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Epidemiology of Human Papillomavirus and the Role of the Cytokines TNF-Alpha and IL-18 in Sexually Active Young Congolese Women

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Abstract

Human papillomavirus (HPV) infection is a virosis that affects women with genotypes that can lead to cervical cancer under the impetus of certain external factors such as early sexual intercourse, prolonged use of oral contraceptives and expression of cytokines at high concentrations. This study, which took place in Pointe-Noire and Brazzaville, Republic of Congo, between 7 December 2019 and 20 September 2021, involved a population of 250 young women in schools and in medical consultations. The prevalence of HPV using the GèneXpert real-time PCR technique is estimated at 38%, with a predominance of genotype 16 (38.9%). Concentrations of the cytokines TNFα and IL18 were measured using the ELISA technique, giving concentrations of 266.2 ng/mL and 89.5 ng/mL respectively. We noted a significant association between HPV carriage and the two cytokines in our study ($P = 0.00017$ and $P < 0.0001$ for TNFα and IL18 respectively) as the median concentrations of the two cytokines were significantly higher in participants infected with HPV compared with those who were not. Analysis of the area under the curve (AUC) values for TNFα (0.66) and IL18 (0.75) showed that IL18 was more

sensitive than TNF*α* for the clinical prognosis of participants infected with HPV. This cytokine is a good marker for HPV infection in our study. In conclusion, this study highlights the high prevalence of HPV in young people and the extreme probability of exposure of the participants to cervical cancer. It calls for increased preventive action against HPV in this juvenile population.

Keywords

Epidemiology, Papillomavirus, TNF*α*, IL18, Young Congolese Women, Sexually, Active

1. Introduction

HPV is a small, non-enveloped virus with double-stranded circular DNA, belonging to the *Papillomaviridae* family. Over 200 types have been identified, classified according to their tropism and pathogenicity [1] [2]. HPV infection is common, with 80% of women and men being exposed to it at some point in their lives. Most of the time, the infection causes no symptoms and remains transitory. In fact, in 80% of cases, the immune system eliminates the virus in less than two years, a process known as viral clearance [3] [4]. However, in around 20% of cases of infection with high-risk HPV, the infection may cause precancerous lesions to appear, which may disappear naturally or develop into cancer after several years. This progression depends on the immunological terrain and certain biological factors, such as the expression of a number of cytokines, including TNF-*α*, IL7 and IL18 in high concentrations [5]. Of the 14 high-risk HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), the two most common (HPV 16 and 18) are responsible for 71% of cervical cancers worldwide [6]-[8]. These different types of oncogenic HPV are responsible for more than 582,000 new cases of cervical cancer (UCC), causing around 266,000 deaths each year worldwide [9]. In countries with limited resources, cervical cancer is a real public health problem due to a number of risk factors, including 1) early sexual debut, 2) lack of screening policy, 3) high frequency of sexually transmitted infections, 4) multiple sexual partners and 5) lack of vaccination policy against oncogenic HPV in young people. It is in these conditions that HPV infection occurs rapidly after first intercourse (3 or even 4 years after first intercourse).

In Africa, the prevalence of HPV is high among young people, with carriage between the ages of 20 and 25 [10]-[12]. In the Republic of Congo, numerous studies show that genotypes 16 and 33 are the most frequently reported, while genotype 18 comes third. However, epidemiological and molecular data on young women are limited or non-existent [13]. However, a few studies carried out in the south of the country have shown significant prevalence of HPV in adult women, with a wide range of genotypes [14] [15]. The local context shows a lack of scientific information on the molecular epidemiology of HPV infections in young people,

and the new national cancer control programme is still in its infancy.

Numerous studies have been carried out to identify the factors associated with the development of cervical cancer in the presence of HPV. The role of cytokines has been highlighted, in particular TNF-α, IL-7 and IL-18 in increasing the risk of cervical cancer [16]-[18]. However, some studies present conflicting results regarding the link between polymorphism of these cytokines and progression to a tumour phase in HPV-positive women [19] [20]. It is, therefore, crucial to complement genetic association studies with the measurement of these cytokines in order to assess their concentrations [18]. With this in mind, we conducted this study to investigate the distribution of high-risk HPV genotypes in association with the cytokines TNF-α and IL-18 in a population of sexually active young Congolese women.

2. Materials and Methods

2.1. Process and Study Population

This was a cross-sectional, prospective and analytical study conducted on a population of young Congolese women aged between 15 and 35. The population consisted of young secondary school girls from the two cities and those attending gynaecological consultations at the Centre Médical Reine Elisabeth in Brazzaville. The study was conducted in the Republic of Congo, more specifically in the departments of Brazzaville and Pointe-Noire, over a period running from 07 December 2019 to 20 September 2021.

2.2. Data Collection

The participants were interviewed by health professionals, including doctors, nurses and psychologists, in-depth knowledge of cervical cancer, cervical screening and human papillomavirus (HPV). The interviews were conducted in French and in the country's national languages (Lingala or Kituba). At secondary school level, the teachers were made aware of the visits made by these health professionals. At the Centre Médical Reine Elisabeth, participants attending gynaecology consultations were given information by a medical team from the centre. The young women interested in taking part in the study discussed the objectives with the health professionals. The objectives were clearly explained to the participants.

The questionnaire was divided into three sections:

1) Socio-demographic characteristics and sexual behaviour of young women.

2) Level of knowledge about HPV, cervical cancer and the HPV vaccine.

3) Clinical history of young women.

Information was also collected on age, level of education, age of first sexual intercourse, condom use, number of sexual partners and use of contraceptive methods.

2.3. Types of Sample

Vaginal samples were collected. A naked-eye inspection of the anogenital region was performed on the participant in the gynaecological position using a single-

use speculum in order to assess the various aspects of the cervix. A cytobrush sample was taken from the endocervical canal by rotating the cytobrush 3 times to collect the endocervical cells, followed by a thin smear on a slide. The remaining sample was stored in a jar containing BD SurePath™ transport solution (Benex Limited, Dun Laoghaire, Ireland) and frozen at 20°C in the refrigerator pending analysis. Whole blood samples were also collected from the elbow crease using a needle and EDTA tube to collect plasma. After centrifugation at 3000 rpm for 10 minutes, the plasma obtained was stored in dry tubes at −20˚C.

