EUROPEAN JOURNAL OF CANCER 46 (2010) 944–953

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Trop-2 overexpression as an independent marker for poor overall survival in ovarian carcinoma patients

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ARTICLE INFO

Article history:
Received 11 November 2009
Accepted 14 December 2009
Available online 8 January 2010

Keywords:
Trop-2
Epithelial ovarian cancer
Immunohistochemistry
Prognosis
Biomarker

ABSTRACT

Background: Prognostic factors currently available are insufficient to predict the clinical course of epithelial ovarian cancer (EOC). In a previous microarray study we identified the human trophoblast cell surface antigen Trop-2 as one of the top differentially expressed genes in serous papillary EOCs compared to normal human ovarian surface epithelial (HOSE) short-term cultures. The aim of the present investigation was to analyse Trop-2 expression at mRNA and protein level and to assess its prognostic significance in EOC.

Methods: Using quantitative real-time PCR we tested a total of 104 fresh-frozen EOC tissues and 24 HOSE for Trop-2 mRNA expression. Trop-2 protein expression was then examined by immunohistochemistry in matched formalin-fixed paraffin-embedded EOC samples and in 13 normal ovaries. Finally, we correlated Trop-2 expression to EOC conventional clinico-pathological features and patient outcomes.

Results: We found a significant Trop-2 mRNA and protein upregulation in EOCs compared to normal controls ($p < 0.001$). Trop-2 protein overexpression was significantly associated with the presence of ascites ($p = 0.04$) and lymph node metastases ($p = 0.04$). By univariate survival analysis, Trop-2 protein overexpression was significantly associated with decreased progression-free ($p = 0.02$) and overall survival ($p = 0.01$). Importantly, Trop-2 protein overexpression was an independent prognostic marker for shortened survival time in multivariate Cox regression analysis ($p = 0.04$, HR = 2.35, $CI_{95\%} = 1.03–5.34$).

Conclusions: Our results indicate, for the first time, that Trop-2 protein overexpression correlates with an aggressive malignant phenotype and may constitute a novel prognostic factor for EOC. The targeting of Trop-2 overexpression by immunotherapeutic strategies may represent an attractive and potentially effective approach in patients harbouring EOC.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.12.019

1. Introduction

Epithelial ovarian cancer (EOC) represents the most lethal gynecological tumour in developed countries. In 2008, it has been responsible for 21,650 new cancer cases and 15,520 deaths in the United States.[1] Poor prognosis is a reflection of EOC's insidious onset along with the frequent presentation at advanced stage and the development of chemoresistant disease despite high initial response to standard treatment (i.e. cytoreductive surgery and platinum-based chemotherapy).[2]

No combination of known prognostic parameters is able to adequately predict EOC relapse and clinical course,[3] thus the identification of innovative outcome-informative markers would be helpful in planning and individualisation of therapy. With this aim, our group has recently reported the results of an EOC gene expression study through microarray technology in which many cancer-related genes have emerged as promising tumour marker candidates.[4] The gene encoding for human Trop-2 (also termed TACSTD2, GA733-1, M1S1, EGP-1) was found to be highly overexpressed in ovarian serous papillary carcinomas (OSPCs) compared to normal human ovarian surface epithelium (HOSE) cell cultures.

Fornaro et al.[5] cloned the Trop-2 intronless gene, which encodes a 35,709-Da type 1 transmembrane protein, highly homologous to the cell–cell adhesion molecule Trop-1 (Ep-CAM, GA733-2).[6] Trop-2 was originally identified in human placental trophoblast[7] and it has been reported to be highly expressed by various types of human carcinomas, but rarely by normal adult tissues.[8–12]

The functional role of Trop-2 is not determined, although, as a cell surface receptor, it may play a role in regulating the growth of carcinoma cells.[5] Recently, Trop-2 overexpression was reported to be associated with invasive tumour phenotypes and poor prognosis in various type of human carcinomas.[13–16] Its wide distribution and selective expression on epithelial tumour cells, other than its membrane localisation as a cell surface receptor, render Trop-2 an attractive target for antibody-based treatments. In this study we have carefully analysed Trop-2 gene and protein expression in various EOC histological types and in normal controls. Moreover, we have investigated the correlation between Trop-2 expression and clinical factors to determine its clinicopathological significance in EOC. Finally, we have evaluated the prognostic significance of Trop-2 protein expression in a selected group of homogeneously treated EOC patients and in subgroups stratified according to histological types and post-operative residual tumour.

2. Patients and methods

2.1. Patients

Cancer tissue samples were obtained from 104 patients diagnosed with EOC and treated at the Division of Gynecologic Oncology of the University of Brescia (Italy), between June 2002 and June 2007. The study was approved by the Institutional Review Board and written informed consent was obtained from all patients enrolled. Each patient underwent primary cytoreductive surgery including total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and pelvic and periaortic lymph node sampling, with neoplastic cytological evaluation of ascites or peritoneal washing.

Age, histological type, stage, grade, residual tumour, presence of ascites and lymph node involvement were recorded in all cases. Tumour staging was in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria, while tumour grade and histological type were determined following World Health Organisation (WHO) standards; optimal cytoreduction was defined as no macroscopic residual tumour after primary surgery (TR = 0).

