BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2018
VOL. 75, NO. 2, 88–91
https://doi.org/10.1080/09674845.2017.1394052

BIOMEDICAL SCIENCE IN BRIEF

Serum S100 calcium binding protein A4 (S100A4, metatasin) as a diagnostic and prognostic biomarker in epithelial ovarian cancer

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ARTICLE HISTORY Received 17 September 2017; Accepted 29 September 2017

KEYWORDS Epithelial ovarian cancer; prognosis; diagnosis; S100A4

Ovarian cancer is the leading cause of gynaecologic cancer death in developed countries and often presents at an advanced stage. The absence of specific symptoms and lack of reliable early diagnostic methods have resulted in the diagnosis of 70% of patients at an advanced stage, a rate that has remained unchanged for 30 years [1,2]. For patients diagnosed in stage I, the 5-year survival rate ranges from 50 to 90% whereas this rate drops to only 25% when the diagnosis occurs at a later stage. The poor detection of ovarian cancer in early stages is attributed to the lack of symptoms and the absence of reliable serum markers that can detect the disease at its onset. An elevated serum CA125 level can detect recurrent ovarian cancer, but its use in screening the general population is limited by a lack of specificity and modest sensitivity in the detection of early-stage disease as it is overexpressed in normal tissues under several physiological and pathological conditions such as pregnancy, endometriosis, liver cirrhosis and colon cancer [3].

S100A4 (also known as metastasin) is a small, multifunctional, calcium-binding protein with the ability to promote invasion and metastasis in several cancer types [4]. S100A4 can enter intercellular fluid by the tumour cell itself or by cells in the local tumour environment, and can exert multiple functions by interaction with receptors such as RAGE [5,6]. El-Abd et al. reported increased levels of S100A4 mRNA in breast cancer compared to benign breast disease and controls ($n = 20$, 15 respectively), and that increased levels were associated with poor 30-month overall survival. These data suggest that S100A4 mRNA may be a survival marker in breast carcinoma [7]. S100A genes can be useful as potential serological biomarkers for bladder cancer, and combined use of urine cytology with S100A genes can improve the sensitivity for detection of this disease cancer [8].

Recently, S100A4 expression was found to be significantly upregulated in ovarian cancer and associated with the clinical stage of EOC patients [9]. Nuclear S100A4 expression in primary carcinomas, FIGO stage IV and poor response to chemotherapy at diagnosis were associated with worse overall survival [10]. Nuclear expression of S100A4 is involved in the aggressive behaviour of ovarian carcinoma, and S100A4 is an autocrine/paracrine factor that plays an important role in this aggression [11]. However, the relation between serum S100A4 and clinical significance in ovarian cancer is still unknown. To clarify this position, we conducted a case-control and follow-up study to determine the potential diagnostic and prognostic value of serum S100A4 in ovarian cancer.

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the affiliated hospital of Qingdao University, Qingdao, China. A written consent for inclusion was obtained from all participants prior to sample collection. Between 2005 and 2011, 160 consecutive patients with invasive epithelial ovarian carcinoma (EOC), 52 patients with benign ovarian neoplasms (6 benign ovarian teratomas, 3 sclerosant tumours, 14 mucinous cystadenomas, 8 ovarian dermoid cysts, 6 serous cystadenomas, 10 fibromas, and 5 corpus luteum cysts) and 52 age matched female volunteers served as controls at the Department of Gynecology, the Affiliated Hospital of Qingdao University were included in the study. Clinical data were obtained from hospital medical records, including patient age, tumour size, FIGO stage, tumour grade, serum CA-125 level, ascites, lymph node metastasis, relapse status and recurrence. Clinical characteristics of the patients are shown in Table 1. All patients were surgically staged in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria. Patients who underwent preoperative chemotherapy and radiotherapy were excluded. The patients after primary cytoreduction received six cycles of adjuvant chemotherapy with paclitaxel–carboplatin. Patients after primary investigative diagnostic surgery (laparoscopy or laparotomy) received 3–4 courses of neoadjuvant chemotherapy and secondary

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Table 1. Clinicopathological factors associated with serum S100A4.

