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*Article*

# The Role of Cf-HPV DNA as an Innovative Biomarker for Predicting Recurrence or Persistence of Cervical Cancer

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**Abstract:** Background: Cervical cancer is highly prevalent among women in Amazonas, Brazil, mainly due to late-stage diagnosis, which compromises treatment efficacy and survival rates. This highlights the urgent need for less invasive biomarkers to monitor affected patients. Methods: This study employed real-time PCR targeting the E7 gene of HPV types 16 and 18 to analyze plasma samples from 39 cervical cancer patients treated at the Oncology Control Center Foundation in Amazonas, Brazil. Results: cf-HPV 16 DNA was detected in 54% of samples before treatment. Socioeconomic and behavioral data showed that 46.2% of patients had low educational levels, 77% reported low income, 79.5% experienced early sexual activity onset, and 15.4% had never undergone cytological screening. Recurrence or persistence occurred in 30.8% of cases over 4–33 months of follow-up, with cf-HPV DNA detectable (at any time, pre- or post-treatment) in 75% of these cases. Conclusions: cf-HPV DNA in plasma is a promising biomarker for post-treatment surveillance, facilitating earlier detection of recurrence and proactive interventions. Incorporating this biomarker into clinical protocols could enhance outcomes and survival, particularly in underserved regions like the Amazon, where access to healthcare is limited.

**Keywords:** HPV; cell free DNA; cervical cancer; recurrence; persistence; post-treatment surveillance; amazonian population

## 1. Introduction

Cervical cancer (CC) stands out as the fourth most common malignant neoplasm among women globally, constituting a significant public health challenge, especially in developing countries. Annually, approximately 604,127 new cases and 341,831 deaths are recorded worldwide [1,2]. For the triennium 2023-2025, Brazil is estimated to annually register 17,010 new cases of cervical cancer, corresponding to an incidence of age-adjusted 15.38 cases per 100,000 women. In 2020, the country accounted for 6,627 deaths due to this disease, resulting in a mortality rate of 6.12 deaths per 100,000 women. Excluding non-melanoma skin tumors, cervical cancer is the second most common type in the Brazilian Northern Region, with an incidence of 20.48 cases per 100,000 women. Specifically, in the State of Amazonas, the adjusted incidence rate for 2023 is 31.7 new cases per 100,000 women,

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while the adjusted mortality rate was 14.49 deaths per 100,000 women in 2020 [3]. Persistent infection by oncogenic Human Papillomavirus (HPV) is intrinsically linked to the etiology of CC.

In the State of Amazonas, the high morbidity and mortality associated with CC can be attributed to late diagnosis and delayed treatment initiation [4]. Factors such as geospatial, cultural, and environmental challenges, along with insufficient hospital infrastructure, exacerbate the difficulty of accessing health services, especially for women residing in remote areas [5,6]. This reality contributes to the late detection of the disease, increasing the risk of diagnosis at advanced stages, which implies more aggressive treatments and reduces the chances of cure [7–9]. Cervical cancer screening in Brazil is a cytology-based program performed at decentralized Basic Health Units (UBS in the Portuguese acronym) offered by a public health system (SUS in the Portuguese acronym). Recently, Brazilian guidelines are under review with a change to screening based on HPV DNA detection [10].

Recent advances in scientific research have revealed the potential of circulating cell-free DNA (cf-DNA), found in the serum and plasma of affected patients, opening new perspectives for diagnosis and monitoring treatment response [11]. Circulating tumor DNA (ctDNA), a form of cfDNA in plasma, has been shown to contain detailed information about tumor genomic characteristics, highlighting its diagnostic and prognostic value in the clinical management of CC [12].

Recent research, including systematic reviews and meta-analyses, has highlighted the utility of cf-HPV DNA testing for early detection of recurrence or monitoring the treatment response in patients undergoing treatment for CC [13] and head and Neck tumors [14,15]. This study aims not only to analyze the presence of cf-HPV DNA in plasma as a predictive marker of recurrence/persistence of the disease in the treatment follow-up of cervical cancer but also to evaluate its potential to improve clinical monitoring protocols.

## 2. Materials and Methods

### *2.1. Population Study and Samples*

Between August 2020 and September 2022, thirty-nine patients diagnosed with cervical cancer were included in a study conducted at the Oncology Control Center Foundation of the State of Amazonas (FCECON, Manaus, Brazil). Women with metastatic disease at diagnosis were not included. The follow-up of participants was structured in four stages: phase zero (before the start of treatment), phase 1 (about six months after the start of treatment), phase 2 (about nine months after the start of treatment), and phase 3 (about 18 months after the start of treatment). Participants were categorized into two groups, A (IA to IIB) and B (IIIA to IVA), according to the International Federation of Gynecology and Obstetrics (FIGO) classification. All patients were invited to participate in the research, signing Informed Consent Form and completing a questionnaire that collected sociodemographic, clinical, and risk information for HPV infection. Recurrence or persistence of the disease was defined by the presence of specific signs and symptoms, such as persistent vaginal bleeding and abdominal pain, which were verified in the clinical records of the patients, including medical observations and results of histopathological and imaging exams.

