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The potential predictive value of serum sRCAS1 levels for overall survival in endometrial cancer

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ABSTRACT

Objectives: The main aim of the study was to evaluate the impact of levels of serum soluble receptor-binding cancer antigen expressed on SiSo cells (sRCAS1) on the overall survival (OS) rates in patients with endometrial cancer. Furthermore, we analyzed sRCAS1 levels according to the clinicopathological characteristics of the disease.

Material and methods: The study group comprised 43 patients who were being treated for endometrial cancer. We included 10 low-risk, 20 intermediate-risk and 13 high-risk endometrial cancers using the criteria of the European Society for Medical Oncology (ESMO), the European Society for Radiotherapy & Oncology (ESTRO) and the European Society of Gynaecological Oncology (ESGO). Serum sRCAS1 levels were obtained before and after surgery. Serum sRCAS1 levels were assessed using the ELISA method.

Results: In our univariate analysis, both the pre- and post-surgery high sRCAS1 groups of patients with endometrial cancer indicated a shortened OS. However, in our multivariate analysis, when patients' age and disease-related risk was taken into consideration, only the post-surgery sRCAS1 levels remained as independent prognostic factors of a poor OS. Pre-treatment serum sRCAS1 levels were statistically significantly higher than post-surgery sRCAS1 levels; however, the difference between pre- and post-surgery sRCAS1 levels did not influence the patients' OS rate. Pre- and post-surgery sRCAS1 levels did not differ according to tumor grade, stage of the disease or the disease-related risk group.

Conclusions: High post-surgery serum sRCAS1 levels seem to be an independent indicator of shortened overall survival in patients with endometrial cancer.

Key words: sRCAS1; RCAS1; receptor-binding cancer antigen expressed on SiSo cells; endometrial cancer; cancer immunology

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INTRODUCTION

Endometrial cancer is the most common gynecologic cancer and it is the fifth most common cancer in women. Due to the early manifestation of symptoms endometrial cancer possess favorable prognosis, and it is the fourteen cancer in terms of women's mortality [1, 2]. However, even in the cases of localized endometrial cancers (FIGO IA and IB), the 5-year survival rate is 88% for the former (IA) and 75% for the latter (IB) [3]. That means that a significant proportion of the risk factors of patients with early stage endometrial cancer have been underestimated. Endometrial cancer is predominantly diagnosed in elderly patients, who are more prone to the adverse outcomes of adjuvant therapy. Thus, adjusted risk stratification may mean that unnecessary adjuvant therapy is abandoned. On the other hand, the incidence of endometrial cancer is increasing and it is now increasingly being diagnosed in premenopausal women, and even in women younger than 40 years of age (5%) [1]. This group of premenopausal patients is at increased risk of long-term adverse outcomes of radiotherapy. Furthermore, due to the present trend in delayed childbearing, fertility-sparing has become an important consideration in the treatment

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of patients with endometrial cancer [4]. Adjuvant therapy in cases of endometrial cancer is still being debated [5]. Especially the exact role of adjuvant chemotherapy in higher risk patients is still not fully elucidated [6]. Thus, evaluation of all prognostic factors is required before any final decision about adjuvant management can be made [6].

Currently, the prognosis for endometrial cancer is predominantly based on the FIGO stage of the disease, the histopathological type of the tumor, histopathological grade and the lymphovascular space invasion (LVSI) status. Based on these aforementioned factors, the European Society for Medical Oncology (ESMO), the European Society for Radiotherapy & Oncology (ESTRO) and the European Society of Gynaecological Oncology (ESGO) proposed new categories of risk groups to guide adjuvant therapy in patients with endometrial cancer [6]. However, risk stratification may also be evaluated using biological markers of cancerogenesis. For instance, a pooled analysis of PORTEC trial results indicated high L1 cell adhesion molecule (L1CAM) expression as an independent predictor for poor overall survival [7].

