margin, which is present only in surgical specimens; it cannot be evaluated using biopsy specimens, and therefore cannot be used to select the preoperative strategy. However, in T1 ESCC, for which the treatment is complicated, the INF type can be applied in the histopathological evaluation of endoscopically resected specimens to assess the likelihood of metastasis and whether any additional treatment is necessary. This is another reason why we treated T1 ESCC as the subject in our study. Kanda et al. reported that the INFc type was associated with a high frequency of peritoneal recurrence, and the INFa or INFb type was associated with a high frequency of hepatic recurrence in gastric cancer [3]. INFc was also associated with recurrence in bladder cancer and colorectal cancer [4, 5]. Thus, we assumed that it may be helpful for the selection of more aggressive postoperative treatment.

The prognostic value of the INF and TILs has been demonstrated in several studies [2–5, 7–9]. In this study, we evaluated for the first time the combination of the INF and TILs as a histopathological prognostic factor in stage T1 ESCC. We

Table 3 Univariate and multivariate Cox analysis of overall survival in stage T1 ESCC

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (< 65/≥ 65)	1.16	0.68–1.97	0.596			
Gender (female/male)	1.47	0.87–2.48	0.148			
Size (< 20 mm/≥ 20 mm)	1.92	1.07–3.45	0.028	1.70	0.94–3.10	0.082
Depth	2.36	1.10–5.07	0.028	1.30	0.56–3.03	0.547
(m1+m2/m3+sm1/sm2+sm3)	4.10	1.93–8.68	0.000	1.36	0.54–3.44	0.515
Differentiation (well-moderately/poorly)	2.02	1.11–3.68	0.021	1.15	0.61–2.19	0.667
Lymphovascular invasion (absence/presence)	2.55	1.21–5.37	0.013	1.49	0.67–3.34	0.329
Lymph node metastasis (absence/presence)	2.90	1.59–5.29	0.001	1.78	0.92–3.42	0.086
INF (a+b/c)	3.07	1.84–5.11	0.000	2.11	1.16–3.84	0.015
TILs (low-grade/high-grade)	0.33	0.20–0.54	0.000	0.40	0.23–0.72	0.002
TLSs (absence/presence)	0.56	0.33–0.94	0.027	1.12	0.63–2.00	0.704

INFa+b, expansive and intermedia type growth pattern; *INFc*, infiltrative-type growth pattern; *TILs*, tumor-infiltrating lymphocytes; *TLSs*, tertiary lymphoid structures; *HR*, hazard ratio; *CI*, confidence interval

