# Serum HE4 as a prognostic marker in cervical cancer: a retrospective study

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## Abstract

# Background

Human epididymis protein 4 (HE4) is a tumor marker that has been well-investigated in ovarian and endometrial cancers. The aim of this study was to evaluate the prognostic value of serum HE4 as a tumor marker in patients with cervical cancer.

# Methods

Serum HE4 levels from 67 cervical cancer patients were measured by immunoassay before starting primary treatment between September 2014 and May 2018. A mean serum HE4 level of 72.6 pmol/L was used to divide the patients into low and high HE4 groups. The patient characteristics, clinicopathological variables, and survival outcomes were compared between the 2 groups.

# Results

There were 55 (82.1%) patients in the HE4 < 72.6 pmol/L group and 12 (17.9%) patients in the HE4 > 72.6 pmol/L group at the date of diagnosis. Higher HE4 levels were significantly associated with older age at diagnosis (age < 50: 0.0% vs. age ≥ 50: 100.0%; P = 0.002), menopause (premenopause: 8.3% vs. postmenopause: 91.7%; P = 0.009), higher FIGO stage (stage I-II: 25.0% vs. III-IV: 75.5%; P = 0.008), large tumor size (< 4.0cm: 41.7% vs. ≥4.0 cm: 58.3%; P = 0.029), positive lymph node metastasis (negative: 41.7% vs. positive: 58.3%; P = 0.049), and involvement of the parametrium (negative: 25.0% vs. positive: 75.0%; P = 0.006). Higher levels of HE4 was a predictive factor for worse overall survival but not for progression-free survival, although elevated HE4 levels were not found to be independent factors for the prediction of either overall survival or progression-free survival. When subgroup analysis was performed by histological type, similar results were obtained for patients with squamous cell carcinoma.

# Conclusions

Our data revealed that high levels of HE4 expression were correlated with poor OS, indicating that elevated HE4 levels are associated with a poor prognosis for patients with cervical cancer.

## Background

Over the past few decades, efforts have been made to reduce the incidence and mortality of cervical cancer through early detection and prevention. Nevertheless, cervical cancer remains the fourth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in women worldwide (1). Histologically, approximately 80% of cervical cancer cases are squamous cell carcinoma (SCC) and approximately 20% are adenocarcinoma (ADC) (2, 3). Currently, squamous cell carcinoma antigen (SCC-Ag) and cancer antigen 125 (CA 125) are the most commonly used tumor markers for cervical cancer. Increased levels of SCC-Ag are present in only 64% of patients with SCC and 25% of patients with ADC, while increased levels of CA125 are present in only 42.6% of patients with SCC and in 18.9% of patients with ADC (3). Therefore, the identification of novel markers that improve the detection rate of cervical cancer is required.

Cervical cancer is a preventable disease and its morbidity and mortality has been dramatically reduced by the introduction of cervical cytology (4). However, cervical cytology has low sensitivity and a high false positive rate, partially due to inadequate specimen collection (3, 5). Adjunctive tests such as colposcopy and screening for human papillomavirus (HPV) have been suggested to overcome this low accuracy (6). In addition, tumor markers for the diagnosis and follow up of cervical cancer are necessary. SCC-Ag is the most widely used tumor marker in the diagnosis of cervical cancer. Elevated levels of SCC-Ag are related to tumor size and stage of disease before treatment, as well as to the response to treatment (7). However, the diagnostic sensitivity is only 30% for early cervical cancer (8). As well, the utility of SCC-Ag in other histologic types is still unclear. With these limitations, identifying other tumor markers for cervical cancer is highly important.

Human epididymis protein 4 (HE4) is a promising biomarker that has been shown to have great potential for clinical use (9-11). HE4 was first isolated from the epithelial cells of the human epididymis and can also be detected in serum (12). HE4 expression has also been identified in a number of normal human tissues outside of the male reproductive system, as well as in various types of malignancies (13, 14). Previous studies have demonstrated that HE4 levels are higher in certain type of cancer (15-17). Increased HE4 is commonly found in tumors of gynecologic origin, and HE4 is well established in ovary and endometrial cancers (18, 19). It has been reported that serum HE4, both alone and in combination with CA125, has great potential to predict prognosis (20-22).