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1) was discovered in 1996 by Sonoda et al. [8] as a membrane protein present on cervical cancer cells. RCAS1 expression was found on a variety of cells, including T, B, and NK cells, and on macrophages, fibroblasts, and human neoplastic cells [9]. RCAS1 acts through the putative receptor, and its main function is related to the induction of selective immunosuppression. In the study by Nakashima et al. [10–12], authors showed that RCAS1 induces the arrest of cell growth and apoptosis in T, B and NK cells. Furthermore, immunohistochemical studies have shown an association between RCAS1 expression and the number of apoptotic lymphocytes and a negative correlation between RCAS1 expression and the quantity of tumor-infiltrating lymphocytes [13–15]. Despite the immunosuppressive activity of RCAS1, the protein can also contribute to tumor stroma remodelling creating a tumor-friendly microenvironment [16–18]. Specifically, RCAS1 interacts with noncancerous stromal cells (tumor associated macrophages, and cancer associated fibroblasts) to stimulate angiogenesis through VEGF, and its expression correlates with extracellular matrix proteases expression [16, 19–21].

RCAS1 can be secreted in the soluble form (sRCAS1) in the process of ectodomain shedding to the tumor microenvironment, and then to the blood system. Sonoda et al. [22], have shown that sRCAS1 also possesses an immunosuppressive capability, as it induces apoptosis of immune cells. Additionally, in their study of patients with cervical and endometrial cancer, Sonoda et al., showed there was a negative correlation between sRCAS1 and the number of peripheral blood lymphocytes [23].

Numerous studies have shown that tumor RCAS1 expression is an indicator of poor prognoses in patients with cancer [16, 24–29]. However, there is sparse data on serum sRCAS1 levels as a prognostic factor in human malignancies, and most of the data that does exist relates to the evaluation of short term outcomes [23]. From a clinical point of view, the most informative prognostic factors are those that influence patients' overall survival (OS) [28, 30]. To the best of our knowledge, there is no study evaluating the influence of serum sRCAS1 levels on the OS rates of patients with endometrial cancer.

Objectives

The main aim of our study was to evaluate the impact of pre- and post-surgery sRCAS1 levels on the overall survival rates in patients with endometrial cancer. Furthermore, we looked for correlations between sRCAS1 levels and clinicopathological features of the disease.

MATERIAL AND METHODS
Human subject

The study included 43 patients diagnosed with and treated for endometrial cancer. The median patient age was 65 and ranged from 43 to 84. The patients underwent treatment either in the Gynecology and Oncology Department of the Lukaszczyk Oncological Centerin Bydgoszcz or in the Gynecologic Oncology Department of the M. Sklodowska-Curie Memorial Institute in Krakow between 2007 and 2010. The patients were recruited consecutively from patients with newly diagnosed endometrial cancer admitted to our Departments for surgical treatment. Patients with significant co-morbidities, including, autoimmune connective tissue diseases or patients during immunosuppression, were excluded from the study. The study group included 37 patients with endometrioid adenocarcinomas, 2 with serous adenocarcinomas, one with adenosquamous carcinoma, one with clear-cell adenocarcinoma, one with carcinosarcoma and one with an undifferentiated carcinoma. The FIGO stages of the cancer patients were as follows: 1A — 11 patients; 1B — 22 patients; II — 2 patients; IIIA — 4 patients; IIIB —2 patients; and IIIC — 2 patients. Twenty-two tumors had G1 cancer, while 16 and 5 tumors were graded as G2 and G3 respectively. Using the combined consensual criteria of the European Society for Medical Oncology (ESMO), the European Society for Radiotherapy & Oncology (ESTRO) and European Society of Gynaecological Oncology (ESGO), the patients with endometrial cancers were divided into groups relating to the risk level of the disease. The study included 10 low-risk endometrial cancers (endometrioid type, grade 1–2, FIGO IA, LVSI negative), 20 intermediate-risk endometrial cancers (endometrioid type, grade 1–2, FIGO IB, LVSI negative), and 13 high-risk endometrial cancers (FIGO stage II or above, or non-endometrioid types). There were no "high-intermediate", "advanced" or "metastatic" risk groups among the endometrial cancers. For the survival analyses,

both low- and intermediate-risk cancers were included in a single subgroup.

We analyzed sRCAS1 levels in serum blood samples obtained both before and after surgery. Patients were treated with total abdominal hysterectomy with bilateral adnexectomy, and pelvic with/without paraaortic lymphadenectomy. Omentectomy was performed when serous endometrial adenocarcinoma was diagnosed. The low- and intermediate-risk group patients were either carefully followed-up postoperatively or they received brachytherapy. High-risk patients received chemoradiation.

