Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

Human papillomavirus infection maybe not associated with primary lung cancer in the Fujian population of China

Fei He[1,2†], Weimin Xiong[1,2†], Fanglin Yu[3], Rendong Xiao[4], Hailing Ye[1,2], Wenjun Li[1,2], Zhiqiang Liu[5,6], Zhijian Hu[1,2] & Lin Cai[1,2]

1 Department of Epidemiology and Health Statistics, School of Public Health, Fujian Medical University, Fuzhou, China
2 Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fuzhou, China
3 Experiment Center, School of Public Health, Fujian Medical University, Fuzhou, China
4 Department of Thoracic Surgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China
5 The United Innovation of Mengchao Hepatobiliary Technology Key Laboratory of Fujian Province, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou, China
6 The Liver Center of Fujian Province, Fujian Medical University, Fuzhou, China

Keywords
Clinical index; human papillomavirus; lung function; primary lung cancer.

Correspondence
Lin Cai, Department of Epidemiology and Health Statistics, School of Public Health, Fujian Medical University, Fuzhou 350108, China.
Tel: +86 591 2286 2023
Fax: +86 591 2286 2023
Email: cailin_prof@sina.com

†Two authors had same contribution.

Received: 16 October 2019;
Accepted: 29 November 2019.

doi: 10.1111/1759-7714.13282

Thoracic Cancer **11** (2020) 561–569

Abstract

Background: To investigate whether human papillomavirus (HPV) infection is associated with primary lung cancer among the Fujian population.

Methods: HPV infection was detected in 140 pairs of lung cancer tissues and matched paracancerous tissues by examining the 21 clinically relevant HPV types using a combination of viral highly conserved L1 region PCR amplification and specific probe reverse hybridization. Paired χ^2 test was used to analyze differences in detection rates of HPV between lung cancer and paracancerous tissues. Differences in detection rates of HPV in lung cancer tissues were analyzed using χ^2 test or the exact probability method. The rank sum test was used to analyze differences in the distributions of routine indices of blood and pulmonary function in lung cancer tissues between the HPV negative and positive groups.

Results: HPV infection was detected in 13 of the 140 tumor specimens and in 16 of the paired normal lung tissues. There was no significant correlation between HPV infection and lung cancer ($P > 0.05$). The diagnosed HPV infection rates did not differ significantly among lung cancer tissues with different stratification ($P > 0.05$). However, the platelet count, platelet pressure, residual gas volume, functional residual volume, and residual gas volume/lung total distribution may differ between HPV-negative and HPV-positive lung cancer tissues ($0.000625 < P < 0.05$).

Conclusions: We concluded that HPV infection may not be associated with the risk of primary lung cancer in the Fujian population. However, HPV infection may affect platelet and residual lung function in primary lung cancer patients.

Introduction

Lung cancer has become one of the most common malignant tumors to seriously endanger human health. According to the 2018 GLOBOCAN statistics,[1] lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths). Lung cancer is the most commonly diagnosed cancer, the leading cause of cancer death in males, and also the third widely diagnosed cancer and the leading cause of cancer death in females after breast and colorectal cancer. Worldwide, lung cancer remains the leading cause of cancer incidence and mortality, with 2.1 million new lung cancer cases and 1.8 million deaths predicted in 2018, representing close to one in five (18.4%) cancer deaths. Although steady progress in the etiology, diagnosis, and treatment of lung cancer has been made over the past century, the incidence and mortality of lung cancer

remain high. Therefore, the prevention of primary lung cancer is particularly important.

Human papillomavirus (HPV) belongs to the Papillomaviridae family which are small epithelial DNA viruses. HPV is a nonenveloped, double-stranded loop-like epithelial small DNA virus with a diameter of 55 nm.[2] The genome encapsulated by an icosahedral capsid includes early structural genes (E1-E8) and late structural genes (L1, L2).[2] The HPV gene expression and life cycle are tightly controlled by epithelial cell differentiation. Small wounds in the epithelial tissue allow the virus to infect undifferentiated basal cells of stratified squamous epithelium.[3]

An oncogenic virus can induce malignant tumors through its carcinogenic effects on cells,[4] and more than 15% of human cancers are caused by chronic viral infections worldwide[5] with chronic HPV infection accounting for 5% of cancer cases.[6] HPV infection is the causative factor for almost all cervical cancers,[7] most anal-genital cancers, and more than 25% of oropharyngeal cancers.[8] The IARC Carcinogen Rating has classified dozens of HPV types as Class I carcinogens (with sufficient evidence of carcinogenicity in humans), including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. Other HPV types have been classified as Class IIA (most likely carcinogenic in humans), Class IIB (possibly carcinogenic to humans), or Class III (insufficient evidence of carcinogenicity in humans). The carcinogenic potential of high-risk HPV is mainly due to its ability to adhere to skin or internal mucosal tissues and its capacity to persist in infection and induce integration of the HPV gene fragment into the host genome. The expression of HPV E6 and E7 oncoproteins and the inactivation of tumor suppressor genes can accelerate tumorigenesis.