2.4. Cytological Analyses

Thin smears taken from the slides during sampling were used for cytological diagnosis. The slides were stained in accordance with the recommendations of the World Health Organisation (WHO) in relation to the laboratory protocol for Papanicolaou staining [21]. The slides were read by a pathologist and the results were interpreted in accordance with the Bethesda 2001 system.

2.5. Molecular Analysis

After bringing the samples to room temperature and resuspending them using a vortex, a volume of 1000 µl of the suspension was dispensed into ready-to-use cartridges (batch no. 15,402 from the manufacturer Céphéid) for each sample. The cartridges were then placed in the GeneXpert 4-module automated system for 60 minutes, in accordance with the manufacturer's instructions, to detect genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

2.6. Cytokine Assay

The test was carried out on 112 participants who responded positively to the blood sample taken for the ELISA blood test, out of a total of 250 participants. This difference is due to the fact that the blood samples were taken later and not all the participants contacted wanted to return for the blood test. TNFα (Human, PRS-01568 Hu, batch no. 202211) and IL18 (Human, PRS-00857 Hu, batch no. 202211) kits from the manufacturer Human were used for the quantitative determination of these two cytokines according to the manufacturer's recommendations. The principle of this technique is based on the visualisation of an antigen-antibody reaction using a colorimetric enzymatic reaction.

2.7. Statistical Analysis

Statistical analysis for HPV infection was performed using Excel spreadsheet and Stata 13 software. Logistic regression was used to identify the effect of demographic characteristics on the level of knowledge about cervical cancer and HPV. The concordance between the tests was determined by summing the positive and negative results for the two tests divided by the total number of patients and multiplying the result by 100. We assessed the risk (order ratio: OR) with the association of each explanatory variable together with virus carriage using a logistic re-

gression model to take into account independently associated risk factors.

Data from the cytokine analyses were entered, coded and checked for quality and consistency in an Excel spreadsheet and then exported to StatView v5.0 (SAS Institute, Inc., Chicago, IL, USA), SPSS v22 (SPSS, IBM, Inc., Chicago, IL, USA) and GraphPad v8.3 (GraphPad PRISM, Inc., California, USA) for statistical analysis. Quantitative variables were presented in the form of mean, standard deviation (SD) or median with interquartile range (IQR), while qualitative variables were presented in the form of numbers and percentages. The median test was used to compare median values between unpaired groups. The normality of quantitative variables was tested using the Agostino-Pearson test [20]. Pearson's chi-square test of independence (χ^2) and Fisher's exact test were used to compare proportions using Cochran's rule [22]. One-way analysis of variance (ANOVA) and Student's t-test on unpaired series were used to compare mean values between groups. Their non-parametric versions (*i.e.* the Kruskal-Wallis test and the Mann-Whitney test) were used if the quantitative variable did not follow a Gaussian distribution. The area under the curve (AUC) was calculated using *Receiver Operating Curve* (ROC) analysis to assess the clinical utility of TNF-α and IL-18 in detecting HPV-infected individuals.

AUC values, standard error (SE), 95% confidence interval (95% CI was used to identify the strength of the association) and statistical significance level were calculated. An AUC ≥ 0.75 was considered to have good clinical utility [23].

Associations were considered statistically significant for $p < 0.05$.

2.8. Ethical Considerations

This study was carried out in accordance with the ethical guidelines for human research in the Republic of Congo and the 1964 Declaration of Helsinki and its subsequent amendments. The study received approval from the Republic of Congo's Health Sciences Research Ethics Committee.
(n˚033-40MESRSIT/DGRST/CERSSA/-23) and administrative agreements from the Ministry of Primary and Secondary Education and Literacy (n˚216/MEPSA-CAB of 14 August 2020). For children under the age of 18, ethical clearance has been obtained from parents through the Congo Pupils' and Students' Parents' Association. Confidentiality and anonymity of the information provided were guaranteed. Only medical staff were authorised to have access to information that could identify the participant. All participants gave their consent on a duly signed form.

3. Results

A total of 260 young women were approached during the study, and 250 gave their consent to participate. As a result, 250 participants made up the sample for this study.

3.1. Socio-Demographic Characteristics

Table 1 below shows the socio-demographic characteristics of the study participants, divided between the cities of Brazzaville and Pointe-Noire. The majority of

participants were in the 18 - 24 age group, representing 70% of the total population. The participants aged 25 - 35 were exclusively from Brazzaville, which may reflect demographic differences between the two cities. The vast majority of participants had secondary education (96%). There were very few participants with primary education or higher, which could indicate a study population made up mainly of young women in secondary education. The majority of participants' parents were married (51.6%). Cohabitation was also fairly common (30.4%), while parents were less likely to be single (18%). The majority of families have between 2 and 5 children (63.2%). Families with 1 to 2 children represent 26.8% of the total population, while families with 5 to 10 children are less numerous (10%). This first part is the subject of an article. Indeed, we published an initial article on our study focusing solely on socio-demographic characteristics and STIs. This study is the result of our PhD thesis work [7].

Table 1. Socio-demographic characteristics of the study population.

	Brazzaville (n = 176)		Pointe Noire (n = 74)		Total population (n = 250)	
Variables	***n***	**%**	***n***	**%**	***n***	**%**
Age group (years)						
15 - 17	26	74.3	9	25.7	35	14
18 - 24	110	62.9	65	37.1	175	70
25 - 35	40	100	0	0	40	16
Level of study						
Primary	2	1.1	0	0	2	0.8
Secondary	166	94.3	74	100	240	96
Superior	8	4.6	0	0	8	3.2
Parents' marital status						
Single	35	77.8	10	22.2	45	18
Cohabitation	66	86.8	10	13.2	76	30.4
Married	75	58.1	54	41.9	129	51.6
Number of children in the family						
[1 - 2]	40	59.7	127	40.3	67	26.8
]2 - 5]	118	74.7	40	25.3	158	63.2
]5 - 10]	18	72	7	28	25	10

3.2. Molecular Characterisation

The overall prevalence of HPV in the study population was 38% with no significant difference reported when comparing the two study cities or when comparing the different recruitment areas (Table 2). The number of participants with an HPV infection was significantly higher among participants included in the secondary schools than at the Queen Elisabeth Medical Centre. This table shows

that young people from secondary schools were one to seven times more likely to be carriers of HPV than those recruited at the Queen Elizabeth Medical Centre.