A homogeneous group of 95 patients, who received the same post-operative platinum-based chemotherapy, was selected for survival analysis. The patients were followed up from the date of surgery until death or the last observation (median follow-up, 28.5 months, range 7.3–77.7 months). At the time of the last follow-up, 39 patients (41%) were alive without evidence of disease, 12 patients (13%) were alive with disease and 44 patients (46%) were dead of disease (median overall survival, 46 months, $CI_{95\%} = 33–\infty$).

For subgroup analysis, the patients were stratified according to tumour histology (serous type and non-serous type) and post-operative residual disease (TR = 0 and TR > 0).

2.2. Tumour samples and HOSE

All EOC specimens were collected and immediately frozen as previously described.[4] All specimens were reviewed by a staff pathologist and only samples containing at least 70% tumour epithelial cells were used for total RNA extraction. A total of 24 HOSEs were cultured from normal ovaries of patients undergoing hysterectomy and bilateral salpingo-oophorectomy for benign pathologies. The average age of the control group was 55 years (range 47–69 years). HOSEs were generated as previously reported[4] and only cell cultures containing at least 99% epithelial cells were retained for total RNA extraction.

2.3. Total RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted and purified from 128 samples, including 82 primary EOC tissues with different histologies, 22 serous papillary omental metastases and 24 HOSE. Total RNA extraction and quality control were performed as previously reported.[4] Since Trop-2 is an intronless gene, the TaqMan Gene Expression Assay was designed within the exonic region. All RNA samples were then treated with TURBO DNase enzyme (TURBO DNA-free Kit; Ambion, Inc., Applied Biosystem Business, CA) to remove the contaminating DNA eventually present. Four micrograms of total RNA were digested with 2U of TURBO DNase enzyme in a 25-μl reaction for 30 min at 37 °C. The digestion was stopped by adding 2.5 μl of DNase Inactivation Reagent, followed by centrifugation. One micrograms of DNase-treated RNA was reverse transcribed using random hexamers in a final volume of 20 μl according to the SuperScript TM II RT RnaseH-reverse transcriptase protocol (Invitrogen Life Technologies, Carlsbad, CA, USA).

QRT-PCR was performed on the ABI PRISM 7000 Sequence detection System (Applied Biosystem, Applera UK, Cheshire,

UK) using the TaqMan Universal PCR master mix and the following Assay on Demand (Applied Biosystem): Hs00242741_s1 (TACSTD2). Reaction and thermal cycling conditions were performed as previously reported.[4] Negative controls consisting in reactions without reverse transcriptase were included to identify eventual genomic DNA contamination. The data were normalised using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal control.

2.4. *Immunohistochemistry on formalin-fixed tissues*

Immunohistochemistry was performed as previously reported[4] with minor modifications. Antigen retrieval was performed in a water bath containing EDTA buffer, pH 8, at 98 °C. The purified goat polyclonal antibody against the recombinant human Trop-2 extracellular domain (R&D Systems, Inc., Minneapolis, MN; diluted 1:100) was applied for 1 h. This antibody has been previously used in the majority of reports analysing Trop-2 immunohistochemical staining in various types of human carcinomas[13–15] and it was found by the manufacturer to have no cross-reactivity against multiple other adhesion molecules such as rhMCAM, rhNCAM-L1 and rhBCAM.

A secondary biotinylated anti-goat antibody (Vector Laboratories, Burlingame, CA; diluted 1:250) and the streptavidin–biotin complex (StreptABComplex/HRP, Dako, CA, USA) were applied, then 3′3-diaminobenzidine (Dako, CA, USA) was used as chromogen and the sections were counterstained by haematoxylin (Dako).

The slides were analysed at medium/high power view (20× and 40× magnification), and a scoring method based on the intensity of the staining and on the percentage of positive tumour cells was applied as follows: intensity was scored 0 (negative), 1 (weak), 2 (moderate) and 3 (strong), while the percentage of positive cells was scored as 0 (0%), 1 (1–10%), 2 (11–50%) and 3 (51–100%). A single scale with scores 0–9 was obtained multiplying the intensity and the percentage staining score and a total score was calculated grouping score 0 in total score 0, 1–3 in total score 1, 4 and 6 in total score 2 and 9 in total score 3.

2.5. *Statistical analysis*

The association between microarray and qRT-PCR data for Trop-2 gene was evaluated using Spearman rank correlation.

