

ORIGINAL PAPER

RETROSPECTIVE ANALYSIS OF NEGATIVE CYTOLOGY RESULTS CORRELATED WITH A POSITIVE HIGH-RISK IN THE HUMAN PAPILLOMAVIRUS RESULT AND SUBSEQUENT RESULTS OF PATIENTS OVER A PERIOD OF THREE YEARSPAWEŁ GERARD PIOTROWSKI¹, MICHAŁ TEODOR JELEŃ²¹Lab Path Experts, Kozięgłowy, Poland²Department of Immunopathology and Molecular Biology, Medical University, Wrocław, Poland

This research paper evaluates the efficacy of co-testing in precluding cervical cancer, with a particular focus on distinguishable outcomes of the human papillomavirus (HPV) vs. cytology tests.

A retrospective review of 5948 patients, who tested positive for high-risk HPV but showed negative cytologic findings, revealed that 15.006% tested positive in subsequent screenings.

A comparative analysis of various commercial HPV tests highlighted the precision of mRNA-based HPV testing by Aptima (Hologic) in reducing the likelihood of false-negative cytology.

The paper challenges the conviction that a negative cytology alone suffices advocating for a condensed testing interval in instances of positive HPV outcomes, thereby facilitating earlier intervention and optimal preventive care. These findings unveil an exigency for reconsidering preventive strategies based on test outcomes.

Key words: cervical cancer, co-testing, cytology tests, HPV, efficacy, retrospective review, high-risk HPV, mRNA-based HPV testing, false-negative cytology, preventive care.

Introduction

Cervical cancer is a tumour that affects 2.6% of women with respect to all cancers in Poland. Four percent of women die (relative to mortality from all cancers among women) [1]. This cancer ranks sixth in terms of frequency and mortality, and third among women aged 15–44 years, despite an improving trend over the years [1].

Most cases are caused by the human papillomavirus (HPV). In 2018, the World Health Organisation declared its goal to eliminate cervical cancer and issued a global call to action [2]. Success in this regard would lead to the eradication of a cancer that has a global incidence of approximately 530,000 cases

per year, with about half of those cases resulting in death [3].

Cervical cancer is almost entirely caused by a group of closely related small DNA viruses with a highly organised genome of only 8 kb, which have existed for millions of years and found a very specific ecological niche in the human reproductive tract. Preventing all HPV-related cancers would also eliminate an additional 80,000 sexually transmitted cancers worldwide, including those affecting the anus, oral cavity, and throat, accounting for about 5% of all cancers worldwide [3]. A positive result only indicates an increased risk of developing precancerous conditions or cervical cancer, but it does not confirm the disease diagnosis [4]. The high-risk HPV (HR HPV) test is

highly sensitive and detects more precancerous cervical conditions than cytology, but it also detects infections that will never progress to disease [5].

The discovery of HPV16 in Heidelberg in 1983 using Southern blot hybridisation with HPV11 DNA [6] laid the foundation for studying HPV-associated cancers. This was in line with the hypothesis put forth by Harald zur Hausen [7], initiated in 1972, which was based on anecdotal reports linking genital warts to squamous cell carcinoma. This hypothesis was later supported by the finding of koilocytes as cytological evidence of HPV infection in cervical dysplasia [8]. All of this was preceded by a long history of observing a potential link between sexual behaviour and cervical cancer, as well as studying papillomavirus-related tumours in animals and the failure to establish the role of other factors, including sperm DNA and herpes virus. Importantly, further research led to the development of methods for recognising precancerous conditions, which could contribute to early detection [9].

Recently, molecular HPV tests have become an increasingly common method for screening, offering greater reproducibility and the benefits of conducting multiple tests simultaneously. These advantages have led to the approval of HPV testing by the US Food and Drug Administration as an adjunct to cytology or as a co-test. Subsequent pooled studies and meta-analyses of randomised and observational studies have demonstrated better detection rates for HPV-based screening (both alone and in combination) compared to cytology [10-12]. Additionally, considering the ATHENA study, HPV testing was approved as a standalone screening strategy in 2014 [13]. As a result, standalone HPV testing has become the preferred strategy compared to cytology, including in European and American guidelines [9, 13]. In the Netherlands, cytology has already been replaced by standalone HPV testing every 5 years [14].

This study was conducted to predict the effectiveness of co-testing in the prevention of cervical cancer. It primarily focuses on positive HPV test results compared to negative cytological results. The Brandenburg region in Germany, which is the closest and largest neighbouring region to Poland, conducts co-testing. The results may contribute to the development of appropriate preventive measures based on the indicated test outcomes.

