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T Cell Density and Location Can Influence the Prognosis of Ovarian Cancer

Ahmad Al-Attar · Mohamed Shehata · Lindy Durrant · Paul Moseley · Suha Deen · Stephen Chan

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Abstract The aims of this study were to examine the significance of CD3+ cells in patients with epithelial ovarian cancer and to determine their influence on the disease in relation to their location within tumours. A 157-core tissue-microarray constructed from primary ovarian cancer patients treated at Nottingham-University-Hospitals (2000–2007) was stained for the T-cell marker CD3. The number of CD3+ cells in direct contact with tumour cells was counted per tumour area. These were considered as "intra-tumoural T-cells (ITTC)". Cores were divided into CD3 'high' or 'low' density tumours. "Stromal T-cells (STC)" were assigned as 'positive' or 'negative'. The study population had a median follow-up time of 36-months (0–75). The number of ITTC counted in tumour cores ranged between 0 and 184/mm^2. 90-tumours-(57%) were found to be in the "low-density" rubric, while 56-(36%) were of a "high-density" T-cell population. STC were found in 118-cores-(75%)-compared to 22-cores-(14%)-negative cores. Higher number of ITTC correlated with lower-grade-(p=0.045), tumour-type-(p=0.034), and longer-median-survival-times (57-versus 37-months for high-and low-ITTC densities, respectively, p=0.038). This relationship was reversed when tumours were infiltrated by CD3+ cells in the stroma, predicting worse-survival (Log-rank-test, p=0.028). Combining ITTC with STC produced an interesting pattern where the ITTC-low/STC + ve had the worst prognosis (p=0.003). Infiltration of ovarian cancer by T-cells can influence its prognosis depending on the location of these cells (intra-tumoural-versus-stromal). The former predicts improved survival, while the latter is probably contributing to tumour progression and, in turn, worse survival.

Keywords Intra-tumoural T cells · Ovarian cancer · Prognosis · Stromal T cells

A. Al-Attar · M. Shehata · P. Moseley · S. Chan (✉)
Department of Clinical Oncology, Nottingham University Hospitals, NHS Trust–City Campus, Hucknall Road, Nottingham NG5 1PB, UK
e-mail: steve.chan@nuh.nhs.uk

L. Durrant
Department of Academic Oncology, University of Nottingham, Hucknall Road, Nottingham NG5 1PB, UK

S. Deen
Department of Histopathology, Nottingham University Hospitals, NHS Trust–QMC Campus, Derby Road, Nottingham NG7 2UH, UK

Introduction

Over 6,000 women are diagnosed with ovarian cancer in the UK each year [1]. It is the fifth most common cancer in females in the UK causing 5 out of every 100 cancers diagnosed in women. Its incidence has been increasing especially in women over 65. Although overall survival rates have improved slowly since the 1970s, it remains the fourth most common cause of death from cancer in women in the UK.

The immune system has been shown to play an important role in the body's response to this cancer and in shaping its progress. The density of CD3$^+$ cells in a tumour may reflect ongoing immune responses against the tumour cells [2]. The presence of tumour-infiltrating T cells has been described in ovarian cancer [3–5]. Characterisation of tumour-infiltrating T cells in lung cancer has demonstrated that such cells can recognise tumour-related epitopes, displaying an oligoclonal expansion and when isolated in vitro, they were able to affect a tumour-specific cytolysis [6, 7].

The presence of immune cells in the tumour microenvironment however, is not always a reflection of good

prognosis. It is thought that the type and subtype of immune cells will determine whether such 'inflammation' is good or bad in terms of controlling and modifying the growth of cancer cells [8–10]. This dogma is central to the current 'immunoediting' hypothesis proposed by Dunn et al., and tested and supported by many contemporary researchers of the immunobiology of cancer. The basis of this hypothesis revolves around the ability of the immune system to both eliminate tumours and sculpt the immunogenic phenotypes of tumours that eventually form in immunocompetent hosts [11]. Cells of the immune system can also promote tumour cell growth, survival, and angiogenesis through the induction of oncogenic inflammation [12]. It has also been shown that a state of immunosuppression is promoted by the tumorigenic process itself in tumour-bearing hosts [13]. This, in turn, is thought to facilitate neoplastic progression.

The aims of this study were to examine the significance of the presence of CD3+ cells in patients with epithelial ovarian cancer and to determine their influence on survival and other clinico-pathological features in relation to their location in a tumour.