Although it is known that serum levels of HE4 are significantly increased in ovarian and endometrial cancers, research on cervical cancer has been minimal. Some studies have shown that patients affected by cervical cancer may also have higher levels of HE4 (17, 23). However, as these studies were limited by their small sample size, it remains unclear whether HE4 is a valuable marker for cervical cancer. A tumor marker that enables better stratification of patients with cervical cancer may improve individualized primary treatment and would help to prevent over- or under-treatment of these patients. Therefore, we sought to evaluate the prognostic value of serum HE4 as a tumor marker for cervical cancer patients.

# Methods

This retrospective study received approval from the Institutional Review Board of the Seoul National University Bundang Hospital (SNUBH; No. B-1602/336-103) and was performed in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived due to the retrospective nature of the study.

### Study population

We retrospectively reviewed the medical records of 67 cervical cancer patients who were treated at Seoul National University Bundang Hospital, a tertiary hospital in Korea, from September 2014 to May 2018. All patients were restaged based on the revised 2018 International Federation of Gynecology and Obstetrics (FIGO) staging system for cervical cancer (24). We included patients who met the following inclusion criteria: (1) patients who had histologically confirmed cervical cancer of any stage; (2) those who underwent initial treatment, such as radical hysterectomy with lymphadectomy, concurrent chemoradiation therapy, radiation therapy or chemotherapy only; and (3) those who had HE4 levels assessed at diagnosis, before initiation of first treatment. Patients with the following conditions were excluded: (1) patients who had an incomplete treatment for any reason; (2) those who had insufficient clinical and pathological data; and (3) those with other malignancies that had the potential to influence survival outcomes.

For the patients who met the criteria mentioned above, we reviewed the medical records, surgical records, pathological findings, and clinical characteristics. Histology was pathologically evaluated in all patients. Tumor size, lymphovascular space invasion (LVSI), lymph node (LN) metastasis, and involvement of the parametrium were evaluated pathologically only in patients who initially received surgery, while magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography computed tomography were evaluated in patients who received initial treatment other than surgery.

### HE4 Immunoassay

The serum HE4 concentration was measured before initiation of patient treatment using the Architect Analyzer (Abbott Laboratories, USA). This is a two-step immunoassay that quantitatively measures HE4 levels in human serum using chemiluminescent microparticle technology. The inter-assay precision for measurement of HE4 was 3.5% (49.7 pmol/L), 3.6% (168.1 pmol/L) and 3.8% (648.2 pmol/L).

### Statistical analysis

The 67 cervical patients had a mean serum HE4 level of 72.6 pmol/L (standard deviation = 95.0) prior to the initiation of treatment. As no definitive diagnostic thresholds for HE4 have been reported to date, we evaluated the differences in clinicopathological characteristics between patients who had lower HE4 levels (<72.6 pmol/L) and those who had higher HE4 levels (≥72.6 pmol/L) at initial diagnosis.

For comparison of continuous variables, the Student's *t*-test and Mann-Whitney *U* test were used. Ordinal and categorical variables were analyzed using the Pearson's chi-squared test or Fisher's exact test, as applicable. The association between HE4 levels and the survival outcomes of patients with cervical cancer was assessed by comparing patients with lower HE4 levels (<72.6 pmol/L) and those with higher HE4 levels (≥72.6 pmol/L) before treatment started. Progression-free survival (PFS) and overall survival (OS) rates were calculated by the Kaplan-Meier method and the differences between curves were assessed using the log-rank test. Univariate analysis was performed using the Cox proportional hazards model to evaluate the impact of patient characteristics and clinical factors on survival. For multivariate survival analysis, Cox proportional hazards analysis was used to estimate the prognostic effects of several variables. All analyses were performed using SPSS software for Windows (version 25.0; SPSS Inc., Chicago, IL, USA). $P < 0.05$ indicated statistical significance.