Table 2. Prevalence of HPV to the two cities.

Variables	Diagnosis of HPV infection				
	Positive n () 95 (38.0)	**Negative** n (%) 155 (62.0)	**Total** N 250	**ORb$ (IC95%)**	**p-value**
Brazzaville n (%)	66 (37.5)	110 (62.5)			0.887*
LR	27 (48.2)	29 (51.8)		2.646 (1.56 - 4.83)	**0.0001**
LPSB	33 (48.5)	35 (51.5)		7.101 (2.57 - 22.11)	**<0.00001**
CMRE	6 (11.5)	46 (88.5)		1*	
Pointe-Noire n (%)	29 (39.2)	45 (60.8)			
LM	29 (39.2)	45 (60.8)		1.696 (1.21 - 2.51)	**0.0009**

3.2.1. Carriage of HR-HPV According to Socio-Demographic Data

Table 3 shows the potential factors associated with the carriage of HPV infection in our population. The age of the participants, the marital status of the parents and the number of children in the family were significantly associated with HPV carriage. The risk of HPV carriage was reduced by 64% (ORb = 0.36; $p = 0.001$) in women aged between 25 and 35 compared with those aged between 15 and 17. In addition, this risk was reduced by 60% (ORb = 0.40; $p = 0.033$) in single women and 58% (ORb = 0.42; $p = 0.005$) in married women compared with cohabiting women. Finally, the chances of having an HPV infection were reduced in women with 2 - 5 children (ORb = 0.53; $p = 0.047$) or more than 5 children (ORb = 0.49; $p = 0.013$) (Table 3).

Table 3. Bivariate logistic analysis of factors associated with HPV infection according to socio-demographic characteristics.

Variables	Diagnosis of HPV infection		ORb$ (IC 95%)	p-value
	Positive	**Negative**		
n (%)	95 (38.0)	155 (62.0)		
Age group, years				
Teenagers: 15 - 17	16 (45.7)	19 (54.3)	1*	
Young adults: 18 - 24	75 (42.9)	100 (57.1)	0.89 (0.89 - 1.99)	0.891
Adults: 25 - 35	4 (10.0)	36 (90.0)	0.36 (0.17 - 0.70)	**0.001**
Level of study				
			1	
Secondary	75 (36.6)	130 (63.4)	1*	
Superior	4 (50.0)	4 (50.0)	1.72 (0.31 - 9.57)	0.674

Continued

Parents' marital status				
Single	14 (31.1)	31 (68.9)	0.40 (0.17 - 0.94)	**0.033**
Cohabitation	40 (52.6)	36 (47.4)	1*	
Married	41 (31.8)	88 (68.2)	0.42 (0.22 - 0.78)	**0.005**
Number of children in the family				
[1 - 2]	34 (50.7)	33 (49.2)	1*	
]2 - 5]	56 (35.4)	102 (64.6)	0.53 (0.29 - 0.99)	**0.047**
]5 - 10]	5 (20.0)	20 (80.0)	0.49 (0.25 - 0.88)	**0.013**

Note. The table shows the numbers (%) of the data. $^{\$}$ORb: Crude Odds ratio, IC 95%: 95% confidence interval; *, Only on participants aged 18 and over; $p < 0.05$ is considered significant (using Fisher's exact test). 1*: Reference

3.2.2. Carrying HPV According to Knowledge, Attitudes and Practices

Table 4 presents the potential factors for carrying HPV according to the knowledge, attitudes and practices of the study participants. Not knowing what UCC is, not knowing how to prevent it or having between 3 and 5 sexual partners were risk factors for carrying HPV in our population. In fact, the risk of carrying HPV was multiplied by almost 6 times (ORb = 5.61; $p = 0.0001$) in participants with no knowledge of UCC and by almost 7 times (ORb = 6.43; $p = 0.0007$) in those with no means of prevention. In addition, participants with 3 - 5 sexual partners had an almost doubled risk (ORb = 1.86; $p = 0.038$) of carrying HPV compared with those with fewer sexual partners (Table 4).

Table 4. Prevalence of HPV according to knowledge, attitudes and practices.

Variables	Diagnosis of HPV infection			p-value
	Positive	Negative	ORb$^{\$}$ (IC95%)	
Knowledge n (%)				
Real PVH				
No	11 (34.4)	21 (65.6)	1*	
Yes	10 (45.5)	12 (54.5)	1.57 (0.45 - 5.56)	0.590
CCU				
No	90 (43.3)	118 (56.7)	5.61 (2.08 - 19.03)	**0.0001**
Yes	5 (11.9)	37 (88.1)	1*	
Means of prevention				
No	92 (41.8)	128 (58.2)	6.43 (1.89 - 34.12)	**0.0007**
Yes	3 (10.0)	27 (90.0)	1*	
Attitudes and Practices				
Age of 1st sexual intercourse, years				
[11 - 15]	23 (37.1)	39 (62.9)	1*	
]15 - 17]	37 (37.4)	62 (62.6)	1.01 (0.50 - 2.07)	1.000

Continued

]17 - 21]	35 (39.3)	54 (60.7)	1.04 (0.73 - 1.51)	0.917
Condom use				
Always	62 (43.7)	80 (56.3)	1*	
From time to time	7 (26.9)	19 (73.1)	0.47 (0.16 - 1.28)	0.165
Never	26 (31.7)	56 (68.3)	0.60 (0.32 - 1.09)	0.103
Use of oral contraceptives				
No	61 (34.7)	115 (65.3)	0.62 (0.35 - 1.13)	0.126
Yes	34 (45.9)	40 (54.1)	1*	
N sexual partners				
[1 - 2]	51 (33.1)	103 (66.9)	1*	
[3 - 5]	38 (48.1)	41 (51.9)	1.86 (1.03 - 3.38)	**0.038**
[6 - 10]	6 (35.3)	11 (64.7)	1.04 (0.56 - 1.86)	1.000
UCC vaccine				
No	94 (38.1)	153 (61.9)	/	
Yes	1 (33.3)	2 (66.7)	/	

Note. The table shows the numbers (%) of the data. $ORb: Odds ratio, IC 95%: 95% confidence interval; $p < 0.05$ is considered significant (using Fisher's exact test). 1*: reference.