		Serum S100A4 (ng/ml)		
Variable	Number (*n*)	≥107 (*n*)	<107 (*n*)	*p*
Age (year)				0.078
≥60	108	60	48	
<60	52	32	20	
FIGO stage				0.001
I/II	67	21	46	
III/IV	93	71	22	
Grade				0.164
1	58	38	20	
2	53	34	19	
3	49	20	29	
Residual tumour size				0.347
≤1 cm	104	67	37	
>1 cm	56	25	31	
Histology				0.104
Serous type	58	34	24	
Mucinous type	28	15	13	
Endometriod type	31	16	15	
Clear cell type	24	18	6	
Mixed cell type	19	9	10	
Ascites (volume ml)				0.018
≤1000	70	31	39	
>1000	90	61	29	
Lymph node metastasis				<0.001
Yes	64	48	16	
No	96	44	52	
Chemotherapy				0.046
Platinum sensitive	89	47		
Platinum resistant	43	28		
Platinum refractory	28	17		
Recurrence				0.037
Yes	78	50	38	
No	82	42	40	
CA125 (ng/ml)				0.078
≥186	107	58	49	
<186	53	34	19	

cytoreductive surgery, followed by subsequent courses of chemotherapy with paclitaxel–carboplatin. All subjects had no family history of ovarian cancer and no diagnosis of a cancer during follow-up. Blood was collected prior to surgery, plasma was separated by 2 rounds of centrifugation at 1000 × g to remove cellular debris. Serum was stored at −70 °C until S100A4 and CA125 concentrations were measured by ELISA and the electrochemiluminescent immunoassay (ECLIA) according to the manufacturer's protocol (CycLex Co, Ltd, Wuhan, China).

The data are described as median (IQR) if not normally distributed and as mean (SD) if normally distributed. The Kruskal–Wallis test and corresponding *post hoc* analysis and Mann–Whitney test were conducted for comparison between groups. Receiver operating characteristic (ROC) curves for serum S100A4 assessed diagnostic accuracy in distinguishing EOC patients from benign or normal control subjects. ROC area under the curve (AUC) assessed diagnostic accuracy. Kaplan–Meier curves and log-rank tests were utilised for univariable survival analysis. We calculated *P* values to determine whether serum S100A4 related to the histopathologic and clinical features of EOC patients by the χ^2 test. All statistical analyses were performed with SPSS software (version 13.0). All statistical tests were considered statistically significant for a two-sided $p < 0.05$.

The three groups were age matched: 59.8 ± 9.6 years for controls, 58.3 ± 9.2 for benign ovarian disease and 60.6 ± 9.8 for those with EOC ($p = 0.374$). Compared with the benign ovarian neoplasms and control groups, patients with EOC had statistically higher serum S100A4 (median, range) [107 (8.3–1102.4) ng/ml, vs. 23 (2.7–180.4) ng/ml, $p < 0.01$] and [107 (8.3–1102.4) ng/ml, vs. 18 (1.8–163.4) ng/ml, $p < 0.01$]. Using 107 ng/ml as the cut-off value, EOC patients were then divided into group A ($n = 92$) as those S100A4 ≥ 107 ng/ml and group B ($n = 68$) as those with S100A4 < 107 ng/ml. In addition, serum CA125 was (median, range) [186 (5.6–28036.5) ng/ml in patients with EOC. Using 86 ng /ml as the cut-off value, EOC patients were then divided into group A ($n = 103$) as those CA125 ≥ 186 ng/ml and group B ($n = 57$) as those with CA125 < 186 ng/ml.

S100A4 level and clinicopathological features of EOC patients are shown in Table 1. There were significant links between S100A4 and clinicopathological characteristics of EOC, including tumour stage ($P = 0.001$), lymph node metastasis ($P < 0.001$), ascites volume ($P = 0.018$), recurrence ($p = 0.037$) and chemotherapy response ($P = 0.046$). S100A4 was not linked to age, grade, histology, tumour diameter, residual tumour size or serum CA125 level ($P > 0.05$) (Table 1).

ROC curve analysis quantified the diagnostic value the serum S100A4 in discriminating healthy control serum

from EOC serum and benign serum from EOC sera with an AUC of 0.83 (95% CI [0.76–0.91]) and 0.81 (95% CI [0.72– 0.89]), respectively. In addition, serum S100A4 discriminated FIGO stage I/II serum from III/IV serum with an AUC of 0.89 (95% CI [0.81–0.94]). ROC curves were constructed to compare the sensitivity and specificity of S100A4 for distinguishing early late-stage cases from non-cases (healthy and benign controls) with an AUC of 0.85 (95% CI [0.80–0.91]). Using a cut-off that maximises sensitivity + specificity, S100A4 had a sensitivity of 78% and specificity of 92% at a cut-off of 90.8 U/ml.

