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Full length article

Asparaginase-like protein 1 as a prognostic tissue biomarker in clinicopathologically and molecularly characterized endometrial cancer

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ABSTRACT

Objective: Prognostic stratification of endometrial cancer involves the assessment of stage, uterine risk factors, and molecular classification. This process can be further refined through annotation of prognostic biomarkers, notably L1 cell adhesion molecule (L1CAM) and hormonal receptors. Loss of asparaginase-like protein 1 (ASRGL1) has been shown to correlate with poor outcome in endometrial cancer. Our objective was to assess prognostication of endometrial cancer by ASRGL1 in conjunction with other available methodologies.

Study Design: This was a retrospective study of patients who underwent primary treatment at a single tertiary center. Tumors were molecularly classified by the Proactive Molecular Risk Classifier for Endometrial Cancer. Expression of ASRGL1, L1CAM, estrogen receptor, and progesterone receptor was determined by immunohistochemistry. ASRGL1 expression intensity was scored into four classes.

Results: In a cohort of 775 patients, monitored for a median time of 81 months, ASRGL1 expression intensity was related to improved disease-specific survival in a dose-dependent manner ($P < 0.001$). Low expression levels were associated with stage II–IV disease and presence of uterine factors, *i.e.* high grade, lymphovascular space invasion, and deep myometrial invasion ($P < 0.001$ for all). Among the molecular subgroups, low expression was most prevalent in p53 abnormal carcinomas ($P < 0.001$). Low ASRGL1 was associated with positive L1CAM expression and negative estrogen and progesterone receptor expression ($P < 0.001$ for all). After adjustment for stage and uterine factors, strong ASRGL1 staining intensity was associated with a lower risk for cancer-related deaths (hazard ratio 0.56, 95 % confidence interval 0.32–0.97; $P = 0.038$). ASRGL1 was not associated with the outcome when adjusted for stage, molecular subgroups, L1CAM, and hormonal receptors. When analyzed separately within the different molecular subgroups, ASRGL1 showed an association with disease-specific survival specifically in “no specific molecular profile” subtype carcinomas ($P < 0.001$). However, this association became nonsignificant upon controlling for confounders.

Conclusions: Low ASRGL1 expression intensity correlates with poor survival in endometrial cancer. ASRGL1 contributes to more accurate prognostication when controlled for stage and uterine factors. However, when adjusted for stage and other biomarkers, including molecular subgroups, ASRGL1 does not improve prognostic stratification.

Abbreviations: ASRGL1, asparaginase-like protein 1; CI, confidence interval; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; MMR, mismatch repair; NSMP, no specific molecular profile; POLE, polymerase-ϵ; ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer; TCGA, The Cancer Genome Atlas.

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Introduction

Asparaginase-like protein 1 (ASRGL1) is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia [1]. The selective removal of asparagine, an amino acid necessary for growth, may diminish cell proliferation and induce apoptosis [2,3]. Abnormal ASRGL1 expression has been found in various cancers, including breast, ovarian, and prostate cancer [https://www.proteinatlas.org]. Compared with many other cancer types, endometrial cancer frequently exhibits medium or high ASRGL1 expression. ASRGL1 emerges as a predictive and prognostic biomarker in endometrial cancer, with low expression levels associated with lymph node metastasis and an unfavorable prognosis, even when adjusted for traditional clinicopathological risk factors [4–6].

Molecular classification, introduced by The Cancer Genome Atlas (TCGA) Research Network in 2013 [7], revolutionized the approach to risk-stratification in endometrial cancer. Based on molecular signatures, tumors can be subdivided into four subgroups: polymerase-∊ (*POLE*) ultramutated with excellent prognosis; microsatellite unstable hypermutated with intermediate prognosis; copy-number low with intermediate prognosis; and copy-number high with poor prognosis. Due to the heterogeneity of the copy-number low subgroup, it has a more variable prognosis compared with the microsatellite unstable hypermutated subgroup [8]. Consequently, the latter has been associated with a worse outcome in some studies [9–11].

Molecular classification is considered more objective, reproducible, and biologically informative compared with the traditional risk-stratification based on tumor morphological criteria [12]. Moreover, molecular classification has implications for adjuvant therapy, involving de-escalation and escalation of treatments in *POLE* ultramutated and copy-number high subgroups, respectively [13].