Materials and Methods

Patients

A cohort of 157 patients with primary ovarian cancer treated at Nottingham University Hospitals between 2000 and 2007 were identified and included in this study. Staging was performed using the International Federation of Obstetrics and Gynecology (FIGO) criteria. All patients included in this study were treated according to the current standard chemotherapy regimens with either single agent carboplatin in 65 patients (41.4%) or platinum-based combination chemotherapy in 89 patients (56.7%), with 3 patients refusing chemotherapy. Platinum-resistant cases were defined as patients who progressed on first-line platinum chemotherapy during treatment or who relapsed within 6 months after treatment. All patients underwent surgery; over 44% of cases (n=69) were deemed to be suboptimally debulked (tumour remaining <1 cm) after initial surgery. Patients were followed-up by physical examination, computed tomography, and CA-125 levels. Haematoxilin and eosin-stained sections from the tumours of these patients were reviewed by a gynaepathologist blinded to the clinical data and pathological diagnosis. A tissue microarray (TMA) was constructed from the paraffin blocks of these tumours. Areas selected for the TMA core were marked on the corresponding slide where representative areas of the tumour could be sampled. For each tumour, a review of its type and differentiation was also carried out by SD. Clinical data associated with each case was collected and recorded from the patients' notes or via the hospital's electronic records (NotIS). Such information included: patients' age at diagnosis, FIGO stage, extent of surgical cyto-reduction, and the type, duration and response to chemotherapy. Details of adjuvant treatment, disease-free survival (DFS) and overall survival (OS) were documented for all patients. Survival was calculated from the operation date until 30th of May 2008 when any remaining survivors were censored. Median follow up was 36 months.

Ethical approval to collect the samples and relevant data for the study was granted by the Nottinghamshire Local Research Ethics Committee.

TMA Construction

TMA blocks were constructed from the paraffin embedded tumour samples obtained prior to the commencement of any chemotherapeutic agent. Three 0.6 mm cores were obtained from the 'donor' block and transferred to empty 'recipient' blocks using specially designed needles attached to a manual tissue-arrayer (Beecher Instruments, Sun Prarie, Wisconsin, USA).

Array blocks were constructed with 100 cores on the first TMA block and 89 cores on the second. This process was repeated in triplicate to produce three copies of the array. In order to preserve maximum tissue antigenicity, fresh 4 μm sections were cut from each TMA block, in duplicate, and placed on coated glass slides to allow the immunohistochemical technique to be performed.

Immunohistochemistry

Immunohistochemical staining for T cells was performed using a routine avidin-biotin peroxidase method. Tissue array sections were first deparaffinised with xylene, rehydrated through graded alcohols and immersed in a 0.3% hydrogen peroxide in methanol solution for 20 min to block endogenous peroxidase activity. Antigen retrieval was achieved by microwaving the sections (immersed in 500 mls of pH 6.0 citrate buffer). Slides were heated for 20 min in an 800 W microwave as follows: 10 min at high power, followed by 10 min at low power. Normal swine serum (NSS) diluted 1:20 in Tris-buffered saline (TBS) was left incubated over the sections for 10 min in order to block non-specific adsorption of the primary antibody.

TMA sections were incubated with 100 μl of mouse anti-human monoclonal antibody to CD3 (clone SP7, culture supernatant, NeoMarkers, Fremont, California, USA). This was diluted in NSS/TBS at 1:150, which was deemed to produce the optimal staining in the preliminary optimisation runs. The antibody was left to incubate on the sections for 60 min at room temperature. Positive control

tissue comprised whole sections of tonsil. The primary antibody was omitted from the negative control, which was left incubating in only NSS/TBS solution.

Slides were then washed with TBS, followed by a 30 min incubation with 150 μl of biotinylated goat anti-mouse/rabbit immunoglobulin (Dako Ltd, Ely, UK) diluted 1:100 in NSS/TBS. Sections were then washed again in TBS and next incubated with 100 μl of pre-prepared solution of streptavidin-biotin/horseradish peroxidase (HRP) complex (Dako) for 60 min at room temperature. Subsequently, visualisation of CD3+ cells was developed using 3, 3'-Diamino-benzidine tetrahydrochloride (Dako). Sections were lightly counterstained with haematoxylin (Sigma, Dorset, UK) and mounted with DPX (Sigma).

Evaluation of CD3 Staining

Stained slides were digitally scanned and captured using NanoZoomer Scanner (Hamamatsu Photonics Ltd., Hertfordshire, UK) at ×100 and ×200 magnification. Scoring was performed by AA and MS, as follows: each core was examined on a 19" LCD monitor (Dell, Berkshire, UK). A perimeter was drawn around the area of tumour tissue per core and was calculated using a tool embedded in the software package used to view the images (NDP.view software plug-in). The number of CD3+ cells in direct contact with tumour cells was counted. These were considered as "intra-tumoural T cells (ITTC)". The number of ITTC was divided by the tumour area to yield a standardised density of T cells per area. High density was classified as ≥70 cell/mm^2. Low density ITTC included core with <70 (including none) CD3-positive cell per mm^3. All other CD3 positive mononuclear cells not in contact with the tumour were considered as "stromal T cells (STC)" and were assessed as present or absent. The latter system was used for STC because of the superior numbers of cells not in contact with tumour tissue compared to the ones classified as ITTC. Eleven cores were not evaluable for T cell infiltration due to absence of tumour tissue (often the result of loss during the staining process) and were hence excluded from the analysis.

