Original Article

Prognostic impact of tumour-associated B cells and plasma cells in oesophageal and gastric adenocarcinoma

Richard Fristedt[1], David Borg[1], Charlotta Hedner[1], Jonna Berntsson[1], Björn Nodin[1], Jakob Eberhard[1], Patrick Micke[2], Karin Jirström[1]

[1]Department of Clinical Sciences, Division of Oncology and Pathology, Lund University, SE-221 85 Lund, Sweden; [2]Department of Immunology, Genetics and Pathology, Uppsala University, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden

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Correspondence to: Richard Fristedt. Department of Clinical Sciences Lund, Division of Oncology and Pathology, Lund University, SE-221 85 Lund, Sweden. Email: richard.fristedt@med.lu.se.

Background: While it is well established that the cell-mediated immune response plays an important role in cancer progression and spread, the role of the humoral immune response in this regard has been less studied. According to the existing literature, dense infiltration of B cells or plasma cells appears to correlate mainly with an improved prognosis in several types of cancer, but their prognostic impact in oesophageal and gastric cancer has not yet been described.

Methods: Immunohistochemistry was applied on tissue microarrays (TMA) to assess the stromal density of B cells (CD20+) and plasma cells [CD138+ or immunoglobulin kappa C (IGKC+)] in chemo-/radiotherapy-naive tumours from a consecutive cohort of 174 patients with resected oesophageal or gastric adenocarcinoma. Cox proportional hazard's modelling was applied to examine the impact of the investigated markers on overall survival (OS) and time to recurrence (TTR).

Results: In curatively treated patients with oesophageal adenocarcinoma, high expression of IGKC was an independent predictor of a prolonged OS [hazard ratio (HR) 0.10; 95% confidence interval (CI), 0.02–0.57], and TTR (HR 0.15; 95% CI, 0.03–0.71). In curatively treated patients with gastric adenocarcinoma, high expression of IGKC independently predicted a prolonged OS (HR 0.46; 95 % CI, 0.24–0.87) and TTR (HR 0.46; 95% CI, 0.21–0.98). Expression of CD20 was not prognostic, and CD138 expression was only prognostic in unadjusted analysis of TTR in gastric cancer.

Conclusions: These results demonstrate, for the first time, that abundant infiltration of IGKC+ plasma cells independently predicts a prolonged survival in both oesophageal and gastric cancer.

Keywords: Plasma cells; prognosis; gastric; oesophageal; adenocarcinoma

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Introduction

Oesophageal cancer is now the eighth most common type of cancer and sixth most common cause of cancer related deaths, with an estimated number of 400,000 deaths worldwide annually (1). In the westernized world there has been a steady increase in adenocarcinoma of the esophagus, now surpassing squamous cell carcinoma (1). Gastric cancer, while overall declining in the west, is still the fifth most prevalent cancer worldwide, and the third when it comes to cancer related deaths, with an estimated number of 723,000 deaths annually (1).

Tumour-infiltrating immune cells have been shown to influence the prognosis and response to treatment in

several types of cancer (2). Immune system evasion has been labelled an emerging hallmark of cancer (3), but cancer cells may also acquire help from the immune system to enable enhanced growth and metastasis (4). Hence, depending on the context, the many effector cells of the immune system may inhibit or promote tumour progression (5-7).

Hitherto, the role of the innate and cell-mediated immune response in cancer has been extensively studied. Tumour associated macrophages promote angiogenesis, epithelial-mesenchymal transition (EMT) and metastasis and have, consequently, been associated with poor prognosis in different types of cancer (8,9). Abundant T lymphocyte infiltration, in particular cytotoxic CD8+ and memory T cells, has been associated with a favourable clinical outcome in many tumour types (2,10,11). The improved understanding of cancer and immune system interactions is now paving the way for novel immunotherapy treatments, e.g., checkpoint inhibitors.

Thus far, less focus has been rewarded to the involvement of the humoral immune system in tumour development and progression. In solid cancers of the breast, cervix, colorectum and non-small cell lung cancer (NSCLC), tumour-infiltrating B cells (CD20+) have been associated with improved outcomes (12-15).

Ambiguous prognostic data exist for the plasma cell marker CD138 (syndecan-1), which can also be expressed in epithelial tumour cells and other stromal cells (e.g., fibroblasts). Tumour infiltrating CD138+ plasma cells have been associated with an improved prognosis in NSCLC and colorectal cancer (15,16), but linked to poor prognosis in breast cancer (17), and in epithelial ovarian cancer (EOC) (18). In gastric cancer, high stromal CD138 expression (unspecified cell type) has been demonstrated to have a negative impact on clinical outcome (19). In EOC, high CD138 expression in stromal fibroblasts has also been associated with an impaired prognosis (20,21).

