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Full length article

Immunohistochemical and serum profile of squamous cell carcinoma of the vulva: The Dual Vulvar Panel (DVP) project

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ABSTRACT

Introduction: This study aimed to evaluate the expression of selected immunohistochemical (IHC) markers and serum squamous cell carcinoma antigen (SCC-Ag) in vulvar squamous cell carcinoma (VSCC), and to investigate associations with recurrence and death using molecular clustering and diagnostic performance analyses.

Study design: This single-centre prospective study included 27 patients with histologically confirmed VSCC. Tumour specimens were assessed for expression of p16, p53, programmed death-ligand 1 (PD-L1), CD44 and epidermal growth factor receptor (EGFR). Serum SCC-Ag was measured and correlated with clinical outcomes. Statistical analyses comprised Pearson's correlation, logistic regression, receiver operating characteristic curve analysis, diagnostic performance metrics, and unsupervised hierarchical clustering integrating IHC and SCC-Ag data.

Results: No significant associations were observed between individual IHC markers and clinical outcomes. Serum SCC-Ag showed a positive trend towards association with recurrence ($r = 0.462$; $p = 0.071$), with an increased odds ratio (OR) (OR = 2.7). When analysed as a binary variable, SCC-Ag demonstrated sensitivity of 50%, specificity of 76%, and overall accuracy of 70%. As a continuous variable, SCC-Ag achieved an area under the curve value of 0.83. The combination of SCC-Ag and p53 improved sensitivity to 83% and negative predictive value to 89%. Unsupervised hierarchical clustering identified three biological subgroups, with the cluster characterized by high SCC-Ag and EGFR expression and low p16 expression associated with recurrence more frequently.

Conclusion: Serum SCC-Ag showed superior prognostic performance compared with individual IHC markers, and may be useful for postoperative risk stratification in VSCC. Combined biomarker panels, including p53, PD-L1, EGFR and p16, yielded promising sensitivity, supporting future strategies.

Introduction

Vulvar carcinoma is a rare but challenging malignancy, accounting for approximately 3–5% of all female genital tract cancers and predominantly affecting postmenopausal women [1,2]. Despite remaining an uncommon malignancy, the incidence of vulvar carcinoma has increased in recent decades, with the most pronounced increase seen in younger women [1,3–5]. Among the various histological types, vulvar squamous cell carcinoma (VSCC) represents the most common subtype (approximately 85–90% of cases) [2,6]. Traditionally, two pathogenetic pathways have been recognized: the human papillomavirus (HPV)-related pathway, often characterized by p16 overexpression; and the HPV-independent route, commonly marked by p53 mutations and frequently arising in older patients [6–8]. Despite increasing efforts to improve diagnostic accuracy and therapeutic strategies, the prognosis of VSCC is strongly influenced by disease stage at diagnosis and, notably, the presence of nodal metastases [6,9].

Current diagnostic evaluation of vulvar cancer relies on a

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combination of clinical examination, vulvar biopsy and imaging. Histopathological confirmation is essential and is typically achieved through a punch or excisional biopsy under local anaesthesia. Advanced imaging (magnetic resonance imaging and 18F-fluorodeoxyglucose-positron emission tomography/computed tomography) aids in defining the tumour extent and assessing nodal involvement [6,9,10]. In select cases, sentinel lymph node (SLN) biopsy guided by radiotracers or combined radiotracer-and-dye techniques are used to evaluate nodal metastases while sparing patients the morbidity of bilateral inguinal-femoral lymphadenectomy [9,10].