Statistical Analysis

Statistical analysis of the study data was performed using the SPSS package (version 11.5 for Windows, SPSS Inc., Chicago, IL). Pearson χ^2 chi-square tests were used to determine the significance of associations between categorical variables. Mann Whitney U test was used for continuous, non-parametric data. Overall survival calculations included all patients who died during follow up. Survival rates were calculated using the Kaplan-Meier method; differences between groups were tested using the log-rank test. Events for disease-free and overall survival were defined as follows: time of disease relapse or death (for disease free survival) and time of death, (for overall survival) respectively. The Cox proportional-hazards model was used for multivariate analysis in order to determine the relative risk and independent significance of individual factors. In all cases p-values <0.05 were considered as statistically significant.

Results

Study Population

The clinico-pathological characteristics of the patients included in this study are presented in Table 1. The median

Table 1 Patients' clinico-pathological characteristics, with relapse and survival data calculated at the time of data censoring

Characteristics	Frequencies	Percentages
Pathology		
Serous cystadenocarcinoma	88	56
Endometrioid	33	21
Clear cell carcinoma	21	13
Mucinous cystadenocarcinoma	12	8
Other	3	2
Grade		
1	20	13
2	23	14
3	114	73
Residual tumour		
None	88	56
Present	69	44
Stage		
IC	44	28
II	21	13
III	71	45
IV	21	14
Chemotherapy		
Carboplatin monotherapy	65	41
Carboplatin combination therapy	89	57
No chemotherapy	3	2
Platinum sensitivity		
Sensitive	104	66
Resistant	50	32
Unknown	3	2
Relapse status		
Relapsed	75	48
Relapse-free	75	48
Unknown	7	4
Survival status		
Living	103	66
Dead	54	34

age of the study population was 61 years (range 33–87, mean 60 years). Of the 157 patients included in the study, 54 (34%) were dead before the end of the follow-up period. Histologically, most patients were found to have a serous cystadenocarcinoma (56%), followed by endometrioid (21%), clear cell carcinoma (13%), mucinous cystadenocarcinoma (8%) or other types (2%). Tumours displayed poor hisotologic differentiation in 73% of cases. Most tumours were classified as FIGO stage III (45%).

ITTC and STC in Ovarian Cancer

ITTC and STC in ovarian cancer: CD3 positive tumour-infiltrating T cells were detected in tumour and in peri-tumoural stroma (Fig. 1). The number of ITTC counted in tumour cores ranged between 0 and 184 per square millimetre, with a median of 30. The average number of cells was 70/mm^3. This was used as a cut-off point between cores with low (<70/mm^3) and high density (≥70/mm^3) T