# Results

In this study, we investigated the HE4 levels in 67 cervical cancer patients. The overall patient characteristics are presented in Table 1. The mean age of the population was 53.4 ± 13.1 years. There were 28 (41.8%) premenopausal patients and 39 (58.2%) postmenopausal patients. The most common histological type was SCC (76.1%), followed by ADC (17.9%), adenosquamous cell carcinoma 1 (1.5%) and others (3.0%). A total of 40 (50.7%) patients were stage I, 4 (6.0%) patients were stage II, 17 (25.4%) patients were stage III, and 6 (9.0%)

patients were stage IV. Over half of the patients underwent surgery (67.2%) for primary treatment, and 29 (43.3%) had adjuvant treatment after initial therapy regardless of the type of primary treatment. During a median length of observation of 43.8 months (range: 9.23-60.88 months), 11 patients (16.4%) experienced disease recurrence and 12 patients (17.9%) died.

The mean concentration of serum HE4 was used to discriminate between low and high HE4 levels. There were 55 (82.1%) patients with mean HE4 levels below 72.6 pmol/L and 12 (17.9%) patients with mean HE4 levels above 72.6 pmol/L at the date of diagnosis. In our analysis, higher HE4 levels were significantly associated with older age (≥50) at diagnosis, menopause, higher FIGO stage, tumor size, presence of LVSI, positive LN metastasis and involvement of the parametrium (Table 2). There was no statistically significant association between HE4 and either adjuvant treatment or recurrence.

Fig. 1 shows the PFS and OS of patients for each group. There was no significant difference in PFS between the two groups (hazard ratio [HR]: 0.650; 95% confidence interval [CI]: 0.145-2.921; *P*=0.572) (Fig. 1A). However, patients with higher HE4 levels had significantly worse OS than those with lower HE4 levels (HR: 3.726; 95% CI: 1.182-11.747; *P*=0.016) (Fig. 1B). We also performed subgroup analysis of patients with the SCC histological type (n=50), and the results were consistent with those of the overall study population. There was no significant difference observed in PFS (HR: 0.974; 95% CI: 0.198-4.805; *P*=0.975) (Fig. 1C), but patients with higher HE4 levels were associated with worse OS than those with lower HE4 levels (HR: 5.449; 95% CI: 1.460-20.332; *P*=0.005) (Fig. 1D).

In order to assess the prognostic significance of clinicopathologic variables of PFS and OS, we performed univariate Cox analysis. FIGO stage, tumor size, LN metastasis and involvement of parametrium were identified as unfavorable factor for PFS and OS (Table 3). In addition, higher HE4 levels were significantly associated with poor clinical outcomes for OS (HR: 3.726; 95% CI: 1.182-11.747; *P*=0.025; Table 3). On multivariate analysis, higher HE4 levels did not remained as an independent indicator of OS (HR: 1.899; 95% CI: 0.507-7.116; *P*=0.342; Table 3). The results were similar in the subgroup analysis for the SCC histological type. Univariate analysis revealed that high levels of HE4 were associated with poor prognostic factor for OS (HR: 5.449; 95% CI: 1.460-20.332; *P*=0.012; Table 4), whereas multivariate analysis revealed that high levels of HE4 did not influence OS (HR: 2.856; 95% CI: 0.663-12.294; *P*=0.159; Table 4).

## Discussion

In this study, we found that high expression of HE4 is correlated with poor OS, indicating that HE4 is a potential candidate biomarker for predicting the prognosis of cervical cancer. Several recent studies investigating HE4 have reported that it is an effective tumor marker for predicting prognosis and is associated with poor OS and PFS, especially in ovarian cancer (25). Our findings revealed that, in cervical cancer patients, elevated serum HE4 levels were significantly correlated with FIGO stage as well tumor size, LVSI, LN metastasis, and parametrium involvement, but not with histological type. Moreover, we found that in univariate analysis serum HE4 levels were associated with worse OS. However, HE4 levels were not found to be an independent prognostic factor for PFS. Similar results were observed in the subgroup analysis of patients with histologically confirmed SCC.