Immunoglobulin kappa C (IGKC), exclusively expressed in plasma cells, has been associated with an improved prognosis in colorectal cancer, both at the protein expression and gene expression levels (15,22), NSCLC (16) and breast cancer (14), including being predictive for chemotherapy response in the latter (gene expression) (22). To the best of our knowledge, the prognostic significance of IGKC in oesophageal and gastric cancer has not yet been reported. The aim of this study was therefore to investigate the expression and prognostic impact of B cells (CD20+) and plasma cells (CD138+ or IGKC+) in tumours from a consecutive cohort of 174 patients with resected oesophageal and gastric adenocarcinoma.

Methods

Study design and participants

The cohort is a consecutive series of 174 patients with chemo-/radiotherapy-naive oesophageal and gastric adenocarcinoma subjected to surgical resection at the University Hospitals of Lund and Malmö between 1 January, 2006 and 31 December, 2010. The cohort has been described previously (23-26), and a new follow-up has been performed until 31 December, 2014, with additional re-examination of some of the clinicopathological data (27). Tumour stage was classified according to TNM 7. Residual tumour status was classified as: R0 = no residual tumour, R1 = microscopic residual tumour (within 1mm of the resection margin), or R2 = macroscopic residual tumour. Three patients included in the cohort had metastatic disease and were resected in order to palliate symptoms from the primary tumour. Only 7.5% of the patients received adjuvant treatment (chemoradiotherapy). Clinical data and information on recurrence and cause of death was obtained from medical charts.

Tissue microarray (TMA) construction

All cases were histopathologically re-evaluated on haematoxylin and eosin stained sections. TMAs were constructed using a semi-automated arraying device (TMArrayer, Pathology Devices, Westminster, MD, USA). Duplicate tissue cores (1 mm) were obtained from non-necrotic areas in the primary tumours, whereby each core, whenever possible, was obtained from two different donor blocks, as previously described (23,25,28).

Immunohistochemistry and staining evaluation

For immunohistochemical (IHC) analysis of CD20 and CD138, 4 µm TMA sections were pre-treated using ULTRA Cell Condition Solution 1, pH 8.5 (Ventana Medical Systems Inc., Tucson, AZ, USA), for heat induced epitope retrieval, and stained with the ready-to-use monoclonal antibodies CD20cy Clone L6 and CD138 clone MI15 in a Ventana BenchMark stainer (Ventana Medical Systems Inc.). The antibody-antigen complex was visualized with ultraView Universal DAB Detection kit (Ventana Medical Systems, Inc.).

For analysis of IGKC, the TMA slides were manually deparaffinised in xylene, rehydrated in graded alcohol and blocked for endogenous peroxidase in 0.3%

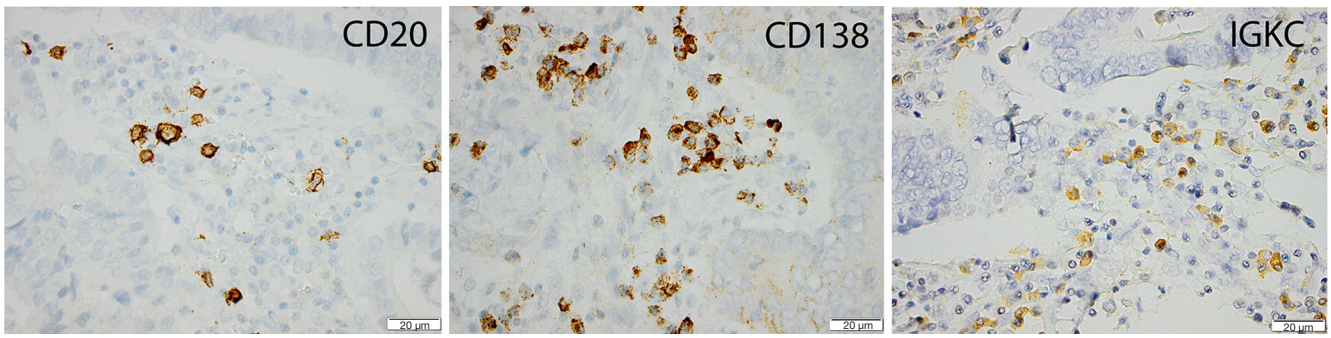

Figure 1 Sample immunohistochemical images of the investigated markers in a T3 N0 M0 oesophageal adenocarcinoma. Total immunoscores for CD20, CD138 and IGKC were 39, 60 and 31.5, respectively. IGKC, immunoglobulin kappa C.