For early-stage VSCC [generally International Federation of Gynecology and Obstetrics (FIGO) stages I–II], wide local excision or radical local excision with SLN biopsy (or lymphadenectomy if indicated) remains the cornerstone of management, often providing a high likelihood of long-term survival [7,10]. More advanced disease (FIGO stages III–IV) typically requires a multi-modal approach that may include radical surgery with inguino-femoral lymph node dissection, followed by adjuvant radiotherapy or chemoradiotherapy, depending on pathological risk factors such as lymph node positivity or extracapsular spread [11,12]. In locally advanced cases that are not suitable for upfront major surgery, neoadjuvant chemotherapy or chemoradiotherapy may be employed to shrink the tumour prior to definitive resection [13]. Despite these strategies, outcomes can remain poor for recurrent or metastatic VSCC, underscoring the need for novel therapeutic targets.

Immunohistochemical (IHC) evaluations have emerged as valuable tools for improving diagnostic precision, and potentially guiding targeted treatments in vulvar carcinoma. Classical markers include p16, a surrogate for HPV-driven oncogenesis, and p53, which is often mutated in HPV-independent tumours and has been linked to worse prognosis [3,5,14]. Additional molecules investigated in VSCC include: CD44, a cell-surface glycoprotein considered as a cancer stem cell marker in several malignancies, and hypothesized to play a role in cell adhesion and metastasis; epidermal growth factor receptor (EGFR), overexpressed in numerous squamous tumours, including VSCC, and a promising target for anti-EGFR agents [3,15]; and programmed death-ligand 1 (PD-L1), which may be expressed by tumour cells or immune-infiltrating cells, and associated with immune escape mechanisms in various cancers, with recent data suggesting how its positivity could pave the way for immune checkpoint inhibitor therapies in selected cases [3,16].

These markers, when examined together via an IHC ‘panel’, can better characterize the molecular landscape of a given tumour, and help to identify subgroups of patients who may respond to targeted or immunomodulatory approaches [3,17].

In parallel to IHC profiling, monitoring of serum squamous cell carcinoma antigen (SCC-Ag) has been widely investigated in other squamous malignancies, such as cervical and head and neck cancers, where elevated preoperative SCC-Ag may correlate with disease burden or increased risk of recurrence [18]. In vulvar carcinoma, recent interest has turned to whether SCC-Ag may also serve as a useful adjunctive biomarker, either for early detection of tumour progression or as a prognostic indicator. Preliminary findings suggest that evaluating SCC-Ag pre-operatively, in combination with IHC results (e.g. p16, p53, CD44, EGFR and PD-L1), could refine risk stratification and guide decisions on surgical extent or adjuvant therapy [6]. The purpose of this study was to create an IHC and serum profile of VSCC, and investigate correlation with clinical outcomes and molecular clustering [19].

Materials and methods

Setting and participants

This prospective study was conducted at "DAI Materno Infantile" of Azienda Ospedaliera Universitaria "Federico II" of Naples. All consecutive patients with histologically proven VSCC referred to the study institution from March 2023 to October 2025 were included.

Surgical and clinical management of the patients had been decided on the basis of specific medical, radiological and histopathological features, in accordance with the European Society of Gynaecological Oncology (ESGO) guidelines [20] and after discussion by a dedicated tumour board, supported by the institutional cancer multi-disciplinary team.

Written informed consent was obtained from all enrolled patients. Clinical data were retrieved from patients’ medical records, collected using an Excel file, and handled in accordance with data protection regulations. All patients were operated on by the same surgeon (LDC).

Patients were excluded in cases of incomplete clinical reports, high anaesthetic risk (ASA IV), concomitant neurological and/or psychiatric disorders, and lack of a signed consent form.

All patients underwent strict assessment by two experienced pathologists (CM and LI). Formalin-fixed, paraffin-embedded tissue blocks of 27 cases of VSCC were obtained from the archives of the Department of Advanced Biomedical Sciences, Pathology Section at the study hosptial. SCC-Ag analysis was undertaken by the Department of Translational Medical Sciences at the study hospital.

The study design and procedures involving collection and handling of tissue samples were performed in accordance with the Declaration of Helsinki [21], in agreement with current Italian law, and the guidelines of the institutional ethics committee.