Material and methods

The human papillomavirus screening combined with cytological screening (co-testing) appears to be the most reasonable method for early detection of cervical cancer. However, many cases show positive HR HPV results but negative cytological results. Although one does not exclude the other, it is worth studying such cases and paying attention to further diagnostic procedures.

For this purpose, a detailed analysis of the medical history of 5948 patients who underwent testing in 2020 was conducted. These patients had negative cytological results but positive HR HPV results. The data were obtained from an institute that conducts over half of the cytological tests in the Brandenburg region (Germany), indicating a highly reliable test group. The institute's management requests anonymity for the facility to protect data privacy. The institute was chosen due to access to data resulting from a long-standing collaboration and a representative database. Results from the year 2020 were collected for a specific reason: this was the time when co-testing became fully implemented in Germany as the primary screening method [5]. The observation of patients' histories was conducted from the beginning of 2020 until April 2023. The human papillomavirus tests were mainly performed using Aptima (Hologic) and PapilloCheck (Greiner) methods.

Results

In 2020, the Institute conducted 114,426 cytological tests (the entire region conducted 219,069), involving 108,947 patients. 96.831% of the results were evaluated as negative (region's data: 96.819%). 62% of histologically confirmed negative results turned out to be positive.

Out of the patients with negative cytological results who were observed and tested positive for HPV, a total of 5948 patients were included. Among this group, 4425 patients had a subsequent history of cytological and HPV testing over the next 3 years. 15.006% of the study group obtained a positive result in the subsequent tests. Table I presents the quantities of individual results in relation to cytological tests, correlated with HPV testing.

Figure 1 shows the relative comparison of all the data examined against the results of cytological tests carried out since 2020.

Among the examinations not confirmed by histopathology, 11 women underwent hysterectomy at that time, and 3 underwent examination after hysterectomy.

In addition, it is worth paying attention to the comparative results of the Greiner and Aptima tests. Both tests were used as primary HPV tests. In total, 208 mixed tests of Greiner (G) vs. Aptima (A) were performed in different orders, at different time intervals, which were later confirmed histopathologically as negative (Fig. 2, 3, Table II).

Discussion

Conventional cytological diagnostics is significantly less sensitive than liquid-based cytology or HPV testing [15]. Currently, the recommended screening interval for women with a negative cytological result

Table I. Number of positive cytology results in the next three years following the initial Cytology –/HR HPV+ result

PARAMETERS	HP CONFIRMED, N = 472	HP NOT CONFIRMED, N = 192	
CIN1	208	157	Patient with LSIL
CIN1-2	13	23	Patient with ASC-H
CIN2	126	12	Patient with HSIL
CIN2-3	15		
CIN3	97		
SCC	1		
AdCa <i>in situ</i>	4		
AdCa	6		
VIN2	1		
VaIN1	1		
HP		4425	
Patient count with PapTest –/HPV+ and further history			
Positive PapTest results in 3 years			664

ASC-H – atypical squamous cells – cannot exclude high-grade squamous epithelial lesion, HIST-PAT – histopathology, HP – human papilloma, HSIL – high-grade squamous epithelial lesion, LSIL – low-grade squamous epithelial lesion, SCC – squamous cell carcinoma

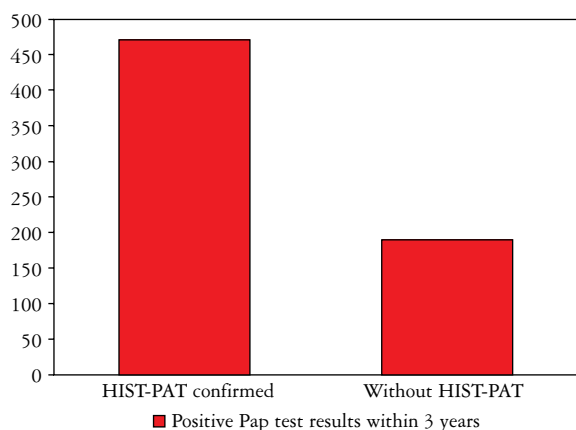


Fig. 1. Breakdown of positive results in the next 3 years after the initial result Cytology –/HR HPV+
HIST-PAT – histopathology

