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Original Article

The serum CK17 and CK19 expressions in cervical cancer patients and their prognostic value

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Abstract: Objective: This study was designed to quantify the serum CK17 and CK19 expressions in cervical cancer (CC) patients and determine their predictive value. Methods: A total of 124 CC patients admitted to Zhejiang North Medical Center (Huzhou Central Hospital) between November 2014 and November 2017 were recruited for the study and placed in a research group (the Res group), and 99 healthy individuals during the same period were also recruited for the study and placed in a control group (the Con group). Their serum CK17 and CK19 expressions were quantified, and the diagnostic significance of the two for CC was analyzed. Additionally, the patients were followed up for three years. The patients were then assigned to favorable and unfavorable prognosis groups, and then the predictive significance of CK17 and CK19 for such patients was evaluated. Results: The Res group presented significantly higher serum CK17 and CK19 expression levels than the Con group, and the two factors were positively associated. Additionally, neither of the AUCs for serum CK17 and CK19 in identifying CC were less than 0.800, and the AUC of the combination of the two in identifying it was not smaller than 0.900. The AUC of the combination of serum CK17 and CK19 in identifying unfavorable CC prognoses was approximate 0.850, and high expression levels CK17 and CK19 were closely related with low three-year overall survival rates. Conclusion: Serum CK17 combined with serum CK19 is of great diagnostic and predictive significance for CC.

Keywords: Cervical cancer, CK17, CK19, prognosis, serum

Introduction

Cervical cancer (CC), a significant cause of death among females, shows an increasing morbidity among developing countries [1, 2]. The difficulty of its therapy primarily results from its low early diagnosis rate that gives rise to unfavorable prognoses and heavily compromises patients' survival [3]. According to CC epidemiological data, there are approximately 569,847 new cases of CC and 311,365 deaths from it worldwide each year [4]. In terms of its etiology, CC is known to be strongly related to human persistent papillomavirus (HPV) infection, and it primarily takes its toll on the reproductive health of middle-aged women [5]. Currently, the screening test accuracy of CC still needs improvement, and pursuing efficient indices for screening CC based on biological indices helps maintain women's reproductive health [6, 7].

CK17 is a basic/myoepithelial cytokeratin strongly associated with the unfavorable prognoses of many malignancies, such as gastric cancer, ovarian cancer, breast cancer, and papillary thyroid cancer [8, 9]. At the current stage, recognized as an unfavorable biological index of CC, CK17 is able to mediate the characteristics of cancer stem cells and lymphatic metastasis *in vivo* during CC pathogenesis. Expressing CK17 will worsen CC by inducing the above process [10]. CK19 and CK17 are both stem cell markers of hair follicle lesions after hair loss, and have a great potential to treat hair loss [11]. Some studies have pointed out that CK19 is a guiding marker of lymph node micrometastasis in early CC and is associated with CC malignant transformation caused by integration with the HPV virus [12, 13].

Currently, studies on serum CK17 and CK19 in CC and their predictive significance for prognosis are scarce. There, with the two aspects as our main research directions, we conducted an in-depth analysis, which is of clinical significance for screening CC and determining its prognosis.

Materials and methods

General materials

A total of 124 female patients with CC admitted to Zhejiang North Medical Center (Huzhou Central Hospital) between November 2014 and November 2017 were recruited for the study and placed in the research group (the Res group, (46.84±17.37) years old on average), and 99 healthy female individuals during the same span were also recruited for the study and placed into the control group (the Con group, (48.49±16.96) years old on average), and their serum was collected. Approval for this study was obtained from the Ethics Committee of Zhejiang North Medical Center (Huzhou Central Hospital), and signed informed consent documents were obtained from all the participants or their guardians after they were apprised of the study. Additionally, the study was carried out in strict accordance with the *Declaration of Helsinki.*

Inclusion and exclusion criteria

Inclusion criteria: Patients confirmed to have CC [14], patients who had not received any associated therapy, and patients with detailed follow-up data.

Exclusion criteria: Patients with severe comorbid organ or systemic disease, malignancies, or infectious diseases, pregnant or lactating women.

