Comparison of Classical and Secondary Cytologic Criteria Relative to Hybrid Capture for Diagnosing Cervical-vaginal Infection by *Human Papillomavirus*

Comparação entre os critérios citológicos clássicos e secundários para o diagnóstico de infecção cérvico-vaginal por papiloma vírus humano em relação à captura híbrida

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Abstract

Objective To compare the diagnostic accuracy of the classic Meisels cytologic criteria and the Schneider secondary criteria relative to the hybrid capture method for diagnosing HPV infection.

Methods This was a retrospective study performed at a public university hospital. A total of 41 patients with a cytologic diagnosis of HPV infection and 40 HPV-negative patients were selected for review of the cervical-vaginal smears seeking to classical and secondary criteria. A single pathologist reviewed the slides in search of the criteria. The classical and secondary cytologic criteria were compared with the hybrid capture for diagnosing HPV infection. Bartleti test was applied for the age analysis, and Fisher's exact test was used to compare proportions. The tests were considered significant when the probability of rejecting the null hypothesis was less than 5% ($p < 0.05$).

Results The Meisels criteria were less sensitive (34.0%) than the secondary Schneider criteria (57.5%) when compared with the hybrid capture ($p < 0.0001$), although the specificity of the former criteria was non-significantly higher (91.2% and 67.7%, respectively). In cases of moderate or intense inflammation, the sensitivity and specificity of the Schneider criteria were decreased, 33.3% and 50.0% respectively ($p = 0.0115$).

Keywords
- HPV
- cervical neoplasia
- vaginal smears
- papillomavirus
- DNA

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Conclusions Compared with hybrid capture for diagnosis of HPV infection, the sensitivity of the secondary Schneider criteria was higher than the classical Meisels criteria. Moderate or intense inflammation reduces the sensitivity and specificity of the secondary Schneider criteria for diagnosing HPV infection using the hybrid capture as the gold standard.

Resumo

Objetivo Comparar a acurácia diagnóstica dos critérios citológicos clássicos de Meisels com a dos critérios secundários de Schneider em relação a captura híbrida para o diagnóstico de infecção pelo HPV.

Métodos Trata-se de estudo retrospectivo realizado em hospital público universitário. Quarenta e uma pacientes com diagnóstico citológico de infecção pelo HPV e 40 pacientes HPV-negativas foram selecionadas para avaliação dos esfregaços cervicais-vaginais em busca dos critérios clássicos e secundários. Um único patologista reviu as lâminas. Os critérios citológicos clássicos e secundários foram comparados com a captura híbrida para o diagnóstico de infecção pelo HPV. O teste de Bartleti foi aplicado para a análise das idades e o teste exato de Fisher para comparar proporções. Os testes foram considerados significativos quando a probabilidade de rejeitar a hipótese de nulidade foi menor que 5% ($p < 0,05$).

Resultados Os critérios de Meisels foram menos sensíveis (34,0%) que os secundários de Schneider (57,5%) quando comparados com a captura híbrida ($p < 0,0001$), embora a especificidade dos critérios de Meisels não tenha sido significativamente superior (91,2% e 67,7%, respectivamente). Em casos de inflamação moderada ou intensa, a sensibilidade e especificidade dos critérios secundários de Schneider foram diminuídas, 33,3% e 50,0%, respectivamente ($p = 0,0115$).

Conclusões Comparado a captura híbrida para o diagnóstico da infecção pelo HPV, a sensibilidade dos critérios secundários de Schneider foi maior que a dos critérios clássicos de Meisels. Inflamação moderada ou intensa reduziu a sensibilidade e especificidade dos critérios secundários de Schneider para o diagnóstico de infecção pelo HPV utilizando a captura híbrida como padrão-ouro.

