


# Diagnostic Value of Vaginal Microecology, Serum miR-18a, and PD-L1 for Identifying HPV-Positive Cervical Cancer

Technology in Cancer Research & Treatment  
Volume 20: 1-7  
© The Author(s) 2021  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1533033821995281  
journals.sagepub.com/home/tct  


Yumei Zhang<sup>1</sup>, Sujuan Qiu<sup>2</sup>, Yueli Guo<sup>3</sup>, Jiaqin Zhang<sup>1</sup>,  
Xiaoqing Wu<sup>4</sup>, and Guolin Hong<sup>1</sup> 

## Abstract

**Objective:** We aimed to investigate the diagnostic value of the vaginal microecology, serum miR-18a, and programmed death ligand-1 (PD-L1) for human papillomavirus (HPV)-positive cervical cancer. **Methods:** Eighty-four patients with HPV-positive cervical cancer were assigned to the observation group, 107 HPV-positive patients without cervical cancer were assigned to the positive group, and 191 healthy women were assigned to the control group. Vaginal microecology and serum levels of miR-18a and PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells were compared among the 3 groups. The observation group was further divided into subgroups according to patients' characteristics for comparison. The diagnostic value of miR-18a and PD-L1 for HPV-positive cervical cancer was investigated. **Results:** Women in the control group had better vaginal microecology and lower levels of miR-18a and PD-L1 than those in the observation and the positive groups (all  $P < 0.05$ ). Compared with the positive group, the observation group had similar vaginal microecology (all  $P > 0.05$ ) but higher levels of miR-18a and PD-L1 (all  $P < 0.05$ ). Moreover, the patients at stage III had higher levels of miR-18a and PD-L1 than those at stage I and II (all  $P < 0.05$ ). The values of area under the curve for miR-18a and PD-L1 in the diagnosis of HPV-positive cervical cancer were over 0.8 (all  $P < 0.001$ ). **Conclusion:** Patients with HPV-positive cervical cancer have vaginal microbial dysbiosis and high serum levels of miR-18a and PD-L1. miR-18a and PD-L1 have diagnostic value for identifying HPV-positive cervical cancer.

## Keywords

vaginal microecology, programmed death ligand-1, human papillomavirus, cervical cancer serum miR-18a

## Abbreviations

PD-L1, programmed death ligand-1; HPV, human papillomavirus; PD-I, programmed death-I; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; BMI, body mass index; miRNA, microRNA

Received: July 29, 2020; Revised: November 20, 2020; Accepted: January 25, 2021.

## Introduction

Human papillomavirus (HPV) is mainly transmitted through sexual intercourse. In most cases, the infection can be cleared spontaneously, whereas in some cases, the infection can stay. It is believed that high-risk HPV when remaining at a high level in human body for a long time, can lead to cervical intraepithelial neoplasia or cervical cancer.<sup>1</sup> Epidemiological data have revealed that there were about 527,600 new cases of cervical cancer and 265,700 deaths from cervical cancer in 2012 worldwide. Moreover, the morbidity of cervical cancer keeps increasing, and patients affected are becoming younger.<sup>2</sup> Although studies have demonstrated that high-risk HPV

<sup>1</sup> Department of Laboratory Medicine, Xiamen Key Laboratory of Genetic Testing, The First Affiliated Hospital of Xiamen University, Xiamen, Fujian Province, China

<sup>2</sup> Department of Laboratory Medicine, Xinglin Branch of the First Affiliated Hospital of Xiamen University, Xiamen, Fujian Province, China

<sup>3</sup> Department of Medical Technology, Zhangzhou Health Vocational College, Zhangzhou, Fujian Province, China

<sup>4</sup> Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian Province, China