However, evidence for the association between HPV infection and lung cancer has been inconsistent. Some scholars believe that high-risk HPV infection is also closely related to the occurrence of lung cancer due to the high affinity for HPV on squamous epithelium and major tissue types of bronchi and lung epithelium.[3] HPV is the second most common cause of lung cancer after cigarette smoking[9] and has been proven to be involved in human bronchioloalveolar carcinoma. It has been reported that the pathology of goat lung adenomatosis caused by the Jaagsiekte sheep retrovirus is similar to that of human bronchoalveolar carcinoma; however, evidence linking the two diseases remains insufficient.[10] It was first proposed that HPV may be involved in the development of bronchial squamous cell lesions in 1979.[11] The morphological changes of HPV and epithelial keratoses were found in bronchial lung cancer tissues and were very similar to the HPV genital warts lesions diagnosed in the female reproductive tract. However, the role of HPV infection in the development of lung cancer has recently caused widespread controversy. A meta-analysis showed that HPV infection rates in lung cancer patients ranged from 0%–78.3%.[12] Differences in geographical regions, patient races, detection methods, histological types, and samples may be sources of heterogeneity. Because normal lung tissue is difficult to obtain, most studies only examined HPV infection in lung cancer tissue.[13]

The aim of this study was to explore the true relationship between HPV infection and lung cancer in the Fujian population, as well as the effect of HPV infection on routine indices of blood and pulmonary function in lung cancer patients. We detected HPV infection in lung cancer tissues and self-matched adjacent normal tissues and also collected data for relevant clinical indicators in lung cancer patients.

Methods

Study population

Patients who were newly pathologically diagnosed with primary lung cancer in the Department of Thoracic Surgery of the First Affiliated Hospital affiliated with Fujian Medical University from November 2013 to May 2015 and Fujian Cancer Hospital from May 2014 to May 2016 were recruited into the study. Pulmonary cancer tissues and self-matched adjacent normal tissues (more than 5 cm away from the cancer tissues as autologous controls) were collected during surgery. All tissue specimens were transferred in cryovials into storage at −80°C within 10 minutes after the operation.

The study was approved by the Ethics Committee of Fujian Medical University. All participants signed an informed consent form.

Clinical data and demographic information collection

The clinical information of the included patients, including pathological type, clinical stage, tumor site, lymph node metastasis, blood indexes, and lung function indexes, were extracted from electronic medical records. The smoking and drinking statuses of the included patients were also collected through one-on-one interviews. "Smoking" was defined as cumulative smoking of more than 100 cigarettes, and smoking packs--years = daily cigarettes smoked ÷20 × years of smoking; "Drinking" was defined as drinking alcoholic beverages at least once a month and lasting for more than six months.[14]

DNA extraction and HPV microarray detection

The genomic DNA of cancerous and paracancerous tissues was extracted using the Mucosal Cell DNA Extraction Kit (Guangdong Hybribio Co., Ltd., Guangdong, China), according to the manufacturer's instructions. In brief, frozen tissues were cut into small pieces in sterile Tris-EDTA Buffer on ice and rinsed three times with fresh ice-cold sterile TE buffer. The tissues were then transferred into a 1.5 mL centrifuge tube with 500 μL of sterile TE buffer and electrically homogenized on ice. The concentration and purity of extracted DNA products were determined using a DeNovix NS-11 ultra-violet spectrophotometer.

The Hybribio HPV Test Kit was used to identify the presence of any of the 21 different HPV infections by combination of the high sensitivity of polymerase chain reaction (PCR) technology with the high-throughput and high-specificity of a diversion hybrid gene chip technology for the rapid diagnosis (sensitivity was 96.61% and specificity 95.74%). The 21 common HPV types including 15 high-risk types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and six low risk types (HPV6, 11, 42, 43, 44, CP8304 (81) could be detected at the same time with this method.

HPV genotyping

The genotyping results were judged blindly. Clear visible blue-violet dots represented positive results. The HPV virus types represented by the positive points were determined according to the strip HPV classification diagram (Fig 1). If one or more HPV typing point was positive, this indicated that the patient was confirmed as having this HPV type, in either a single or mixed HPV infection. To prevent cross-contamination, the entire process was performed in three separate areas: the pre-PCR area for sample preparation and DNA extraction; the PCR area for PCR amplification; and the post-PCR area for diffusion hybridization assay. Each hybridization membrane had dual quality control points for biotin (DNA control) and an internal control (IC) point (for monitoring the effectiveness of PCR reactions). The results were normal only when both the biotin point and the IC dots were colored at the same time. Simultaneous detection of cervical cytological brush samples served as negative and positive controls (Fig 1).

Statistical analysis

All statistical analyses were completed using SPSS 24.0. A paired χ^2 test was used to compare the HPV diagnosis rates between lung cancer and adjacent normal tissue samples. The differences in HPV diagnosis rates among lung cancer tissues of different histological type as well as those according to gender, age, clinical stage, smoking habits, and alcohol consumption were analyzed using χ^2 test or Fisher's exact probability method. The results for blood indexes were used to divide the study population into a normal group and an abnormal group according to the clinical reference value. Based on lung function, patients were divided into a high or low group according to the median of the clinical predictive value. The positive rates of HPV in the two groups were analyzed using the χ^2 test or Fisher's exact test. The differences in the distribution of blood index and lung function parameters in lung cancer tissues between the HPV-negative group and HPV-positive group were analyzed using a two-sample rank sum test. All *P*-values were based on a two-sided test, and since 80 statistical tests were performed in the current study, the level of statistical significance, α, was set at 0.000625.