In univariate analysis, the disease-free survival of EOC patients was linked to S100A4 level [hazard ratio (HR) 2.26: 95% CI: 1.35–4.76, $p = 0.024$], FIGO stage [HR 3.26: 95% CI: 2.84–8.47, $p = 0.003$], chemotherapy [HR 1.28: 95% CI: 0.96–3.64, $p = 0.047$] and lymph node metastasis [HR 3.56: 95% CI: 3.07–9.16, $p = 0.001$], whereas the overall survival was linked with S100A4 level [HR 2.47:95% CI: 1.64–5.38, $p = 0.018$], FIGO stage [HR 3.83:95% CI: 3.15–10.6, $p = 0.001$], tumour grade [HR 1.23:95% CI: 0.94–3.46, $p = 0.017$] and lymph node metastasis [HR 3.87:95% CI: 3.32–12.4, $p = 0.001$] in overall survival. Multivariate analysis using Cox regression model indicated that S100A4 level [HR 2.96:95% CI: 2.13–5.85, $p = 0.004$] and FIGO stage [HR 2.89:95% CI: 2.31–9.48, $p = 0.002$] may serve as independent prognostic factors for disease-free survival; S100A4 level [HR 2.16:95% CI: 1.83–5.05, $p = 0.033$] and FIGO stage [HR 2.36:95% CI: 1.89–7.46, $p = 0.015$] may serve as independent prognostic factors for overall survival. Kaplan-Meier survival curves also demonstrated that EOC patients with high S100A4 level and advanced FIGO stage presented a significantly unfavourable disease-free survival time and overall survival time (Figure 1).

S100A4 is associated with both non-malignant and malignant human diseases. Several groups have reported a role of S100A4 in inflammation. S100A4 was also shown to be commonly overexpressed in cardiac hypertrophy. The expression of S100A4 in this model of tissue injury was linked to general elevated expression of cell growth-related proteins, leading to tissue remodelling during reconstitution of the myocardium. S100A4 has a profound impact in many types of solid cancers, where its upregulation causes tumour progression and metastasis formation. S100A4 expression levels in tumours are considered as a biomarker for the prognosis of both metachronous metastasis and survival of cancer patients [10,11].

Numerous reports have showed that S100A4 are also present in various biological fluids including blood and the levels of S100A4 are linked to the diagnosis and prognosis for disease. S100A4 is also secreted into the