hydrogen peroxide. For antigen retrieval, the slides were immersed in citrate buffer pH 6.7 and microwaved for 15 min. Automated IHC staining was performed using the Autostainer Link 48 (Dako; Glostrup, Copenhagen, Denmark) and a polyclonal rabbit anti-human kappa light chain antibody (Dako, A019; 1:40,000). The slides were incubated with the secondary antibody (EnVision™ FLEX, Rabbit/Mouse, K8000, Dako) for 30 min at RT and developed using diaminobenzidine (DAB). All TMA slides were counterstained with Mayer's haematoxylin (Sigma-Aldrich).

CD20, CD138 and IGKC were exclusively expressed in the cytoplasm and cell membrane of the lymphocytes. The staining was assessed by two independent observers, RF and KJ. Discrepant scores were discussed in order to reach consensus. The estimated fraction of stained cells was denoted as 0.0–1.0 (1=100%) and the staining intensity was denoted as 0= negative, 1= weak, 2= moderate and 3= strong intensity. A multiplier of the fraction (0.0–1.0) and intensity (0–3) was calculated for each core and a mean value of the annotated cores was used in the analyses.

The presence of CD20+ lymphoid islets (15) was also annotated as either absent or, when present, the total number of islets.

Statistical analysis

Non-parametric Wilcoxon signed-rank, Mann-Whitney U and Kruskal-Wallis tests were applied for analyses of differences in the distribution of CD20, CD138 and IGKC expression in relation to clinicopathological characteristics. Time to recurrence (TTR) was defined as the date of surgery to the date of loco-regional or distant recurrence. The median value was used for prognostic cut-off. Kaplan Meier analysis and the log rank test were applied to estimate differences in 5-year overall survival (OS) and TTR in strata according to high and low density of CD20+, CD138+ and IGKC+ cells, respectively. Hazard ratios (HR) and confidence intervals (CI) at the 95% level for death and recurrence within 5 years were calculated by Cox proportional hazard's regression in both univariable and multivariable analysis, adjusted for age, sex, tumour (T-) stage, nodal (N-) stage, tumour grade and resection margins. All tests were two sided. P values <0.05 were considered significant. All statistical analyses were performed using IBM SPSS Statistics version 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Interrelationship of IGKC, CD20 and CD138 expression and associations with clinicopathological parameters

Sample IHC images are shown in *Figure 1*. CD20 expression could be assessed in 170 (97.7%) cases and CD138 in 172 (98.8%) cases. IGKC expression could be determined in a total of 173 (99.4%) cases and correlations between the investigated immune markers were found to be moderately strong, as shown in *Table 1*. In oesophageal adenocarcinoma, CD20 expression was significantly associated with IGKC expression (R=0.431, P<0.001), but not with CD138 expression. There was a significant correlation between expression of CD138 and IGKC (R=0.459, P≤0.001). In gastric adenocarcinoma, there was a significant correlation between expression of CD20 and IGKC (R=0.431, P<0.001), but not between CD20 and CD138. The correlation coefficient for CD138 and IGKC was also moderately strong (R=0.425, P≤0.001).

Associations of the investigated immune cell markers

Table 1 Interrelationship between immune cell-specific CD20, CD138, and IGKC expression

Marker	Oesophageal adenocarcinoma			Gastric adenocarcinoma		
	CD20	CD138	IGKC	CD20	CD138	IGKC
CD20						
R		0.143	0.431**		0.137	0.460**
P		0.236	<0.001		0.177	<0.001
n		70	70		99	101
CD138						
R	0.143		0.459**	0.137	0.425**	
P	0.236		<0.001	0.177	<0.001	
n	70		71	99	101	
IGKC						
R	0.431**	0.459**		0.460**	0.425**	
P	<0.001	<0.001		<0.001	<0.001	
n	70	71		101	101	

*, significance at the 5% level; **, significance at the 1% level. R, Spearman's correlation coefficient; P, P value; n, number of cases available for analysis; IGKC, immunoglobulin kappa c.

and patient characteristics in oesophageal and gastric adenocarcinoma are shown in *Tables 2* and *3*, respectively. In oesophageal adenocarcinoma, there were significant associations between high density of CD138+ cells and less advanced N-stage (P=0.048) and M-stage (P=0.047). In gastric adenocarcinoma, high density of CD20+ cells was significantly associated with less advanced N-stage (P=0.018) and low tumour grade (P=0.007), while high CD138 as well as IGKC expression was significantly associated with less advanced T-stage (P=0.037 and P=0.008, respectively).