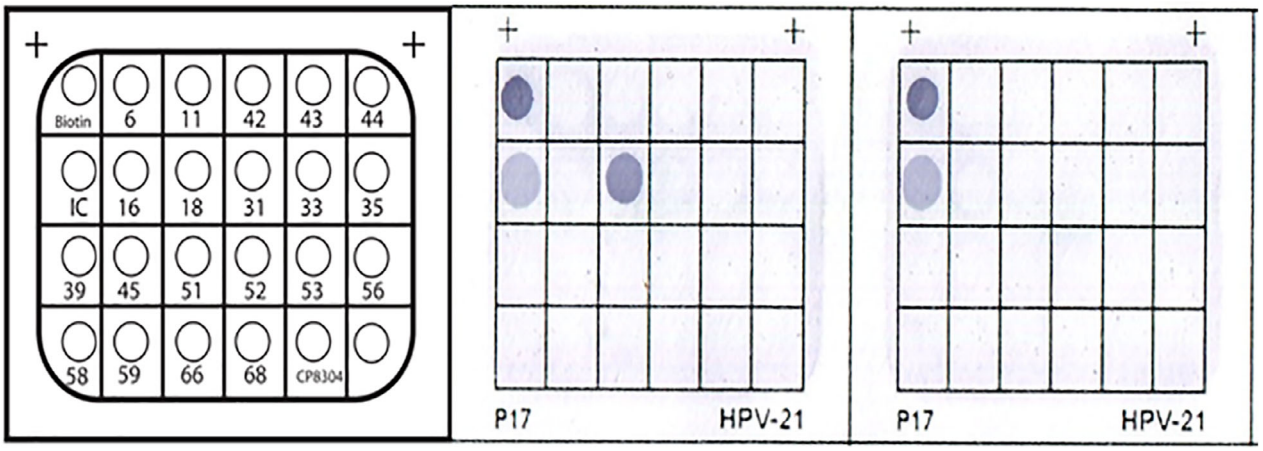

Figure 1 HPV typing diagram, cervical cytoplasmic brush positive (HPV18) and negative controls.

Results

Patient characteristics

A total of 140 patients with primary lung cancer were included in this study. The mean age at diagnosis was 58.84 ± 9.15 years (range, 38–82 years). A total of 38 (27.14%) had squamous cell carcinoma, 88 (62.86%) adenocarcinoma, seven (5.00%) adenosquamous carcinoma, two (1.43%) large cell carcinoma, one (0.71%) small cell carcinoma, two (1.43%) carcinoma sarcomatodes, and two (1.43%) had pleomorphic carcinomas. Overall, 61.43% of the patients were nonsmokers and 92.14% were nondrinkers.

HPV infection rates in lung cancer and adjacent normal tissues

Biotin points and IC points were both developed at the same time for all samples. The positive detection rate of HPV infection in lung cancer tissues was 9.29%. The specific types of HPV infection in the 13 positive cases were: four HPV16, three HPV42, two HPV18, two HPV51, one HPV44, and one multiple infection (HPV35, 42, 44). The positive detection rate of HPV infection in the adjacent normal tissues was 11.43%. The specific types of HPV infection in the 16 positive cases were: seven HPV16, four HPV18, three HPV42, one HPV6, and one multiple infection (HPV18, 33). The positive detection rate of HPV infection did not differ significantly between lung cancer and adjacent normal tissues ($P = 0.607$).

Stratification analysis of HPV infection in lung cancer

Positive detection rates of HPV infection in lung cancer tissues were stratified based on gender, age, smoking, alcohol consumption, pathological type, clinical stage, and lymph node metastasis. As shown in Table 1, the differences in HPV infection rates according to these factors were not statistically significant ($P > 0.05$).

Correlation between clinical indicators and HPV infection rate in lung cancer tissues

The χ^2 test or Fisher's exact test showed no significant difference in the positive detection rates of HPV infection between the normal blood index and abnormal blood index groups. There were no significant differences in functional residual capacity (FRC) and residual volume/total lung capacity (RV/TLC) between the low and high lung function groups ($0.000625 < P < 0.05$). The results may indicate that the positive detection rates of HPV infection in the low FRC and RV/TLC groups were higher than those in the high FRC and RV/TLC groups, respectively (Tables 2 and 3).