Surgical management

For early-stage disease (FIGO stages I–II), patients underwent wide local excision or partial vulvectomy with the aim of achieving tumour-free margins, with or without SLN biopsy. SLN biopsy was considered in patients with unifocal tumours $\leq$4 cm, located >2 cm from the midline, with no clinical or imaging evidence of groin lymph node involvement and no previous vulvar surgery. In cases where the tumour was located within 2 cm of the midline, bilateral SLN mapping was performed, in accordance with the ESGO guidelines [20]. SLN biopsy was performed using pre-operative radiotracer injection (technetium-99 m) and intra-operative detection with a gamma probe. Primary inguino-femoral lymphadenectomy was performed in patients who were not eligible for SLN biopsy.

For locally advanced disease (FIGO stage III), radical vulvectomy with inguino-femoral lymphadenectomy was performed, followed by adjuvant radiotherapy or chemoradiotherapy according to pathological risk factors, including lymph node involvement and extracapsular spread.

Histopathological and serum findings

For each case, formalin-fixed, paraffin-embedded tumour tissue was selected and used to obtain serial sections. Serial 4-μm sections were cut from paraffin blocks using an ordinary microtome, and mounted on TOMO IHC Adhesive Glass Slides (Matsunami Glass Ind., Kishiwada, Japan) for the IHC evaluation of p53 (mouse monoclonal antibody, clone Bp53-11; Ventana Medical Systems, Tucson, AZ, USA), p16 INK4a (mouse monoclonal, clone E6H4; Ventana Medical Systems), EGFR (rabbit monoclonal antibody, clone EP38Y; Epredia, Portsmouth, NH, USA), CD44 (mouse monoclonal antibody, clone MRQ-13; Cell Marque, Rocklin, CA, USA) and PD-L1 (rabbit monoclonal antibody, clone SP 263; Ventana Medical Systems). IHC staining was performed using the Benchmark Ultra system (Ventana Medical Systems) with the OptiView DAB IHC Detection Kit for p53, p16 INK4a, PD-L1 and EGFR, and the UltraView Universal DAB Detection Kit for CD44, in accordance with the manufacturers’ recommendations.

Positivity for p53 was visualized as nuclear staining. The level of immunostaining was classified as: mutated, characterized by overexpression (strong diffuse nuclear immunoreactivity in all tumour cells); null (complete absence of nuclear immunoreactivity in all tumour cells); or wild type (variable nuclear immunoreactivity in tumour cells).

Positivity for PD-L1 was visualized as membranous staining. The

level of immunostaining was scored using the combined positive score, calculated by dividing the number of PD-L1-stained cells (including tumour cells, lymphocytes and macrophages) by the total number of viable tumour cells, then multiplying by 100. The specific cut-off value for positivity was ≥1.

Positivity for EGFR was visualized as membranous staining. The level of immunostaining was scored by assessing the intensity and extent of EGFR staining in tumour cells. A score of 0 indicated no staining or faint staining in <10% of tumour cells, a score of 1+ indicated weak staining in ≥10% of tumour cells, a score of 2+ indicated moderate staining in ≥10% of tumour cells, and a score of 3+ indicated strong staining in ≥10% of tumour cells.

Positivity for CD44 and p16INK4a was visualized as membranous staining. The level of immunostaining was scored using an intensity scale such as none, mild, moderate and strong.

Serum concentrations of SCC-Ag were measured using the Atellica IM SCC assay on the Atellica IM Analyzer (Siemens Healthineers, Erlangen, Germany), a fully automated chemiluminescent immunoassay system. The assay is based on a two-site sandwich immunoassay - technique that employs monoclonal antibodies specific to SCC-Ag. Venous blood samples were collected in serum separator tubes, allowed to clot for 30 min at room temperature, and centrifuged at 1500 x *g* for 10 min. Serum was aliquoted and stored at –80°C until analysis. All samples were measured in accordance with the manufacturer's instructions.