## Corresponding Author:

Guolin Hong, Department of Laboratory Medicine, Xiamen Key Laboratory of Genetic Testing, The First Affiliated Hospital of Xiamen University, No.55 Zhenhai Road, Siming District, Xiamen 361003, Fujian Province, China.  
Email: hongguolin1yxd@163.com



infection is the main cause of cervical cancer, the pathogenesis and prognosis of this disease are also associated with other factors such as vaginal microecology, inflammatory factors, genetic factors, and immune function of the body.<sup>3,4</sup> In recent years, surgical methods, chemotherapy, and radiotherapy have become the main methods for treating early cervical cancer, and the 5-year survival rate can exceed 90%. However, in patients with advanced or recurrent cervical cancer, the 5-year survival rate is less than 20% and 5%, respectively.<sup>5</sup> Therefore, it is of great significance to find out a specific marker for detecting the occurrence and prognosis of cervical cancer.

Programmed death-1 (PD-1) and its ligand, programmed death ligand-1 (PD-L1), are negative immunoregulatory factors discovered in recent years. Activations of PD-1 and PD-L1 can prevent the activation of T cells in the tumor microenvironment, thereby helping the tumor cells to escape the immune surveillance and killing.<sup>6</sup> Studies have shown that PD-L1 is highly expressed in a variety of solid tumors.<sup>7</sup> microRNA (miRNAs) is an endogenous non-coding RNA at a length of 19-25 nucleotides. It can negatively regulate the target genes after transcription and has a regulatory role in the occurrence, development, and invasion of the tumor cells.<sup>8,9</sup> Since miRNAs can exist in body fluids in an abundant and stable form, serum and plasma can be used as test specimen instead of primary tumor tissue as the testing can be noninvasive and convenient in the early diagnosis of tumor. Research has revealed that miR-18a is overly expressed in many solid carcinomas, such as liver cancer and esophageal cancer, and high level of miR-18a can be detected in patients' serum, suggesting that miR-18a may be a novel biomarker in tumor diagnosis and the prognosis assessment.<sup>10</sup> Since cervical cancer is also solid cancer, whether there is an over-expression of miR-18a in patients with cervical cancer and the diagnostic significance of miR-18a in HPV-positive cervical cancer drew our attention. Therefore, in this study, we investigated the diagnostic value of vaginal microecology, miR-18a, and PD-L1 for identifying HPV-positive cervical cancer.

## Materials and Methods

### Participants

Of the patients treated in our hospital from January 2017 to January 2019, 84 patients with HPV-positive cervical cancer were assigned to the observation group, and 107 HPV-positive patients without cervical cancer were assigned to the positive group. Meanwhile, 191 healthy women were assigned to the control group. The study was approved by the Ethics Committee of our hospital, and informed consent was obtained from the participants and their family members. See Supplementary File.

### Inclusion and Exclusion Criteria

**Inclusion criteria.** Inclusion criteria for the control group were as follows: 1) women who had normal menstrual cycle; 2) women

who had a sexual partner or history of sexual intercourse; 3) women who did not receive any antimicrobial medicine over the past 3 months.<sup>5</sup>

**Inclusion criteria for the positive group** were: 1) women who had positive HPV-DNA test result, and the HPV type was HPV16 or 18; 2) women who did not receive any antimicrobial medicine over the past 3 months; 3) women who had normal menstrual cycle; 4) women who had a sexual partner or history of sexual intercourse.

**Inclusion criteria for the observation group** were: 1) women who had cervical cancer confirmed by pathological examination, and the HPV type was HPV-16 or HPV-18; (2) women who did not receive any radiotherapy, chemotherapy, or immunotherapy; 3) women who did not have other malignant tumors or other infectious diseases; 4) women who had a sexual partner and sexual intercourse.

**Exclusion criteria.** Exclusion criteria for the control group were: 1) women who had other severe diseases such as immune or endocrine system diseases; 2) women who were menstruating or had irregular vaginal bleeding; 3) women who were pregnant or lactating; 4) women with poor compliance; 5) women who received uterine or cervical surgery in the past; 6) women with vaginitis or other inflammatory diseases that may affect the results of the study.