The significance of ASRGL1 in risk-stratifying endometrial cancer has not been thoroughly investigated beyond conventional risk factors. Thus, we aimed to elucidate risk-stratification based on ASRGL1 expression within a sizable institutional dataset distinguished by detailed clinicopathological and follow-up information, molecular subtype classification, and enrichment with annotations from the most established tissue biomarkers.

Material and methods

This was a retrospective cohort study of patients who underwent surgical treatment for stage I–IV endometrial carcinoma at the Department of Obstetrics and Gynecology, Helsinki University Hospital, between January 1, 2007, and December 31, 2012. The study was approved by the Helsinki University Hospital institutional review board and the National Supervisory Authority for Welfare and Health.

Clinicopathological data were abstracted from institutional medical and pathology records. Details regarding the cause of death were supplemented from death certificates derived from Statistics Finland when the information was not available in medical records. Stage was determined according to the International Federation of Gynecology and Obstetrics guidelines revised in 2009 [14]. Lymphovascular space invasion was defined as the presence of adenocarcinoma, of any extent, in endothelium-lined channels of uterine specimens outside the tumor.

We performed immunohistochemistry on multicore tissue microarray slides, prepared as described before [15]. The following monoclonal antibodies were used for chromogenic immunohistochemistry: MLH1 (ES05, Dako, Santa Clara, CA); MSH2 (G219-1129, BD Biosciences, San Jose, CA); MSH6 (EPR3945, Abcam, Cambridge, UK); PMS2 (EPR3947, Epitomics, Burlingame, CA); p53 (DO-7, Dako); L1 cell adhesion molecule (L1CAM, clone 14.10, Covance, Princeton, NJ); estrogen receptor alpha (SP1, Roche Diagnostics, Indianapolis, IN); progesterone receptor (clone 16, Novocastra Laboratories, Newcastle, UK); and ASRGL1 (HPA029725, Atlas Antibodies, Stockholm, Sweden).

Mismatch repair (MMR) protein status was considered deficient when we observed a complete loss of nuclear expression in carcinoma cells of one or more MMR proteins (MLH1, MSH2, MSH6, PMS2). Abnormal p53 staining was defined as strong and diffuse nuclear staining or completely negative ('null') staining in carcinoma cells. Weak and heterogeneous staining was classified as wild-type (wt) expression. Stromal cells and inflammatory cells served as internal controls for MMR and p53 stainings. L1CAM expression was scored as reported earlier, with $\geq$10 % of membranous staining considered positive [15–17]. We adopted a 10 % cut-off for estrogen receptor and progesterone receptor positivity based on a previous study on endometrial cancer [18]. ASRGL1 staining intensity was semi-quantitatively assessed in four classes (negative, weak, moderate, and strong). Similarly, the fraction of ASRGL1-immunoreactive tumor cells was scored in four classes (0–1 %, 2–50 %, 51–75 %, and >75 %).

POLE exonuclease domain mutation screening of pathogenic hot spots in exons 9, 13, and 14 was performed by direct sequencing [19]. Only samples with high quality sequence for all the four *POLE* hot spots examined were included in the study.

Molecular subgroups were recapitulated using the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) [20]. Tumors were classified as *POLE* mutant (*POLE*mut); MMR deficient (MMRd, surrogate to microsatellite unstable hypermutated in the TCGA classification system [7]); "no specific molecular profile" (NSMP, surrogate to copy-number low); and p53 abnormal (p53abn, surrogate to copy-number high). In the ProMisE classifier [20], tumors are classified in a stepwise fashion. The first subgroup assignment is based on MMR status. Tumors with intact MMR proteins undergo *POLE* mutational analysis and *POLE*wt tumors are classified as p53abn or NSMP.

Chi-squared test was used for comparison of categorical variables. Cohenskappa statistics were calculated to measure the agreement of ASRGL1 staining intensity and frequency. Areas under curve were compared with the 2-tailed receiver operating characteristic curve area comparison test. Disease-specific survival was calculated as the time from surgery to death from endometrial cancer. Survival times were estimated using the Kaplan-Meier method. Differences between groups were compared using the log rank test. Hazard ratios (HR) for cancer-related mortality were estimated using univariable and multivariable Cox regression analyses. Statistical significance was set at $P < 0.05$. Data were analyzed using the Statistical Package for the Social Sciences version 29 software (IBM Corp., Armonk, NY).