Blood samples from patients treated surgically were collected directly prior to surgery and on the fourth day following hysterectomy. Blood samples were collected in a serum collection tube. A clot was permitted to form at room temperature for 30–60 minutes. The tube was placed on ice for 30 minutes to allow the clot to contract. The serum samples were then centrifuged at 3000x for 10 minutes at room temperature. Next, 1.0–2.0 mL samples were collected from the supernatants and stored at -80°C.

Each of the patients gave their written informed consent. Prior to the study, we obtained approval from the Jagiellonian University Ethical Committee (KBET/135/B/2007). Information on any patients who died was retrieved from the database of the Cuiavia-Pomerania and Lesser Poland National Health System of Poland. We analyzed patients' overall survival (OS) rates with respect to pre- and post-interventional sRCAS1 levels as well as with respect to the differences between pre- and post-surgery levels. The median follow-up period for patients was 1593 days (range 138–2468).

sRCAS1 levels assessment

The analysis of sRCAS1 concentrations in the serum samples was performed in the Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University. We used a commercially available ELISA kit for sRCAS1 detection (Medical & Biological Laboratories Co. Ltd, Nagoya, Japan). Briefly, the diluted serum samples were pre-treated with neuraminidase and incubated in the wells of the plates coated with anti-human RCAS1 monoclonal antibodies. After washing, the wells were incubated with biotin conjugated anti-RCAS1 antibodies, and this procedure was followed by a second washing and incubation of the wells with a streptavidin-peroxidase conjugate. Following a third washing, color reaction was developed using the tetramethylbenzidine/hydrogen peroxide substrate. The reaction was stopped by acidification of the contents of the wells, and the plates were then read at 450 nm on a microplate reader. The plates were individually calibrated by a quantitative sRCAS1 reference standard provided by the manufacturer of the set and expressed in arbitrary units (U/mL). The correlation coefficient of the dose-response curves we obtained was above 0.99.

Statistical analysis

The distribution of variables in the study groups was verified using the Shapiro-Wilk test. Parametric or non-parametric tests were used for evaluation according to data distribution. The difference between pre- and post-surgery sRCAS1 levels was investigated using Wilcoxon matched-pairs test. The differences between sRCAS1 levels according to tumor grade were investigated with the use of Kruskal-Wallis test, both in pre- and post-intervention group. The differences in sRCAS1 levels between low/intermediate and high-risk patients, and between FIGO I and FIGO II/III stage disease were evaluated using Mann-Whitney test, both in pre-and post-intervention group. The correlation between patient age and sRCAS1 levels was evaluated using Spearman Rank correlation. Survival analysis was conducted using Kaplan-Meier survival curves. The cut-offs between "high" and "low" pre- and post-surgery sRCAS1 levels were determined following OS analyses; namely that we have analyzed different cut-offs, and the level with the lowest P-value was chosen. Multivariate survival analysis was conducted using Cox proportional-hazards regression with the stepwise entering method.

RESULTS
Survival analysis

Patients' pre-treatment sRCAS1 levels below 10 U/mL (36 women) were associated with statistically significant longer overall survival when compared with patients with pre-treatment sRCAS1 levels above 10 U/mL (7 women). Similarly, postoperative sRCAS1 levels below 8 U/mL (33 women) indicated longer OS when compared with patients with sRCAS1 levels above 8 U/mL (10 women). The variation of sRCAS1 levels after treatment was not associated with patients' survival. Serum sRCAS1 levels were found to be decreased more than 10% in 20 women, while 23 patients had either stable or increased sRCAS1 levels after treatment (this subgroup included patients either with a decrease of less than 10% or a stable level, or an increased sRCAS1 level). Patients with sRCAS1 levels decreasing more than 10% had median survival not significantly different from that of the rest of the group.

We have found the patient survival to be related with patients' age. Patients older than 65 (23 women) had significantly shortened survival when compared with patients younger than 65 (20 women).

Patients with high-risk endometrial cancers (13 patients) had significantly shortened survival when compared with the patients with low- and intermediate-risk endometrial cancers (30 patients). In the multivariate survival analysis, only postoperative sRCAS1 levels (P = 0.03), patients' ages (P = 0.02), and the risks group according to ESMO-ESGO-ESTRO criteria (P = 0.02) were independent predictors of patients' overall survival rates.