Follow-up

The patients were followed up by accessing their medical records or by using their follow-up data for three years once every three months, and their survival or disease progress was recorded. Overall survival (OS) refers to the period from the day of diagnosis to the death day or the final day of the follow-up.

Determination means

Elbow venous blood (5 mL) was sampled from each participant who had experienced 8 h fasting early in the morning and then placed in coagulation-promoting tubes, and then transferred to new tubes, followed by 10 min centrifugation (1500×g, 4°C), after which the obtained supernatant was stored at -80°C for subsequent analysis. Afterwards, the serum CK17 and CK19 levels were quantified using ELISA in strict accordance with the guidelines of the CK17 and CK19 assay kits (A6321 and ABE10596, Walan Biotechnology Co., Ltd., Shanghai, CN) [15]. Finally, a spectrophotometer (UV5Nano, ZEPING Bioscience & Technologies Co., Ltd., Beijing, CN) was used to determine the CK17 and CK19 concentrations.

Statistical analysis

We used GraphPad Prism 7.0 and SPSS 22.0 (Baiao Yijie Technology Co., Ltd., Beijing, China) for the data analysis. The inter-group comparisons of the enumeration data, expressed as cases/percentages (n/%), were performed using chi-square tests, and the data with a theoretical frequency in the chi-square tests lower than 5 were analyzed using continuity correction chi square tests. The inter-group comparisons of the measurement data, expressed as the mean ± SEM, were performed using independent-samples T tests. ROC curve were used to evaluate the diagnostic and prognostic significance of CK17 and CK19 for CC, and Pearson's coefficient was used to analyze the association between the CK17 and CK19 expressions. The Kaplan-Meier method was used to calculate the OS, and log-rank tests were used to evaluate the inter-group survival-time differences. $P<0.05$ indicated a significant difference.

Results

Baseline data

There were no significant differences between the two groups in terms of age, mean age, HPV infection, lymph node metastasis (LNM), TNM stage, pathological grade, tumor size, smoking history, abortion history, or place of residence (all $P>0.05$) (**Table 1**).

The upregulation of serum CK17 and CK19 in CC patients

We quantified the serum CK17 and CK19 levels in the Res group (n=124) and the Con group (n=99). We found that the serum CK17 and CK19 expressions in the Res group were significantly higher than they were in the Con group ($P<0.05$) (**Figure 1**).

Table 1. Baseline data of the two groups [n (%), mean ± SEM]

Factor	n	The research group (n=124)	Control group (n=99)	χ^2/t	*P*-value
Age (Y)				0.541	0.462
<50	121	70 (56.45)	51 (51.52)		
≥50	102	54 (43.55)	48 (48.48)		
Mean age (Y)	223	46.84±17.37	48.49±16.96	0.712	0.477
HPV infection				2.899	0.089
No	22	16 (12.90)	6 (6.06)		
Yes	201	108 (87.10)	93 (93.94)		
Lymph node metastasis				0.678	0.410
No	108	57 (45.97)	51 (51.52)		
Yes	115	67 (54.03)	48 (48.48)		
TNM stage				3.760	0.053
I-II	101	49 (39.52)	52 (52.53)		
III-IV	122	75 (60.48)	47 (47.47)		
Pathological grade				0.212	0.646
G1	78	45 (36.29)	33 (33.33)		
G2-G3	145	79 (63.71)	66 (66.67)		
Tumor size (cm)				0.163	0.686
<4	107	58 (46.77)	49 (49.49)		
≥4	116	66 (53.23)	50 (50.51)		
Smoking history				2.045	0.153
No	153	90 (72.58)	63 (63.64)		
Yes	70	34 (27.42)	36 (36.36)		
Abortion history				0.736	0.391
No	164	94 (75.81)	70 (70.71)		
Yes	59	30 (24.19)	29 (29.29)		
Place of residence				0.201	0.654
Rural area	87	50 (40.32)	37 (37.37)		
Urban area	136	74 (59.68)	62 (62.63)		