### *2.2. Biological Samples Collection and Processing*

During the initial phase of the study, before treatment, tissue collection was performed as clinical viability either at the patient's first outpatient visit or during surgical procedures. Tumor tissue fragments, measuring approximately 3 to 5 mm, were collected, and stored in dry plastic microtubes, free of DNase and RNase, and kept at -30 °C until processing. Simultaneously, a blood sample was collected in PPT™ (Plasma Preparation Tube) PLUS (BD Vacutainer®, USA) with a 5mL separator gel, through venous puncture. These samples were processed to obtain plasma by centrifugation at 1,400-1,600x g for ten minutes, no more than two hours after collection. Plasma was separated into aliquots and stored at -30°C. To ensure complete removal of cellular debris before DNA extraction, a second centrifugation at 16,000x g for ten minutes at 4°C was performed. For cases without an indication for surgical treatment and tissue fragment collection, cervical-vaginal material was

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collected by the medical doctor in the outpatient clinic using a Cervex-Brush® Combi (Rovers® Medical Devices B.V The Netherlands). After collection, the removable head of the brush was placed in a bottle containing 4M guanidine thiocyanate preservative solution and vigorously shaken for 15 seconds, then the sample was separated into aliquots and stored at -30°C. During follow-up, blood samples were collected as patients visited the hospital for any medical, dental, psychological, social service, physiotherapy consultations, or during home visits conducted by the FCECON social service.

Molecular screening for HPV 16 and 18 DNA was initially conducted on tumor tissue samples or cervicovaginal material. Only patients whose tissue samples were positive for HPV 16 and/or 18 DNA proceeded to plasma analysis and subsequent follow-up.

*2.3. DNA Extraction*

Tissue- DNA extraction from frozen tissues was performed using the DNeasy® Blood & Tissue Kit (QIAGEN Inc., USA), following the manufacturer's recommendations. The final volume of extracted DNA was 200 µl, and the aliquot was stored at -30ºC.Plasma and cervicovaginal Material - For the extraction of DNA from frozen plasma and cervicovaginal fluid, the ReliaPrep™ Blood gDNA Miniprep System (Promega Inc., USA) was used, according to the manufacturer's recommendations. The final volume of extracted DNA from plasma was 40 µl and from cervicovaginal material was 60 µl. The extracted DNA was stored at -30ºC.

*2.4. Human β-actin PCR*

To ensure the quality of DNA extraction, the human β-actin gene was amplified by real-time polymerase chain reaction (qPCR) using Primer F: (5′ CCATCTACGAGGGGTATGC'3) and Primer R: (5′ GGTGAGGATCTTCATGAGGTA'3) and probe (5'VIC-CCTGCGTCTGGACCTGGCTG-NFQ 3') (Life Technologies, São Paulo, Brazil). The qPCR reaction was prepared with a final volume of 10µL, containing 1X TaqMan master mix (Applied Biosystems, Foster City, CA), 300 nM each forward and reverse primer, 100nM of TaqMan fluorogenic probe, and 50-100 ng of DNA for tissue samples. The DNA extracted from plasma could not be quantified, and the same volume of extracted DNA applied to tissue samples was used to plasma sample protocol. The amplification protocol started at 50°C for two minutes; followed by 95°C for ten minutes and continued with 40 cycles of 95°C for 15 seconds, 55°C for one minute, and 60°C for one minute.

*2.5. E7 HPV16/HPV18 Type-Specific Quantitative Real-time PCR (qPCR)*

All samples were subjected to quantitative real-time PCR (qPCR) assays based on TaqMan technology, targeting the E7 genes of HPV16 and HPV18, using the QuantStudio™ 5 system (Thermo Fisher Scientific© Inc, USA). Assays and controls were performed in duplicate.

HPV16-E7 – using the forward primer (5'GATGAAATAGATGGTCCAGC3') and reverse primer (5'GCTTTGTACGCACAACCGAAGC3') and the probe (5'FAM-CAAGCAGAACCGGACAG-MGB-NFQ), in a final reaction volume of 12.5 µL. The mixture for each qPCR reaction contained 1X TaqMan master mix (Applied Biosystems, Foster City, CA), 300 nM of each forward and reverse primer, 100 nM of TaqMan fluorogenic probe, and between 50-100 ng of DNA for tissue samples. The DNA extracted from plasma could not be quantified, and the same volume of extracted DNA applied to tissue samples was used to plasma sample protocol. Cycling conditions were 50°C for two minutes, followed by 95°C for ten minutes, and 40 cycles of 95°C for 15 seconds, 55°C for one minute, and 60°C for one minute. minute [16]. As a positive control, 139 ng DNA from the SiHa cell line, containing 1-2 copies of HPV 16 per cell, was used.