Survival curves are presented in Figure 1. The results of our analysis are summarized in Table 1.
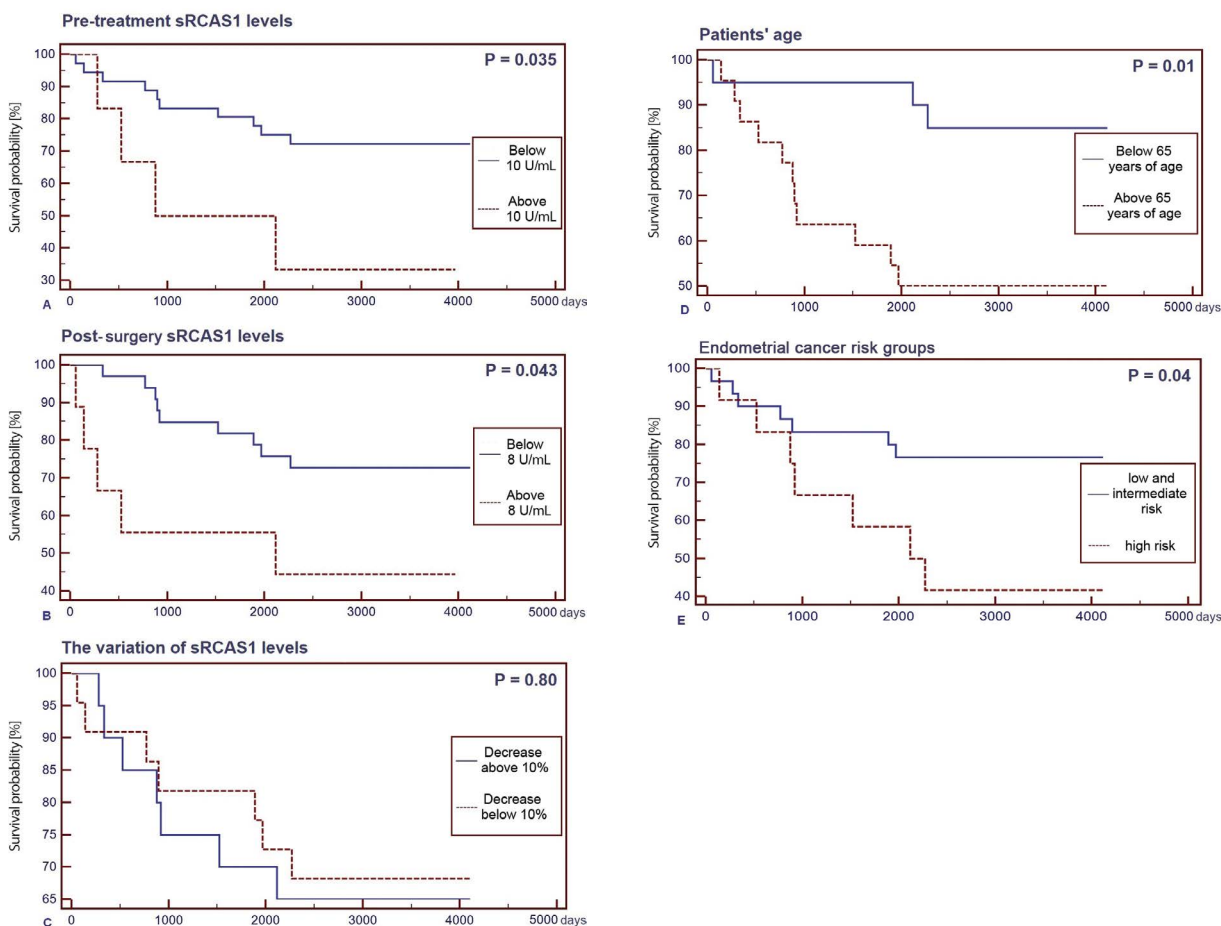
Figure 1. Analysis of patients' survival. **A)** Pre-treatment sRCAS1 levels. Group 1) below 10 U/mL (median survival: 3853 days, range 55–4117); Group 2) above 10 U/mL (1496 days, range 279–3967, P = 0.035). **B)** Postoperative sRCAS1 levels. Group 1) below 8 U/mL (median survival: 3855 days, range 336–4117); Group 2) above 8 U/mL (2116, range 55–3967; P = 0.043). **C)** Variation of sRCAS1 levels after treatment — the difference between postoperative and pre-surgery levels. Group 1) sRCAS1 levels decrease above 10% (median survival: 3889 days range 279–4104); Group 2) decrease below 10% and stable or increased sRCAS1 levels(3826 days, range 55–4117; P = 0.80). **D)** Survival according to patient age. Group 1) patients younger than 65 (median survival 3917 days, range 55–4117); Group 2) patients older than 65 (2854 days, range 138–4111, P = 0.01). **E)** Survival according to disease specific risk. Group 1) low- and intermediate-risk endometrial cancers (median survival: 3904 days, range 336–4084); Group 2) high-risk endometrial cancers (2975 days, range 55–4117; P = 0.04).