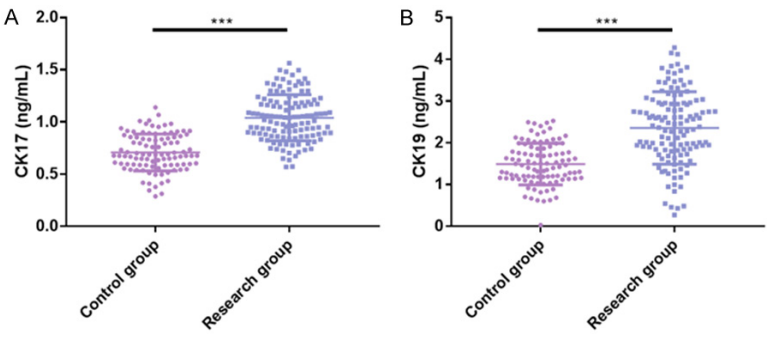

Figure 1. The up-regulation of serum CK17 and CK19 in patients with CC. A. The research group showed significantly higher serum CK17 levels than the control group. B. The research group showed significantly higher serum CK19 levels than the control group. Note: ***P<0.001.

The positive association between the serum CK17 and CK19 levels in CC patients

According to our Pearson's coefficient-based analysis of the association between the serum CK17 and serum CK19 levels in CC patients, there was a significantly positive association between them (r=0.745, P<0.001) (**Figure 2**).

The significance of the serum CK17 and CK19 levels in diagnosing CC

We studied the diagnostic significance of the serum CK17 and CK19 levels for diagnosing CC using ROC curves and found that the AUC, optimal cut-off, sensitivity, and specificity of the serum CK17 level in diagnosing CC were 0.878 (95% CI: 0.835-0.921), 0.91 ng/mL, 68.55%, and 89.90% respectively. For serum CK19, the values were 0.804 (95% CI: 0.747-0.861), 1.79 ng/mL, 76.61%, and

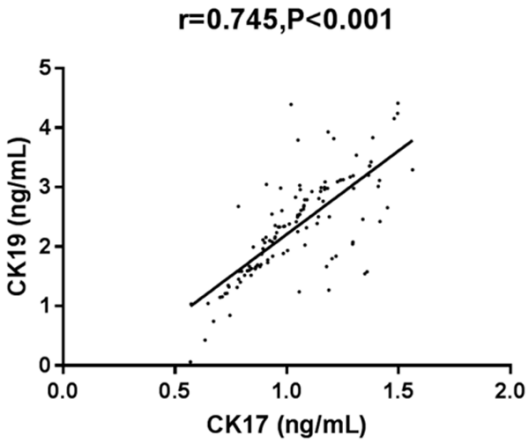

Figure 2. The positive association of serum CK17 with serum CK19 in patients with CC (r=0.745, P<0.001).

73.74%, respectively, and for serum CK17 combined with CK19 in diagnosing CC, the values were 0.926 (95% CI: 0.894-0.959), 0.56 ng/mL, 84.68%, and 86.87%, respectively (**Figure 3** and **Table 2**).

The predictive significance of the serum CK17 and CK19 levels for determining the prognosis of CC

The successful three-year follow-up of the 134 patients revealed that their three-year OS was 50.00% (62/124). The deceased patients and those whose diseases deteriorated were assigned to an unfavorable prognosis group (UPG, n=62), and the rest to a favorable prognosis group (FPG, n=62). As the data show, the UPG group had significantly higher expressions of serum CK17 and CK19 than the FPG (P<0.05). The ROC analysis revealed that the AUC, optimal cut-off, sensitivity, and specificity of the serum CK17 in identifying CC were 0.805 (95% CI: 0.729-0.881), 0.85 ng/mL, 88.71%, and 59.68%, respectively; the AUC, optimal cut-off, sensitivity, and specificity of serum CK19 in diagnosing CC were 0.801 (95% CI: 0.718-0.884), 1.94 ng/mL, 75.81%, and 80.65%. The AUC, optimal cut-off, sensitivity, and specificity of serum CK17 combined with serum CK19 in diagnosing CC were 0.880 (95% CI: 0.818-0.942), 0.68 ng/mL, 72.58%, and 96.77% (**Figure 4** and **Table 3**).

The association of the serum CK17 and CK19 levels with the three-year OS of CC patients

Survival curves were drawn with the median serum CK17 and CK19 expression levels as the critical points (0.97 ng/mL and 1.82 ng/mL, respectively). The curves revealed a strong association between low serum CK17 and CK19 and comparatively high three-year OS among the CC patients (P<0.001) (**Figure 5**).