Results

A total of 775 patients were included in the study, with a median follow-up time of 81 months (range 1–136). Clinicopathological findings and details regarding surgical and adjuvant therapy are shown in Table 1.

Information on lymphovascular space invasion was unavailable for nine patients, and the depth of myometrial invasion for one patient. Molecular classification by ProMisE was accessible for 561 cases. Data on L1CAM, estrogen receptor, and progesterone receptor staining were not available for 27, 36, and 42 cases, respectively.

Negative ASRGL1 expression was observed in 12.0 % ($n = 93$) of the cases, while weak, moderate, and strong expressions were observed in 15.4 % ($n = 119$), 28.0 % ($n = 217$), and 44.6 % ($n = 346$) of the cases, respectively. The distribution for ASRGL1 staining frequencies was as follows: 12.0 % ($n = 93$) for 0–1 %, 7.4 % ($n = 57$) for 2–50 %, 11.9 % ($n = 92$) for 51–75 %, and 68.8 % ($n = 533$) for > 75 %. ASRGL1 staining intensity showed moderate agreement with staining frequency (kappa 0.446, $P < 0.001$) [21].

Kaplan-Meier curves illustrated a dose-dependent relationship between ASRGL1 staining intensity and frequency, and improved disease-specific survival (Fig. 1). The area under curve for disease-specific survival was 0.683 (95 % confidence interval [CI] 0.632–0.734) for ASRGL1 intensity and 0.640 (95 % CI 0.585–0.695) for ASRGL1 frequency. Although the difference was not statistically different ($P =$

Table 1
Clinicopathological data (n = 775).

Age (years) [median (interquartile range)]	67 (60–75)
Body mass index (kg/m^2) [median (interquartile range)]	27.3 (23.7–32.4)
Pelvic lymphadenectomy (number of cases, percent)	424 (54.7 %)
Pelvic and *para*-aortic lymphadenectomy (number of cases, percent)	114 (14.7 %)
Histology (number of cases, percent)	
Endometrioid carcinoma	685 (88.4 %)
Clear cell carcinoma	34 (4.4 %)
Serous carcinoma	26 (3.4 %)
Undifferentiated carcinoma	13 (1.7 %)
Carcinosarcoma	16 (2.1 %)
Neuroendocrine carcinoma	1 (0.1 %)
Grade (number of cases, percent) (for endometrioid only, n = 685)	
Grade 1	392 (57.2 %)
Grade 2	194 (28.3 %)
Grade 3	99 (14.5 %)
FIGO 2009 stage (number of cases, percent)	
IA	421 (54.3 %)
IB	164 (21.2 %)
II	50 (6.5 %)
IIIA	36 (4.6 %)
IIIB	5 (0.6 %)
IIIC1	45 (5.8 %)
IIIC2	25 (3.2 %)
IVA	0 (0 %)
IVB	29 (3.7 %)
Adjuvant therapy	
Vaginal brachytherapy	372 (48.0 %)
Whole pelvic radiotherapy	103 (13.3 %)
Chemotherapy	32 (4.1 %)
Chemotherapy and vaginal brachytherapy/whole pelvic radiotherapy	149 (19.2 %)

0.265), ASRGL1 staining intensity was selected for further analyses.

Low ASRGL1 expression levels were associated with stage II–IV disease and presence of uterine risk factors, *i.e.* high grade endometrioid and nonendometrioid histology, lymphovascular space invasion, and deep myometrial invasion (Table 2). Among the molecular subgroups, low expression was most prevalent in p53abn subtype carcinomas (Fig. 2). Low ASRGL1 was also associated with positive L1CAM expression and negative estrogen and progesterone receptor expression (Fig. 2).

Similar to stage and uterine risk factors, weak to strong ASRGL1 staining demonstrated an association with disease-specific survival in a univariable Cox regression analysis (Table 3). The associations for stage, uterine risk factors, and strong ASRGL1 staining remained significant in a multivariable analysis, whereas the effects of weak and moderate ASRGL1 staining disappeared (Table 3).

Molecular classification by ProMisE, along with biomarkers L1CAM, estrogen receptor, and progesterone receptor exhibited an association with disease-specific survival in univariable analyses (Table 4). In a multivariable model that included molecular classification and biomarkers L1CAM, hormonal receptors, and ASRGL1 as independent covariates, only molecular classification showed a significant effect on the outcome (Table 4).