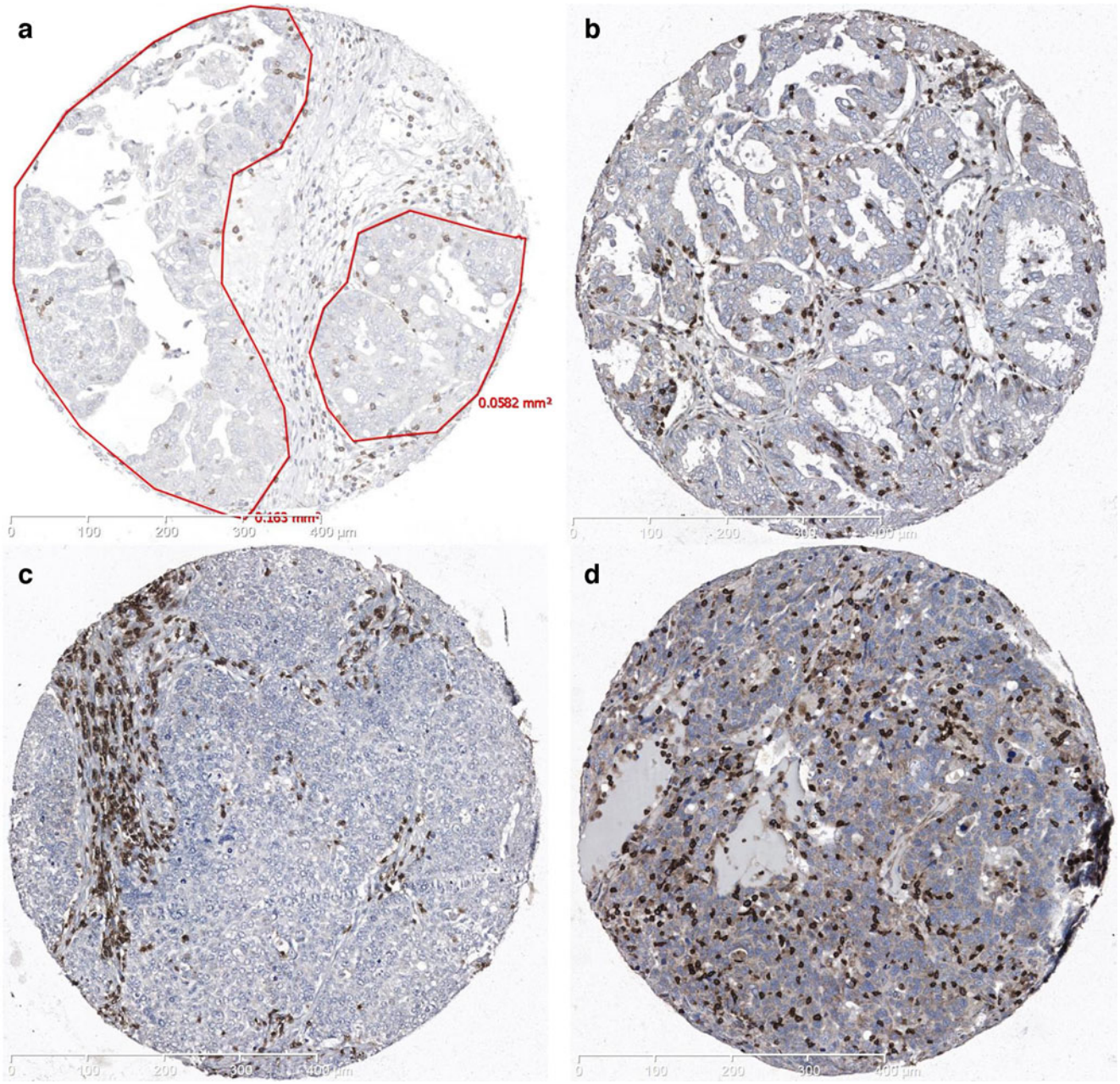


Fig. 1 Representative pictures of the immunohistochemical staining of tumour-infiltrating T-lymphocytes in ovarian cancer tissue. CD3+ cells were observed both in cancer stroma (STC) and within cancer epithelium (ITTC). In core **a** a perimeter is drawn around the tumour, and the area measured in mm^2, demonstrating the method used to calculate ITTC per area. Core **b** is representative of low ITTC count; core **c** shows high STC with low ITTC; and core **d** represents high ITTC infiltration

Table 2 The distribution of T-cells within the tumour mass (ITTC), in the stromal tissue (STC) and a combined score for both locations

	Frequency	Percent
ITTC		
Low density	90	57
High density	56	36
Not assessed	11	7
Total	157	100
STC		
Negative	22	14
Positive	118	75
Not assessed	17	11
Total	157	100
T cells in both locations		
Low density ITTC / Negative STC	21	13.4
High density ITTC / negative STC	1	0.6
Low density ITTC / positive STC	64	40.8
High density ITTC / positive STC	54	34.4
Not assessed	17	10.8
Total	157	100

cell infiltration. Using this division, 90 tumours (57%) were found to be in the "low density" rubric, while 56 (36%) were deemed to have a "high density" T cell population (Table 2). STC were found in 118 cores (75%) compared to 22 cores (14%) where no stromal T cells were found (Table 2).

Correlation of Tumour-Infiltrating T Cells with Clinico-Pathological Parameters

Patients with well differentiated tumours were less likely to have ITTC cells compared to patients with poorly differentiated tumours. Table 3 shows ratios of high ITTC in case of 3/17 (18%), 7/21 (33%) and 46/108 (43%) patients with well, moderately and poorly differentiated tumours, respectively (χ^2 test for trend; $p=0.045$). However, no associations were seen between ITTC and stage or the extent of surgical debulking. Tumours resistant to platinum-based chemotherapy displayed a trend to have less T cells within the tumour areas, but this did not reach statistical significance ($p=0.093$). The number of ITTC was noticeably lower in mucinous adenocarcinomas than other EOC

Table 3 Correlation of T cell infiltrate in ovarian tumours with clinico-pathological variables; a cross-tabulation using chi-square to test significance ($p \leq 0.05$)

	ITTC		*p*-value	STC		p-value
	Low	High		Negative	Positive	
Age						
≤55	29	18	0.9	5	39	0.338
>55	61	38		17	79	
Tumour type						
Serous	49	36	0.034	6	76	0.002
Mucinous	10	1		3	6	
Endometrioid	14	15		5	23	
Clear cell	15	4		8	11	
Others	2	0		0	2	
Grade						
Well	14	3	0.045[a]	3	12	0.996
Moderate	14	7		2	19	
Poor	62	46		17	87	
Cytoreduction						
Optimal	52	36	0.435	15	70	0.435
Sub-optimal	38	20		7	48	
Stage						
I	28	13	0.553	8	31	0.55
II	9	9		4	14	
III	43	26		8	57	
IV	10	8		2	16	
Platinum sensitivity						
Sensitive	58	42	0.093	17	79	0.391
Resistant	32	12		5	37	

[a] Chi-square test for trend

types (p=0.034). Although a statistically significant correlation was also found between tumour type and STC, no clear differences in STC distribution patters among different tumour types was noteworthy. Table 3 shows the relationship of T cells to the clinico-pathological variables examined in this study.