sRCAS1 levels and clinicopathological features of the disease

Pre-treatment serum sRCAS1 levels were statistically significantly higher than postoperative sRCAS1 levels (7.35 U/mL, range 3.40–66.16 compared with 6.85 U/mL, range 3.76–31.78, respectively; P = 0.0001).

We did not observe differences in pre- and post-surgery sRCAS1 levels regarding the grade of the tumor. Similarly, pre- and post-surgery sRCAS1 levels were not related either with FIGO stage of the disease or with the risk group. The results of the sRCAS1 assessment regarding the clinicopathological features of the disease we analyzed are summarized in Table 2.

The low-intermediate and high-risk groups of patients were not different in terms of patient age

(60.4 ± SD 9.26 compared with 60.52 ± SD 10.34, respectively; P = 0.37).

Pre-treatment sRCAS1 levels were positively correlated with patients' age (R Spearman = 0.37, P = 0.01). Similarly, postoperative sRCAS1 levels were positively correlated with patients' ages (R Spearman = 0.36, P = 0.02).

DISCUSSION

In the present study, our univariate survival analysis has shown that high sRCAS1 levels in both pre- and postoperative patients were predictive factors of shortened survival rates for endometrial cancer patients. However, when other prognostic factors, like disease specific risk and patients' ages were taken into consideration, only high postoperative sRCAS1 levels was associated with patients' overall

Table 1. Survival analysis according to pre- and post-surgery serum sRCAS1 levels

Univariate Survival Analysis

	Groups	Survival	P-Value
Pre-treatment sRCAS1 levels	below 10 U/mL (36 patients)	3853 days, range 55 –4117	P = 0.035
	above 10 U/mL (7 patients)	1496 days, range 279–3967	
Postoperative sRCAS1	below 8 U/mL (33 patients)	3855 days, range 336–4117	P = 0.043
	above 8 U/mL (10 patients)	2116, range 55–3967	
Variation insRCAS1 levels after treatment	decrease above 10% (20 patients)	3889 days, range 279–4104	P = 0.80
	decrease below 10% and stable or increased sRCAS1 levels (23 patients)	3826 days, range 55–4117	
Patients' age	older than 65 (23 patients)	2854 days, range 138–4111	P = 0.01
	younger than 65 (20 patients)	3917, range 55–4117	
Risk group	Low- and intermediate-risk (30 patients)	3904 days, range 336–4084	P = 0.04
	High-risk (13 patients)	2975 days, range 55–4117	

Multivariate Survival Analysis

	P-value	95% CI	P-Value
Patients' age	P = 0.02	1.34–17.83	P = 0.0025
Risk group	P = 0.02	1.16-10.14	
Pre-treatment sRCAS1 levels	Not included		
Postoperative sRCAS1	P = 0.03	1.12–10.77	

Risk associated with the disease was stratified according to ESMO-ESGO-ESTRO criteria. We have used Cox proportional-hazards regression multivariate survival with the stepwise entering method. Pre-treatment sRCAS1 levels were not included in the calculation due to the nonsignificant impact on patients' survival rates in the multivariate analysis.