**Exclusion criteria for the positive group** were: 1) women with cervical cancer; 2) women who had other severe diseases such as immune or endocrine system diseases; 3) women who were menstruating or had irregular vaginal bleeding; 4) women who were pregnant or lactating; 5) women with poor compliance; 6) women who received uterine or cervical surgery in the past; 7) women with vaginitis or other inflammatory diseases that may affect the results of the study.

**Exclusion criteria for the observation group:** 1) women who received radiotherapy, chemotherapy, or immunotherapy; 2) women who had other severe diseases such as immune or endocrine system diseases; 3) women who were menstruating or had irregular vaginal bleeding; 4) women who were pregnant or lactating; 5) women with poor compliance; 6) women who received uterine or cervical surgery in the past; 7) women with vaginitis or other inflammatory diseases that may affect the results of the study.

### Outcome Measures

**Main outcome measures.** The main outcome measures included the serum levels of miR-18a and PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as vaginal microecology (cleanliness, presence of candida and trichomonas, pH value, lactobacillus level, and microbiota diversity and density). The diagnostic performance of the serum levels of miR-18a and PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in detecting HPV-positive cervical cancer was analyzed using receiver operating characteristic (ROC) curves.

**Table 1.** Classification of the Results of the Markers.

Criteria	
Cleanliness	
I	Most of the bacteria are vaginal bacillus and there are a large number of epithelial cells.
II	Part of the bacteria are vaginal bacillus and there are some epithelial cells, pyocytes, and other bacteria.
III	A small amount of vaginal bacillus and epithelial cells and a large number of pyocytes and other bacteria.
IV	No vaginal bacillus, a large number of pyocytes and other bacteria.
Candida	
Positive	Spores and hyphae can be observed under the microscope.
Negative	Spores and hyphae cannot be observed under the microscope.
Trichomonas	
Positive	Trichomonas vaginalis can be observed under the microscope.
Negative	Trichomonas vaginalis cannot be observed under the microscope.
Lack of lactobacilli	
Positive	≤ 30 / HPF
Negative	> 30 / HPF
Microbiota diversity	
I	1-3 types of bacteria identified.
II	4-6 types of bacteria identified.
III	7-10 types of bacteria identified.
IV	No less than 11 types of bacteria identified.
Microbiota density	
I	An average of 1-9 bacteria in one field.
II	An average of 10-99 bacteria in one field.
III	No less than 100 bacteria in one field.
IV	The bacteria agglomerate.

Abbreviation: HPF, high power field.

**Secondary outcome measures.** Patients in the observation group were further divided into subgroups based on their age, pathological type, differentiation degree, tumor size, vascular invasion, and clinical stage. The expression levels of serum miR-18a and PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells were compared between the groups.

## Methods

### Vaginal Microecological Examination

On the next day after grouping, the vaginal secretion of each participant was collected using 3 disposable swabs. One swab was used to make Wright-Giemsa stained dried smear for detecting the lactobacillus level and microbiota diversity and density under the oil immersion lens; another swab was placed in 0.85% sodium chloride for measuring the cleanliness of the

**Table 2.** Primer Sequences.

Primer	Sequence
miR-18a forward primer	5'-TAAGTGTGCGCATCGTGAGCG ATAG-3'
snRNA U6 forward primer	5'-CTCGCTTCGGCAGCACA-3'
snRNA U6 reverse primer	5'-AACGCTTCACGAATTTGCGT-3'

secretion and levels of candida, trichomonas, and lactobacillus; the third swab was used to detect PH value of the vaginal secretion.

The interpretation of the results is listed in Table 1.<sup>11</sup> The microbiota diversity or density was considered as positive if its grade was II or above.