Prognostic Significance of CD3 ± Cells

Contrasting survival differences were observed based on the location of T cell infiltration, being intra-tumoural or stromal. Using the Mann-Whitney U test, we noticed a significantly longer overall and progression-free survival between patients with high compared to low ITTC (p= 0.032 and 0.020, respectively). Figure 2 shows a box and whisker plot of the distribution of survival times between the two groups. On a Kaplan-Meier curve, lines representing the two populations separate early on, as seen in Fig. 3a, with a 20 month improved median survival in the ITTC-high group. This difference was confirmed statistically using the Log-rank test (statistic value=4.3, p=0.038). A similar separation of the curves was seen when 'disease progression' was analysed as an endpoint; however, it did not reach statistical significance on the Log rank test (Fig. 3b). Stromal T cell infiltration consistently displayed an opposite effect on prognosis to that seen with ITTC. Figure 3c and d show the influence of STC on overall and progression-free survival using Kaplan-Meier graphs. Using the Log-rank test, a statistically significant worse overall and progression-free survival was noticed in patients who had positive STC infiltration compared to those who had none (p=0.028 and 0.046, respectively). A summary of patient survival times, 95% confidence intervals and p-values based on T cell distribution is shown in Table 4. Standard prognostic clinico-pathological factors (e.g. stage and residual disease) are also shown in Table 4 for comparison. T cell Differences in median survival times could not be calculated because less than 50% of the cases had an event in the STC-negative group. Interestingly, when the T cell distribution was considered as a whole, the group that had ITTC-low/STC + ve fared worst among the other combinations (p=0.003). This is shown in Fig. 4.

Discussion

Tumours in which the interactions between the tumour cells and the host's defence systems correlate with clinical

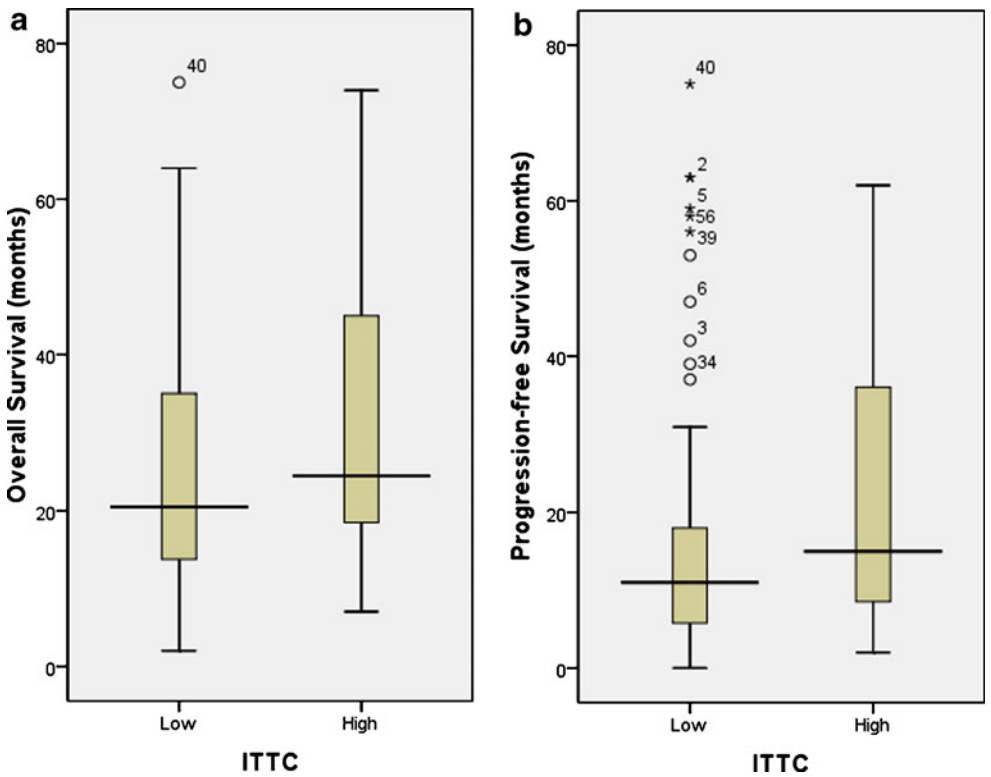
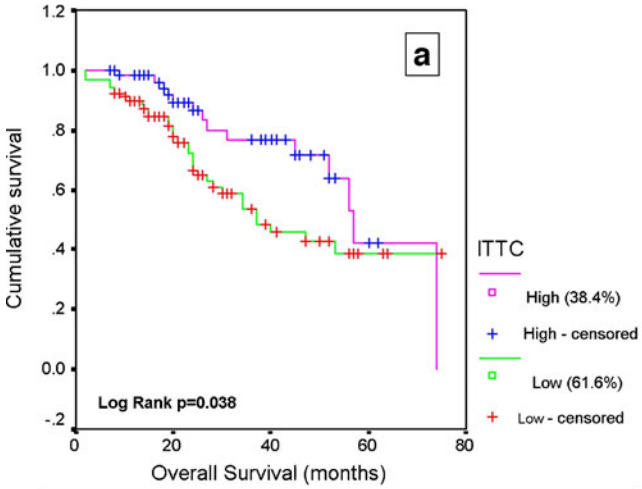
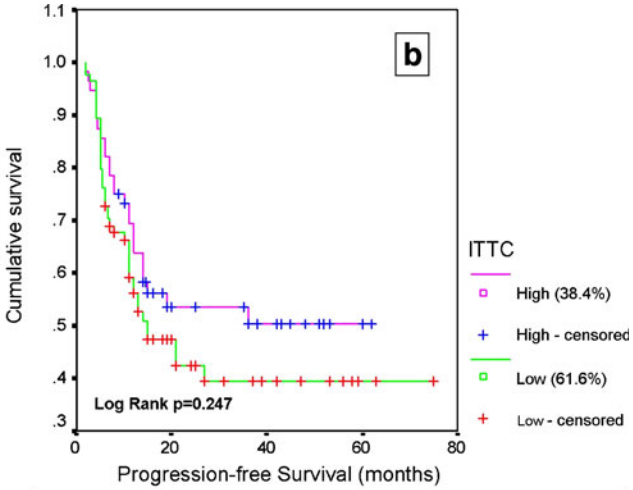