Ethical approval and consent to participate

The study was approved by the Ethics Committee of University "Federico II" – AORN "A. Cardarelli" of Naples (Protocol No. 138/23). All patients provided written informed consent, in accordance with the regulations of the study institution. The study was conducted in accordance with ethical standards, including the principles outlined in the Declaration of Helsinki [20], ensuring the protection of participants' rights and data privacy.

Statistical analysis

Statistical analysis was performed using SPSS Version 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism Version 9 (GraphPad Software, San Diego, CA, USA). Continuous variables have been reported as mean ± standard deviation (SD) or median (range), as appropriate, and categorical variables have been expressed as frequency and percentage. The distribution of continuous variables was assessed using the Shapiro–Wilk test.

Comparisons between categorical variables were performed using Chi-squared test or Fisher's exact test, as appropriate. Associations between biomarkers and clinical outcomes were explored using Pearson's correlation analysis and logistic regression models, with results expressed as odds ratio (OR) and 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminative ability of serum SCC-Ag for the composite endpoint of recurrence within 12 months and/or death.

Exploratory unsupervised hierarchical clustering was conducted based on IHC marker expression and serum SCC-Ag, using standardized values. All statistical tests were two-sided, and $p < 0.05$ was considered to indicate significance.

Results

The demographic and clinical characteristics of the study population are summarized in Table 1. The mean ± SD age of the study cohort was 67.6 ± 10.6 years, with a mean ± SD body mass index of 31.3 ± 4.7 kg/m^2. Most patients presented with FIGO stage II (48%) or III (18%) disease and high-grade histology (G3, 70%). The majority of tumours were unifocal (85%), laterally located (69%), and of the keratinizing subtype (55%), followed by non-keratinizing (34%) and basaloid (11%) subtypes.

Table 1
Clinical and pathological characteristics of the study population ($n = 27$).

Variable	Value
Patient characteristics	
Age, years (mean ± SD)	67.6 ± 10.6
Body mass index, kg/m^2 (mean ± SD)	31.3 ± 4.7
Age at menarche, years (mean ± SD)	12.8 ± 1.3
Parity ≥ 1, *n* (%)	21 (78%)
Smoking status, *n* (%)	
Yes	8 (29%)
No	19 (71%)
Family history of cancer, *n* (%)	9 (33%)
Comorbidities, *n* (%)	
Cardiovascular disease	16 (60%)
Diabetes mellitus	3 (11%)
Multiple comorbidities	7 (26%)
Tumour characteristics	
Tumour focality, *n* (%)	
Unifocal	23 (85%)
Multi-focal	4 (15%)
Tumour location, *n* (%)	
Lateral	18 (69%)
Anterior/central/posterior	9 (31%)
Histological subtype, *n* (%)	
Keratinizing squamous cell carcinoma	15 (55%)
Non-keratinizing squamous cell carcinoma	9 (34%)
Basaloid squamous cell carcinoma	3 (11%)
FIGO stage, *n* (%)	
I	9 (33%)
II	13 (48%)
III	5 (18%)
Tumour grade, *n* (%)	
G1–G2	8 (30%)
G3	19 (70%)
Depth of invasion, mm (mean ± SD)	5.7 ± 4.3
Largest tumour diameter ≥ 4 cm, *n* (%)	5 (18%)
Lymphovascular space invasion, *n* (%)	5 (18%)
Surgical treatment	
Type of vulvar surgery, *n* (%)	
Partial vulvectomy	10 (37%)
Radical vulvectomy	17 (63%)
Nodal assessment, *n* (%)	
Sentinel lymph node biopsy	4 (15%)
Lymphadenectomy	14 (52%)
None	9 (33%)
Adjuvant treatment, *n* (%)	
Radiotherapy ± chemotherapy	4 (15%)
None	23 (85%)
Biomarker profile and outcomes	
p16 positive, *n* (%)	16 (59%)
p53 mutated, *n* (%)	11 (41%)
Serum SCC-Ag ≥1.9 ng/ml, *n* (%)	8 (29.6%)
Recurrence within 12 months, *n* (%)	5 (18.5%)
Death, *n* (%)	2 (7.4%)
Composite endpoint (recurrence and/or death), *n* (%)	6 (22.2%)

Data are reported as mean ± standard deviation or *n* (%), as appropriate.
FIGO, International Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma; SCC-Ag, squamous cell carcinoma antigen; SD, standard deviation.