### Detection of Serum Levels of Mir-18a and PD-L1 on the Surface of CD4<sup>+</sup> and CD8<sup>+</sup> T Cells

The fasting venous blood samples (3 ml \* 2 tubes) were drawn from each participant the next morning after grouping. The blood samples were centrifuged at 3,000 rpm for 15 min. After separating the supernatant, the percentage of PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the peripheral blood was detected using a flow cytometer (BD FACSCalibur, USA). Meanwhile, total mRNAs were extracted from the blood sample in another tube for detecting the expression levels of miR-18a by RT-PCR (PCR kit: Takara, Japan). The primers (designed and synthesized by Dingguo Biotech, Beijing, China) are listed in Table 2. The universal downstream primers of miR-18a were from the reverse transcription kit (Mir-XTM miRNA First Strand kit, Takara, Japan). The reverse transcription and RT-PCR were performed according to the manufacturer's instructions of the kits (RT-PCR kit: SYBR RT-PCR kit, Takara, Japan). The reaction conditions of the PCR were as follows: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, and annealing at 60°C for 30 s; the cycles were repeated 35 times. The relative expression levels of miR-18a and U6 in each group were measured using the 2<sup>-ΔΔCt</sup> method with GADPH as the internal reference. Each sample was tested 3 times to calculate the average value.

### Statistical Analysis

SPSS 22.0 was applied for statistical analysis. Count data are presented as number or percentage and were compared by chi-square test and partitions of chi-square ( $\alpha = 0.0167$ ); measurement data are presented as mean ± standard deviation and were compared by one-way analysis of variance and Bonferroni post-hoc test. ROC curve was plotted to analyze the clinical value of serum miR-18a and PD-L1 in the diagnosis of HPV-positive cervical cancer. P < 0.05 indicated a statistically significant difference.

**Table 3.** Baseline Data in the 3 Groups.

	Observation group (n = 84)	Positive group (n = 107)	Control group (n = 191)	$\chi^2/t$	P
Age (year)	41.23 ± 3.34	41.09 ± 3.81	41.01 ± 4.42	0.087	0.917
Number of childbirths					
0	2	2	9	4.721	0.58
1	43	56	109		
2	36	47	70		
3	3	2	3		
Marital status					
Married	69	85	155	2.412	0.878
Divorced	12	17	27		
Widowed	3	5	7		
Unmarried	0	0	2		
Menopause					
Yes	3	3	5	0.193	0.908
No	81	104	186		
BMI (kg/m <sup>2</sup> )	23.78 ± 3.35	23.41 ± 3.57	23.82 ± 3.94	0.445	0.641

Abbreviations: HPV, human papillomavirus; BMI, body mass index.

**Table 4.** Vaginal Microecology in the 3 Groups.

		Observation group (n = 84)	Positive group (n = 107)	Control group (n = 191)	$\chi^2$	P
Cleanliness	I, II	41	56	161	49.157	<0.001
	III, IV	43**	51**	30		
PH	≤4.5	46	61	159	33.588	<0.001
	>4.5	38**	46**	32		
Candida	Negative	75	95	177	1.555	0.495
	Positive	9	12	14		
Trichomonas	Negative	81	104	186	0.193	0.908
	Positive	3	3	5		
Lack of lactobacillus	Negative	12	23	168	186.914	<0.001
	Positive	72**	84**	23		
Microbiota diversity	Negative	61	73	23	133.613	<0.001
	Positive	23**	34**	168		
Microbiota density	Negative	69	85	27	169.492	<0.001
	Positive	15**	22**	164		

Note. \*\*P < 0.01 vs. the control group.

## Results

### Baseline Data

No intergroup differences were found in the baseline data (all P > 0.05). See Table 3.

### Vaginal Microecology in the 3 Groups

There were no intergroup differences in the presence of candida and trichomonas (both P > 0.05). However, the patients in the control group had better vaginal microecology in terms of cleanliness, pH value, lactobacillus level, and microbiota diversity and density than the patients in the observation and the positive groups (all P < 0.05). Meanwhile, no differences were found in the microecology between the observation and the positive groups (all P > 0.05). See Table 4.