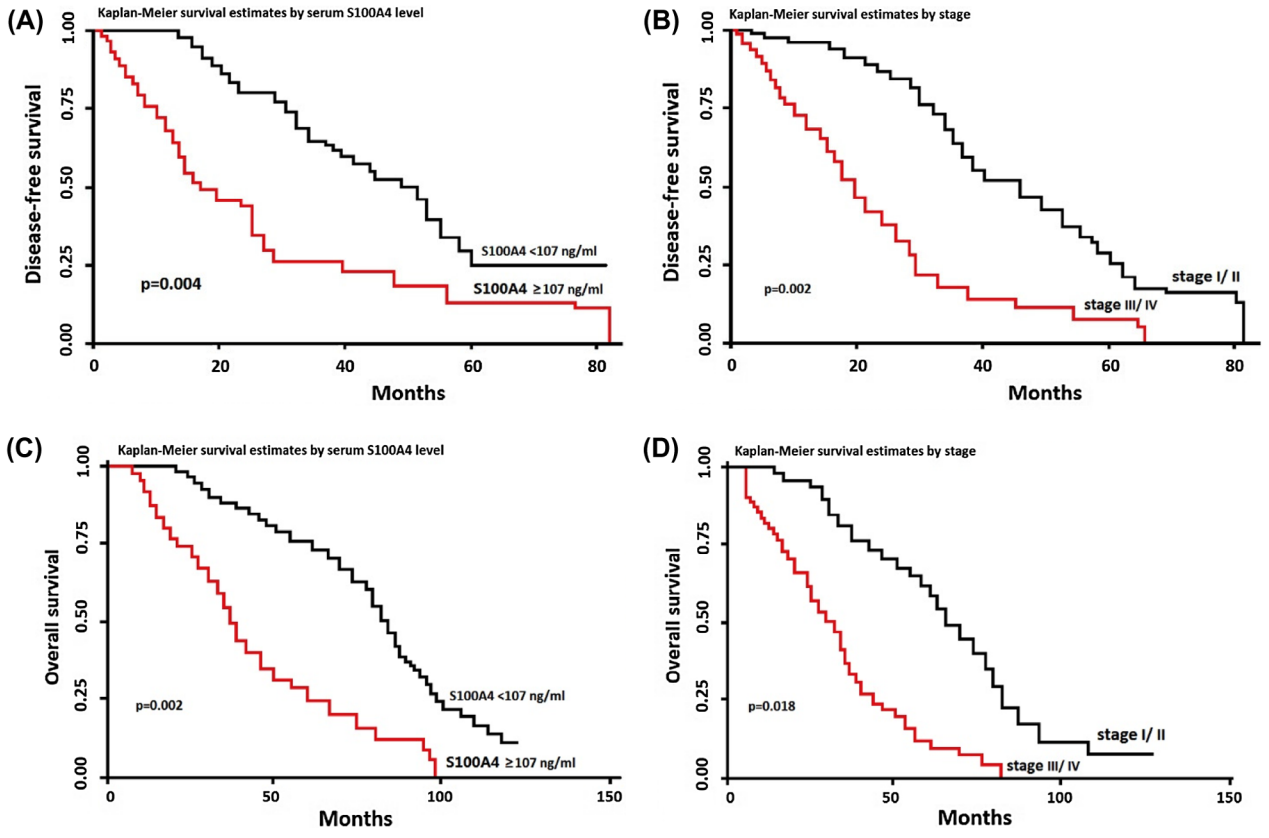

Figure 1. Survival analysis of EOC patients by Kaplan-Meier. (A) Disease-free survival rate in patients with high serum S100A4 level was significantly lower than that in patients with a low serum S100A4 level. (B) Disease-free survival rate in patients with I/II of FIGO stage was significantly lower than that in patients with III/IV of FIGO. (C) Overall survival rate in patients with high serum S100A4 level was significantly lower than that in patients with a low serum S100A4 level. (D) Overall survival rate in patients with I/II of FIGO stage was significantly lower than that in patients with III/IV of FIGO.

intercellular fluid-by the tumour cell itself or by cells in the local tumor environment [5,6]. Previous studies have reported that high serum S100A4 mRNA level reflects poor prognosis and so could be a diagnostic marker in certain cancers [7,8].

Our first major result is that S100A4 in patients with EOC is higher than in serum of healthy controls and benign ovarian disease, consistent with reports showing that the S100A4 protein in EOC tissues is highly upregulated compared to that tissues of benign ovarian disease [12]. We have also shown that high S100A4 level in EOC was linked to certain clinical pathologic parameters, including FIGO stage, lymph node metastasis, the ascites volume and recurrence. We also found that high serum S100A4 level in EOC was associated with chemoresistance, consistent with reports that high S100A4 protein expression in cancer tissues is associated with chemoresistance [13]. Therefore, serum levels of S100A4 are useful for predicting the prognosis and chemosensitivity of patients with EOC.

The detection of CA125 has been used in the follow-up of ovarian cancer. CA125 is not cancer-specific; it is also elevated in benign ovarian neoplasms and endometriosis, needing further investigation by imaging. In addition, some EOCs at early-stage do not generate sufficient CA125 for diagnostic purposes, leading to the view that serum CA125 has no clinical value for the follow-up monitoring the recurrence for postoperative patients with EOC [14]. We found that serum S100A4 discriminates healthy controls and benign disease from EOC, and that it distinguishes early late-stage cases from healthy and benign controls. These results point to a potential use of serum S100A4 for diagnosis of EOC, especially in early stages of EOC.

Kaplan-Meier analysis demonstrated that the life span of patients with high S100A4 is shorter than that of patients with low levels. Univariate analysis showed that serum S100A4, FIGO stage and lymph node metastasis were linked to life span (both in disease-free survival and overall survival) of EOC patients. Multivariate analysis further confirmed showed that high S100A4 and advanced FIGO stage independently predicted unfavourable disease-free survival and overall survival of EOC patients.

This work represents an advance in biomedical science because it shows that, unlike CA-125, serum S100A4 can discriminate between normal and EOC patients, especially in early-stage disease, and may be a novel diagnostic and prognostic marker for ovarian cancer.

Disclosure statement

No potential conflict of interest was reported by the authors.

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