The variation in Trop-2 gene expression measured by qRT-PCR between EOCs and HOSE was evaluated using a *t*-test (after log transformation), while the difference in immunohistochemical staining between groups, considered on the ordinal scale, was investigated using Wilcoxon–Mann–Whitney test. The variation among EOC histotypes in Trop-2 gene expression was evaluated using a standard parametric ANOVA, while differences in Trop-2 protein expression were tested with a non-parametric one-way ANOVA. Approximate *p*-values were computed through Monte Carlo resampling (using $B = 9999$ replications). Multiple comparison correction was applied for paired tests using a step-down procedure. The correlation between Trop-2 expression measured by qRT-PCR and IHC staining was evaluated by means of the polyserial correlation coefficient. The association between Trop-2 mRNA expression and clinicopathologic parameters was investigated with an ANOVA (after log transformation). The association between IHC, coded as $\leqslant 1$, 2 and 3, and clinical covariates was evaluated by means of Kruskal–Wallis and Wilcoxon–Mann–Whitney tests. For survival analysis, three end-points (cancer relapse, cancer progression and death due to cancer) were used to calculate disease-free survival (DFS), progression-free survival (PFS) and overall survival (OS), respectively. DFS was defined as the time interval between the date of surgery and the date of identification of disease recurrence, PFS was defined as the time interval between the date of surgery and the date of identification of progressive disease (disease not treatable with curative intent) and OS was defined as the time interval between the date of surgery and the date of death. For all three end-points the last date of follow-up was used for censored subjects. Survival models were fitted using the Cox proportional hazard models, while survival curves were drawn based on the Kaplan–Meier methods. The effect of Trop-2 qRT-PCR expression on prognosis was evaluated categorising the qRT-PCR values in tertiles computed on the whole cohort (low: <891; medium: 891–1782.9; high: >1782.9), while immunostaining was analysed both as a continuous variable and as $\leqslant 1$, 2 and 3. In all analyses, a p value < 0.05 was considered significant. All statistical analyses were performed using the R language.

3. Results

3.1. *Evaluation of Trop-2 gene expression by quantitative RT-PCR*

QRT-PCR was used to validate microarray data for Trop-2 gene in the same 19 EOCs (OSPCs) and 15 HOSEs analysed in our previous paper.[4] QRT-PCR results significantly correlated to the microarray data ($p < 0.05$; rs = 0.48).

QRT-PCR analysis was then extended to a total of 82 primary EOCs with different histologies, 22 serous papillary omental metastases and 24 HOSEs. Trop-2 mRNA expression was significantly higher in patients with EOC compared to HOSE (fold change = 84.8; $p < 0.001$). The optimal cutoff point was determined by means of a receiver-operating characteristic curve, and it was set at relative quantification equal to 103.87. According to the chosen threshold, 23 of 24 HOSEs were negative for Trop-2 mRNA expression, while 103 of 104 tumour samples resulted positive. Therefore, the sensitivity and specificity of the test were 99% and 96%, respectively. All EOC histological types displayed a significant higher Trop-2 mRNA expression compared to HOSE ($p < 0.01$), while no significant difference among EOC histological types was detected ($p = 0.19$).

3.2. *Validation of Trop-2 protein expression by immunohistochemical staining*

To confirm Trop-2 gene expression results at the protein level, immunohistochemistry for Trop-2 was carried out on 82 primary EOCs of different histological types, 22 omental metastases of serous papillary ovarian carcinoma and 13 normal

ovaries. A positive membrane staining for Trop-2 was detected in 96 of 104 (92%) ovarian cancer specimens, while only 2 of 13 (15%) normal ovaries showed a very weak immunoreactivity for Trop-2 localised in the surface epithelium ($p < 0.001$, Fig. 1). The majority of primary EOCs of serous papillary, endometrioid, mucinous and mixed type, as well as serous papillary omental metastases showed a strong to moderate intensity staining for Trop-2 (Table 1, Fig. 1B, C, E, G and H), while the majority of primary clear cell and undifferentiated EOCs showed a weak staining (Table 1, Fig. 1D and F). In all EOCs, Trop-2 immunoreactivity was localised exclusively to the membrane of neoplastic epithelium, while tumoural stromal cells were regularly negative (Fig. 1B–H). A fine diffuse cytoplasmic staining was only detected in mucinous EOC (Fig. 1E). Significant differences in Trop-2 expression were found between clear cell carcinomas and both mucinous ($p = 0.02$) and serous papillary carcinomas ($p \leqslant 0.01$). Moreover, serous papillary EOC showed a significant Trop-2 overexpression compared to undifferentiated EOC ($p = 0.03$). Finally, qRT-PCR data for Trop-2 mRNA expression were significantly correlated to the IHC results performed in paired tumour samples ($p < 0.01$, rs = 0.26).

3.3. Trop-2 expression and clinicopathologic variables

Trop-2 protein expression was significantly associated with histological type (Kruskal–Wallis test $p < 0.01$), presence of ascites (Wilcoxon–Mann–Whitney test $p = 0.04$) and lymph node metastases (Wilcoxon–Mann–Whitney test $p = 0.04$) (Table 1). No significant correlation was found between Trop-2 mRNA expression and clinicopathological variables (Table 2).

3.4. Trop-2 expression and patient survival

As expected, known EOC clinical prognostic factors such as FIGO stage, histological type, residual tumour, presence of ascites and lymph node involvement showed a statistically significant association with OS and PFS in univariate analyses ($p < 0.05$, Table 3). In addition, Trop-2 protein overexpression showed a significant association with a poor OS ($p = 0.01$) and shorter PFS ($p = 0.02$) (Table 3). Kaplan–Meier survival curves show that OS and PFS significantly decreased in patients with high Trop-2 protein expression (score = 3) compared to those with none or weak Trop-2 protein expression (score $\leqslant$ 1) (Fig. 2A and B). In the subset of patients with none

Table 1 – Clinical and pathologic characteristics of 104 epithelial ovarian cancer patients and their association to Trop-2 protein expression.