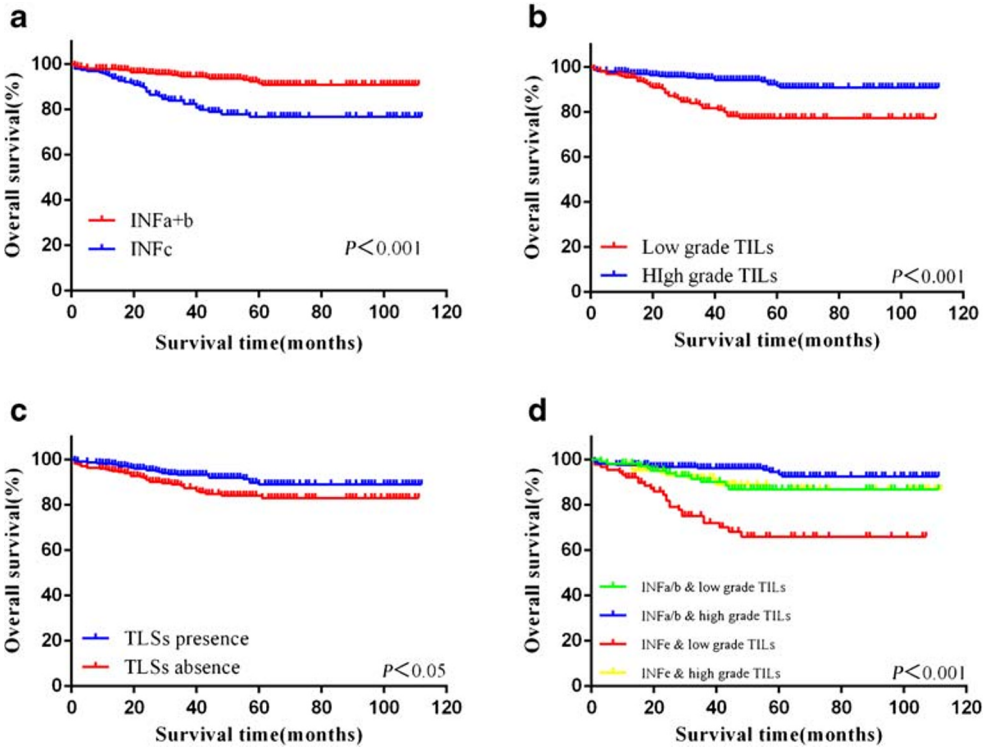

Fig. 1 Kaplan-Meier survival curve of overall survival of patients with stage T1 ESCC stratified by the following histologic parameters. **a** INF type. **b** TILs. **c** TLSs. **d** INF and TILs

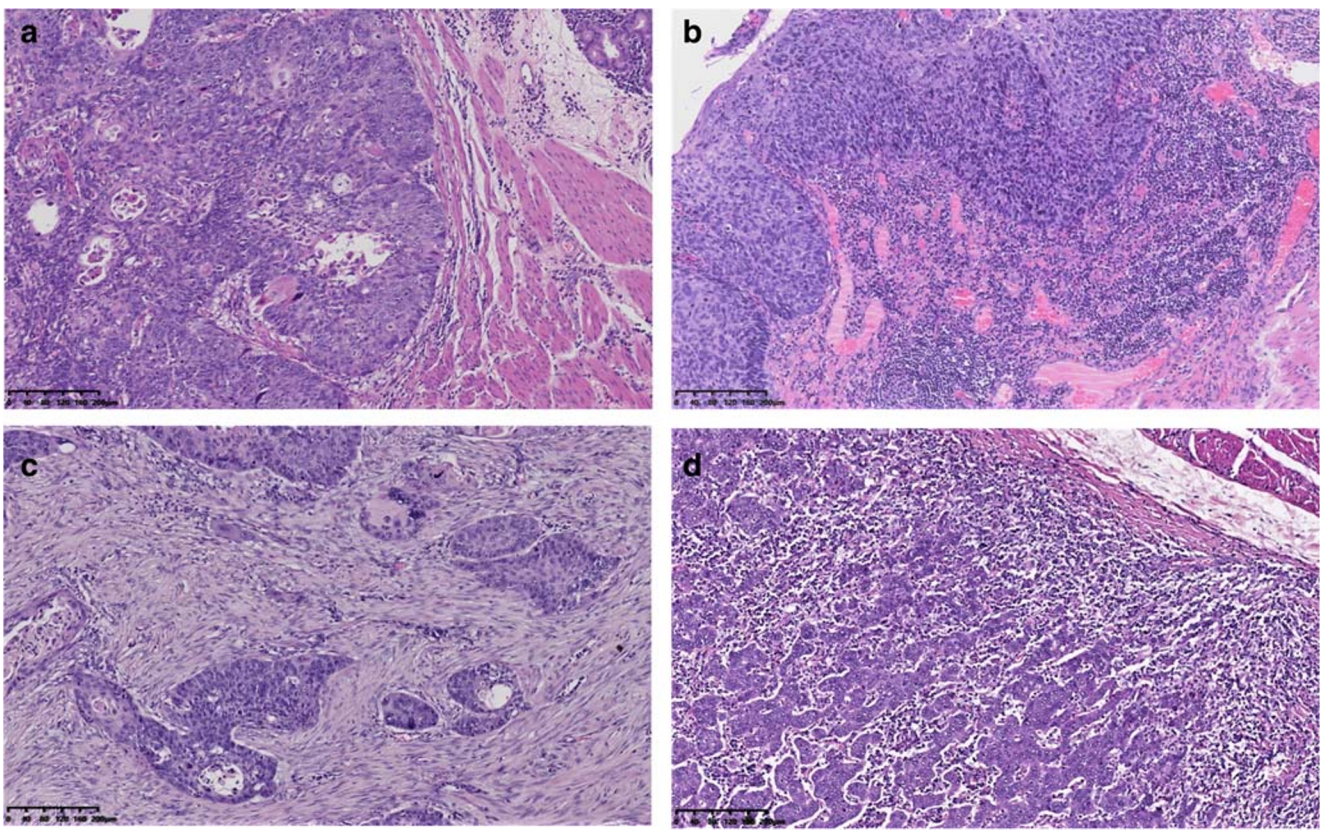

Fig.2 A novel histologic risk stratification model based on hematoxylin and eosin–stained slices. **a** Tumors with INFa/b and low-grade TILs. **b** Tumors with INFa/b and high-grade TILs. **c** Tumors with INFc and low-grade TILs. **d** Tumors with INFc and high-grade TILs