Table 1 Stratified analysis of HPV infection in lung cancer

Features	HPV DNA (*n* [%]) +	HPV DNA (*n* [%]) –	χ^2	*P*-value
Gender			0.002	0.962
Male	8 (61.5)	79 (62.2)		
Female	5 (38.5)	48 (37.8)		
Age (years)			0.763	0.382
<60	8 (61.5)	62 (48.8)		
≥60	5 (38.5)	65 (51.2)		
Smoking			1.411	0.235
Yes	7 (53.8)	47 (37.0)		
No	6 (46.2)	80 (63.0)		
Smoking (pack/year)			5.002†	0.062†
0	6 (46.2)	80 (63.0)		
≤20	4 (30.8)	11 (8.7)		
>20	3 (23.0)	36 (28.3)		
Drinking			0.318‡	0.573‡
Yes	0 (0.0)	11(8.7)		
No	13 (100.0)	116 (91.3)		
Histological type			0.814†	0.820†
Adenocarcinoma	8 (61.5)	80 (63.0)		
Squamous cell carcinoma	3 (23.1)	35 (27.6)		
Others§	2 (15.4)	12 (9.4)		
Clinical stage¶			1.013†	0.823†
I	5 (38.5)	60 (47.6)		
II	3 (23.0)	20 (15.9)		
III	5 (38.5)	42 (33.3)		
IV	0 (0.0)	4 (3.2)		
Lymph node metastasis¶			0.496	0.481
Yes	7 (53.8)	55 (43.7)		
No	6 (46.2)	71(56.3)		

†Fisher's exact test. ‡Continuity correction χ^2 test. §Including adenosquamous, large cell, small cell, sarcomatoid, and pleomorphic carcinomas. ¶Lack of one case.

Association between HPV infection and clinical index distribution in lung cancer

Independent sample Mann-Whitney U test showed that the distribution of platelet counts, plateletcrit, RV, RV measured/predicted values, FRC, FRC measured/predicted values, RV/TLC and RV/TLC measured/predicted values did not differ significantly ($0.000625 < P < 0.05$) between the HPV-negative and HPV-positive groups. The results indicated that platelet count and plateletcrit in the HPV-positive group were higher than those in the HPV-negative group (Table 4); RV, RV measured/predicted values, FRC, FRC measured/predicted values, RV/TLC, and RV/TLC measured/predicted values in the HPV-positive group were lower than those in the HPV-negative group (Table 5).

Table 2 The relationship between blood index and HPV infection in lung cancer

Blood index	HPV DNA (*n* [%]) +	HPV DNA (*n* [%]) –	χ^2	*P*-value
White blood cell count†			0.075‡	0.784‡
Normal	10 (76.9)	106 (84.1)		
Abnormal	3 (23.1)	20 (15.9)		
Neutrophil absolute value†			0.319‡	0.572‡
Normal	9 (69.2)	101(80.2)		
Abnormal	4 (30.8)	25 (19.8)		
Mononuclear cell absolute value§			0.000‡	1.000‡
Normal	11 (84.6)	109 (87.2)		
Abnormal	2 (15.4)	16 (12.8)		
Lmphocyte cell absolute§			–	0.598¶
Normal	13 (100.0)	115 (92.0)		
Abnormal	0 (0.0)	10 (8.0)		
Hemoglobin†			1.417‡	0.234‡
Normal	12 (92.3)	92 (73.0)		
Abnormal	1 (7.7)	34 (27.0)		
Red blood cell count†			2.666‡	0.103‡
Normal	13 (100.0)	96 (76.2)		
Abnormal	0 (0.0)	30 (23.8)		
Hematocrit†			0.298‡	0.585‡
Normal	9 (69.2)	72 (57.1)		
Abnormal	4 (30.8)	54 (42.9)		
Blood cell count†			0.177‡	0.674‡
Normal	9 (69.2)	99 (78.6)		
Abnormal	4 (30.8)	27 (21.4)		
Thrombocytocrit†			0.936‡	0.333‡
Normal	9 (69.2)	106 (84.1)		
Abnormal	4 (30.8)	20 (15.9)		
Total protein§			0.000‡	1.000‡
Normal	11(84.6)	110 (88.0)		
Abnormal	2 (15.4)	15 (12.0)		
Albumin§			0.000‡	1.000‡
Normal	11(84.6)	106 (84.8)		
Abnormal	2 (15.4)	19 (15.2)		
Globulin§			1.438‡	0.230‡
Normal	13 (100.0)	104 (83.2)		
Abnormal	0 (0.0)	21 (16.8)		

†There was one case in which the blood index was not detected at admission. ‡Continuity correction χ^2test. §There were two cases in which the blood index was not detected at admission. ¶Fisher's exact test.

Table 3 The relationship between pulmonary function with HPV infection in lung cancer

Pulmonary function	HPV DNA (*n* [%]) +	HPV DNA (*n* [%]) –	χ^2	*P*-value
VT (L)†			0.000‡	1.000‡
Low	1 (8.3)	11 (10.3)		
High	11 (91.7)	96 (89.7)		
BF (L/min)†			0.449‡	0.503‡
Low	6 (50.0)	38 (35.5)		
High	6 (50.0)	69 (64.5)		
MV (L/min)†			0.000‡	1.000‡
Low	1 (8.3)	9 (8.4)		
High	11(91.7)	98 (91.6)		
VC MAX (L)			0.049‡	0.825‡
Low	9 (75.0)	88 (82.2)		
High	3 (25.0)	19 (17.8)		
FVC (L)†			0.535‡	0.464‡
Low	8 (66.7)	86 (80.4)		
High	4 (33.3)	21(19.6)		
FEV1 (L)†			0.419‡	0.518‡
Low	8 (66.7)	85 (79.4)		
High	4 (33.3)	22 (20.6)		
FEV1/VC MAX (%)†			1.717	0.19
Low	4 (33.3)	57 (53.3)		
High	8 (66.7)	50 (46.7)		
PEF (L/second)†			1.967‡	0.161‡
Low	12 (100.0)	84 (78.5)		
High	0 (0.0)	23 (21.5)		
MMEF 75/25 (L/second)†			–	0.596§
Low	12(100.0)	98 (91.6)		
High	0 (0.0)	9 (8.4)		
RV (L)¶			0.501‡	0.479‡
Low	6 (50.0)	36 (35.0)		
High	6 (50.0)	67 (65.0)		
TLC (L)¶			0.329‡	0.566‡
Low	8 (66.7)	81 (78.6)		
High	4 (33.3)	22 (21.4)		
FRC (L)††			4.53	0.033
Low	10 (83.3)	52 (51.0)		
High	2 (16.7)	50 (49.0)		
RV/TLC (%)¶			9.244‡	0.002‡
Low	6 (50.0)	12 (11.7)		
High	6 (50.0)	91 (88.3)		