Discussion

CC, a pervasive gynecological tumor with a high mortality and morbidity, poses a public health challenge, has a negative societal impact, and severely impairs women's physical and mental health [16]. Giving priority to the screening of CC and to potential novel biomarkers for determining its prognosis is of profound significance for the early diagnosis of tumors [17]. Serum indices are likely to be the preferred tools for CC screening in the future because of their advantages of being non-invasive, convenient, and cheap [18, 19]. This study started with the serum biological indices, aiming to offer novel insights for the efficient screening and prognosis of CC.

A growing number of scholars now focus on the in-depth analysis of the diagnostic value of CK17 and CK19. Podoll and team members [20] reported that CK17 can be used for diagnosing anal genital diseases like differentiated vulvar intraepithelial tumors as an immunohistochemical index. One study by Liang and team members [21] pointed out that CK17 can used to screen adenoid basal carcinoma of the cervix as an auxiliary index, and it plays a role in cases of ambiguous histological examination results. There are some intriguing reports on CK19. For instance, Domagala and team members [22] revealed the beneficial impact of Nuclear CK19 immuno-positive pseudoinclusion bodies on improving the diagnostic efficiency of papillary thyroid carcinoma. Winter and team members [23] pointed out the promotion of one-step nucleic acid amplification of CK19 on LNM in cases with prostate cancer. All the above studies have verified the diagnostic significance of CK17 and CK19 in malignancies such as reproductive tumors. In our study, the Res group presented significantly higher expressions of serum CK17 and CK19 than the Con group, indicating that the abnormal dynamic changes of CK17 and CK19 as serum indices probably help indicate the development of CC. Additionally, our analysis of the association between the serum CK17 and serum CK19 levels revealed that they were strongly positively associated, suggesting that they play a potential synergistic role in the progression of CC. In

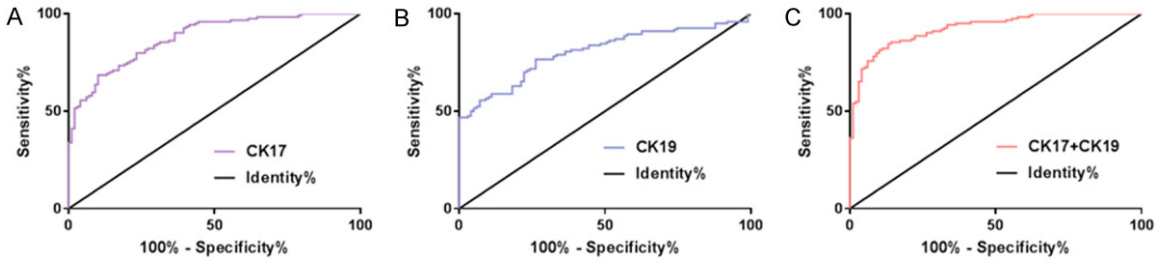

Figure 3. The AUCs of serum CK17 and CK19 in identifying CC.

Table 2. The ROC parameters of the serum CK17 and CK19 levels in identifying CC

Index	AUC	95% CI	Standard error	cutoff value (ng/mL)	Sensitivity (%)	Specificity (%)
CK17	0.878	0.835-0.921	0.022	0.91	68.55	89.90
CK19	0.804	0.747-0.861	0.030	1.79	76.61	73.74
CK17+CK19	0.926	0.894-0.959	0.017	0.56	84.68	86.87

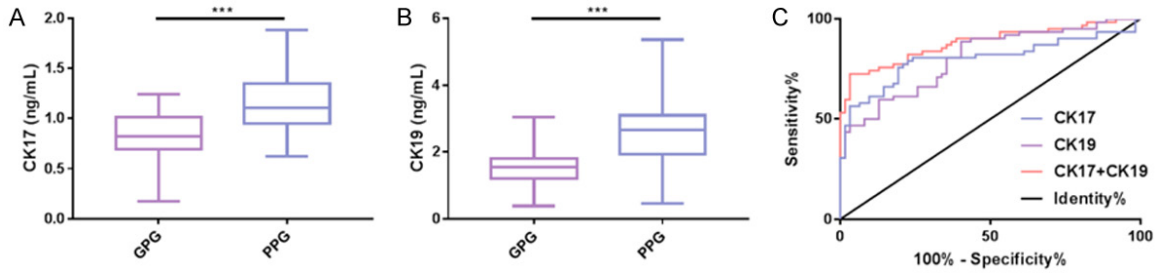

Figure 4. The ROC curves of serum CK17 and CK19 in forecasting the prognosis of CC.