During follow-up, five of 27 patients (18.5%) experienced clinical recurrence within 12 months, and two patients (7.4%) died. One patient experienced both recurrence and death. Overall, six patients (22.2%) met the composite endpoint of early recurrence and/or death.

IHC profiling showed p16 positivity, indicative of HPV-associated disease, in 16 of 27 cases (59%), while p53 mutation, consistent with HPV-independent carcinogenesis, was observed in 11 cases (41%). CD44 expression was uniformly positive across all tumours (100%), limiting its discriminatory value. PD-L1 expression was detected in 87% of p16-positive tumours and 82% of p53-mutated tumours, while EGFR overexpression was observed in 93% and 90% of these subgroups, respectively (Fig. 1). However, Chi-squared test and Fisher's exact test did not

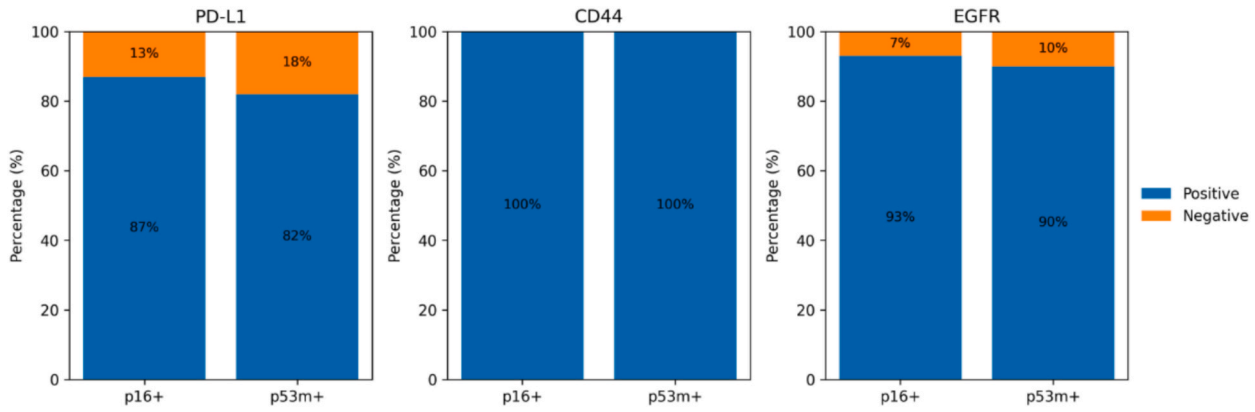

Fig. 1. Distribution of programmed death-ligand 1 (PD-L1), CD44 and epidermal growth factor receptor (EGFR) expression according to p16 and p53 status in vulvar squamous cell carcinoma.

reveal any significant association between individual IHC markers and clinical outcomes, including recurrence or death ($p > 0.05$; Table 1).

Correlation analyses demonstrated a positive, although not significant, association between serum SCC-Ag level and recurrence (Pearson $r = 0.462$; $p = 0.071$). p53 mutation status showed a non-significant trend towards association with death ($p = 0.091$). When SCC-Ag was analysed as a continuous predictor, logistic regression models indicated an increased but non-significant risk of recurrence (OR = 2.7, 95% CI 0.75–9.68; $p = 0.127$) and death (OR = 2.5, 95% CI 0.56–11.23; $p = 0.228$). PD-L1 showed a moderate, non-significant correlation with death ($r = 0.326$; $p = 0.104$), while p16 expression demonstrated a negative, non-significant correlation with death ($r = –0.31$; $p = 0.116$). Correlations of EGFR and p53 with clinical outcomes were weak and non-significant. CD44 was excluded from correlation analyses due to its uniform expression across the cohort (Table 2).