3.2.3. Risk Factors Independently Associated with HPV Carriage

Only variables significantly associated in the univariate analysis were included in the multivariate analysis. Only the marital status of the parents ("cohabiting") and the number of sexual partners of the participants between 3 and 5 were risk factors independently associated with the carriage of HPV infection in this population (Table 5).

Table 5. Multivariate analysis.

	Multivariate analysis	
Variables	**OR_a (IC95%)**	***P***
Parents' marital status		
Single	1	
Cohabitation	**3.18 (1.21 - 8.38)**	**0.01**
Married	1.05 (0.44 - 2.48)	0.91
Number of sexual partners		
[1 - 2]	1	
[3 - 5]	**3.29 (1.67 - 6.46)**	**0.0006**
[6 - 10]	1.46 (0.45 - 4.76)	0.53

Note. ORa: Adjusted Odds Ratio, IC 95%: 95% Confidence Interval; Factors associated with HPV infection were identified using multivariate logistic regression analyses (variables that were statistically significant in the univariate analysis were retained in the construction of the multivariate logistic model). The threshold for statistical significance was set at $p < 0.05$.

3.2.4. Prevalence of Different HPV Genotypes According to Study Area

Figure 1 shows the distribution of the different genotypes obtained.

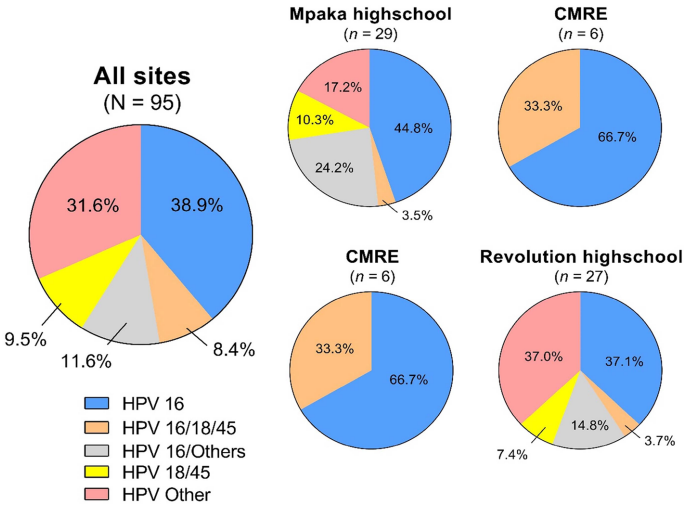

Figure 1. Distribution of different HR-HPV genotypes according to study sites. **Note.** Others: genotypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68 grouped in the Xpert cartridge; CMRE, Queen Elisabeth Medical Centre.

Overall, the HPV16 genotype (56/95; 58.9%) was the most common in the 95 HPV-positive patient samples, of which 38.9% (37/95) were mono-infected. The distribution of the different genotypes differed statistically between the samples collected in Brazzaville and those collected in Pointe Noire ($p = 0.042$). With the exception of samples with co-infection of HPV16/other genotypes (genotypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68 grouped in the Xpert cartridge), which were more prevalent in samples collected in Pointe Noire, all other genotypes were more prevalent in samples collected in Brazzaville. Only samples mono-infected with the PVH16 genotype and samples co-infected with genotypes of the PVH16/48/45 type were found at the CMRE.

3.2.5. Prevalence of Different HPV Genotypes According to Cytological Results

Almost all the participants had normal cytology. On inspection with the naked eye, the cervix was normal in all the young women in our study population. In 1.2%, it was not possible to determine the cytological classification according to the Bethesda 2001 scale (Table 6) [24].

Table 6. Distribution of different HPV genotypes according to cytology results.

Cytology	Total	PVH+	HPV genotypes				
			16	16/18/45	16/Other	18/45	Other[£]
CMASI (ASCUS) n (%)	3 (1.2)	0	0	0	0	0	0
Normal n (%)	247 (98.8)	95	37 (38.9)	8 (8.4)	11 (11.6)	9 (9.5)	30 (31.6)

Note. PVH+: Presence of human papilloma virus infection; CMASI: Atypical squamous cells of undetermined significance; ASCUS: CMASI in English.

3.3. Cytokine Assay

In the present study, we measured the concentrations of the cytokines TNF α and IL18 in 112 participants who agreed to have blood samples taken. We obtained an average of 266.2 ng/mL and a median of 201.3 (23.57 - 670.5) for TNF α and an average of 89.5 ng/mL and a median of 67.3 (5 - 288.1) for IL18.

3.3.1. TNFα Concentration as a Function of HPV Carriage and Genotypes

Analysis of plasma TNF-α levels as a function of HPV infection and different HPV serotypes is shown in Figure 2. Overall, plasma TNF-α levels were significantly higher in participants infected with HPV than in those not infected (Figure 2(a)) (p = 0.00017). The same trend was observed for genotype 16 (Figure 2(a)) (p = 0.02). In contrast, plasma concentrations of TNFα were similar between participants infected or not with the PVH-18/45 or PVH-Other genotypes (Figure 2(c) and Figure 2(d)).