†This indicator was not detected in 21 patients on admission. ‡Continuity correction χ^2 test. §Fisher's exact test. ¶This indicator was not detected in 25 patients on admission. ††This indicator was not detected in 26 patients on admission. BF, respiratory rate; FEV1, forced expiratory volume in one second; FRC, functional residual volume; FVC, forced vital capacity; MMEF, maximum expiratory midstream flow; MV, ventilation; PEF, expiratory peak flow velocity; RV, residual gas volume; TLC, total lung volume; VC MAX, maximum vital capacity; VT, tidal volume.

Discussion

In the present study, 140 pairs of self-matched lung cancer and adjacent normal tissues were analyzed for HPV infection. The results indicated that the positive detection rate of HPV infection in lung cancer tissues was 9.29%, and in paratumor normal tissues was 11.43%. There was no statistically significant difference in the positive detection rates of HPV infection between the lung cancer and paratumor tissues. We further performed a stratification

Table 4 Association of HPV infection and blood index distribution differences in lung cancer

	HPV (+)	HPV (−)	
Blood index	P_{50} (P_{25}, P_{75})	P_{50} (P_{25}, P_{75})	*P*†
White blood cell count (10^9/L)	7.71 (5.30, 9.52)	6.67 (5.49, 8.70)	0.882
Neutrophil absolute value (10E9/L)	4.23 (3.30, 6.89)	4.16 (3.00, 6.00)	0.517
Mononuclear cell absolute value (10E9/L)	0.37 (0.34, 0.55)	0.45 (0.33, 0.59)	0.643
Lymphocyte cell absolute value (10E9/L)	1.67 (1.37, 2.19)	1.80 (1.50, 2.28)	0.59
Eosinophil absolute value (10E9/L)	0.10(0.08, 0.12)	0.13(0.08, 0.22)	0.12
Basophil absolute value (10E9/L)	0.02 (0.01, 0.03)	0.03 (0.02, 0.04)	0.191
Hemoglobin (g/L)	138.0 (125.0,145.0)	135.5 (123.5, 145.0)	0.712
Red blood cell count (10E12/L)	4.40 (4.16, 4.74)	4.44 (4.17, 4.77)	0.885
Hematocrit (%)	0.40 (0.37, 0.44)	0.40 (0.38, 0.42)	0.775
Blood platelet count (10E9/L)	274.0 (245.5, 340.5)	232.5 (190.8, 296.5)	0.031
Thrombocytocrit (%)	0.24 (0.22, 0.33)	0.20 (0.17, 0.25)	0.017
Total protein (g/L)	69.50 (66.45, 74.10)	69.50 (64.40, 72.80)	0.63
Albumin (g/L)	42.20 (36.50, 45.25)	38.80 (36.25, 43.10)	0.257
Globulin (g/L)	29.80 (26.30, 32.40)	29.40 (25.90, 33.10)	0.878
Albumin/globulin	1.31(1.20, 1.58)	1.32 (1.14, 1.58)	0.538

†Mann-Whitney U test.

analysis for HPV infection in lung cancer tissues based on gender, age, smoking, alcohol consumption, pathological type, clinical stage and lymph node metastasis, and the results showed no statistically significant differences in the detection rates according to these factors ($P > 0.05$). Differences in routine blood and biochemical parameters between the HPV-negative and HPV-positive lung cancer tissue groups were compared, and the distributions of platelet count and plateletcrit were found to differ differences between these groups ($0.000625 < P < 0.05$). The distributions of RV, FRC and RV/TLC also differed between the HPV-negative and HPV-positive groups ($0.000625 < P < 0.05$). Moreover, differences in FRC and RV/TLC were observed between the low and high lung function groups ($0.000625 < P < 0.05$). Finally, the positive detection rate of HPV infection was higher in the low FRC and RV/TLC groups than in the high FRC and RV/TLC groups, respectively.