HPV18-E7 – the assay included the forward primer (5'AAGAAAACGATGAAATAGATGGA3') and reverse primer (5'GGCTTCCACCTTACAACACA3') and the probe (5'VIC-AATCATCAACATTTACCAGCC-MGBNFQ3') in a final volume of 12.5 µL. The composition of the qPCR reaction was the same as the assay for HPV16-E7. As positive control, 75 ng of DNA from the HeLa cell line, containing 10-20 copies of HPV 18 per cell, was used. Purified DNA from SiHa and HeLa cell lines were provided by one of the authors (JE Levi)

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HPV 16 Viral Load in Plasma Sample – A standard curve was constructed using DNA extracted from SiHa cells (one copy of HPV 16 per cell) at a concentration of 55.6 ng/µL. Serial dilutions (1:10) were prepared to generate the standard curve, in accordance with the MIQE guidelines (Minimum Information for Publication of Quantitative real-time PCR Experiments) [17].

*2.6. Statistical Analysis*

Descriptive analyses were presented as absolute frequencies (n) and relative percentages (%), using tables and graphs. The detection of circulating HPV DNA in plasma, before and during treatment, was visualized through Swimmer Plots, created in Microsoft Excel (version 2307 build 16626.20132) and Adobe Illustrator programs (version 26.5.0.223,2022). Survival was analyzed by the Kaplan-Meier method, with comparisons between groups performed by the Log-Rank test. Survival time was calculated from the start of treatment to death or the last follow-up date. For patients who did not start treatment, the date of inclusion in the study was considered the starting point. The analysis of the time until the start of treatment ($\Delta t$) was based on the interval from the first consultation to the start of treatment. The association between clinical and histopathological characteristics of patients and the presence of markers during follow-up was evaluated by Pearson's Chi-Square test, using IBM SPSS Statistics software version 21, with a significance level of 5%.

## 3. Results

This study included 39 cervical cancer patients treated at FCECON from August 2020 to September 2022, divided into two groups according to FIGO staging: Group A (13 patients, FIGO IA to IIB) and Group B (26 patients, FIGO IIIA to IVA). The sociodemographic analysis revealed an age range from 25 to 80 years, with an average of 48.4 years (±13.4). About 46.2% of the participants had education up to incomplete elementary school and 77.0% had no income or received up to one minimum wage (< U$286.00) (Table 1).

**Table 1.** Sociodemographic and economic characterization of patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

| Variables | n (39) | % |
|---|---|---|
| **Age Range** | | |
| 21- 40 | 14 | 35.9 |
| 41- 55 | 17 | 43.6 |
| 56- 65 | 2 | 5.1 |
| > 65 | 6 | 15.4 |
| **Ethnic group** | | |
| White | 3 | 77 |
| Black | 1 | 2.6 |
| Brown | 35 | 89.7 |
| **Education level** | | |
| Illiterate | 6 | 15.4 |
| Incomplete fundamental | 12 | 30.8 |
| Complete fundamental | 1 | 2.6 |
| Full high school | 15 | 38.5 |
| Incomplete higher education | 1 | 2.6 |
| Complete higher education | 4 | 10.3 |
| **Marital status** | | |

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| | | |
|---|---|---|
| Single | 14 | 35.9 |
| Married | 17 | 43.6 |
| Divorced | 2 | 5.1 |
| Widow | 6 | 15.4 |
| **Place of birth** | | |
| Capital (Manaus) | 9 | 23.1 |
| Interior of Amazon State | 19 | 48.7 |
| Other States of Brazil | 11 | 28.2 |
| **Residential history (last five years)** | | |
| Capital | 25 | 64.1 |
| Interior of Amazon State | 11 | 28.2 |
| Other States os Brazil | 3 | 7.7 |
| **Family income** | | |
| No economic income | 15 | 38.5 |
| Until 1 MW | 15 | 38.5 |
| 2 - 3 MW | 8 | 20.5 |
| > 3 MW | 1 | 2.6 |

**MW:** Minimum Wage in Brazil (U$ 286.00).

Risk factors for cervical cancer (CC) included the onset of sexual activity between 12 and 17 years (79.5%), having between two and five sexual partners (61.5%), and occasional use of condoms (61.5%). In Brazil, screening for cervical cancer is carried out mainly through the Pap smear, recommended for women between 25 and 64 years old who have already started their sexual life. It was observed that 43.6% underwent screening above the recommended by Brazilian guidelines while 46.1% underwent fewer exams than indicated. Sexually transmitted infections were reported by 15.4% of patients, including one case of HIV (Table 2). Barriers to CC screening such as inhibition, shame, fear, or lack of time were reported by 59.1% of participants (Figure S1- Supplementary material).