### The Expression Levels of Serum MIR-18a and PD-L1 on the Surface of CD4<sup>+</sup> and CD8<sup>+</sup> T Cells in the 3 Groups

Compared with the control group, the expression levels of serum miR-18a and PD-L1 on the surface of the CD4<sup>+</sup> and CD8<sup>+</sup> T cells were much higher in the observation and the positive groups (all P < 0.05). Moreover, the observation group had higher levels of these markers than the positive group (all P < 0.05). See Table 5.

### The Expression Levels of Serum miR-18a and PD-L1 in Patients with HPV-Positive Cervical Cancer

The observation group was further divided into subgroups of age, pathological type, differentiation, tumor size, vascular infiltration, and clinical-stage for comparison of miR-18a and PD-L1 levels. The patients at stage III had higher levels of miR-18a and PD-L1 levels than patients at stage I-II (all P < 0.05). No

differences were observed in the levels of these markers between patients with different characteristics in other subgroups (all  $P > 0.05$ ). See Table 6.

### ROC Curve Results

The values of the area under the curve (AUC) of miR-18a and PD-L1 in the diagnosis of HPV-positive cervical cancer were all over 0.700. See Table 7 and Figure 1.

**Table 5.** The Expression Levels of Serum miR-18a and PD-L1 on the Surface of CD4<sup>+</sup> and CD8<sup>+</sup> T Cells in the 3 Groups.

	CD4 <sup>+</sup> PD-L1 (%)	CD8 <sup>+</sup> PD-L1 (%)	miR-18a
Observation group (n = 84)	10.91 ± 3.58* <sup>#</sup>	11.37 ± 4.18* <sup>#</sup>	5.11 ± 1.75* <sup>#</sup>
Positive group (n = 107)	6.31 ± 4.55*	6.98 ± 3.39*	3.07 ± 1.55*
Control group (n = 191)	3.61 ± 2.44	5.51 ± 3.29	1.73 ± 1.17
F	134.958	80.667	166.054
P	<0.001	<0.001	<0.001

\* $P < 0.05$  vs. the control group; <sup>#</sup> $P < 0.05$  vs. the positive group.

### Discussion

Studies have shown that vaginal microbial dysbiosis is closely associated with HPV infection. The vaginal infection caused by microorganisms can lead to the damage of reproductive tract mucosa, thereby increasing the risk of HPV invasion. Moreover, HPV infection can exert a negative impact on the mucosal metabolism and immune system to further damage the vaginal microecology, thus forming a vicious circle.<sup>12,13</sup> It has been reported that vaginal microbial dysbiosis is one of the main factors affecting the clearance rate of HPV. Lactobacilli are the predominant bacteria that help to maintain normal vaginal microecology. Studies have demonstrated that the dysbiosis of vaginal microecology is closely related to bacterial vaginosis.<sup>14</sup> The lack of lactobacilli can weaken the body's ability to inhibit the pathogenic microorganisms, thus increasing the risk of HPV infection and reproductive tract inflammation and even causing cervical lesions in some severe cases.<sup>15</sup> Liu *et al.*,<sup>16</sup> after diagnosing 4,290 patients with bacterial vaginosis, found that bacterial vaginosis is closely associated with high-risk HPV infection. In our study, we found that compared with healthy women, patients who were HPV positive alone or who had HPV-positive cervical cancer were lack of lactobacilli and had much higher microbiota density and diversity, which aligns