Regarding the molecular subgroups, the Kaplan-Meier method revealed an association between ASRGL1 staining intensity and disease-specific survival solely within the NSMP subgroup (Fig. 3). The *POLE*mut subgroup showed no events, thus precluding analysis. After adjusting for stage, lymphovascular space invasion, and deep myometrial invasion, ASRGL1 no longer exhibited a significant effect on disease-specific survival in NSMP subtype endometrial carcinomas (Table 5).

Discussion

In the present study, we observed a dose-dependent correlation between ASRGL1 expression levels and improved disease-specific survival in endometrial cancer. This association persisted even after adjustment for stage and uterine risk factors. In contrast, ASRGL1 did not exhibit a significant association with disease-specific survival after adjustment for stage, molecular subgroups, and other biomarkers, *i.e.* L1CAM, estrogen receptor, and progesterone receptor.

When analyzed separately within the different molecular subgroups,

Table 2
Clinicopathological data according to ASRGL1 staining intensity (n = 775).

	ASRGL1 negative (n = 93)	ASRGL1 weak (n = 119)	ASRGL1 moderate (n = 217)	ASRGL1 strong (n = 346)	P
Stage II–IV	47 (50.5 %)	38 (31.9 %)	54 (24.9 %)	52 (15.0 %)	**<0.001**
Histology					**<0.001**
Endometrioid grade 1–2	22 (23.7 %)	59 (49.6 %)	179 (82.5 %)	326 (94.2 %)	
Endometrioid grade 3	33 (35.5 %)	27 (22.7 %)	23 (10.6 %)	16 (4.6 %)	
Nonendometrioid	38 (40.9 %)	33 (27.7 %)	15 (6.9 %)	4 (1.2 %)	
Lymphovascular space invasion	42 (46.2 %) [1]	44 (37.3 %) [2]	48 (22.5 %) [3]	59 (17.2 %) [1]	**<0.001**
Myometrial invasion ≥ 50 %	56 (60.2 %)	62 (52.1 %)	79 (36.6 %) [2]	98 (28.3 %)	**<0.001**

Abbreviation: ASRGL1, asparaginase-like protein 1.

[1] Data missing for 2 patients; [2] Data missing for 1 patient; [3] Data missing for 4 patients.

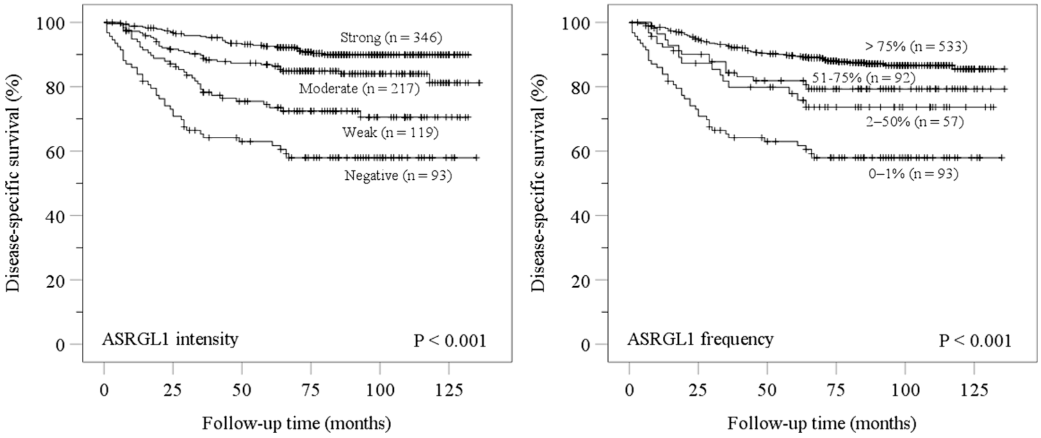

Fig. 1. Kaplan-Meier endometrial cancer-specific survival analyses based on the intensity and frequency of asparaginase-like protein 1 expression.