Careful attention is required in interpreting serum HE4 levels, as they can be affected by numerous factors (26). Previous studies have shown that age, menopausal status, smoking, renal function, chronic liver disease, ethnicity, and detection method may influence serum HE4 levels (17, 27-29). Thus, our results should be interpreted with caution. At current, there is no consensus established for optimal baseline serum HE4 cutoff values (30). Based on previous published investigations, a serum HE4 cut-off value of 70 pmol/L yields the best sensitivity and specificity (26). The positive serum HE4 cut-off values previously identified in ovarian and endometrial cancer patients were found to be similar to the mean value of HE4 in our study (23, 31).

This study has several limitations. First, the existence of underlying selection bias is possible due to the retrospective design. Second, the sample size of the study population as well as the number of recurrence and death events might be insufficient for a comparison of survival outcomes between lower and higher serum levels of HE4. Third, no subgroup analysis was performed for the ADC histology type because of the small sample size of this population. Lastly, we did not attempt any stratification of the study population.

## Conclusions

In conclusion, our study showed that elevated serum HE4 was associated with poor prognostic factors for cervical cancer and was correlated with poor OS, suggesting the potential of HE4 as a novel biomarker for predicting the survival of patients with cervical cancer.

## Abbreviations

HE4
Human Epididymis protein 4

SCC
Squamous Cell Carcinoma
ADC
Adenocarcinoma
SCC-Ag
Squamous Cell Carcinoma Antigen
CA 125
Cancer Antigen 125
HPV
Human Papillomavirus
FIGO
International Federation of Gynecology and Obstetrics
LVSI
Lymphovascular Space Invasion
LN
Lymph Node
MRI
Magnetic Resonance Imaging
CT
Computed Tomography
PFS
Progression-Free Survival
OS
Overall Survival
HR
Hazard Ratio

## Declarations

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of the Seoul National University Bundang Hospital. . The requirement for informed consent was waived due to the retrospective nature of the study.

### CONSENT FOR PUBLICATION

Not applicable.

### AVAILABILITY OF DATA AND MATERIALS

The data generated or analysed during this study are available from the corresponding author upon reasonable request.

### COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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The authors have no support or funding to report.

### AUTHOR CONTRIBUTIONS

Conceptualization: WY Hwang, JH No; Methology: WY Hwang, JH No; Data acquisition: WY Hwang; Validation: WY Hwang, JH No; Formal analysis and investigation: all authors; Writing - original draft: WY Hwang; Writing – review & editing: all authors; Supervision: JH No

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# Tables

**Table 1.** Characteristics of the overall study population (*N*=67)

| Characteristics | *N* (%) |
|---|---|
| Age, years | |
| Mean±SD | 53.4 ± 13.2 |
| Menopause | |
| Premenopause | 28 (41.8) |
| Postmenopause | 39 (58.2) |
| Histology | |
| Squamous cell carcinoma | 50 (74.6) |
| Adenocarcinoma | 15 (22.4) |
| Adenosquamous cell carcinoma | 1 (1.5) |
| Others | 1 (1.5) |
| FIGO Stage | |
| I | 40 (59.7) |
| II | 4 (6.0) |
| III | 17 (25.4) |
| IV | 6 (9.0) |
| Initial Treatment | |
| Operation | 45 (67.2) |
| CCRT | 16 (23.9) |
| RTx | 2 (3.0) |
| CTx | 4 (6.0) |
| Adjuvant Treatment | |
| Yes | 29 (43.3) |
| No | 38 (56.7) |
| Recurrence | |
| Yes | 15 (22.4) |
| No | 52 (77.6) |