Table 2. Pre- and postoperative sRCAS1 levels according to the tumor grade, FIGO stage of the disease and the risk group related to endometrial cancer

	Pre-treatments sRCAS1 levels Median U/mL (range)	P-Value
G1	7.35 (4.16–10.75)	
G2	6.52 (3.40–66.16)	P = 0.23
G3	7.82 (6.56–31.23)	
FIGO I	6.94 (3.40–42.25)	P = 0.17
FIGO II - III	7.75 (3.91–66.16)	
Low- and intermediate-risk	6.96 (3.4-42.25)	P = 0.78
High-risk	7.69 (4.16–42.25)	

	Postoperative sRCAS1 levels Median U/mL (range)	P-value
G1	6.73 (3.76–17.15)	
G2	6.51 (3.76–31.78)	P = 0.73
G3	6.13 (5.80–23.08)	
FIGO I	6.69 (3.74–23.08)	P = 0.37
FIGO II - III	6.76 (5.54–31.78)	
Low- and intermediate-risk	6.56 (3.76–17.15)	P = 0.23
High-risk	6.75 (5.54–31.78)	

Risks groups associated with the disease were stratified according to ESMO-ESGO-ESTRO criteria

survival rates. Our results may indicate, that the intensity of the selective suppression of the host's immune system and the tumor stroma modulation related to RCAS1 function are reflected in the patients' prognoses. In an earlier research study, Sonoda et al. [31], showed that high tumor RCAS1 expression was associated with shortened OS. In that study, patients' survival was progressively correlated with the degree of RCAS1 expression. Similarly, RCAS1 expression was an independent prognostic factor in multivariate analysis [31]. From a clinical point of view, analysis of serum markers is more feasible and less subjective when compared with immunohistochemical tumor analysis. Additionally, serum sRCAS1 levels are also available for patients who are not operated upon. Thus, for practical purposes, serum sRCAS1 levels seem to be more a useful prognostic factor when compared with evaluations of tumor RCAS1 expression.

RCAS1 expression has been shown to progress from the normal endometrium, through premalignant lesions, to invasive carcinomas [32, 33]. Sonoda et al. [31], showed there is an association between RCAS1 expression in endometrial cancer and the clinical stage of the disease. Similarly, Zhou et al. [33], observed higher RCAS1 expression in endometrial cancer characterized by deep myometrial and vascular invasion. In an earlier study by Sonoda et al. [32], the authors reported that there was higher RCAS1 expression in grade 3

endometrial cancers when compared with grade 1 and 2 tumors. However, it seems that there is lack of association between serum sRCAS1 levels and the clinicopathological characteristics of endometrial cancer. Sonoda et al. [23], have evaluated serum sRCAS1 levels in fifty patients with endometrial cancer. These authors showed that there were higher pretreatment sRCAS1 levels in the patients with endometrial cancer compared with those of the healthy controls. However, serum sRCAS1 concentrations were not correlated with clinical stage, histologic type, grade, or lymph node metastasis [23]. This finding concurs with our observations. Namely, we did not observed differences between pre- and postoperative sRCAS1 levels according to tumor grade or the FIGO stage of the disease. Furthermore, in our study, we have found no association between sRCAS1 levels and the risk groups according to ESMO-ESGO-ESTRO criteria. This observation is very important, because it suggest that serum sRCAS1 levels may serve as a prognostic factor independent from clinical features of the disease. However, the results of our study should be interpreted with caution because of the small number of cases.

Sonoda et al. [23], showed there are decreased levels of sRCAS1 after successful treatment. We also observed decreased levels of sRCAS1 following hysterectomy. However, we did not observe any relationship between the degree of sRCAS1 level changes and the patients' prognoses. Therefore, we conclude that while sRCAS1 levels decrease after treatment for endometrial cancer, patients' prognoses are more related to the absolute levels of sRCAS1.

We observed a strong correlation between both pre- and postoperative sRCAS1 and patient age. In the study by Sonoda et al. [23], serum sRCAS1 levels were not associated with patient age. The possible explanation of this discrepancy is that Sonoda et al. studied a single group combining endometrial and cervical cancer patients, although these two cancers occur in different age groups. However, another study by Sonoda et al. [31], reported a strong and positive correlation between tumor RCAS1 immunoreactivity and patient age. Patients' age is in general regarded as a negative prognostic factor in endometrial cancer [34, 35]. Our results confirm this observation, because advanced age was associated with shortened survival. Although in our study sRCAS1 levels were correlated with patient age, in our multivariate analyses, both patient age and sRCAS1 levels were shown to be independent prognostic factors of patient OS.

In conclusion, we have found that elevated serum sRCAS1 were shown to be associated with shortened overall survival in patients with endometrial cancer. However, more prospective studies are needed to confirm or reject the hypothesis that the serum sRCAS1 levels could be used to predict the OS in women treated for endometrial cancer.

Conflicts of interest

The authors declare no conflict of interests.

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