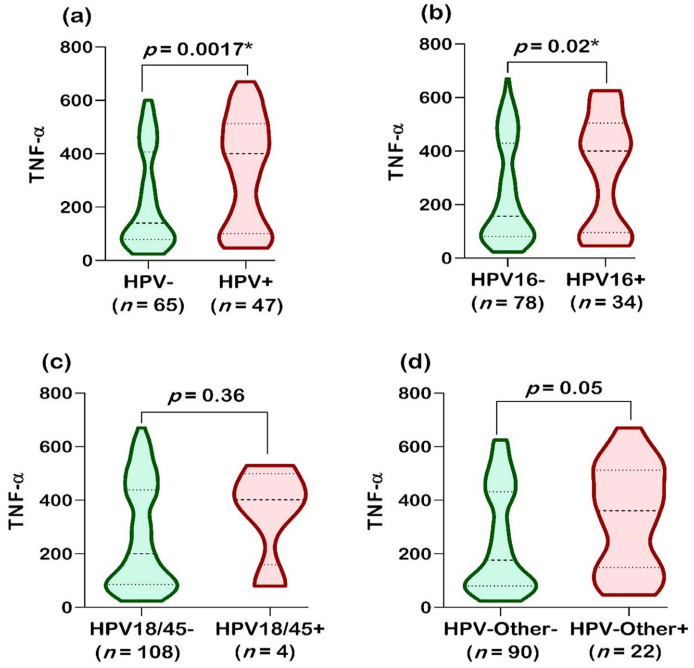

Figure 2. Violin diagrams of TNFα variation as a function of HPV and genotypes. **Note.** HPV: Human papillomavirus, TNF-α: Tumour necrosis factor. Data are presented as mean value and standard deviation. The shape of the fiddle diagrams indicates the distribution of the data. Student's t-test was used to compare groups. *statistically significant at a two-sided $p < 0.05$

3.3.2. IL18 Concentrations as a Function of HPV Carriage and Genotypes

Analysis of the mean concentration of the cytokine IL18 was much lower than that observed for the cytokine TNFα (Figure 3). We observed that participants infected with HPV ($p < 0.0001$) or HPV16 (p = 0.0002) had higher plasma levels of IL18 than uninfected participants (Figure 3(a) and Figure 3(b)). In addition, a statistically significant increase in IL-18 levels was observed in HPV-positive women (p = 0.0002) (Figure 3(d)).

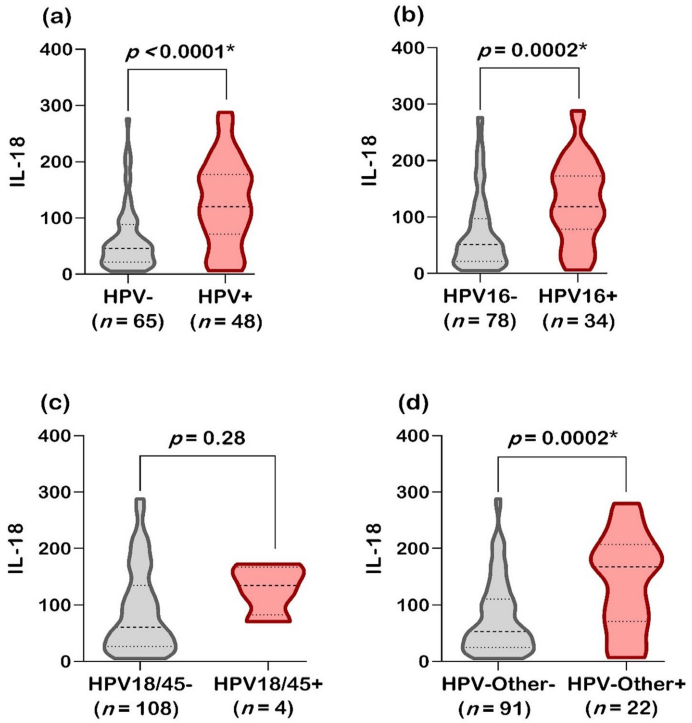

Figure 3. Violin diagrams of IL-18 variation as a function of HPV infection and serotypes.
Note. HPV: Human papillomavirus, IL-18: Interleukin 18. Data are presented as mean value and standard deviation. The shape of the fiddle diagrams indicates the distribution of the data. Student's t-test was used to compare groups. *statistically significant at a two-sided $p < 0.05$.

3.3.3. TNFα Concentration as a Function of HPV Infection Status and Participant Characteristics

Analysis of plasma TNFα levels as a function of HPV infection and serotypes after stratification for four variables, namely localities, age, oral contraception and number of sexual partners, is presented in **Figure 4**. These possibly confounding variables did not alter the effect of HPV infection or HPV genotypes on plasma TNFα levels. Overall, TNFα levels were statistically significantly higher in HPV-infected participants, irrespective of the terms of the four variables (**Figure 4**).

3.3.4. IL-18 Concentration as a Function of HPV Infection Status and Participant Characteristics

Similar to the results presented for TNFα, plasma IL-18 levels were significantly higher in HPV-infected participants than in uninfected participants, irrespective of variables such as locality, age, oral contraception and number of sexual partners (**Figure 5**).

3.3.5. Clinical Significance of TNF-α and IL-18 for the Prognosis of HPV Infection

Analysis of the area under the curve (AUC) values for TNFα and IL18 showed that IL18 was more sensitive than TNFα for the prognosis of participants infected with HPV or one of its genotypes (**Figure 6**). Indeed, only IL18 reached the minimum threshold of 0.75 for good clinical utility with an AUC value of 0.75 (ES = 0.05; IC

95%: 0.65 - 0.85; $p < 0.0001$) (Figure 6(a)). AUC values for IL18 decreased when analysing HPV16 genotypes (AUC = 0.72; ES = 0.05; 95% CI: 0.61 - 0.83; $p < 0.0001$), PVH18/45 (AUC = 0.71; ES = 0.07; CI95%: 0.58 - 0.85; $p = 0.14$), and PVH-Other (AUC = 0.72; ES = 0.07; CI95%: 0.59 - 0.86, $p = 0.001$) (Figures 6(b)-(d)).