Prognostic significance of CD20, CD138 and IGKC expression in oesophageal and gastric adenocarcinoma

Kaplan-Meier curves for OS according to the investigated markers in all patients with oesophageal adenocarcinoma are shown in *Figure 2*. Whereas there were no significant associations between OS and high CD20 or CD138 expression, there was a significantly prolonged OS for patients with tumours displaying high IGKC expression (P=0.003). As shown in *Table 4*, in patients with M0/R0 oesophageal cancer, the association of high IGKC expression with a reduced risk of death was significant in both univariable and multivariable Cox regression analysis (HR 0.21; 95% CI, 0.08–0.60 and HR 0.10; 95% CI, 0.02–0.57, respectively). With regards to TTR, KM analysis revealed that a high IGKC expression was significantly associated with a prolonged TTR in all patients with oesophageal adenocarcinoma (P=0.003, data not shown). In patients with M0/R0 oesophageal cancer, the association of high IGKC expression with a reduced risk of recurrence was significant in both univariable and multivariable Cox regression analysis (HR 0.20; 95% CI, 0.06–0.65, and HR 0.15; 95% CI, 0.03–0.71, respectively) (*Table 4*).

Kaplan-Meier curves for OS according to the investigated markers in all patients with gastric adenocarcinoma are shown in *Figure 3*. There were no significant associations between high CD20 expression and OS; however, there was a significantly prolonged OS for patients with tumours displaying high CD138 expression (P=0.002), and a non-significant trend towards an improved OS for patients with tumours displaying high IGKC expression with (P=0.083). As shown in *Table 4*, in patients with M0/R0 gastric cancer, the association of high CD138 expression with a reduced risk of death was significant only in univariable and borderline significant in multivariable Cox regression analysis (HR 0.50; 95% CI, 0.27–0.90 and HR 0.55; 95% CI, 0.30–1.00, respectively). The association

Table 2 Associations between IGKC, CD20, CD138 expression and clinicopathological parameters in oesophageal adenocarcinoma

Factor	Total CD20+			Total CD138+			Total IGKC+		
	n	Median (range)	P value	n	Median (range)	P value	n	Median (range)	P value
Age			0.561			0.909			0.770
≤70	39	5.00 (0.00–165.00)		40	2.00 (0.00–150.00)		40	2.75 (0.00–41.00)	
>70	31	6.25 (0.00–120.00)		31	1.50 (0.00–50.00)		31	2.50 (0.00–65.00)	
Gender			0.510			0.709			0.305
Female	6	27.25 (2.00–165.00)		6	5.25 (0.00–45.00)		6	10 (0.00–35.00)	
Male	64	5.00 (0.00–120.00)		65	1.50 (0.00–150.00)		65	2.50 (0.00–65.00)	
T-stage			0.507			0.579			0.221
1	8	5.12 (0.00–120.00)		9	29.00 (0.00–35.00)		9	12.00 (0.00–41.00)	
2	11	5.25 (0.00–165.00)		11	1.50 (0.00–16.50)		11	1.50 (0.00–18.00)	
3	44	5.62 (0.00–112.50)		44	1.25 (0.00–150.00)		44	2.37 (0.00–65.00)	
4	6	2.50 (0.00–21.25)		6	2.50 (0.00–28.75)		6	0.87 (0.00–23.00)	
N-stage			0.060			0.048			0.069
0	16	24.00 (0.00–165.00)		6	2.50 (0.00–28.75)		17	11.00 (0.00–41.00)	
1	15	4.00 (0.00–63.00)		15	1.00 (0.00–50.00)		15	2.25 (0.00–31.00)	
2	17	3.50 (0.00–82.50)		17	0.00 (0.00–37.50)		17	0.00 (0.00–52.50)	
M-stage			0.404			0.047			0.298
0	60	5.25 (0.00–165.00)		17	0.00 (0.00–37.50)		61	3.00 (0.00–65.00)	
1	10	2.25 (0.00–46.25)		10	0.00 (0.00–12.50)		10	1.00 (0.00 –30.00)	
Tumour grade			0.535			0.814			0.108
Low	28	1.75 (0.00–63.00)		31	4.00 (0.00–62.50)		31	4.00 (0.00–52.50)	
High	72	16.25 (0.00–150.00)		40	1.50 (0.00–150.00)		40	1.50 (0.00–65.00)	
Resection margin			0.591			0.847			0.838
R0	44	5.25 (0.00–165.00)		45	1.50 (0.00–150.00)		45	3.00 (0.00–52.50)	
R1 & 2	26	4.00 (0.00–108.00)		26	2.50 (0.00–50.00)		26	2.00 (0.00–65.00)	