Abbreviations: SD, standard deviation; FIGO, International Federation of Gynecology and Obstetrics; CCRT, concurrent chemoradiation therapy; RTx, Radiation therapy; CTx, Chemotherapy

**Table 2.** Distribution of study population according to levels of HE4

| Chararcteristics | HE4<72.6 pmol/L (N=55) | HE4≥72.6 pmol/L (N=12) | *P* value |
|---|---|---|---|
| Age, years | | | 0.002* |
| <50 | 26 (47.3) | 0 (0.0) | |
| ≥50 | 29 (52.7) | 12 (100.0) | |
| Menopause | | | 0.010* |
| Yes | 28 (50.9) | 11 (91.7) | |
| No | 27 (49.1) | 1 (8.3) | |
| Histology | | | 0.270* |
| SCC | 39 (70.9) | 11 (91.7) | |
| Non-SCC | 16 (29.1) | 1 (8.3) | |
| FIGO Stage | | | 0.017* |
| I-II | 40 (72.7) | 4 (33.3) | |
| III-IV | 15 (27.3) | 8 (66.7) | |
| Tumor size, cm | | | 0.029* |
| <4.0 | 43 (78.2) | 5 (41.7) | |
| ≥4.0 | 12 (21.8) | 7 (58.3) | |
| LVSI | | | 0.004** |
| Negative | 25 (45.5) | 2 (16.7) | |
| Positive | 17 (30.9) | 1 (8.3) | |
| Unknown | 13 (23.6) | 9 (75.0) | |
| LN metastasis | | | 0.049* |
| Negative | 40 (72.7) | 5 (41.7) | |
| Positive | 15 (27.3) | 7 (58.3) | |
| PM involvement | | | 0.006* |
| Negative | 40 (72.7) | 3 (25.0) | |
| Positive | 15 (27.3) | 9 (75.0) | |
| Adjuvant Treatment | | | 0.604 |
| Yes | 23 (41.8) | 6 (50.0) | |
| No | 32 (58.2) | 6 (50.0) | |
| Recurrence | | | 0.721* |
| Yes | 13 (23.6) | 2 (16.7) | |
| No | 42 (76.4) | 10 (83.3) | |

Abbreviations: HE4, human epididymis protein 4; SCC, squamous cell caricinoma; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; LN, lymph node; PM, parametrium

*P values were calculated by Fisher's exact test.

**Table 3.** Factors associated with survival in the overall population

| Variables | Progression-free survival | | | | | | Overall survival | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate analysis | | | Multivariate analysis | | | Univariate analysis | | | Multivariate analysis | | |
| | HR | 95% CI | *P* value | HR | 95% CI | *P* value | HR | 95% CI | *P* value | HR | 95% CI | *P* value |
| Age | | | 0.115 | | | | | | 0.054 | | | |
| <50 | 1 | | | | | | 1 | | | | | |
| ≥50 | 2.770 | 0.781-9.819 | | | | | 7.491 | 0.967-58.038 | | | | |
| Histologic type | | | 0.141 | | | | | | 0.944 | | | |
| Non-SCC | 1 | | | | | | 1 | | | | | |
| SCC | 0.459 | 0.163-1.294 | | | | | 0.954 | 0.258-3.525 | | | | |
| FIGO stage | | | 0.002 | | | | | | 0.001 | | | |
| I-II | 1 | | | | | | 1 | | | | | |
| III-IV | 6.475 | 2.037-20.576 | | | | | 11.875 | 2.595-54.334 | | | | |
| Tumor size | | | 0.014 | | | 0.246 | | | 0.001 | | | 0.014 |
| <4.0cm | 1 | | | 1 | | | 1 | | | 1 | | |
| ≥4.0cm | 3.603 | 1.301-9.981 | | 1.980 | 0.624-6.285 | | 9.738 | 2.619-36.201 | | 7.035 | 1.489-33.227 | |
| LN metastasis | | | 0.001 | | | 0.017 | | | 0.011 | | | 0.370 |
| No | 1 | | | 1 | | | 1 | | | 1 | | |
| Yes | 6.516 | 2.054-20.669 | | 5.337 | 1.349-21.111 | | 4.707 | 1.416-15.650 | | 1.975 | 0.446-8.743 | |
| PM involvement | | | 0.031 | | | 0.869 | | | 0.019 | | | 0.800 |
| No | 1 | | | 1 | | | 1 | | | 1 | | |
| Yes | 3.144 | 1.113-8.879 | | 0.898 | 0.249-3.236 | | 4.213 | 1.267-14.010 | | 0.804 | 0.149-4.344 | |
| HE4 | | | 0.575 | | | | | | 0.025 | | | 0.342 |
| <72.6 | 1 | | | | | | 1 | | | 1 | | |
| ≥72.6 | 0.650 | 0.145-2.921 | | | | | 3.726 | 1.182-11.747 | | 1.899 | 0.507-7.116 | |