		Trop-2 protein expression			
	Total	Score $\leqslant$ 1 *n*(%)	Score = 2 *n*(%)	Score = 3 *n*(%)	*p*-Value
Age at diagnosis (y)					
$\leqslant$40	9	4(44.4)	4(44.4)	1(11.1)	0.59*
>40	95	35(36.8)	44(46.3)	16(16.8)	
Histological-type					
Serous papillary	31	10(32.3)	15(48.4)	6(19.4)	
Endometrioid	18	6(33.3)	10(55.6)	2(11.1)	
Clear cell	11	8(72.7)	3(27.3)	0	
Mucinous	3	0	3(100)	0	<0.01**
Mixed	14	5(35.7)	7(50)	2(14.3)	
Undifferentiated	5	5(100)	0	0	
Serous papillary omental metastases	22	5(22.7)	10(45.5)	7(31.8)	
FIGO stage					
>IIB	81	29(35.8)	36(44.4)	16(19.8)	
$\leqslant$IIB	23	10(43.5)	12(52.2)	1(4.3)	0.20*
Histological grade					
G1	6	2(33.3)	4(66.7)	0	
G2 + G3	98	37(37.8)	44(44.9)	17(17.3)	0.75*
Residual tumour (TR), cm					
TR = 0	42	18(42.9)	21(50)	3(7.1)	
TR > 0	62	21(33.9)	27(43.5)	14(22.6)	0.10*
Presence of ascites					
No	43	21(48.8)	17(39.5)	5(11.6)	
Yes	61	18(29.5)	31(50.8)	12(19.7)	0.04*
Lymph nodal involvement					
Negative	52	24(46.2)	21(40.4)	7(13.5)	
Positive	28	6(21.4)	16(57.1)	6(21.4)	
Missing	24	9(37.5)	11(45.8)	4(16.7)	0.04*

* Wilcoxon–Mann–Whitney test.
** Kruskal–Wallis test.