Palavras-chave
- HPV
- neoplasia do colo do útero
- esfregaço vaginal
- papilomavírus
- DNA

Introduction

Cervical cancer is the third most common cancer among women. In developed countries, the diagnosis is made earlier; consequently, the 5-year survival rate is higher. In the last few decades, the incidence of cervical cancer has decreased in countries with effective screening systems, probably as result of the early treatment of precancerous lesions. Thus, it is important to understand the minimal abnormalities in cervical-vaginal cytology.[1–3]

With the development of molecular biological techniques, epidemiological and laboratory findings have identified *human papillomavirus* (HPV) as the principal agent involved in the geneses of cervical cancer and cervical intraepithelial neoplasia. This evidence has increased the importance of the morphological identification of HPV infection in cervical-vaginal cytology.[1,4–8] Classically, the natural history of cervical cancer starts with infection by HPV. Subsequent progressive intraepithelial transformations can evolve into invasive neoplasia in the long term. This connection has raised interest in establishing and perfecting the diagnosis of this infection, as well as identifying risk factors for it, to detect populations susceptible to HPV-induced cervical carcinogenesis.[4]

Although cellular biological tests are more sensitive in diagnosing HPV infection than cytological tests, access become larger and the cost somewhat lower, some factors limit their routine use, including cost and methodological difficulty, particularly in poorer countries.[9,10] Thus, the cytologic Papanicolaou exam (also known as the Pap smear) remains the main screening method for cervical cancer.[3,11] Where as Papanicolaou first described the exfoliated squamous cells of vaginal and cervical condyloma acuminatum,[12] it was Meisels and Fortin[13] and Meisels, Fortin and Roy[14] who identified the cell changes that, currently, are considered pathognomonic for HPV infection (i.e., classic koilocytosis and dyskeratosis). These criteria for HPV infection are very specific, but not very sensitive.[3,11] To increase the sensitivity of the test without substantially reducing specificity, various authors have sought other "secondary" cytologic indices.[3,15–19]

Among the secondary criteria proposed, the most accepted are those of Schneider et al. (1987),[15] which include:

slight koilocytosis or an outline of koilocytosis, slight dyskeratosis, cleared cytoplasm, keratin hyaline granules, condensation of filaments in the cytoplasm, fusiform cells, hyperchromatic nuclei, bi- or multinucleation, and perinuclear halo.

Nevertheless, there is still some controversy about the use of secondary criteria.[16–19] Thus, we wanted to compare the use of classical and secondary cytologic criteria to the hybrid capture (HC) molecular biological test for diagnosing HPV infection. The HC method was chosen because it is easily performed, yields rapid results, has good sensitivity for latent, subclinical, and clinical infections, and can detect HPV infection anywhere in the woman's lower genital tract.[5,9,20]

The objective of this study was to compare the classic cytologic Meisels criteria (CMC)[13,14] and the secondary criteria proposed by Schneider et al.[15] (SSC) to the molecular biological HC method for diagnosing HPV infection.

Methods

The Research Ethics Committee at the Universidade Federal Triângulo Mineiro approved this research (protocol no. 0281 on 12.20.2002). We performed a pilot study to determine the sample size. The sample calculation was performed for two proportions, following Arango.[21] Applying the formula to compare two samples, we calculated $n = 27$. Using the value 1.96 with an α level of 0.05 and β level of 0.084, we determined that the sample size for this study was sufficient to find significant differences when they actually exist.

This retrospective study included 41 patients with a cytologic diagnosis of HPV infection by Pap smear. Patients received care between June 2000 and October 2002 at the Gynecology and Obstetrics outpatient clinic of a public university hospital. All patients signed an informed consent form to demonstrate that they agreed to participate in the study. These cases were paired with 40 control cases with a normal routine or inflammatory cervical-vaginal cytology, collected during the same sample period. When the 81 patients returned to the clinic, we collected material to perform the HC test to diagnose HPV infection.

The same observer reviewed the slides according to the CMC[13,14] and SSC.[15] The observer was unaware of the previous results of the Papanicolaou and HC exams. We considered the cytology as positive for a diagnosis of HPV infection based on the CMC when classic koilocytosis or dyskeratosis was present. The cytology was considered positive according to the SSC when a minimum of five of the nine criteria were present.