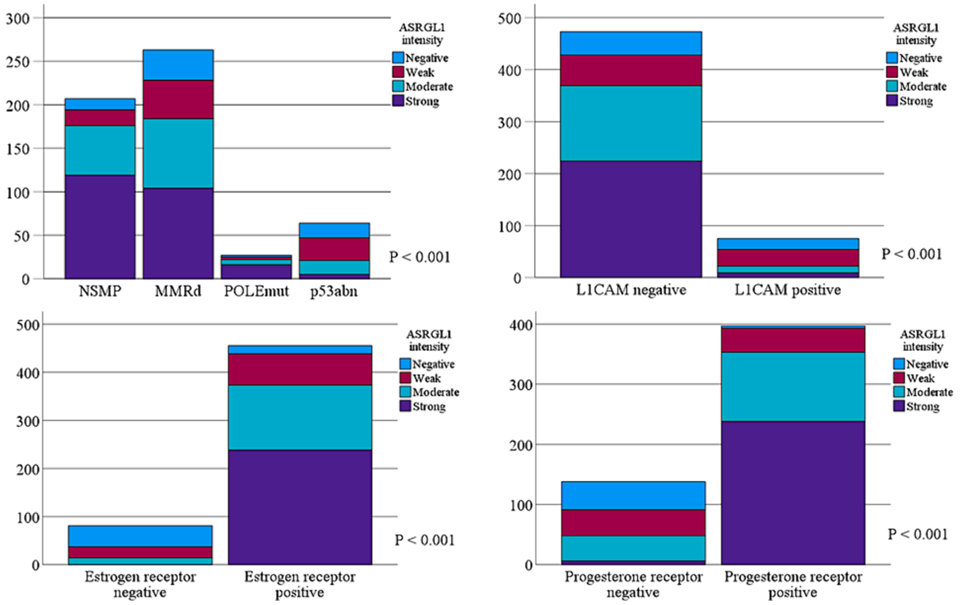

Fig. 2. Relationship between asparaginase-like protein 1 and other biomarkers in endometrial cancer. Abbreviations: ASRGL1, asparaginase-like protein 1; L1CAM, L1 cell adhesion molecule; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; *POLE*mut; polymerase-ϵ mutant; p53abn, p53 abnormal.

Table 3
Univariable and multivariable Cox regression disease-specific survival analyses considering stage, uterine risk factors, and ASRGL1 staining intensity.

		Univariable (n = 775)		Multivariable (n = 765)	
		N deaths = 136		N deaths = 135	
	N	HR (95 % CI)	P	HR (95 % CI)	P
Stage II–IV	191	6.0 (4.2–8.4)	**<0.001**	2.6 (1.8–3.9)	**<0.001**
Histology			**<0.001**		**0.003**
Endometrioid grade 1–2	586	1		1	
Endometrioid grade 3	99	4.5 (2.9–6.8)	**<0.001**	2.1 (1.3–3.3)	**<0.001**
Nonendometrioid	90	6.2 (4.1–9.2)	**<0.001**	2.4 (1.5–3.8)	**<0.001**
Lymphovascular space invasion [1]	193	4.4 (3.1–6.1)	**<0.001**	2.2 (1.5–3.2)	**<0.001**
Myometrial invasion ≥ 50 % [2]	295	4.1 (2.8–5.9)	**<0.001**	1.6 (1.0–2.4)	**0.029**
ASRGL1 intensity			**<0.001**		0.198
Negative	93	1		1	
Weak	119	0.58 (0.36–0.93)	**0.025**	0.84 (0.52–1.4)	0.479
Moderate	217	0.31 (0.19–0.49)	**<0.001**	0.68 (0.41–1.1)	0.132
Strong	346	0.17 (0.11–0.28)	**<0.001**	0.56 (0.32–0.97)	**0.038**

Abbreviations: ASRGL1, asparaginase-like protein 1; CI, confidence interval; HR, hazard ratio.

[1] Data missing for 9 patients; [2] Data missing for 1 patient.

Table 4
Univariable and multivariable Cox regression disease-specific survival analyses considering stage and molecular biomarkers.