ROC analysis confirmed the discriminative ability of serum SCC-Ag for the composite endpoint of recurrence within 12 months and/or death, yielding an area under the curve of 0.83 (Table 2). Among the evaluated biomarkers, serum SCC-Ag demonstrated the highest discriminative performance, whereas individual IHC markers showed limited prognostic utility.

Unsupervised hierarchical clustering based on IHC markers and serum SCC-Ag identified three molecular clusters (Fig. 2). Cluster 1 was characterized by high expression of either p16 or p53, with generally low levels of EGFR and SCC-Ag. Cluster 2 showed moderate PD-L1 and CD44 expression with variable levels of EGFR. Cluster 3 was defined by high expression of EGFR and SCC-Ag, combined with low or absent p16 expression. Notably, Cluster 3 was associated more frequently with

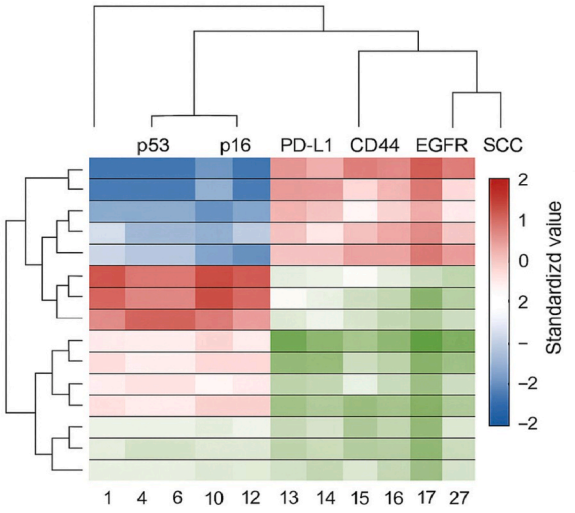

Fig. 2. Exploratory unsupervised hierarchical clustering of immunohistochemical markers and serum squamous cell carcinoma antigen (SCC-Ag) in vulvar squamous cell carcinoma. The heatmap displays standardized values (z-scores) of p53, p16, programmed death-ligand 1 (PD-L1), CD44, epidermal growth factor receptor (EGFR) and SCC-Ag across the study cohort.

Table 2
Association of serum squamous cell carcinoma antigen (SCC-Ag) and immunohistochemical markers with clinical outcomes in vulvar squamous cell carcinoma (composite endpoint: recurrence within 12 months and/or death).

Biomarker	Analysis	Estimate	*p*-value	Sensitivity (%)	Specificity (%)	NPV (%)	Accuracy (%)	AUC
SCC-Ag (continuous)	Pearson correlation	$r = 0.46$	0.071	NA	NA	NA	NA	NA
SCC-Ag (continuous)	Logistic regression	OR 2.7 (95% CI 0.75–9.68)	0.127	NA	NA	NA	NA	NA
p53 mutation	Logistic regression	OR 0.96 (95% CI 0.13–6.98)	0.97	50	62	81	59	0.56
p16 positivity	Logistic regression	OR 1.04 (95% CI 0.14–7.53)	0.97	50	38	73	41	0.44
PD-L1 positivity	Logistic regression	OR 0.63 (95% CI 0.05–7.75)	0.72	83	14	75	30	0.49
EGFR overexpression	Logistic regression	OR 0.19 (95% CI 0.01–3.72)	0.27	83	5	50	22	0.44
SCC-Ag (≥1.9 ng/ml)	Diagnostic performance	NR	NR	50	76	84	70	0.83
SCC-Ag + p53	Combined panel	NR	NR	83	38	89	48	NA

AUC, area under the receiver operating characteristic curve; CI, confidence interval; EGFR, epidermal growth factor receptor; NA, not applicable; NPV, negative predictive value; NR, not reported; OR, odds ratio; PD-L1, programmed death-ligand 1.
Serum SCC-Ag was analysed as a continuous variable for association analyses, and dichotomized using a cut-off of 1.9 ng/ml for diagnostic performance.

recurrence and aggressive clinicopathological features, suggesting the presence of biologically and potentially clinically relevant subgroups within VSCC.