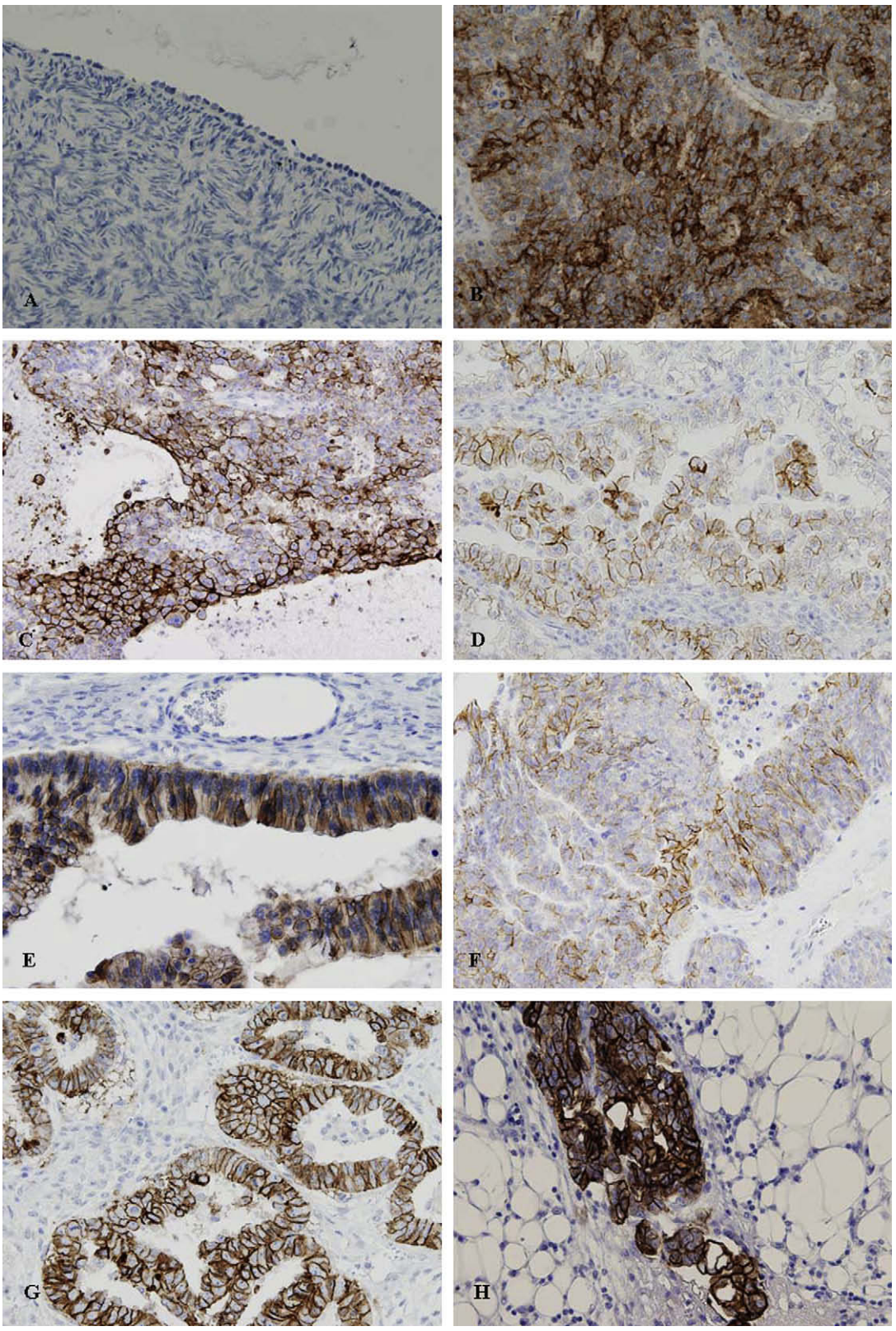

Fig. 1 – Representative immunohistochemical staining for Trop-2. Normal ovary is negative for Trop-2 (A). Positive Trop-2 membrane staining (B–H): serous papillary (B), endometrioid (C), clear cell (D), mucinous (E), undifferentiated (F), mixed (G) primary EOCs and serous papillary omental metastases (H). (Original magnification 20×.)

or weak Trop-2 protein expression (score $\leqslant 1$), median OS time was 57 months and decreased to 35 months for score 2 and 16 months for score 3. Similarly, the median PFS for the group with low or negative Trop-2 protein expression

Table 2 – Clinical and pathologic characteristics of 104 epithelial ovarian cancer patients and their association with Trop-2 mRNA expression.

Parameters	Fold change	95%CI	*p*-Value
Age at diagnosis (y)			
>40 versus ⩽40	1.139	0.582–2.227	0.7
Histological type	–	–	0.19
FIGO stage			
⩽IIB versus >IIB	0.802	0.510–1.261	0.34
Histological grade			
G2 + G3 versus G1	0.548	0.246–1.220	0.14
Residual tumour (TR), cm			
TR > 0 versus TR = 0	1.147	0.781–1.684	0.48
Presence of ascites			
Yes versus no	1.340	0.917–1.957	0.13
Lymph node involvement			
Positive versus negative	0.960	0.609–1.512	0.86

(score ⩽ 1) was 57 months and decreased to 38 months for score 2 and 16 months for score 3. Regarding DFS, FIGO stage, residual tumour, presence of ascites and lymph node involvement were of prognostic significance, whereas Trop-2 immunostaining was not. No significant correlation was found between Trop-2 mRNA expression and survival variables (Table 3).

FIGO stage, residual tumour, presence of ascites and Trop-2 immunoreactivity were included in a multivariate analysis. Trop-2 protein overexpressions, along with advanced FIGO stage, were identified to be independent predictive factors for poor OS (both $p = 0.04$, Table 3). Moreover, Trop-2 protein immunostaining showed to be marginally significant as prognostic factor for shorter PFS ($p = 0.07$, Table 3). Regarding DFS, FIGO stage and presence of ascites were of prognostic significance, whereas Trop-2 protein expression was not (Table 3). Furthermore, we performed separate analyses in subgroups of patients stratified according to histological type and residual tumour. Univariate analysis revealed that patients with serous type tumour and strong overexpression of Trop-2 protein (score 3) had a significant poor OS and shorter PFS ($p = 0.03$) than patients with none or weak expression of Trop-2 (score ⩽ 1) (Fig. 3A). In multivariate analyses, Trop-2 overexpression retained its significance as an independent prognostic factor for poor OS ($p = 0.04$), while remained marginally significant for shorter PFS ($p = 0.07$). Additionally, in the subgroup of patients with suboptimal debulking, univariate analysis revealed that Trop-2 protein overexpression was significantly associated with poor OS ($p = 0.01$) and shorter PFS ($p < 0.01$) (Fig. 3B). The survival differences, either for OS or for PFS, remained significant in multivariate analyses ($p < 0.01$ and $p = 0.02$, respectively). Finally, the patients with non-serous carcinomas and those optimally debulked did not show a significant association between Trop-2 protein expression and survival variables in univariate analysis model.

4. Discussion

In the present study we show that tumour-associated calcium signal transducer 2 (Trop-2) overexpression represents an independent prognostic factor for overall survival in ovarian

Table 3 – Univariate and multivariate analyses of OS, DFS and PFS in relation to clinical parameters and Trop-2 expression.