**Table 2.** Characterization of behavioral and risk factors of patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

| Variable | n (39) | % |
|---|---|---|
| **Sexual debut (age)** | | |
| 12-14 | 12 | 30.8 |
| 15 -17 | 19 | 48.7 |
| From 18 years old | 7 | 17.9 |
| Not informed | 1 | 2.6 |
| **Sexual Partners** | | |
| Only 1 | 4 | 10.3 |
| 2 - 5 | 24 | 61.5 |
| 6 - 10 | 8 | 20.5 |
| > 10 | 1 | 2.6 |
| Unknown | 2 | 5.1 |
| **Condom Use** | | |

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| | | |
|---|---|---|
| Sometimes | 24 | 61.5 |
| Always | 1 | 2.6 |
| Never | 14 | 35.9 |
| **Screening by cytology** | | |
| Every 6 months | 3 | 7.7 |
| Once per year | 9 | 23.1 |
| 2 in 2 years | 5 | 12.8 |
| Once in More than 3 years | 12 | 30.7 |
| Never | 6 | 15.4 |
| Not informed | 4 | 10.3 |
| **History of smoking** | | |
| Yes | 15 | 38.5 |
| No | 24 | 61.5 |
| **STI** | | |
| Yes | 6 | 15.4 |
| No | 23 | 59.0 |
| Don't know | 10 | 25.6 |
| **Type of STI** | **n (6)** | **%** |
| HIV | 1 | 16.7 |
| Syphilis | 1 | 16.7 |
| Unknow | 4 | 66.7 |

**STI:** Sexually transmitted infection; **HIV:** Human immunodeficiency virus.

From a histological point of view, 89.7% of diagnoses were squamous cell carcinoma, and most (66.7%) were in advanced stages (III and IV) according to the FIGO classification. Most (74.4%) patients were treated with chemotherapy and radiotherapy, although three did not receive treatment due to various reasons, including indication for palliative treatment and financial difficulties for transportation to the treatment unit (Table S1 Supplementary material).

The therapeutic analysis showed that 12 patients (30.8%) presented recurrence or persistence of the disease, and four (10.3%) died (Table 3).

**Table 3.** Frequency of recurrence/persistence cases in patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

| Variables | | % |
|---|---|---|
| **Relapse/Persistence** | **(n=39)** | |
| Yes | 12 | 30.8 |
| No | 24 | 61.5 |
| No treatment | 3 | 7.7 |
| **FIGO** | **(n = 12)** | **%** |
| Group A (I/II) | 1 | 2.6 |
| Group B (III/IV) | 11 | 28.2 |
| **Outcome** | **(n=39)** | |
| Death | 4 | 10.3 |

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| | | |
|---|---|---|
| Alive | 35 | 89.7 |

**FIGO**: International Federation of Gynecology and Obstetrics.

The detection of HPV in plasma revealed that 21 (53.8%) of the samples were positive for HPV 16 and 18 at the beginning of the study. In the follow-up, the proportion of patients with detectable circulating HPV DNA reduced to 16.7% after 18 months of treatment, without a statistically significant difference over time (Table 4).

**Table 4.** Frequency of cf HPV16 DNA positivity in patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

| cf HPV16 DNA | | | | | | | |
|---|---|---|---|---|---|---|---|
| **TIME** | **Patients (n)** | **%** | **Detectable** | **%** | **Undetectable** | **%** | ***p**** |
| **$T_0$** | 39 | 100 | 21 | 53.8 | 18 | 46.2 | |
| **$T_1$** | 7 | 17.9 | 2 | 28.6 | 5 | 71.4 | 0.410 |
| **$T_2$** | 11 | 28.2 | 2 | 18.2 | 9 | 81.8 | 0.046 |
| **$T_3$** | 6 | 15.4 | 1 | 16.7 | 5 | 83.3 | 0.187 |

**cf HPV16 DNA**: cell free HPV16 DNA. * Significant p-value for p<0.05 (5%).

The relationship between FIGO staging and the presence of cf-HPV DNA indicated that patients in advanced stages had a higher frequency of detection (81.0%), with statistical significance (p=0.041). Recurrence or persistence was observed in 33.3% of women with detectable cf-HPV DNA, of which 19% died (Table 5).

**Table 5.** Relationship between the clinical characteristics of patients with cf HPV16 DNA, treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

| **Variables** | **cf HPV16 DNA** | | | | **n (39)** | ***p**** |
|---|---|---|---|---|---|---|
| | **Detectable (n=21)** | **%** | **Undetectable (n =18)** | **%** | | |
| **FIGO** | | | | | | |
| Group A | 4 | 19.0 | 9 | 50.0 | 13 | *0.041** |
| Group B | 17 | 81.0 | 9 | 50.0 | 26 | |
| **Histology** | | | | | | |
| AC | 2 | 9.5 | 2 | 11.1 | 4 | *0.636* |
| SCC | 19 | 90.5 | 16 | 88.9 | 35 | |
| **Relapse/Persistence** | | | | | | |
| Yes | 6 | 28.6 | 6 | 33.3 | 12 | *0.491* |
| No | 15 | 71.4 | 12 | 66.7 | 27 | |
| **Outcome** | | | | | | |
| Death | 4 | 19.0 | 0 | 0.0 | 4 | *0.073* |
| Survivor | 17 | 81.0 | 18 | 100.0 | 35 | |