To further assess clinical applicability, diagnostic performance analyses were conducted using the composite endpoint of recurrence and/or death. When SCC-Ag was dichotomized (≥1.9 ng/ml), it demonstrated balanced diagnostic performance, with sensitivity of 50%, specificity of 76% and overall accuracy of 70%. Among combined biomarker panels, SCC-Ag plus p53 achieved higher sensitivity (83%) and a negative predictive value of 89%, supporting its potential utility in identifying patients at low risk of early adverse outcomes (Table 3). Notably, combinations including PD-L1, EGFR and p16 yielded overall satisfactory results, supporting the feasibility of alternative multi-parametric biomarker panels and their potential exploitation in future patent and translational applications.

Overall, these findings indicate that serum SCC-Ag represents the most reliable single biomarker for prognostic stratification in VSCC. While integration with IHC markers and exploratory molecular clustering provides additional biological insight, it does not enhance diagnostic performance substantially beyond SCC-Ag alone.

Discussion

Vulvar carcinoma is a rare malignancy, accounting for approximately 3–5% of all female genital tract cancers, that predominantly affects postmenopausal women [22]. VSCC represents nearly 90% of cases, and is classically divided into HPV-associated and HPV-independent pathways, characterized by p16 overexpression and p53 mutation, respectively [23]. Despite advances in surgical techniques and multi-modal treatment strategies, prognosis remains closely linked to FIGO stage and lymph node involvement [24,25], underscoring the need for additional tools to improve postoperative risk stratification.

In this prospective cohort, the prognostic value of selected serum and IHC biomarkers in relation to early recurrence and death was investigated. Among all variables investigated, serum SCC-Ag consistently emerged as the most informative biomarker. Although logistic regression analyses did not reach significance, likely due to the limited sample size inherent to this rare disease, SCC-Ag showed a consistent directional association with adverse outcomes, and the highest discriminative ability for the composite endpoint of recurrence and/or death. These findings support a potential role for SCC-Ag as a pragmatic adjunct in the postoperative surveillance of VSCC.

SCC-Ag is a well-established biomarker in squamous cell carcinoma of the cervix and head and neck, and has been explored increasingly in vulvar cancer. Previous studies have associated elevated SCC-Ag with increased tumour burden, nodal involvement, and higher risk of recurrence. In line with these studies, the present results suggest that SCC-Ag may provide clinically meaningful prognostic information in VSCC. Importantly, SCC-Ag maintained a consistent performance when analysed using different analytical approaches, supporting its robustness. Nevertheless, serum SCC-Ag should be interpreted with caution, as it may be influenced by benign inflammatory or dermatological conditions.

In contrast, individual IHC markers, including p16, p53, PD-L1 and EGFR, demonstrated limited standalone prognostic value. CD44 expression was uniformly positive across the cohort, precluding any discriminatory role. These findings are consistent with previous reports highlighting the heterogeneity and limited reproducibility of tissue-based biomarkers in rare gynaecological malignancies [6]. However, the observed variability in PD-L1 and EGFR expression suggests that these markers may still be of interest in specific biological or therapeutic contexts, particularly in the era of immunotherapy.

ROC analysis further reinforced the relative strength of SCC-Ag, which achieved the highest discriminative performance among the evaluated biomarkers. While the combination of SCC-Ag with IHC markers led to a modest improvement in sensitivity, this occurred at the expense of specificity and overall accuracy, limiting immediate applicability in routine clinical practice. Notably, the combination of SCC-Ag with p53 showed a high negative predictive value, suggesting potential utility in identifying patients at low risk of early adverse outcomes, particularly in follow-up settings where ruling out recurrence is clinically relevant.