Variables	OS				DFS				PFS			
	N	HR	95%CI	*p*	N	HR	95%CI	*p*	N	HR	95%CI	*p*
Univariate analysis												
Trop-2 mRNA RQ												
High versus medium and low	95	0.85	0.43–1.65	0.63	69	0.86	0.41–1.79	0.69	94	1.01	0.56–1.83	0.96
Trop-2 IHC												
In continuous	95	2.83	1.22–6.53	0.01	69	1.53	0.57–4.13	0.39	94	2.47	1.11–5.48	0.02
Trop-2 IHC												
Score = 3 versus score ⩽ 1	95	2.87	1.27–6.44	0.01	69	1.41	0.45–4.33	0.54	94	2.54	1.17–5.52	0.02
Histological-type												
Non-serous versus serous	95	0.37	0.19–0.73	<0.01	69	1.77	0.91–3.44	0.08	94	0.495	0.27–0.88	0.01
Age												
⩽40 versus >40	95	1.07	0.33–3.49	0.90	69	0.88	0.21–3.69	0.86	94	1.20	0.43–3.36	0.71
FIGO stage												
⩽IIB versus >IIB	95	0.07	0.009–0.50	<0.01	69	0.10	0.02–0.45	<0.01	94	0.05	0.007–0.38	<0.01
Residual tumour												
TR = 0 versus TR > 0	95	0.28	0.12–0.60	<0.01	69	0.49	0.24–0.99	0.04	94	0.28	0.14–0.57	<0.01
Presence of ascites												
No versus yes	95	0.39	0.20–0.77	<0.01	69	0.35	0.17–0.72	<0.01	94	0.39	0.21–0.73	<0.01
Lymph node involvement												
Positive versus negative	75	2.33	1.10–4.92	0.02	59	2.63	1.28–5.40	<0.01	75	1.99	1.03–3.84	0.03
Multivariate analysis[*]												
Trop-2 IHC												
Score = 3 versus score ⩽ 1	95	2.35	1.03–5.34	0.04	69	0.99	0.31–3.14	0.99	94	2.06	0.93–4.53	0.07

Abbreviations: HR = hazard ratio, 95%CI = 95% confidence interval, IHC = immunohistochemistry, and RQ = relative quantification.

[*] Multivariate model accounted for FIGO stage, residual tumour and presence of ascites.

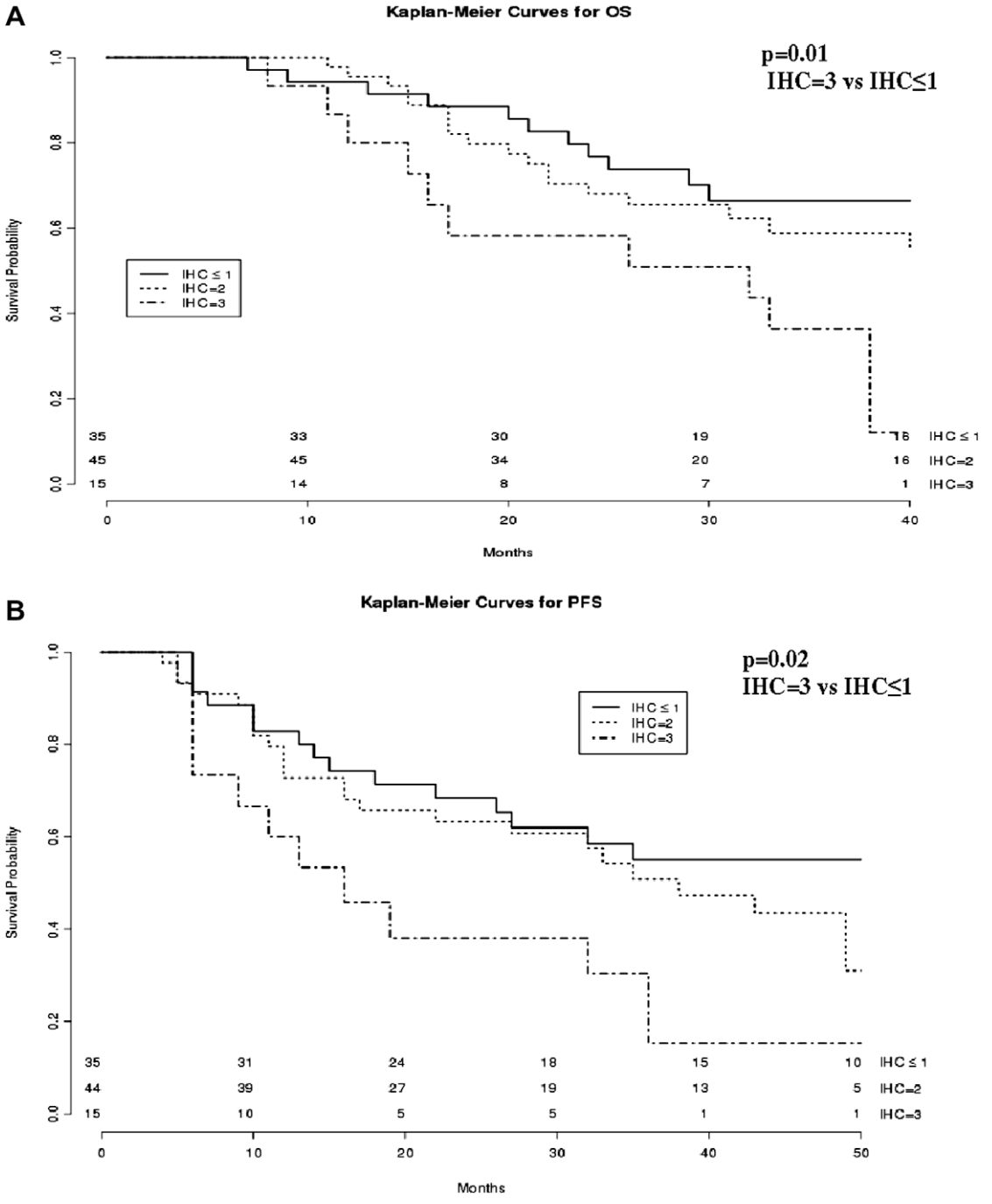

Fig. 2 – Kaplan-Meier survival curves for EOC patients according to immunoexpression of Trop-2 on the entire patient cohort. (A) Overall survival (OS) and (B) progression-free survival (PFS).