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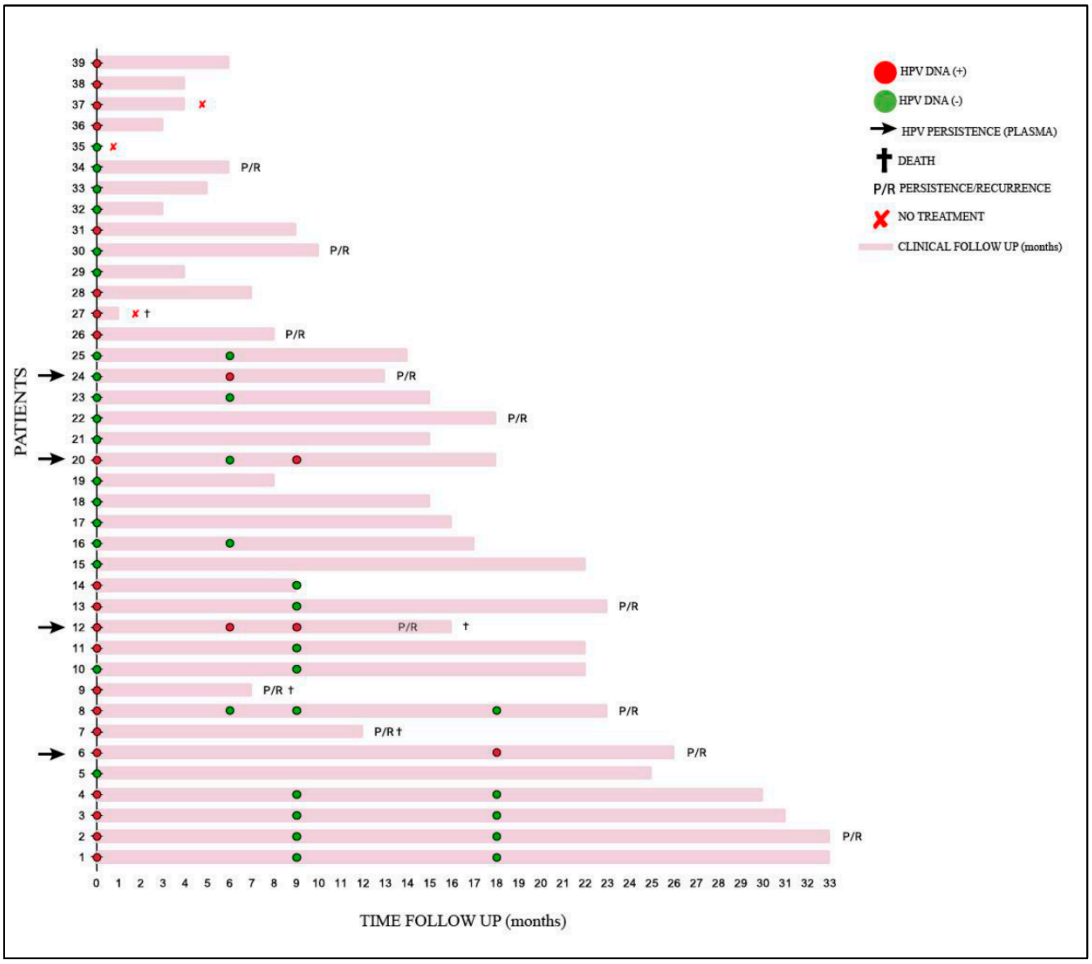
Significant *p*-value for p<0.05 (5%). Pearson Chi-Square Test. **cf HPV16 DNA**: Cell free HPV16 DNA; **AC**: Adenocarcinoma; **SCC:** Squamous Cell Carcinoma.

It was not possible to collect plasma from all patients for the detection of cf-HPV DNA during follow-up, but 11 of the 16 monitored patients were positive for cf-HPV DNA at diagnosis, and four remained positive after treatment. Individual cases highlighted include variations in HPV viral load and its relationship with clinical recurrence or persistence of the disease. The trajectory of the 39 patients included in the study can be verified in Figures 1A and 1B.