**Table 6.** The Expression Levels of Serum miR-18a and PD-1 in Patients With HPV-Positive Cervical Cancer.

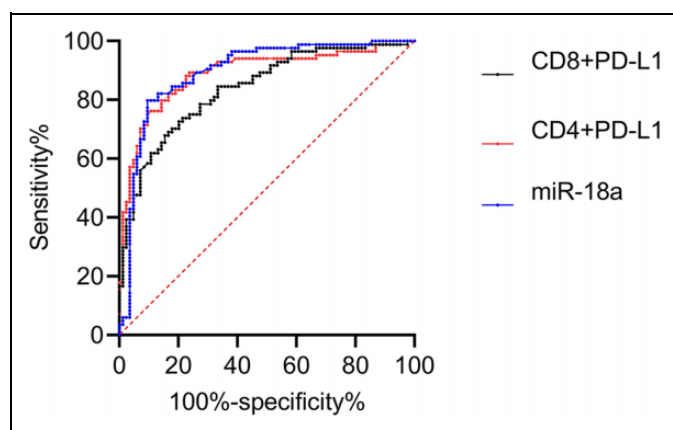
	CD4 <sup>+</sup> PD-L1 (%)	CD8 <sup>+</sup> PD-L1 (%)	miR-18a
Age			
≤40 (n = 24)	10.35 ± 4.94	10.98 ± 6.55	5.22 ± 4.43
>40 (n = 60)	10.68 ± 5.22	10.81 ± 6.23	5.12 ± 4.75
Pathological type			
Squamous cell carcinoma (n = 68)	10.45 ± 5.23	11.02 ± 6.87	5.19 ± 4.52
Adenocarcinoma (n = 16)	10.77 ± 5.29	10.84 ± 6.62	5.11 ± 4.37
Differentiation degree			
Moderately or well differentiated (n = 64)	10.41 ± 5.29	10.99 ± 6.51	5.24 ± 4.17
Poorly differentiated (n = 20)	10.61 ± 5.52	10.91 ± 6.87	5.09 ± 4.42
Tumor size (cm)			
<4 (n = 62)	10.46 ± 5.21	11.04 ± 6.67	5.21 ± 4.55
≥4 (n = 22)	10.74 ± 5.24	10.89 ± 6.62	5.11 ± 4.33
Vascular infiltration			
Positive (n = 61)	10.49 ± 5.22	11.01 ± 6.49	5.25 ± 4.21
Negative (n = 23)	10.59 ± 5.51	10.93 ± 6.67	5.11 ± 4.43
Clinical stage			
I, II (n = 49)	7.49 ± 3.55*	7.49 ± 4.33*	3.98 ± 2.31*
III (n = 35)	12.48 ± 6.61	12.28 ± 6.17	7.12 ± 4.32

Note. \* $P < 0.05$  (comparison of the levels of the markers between different clinical stages and between different HPV types). Abbreviations: HPV, human papillomavirus.

**Table 7.** ROC Curve Results.

	Cut-off value	AUC	95% CI	Sensitivity	Specificity	P
CD4 <sup>+</sup> PD-L1 (%)	7.543	0.844	0.783, 0.905	0.893	0.813	<0.001
CD8 <sup>+</sup> PD-L1 (%)	6.516	0.837	0.775, 0.899	0.940	0.785	<0.001
miR-18a	3.162	0.856	0.800, 0.912	0.952	0.757	<0.001

Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.



**Figure 1.** ROC curve results. ROC, receiver operating characteristic.

with the results of the previous studies. However, the correlations of trichomonas vaginalis and candida with HPV infection have not been elucidated. The results of our study showed no intergroup differences in the positive rates of trichomonas and candida among the 3 groups, and this result might be influenced by the sample size, sample source, and testing methods. Therefore, more studies need to be carried out in the future for verification.

PD-L1 level is closely related to the viral load of high-risk HPV. Persistent infection of high-risk HPV can inhibit the local immunity of the cervix transformation zone against HPV, and HPV may evade the host's immune response by activating the immunosuppression of PD-1 / PD-L1, which eventually leads to the cervical disease.<sup>17</sup> It has been reported that the levels of PD-1 and PD-L1 in T-lymphocytes of patients with cervical lesions are markedly increased.<sup>18</sup> The results of our study showed that compared with the healthy women, the HPV-positive patients without cervical cancer and the patients with HPV-positive cervical cancer had much higher levels of PD-L1 on the surface of T-lymphocytes; moreover, the magnitude of increase in the PD-L1 levels was greater in the patients with HPV-positive cervical cancer than in the HPV-positive patients without cervical cancer. Also, the levels of PD-L1 in the patients with high-risk HPV were higher than those with low-risk HPV. These results are consistent with the previous studies. In addition, we also investigated the clinical significance of PD-L1 in the diagnosis of HPV-infected cervical cancer. The ROC curve results showed high sensitivity and specificity of PD-L1 on the surface of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and demonstrated the clinical value of PD-L1 in the diagnosis of HPV-infected cervical cancer.