IGKC, immunoglobulin kappa C.

of high IGKC expression with a reduced risk of death was however significant in both univariable and multivariable Cox regression analysis (HR 0.55; 95% CI, 0.31–0.99 and HR 0.46; 95% CI, 0.26–0.87, respectively). With regards to TTR in patients with M0/R0 oesophageal cancer, neither CD20 nor CD138 expression was prognostic, but high IGKC expression was significantly associated with a reduced risk of recurrence in both univariable and multivariable Cox regression analysis (HR 0.20; 95% CI, 0.06–0.65 and HR 0.15; 95% CI, 0.03–0.71, respectively) (*Table 4*). In patients with M0/R0 gastric cancer, neither CD20 nor CD138 expression was prognostic in relation to TTR, and high IGKC expression was only prognostic in multivariable analysis (HR 0.45; 95% CI, 0.21–0.98).

CD20+ B cell islets were present in 11 (6.3%) of the tumours, and the median was 1 islet per tumour (range, 0–3). In the Kaplan-Meier analysis there was a trend, however non-significant, towards an improved survival for patients with tumours displaying B cell islets as compared with tumours having none.

Table 3 Associations between CD20, CD138, IGKC expression and clinicopathological parameters in gastric adenocarcinoma

Factor	Total CD20+			Total CD138+			Total IGKC+		
	n	Median (range)	P value	n	Median (range)	P value	n	Median (range)	P value
Age			0.511			0.478			0.734
≤70	45	15.00 (0.00–120.00)		45	5.00 (0.00–150.00)		46	4.50 (0.00–55.00)	
>70	55	6.00 (0.00–150.00)		56	3.50 (0.00–97.50)		56	3.50 (0.00–95.00)	
Gender			0.624			0.497			0.379
Female	33	4 (0.00–120.00)		34	4.00 (0.00–150.00)		34	3.75 (0.00–70.00)	
Male	67	7.50 (0.00–150.00)		67	4.00 (0.00–97.50)		68	4.25 (0.00–95.00)	
T-stage			0.139			0.037			0.008
1	9	27.00 (0.00–106.25)		9	22.50 (0.00–150.00)		9	12.00 (4.50–95.00)	
2	19	8.00 (0.00–51.00)		20	12.00 (0.00–90.00)		21	5.00 (0.00–70.00)	
3	49	6.00 (0.00–68.75)		49	2.50 (0.00–105.00)		49	0.50 (0.00–55.00)	
4	21	4.00 (0.00–150.00)		21	1.50 (0.00–90.00)		21	4.00 (0.00–24.00)	
N-stage			0.018			0.627			0.207
0	40	20.00 (0.00–106.25)		40	4.00 (0.00–150.00)		41	4.50 (0.00–95.00)	
1	14	2.00 (0.00–150.00)		15	0.00 (0.00–35.00)		15	0.75 (0.00–35.00)	
2	24	2.00 (0.00–46.50)		24	5.00 (0.00–97.50)		24	0.50 (0.00–43.50)	
M-stage			0.261			0.842			0.087
0	88	7.75 (0.00–150.00)		89	4.00 (0.00–150.00)		90	4.50 (0.00–95.00)	
1	12	5.00 (0.00–52.50)		12	2.75 (0.00–90.00)		12	0.37 (0.00–10.00)	
Tumour grade			0.007			0.407			0.325
Low	28	1.75 (0.00–63.00)		29	2.00 (0.00–97.50)		29	1.50 (0.00–95.00)	
High	72	16.25 (0.00–150.00)		72	5.00 (0.00–150.00)		73	4.50 (0.00–70.00)	
Resection margin			0.172			0.920			0.834
R0	74	6.00 (0.00–106.25)		75	4.00 (0.00–150.00)		76	4.25 (0.00–95.00)	
R1 & 2	26	15.00 (0.00–150.00)		26	2.50 (0.00–90.00)		26	3.75 (0.00–55.00)	

IGKC, immunoglobulin kappa C.

Discussion

The numerous studies investigating the prognostic effect of the cellular immune system in different forms of cancer have recently been supplemented by research exploring the role of the humoral immune system. To the best of our knowledge, this is the first report to describe the prognostic impact of IGKC in oesophageal and gastric adenocarcinoma, with the results demonstrating that abundant IGKC+ plasma cell tumour infiltration is an independent predictor of an improved OS and TTR in both oesophageal and gastric adenocarcinoma.