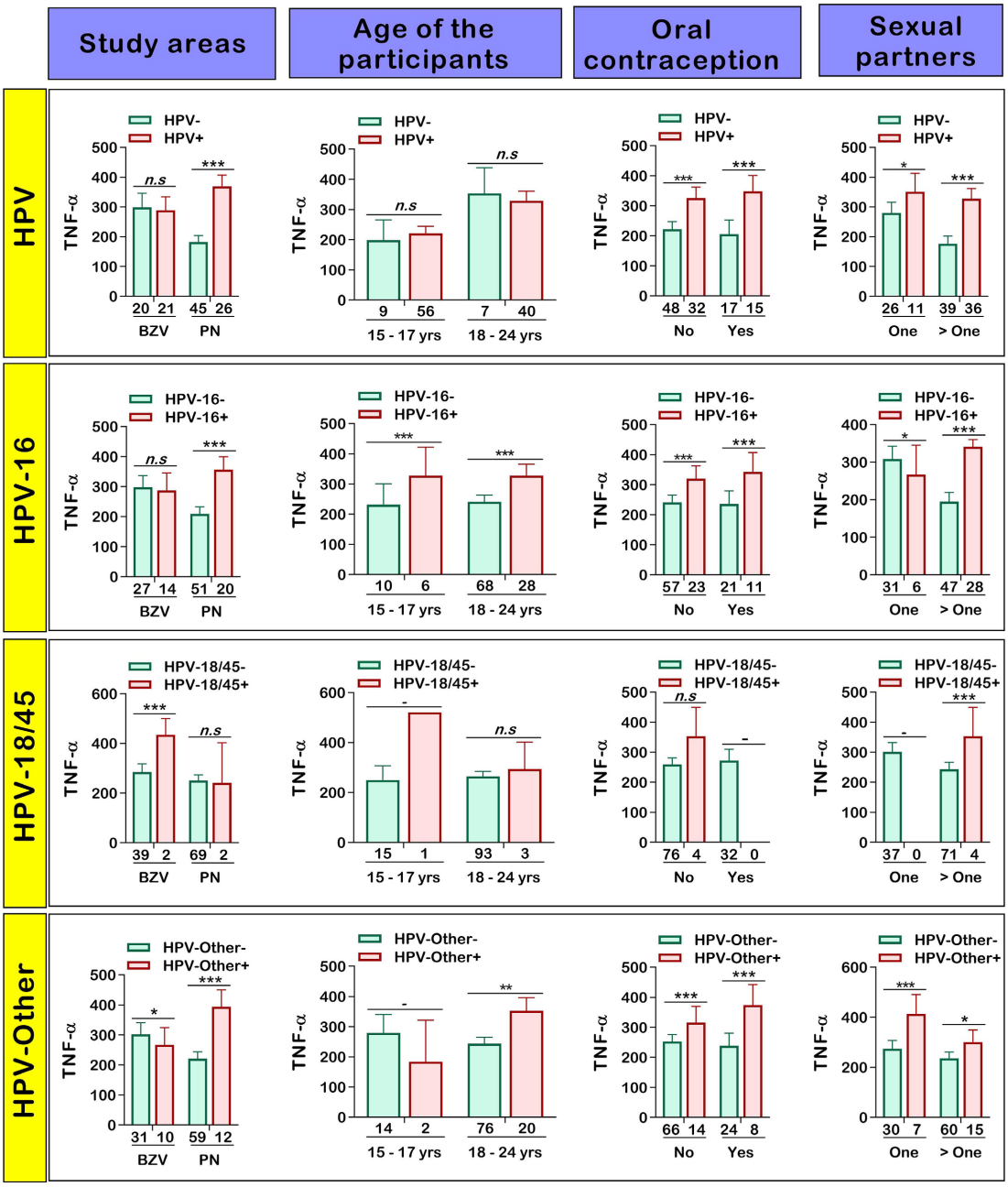

Figure 4. TNF-α concentration as a function of HPV infection and participant details. **Note.** HPV: Human papillomavirus, BZV: Brazzaville, PN: Pointe-Noire, TNF-α: Tumour necrosis factor, n.s: Not significant. Data are presented as mean and standard deviation. The numbers under each bar represent the number of participants analysed in each modality of the variables tested (study areas, age, oral contraception, and number of sexual partners). The Student's t-test on unpaired series and the non parametric Mann-Whitney test were used to compare the groups. Statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.0001.0$