(**A**)

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| Patient ID | Age (years) | cf HPV DNA positive/viral load copies/ml of plasma (qPCR) | | | | FIGO staging | Histology | Death | Persistense/Recurrence time until diagnostic (months) |
|---|---|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | T3 | | | | |
| **2** | 34 | 1.94 | NSC | Undetectable | Undetectable | IIIC1 | SCC | No | 33 |
| **6** | 53 | 40.33 | NSC | NSC | 5,13 | IVA | SCC | No | 26 |
| **7** | 51 | 1.12 | NSC | NSC | NSC | IVA | SCC | Yes | 12 |
| **8** | 26 | 7.47 | Undetectable | Undetectable | Undetectable | IIIC2 | SCC | No | 23 |
| **9** | 48 | 486.23 | NSC | NSC | NSC | IIIB | SCC | Yes | 7 |
| **12** | 41 | 54.72 | 6,12 | 68,09 | NSC | IIIC1 | SCC | Yes | 14 |
| **13** | 61 | 43.4 | NSC | Undetectable | NSC | IIIC1 | SCC | No | 23 |
| **22** | 38 | Undetectable | NSC | NSC | NSC | IIB | SCC | No | 18 |
| **24** | 52 | Undetectable | 0,92 | NSC | NSC | IIIB | SCC | No | 13 |
| **26** | 62 | 1.53 | NSC | NSC | NSC | IIIC1 | SCC | No | 8 |
| **30** | 31 | Undetectable | NSC | NSC | NSC | IIIB | SCC | No | 10 |
| **34** | 74 | Undetectable | NSC | NSC | NSC | III | SCC | No | 6 |

(**B**)

**Figure 1.** (**A**) Clinical follow-up of 39 patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil. Each line corresponds to one patient (n= 39); In red (•) and green (•) correspond to plasma samples positive for circulating HPV DNA and negative for circulating HPV DNA, respectively. Patients with detectable circulating HPV DNA in their samples during treatment are indicated by a black arrow; (✝) patients died; P/R patients presented persistence/relapse; (X) patients did not treatment. (**B**) Clinicopathological characteristics of patients who presented recurrence/persistence. **cf HPV16 DNA**: Cell free HPV16 DNA; **qPCR**—Quantitative Polymerase Chain Reaction; **T0**—Timer 0 (before starting treatment); **T1**—Timer 1 (6 months after starting treatment); **T2**—Timer 2 (9 months after starting treatment); **T3**—Timer 3 (18 months after starting treatment); **FIGO**—International Federation of Gynecology and Obstetrics; **NSC**—Sample not collected; **SCC**—Squamous Cell Carcinoma.

The Kaplan-Meier survival curve suggested a trend of lower survival for patients with detectable cf-HPV DNA, although the difference did not reach statistical significance (p=0.073) (Figure 2). The interval between the first consultation and the start of treatment ranged from one to 29 months, with an average of 6 months (±5 months), indicating a varied distribution of time until treatment among patients.

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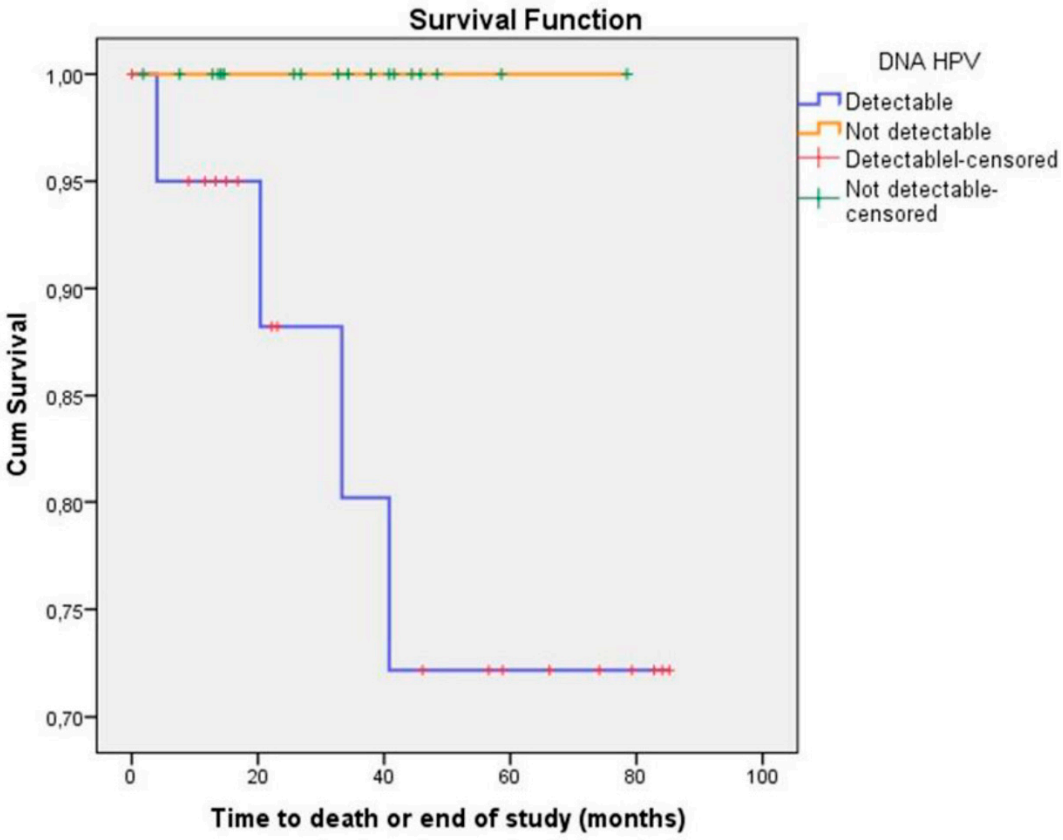


**Figure 2.** Survival analysis of women monitored through cf-HPVDNA testing after the initiation of cervical cancer treatment at FCECON, Manaus – AM, Brazil, from August 2020 to September 2022.