The relationship between the potential carcinogenicity of HPV and the development of various cancers has been studied for nearly half a century. It has been demonstrated that HPV is closely related to cancers of the reproductive system[15]; however, whether HPV is related to nonreproductive malignancy has not yet been elucidated, especially for respiratory malignancies. Notably, HPV has a high degree of affinity to the squamous epithelial mucosa and can cause abnormal proliferation of the host mucosa.[16] Therefore, HPV infection is likely to be closely related to the occurrence and development of primary lung cancer. Our previous meta-analysis also suggested that the positive detection rate of HPV infection in lung cancer tissues was 2.59 times that in paracancerous tissues.[17] Galvan *et al.*[18] who used the same detection methods to identify HPV DNA in 100 pairs of lung cancer tissues and matched adjacent normal tissues, found that all the tumor samples were free of HPV infection, and deemed that HPV infection was not associated with the risk of lung cancer in a European population. Other studies have reported that the positive detection rate of HPV infection in lung cancer tissues in Asian populations (28.1%–35.7%) is generally higher than in European (8.4%–17%) and American (15%–21.3%) populations.[9,19] After adjustment for age, gender, smoking status and tumor stage, the incidence of HPV infection in lung cancer tissues in Asian populations was 4.60%, and the incidence of HPV infection in lung cancer tissues was higher than that in normal lung tissues.[16] These differences in the association between HPV and lung cancer may be related to differences in geographic location, gender, age, smoking status, alcohol consumption, pathological type, clinical stage and sample size.

The results of our current study were similar to those of a previous report which showed that HPV16/18 infection was not related to the clinicopathological parameters of age, sex, smoking status, histological type, tumor stage, and grade.[16,20] The HPV infection rate in lung cancer patients who smoke was found to be higher than that in nonsmoking patients.[21] Research also showed that carcinogens in tobacco smoke increased HPV16/18 virus synthesis and interaction with the HPV16 E6/E7 oncoprotein, resulting in malignant proliferation of lung cells.[22] The HPV infection rates in nonsmoking lung cancer patients in Asia[23] and Europe[19] are significantly higher than those in patients who smoke; however, some studies have reported that the difference in HPV infection rates between smokers and nonsmokers is not statistically significant.[19,24] Whether HPV infection is a risk factor for lung cancer in smoking

Table 5 Association of HPV infection and lung function distribution in lung cancer

	HPV (+)	HPV (−)	
Lung function index	P_{50} (P_{25}, P_{75})	P_{50} (P_{25}, P_{75})	*P*†
VT (L)	0.70 (0.57, 0.84)	0.72 (0.54, 1.01)	0.944
VT Real/pre	151.00 (128.43, 267.53)	168.20 (131.50, 222.30)	0.603
BF (L/min)	21.99 (14.30, 35.10)	23.44 (17.43, 33.12)	0.590
BF Real/pre	109.90 (71.481 86.25)	117.20 (87.20, 165.60)	0.691
MV (L/min)	15.63 (11.47, 27.75)	17.82 (12.73, 24.64)	0.704
MV Real/pre	217.70 (127.70, 252.20)	201.70 (147.20, 291.40)	0.916
VC MAX (L)	3.06 (2.22,3.38)	2.90 (2.48, 3.35)	0.846
VC MAX Real/pre	88.05 (73.33, 101.05)	85.20 (76.50, 97.10)	0.808
IC (L)	1.98 (1.78, 3.12)	2.37 (1.93, 2.74)	0.982
IC Real/pre	103.90 (90.30, 127.95)	96.60 (84.20, 108.10)	0.275
ERV (L)	0.44 (0.19, 0.97)	0.64 (0.32, 0.87)	0.304
ERV Real/pre	38.70 (16.75, 101.50)	65.40 (36.73, 89.00)	0.368
FVC (L)	2.91(2.15, 3.26)	2.77 (2.39, 3.26)	0.791
FVC Real/pre	91.20 (73.85, 106.15)	83.70 (75.10, 97.00)	0.308
FEV1 (L)	2.36 (1.79, 2.65)	2.22 (1.78, 2.57)	0.781
FEV1 Real/pre	92.20 (80.38, 100.70)	81.80 (73.10, 94.90)	0.098
FEV1/FVC (%)	82.60 (77.67, 84.92)	79.83 (73.46, 86.76)	0.354
FEV1/VC MAX (%)	81.17 (75.50, 82.26)	77.32 (71.57, 82.96)	0.419
FEV1/VC MAX Real/pre	104.40 (98.83, 107.78)	99.50 (93.10, 108.00)	0.274
PEF (L/second)	5.34 (3.72, 6.18)	5.44 (4.33, 7.03)	0.325
PEF Real/pre	79.00 (63.75, 83.48)	79.30 (63.80, 97.30)	0.445
MMEF 75/25 (L/second)	1.86 (1.46, 2.49)	1.75 (1.27, 2.51)	0.825
MMEF 75/25 Real/pre	63.20 (48.43, 76.83)	55.10 (38.30, 79.00)	0.615
RV (L)	1.70 (1.54, 2.31)	2.37 (2.00, 2.65)	0.015
RV Real/pre	95.20 (73.03, 110.40)	111.20 (94.00, 129.00)	0.033
TLC (L)	4.51(3.81, 5.25)	4.90 (4.53, 5.55)	0.107
TLC Real/pre	90.75 (75.30, 101.25)	88.40 (80.10, 97.60)	0.837
FRC (L)	2.28 (1.93, 3.04)	3.01 (2.67, 3.57)	0.020
FRC Real/pre	84.45 (63.00, 96.88)	99.65 (84.98, 112.95)	0.032
RV/TLC (%)	39.37 (35.52, 48.35)	46.34 (41.62, 52.78)	0.044
RV/TLC Real/pre	100.00 (97.23, 116.48)	124.60 (108.40, 136.90)	0.008