Abbreviations: HR, hazard ratio; CI, confidence interval; SCC, squamous cell caricinoma; FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; PM, parametrium; HE4, human epididymis protein 4

**Table 4.** Factors associated with survival in patients with squamous cell carcinoma

| Variables | Progression-free survival | | | | | | Overall survival | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate analysis | | | Multivariate analysis | | | Univariate analysis | | | Multivariate analysis | | |
| | HR | 95% CI | *P* value | HR | 95% CI | *P* value | HR | 95% CI | *P* value | HR | 95% CI | *P* value |
| Age | | | 0.115 | | | | | | 0.181 | | | |
| <50 | 1 | | | | | | 1 | | | | | |
| ≥50 | 5.324 | 0.665-42.615 | | | | | 44.538 | 0.171-11630.901 | | | | |
| FIGO stage | | | 0.018 | | | 0.021 | | | 0.007 | | | 0.033 |
| I-II | 1 | | | 1 | 1 | | 1 | | | 1 | | |
| III-IV | 6.846 | 1.393-33.642 | | 18.476 | 1.561-218.736 | | 17.443 | 21.178-139.673 | | 20.833 | 1.287-337.164 | |
| Tumor size | | | 0.103 | | | | | | 0.001 | | | 0.098 |
| <4.0cm | 1 | | | | | | 1 | | | 1 | | |
| ≥4.0cm | 3.009 | 0.801-11.297 | | | | | 14.755 | 3.045-71.493 | | 4.577 | 0.753-27.801 | |
| LN metastasis | | | 0.046 | | | 0.329 | | | 0.027 | | | 0.148 |
| No | 1 | | | 1 | | | | | | 1 | | |
| Yes | 4.203 | 1.024-17.260 | | 0.334 | 0.037-3.014 | | 4.801 | 1.200-19.212 | | 0.251 | 0.039-1.629 | |
| PM involvement | | | 0.256 | | | | | | 0.061 | | | |
| No | 1 | | | | | | 1 | | | | | |
| Yes | 2.159 | 0.573-8.142 | | | | | 3.756 | 0.939-15.031 | | | | |
| HE4 | | | 0.975 | | | | | | 0.012 | | | 0.159 |
| <72.6 | 1 | | | | | | 1 | | | 1 | | |
| ≥72.6 | 1.026 | 0.208-5.060 | | | | | 5.449 | 1.460-20.332 | | 2.856 | 0.663-12.294 | |

Abbreviations: HR, hazard ratio; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; PM, parametrium; HE4, human epididymis protein 4