B cells are known to be antibody producers but they are also, alongside macrophages and dendritic cells, antigen-presenting cells with the ability to supply co-stimulatory signals for T-cells (29-31). The existence of T cells and B cells in conjunction is necessary for an effective anti-tumour immunity, as evidenced by the improved prognosis in tumours with a high number of CD8+, CD4+ and CD20+ lymphocytes (32-34). The recent report by Kroeger *et al.* in ovarian cancer (34), demonstrating that CD8+ T cells

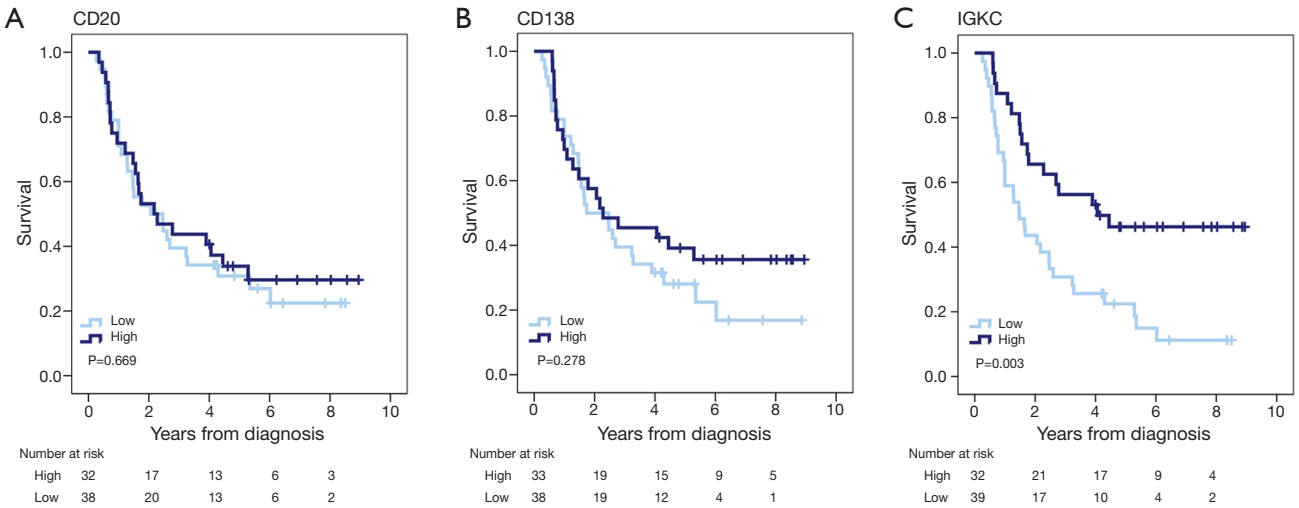

Figure 2 Kaplan-Meier estimates of overall survival according to expression of B cell and plasma cell markers in patients with oesophageal adenocarcinoma. Overall survival according to high and low expression of (A) CD20, (B) CD138 and (C) IGKC in all patients with oesophageal adenocarcinoma. IGKC, immunoglobulin kappa C.

do not carry any prognostic value without the presence of dense infiltration of plasma cells (IgG+) in ovarian cancer further corroborates the importance of an intact immune system and more specifically the significance of the humoral immune response.

The auspicious impact of tumour infiltrating plasma cells has previously been reported in NSCLC, colorectal and breast cancer (22). Our data on esophageal and gastric adenocarcinoma, demonstrating dense plasma cell infiltration to improve OS, is in line with the vast majority of previous research.

The antibody dependent activation of CD8+ T cells and natural killer cells, and the following cellular cytotoxicity is a plausible explanation for the positive impact on survival seen with an increased number of tumour infiltrating plasma cells. Furthermore, plasma cells have been shown to be able to activate the complement system (35) and to competitively inhibit myeloid derived stem cells (36), thereby promoting tumour cell destruction and creating a favourable inflammatory microenvironment, respectively. On the other hand, Mohammed *et al.* (17) reported that breast cancer patients with tumours displaying a high proportion of tumour infiltrating plasma cells (CD138+) had a significantly decreased OS, in line with recent findings in EOC (18). One explanation for this may be the presence of regulatory plasma cells with the ability to inhibit the T cell response through cytokines IL-10 (37) and IL-35 (38). Moreover, CD138 has also been proposed as a novel target for immunotherapy in metastatic breast cancer (39). Notably, these are two of very few studies to demonstrate an adverse relationship between high plasma cell infiltration and survival, although plasma cell infiltration may vary in different cancer types, and its effect on survival accordingly.