†Mann-Whitney U test. Actual/pre (%), indicating the measured value of lung function than the predicted value; BF, respiratory rate; ERV, compensatory expiratory volume; FEV1, forced expiratory volume in one second; FRC, functional residual volume; FVC, Forced vital capacity; IC, deep inspiratory volume; MMEF, maximum expiratory midstream flow; MV, ventilation; PEF, expiratory peak flow velocity; RV, residual gas volume; TLC, total lung volume; VC MAX, maximum vital capacity; VT, tidal volume.

patients remains controversial. Our present study did not find a significant association between smoking status of lung cancer patients and HPV infection, and a further study with an increased sample size is needed.

It has been reported that HPV infection is closely related to lung squamous cell carcinoma,[25] and studies have also found that HPV-positive lung cancers were all lung adenocarcinoma.[26] However, a meta-analysis[27] showed that high-risk HPV infection was significantly associated with the risk of lung squamous cell cancer, but not with lung adenocarcinoma. Our study did not find significant differences in HPV infection rates among different histopathological types of lung cancer.

The platelet count is usually elevated in patients with lung cancer and significantly higher than that in patients with benign lung disease.[28] Our current study indicated that HPV infection increased the platelet count and platelet pressure in lung cancer patients. A previous meta-analysis showed that platelet elevation promotes blood metastasis in cancer cells and is associated with a poor prognosis in lung cancer.[29] However, the overall survival rate of patients with HPV-positive lung cancer was not significantly different from that of HPV-negative patients.[30] The relationship between HPV infection, platelet elevation, and prognosis of lung cancer still needs further exploration.

Pulmonary function was significantly affected in patients with lung infections,[31,32] and no reports have linked HPV infection with pulmonary function in any population. The results of our current study suggest

that HPV infection may affect lung function, resulting in relatively lower RV, FRC and RV/TLC values. The mechanism may be related to HPV infection-induced bronchial epithelial condyloma lesions,[11,33] which in turn cause aggravated airway obstruction and reduced lung residual air volume.

Possible factors contributing to the inconsistent results for the association between HPV infection and lung cancer include differences in: (i) sample types (blood or tissues); (ii) handling methods (freshly frozen or embedded in paraffin); (iii) detection methods (PCR or in situ hybridization); (iv) study populations (age, gender, smoking and drinking history, histopathological type, pathological stage, race, etc.); and (v) sample collection time (before, during or after treatment). Therefore, the following measures were used in the present study to improve the reliability of the results: (i) Standardization of the sample collection procedure which involved immediate placement in a −80°C freezer after surgery to minimize contamination and nucleic acid degradation; (ii) detection of multiple HPV types using conductivity-guided hybridization with high sensitivity and specificity; (iii) use self-matched normal tissues next to the cancer tissues as control samples. The age, gender, smoking and drinking history, histopathological type, pathological stage, and race were matched to avoid the effects of differences in the basic demographic data and (iv) control for potential influencing factors with none of the sampled patients having had radiotherapy or chemotherapy before surgery.

However, the sample size in our current study was small, and the causal link between HPV infection and lung cancer could not be obtained due to the time-series limitations of the case-control study itself. The development of international standard laboratory methods is needed to enable analysis of multisite and multicenter experimental results. In the meantime, well-designed cohort or randomized controlled studies are needed to further clarify the relationship between HPV infection and the risk of primary lung cancer.

In conclusion, HPV infection was not found to be significantly associated with the risk of primary lung cancer in the Fujian population. However, even if the number of HPV positive patients is low, there may be a tendency for HPV infection to affect platelet and residual lung function in primary lung cancer patients.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Grant Number 81402738), Fujian Program for Outstanding Young Researchers in University awarded by Education Department of Fujian (Grant Number 2017B019) and the National Key Research and Development Program of China (Grant Number 2017YFC0907100).

Disclosure

The authors declare that they have no competing interests.