Table 3. The ROC parameters of the serum CK17 and CK19 levels for determining the prognosis of CC

Index	AUC	95 %CI	Standard error	Cutoff value (ng/ml)	Sensitivity (%)	Specificity (%)
CK17	0.805	0.729-0.881	0.039	0.85	88.71	59.68
CK19	0.801	0.718-0.884	0.042	1.94	75.81	80.65
CK17+CK19	0.880	0.818-0.942	0.032	0.68	72.58	96.77

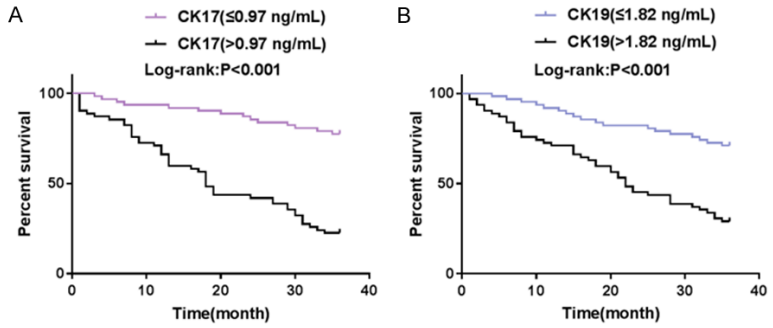

Figure 5. The association of the serum CK17 and CK19 levels with the three-year OS of patients with CC. A. The strong association of low serum CK17 levels with the comparatively high three-year OS among cases with CC. B. The strong association of low serum CK19 levels with comparatively high three-year OS among CC patients.

our evaluation of CC diagnosis, the AUCs of serum CK17 and CK19 were found to be 0.878 and 0.804, respectively, the AUC of serum CK17 combined with serum CK19 to be up to 0.926, the sensitivity of the combination to be 84.68%, and the specificity of the combination to be 86.87%. The results imply the significantly high diagnostic values of serum CK17 + serum CK19 in identifying CC, with both high sensitivity and specificity.

CK17 also has an enormous potential in determining the prognosis of many malignancies. For instance, one study by Carrasco and team members [24] has indicated that CK17 can be used to predict the differentiation of gallbladder cancer as a gastrointestinal tumor-associated protein. Another study by Liu and team members [25] revealed that there is a strong association between CK17 in cases with triple negative breast cancer and the progression of the cancer, and it can help indicate an unfavorable prognosis, a high differentiation degree and axillary LNM. Coincidentally, CK19 is also of potential prognostic significance in malignancies. For instance, Yang and team members [26] reported CK19 as an independent prognostic marker of esophageal squamous cell carcinoma, and its ability to help forecast the survival of patients with this disease. Moreover, reportedly, the combination of CK19 with Hep Par1 can augment the ability to forecast the prognosis of hepatocellular carcinoma [27]. In our study, the three-year OS of patients with CC was 50.00%, similar to the result of Kubota and team members (47.00%) [28]. The AUC of serum CK17 or CK19 in predicting the prognosis of CC was approximate 0.800, and the AUC of the combination was up to 0.880, which suggests the high value of their combination in determining the CC prognosis. Finally, our three-year OS curve analysis revealed a strong association of the high expressions of CK17 and CK19 with the comparatively three-year low OS, indicating that CK17 and CK19 have a potential in predicting the 3-year OS of CC patients.