## 4. Discussion

This study evaluated the use of cell free HPV DNA (Cf-HPV DNA) as a prognostic marker for disease recurrence or persistence in patients with cervical cancer after treatment. The research focused on patients from the Northern region of Brazil, where the prevalence of cervical cancer is high, often exacerbated by difficulties in accessing diagnosis and treatment. Delays in the diagnosis and treatment of cervical cancer can be attributed to multiple factors, including limitations of patients, health professionals, and the structure of health services [18,19]. The COVID-19 pandemic in 2020 exacerbated these challenges, impacting the screening and diagnosis of cervical lesions and highlighting the fragilities of the Brazilian health system, especially in the Northern Region, marked by pronounced social inequalities.

The state of Amazonas, with its vast geographical area (1,559,159,148 km2) and cultural, geospatial, and environmental challenges, exemplifies the difficulties in accessing health services. Only five health units in the SUS offers colposcopy exams, all located in the capital, Manaus, leaving women from the interior at a disadvantage. It is important to mention that 71.5% of the Brazilian population depends on access to the public health service [20,21]. This scenario contributes to the late diagnosis of the disease, as observed in 26 (66.7%) of the patients in this study, classified in group B (FIGO IIIA to IVA), with a prevalence of stage IIIC1 (23.1%), aligning with literature indicating diagnosis at advanced stages in about 50% of cases [22].

This study revealed an average age of 48.4 years (±SD 13.4) among patients, aligning with literature indicating the prevalence of cervical cancer between 45 and 55 years [23]. However, it was notable that 35.9% of patients were between 21 and 40 years, reflecting similar findings by Moysés et al. [24] in 2019, which described a predominant age profile between 35 and 39 years for cervical cancer in Manaus.

The association between low educational level and increased risk for CC suggests a gap in recognizing the importance of preventive exams and in actively seeking screening and treatment by

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these women [25]. This correlation was evidenced in a cross-sectional study with 451 women in Kenya, where 49.2% of participants reported low literacy or only primary education, and in qualitative research in Lagos, Nigeria, which found 60% of women with non-formal education or primary education [26,27]. In Brazil, a prospective study with 631 patients from various regions also associated lower education with not performing the Pap smear test [28].

In this context, 46.2% of women in this study reported being illiterate or having incomplete elementary education. This data is corroborated by a previous study in Manaus, where 27.2% of patients treated for CC at FCECON presented low educational levels [24]. Similarly, Lucena et al. [29], in a study conducted in Porto Velho, Rondônia (north of Brazil), found that 34.3% of women had low education, reinforcing the prevalence of low educational level in the female population of the Northern Region of Brazil.

Screening for precursor lesions is challenging in the Amazon Region due to geographical isolation, especially for women in riverside communities. This study revealed that 48.7% of patients reported being from the interior of the State. However, when approached housing local in the last five years, 64.1% reported living in the capital, however, in some situations where contact with the patient was necessary, there were difficulties in finding some addresses. A similar situation was observed in a study of women with cervical cancer treated at the oncological hospital in Manaus, in which the reference to residence was also inaccurate, as some of them indicated an address in the urban area despite coming from rural areas or municipalities in the interior of the country. Amazonas. In this situation, it can be considered that these patients do not want to be contacted; sometimes due to the stigma of the disease, as CCU affects an organ full of symbolism for women, as it involves issues inherent to sexuality, femininity and reproduction [5,30].

Economically, 77.0% of women reported having no family income or an income of up to one minimum Brazilian wage (U$ 286.00), highlighting the influence of socioeconomic condition on the performance of screening exams and on the incidence and mortality of the disease [33,34]. Early onset of sexual activity was observed as a risk factor for the development of CC, aligned with studies showing an association between early onset of sexual activity and higher risk of cervical cancer [33–35].

The study also highlighted the low adherence to the Pap smear test, with 68.2% of women reporting barriers such as inhibition, shame, fear, lack of time or carelessness with health, emphasizing the importance of approaches that consider cultural and emotional barriers to screening [36–40].