carcinoma (EOC). In analogy with the previously reported data[17,18] Trop-2 mRNA and protein were overexpressed in EOCs compared to normal human ovarian surface epithelial (HOSE) short-term cultures. Moreover, in accordance with Köbel et al.,[19] higher levels of Trop-2 protein were detected in serous papillary carcinoma compared to clear cell carcinoma. Trop-2 protein expression was significantly associated with tumour histology, along with the presence of ascites and lymph node involvement, suggesting a relation between biological aggressiveness and Trop-2 overexpression in EOCs. In univariate analysis Trop-2 protein overexpression significantly correlated with poor PFS and OS and, by multivariate analysis, it was shown to be an independent prognostic predictor of OS in EOC patients. Moreover, in the subgroup of patients either with serous EOC or with suboptimal surgical debulking, Trop-2 overexpression identified patients with worse prognosis, since it was significantly correlated with poorer OS in both univariate and multivariate analyses.

The reason why Trop-2 immunohistochemical staining was found to be an independent prognostic marker while

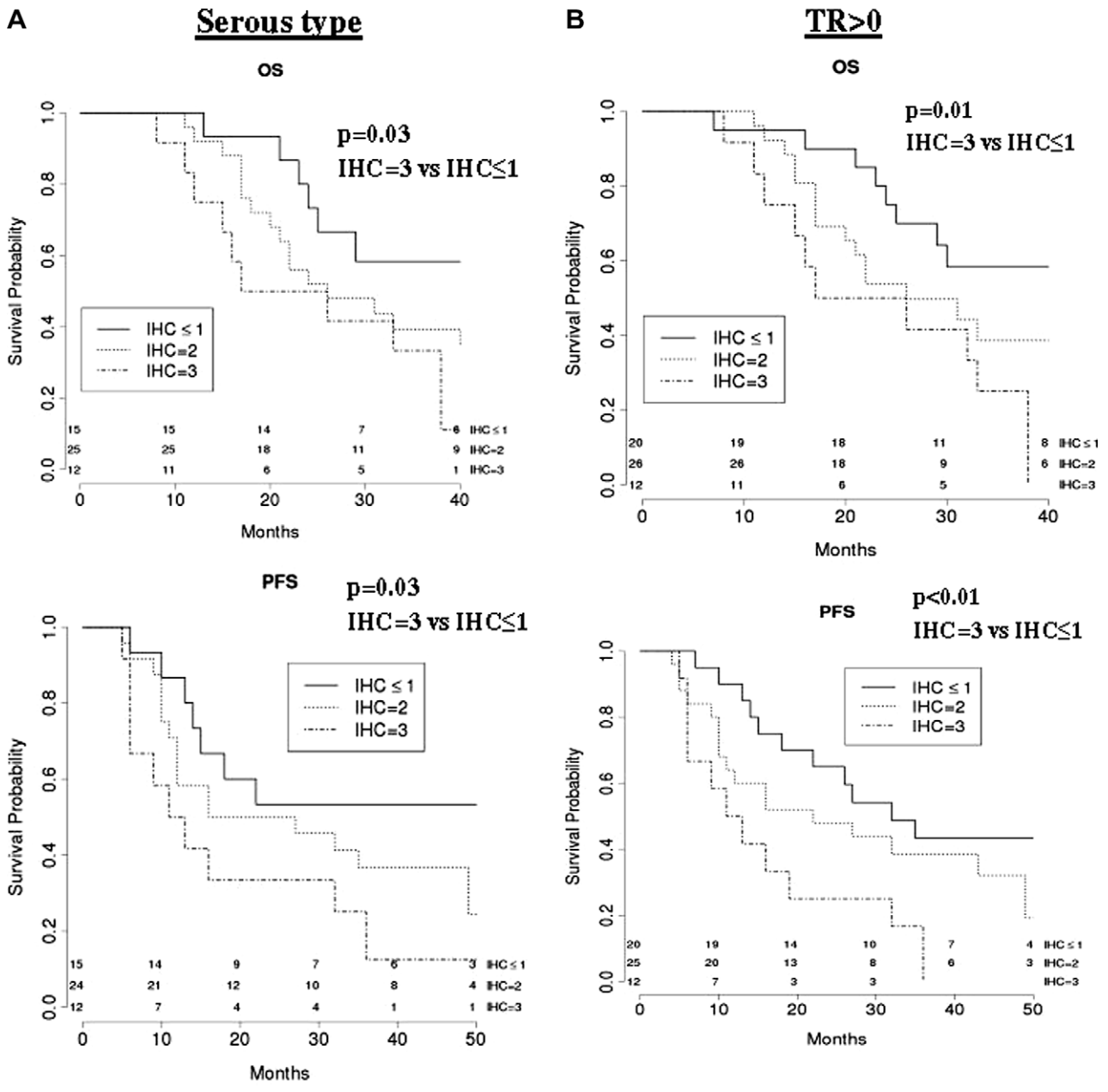

Fig. 3 – Kaplan-Meier survival curves for EOC patients according to Trop-2 immunoexpression stratified for histological type and residual tumour. Kaplan–Meier OS and PFS curves in the subgroup of patients with serous-type carcinoma (A) and in the subgroup of patients with suboptimal debulking (B).