Our study verified the up-regulation of serum CK17 and CK19 in patients with CC and the possible function of their combination in helping determine the prognosis of CC. Nevertheless, this study still has room for improvement. First, we can supplement our analysis of the influencing factors on the prognosis of CC and further verify whether CK17 and CK19 are potential prognostic markers of such patients. Second, we can conduct basic research to further clarify the potential regulatory mechanism of CK17 and CK19 in the process of CC. Finally, we can analyze the association of the two with the pathological parameters of CC patients to further explore the potential clinical significance of the two. We will continue to improve our study in the future.

To sum up, we have proposed for the first time the role of serum CK17 and serum CK19 as an auxiliary index for screening and determining the prognosis of CC, which will offer clinical information for the evaluation and prognosis analysis of CC.

Disclosure of conflict of interest

None.

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References

[1] Moga MA, Dima L, Balan A, Blidaru A, Dimienescu OG, Podasca C and Toma S. Are bioactive molecules from seaweeds a novel and challenging option for the prevention of hpv infection and cervical cancer therapy?-A review. Int J Mol Sci 2021; 22: E629.

[2] Tan X, Li K, Zhang J, Wang W, Wu B, Wu J, Li X and Huang X. Automatic model for cervical cancer screening based on convolutional neural network: a retrospective, multicohort, multicenter study. Cancer Cell Int 2021; 21: 35.

[3] Pulati N, Zhang Z, Gulimilamu A, Qi X and Yang J. HPV16(+) -miRNAs in cervical cancer and the anti-tumor role played by miR-5701. J Gene Med 2019; 21: e3126.

[4] Lin W, Wang Y, Liu Z, Chen B, Yuan S, Wu B and Gong L. Inequalities in awareness and attitude towards HPV and its vaccine between local and migrant residents who participated in cervical cancer screening in Shenzhen, China. Cancer Res Treat 2020; 52: 207-217.

[5] Arbyn M, Weiderpass E, Bruni L, de Sanjose S, Saraiya M, Ferlay J and Bray F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health 2020; 8: e191-e203.

[6] Zheng M, Hou L, Ma Y, Zhou L, Wang F, Cheng B, Wang W, Lu B, Liu P, Lu W and Lu Y. Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. Mol Cancer 2019; 18: 76.

[7] Jiao X, Zhang S, Jiao J, Zhang T, Qu W, Muloye GM, Kong B, Zhang Q and Cui B. Promoter methylation of SEPT9 as a potential biomarker for early detection of cervical cancer and its overexpression predicts radioresistance. Clin Epigenetics 2019; 11: 120.

[8] Kim K, Lee HW, Chae SW, Kim DH, Do IG, Lee HJ, Do SI, Min KW, Pyo JS, Shin JH and Sohn JH. Cytokeratin 17 expression is associated with poor prognosis in gallbladder adenocarcinoma. Appl Immunohistochem Mol Morphol 2017; 25: 346-350.

[9] Kim HS, Lee JJ, Do SI, Kim K, Do IG, Kim DH, Chae SW and Sohn JH. Overexpression of cytokeratin 17 is associated with the development of papillary thyroid carcinoma and the presence of lymph node metastasis. Int J Clin Exp Pathol 2015; 8: 5695-5701.

[10] Wu L, Han L, Zhou C, Wei W, Chen X, Yi H, Wu X, Bai X, Guo S, Yu Y, Liang L and Wang W. TGF-beta1-induced CK17 enhances cancer stem cell-like properties rather than EMT in promoting cervical cancer metastasis via the ERK1/2-MZF1 signaling pathway. FEBS J 2017; 284: 3000-3017.

[11] El Sakka D, Gaber MA, Abdou AG, Wahed MA, Saleh AA and Shehata W. Stem cell markers (Cytokeratin 17 and Cytokeratin 19) in scarring and nonscarring alopecia. J Cutan Aesthet Surg 2016; 9: 165-171.

[12] Zhang F, Liu D, Lin B, Hao Y, Zhou D, Qi Y and Zhang S. Expression of high-risk HPV DNA and CK19 in pelvic lymph nodes in stage Ia-IIa cervical cancer and their clinical value. Oncol Rep 2012; 27: 1801-1806.

[13] Lee H, Lee H and Cho YK. Cytokeratin7 and cytokeratin19 expression in high grade cervical intraepithelial neoplasm and squamous cell carcinoma and their possible association in cervical carcinogenesis. Diagn Pathol 2017; 12: 18.