Our findings are in concordance with studies on NSCLC (16,22), colorectal cancer (22,34), ovarian cancer (33) and breast cancer (22), all reporting ameliorated OS rates with high levels of plasma cells. Hitherto, only the tumour cell-specific expression of CD138 has been shown to be associated with more advanced TNM stage and poor clinical outcome in gastric cancer (19), similarly to findings in colorectal cancer (40).

There were significant intercorrelations between all three herein investigated markers, which are in consistency with previous findings in NSCLC (16). Of note, CD138 is a less specific plasma cell marker than IGKC (22), i.e., it may also be expressed in normal and malignant epithelial cells as well as in stromal fibroblasts.

There are somewhat conflicting data on the prognostic impact of CD20+ tumour infiltrating lymphocytes (TIL) in the existing literature. In a report by Shah *et al.* (41), mice completely lacking CD20+ TIL showed an improved anti-tumour cell response, suggesting that lifting CD20+ TIL inhibition enabled CD8+ TIL and Th1 (CD4+) cytokine anti-tumour responses. Similar data have been reported by Julien *et al.* (42). However, in a report on serous EOC, CD20+ TIL, when coupled with CD8+ TIL, were found

Table 4 Cox proportional hazards analysis of the impact of CD20, CD138 and IGKC expression on time to recurrence and overall survival in oesophageal and gastric adenocarcinoma, respectively

Group	TTR			OS		
	n [events]	HR (95 % CI)	P	n [events]	HR (95 % CI)	P
Oesophageal						
CD20						
Univariable						
Low	17 [7]	1.00		20 [10]	1.00	
High	15 [7]	0.88 (0.31–2.73)	0.878	18 [18]	1.17 (0.49–2.81)	0.726
Multivariable						
Low	16 [7]	1.00		19 [10]	1.00	
High	15 [6]	1.17 (0.33–4.12)	0.805	18 [10]	1.23 (0.41–3.71)	0.716
CD138						
Univariable						
Low	15 [8]	1.00		20 [13]	1.00	
High	18 [5]	0.40 (0.13–1.25)	0.116	19 [7]	0.39 (0.16–1.00)	0.050
Multivariable						
Low	15 [8]	1.00		20 [13]	1.00	
High	17 [5]	1.62 (0.49–5.31)	0.426	18 [7]	0.73 (0.24–2.21)	0.575
IGKC						
Univariable						
Low	14 [9]	1.00		20 [15]	1.00	
High	19 [4]	0.20 (0.06–0.65)	0.008	19 [5]	0.21 (0.08–0.60)	0.003
Multivariable						
Low	13 [9]	1.00		19 [15]	1.00	
High	19 [4]	0.15 (0.03–0.71)	0.017	19 [5]	0.10 (0.02–0.57)	0.009
Gastric						
CD20						
Univariable						
Low	30 [16]	1.00		32 [23]	1.00	
High	32 [13]	0.55 (0.26–1.14)	0.110	38 [24]	0.73 (0.41–1.29)	0.278
Multivariable						
Low	30 [16]	1.00		32 [23]	1.00	
High	32 [13]	0.92 (0.42–2.03)	0.834	38 [24]	0.98 (0.51–1.87)	0.952
CD138						
Univariable						
Low	29 [14]	1.00		31 [24]	1.00	
High	34 [15]	0.77 (0.37–1.60)	0.481	40 [23]	0.50 (0.27–0.90)	0.021

Table 4 (*continued*)

Table 4 (*continued*)

Group	TTR			OS		
	n [events]	HR (95 % CI)	P	n [events]	HR (95 % CI)	P
Multivariable						
Low	29 [14]	1.00		31 [24]	1.00	
High	34 [15]	1.17 (0.51–2.65)	0.711	40 [23]	0.0.55 (0.30–1.00)	0.052
IGKC						
Univariable						
Low	30 [16]	1.00		34 [25]	1.00	
High	34 [13]	0.53 (0.25–1.10)	0.090	38 [22]	0.55 (0.31–0.99)	0.047
Multivariable						
Low	30 [16]	1.00		34 [25]	1.00	
High	34 [13]	0.45 (0.21–0.98)	0.043	38 [22]	0.46 (0.24–0.87)	0.018

IGKC, immunoglobulin kappa C; TTR, time to recurrence; OS, overall survival; HR, hazard ratio; CI, confidence interval.