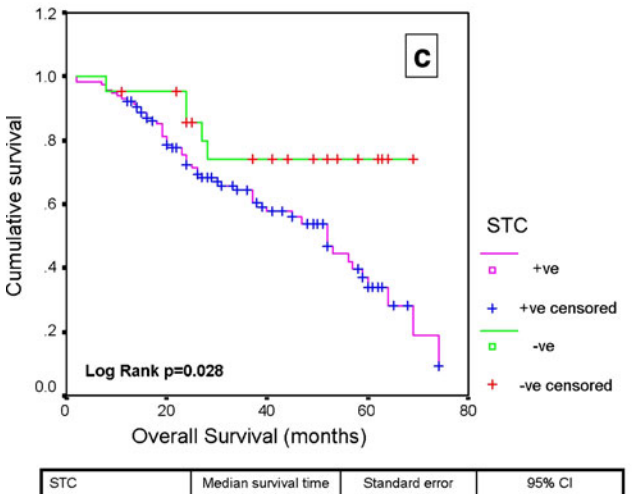
Fig. 2 Box-and-whisker plots depicting survival times: **a** Ovarian cancer patients whose tumours contained high levels of CD3 positive ITTC had an average duration of overall survival of 31.3 months, while the cancer patients whose tumours displayed low ITTC had an average duration of overall survival of 26.1 months (p=0.032). **b** Ovarian cancer patients whose tumours contained high levels of CD3 positive ITTC had an average duration of progression-free survival of 21.6 months, while the cancer patients whose tumours displayed low ITTC had an average duration of overall survival of 15.9 months (p= 0.02). Statistical values obtained using Mann-Whitney U test



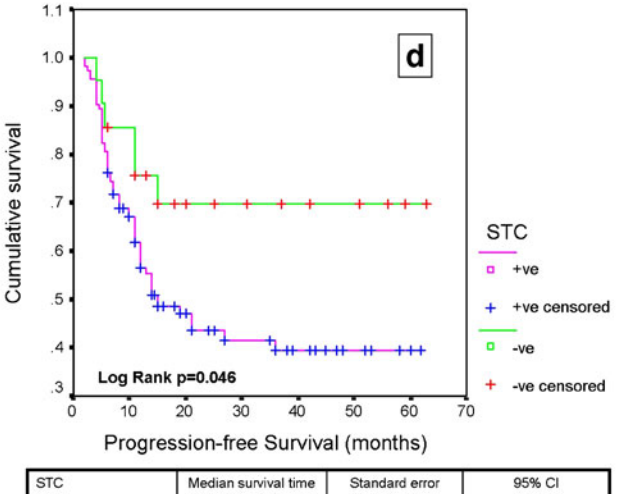
ITTC	Median survival time (months)	Standard error	95% CI
High	57	3.3	50.5-63.5
Low	37	7.5	22.3-51.7



ITTC	Median survival time (months)	Standard error	95% CI
High	-	-	-
Low	15	3.9	7.3-22.7



STC	Median survival time (months)	Standard error	95% CI
Negative (-ve)	-	-	-
Positive (+ve)	52	4.25	43.7-60.3



STC	Median survival time (months)	Standard error	95% CI
Negative (-ve)	-	-	-
Positive (+ve)	15	3.4	8.4-21.6

Fig. 3 Kaplan-Meier graphs and survival functions for ITTC and STC in ovarian cancer. Figures **a** and **b** represent overall and progression-free survival respectively in patients according to the level of ITTC infiltrate. Figures **c** and **d** represent overall and progression-free survival respectively in patients according to the presence (+ve) or absence (-ve) of STCs. Median values are only calculated when >50% of patients 'have an event'. Log Rank test p-values are shown at the bottom of the graph

outcome include vertical-growth phase melanoma and breast, prostate, renal-cell, oesophageal, and colorectal carcinomas [14–18]. Ovarian carcinoma is thought to display similar interactions that affect disease progression. Controversy still surrounds the role and prognostic significance of T cells within a tumour microenvironment. Many studies have shown that it is an indication of the host immune response to tumour antigens [5, 16, 19] and a reflection of the dynamic process of cancer immunoediting [11]. Studies of ovarian cancer have shown that immune mechanisms can attack cancer cells in spite of the deployment of different mechanisms by these tumours aimed at escaping immune surveillance [20–26].