We also analyzed the presence of inflammation and infection by other agents. To diagnose inflammation, we analyzed the presence of neutrophils and cell changes, such as an increase in nuclear volume, binucleation, hyperchromasia, margination of chromatin, small perinuclear halo, and cytoplasmic vacuolization.[3] We classified the inflammatory process as slight, moderate or intense, based on the intensity of the inflammatory exudate and the frequency of inflammation-related cell changes. Diagnosis of infection was based only on the morphological identification of the agent.[22]

The obtained results were entered into a database for statistical analysis via *Microsoft Access* 2000®. GRAPHPAD INSTAT® (version 3.0) was used to perform the statistical calculations. Bartleti test was applied for the age analysis, and Fisher's exact test was used to compare proportions. The tests were considered significant when the probability of rejecting the null hypothesis was less than 5% ($p < 0.05$).

Results

In the HC exam, 47 (58%) of the 81 cases tested positive for HPV infection. The age of patients with positivity for HPV-DNA by HC ranged from 14 to 47 years, with an average of 24.1 ± 6.5 years. In the HPV-DNA–negative group, the age ranged from 14 to 50 years, with an average age of 25.18 ± 6.56 ($p = 0.4693$ between groups).

Using the CMC and considering the CH as gold standard for diagnosing HPV infection, of the 81 cases, 19.8% were true positives and 38.3% true negatives. The specificity was 91.2% and the sensitivity by 34% (►**Table 1**).

Using the SSC, 33.3% of the 81 cases were classified as true positives and 23 (28.39%) as true negatives. The sensitivity was 57.5%, and the specificity 67.7% (►**Table 2**).

All cases that were positive by HC and CMC were also positive by SSC (►**Table 3**). Thus, use of the SSC increased the sensitivity for diagnosing HPV infection from 34% to 57.5% ($p< 0.0001$). The difference in specificity between the SSC and CMC was not statistically significant.

The most frequently encountered SCC were: bi-or multinucleation (68 cases), nuclear hyperchromasia (61 cases), perinuclear halo (58 cases), slight koilocytosis (54 cases), and dyskeratosis (36 cases). These criteria were also common in

Table 1 Cytologic analysis of 81 cases based on the classic Meisels criteria (CMC), using hybrid capture (HC) as the gold standard for diagnosing HPV infection in patients accompanied on ambulatory of Universidade Federal do Triângulo Mineiro

	Positive HC n (%)	Negative HC n (%)	Total n (%)
Positive CMC	16 (34.1)	3 (8.8)	19 (23.4)*
Negative CMC	31 (65.9)	31 (91.2)	62 (76.6)
Total	47 (100.0)	34 (100.0)	81 (100.0)

Fisher's exact test; *$p = 0.0087$.

Table 2 Cytologic analysis of 81 cases based on Schneider's secondary criteria (SSC), relative to hybrid capture (HC) as the gold standard for diagnosing HPV infection in patients accompanied on ambulatory of Universidade Federal do Triângulo Mineiro

	Positive HC n (%)	Negative HC n (%)	Total n (%)
Positive SSC	27 (57.4)	11 (32.4)	38 (46.9)*
Negative SSC	20 (42.6)	23 (67.6)	43 (53.1)
Total	47 (100.0)	34 (100.0)	81 (100.0)

Fisher's exact test; *$p = 0.0417$.

Table 3 Distribution of the 47 HPV-DNA–positive cases, according to cytologic analysis by Schneider's secondary criteria (SSC) and the classic Meisels criteria (CMC) in patients accompanied on ambulatory of Universidade Federal do Triângulo Mineiro

	Positive SSC n (%)	Negative SSC n (%)	Total n (%)
Positive CMC	16 (59.2)	0	16 (34.0)*
Negative CMC	11 (40.8)	20 (100.0)	31 (66.0)
Total	27 (100.0)	20 (100.0)	47 (100.0)

Fisher's exact test; *$p < 0.0001$.

cases with a negative HC result, without any significant difference. Cleared cytoplasm was the only criteria that, in isolation, showed a significant difference for the groups with positive and negative HC results; however, it was present in only 30.86% of positive cases and was a common finding (11.12%) in negative cases.

Some of the SSC were also often found in cases of inflammation or infection, especially in more accentuated cases, which may have been related to the increased number of false positives. In reviewing the slides, 76 of the 81 cases (93.8%) showed varying degrees of inflammation, including 81.5% of slight, 14.5% of moderate, and 4% of intense inflammation. Among patients with inflammatory changes, most had non-specific inflammation (lactobacilli), 15.8% had a diagnosis of infection by *Gardnerella vaginalis*, 14.5% had a diagnosis of infection by *Candida sp*, and 6.6% had predominance of cocobacilli.