Women living with HIV/acquired immunodeficiency syndrome (AIDS), especially those with immunosuppression, have a higher susceptibility to persistent infections by high-risk HPV types, increasing the risk of developing cervical dysplasia and cervical cancer [41]. This study identified one patient with HIV infection, highlighting that HIV-positive women have four to five times more chances of developing cervical cancer (CC), as evidenced by Clifford et al. (2017) [42], who found a high prevalence of HPV types 16, 18, and 45 in women with invasive cervical cancer. Bowden et al. (2023) [43] also highlighted that HIV positivity decreases the likelihood of eliminating high-risk HPV. In Belém, Pará (north region of Brazil), a study with HIV-positive women showed that 63.3% were infected with HPV, with 40.4% positive for high-risk HPV 16 and 12.8% for HPV 52, indicating that these patients may have a suboptimal response to conventional treatments for CC [44–47].

In this study, squamous cell carcinoma (SCC) was the most common histological type (89.7%), followed by adenocarcinoma (ADC) (10.3%), aligning with findings by de Sanjose et al. (2010) [48], who analyzed cervical cancer samples from 38 countries and found a predominance of SCC. Other Brazilian studies corroborate these results, showing a similar prevalence of SCC in cervical cancer diagnoses [35,49,50]. Considering that approximately 35% of CC cases are diagnosed at advanced stages (III and IV) in Brazil [51], this research found a significant proportion of patients (23.1%) at an advanced stage (IIIC1), reflecting the influence of socioeconomic and demographic factors on accessibility to health services and, consequently, on the stage at which the disease is diagnosed [52].

Three patients (7.7%) did not receive treatment due to various reasons, including indication for palliative care, lack of financial resources for transportation, and decision to return to their

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hometown. These findings highlight how low socioeconomic status can prevent access to adequate information about CC and necessary treatment [53], leading to decisions that compromise the patient's prognosis.

The detection of cf-HPV 16 DNA in plasma samples was observed in 53.8% of patients at diagnosis, while genotype 18 was not detected. These data are consistent with previous studies reporting variations in the prevalence of HPV genotypes [54–56]. A systematic review revealed that most recurrences/ persistence of the disease was detected within two to five years after primary treatment for CC [57], corroborating the importance of continuous monitoring for early detection of recurrences.

This study also highlighted the potential of cf-HPV DNA as a biomarker for therapeutic monitoring and early detection of recurrence/persistence of the disease, as the presence of cf-HPV DNA in plasma was identified months before the clinical diagnosis of recurrence in some patients. Furthermore, the research emphasized the importance of the quality and availability of health services in the prognosis of patients, stressing the need to ensure access to treatment within the deadlines established by Brazilian legislation, especially considering the challenges faced by women with CC in accessing timely treatments [58,59].

In summary, the results of this study underline the complexity of managing women with cervical cancer and the need for screening and treatment strategies adapted to the socioeconomic and geographical particularities of Brazil, aiming to improve the prognosis and quality of life of these patients.

The SARS-CoV-2 pandemic posed significant challenges to women's access to the Oncology Control Center Foundation (FCECON), directly affecting sampling and the continuity of biological sample collections as planned. This interruption resulted in an incomplete analysis of the four phases envisaged in the study, restricting the robustness of the analyses due to the limited sample size. Additionally, the consultation and interpretation of patient records, essential for identifying cases of recurrence or persistence of the disease, were hindered by the inconsistency of records made by the care team, with the absence of crucial information. Another limiting factor was the follow-up period ranging from 6 to 18 months, which may have affected the survival analysis of patients.

## 5. Conclusions

The investigation into the presence of cell free HPV DNA in patients with post-treatment cervical cancer indicates a possible correlation with the risk of recurrence or persistence of the disease. Cf-HPV DNA was detected in the plasma of 53.8% of patients before the start of treatment. Notably, the presence of cf-HPV DNA was identified up to six months before the clinical characterization of recurrence or persistence of the disease. Additionally, a trend indicating that patients with cf-HPV DNA in plasma have a reduced survival was observed. These findings underline the potential utility of cf-HPV DNA as a biomarker for early monitoring of recurrence or persistence of the disease in patients treated for cervical cancer, although the methodological limitations of the study require caution in interpreting the results and highlight the need for more comprehensive future research.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1. Reason why patients with cervical cancer, treated at FCECON from August 2020 to September 2022, Manaus – AM, Brazil, did not routinely perform preventive care (n=22); Table S1. Clinical characterization of patients with cervical cancer treated at FCECON, from August 2020 to September 2022, Manaus – AM, Brazil.

**Author Contributions:** K.L.T., V.C.A.M., J.E.L and M.P. were responsible for the study set-up; M.P, L.L.M.S.D, L.S.P., F.N.O.G., H.H.M.F.R., M.P.O., J.S.S., H.F.F and D.L.L. were accountable for data and specimen collection. M.P., V.C.A.M., H.H.M.F.R., F.N.O.G. and M.P.O were responsible for molecular analysis. M.P. performed data analysis. The first draft was written by M.P., with contributions from K.L.T., V.C.A.M and J.E.L. All authors were actively involved in interpreting the data, creating and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

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