demonstrated that INFc patients with high-grade TILs had a better prognosis than patients with low-grade TILs; the same was also true for INFa or INFb patients. Using this novel risk stratification model, we can assign patients based on the findings of a microscopic evaluation with HE staining alone, making it a useful and practical system that can be applied in any pathology laboratory and that requires no special ancillary testing. This prognostic impact was independent of the tumor–node–metastasis (TNM) stage, so it may be able to supplement the current TNM staging system.

This study has several limitations. First, it is a retrospective observational study that enrolled stage T1 ESCC patients who underwent esophagectomy and regional lymphadenectomy. As endoscopic resections were increasingly performed in tumors confined to the mucosa, esophagectomy was only performed in patients with poorly differentiated, larger lesions, or suspected LN metastasis by examination. Thus, a selection bias may exist due to surgical indications and patients' decisions. Second, the number of events expected in OS was low, and the analysis may be under-powered. Therefore, more studies and a larger sample size will be necessary in future research. Third, other immune cells, such as neutrophilic and eosinophilic granulocytes and macrophages, etc., were not addressed in this study; however, they may be involved in immune microenvironment.

In conclusion, INFc is an independent risk factor for lymph node metastasis and an independent yet inferior prognostic factor of OS in T1 stage ESCC. Furthermore, it is associated with immunosuppressive low-grade TILs and the absence of TLSs, and the combination of the INF and TILs is useful for the risk stratification of prognosis in stage T1 ESCC.

Authors' contributions Jin Mulan, Chen Hong, and Wang Ying conceived and designed the study, and revised the manuscript. Zhang Yong acquired clinical data and revised the manuscript. Xu Enwei, Yang Xuanqin, and Zhao Yuanyuan evaluated histological findings. Zhao Yuanyuan integrated the data, performed the statistical analysis, and wrote the manuscript. All authors approved the final manuscript for submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted with the approval of the ethics committee of the Shanxi cancer hospital, China (Reference number 201990).