Exploratory unsupervised hierarchical clustering analysis provided additional biological insight by revealing patterns of biomarker co-expression suggestive of distinct subgroups within VSCC. In particular, tumours characterized by high SCC-Ag and EGFR expression and low p16 expression were more frequently associated with recurrence and aggressive features. These findings should be considered hypothesis-generating, and support a multi-dimensional approach to risk assessment that integrates serum biomarkers with tissue profiling.

The main strengths of this study include its prospective design, the integrated evaluation of serum and IHC biomarkers, and the application of both conventional and exploratory analytical methods. Limitations include the single-centre setting and small sample size, which limited the generalizability of the findings and constrained the statistical power. Nonetheless, despite these limitations, the results consistently highlight serum SCC-Ag as the most robust and accessible biomarker for prognostic stratification in VSCC.

Table 3
Diagnostic performance of biomarkers for vulvar squamous cell carcinoma.

Marker	Sensitivity	Specificity	PPV	NPV	Accuracy	LR+	LR−
p53	0.50	0.62	0.27	0.81	0.59	1.31	0.81
p16	0.50	0.38	0.19	0.73	0.41	0.81	1.31
PD-L1	0.83	0.14	0.22	0.75	0.30	0.97	1.17
EGFR	0.83	0.05	0.20	0.50	0.22	0.88	3.40
SCC-Ag	0.50	0.76	0.38	0.84	0.70	2.10	0.66
SCC-Ag + p53	0.83	0.38	0.28	0.89	0.48	1.35	0.44
SCC-Ag + p16	0.67	0.38	0.24	0.80	0.44	1.08	0.88
SCC-Ag + PD-L1	0.83	0.10	0.21	0.67	0.26	0.92	1.75
SCC-Ag + EGFR	1.00	0.05	0.23	1.00	0.26	1.05	0.00
Discriminative ability of biomarkers							
Marker			AUC				
p53			0.560				
p16			0.440				
PD-L1			0.488				
EGFR			0.440				
SCC-Ag			0.833				

Diagnostic performance was calculated against the composite endpoint of recurrence within 12 months and/or death.
AUC, area under the receiver operating characteristic curve; EGFR, epidermal growth factor receptor; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; PD-L1, programmed death-ligand 1; SCC-Ag, squamous cell carcinoma antigen.

This study supports a predominant role for serum SCC-Ag in the prognostic assessment of VSCC. While IHC markers and exploratory clustering analyses offer complementary biological insights, SCC-Ag alone currently represents the most practical and reliable tool for post-operative risk stratification. Larger, multi-centre prospective studies are warranted to validate these findings, and to define the optimal integration of biomarker-based approaches into clinical decision-making for this rare malignancy.

Conclusion

Serum SCC-Ag demonstrated the most consistent prognostic performance among the evaluated biomarkers in VSCC. Compared with individual IHC markers, SCC-Ag showed a more balanced diagnostic profile, while its combination with p53 improved sensitivity and negative predictive value, albeit at the expense of specificity. Importantly, the overall satisfactory performance observed across other combined panels, including PD-L1, EGFR and p16, supports the concept that multiparametric biomarker approaches may enhance test sensitivity and provide complementary prognostic information. These findings suggest that the integration of combined serum and IHC panels could progressively become part of routine clinical practice, even for rare tumours such as VSCC, fostering more refined postoperative risk stratification. Further prospective, multi-centre studies are warranted to validate these promising findings.

CRediT authorship contribution statement

Luigi Della Corte: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mario Palumbo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chiara Mignogna:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Silvia Varricchio:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **Gianluigi Carbone:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Rosa Sirica:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Luigi Insabato:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Daniela Terracciano:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Giuseppe Bifulco:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to Participate

The study was approved by the Local Ethics Committee of the University "Federico II" - AORN "A. Cardarelli" of Naples (Protocol Number 138/23).

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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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