miR-18a belongs to the miR-17-92 cluster. It has been revealed that miR-18a can be a tumor suppressor gene. By regulating its downstream target gene, miR-18a can inhibit the *in vitro* proliferation of tumor cells, induce G1-phase arrest, and promote the apoptosis of tumor cells.<sup>19,20</sup> However, some studies have found that the expression levels of miR-18a are increased in breast cancer, glioblastoma, and other tumors.<sup>21</sup> The overexpression of miR-18a can activate cyclinD1 via the

PTEN-PI3K-AKTmTOR pathway, thereby promoting the proliferation of esophageal cancer cells.<sup>22</sup> The results of our study showed that the serum levels of miR-18a increased markedly in the HPV-positive patients and the patients with HPV-infected cervical cancer, and the levels of miR-18a in the patients with high-risk HPV were higher than those with low-risk HPV. These results suggest that miR-18a may participate in the pathogenesis of HPV-mediated cervical cancer. The ROC curve result showed high diagnostic performance of serum miR-18a. Since the sampling and testing methods are easy to be performed, miR-18a may serve as a good biomarker in the screening for HPV-infected cervical cancer.

In conclusion, patients with HPV-positive cervical cancer can have vaginal microbial dysbiosis and markedly increased serum levels of miR-18a and PD-L1. Moreover, the levels of PD-L1 and miR-18a rise with the increase of the HPV risk level. miR-18a and PD-L1 have clinical value in the diagnosis of HPV-positive cervical cancer. However, since the sample size was small and the levels of various indicators were not compared between patients with different HPV types, more studies need to be carried out in the future for further verification.

#### Authors' Note

Yumei Zhang, Sujuan Qiu, and Guolin Hong are co-first authors and contributed equally to this work. The study was approved by the Ethics Committee of our hospital (approval number of the institutional review board: 2016052). All informed consent was obtained from the participants and their family members.

#### Acknowledgments

This work was supported by the National Natural Science Foundation of China (81772287).


#### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

#### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Received financial support from National Natural Science Foundation of China (81772287).

#### ORCID iD

Guolin Hong  <https://orcid.org/0000-0003-0641-9438>

#### Supplemental Material

Supplemental material for this article is available online.

#### References

- Zhang G, Lang J, Shen K, Zhu L, Xiang Y. High-risk human papillomavirus infection clearance following conization among patients with cervical intraepithelial neoplasm grade 3 aged at least 45 years. *Int J Gynaecol Obstet.* 2017;136(1):47-52. doi: 10.1002/ijgo.12000