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J (2016) Cancer statistics in China, 2015. CA Cancer J Clin 66(2):115–132. https://doi.org/10.3322/caac.21338
2. Ito E, Ozawa S, Kijima H, Kazuno A, Nishi T, Chino O, Shimada H, Tanaka M, Inoue S, Inokuchi S, Makuuchi H (2012) New invasive patterns as a prognostic factor for superficial esophageal cancer. J Gastroenterol 47(12):1279–1289. https://doi.org/10.1007/s00535-012-0587-y
3. Kanda M, Mizuno A, Fujii T, Shimoyama Y, Yamada S, Tanaka C, Kobayashi D, Koike M, Iwata N, Niwa Y, Hayashi M, Takami H, Nakayama G, Sugimoto H, Fujiwara M, Kodera Y (2016) Tumor infiltrative pattern predicts sites of recurrence after curative gastrectomy for stages 2 and 3 gastric cancer. Ann Surg Oncol 23(6):1934–1940. https://doi.org/10.1245/s10434-016-5102-x
4. Morikawa T, Kuchiba A, Qian ZR, Mino-Kenudson M, Hornick JL, Yamauchi M, Imamura Y, Liao X, Nishihara R, Meyerhardt JA (2012) Prognostic significance and molecular associations of tumor growth pattern in colorectal cancer. Ann Surg Oncol 19(6):1944–1953. https://doi.org/10.1245/s10434-011-2174-5
5. López JI, Angulo JC (2009) Growth pattern in superficial urothelial bladder carcinomas. Histological review and clinical relevance. Int Urol Nephrol 41(4):847–854. https://doi.org/10.1007/s11255-009-9537-x
6. Ebisumoto K, Okami K, Ogura G, Sakai A, Sugimoto R, Saito K, Kaneda S, Hanakita T, Nakamura N, Iida M (2015) The predictive role of infiltrative growth pattern in early pharyngeal cancers. Acta Otolaryngol 135(11):1172–1177. https://doi.org/10.3109/00016489.2015.1072872
7. Sudo T, Nishida R, Kawahara A, Saisho K, Mimori K, Yamada A, Mizoguchi A, Kadoya K, Matono S, Mori N, Tanaka T, Akagi Y (2017) Clinical impact of tumor-infiltrating lymphocytes in esophageal squamous cell carcinoma. Ann Surg Oncol 24(12):3763–3770. https://doi.org/10.1245/s10434-017-5796-4
8. Brambilla E, Le Teuff G, Marguet S, Lantuejoul S, Dunant A, Graziano S, Pirker R, Douillard JY, Le Chevalier T, Filipits M, Rosell R, Kratzke R, Popper H, Soria JC, Shepherd FA, Seymour L, Tsao MS (2016) Prognostic effect of tumor lymphocytic infiltration in resectable non-small-cell lung cancer. J Clin Oncol 34(11):1223–1230. https://doi.org/10.1200/JCO.2015.63.0970
9. Lu J, Chen XM, Huang HR, Zhao FP, Wang F, Liu X, Li XP (2018) Detailed analysis of inflammatory cell infiltration and the prognostic impact on nasopharyngeal carcinoma. Head Neck 40(6):1245–1253. https://doi.org/10.1002/hed.25104
10. Vayrynen JP, Tuomisto A, Klintrup K, Makela J, Karttunen TJ, Makinen MJ (2013) Detailed analysis of inflammatory cell infiltration in colorectal cancer. Br J Cancer 109(7):1839–1847. https://doi.org/10.1038/bjc.2013.508
11. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH (2019) Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer 19(6):307–325. https://doi.org/10.1038/s41568-019-0144-6
12. Japan Esophageal S (2017) Japanese classification of esophageal cancer, 11th edition: part I. Esophagus 14(1):1–36. https://doi.org/10.1007/s10388-016-0551-7
13. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S, International TWG (2015) The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs working group 2014. Ann Oncol 26(2):259–271. https://doi.org/10.1093/annonc/mdu450
14. Zlobec I, Lugli A (2009) Invasive front of colorectal cancer: dynamic interface of pro-/anti-tumor factors. World J Gastroenterol 15(47):5898–5906. https://doi.org/10.3748/wjg.15.5898
15. Tallerico R, Todaro M, Di Franco S, Maccalli C, Garofalo C, Sottile R, Palmieri C, Tirinato L, Pangigadde PN, La Rocca R,

Mandelboim O, Stassi G, Di Fabrizio E, Parmiani G, Moretta A, Dieli F, Karre K, Carbone E (2013) Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. J Immunol 190(5): 2381–2390. https://doi.org/10.4049/jimmunol.1201542

16. Max N, Harbaum L, Pollheimer MJ, Lindtner RA, Kornprat P, Langner C (2016) Tumour budding with and without admixed inflammation: two different sides of the same coin? Br J Cancer 114(4):368–371. https://doi.org/10.1038/bjc.2015.454
17. Ulase D, Heckl S, Behrens HM, Kruger S, Rocken C (2020) Prognostic significance of tumour budding assessed in gastric carcinoma according to the criteria of the international tumour budding consensus conference. Histopathology 76(3):433–446. https://doi.org/10.1111/his.13997
18. van Wyk HC, Park JH, Edwards J, Horgan PG, McMillan DC, Going JJ (2016) The relationship between tumour budding, the tumour microenvironment and survival in patients with primary operable colorectal cancer. Br J Cancer 115(2):156–163. https://doi.org/10.1038/bjc.2016.173
19. Koelzer VH, Dawson H, Andersson E, Karamitopoulou E, Masucci GV, Lugli A, Zlobec I (2015) Active immunosurveillance in the tumor microenvironment of colorectal cancer is associated with low frequency tumor budding and improved outcome. Transl Res 166(2):207–217. https://doi.org/10.1016/j.trsl.2015.02.008
20. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, Bruneval P, Trajanoski Z, Fridman WH, Pages F, Galon J (2011) Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 29(6):610–618. https://doi.org/10.1200/JCO.2010.30.5425