## Figures

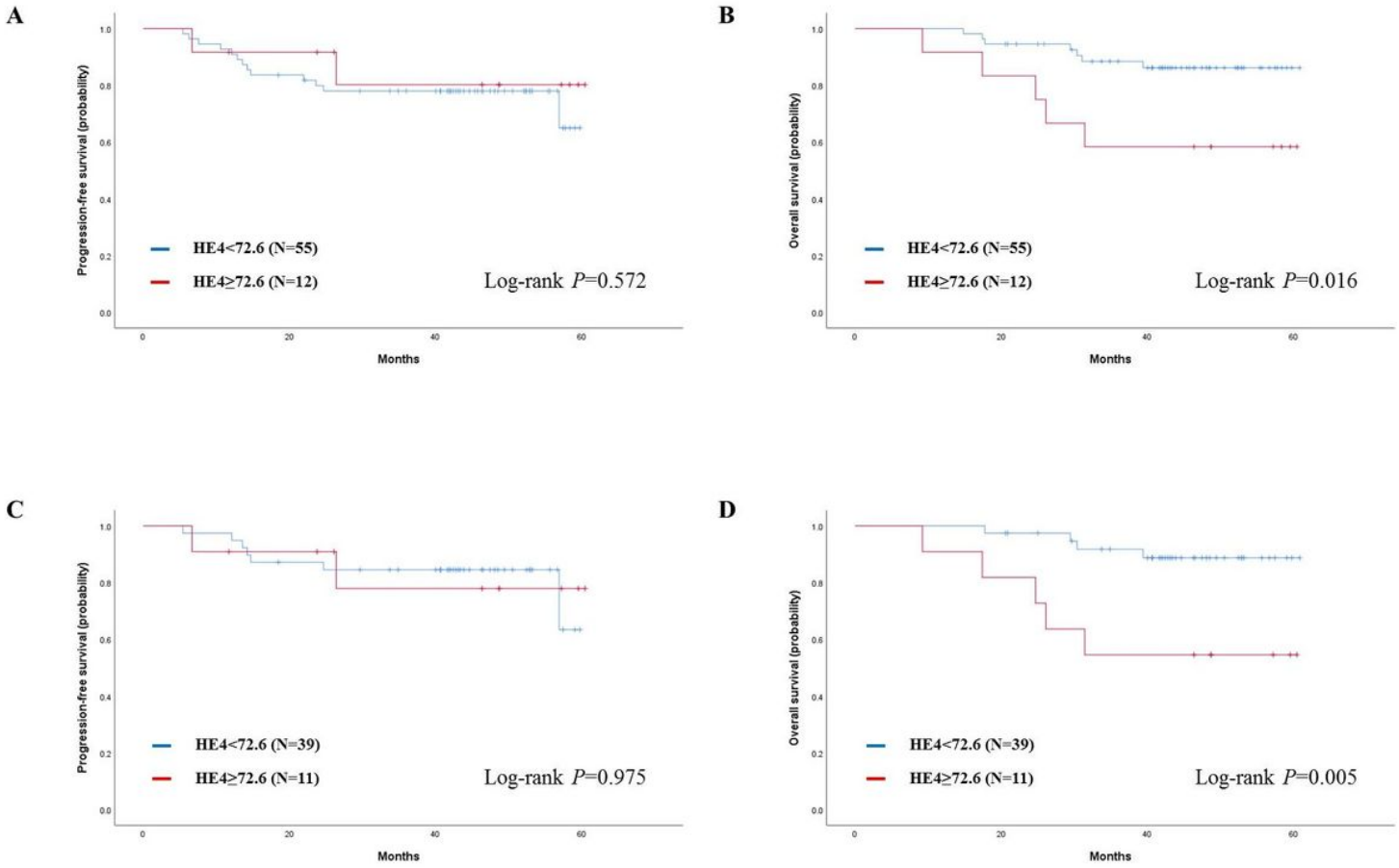


**Figure 1**

Comparisons of survival outcomes between patients with low (<72.6 pmol/L) and high (≥72.6 pmol/L) HE4. (A) Progression-free survival in the entire cohort; (B) Overall survival in the entire cohort; (C) Progression-free survival in patients with squamous cell carcinoma; (D) Overall survival in patients with squamous cell carcinoma.