[14] Stumbar SE, Stevens M and Feld Z. Cervical cancer and its precursors: a preventative approach to screening, diagnosis, and management. Prim Care 2019; 46: 117-134.

[15] Ong DSY, de Man SJ, Lindeboom FA and Koeleman JGM. Comparison of diagnostic accuracies of rapid serological tests and ELISA to molecular diagnostics in patients with suspected coronavirus disease 2019 presenting to the hospital. Clin Microbiol Infect 2020; 26: 1094.e7-1094.e10.

[16] Ou R, Lv J, Zhang Q, Lin F, Zhu L, Huang F, Li X, Li T, Zhao L, Ren Y and Xu Y. circAMOTL1 motivates AMOTL1 expression to facilitate cervical cancer growth. Mol Ther Nucleic Acids 2020; 19: 50-60.

[17] Farzanehpour M, Mozhgani SH, Jalilvand S, Faghihloo E, Akhavan S, Salimi V and Azad TM. Serum and tissue miRNAs: potential biomarkers for the diagnosis of cervical cancer. Virol J 2019; 16: 116.

[18] Du S, Zhao Y, Lv C, Wei M, Gao Z and Meng X. Applying serum proteins and microrna as novel biomarkers for early-stage cervical cancer detection. Sci Rep 2020; 10: 9033.

[19] Jiang W, Pan JJ, Deng YH, Liang MR and Yao LH. Down-regulated serum microRNA-101 is associated with aggressive progression and poor prognosis of cervical cancer. J Gynecol Oncol 2017; 28: e75.

[20] Podoll MB, Singh N, Gilks CB, Moghadamfalahi M and Sanders MA. Assessment of CK17 as a marker for the diagnosis of differentiated vulvar intraepithelial neoplasia. Int J Gynecol Pathol 2017; 36: 273-280.

[21] Liang Y, Lu B and Zhou C. Cervical adenoid basal carcinoma: clinicopathologic features of 9 cases with reference to CK17 and Ki-67 expression. J Low Genit Tract Dis 2019; 23: 77-81.

[22] Domagala P and Domagala W. Nuclear CK19-immunopositive pseudoinclusions as a new additional objective diagnostic feature of papillary thyroid carcinoma. Pol J Pathol 2020; 71: 1-6.

[23] Winter A, Engels S, Goos P, Suykers MC, Henke RP, Gerullis H and Wawroschek F. Detection of CK19 mRNA using one-step nucleic acid amplification (OSNA) in prostate cancer: preliminary results. J Cancer 2018; 9: 4611-4617.

[24] Carrasco C, Tittarelli A, Paillaleve N, Pozo MD, Rojas-Sepulveda D, Barria O, Fluxa P, Hott M, Martin C, Quezada C and Salazar-Onfray F. The evaluation of 17 gastrointestinal tumor markers reveals prognosis value for MUC6, CK17, and CD10 in gallbladder-cancer patients. Diagnostics (Basel) 2021; 11: 153.

[25] Liu ZB, Wu J, Ping B, Feng LQ, Shen ZZ and Shao ZM. Expression of CK5/6 and CK17 and its correlation with prognosis of triple-negative breast cancer patients. Zhonghua Zhong Liu Za Zhi 2008; 30: 610-614.

[26] Yang ZY, Zhang HY, Wang F, Ma YH, Li YY, He HL, Wang C and Li SS. Expression of cytokeratin(CK)7, CK8/18, CK19 and p40 in esophageal squamous cell carcinoma and their correlation with prognosis. Zhonghua Bing Li Xue Za Zhi 2018; 47: 834-839.

[27] Jin Y, Liang ZY, Zhou WX and Zhou L. Combination with CK19 might increase the prognostic power of hep Par 1 in hepatocellular carcinoma after curative resection. J Invest Surg 2018; 31: 412-419.

[28] Kubota H, Tsujino K, Sulaiman NS, Sekii S, Matsumoto Y, Ota Y, Soejima T, Yamaguchi S and Sasaki R. Comparison of salvage therapies for isolated para-aortic lymph node recurrence in patients with uterine cervical cancer after definitive treatment. Radiat Oncol 2019; 14: 236.