		Univariable (n = 775)		Multivariable (n = 506)	
		N deaths = 136		N deaths = 93	
		HR (95 % CI)	P	HR (95 % CI)	P
Stage II–IV	191	6.0 (4.2–8.4)	**<0.001**	4.5 (3.0–6.9)	**<0.001**
ProMisE [1]			**<0.001**		**0.020**
NSMP	207	1		1	
MMRd	263	1.9 (1.2–3.2)	**0.008**	1.9 (1.2–3.3)	**0.012**
*POLE*mut	27	0 (not calculable)	0.954	0 (not calculable)	0.955
p53abn	64	4.9 (2.8–8.5)	**<0.001**	2.8 (1.4–5.4)	**0.003**
L1CAM [2]	111	4.0 (2.8–5.7)	**<0.001**	1.6 (0.91–3.0)	0.097
Estrogen receptor [3]	631	0.25 (0.17–0.36)	**<0.001**	0.66 (0.34–1.3)	0.227
Progesterone receptor [4]	550	0.29 (0.20–0.41)	**<0.001**	0.78 (0.43–1.4)	0.422
ASRGL1 intensity			**<0.001**		0.953
Negative	93	1		1	
Weak	119	0.58 (0.36–0.93)	**0.025**	1.0 (0.52–2.1)	0.909
Moderate	217	0.31 (0.19–0.49)	**<0.001**	0.94 (0.44–2.0)	0.865
Strong	346	0.17 (0.11–0.28)	**<0.001**	0.85 (0.35–2.1)	0.721

Abbreviations: ASRGL1, asparaginase-like protein 1; CI, confidence interval; HR, hazard ratio; L1CAM, L1 cell adhesion molecule; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; *POLE*mut; polymerase-ϵ mutant; p53abn, p53 abnormal.

[1] Data missing for 214 patients; [2] Data missing for 27 patients; [3] Data missing for 36 patients; [4] Data missing for 42 patients.

ASRGL1 showed an association with disease-specific survival specifically in NSMP subtype carcinomas. However, this association became nonsignificant upon controlling for confounders.

Interestingly, strong ASRGL1 staining intensity was consistently linked to low-risk characteristics of endometrial cancer, including localized disease, lack of uterine risk factors, lower prevalence of the p53abn molecular subgroup, and a favorable biomarker profile. Most notably, strong ASRGL1 staining intensity was observed in 94.2 % of grade 1–2 endometrioid carcinomas, reinforcing the hypothesis that ASRGL1 plays a critical role in maintaining less aggressive tumor behavior. Translational studies are needed to elucidate the biological implications of reduced ASRGL1 expression on the aggressiveness of endometrial cancer. Understanding whether the loss of ASRGL1 expression directly contributes to tumor progression or is merely a consequence of other molecular changes will be crucial. Further, investigating the mechanisms behind ASRGL1 expression regulation could provide insights into new therapeutic strategies and improve prognosis prediction in endometrial cancer.

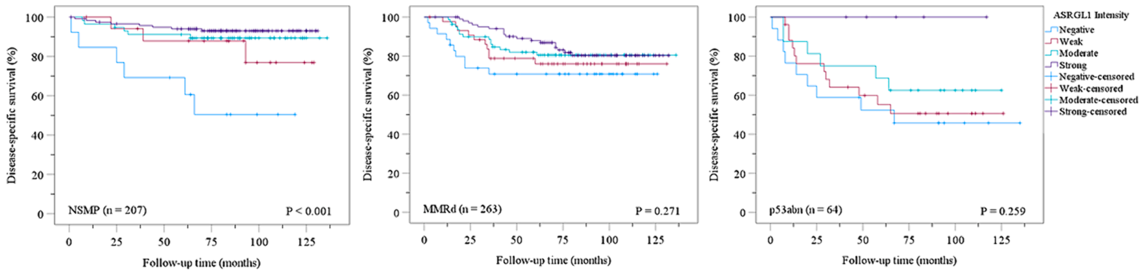

Fig. 3. Kaplan-Meier endometrial cancer-specific survival analyses based on the intensity of asparaginase-like protein 1 expression within various molecular subgroups. The polymerase-ϵ mutant subgroup showed no events, hence analysis was not feasible. Abbreviations: ASRGL1, asparaginase-like protein 1; MMRd, mismatch repair deficient; NSMP, no specific molecular profile; p53abn, p53 abnormal.

Table 5
Multivariable Cox regression disease-specific survival analysis considering stage, uterine risk factors, and ASRGL1 staining intensity in NSMP subtype endometrial carcinomas.