We separated the cytologies into two groups: cases with slight or no inflammation, and cases with moderate or intense inflammation. Of the 67 cases with slight or no inflammation, 47.8% had five or more SSC (i.e., were considered positive for HPV infection by SSC). Of these cases, 21.9% had a negative HC result, and 78.1% had a positive HC result. The sensitivity of the SSC relative to HC was 60.98%, and the specificity was 73.08%. There was a statistically significant difference ($p = 0.0115$) between the groups, indicating that the SSC were good indicators of infection by HPV in cases with slight or no inflammation.

Among the 14 cases with moderate or intense inflammation, 42.9% were considered positive for HPV infection, according to the SSC. Of these 6 cases, 33.3% had a positive HC result, and 66.7% had a negative HC result. The sensitivity of the SSC in relation to HC was 33.3%, and the specificity 50%. The sensitivity and specificity of the SSC in more intense inflammatory situations was lower.

Discussion

The Pap test has worked as well as it has despite the poor sensitivity of a single test because it is repeated periodically during the span of a woman's lifetime.[23]

Despite the high specificity, the sensitivity of the CMC is low. Compared with HC for diagnosis of HPV infection, the sensitivity of the SSC was higher than CMC, but the specificity was lower, agreement with other authors.[15–18] Thus, the secondary criteria described by Schneider et al.[15] appear to have a better ability to detect HPV infection in true-positive patients.

Other authors found sensitivity for non-classic criteria of only 15.8% and the specificity of 100% in samples previously diagnosed by polymerase chain reaction (PCR). However, they used different cytologic criteria from our study, examining only nuclear hyperchromasia, pleomorphism, and the nucleus/cytoplasm relationship in HIV infected patients.[24] In other study, after including secondary criteria, they observed that the diagnostic frequency for HPV using cytology increased from 24.4% to 75.6%.[18] Again, these authors used different secondary cytologic criteria from those used in our study. In comparing cytology with molecular hybridization, the first study obtained an agreement of 48% when they used only koilocytosis as a cytologic criterion. However, when they also included dyskeratosis, dyskariosis, binucleation, and multinucleation agreement increased to 75%.[24] Different research indicated that the inclusion of non-classical cytomorphologic signs increased the sensitivity of the cytologic test for detecting HPV when compared with PCR, although they used monobed, rather than conventional, cytology.[17] Using PCR for HPV 16, an study concluded that the classic cell changes were not the only ones that permitted a diagnosis of HPV infection.[11]

We found a reduction in the specificity and sensitivity of the SSC in cases with moderate or intense inflammation. This finding probably stems from the superimposition of cytologic changes related to inflammation with certain secondary criteria involved in the HPV diagnosis. Moreover, the inflammatory exudate may have made it difficult to visualize the cytologic criteria.[3] Thus, we believe that it is better not to use the SSC in cases with moderate or intense inflammation, to avoid an increase in the number of false positives.

Studing the importance of the application of the non-classical criteria compared with HC, they found inflammation in 61.5% of cases, although they did not classify the intensity of inflammation.[19] Other research identified inflammation in only 4.9% of cases when comparing non-classical criteria with PCR.[18] Nevertheless, their findings conflict with those in the literature, as inflammation is a very common finding in cervical-vaginal cytology, particularly unspecific cervicitis in young patients.[3,4,22] In addition, the authors did not classify the intensity of the inflammation.

Despite the introduction of the HPV vaccines, screening programs using cervical-vaginal cytology must continue. The vaccine does not completely protect against cervical cancer and is still not universally used.[25–27]

Compared with HC for diagnosis of HPV infection, the sensitivity of the SSC was higher than CMC. Moderate or intense inflammation reduces the sensitivity and specificity of the SSC for diagnosing HPV infection using the HC as the gold standard. We believe that, with greater use of the vaccine against HPV, there will be an increase in the number of cytologies with minimal cell changes, making it even more important to recognize the non-classical changes that are associated with HPV infection.

Conflicts of Interest

None.

Acknowledgments

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