2. Poirson J, Biquand E, Straub ML, et al. Mapping the interactome of HPV E6 and E7 oncoproteins with the ubiquitin-proteasome system. *FEBS J*. 2017;284(19):3171-3201. doi:10.1111/febs.14193
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30. doi:10.3322/caac.21387
4. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115-132. doi:10.3322/caac.21338
5. Tewari KS, Sill MW, Penson RT, et al. Bevacizumab for advanced cervical cancer: final overall survival and adverse event analysis of a randomised, controlled, open-label, phase 3 trial (Gynecologic Oncology Group 240). *Lancet*. 2017;390(10103):1654-1663. doi:10.1016/s0140-6736(17)31607-0
6. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Transl Med*. 2016;8(328):328rv4. doi:10.1126/scitranslmed.aad7118
7. Dermiani FK, Samadi P, Rahmani G, Kohlan AK, Najafi R. PD-1/PD-L1 immune checkpoint: potential target for cancer therapy. *J Cell Physiol*. 2019;234(2):1313-1325. doi:10.1002/jcp.27172
8. Greenhill C. Non-coding RNA: exosomal microRNAs as novel adipokines. *Nat Rev Genet*. 2017;18(4):212. doi:10.1038/nrg.2017.14
9. Karakas M, Schulte C, Appelbaum S, et al. Circulating microRNAs strongly predict cardiovascular death in patients with coronary artery disease—results from the large AtheroGene study. *Eur Heart J*. 2017;38(7):516-523. doi:10.1093/eurheartj/ehw250
10. Dong P, Xiong Y, Yu J, et al. Control of PD-L1 expression by miR-140/142/340/383 and oncogenic activation of the OCT4-miR-18a pathway in cervical cancer. *Oncogene*. 2018;37(39):5257-5268. doi:10.1038/s41388-018-0347-4
11. Xie X, Gou WL. *Obstetrics and Gynecology*. 8th ed. People's Medical Publishing House. 2013.
12. Munson E, Munson KL, Schell RF. Clinical laboratory assessments for mycoplasma genitalium in a high-prevalence sexually-transmitted infection community reveal epidemiologic dichotomies with *Trichomonas vaginalis*. *Expert Rev Anti Infect Ther*. 2017;15(2):133-145. doi:10.1080/14787210.2017.1265887
13. Maria de Castro-Sobrinho J, Helena Rabelo-Santos S, Ribeiro Figueiredo-Alves R, et al. Bacterial vaginosis and inflammatory response showed association with severity of cervical neoplasia in HPV-positive women. *Diagn Cytopathol*. 2017;45(5):474-476. doi:10.1002/dc.23641
14. Mitchell CM, Haick A, Nkwopara E, et al. Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women. *Am J Obstet Gynecol*. 2015;212(5):611.e1-9. doi:10.1016/j.ajog.2014.11.043
15. Kyrgiou M, Mitra A, Moscicki AB. Does the vaginal microbiota play a role in the development of cervical cancer? *Transl Res*. Jan 2017;179:168-182. doi:10.1016/j.trsl.2016.07.004
16. Liu J, Liu W, Liu Y, Zhou X, Zhang Z, Sun Z. Prevalence of microorganisms co-infections in human papillomaviruses infected women in Northern China. *Arch Gynecol Obstet*. 2016;293(3):595-602. doi:10.1007/s00404-015-3826-7
17. Yang W, Lu YP, Yang YZ, Kang JR, Jin YD, Wang HW. Expressions of programmed death (PD)-1 and PD-1 ligand (PD-L1) in cervical intraepithelial neoplasia and cervical squamous cell carcinomas are of prognostic value and associated with human papillomavirus status. *J Obstet Gynaecol Res*. 2017;43(10):1602-1612. doi:10.1111/jog.13411
18. Yang-Chun F, Zhen-Zhen C, Yan-Chun H, Xiu-Min M. Association between PD-L1 and HPV status and the prognostic value for HPV treatment in premalignant cervical lesion patients. *Medicine*. 2017;96(25): e7270. doi:10.1097/md.00000000000007270
19. Wu W, Zhou X, Yu T, et al. The malignancy of miR-18a in human glioblastoma via directly targeting CBX7. *Am J Cancer Res*. 2017;7(1):64-76.
20. Zhang W, Lei C, Fan J, Wang J. miR-18a promotes cell proliferation of esophageal squamous cell carcinoma cells by increasing cyclin D1 via regulating PTEN-PI3K-AKT-mTOR signaling axis. *Biochem Biophys Res Commun*. 2016;477(1):144-149. doi:10.1016/j.bbrc.2016.06.034
21. Fan YX, Dai YZ, Wang XL, Ren YQ, Han JJ, Zhang H. MiR-18a upregulation enhances autophagy in triple negative cancer cells via inhibiting mTOR signaling pathway. *Eur Rev Med Pharmacol Sci*. 2016;20(11):2194-2200.
22. Li X, Zhang Z, Li Y, et al. miR-18a counteracts AKT and ERK activation to inhibit the proliferation of pancreatic progenitor cells. *Sci Rep*. 2017;7:45002. doi:10.1038/srep45002