Others have shown that inflammatory infiltrate in the tumour microenvironment induces tumour-promotion and drives the proliferation and survival of malignant cells via promoting angiogenesis and metastasis, dampening the adaptive immune responses, and negatively affecting the sensitivity of cancer cells to chemotherapeutic agents [27].

It is vital to keep in mind that outcomes such as survival indices and the extent of tumour cyto-reduction depend not only upon the type of host response but on the intensity of

Table 4 Univariate correlations of overall patient survival with the standard clinico-pathological criteria (stage, grade, residual disease and platinum sensitivity) and T cell distribution, showing median survival time in months, 95% confidence intervals and Log-rank test p-values. Significant p-values (<0.05) are shown in bold

Factor	Median survival time (months)[a]	95% Confidence intervals	p-value (Log rank test)
Stage			
I and II	74	8.6–139.4	<0.001
III and IV	31	22.2–39.8	
Grade			
1 and 2	–	–	0.047
3	45	−27.5−62.5	
Residual disease			
None	–	–	<0.001
Present	34	22.4–45.6	
Platinum sensitivity			
Sensitive	74	50.1–97.9	<0.001
Resistant	21	17.2–24.8	
ITTC			
Sparse	37	22.3–51.7	0.038
Dense	57	50.5–63.5	
STC			
Negative	–	–	0.028
Positive	52	43.7–60.3	

[a] Median values and 95% confidence intervals cannot be calculated when <50% of cases are dead

such response and the tumour's own biology [4]. Recently, efforts have been made to better understand the T-cell responses to ovarian cancer, which can potentially lead to improved clinical outcomes by amplifying host immunity [28]. In-vitro and in-vivo evidence exist demonstrating oligo-clonal/polyclonal expansion, recognition of tumour antigens and a tumour-specific cytolytic activity of tumour-associated lymphocytes in ovarian carcinoma [7, 29–31].

In this study, we tried to investigate the impact of T cell location within the tumour on prognosis. This was done in order to differentiate between tumours where the immune system has managed to infiltrate into the cancer cell border. Such tumours were shown to have T cell infiltrates in direct contact with cancerous cells, and may be considered as "immune-accessible tumours". On the other hand, these tumours which displayed T-cell infiltrates in the stromal compartment with no or little cells in direct contact with the tumour were seen as "immune-inaccessible tumours". Our data show that *high* ITTC predicted longer survival, inasmuch as *no* STC did (p=0.038 and 0.028, respectively). When both locations were taken into account, patients whose tumours had low ITTC and were positive for STC were in the worst survival cluster. The small number of cases resulting from subset analysis meant that the opposite possibility (high ITTC and negative STC) had only one event, which *anecdotally* demonstrated longer survival than any other combination.

Multivariate analysis failed to show the persistence of a statistical significance for T cell infiltrate, independent of stage and platinum sensitivity, in this cohort of patients (HR for high vs. low ITTC=0.68, 95% CI=0.42–1.12). However, ITTC was shown to correlate inversely with grade where poorly differentiated tumours, which have worse prognosis, were more frequently associated with high ITTC density, yet the latter was shown to have a positive influence on survival. This may indicate that T cells affect prognosis independently from the traditional pathological criteria, such as histological grade.

The results of T-cell staining pattern in this cohort of patients compares well with some of the recent publications. Zhang et al found high T-cell infiltrate in 54.8% of tumours using 186 frozen sections. They showed over 30% improved overall survival in this group over the patients whose tumours did not display significant ITTC infiltrate [5]. They did not however take STC into account in their analysis. Sato et al did address this issue, but could not find a significant correlation between either ITTC or STC with survival [3]. Results from a study of 119 ovarian cancer patients by Shah and colleagues were similar to those of Sato et al in terms of predicting survival [4], although they did show that patients with greater ITTC were more likely to be optimally cyto-reduced (p=0.005). Both studies attribute the different results they got from those of Zhang's report to the methodologic differences and the differences in chemo-therapeutic regimens among the different studies.