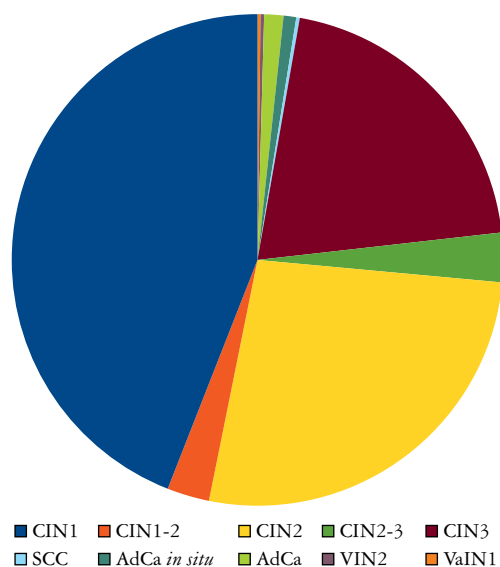


Fig. 2. Division of histologically confirmed cytology results
SCC – squamous cell carcinoma

is 3 years [16]. However, the aforementioned studies indicate that this period may be too long. The results suggest that during this time, 75% of women in Germany undergoes private cytological examinations, often combined with HPV testing. Among these women, as many as 15% receive a positive result within the 3-year period. In slightly over half of the patients, high-grade changes were detected and histologically confirmed, while 2% showed neoplastic changes. In this case, it cannot be determined what the future outcomes will be for women who undergo cytological testing again after the recommended 3-year interval.

It should also be noted that the percentage of women tested with a positive HR HPV test and a negative cytological result is only 5.46% of all patients, while according to data presented on 10 March 2023, in

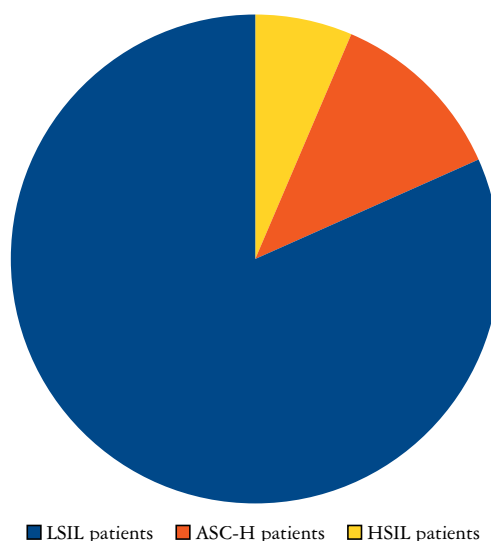


Fig. 3. Division of cytological results without subsequent histopathological confirmation
ASC-H – atypical squamous cells – cannot exclude high-grade squamous epithelial lesion, HSIL – high-grade squamous epithelial lesion, LSIL – low-grade squamous epithelial lesion

Poland, the percentage of similar results is as high as 16.5% [17]. This would imply that approximately 1.5% of women out of 100,000 would be assessed as negative in the cytological examination, despite the likelihood of neoplastic changes already occurring.

It is also confirmed that a positive HPV test result should lead to a shorter interval for cytological testing due to the high risk of neoplastic changes. It may seem that, despite everything, a negative cytology result remains the primary determinant of a patient's health level when deciding to postpone their next visit to the gynaecologist. However, this would require confirmation through appropriate examinations. With regard to cytological testing, even if no changes are observed, based on the HPV test history, it would be advisable to assign an ASC-US status to such a test or create a separate record that requires shorter-term follow-up and is equivalent to low-grade neoplastic changes. Although a comment on post-treatment follow-up can be added, it is probably insufficient to influence the decision regarding cytological and HPV testing within a shorter interval than recommended.

Conclusions

It is also worth considering the data confirming negative results through subsequent HPV tests from different manufacturers. The PapilloCheck test from Greiner Bio-One detects the E1 region of the HPV virus [18]. This region is responsible for the production of the E1 protein and is the only protein of this virus with enzymatic activity. The main known function of this protein is the regulation of viral DNA replication [19, 20]. On the other hand, the Aptima test from Hologic detects the E6/E7 regions, which are the major oncogenes of the HPV virus. Manipulation of these genes is currently the most successful form of therapy for cervical cancer [21]. An important distinction is that the PapilloCheck test is a DNA-based test, while the Aptima test is based on mRNA. Comparative results of both tests demonstrate that the Aptima mRNA-based HPV test from Hologic is significantly more accurate in ruling out false-negative cytology results.

In Poland, HPV testing has not been reimbursed thus far, while the HPV vaccine has been reimbursed since 1 November 2021 [22].

The authors declare no conflict of interest.

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Table II. Greiner and Aptima test comparison

GREINER AND APTIMA TESTS	N	%
G+/A-	108	51.92
A+/G-	64	30.77
A+/G+	36	17.31

*HPV – the human papillomavirus
G–/A results were not verifiable due to the nature of the study, which focused on positive HPV test results.*

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