References

1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394–424.
2 Psyrri A, Boutati E, Karageorgopoulou S. Human papillomavirus in head and neck cancers: Biology, prognosis, hope of treatment, and vaccines. *Anticancer Drugs* 2011; **22**: 586–90.
3 Tommasino M. The human papillomavirus family and its role in carcinogenesis. *Semin Cancer Biol* 2014; **26**: 13–21.
4 zur Hausen H. Viruses in human cancers. *Science* 1991; **254**: 1167–73.
5 de Martel C, Ferlay J, Franceschi S e a. Global burden of cancers attributable to infections in 2008: A review and synthetic analysis. *Lancet Oncol* 2012; **13**: 607–15.
6 De Flora S, La Maestra S. Epidemiology of cancers of infectious origin and prevention strategies. *J Prev Med Hyg* 2015; **56**: E15–20.
7 Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. *Lancet* 2013; **382**: 889–99.
8 Grulich AE, Jin F, Conway EL, Stein AN, Hocking J. Cancers attributable to human papillomavirus infection. *Sex Health* 2010; **7**: 244–52.
9 Klein F, Amin Kotb WF, Petersen I. Incidence of human papilloma virus in lung cancer. *Lung Cancer* 2009; **65**: 13–8.
10 Mornex JF, Thivolet F, de las Heras M, Leroux C. Pathology of human bronchioloalveolar carcinoma and its relationship to the ovine disease. *Curr Top Microbiol Immunol* 2003; **275**: 225–48.
11 Syrjanen KJ. Condylomatous changes in neoplastic bronchial epithelium. Report of a case. *Respiration* 1979; **38**: 299–304.
12 Srinivasan M, Taioli E, Ragin CC. Human papillomavirus type 16 and 18 in primary lung cancers – a meta-analysis. *Carcinogenesis* 2009; **30**: 1722–8.
13 Syrjanen K. Detection of human papillomavirus in lung cancer: Systematic review and meta-analysis. *Anticancer Res* 2012; **32**: 3235–50.
14 Ribeiro KB, Levi JE, Pawlita M e a. Low human papillomavirus prevalence in head and neck cancer: Results from two large case-control studies in high-incidence regions. *Int J Epidemiol* 2011; **40**: 489–502.
15 Zandberg DP, Bhargava R, Badin S, Cullen KJ. The role of human papillomavirus in nongenital cancers. *CA Cancer J Clin* 2013; **63**: 57–81.

16 Ragin C, Obikoya-Malomo M, Kim S e a. HPV-associated lung cancers: An international pooled analysis. *Carcinogenesis* 2014; **35**: 1267–75.
17 Xiong WM, Xu QP, Li X, Xiao RD, Cai L, He F. The association between human papillomavirus infection and lung cancer: A system review and meta-analysis. *Oncotarget* 2017; **8**: 96419–32.
18 Galvan A, Noci S, Taverna F e a. Testing of human papillomavirus in lung cancer and non-tumor lung tissue. *BMC Cancer* 2012; **12**: 512.
19 Hasegawa Y, Ando M, Kubo A e a. Human papilloma virus in non-small cell lung cancer in never smokers: A systematic review of the literature. *Lung Cancer* 2014; **83**: 8–13.
20 Fei Y, Yang J, Hsieh WC e a. Different human papillomavirus 16/18 infection in Chinese non-small cell lung cancer patients living in Wuhan, China. *Jpn J Clin Oncol* 2006; **36**: 274–9.
21 Carpagnano GE, Koutelou A, Natalicchio MI e a. HPV in exhaled breath condensate of lung cancer patients. *Br J Cancer* 2011; **105**: 1183–90.
22 Munoz JP, Gonzalez C, Parra B *et al*. Functional interaction between human papillomavirus type 16 E6 and E7 oncoproteins and cigarette smoke components in lung epithelial cells. *PLOS One* 2012; **7**: e38178.
23 Dela Cruz CS, Tanoue LT, Matthay RA. Lung cancer: Epidemiology, etiology, and prevention. *Clin Chest Med* 2011; **32**: 605–44.
24 Anantharaman D, Gheit T, Waterboer T e a. No causal association identified for human papillomavirus infections in lung cancer. *Cancer Res* 2014; **74**: 3525–34.
25 Gao ZD, Shao W, Li LI, Xiao-Ye MA, Sun YP. The correlation between HPV infection subtype and lung squamous cell carcinoma in Qingdao region. *Carcinogen Teratogen & Mutagen* 2013; **25**: 111–4.
26 Joh J, Jenson AB, Moore GD e a. Human papillomavirus (HPV) and Merkel cell polyomavirus (MCPyV) in non small cell lung cancer. *Exp Mol Pathol* 2010; **89**: 222–6.
27 Zhai K, Ding J, Shi HZ. HPV and lung cancer risk: A meta-analysis. *J Clin Virol* 2015; **63**: 84–90.
28 Pedersen LM, Milman N. Diagnostic significance of platelet count and other blood analyses in patients with lung cancer. *Oncol Rep* 2003; **10**: 213–6.
29 Zhang X, Ran Y. Prognostic role of elevated platelet count in patients with lung cancer: A systematic review and meta-analysis. *Int J Clin Exp Med* 2015; **8**: 5379–87.
30 Guo L, Liu S, Zhang S e a. Human papillomavirus infection as a prognostic marker for lung adenocarcinoma: A systematic review and meta-analysis. *Oncotarget* 2017; **8**: 34507–15.
31 Chen Z, Gou F, Zhang T, Wang Y, Fang F. Influence of pulmonary infection on respiratory function and peripheral blood inflammatory factors of patients with severe acute pancreatitis. *Chin J Nosocomiol* 2017; **9**: 2050–3.
32 Hou T, Sun Y, Li J e a. The safety of ovarian preservation in stage I endometrial endometrioid adenocarcinoma based on propensity score matching. *Comb Chem High Throughput Screen* 2017; **20**: 647–55.
33 Syrjanen KJ. Bronchial squamous cell carcinomas associated with epithelial changes identical to condylomatous lesions of the uterine cervix. *Lung* 1980; **158**: 131–42.