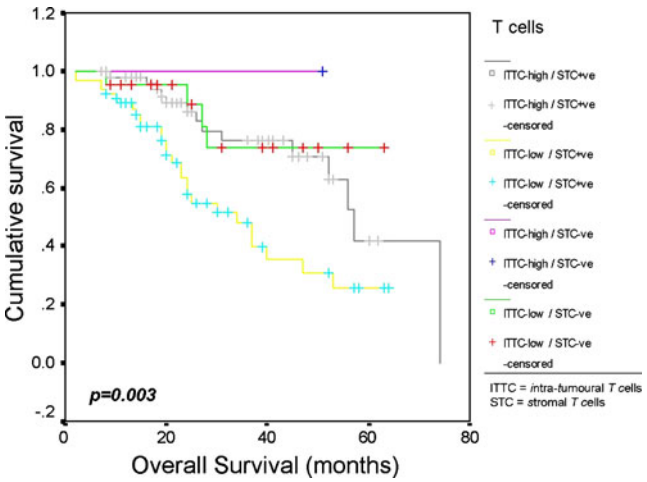


Fig. 4 Kaplan-Meier graph and Log Rank test when tumours were divided into 4 groups: dense ITTC with no stromal cells (ITTC-high/STC-ve), dense ITTC with positive stromal cells (ITTC-high/STC + ve), sparse ITTC with no stromal cells (ITTC-low/STC-ve), and sparse ITTC with positive stromal cells (ITTC-low/STC + ve). Median values are only calculated when >50% of patients 'have an event'. Log Rank test p-values are shown at the bottom of the graph

Reports by Raspollini et al and Tomšová et al however demonstrated a strong prognostic influence of T cells in ovarian cancer [19, 32]. High CD3+ tumour-infiltrating T cells correlated with disease-free interval ($p=0.004$), overall survival ($p<0.001$) and clinical responsiveness to chemotherapy ($p=0.003$) in the study by Raspollini, and were shown to be an independent prognostic factor in Tomšová's work ($p<0.001$) when a cut-off point of 125 cell/mm^2 was employed (HR=0.27, 95%CI=0.15–0.5).

Different tumours are thought to have varying ways of interaction with T cells. One of the important mechanisms by which the immune system eliminates cancer cells is via a CTL-based response to abnormal peptide presented in conjunction with HLA class I antigen on tumour cells. Functional T cells have to be in contact with HLA-I to elicit their function. We have shown before that loss of HLA class I in ovarian cancer correlates with a poor outcome [33]. Absence of the HLA receptor denies T cells the interaction required to kill tumour cells, and is possibly a reason for T cells to leave the tumour milieu. Another reason behind differential infiltration of tumours by these cells can be the insufficient chemoattraction of T cells to the tumour tissue. Homing of inflammatory cells depends on the action of chemokines, "inviting" them to survey the tissue microenvironment [34]. A selection of potential chemokines implicated in the circulation and extravasation of T cells have been investigated, e.g. Zhang et al have shown strong expression of CXCL9 in and around tumour islets [5]. Another possible explanation is the release of chemotoxic substances by the tumour which induces apoptosis of cytotoxic T cells, which has been shown to be Fas-dependent and was observed in some tumour types [24]. The opposing effects of ITTC and STC on prognosis could also be explained, at least in part, by the notion that not all T-lymphocyte subsets contribute equally to prognosis, and that different localisation of CD8+ and CD4+ T cells subsets in tumours can affect different outcomes. Regulatory CD4+ T-lymphocytes (Treg) induce peripheral tolerance and in turn suppress immune responses. This is largely done by secreting transforming growth factor β (TGF- β) and interleukin 10 (IL-10) or by direct cell-cell contact [35]. Studies examining the localisation of Treg cells in a variety of cancer types indicate a propensity to concentrate in the peri-tumoural area [36–39]. This may, in part, explain the negative impact on prognosis seen with STCs.

One limitation of our study was the smaller number of T cells we were able to count in a tumour using the TMA technique. Although the analysis was done using 3 cores from each tumour, the area of tissue available would've still been much smaller than that of a whole mount. However, TMA technology has been verified for the analysis of cellular infiltrate in tumours. Leffers et al analysed the presence and relative influence of tumour infiltrating subsets of T cells using antibodies to CD8, CD45RO and FoxP3 on TMAs of primary ovarian tumour tissue and its omental metastases [40]. Their analysis was carried out on 270 cases and showed that a high number of CD8+ cells were associated with improved survival ($p=0.042$) when counts were divided into tertiles, and patients in the top two were compared to those in the lowest tertile. Although the p values obtained are not as low as that of studies done on whole sections, the numerical power of these TMA studies compensates for the smaller tumour area examined.

In conclusion, the detection of the density and location of CD3+ cells in ovarian cancer can be potentially developed as a routine test to find out not only valuable prognostic data, but also to stratify patients into those who may benefit from immunotherapy, and those in whom the immune system should perhaps be inhibited as it acts as a promoting factor and a driving force to enhance spread and resistance by causing immuno-sculpting. However, elucidation of the phenotype and function of T cells will also be critically important. The absolute numbers of T-cells should be interpreted with caution and with the clear understanding that cases of the immune system succeeding in preventing tumours rarely arrive under a pathologist's microscope. This conclusion is well aligned with the concept of the important role of T cells in anti-tumour immune surveillance.

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