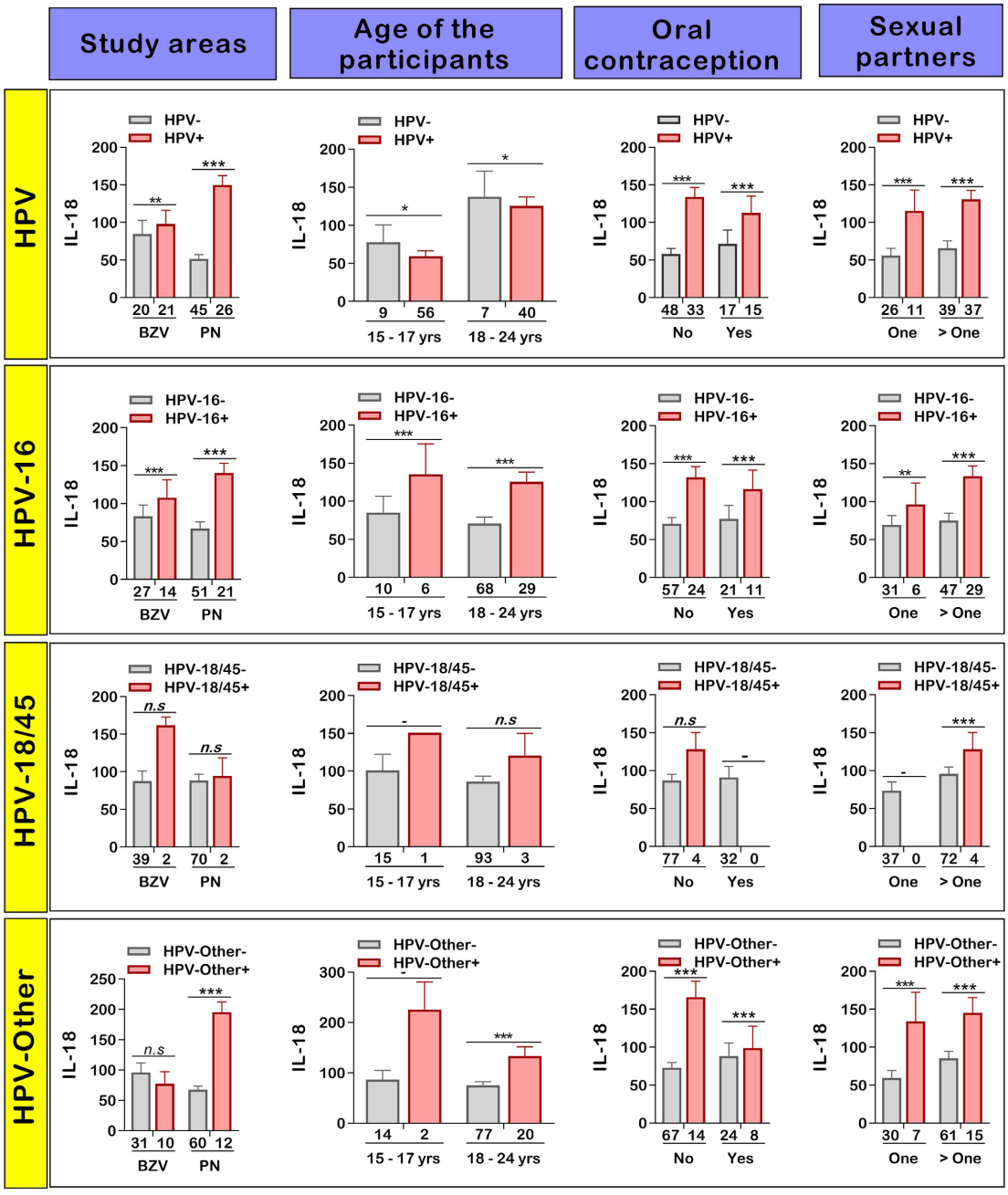

Figure 5. IL-1 concentration as a function of HPV infection and participant details. **Note.** HPV: Human papillomavirus, BZV: Brazzaville, PN: Pointe-Noire, IL: Interleukin, n.s: Not significant. Data are presented as mean value and standard deviation. The numbers under each bar represent the number of participants in each group analysed. Student's t-test and non-parametric Mann-Whitney test were used to compare groups. Statistically significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.0001$.

4. Discussion

HPV infection, which is the main cause of cervical cancer, remains a recurrent infection among sexually active young people. In the Republic of Congo, data on HPV infection and cervical cancer is highly varied, poorly documented and very limited [7] [25]. A few studies carried out in the Congo have focused on the entire

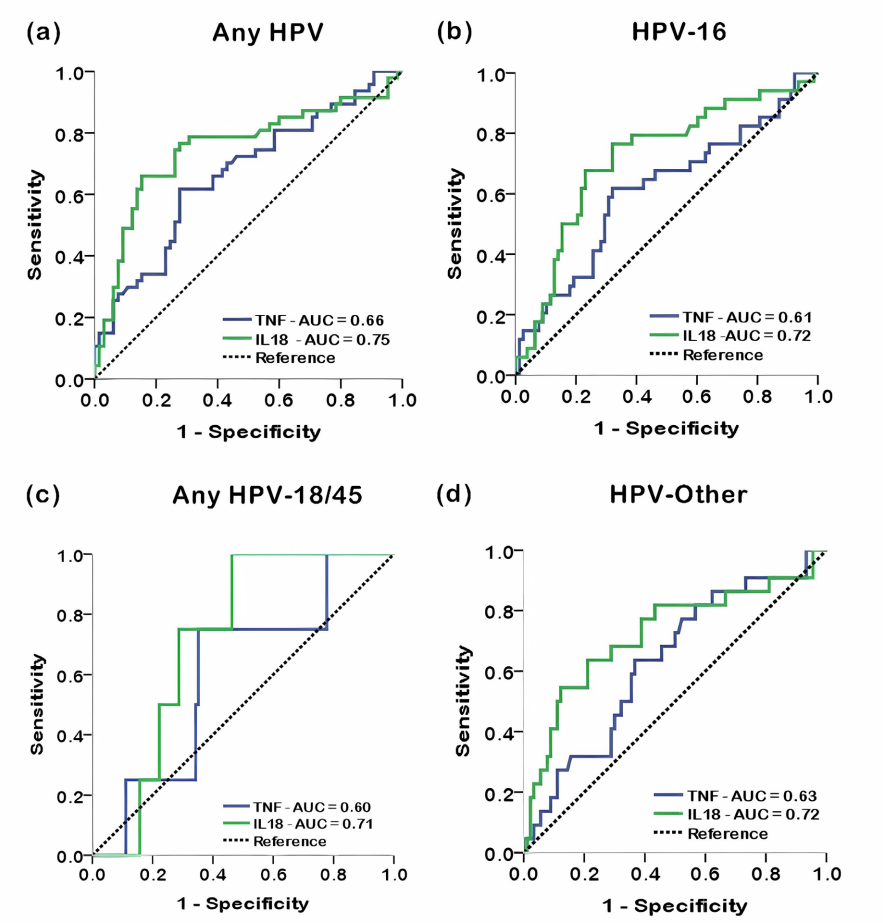

Figure 6. Clinical utility of TNF-α and IL-18 for the prognosis of HPV infection (a), HPV-16 (b), HPV-18/45 (c) and HPV-other (d) using ROC curves. **Note.** HPV: Human papillomavirus, AUC: Area under the curve, IL: Interleukin, TNF-α: Tumour necrosis factor.

female population [26]-[28]. Studies on young girls, who are at risk, are almost non-existent. It was with this in mind that we decided to carry out the present study, to provide new information from an epidemiological and molecular point of view. The aim of the study, which involved 250 young women, was to investigate the distribution of high-risk HPV genotypes in association with the cytokines TNFα and IL18 in a population of sexually active Congolese girls.