mRNA expression was not, it is open to speculation. On the other hand, this result was not unexpected considering the low correlation coefficient between Trop-2 mRNA and protein expression found in our analysis (rs = 0.26). In this regard, a discrepancy between the abundance of mRNA and the encoded protein has been previously reported in several studies[20–22] and it is presumably a result of Trop-2 gene expression biology, suggesting various levels of regulation during protein synthesis (post-transcriptional regulation mechanisms involving mRNA stability, translation initiation and protein stability), a frequent phenomenon in higher organisms.

Our data on the prognostic significance of Trop-2 expression are in contrast with those obtained by Köbel et al.,[19] who failed to identify any significant association with outcome. Although the reason for the discrepancy in results between the two studies is not well understood, several factors may likely explain the different conclusions. These include divergent scoring methods or, more likely, technical differences in sampling, since we evaluated by immunostaining full tumour sections and not duplicate tissue cores.

Consistent with this view, our data support and extend in EOC previously reported results in several other human carcinomas including gastric, pancreatic and colon cancers,[13–16] where high Trop-2 protein expression was found to correlate with a more aggressive tumour phenotype and predict poor OS. The relationship between high Trop-2 expression and

aggressiveness of epithelial neoplasms remains unclear, since the role of this protein in tumourigenesis has not been fully elucidated. It has been suggested that Trop-2, possessing cytoplasmic potential serine and tyrosine phosphorylation sites, might function as cell signal transducer and regulator of tumour cell growth.[6,23] Importantly, a recent paper has evidenced that a large fraction of human cancers expresses a bicistronic CYCLIN D1-TROP2 mRNA chimera, acting as an oncogene able to induce aggressive tumour growth.[18] Another recent study has reported that Trop-2 is necessary for tumourigenesis in colon carcinoma cell lines, showing that Trop-2 targeting with specific antibodies resulted in inhibition of tumour cell migration and invasion; these data, in addition, have suggested a potential therapeutic role for passive immunotherapy with anti-Trop-2 mAbs in colon cancer.[24]

Trop-2 protein has several structural features homologous to the cell-cell adhesion molecule EpCAM,[6] which was found to be overexpressed and to represent a strong prognostic marker for poor survival in ovarian carcinoma.[25] Due to its immunogenicity, EpCAM antigen has been targeted by monoclonal antibodies in several in vitro studies investigating the potential role of anti-EpCAM immunotherapy in EOC.[26,27] Of interest, a phase I/II study on immunotherapy with Catumaxomab, a monoclonal antibody binding simultaneously to EpCAM and to CD3-antigen, has been recently demonstrated to induce tumour cell killing and to prevent ascites accumulation in EOC patients.[28]

In contrast to EpCAM, however, the immunogenic properties of the Trop-2 antigen have so far been investigated only in preclinical studies. In this regard, the murine monoclonal antibody RS7 specifically developed against Trop-2, has been shown to possess pancarcinoma reactivity.[12] Moreover, radiolabelled and applied for tumour imaging, it has been demonstrated to efficiently target tumour cells in several human cancer xenografts and to represent a potential therapeutic tool. In another study, a humanised version of RS7 was successfully generated with promising anti-neoplastic activity in an in vivo breast cancer model.[29–31]

In summary, this study reported for the first time that Trop-2 may represent a marker able to identify patients harbouring a more aggressive EOC phenotype and an independent prognostic factor for OS. If our data will be confirmed on a larger patients' cohort, the evaluation of Trop-2 immunostaining might be useful to select high-risk patients who could benefit from individualised treatments. Furthermore, since Trop-2 protein is overexpressed by EOC cells and rarely by normal tissues, it could represent an attractive target for specific immune-based therapies against EOC with potentially limited toxicity. With this aim, our group is currently applying phage display technology to develop a human antibody in a single-chain fragment format (scFv) specifically directed against Trop-2, as recently reported by our research group against another antigen found highly differentially expressed in ovarian cancer (i.e. claudin-3).[32]

Conflict of interest statement

None declared.

Acknowledgements

This investigation was supported by grants from the Nocivelli, the Camillo Golgi and the Berlucchi Foundations Brescia, Italy and by grants from the Istituto Superiore di Sanità (Programma Italia-USA Farmacogenomica Oncologica, 527B/2A/1 and Programma Onco-tecnologico), Rome, Italy and the Centre for Innovative Diagnostics and Therapeutics (IDET), Brescia, Italy.

We wish to thank Prof Piergiovanni Grigolato and Dr. Carla Donzelli for their support in providing samples. We are also grateful to Mr. Franco Alpi for his essential technical contribution in performing immunohistochemical staining.

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