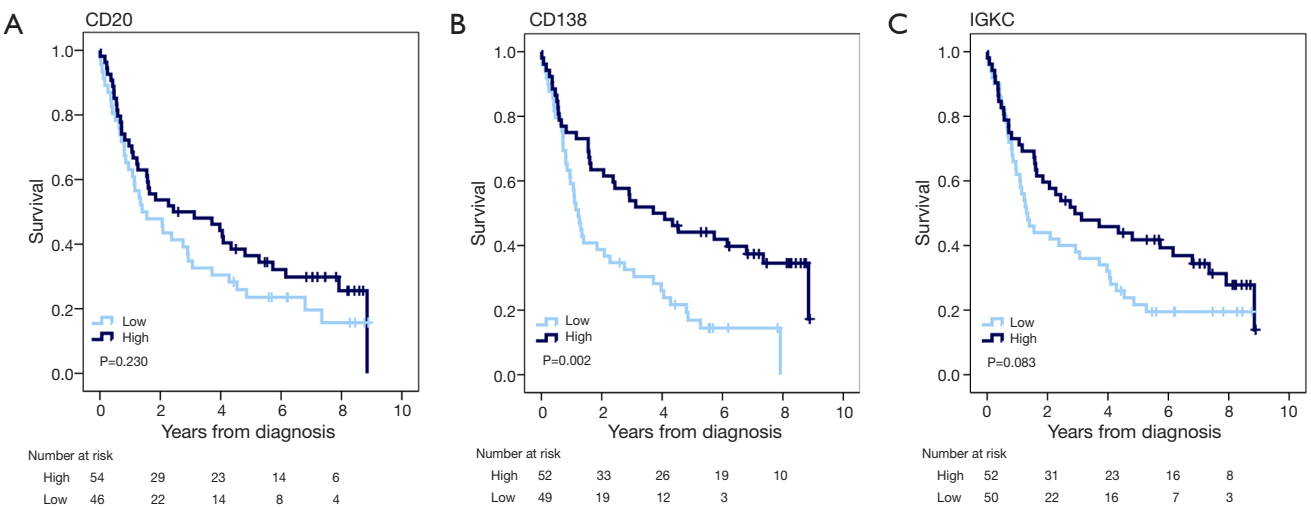

Figure 3 Kaplan-Meier estimates of overall survival according to expression of B cell and plasma cell markers in patients with gastric adenocarcinoma. Overall survival according to high and low expression of (A) CD20, (B) CD138 and (C) IGKC in all patients with gastric adenocarcinoma. IGKC, immunoglobulin kappa C.

to be significantly associated with decreased tumour progression, resulting in an improved OS (43). In the current study, however, there was no significant correlation between CD20+ cell density and survival.

A potential limitation to the present study is the use of TMA. More than fifteen years after its introduction (44), the TMA technique can be considered a well- established platform for tissue biomarker studies, providing similar or even better prognostic information than whole tissue section based analyses (45). However, suboptimal sampling may occur, e.g., of heterogeneously expressed markers. Therefore, whenever possible, tissue cores were obtained from different donor blocks of the primary tumours. Moreover, the use of two 1.0 mm cores can be considered a comparatively generous sampling size and, of note, heterogeneity issues may well arise even with the use of whole tissue sections. Use of the TMA technique may also not be optimal for detection of B cell islets. In the study by

Berntsson *et al.*, which was also based on TMA analysis and wherein a high density of CD20+ B-cells was found to be an independent predictor of a prolonged survival in colorectal cancer, CD20+ B cell islets were found in 8% of cases (15). The corresponding proportion in this study was 11%, and in neither of the studies, a significant association with survival could be found. The small proportion may well be due to use of the TMA-technique, and may also explain the lack of association with prognosis. It is likely that analysis of whole tissue sections slides would improve the detection rate for B cell islets, and hence also demonstrate their prognostic impact in oesophageal and gastric cancer.

Although the study cohort can be considered as medium-sized, the number of cases is more limited when stratifying for tumour location. Therefore, additional validatory studies on independent, and, if possible, larger cohorts are warranted. Another potential caveat is that several tests have been made, which increases the risk for type I errors, i.e., detecting a difference that is coincidental.

Conclusions

This study provides a first description of the prognostic significance of tumour-infiltrating B cells and plasma cells in oesophageal and gastric adenocarcinoma. The strongest association with prognosis was observed for IGKC+ plasma cells, abundant infiltration of which was found to be an independent predictor of a prolonged survival in both types of cancer.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the regional ethics committee at Lund University (No. 445/07), whereby the committee waived the need for consent other than by the option to opt out.

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