As in previous studies, we found that HPV infection is extremely common in sexually active adolescent girls and young adults. The point prevalence of detectable HPV infection in our study ranged from 10% to 45.7%, indicating an overall HPV prevalence of 38%. In Africa, one study reported a prevalence of 48.2% among women aged between 15 and 24, and a prevalence of 50.5% among those aged between 25 and 34 in a meta-analysis [29]. Among women aged 25 and over, several studies conducted in Central Africa show that rates of HPV vary between 12% and 64.4% [30]-[32]. In West Africa, the rate varies from 16.5% to 33.2% [12] [33] [34]. Higher prevalence rates have been reported among adolescent girls in South Africa, ranging from 36.7% to 66.7% [35]-[37]. In East Africa, a study of a

cohort of young women in Tanzania found a higher prevalence (74%) than ours [38]. Thus, comparing our results with those of other studies, although the prevalence of HPV in young girls varies from one region of the world to another, all the studies agree that this infection is more prevalent in young people, who are more exposed to early sexual intercourse with multiple partners. This often exposes them to high levels of STIs. In our study, lack of knowledge about prevention methods and having between 3 and 5 sexual partners were risk factors for carrying HPV. These results corroborate the findings of previous studies, which showed that sex and early sexual activity are widespread in the two cities of Brazzaville and Pointe-Noire [39]. The differences observed in terms of prevalence can also be explained in terms of the average age of the studies, the sample size of the population used by the various authors and the type of population recruited.

In our study, we found that prevalence decreased with age. This confirms the hypothesis of Collins *et al.* and Huraux *et al.* that the time taken to acquire infection after first sexual intercourse is 2.6 months, and that the incidence of infection subsequently decreases with age [40] [41]. This was also demonstrated by Monsonego *et al.*, who established that the prevalence of HPV is highest in women under 25 years of age and decreases with age, reaching a plateau in those over 45 [42]. This could be explained by the simple fact that the older we get, the more aware we become of the existence of infections, and condom use becomes the only way to protect against possible STIs. Also, after puberty, young women tend to limit the number of sexual partners they have

The PVH-HR16 genotype in mono-infection was present in 38.9% of samples, which corroborates previous studies in Africa, Europe, America and Asia, although the rates are variable [12] [35] [42] [43]. However, in the study conducted by Sagna *et al.*, HPV-HR carriage among sexually active adolescent girls in the city of Ouagadougou was predominantly of the PVH-HR52 type, as in the study conducted in Asia, where the PVH-HR16 genotype was only in second place, or in the study by Tounkara *et al.*, in Benin, who reported that the 16 genotype was only in second place after that of PVH-58 ; and in the study by Akouélé *et al.*, where genotype 16 was only in third place behind 56 and 51 [12] [44] [45]. Another study conducted in western Cameroon by Tebeu *et al.* showed a predominance of the PVH-HR18 vaccine genotype [46]. A comparison of our results with those of other authors seems to confirm the growing importance of the PVH-HR16 genotype in the juvenile population in different regions of the world in general and in the Republic of Congo in particular.

In our study, mean plasma levels of the cytokines TNF-α and IL-18 were significantly higher in HPV-infected participants than in uninfected participants. These results corroborate those reported in previous studies conducted in Asia, South America and North America [47]-[49]. These studies show an association between elevated levels of cytokines and susceptibility to HPV infection, as these cytokines can interfere with the immune response against the virus. This may also explain why, in the group of girls without HPV infection, we observed a significantly higher mean level of TNF-α cytokines in girls with a history of STIs than in those

without. Other studies, however, found no association between mean TNF-*α* cytokine levels and HPV carriage [50]-[52]. When comparing patients with and without the HR16-HPV genotypes, we observed a significantly higher mean cytokine level in the group of women with HR16-HPV. Similarly, participants carrying the other HR-HPVs (31, 33, 35, 39, 51, 52, 56, 58, 59, 68 and 68) also had significantly higher mean cytokine levels than those infected with the other HR-HPVs (16, 18/45). While Seung-Hun *et al.* in South Korea and Chagas BS *et al.* in Brazil found no association between mean plasma levels of TNF-*α*, IL-18, IL10 and HPV genotypes [49] [53]. Elevated levels of these cytokines during HR-HPV infection necessitate increased monitoring of the infection to determine how long individuals can remain positive and progress to cervical neoplasia, given that viral escape from immunity plays an important role in the tumour progression of carcinomas. [54]

It should be noted that when the area under the curve was analysed, the cytokine IL-18 was found to be more sensitive in detecting HPV infection than TNF-*α*, making it a good biomarker. This may be due to its immunological capacity to appear earlier than TNF-*α*.

5. Conclusion

In this study, we set out to conduct an epidemiological study of HPV infection and to determine the role of the cytokines TNF-*α* and IL-18 in susceptibility to HPV infection in adolescents (11 - 17 years), young adults (18 - 24 years) and adults (25 - 35 years) in the Republic of Congo. We noted a prevalence of HPV of 38%. Different genotypes of HPV-HR were detected with a variability in their prevalence depending on the sites. The PVH-HR16 type was the most prevalent genotype in the juvenile population with more than half of the specimens positive for this genotype in mono- or co-infection. Elevated levels of the cytokines TNF-*α* and IL-18 were associated with HPV carriage and STIs with respect to the cytokine TNF-*α* in the absence of HPV. IL-18 was more sensitive than TNF-*α* for the prognosis of participants infected with HPV or one of its genotypes, making this molecule a potential biomarker in clinical decision-making. We can say that human papillomavirus infection is highly prevalent among young Congolese women, and that multiple partners and early sexual intercourse are risk factors.

Consent to Publication

Consent to publication was obtained from all those included in the study.

Availability of Data and Equipment

All the data underlying the results described in this article have been presented in full in the manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Abreviations

95% CI	95% confidence interval,
Aor	Adjusted odds ratio,
cOR	Crude odds ratio,
HPV	Human papillomavirus,
TNFα	Tumour necrosis factor,
IL7	Interleukin 7,
IL18	Interleukin 18,
AUC	Area under the curve,
CCU	Cervical cancer,
MEPSA-CAB	Ministry of Primary, Secondary Education and Literacy-Cabinet,
PN	Pointe-Noire,
BZV	Brazzaville,
USA	United of States America.