	NSMP (n = 207)		
	N deaths = 23		
	N	HR (95 % CI)	P
Stage II–IV	48	2.0 (0.68–5.7)	0.213
Histology			**0.028**
Endometrioid grade 1–2	184	1	
Endometrioid grade 3	11	6.4 (1.6–25)	**0.008**
Nonendometrioid	12	3.4 (0.79–15)	0.102
Lymphovascular space invasion	45	2.7 (1.1–7.1)	**0.039**
Myometrial invasion ≥ 50 %	78	2.0 (0.58–7.1)	0.266
ASRGL1 intensity			0.865
Negative	13	1	
Weak	18	0.55 (0.13–2.4)	0.425
Moderate	57	0.97 (0.25–3.7)	0.964
Strong	119	0.89 (0.19–4.2)	0.886

Abbreviations: ASRGL1, asparaginase-like protein 1; CI, confidence interval; HR, hazard ratio; NSMP, no specific molecular profile.

Although current guidelines do not incorporate L1CAM, estrogen receptor, or progesterone receptor in the risk-stratification of endometrial cancer, they show some promise as prognostic indicators. Numerous studies have identified a correlation between high L1CAM expression and poor outcomes [15–17,22], leading to its inclusion as a parameter for patient stratification in adjuvant therapies within the ongoing PORTEC-4a trial [23]. Hormonal receptors may play a role especially in refining the risk-assessment of NSMP subtype endometrial carcinomas that possess a significant overlap with estrogen-related type 1 tumors [24]. However, in our present study, molecular classification was the only independent biomarker-based predictor of poor survival. This observation most probably aligns with our post-surgical risk-stratification approach, where disease stage remains a robust prognostic variable, and the inclusion of multiple biomarkers in the regression model. Notably, when considered as sole biomarkers alongside stage and molecular classification, each of them—L1CAM (HR 2.1, 95 % CI 1.3–3.4; $P = 0.002$), estrogen receptor (HR 0.51, 95 % CI 0.33–0.80; $P = 0.003$), and progesterone receptor (HR 0.50, 95 % CI 0.33–0.77; $P = 0.002$)—individually exhibited significant associations with cancer-related deaths. This finding also extended to ASRGL1 when expressed at moderate (HR 0.55, 95 % CI 0.32–0.96; $P = 0.035$) or strong (HR 0.44, 95 % CI 0.25–0.80; $P = 0.007$) levels.

Our study confirms previous findings showing that low expression of ASRGL1 is associated with poor disease-specific survival in endometrial cancer, even when adjusted for clinicopathological variables [5,6]. Huvila *et al.* created an immunohistochemical biomarker panel for the assessment of endometrial cancer outcome independent of clinicopathological information [25]. They found that a panel combining ASRGL1 and p53 stratifies patients into three risk groups with distinct outcomes: low-risk (p53wt, ASRGL1 > 75 %), intermediate-risk (p53wt, ASRGL1 ≤ 75 % or p53abn, ASRGL1 > 75 %), and high-risk (p53abn, ASRGL1 ≤ 75 %). It is noteworthy that the omission of stage as a risk variable potentially allowed ASRGL1 to emerge as a significant variable in the analysis.

Clearly, the prognostic evaluation of endometrial cancer can be tailored based on the preferences and capabilities of pathology laboratories. Nevertheless, as the accessibility of comprehensive molecular classification gradually increases in clinical settings, *e.g.* by cost-effective alternatives for *POLE* sequencing [26–28], its significance is likely to become more prominent. Importantly, unlike individual tissue biomarkers, molecular classification currently holds implications for adjuvant therapies in alignment with available guidelines [13].

The strengths of our study lie in the thorough description of an unselected patient cohort, coupled with detailed follow-up data. Given the lack of comprehensive validation of ASRGL1 at various cut-off thresholds, we mitigated this limitation by examining its expression in a semiquantitative manner, thereby addressing potential concerns. A notable weakness stems from incomplete data for certain variables, a common challenge inherent in all retrospective studies.

In conclusion, our study establishes a correlation between low ASRGL1 expression and the existence of high-risk clinicopathological and molecular characteristics, as well as diminished survival rates in endometrial cancer. ASRGL1 enhances prognostic accuracy when controlled for stage and uterine risk factors. However, when adjusted for stage and other biomarkers, including molecular subgroups, ASRGL1 does not improve prognostic stratification. This supports the notion that potential biomarkers for endometrial cancer should ideally be validated within the framework of molecular classification.

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CRediT authorship contribution statement

Mikko J. Loukovaara: Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Jutta K. Huvila:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Annukka M. Pasanen:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Ralf C. Bützow:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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