PROTOCOL TITLE 'Incidence of Cervical Cancer in HPV-positive Women with Low-grade Cytological Abnormalities'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AGC: Atypical Glandular Cells of Endocervical Origin

ASC-US: Atypical Squamous Cells of Undetermined Significance

CIN: Cervical Intraepithelial Neoplasia

HPV: Human Papillomavirus

IQR: Interquartile Range

LSIL: Low-grade Squamous Intraepithelial Lesion

NILM: Negative for Intraepithelial Lesion or Malignancy

PALGA: Dutch nationwide pathology databank

SD: Standard Deviation

SUMMARY

Rationale:

Since 2017, the Dutch cervical cancer screening program has adopted human papillomavirus (HPV) testing with referral for colposcopy in all HPV-positive women with cytological abnormalities. However, most low-grade abnormalities — atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells of endocervical origin (AGC), and low-grade squamous intraepithelial lesions (LSIL) — regress spontaneously, which may result in overdiagnosis, overtreatment, and unnecessary pressure on the healthcare system. To evaluate whether follow-up intensity can be safely reduced, it is important to assess the actual cancer risk in this subgroup and compare it with the risk in women with stable negative for intraepithelial lesion or malignancy (NILM) cytology after one year, who are currently returned to the screening program.

Objective:

- Primary objective: To determine the five-year incidence of histologically confirmed cervical cancer in HPV-positive women with low-grade cytological abnormalities detected through the Dutch cervical cancer screening program.
- Secondary objectives:
    - To assess time to cervical cancer diagnosis, all-cause mortality, the type of examination at which the diagnosis was established, and the histological characteristics of the carcinoma (subtype) in this population, within five years after enrollment.
    - To assess cervical cancer risk stratified by histological diagnosis within three months of baseline abnormal cytology (≤ cervical intraepithelial neoplasia (CIN) I vs CIN 2+) and, for the ≤ CIN I group, further stratified by cytology at 12-month follow-up (no or nondiagnostic cytology, ≤ LSIL, HSIL+).
    - To perform a sub-analysis of women with a history of abnormal cytology or histology and assess their five-year cervical cancer risk after enrollement.
    - To evaluate the incidence of cervical cancer in women with stable NILM cytology after one year and compare it with the five-year incidence in women with low-grade cytological abnormalities.

Study design:

Retrospective cohort study based on routinely collected data from the Dutch population-based cervical cancer screening program.

Study population:

Women who participated in the cervical cancer screening program in the Netherlands between January 2017 and December 2018, who:

- Tested HPV-positive with cytology results categorized as ASC-US, AGC, or LSIL according to the Bethesda system, or
- Had stable NILM cytology after one year.

Main study parameters/endpoints:

Primary endpoint: Incidence of histologically confirmed cervical cancer within the five-year screening interval.

Secondary endpoints:

- Time to diagnosis of cervical cancer, all-cause mortality, the type of examination at which the diagnosis was established, and the histological characteristics of carcinoma (subtype), within five years after enrollment.
- Cervical cancer incidence in women with a history of abnormal cytology or histology.
- Cervical cancer risk stratified by histological diagnosis within three months of baseline abnormal cytology (≤ CIN I vs CIN 2+) and, for the ≤ CIN I group, further stratified by cytology at 12-month follow-up (no or nondiagnostic cytology, ≤ LSIL, HSIL +). With a follow-up period of five years and three months after enrollment.
- Cervical cancer incidence in women with stable NILM cytology after one year.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

As this is a retrospective cohort study using existing screening data, no direct burden, risks, or interventions are associated with study participation. The potential benefit lies at the population level: results may provide evidence to optimize follow-up strategies for HPV-positive women with low-grade cytological abnormalities or persistent NILM results, and for those with relevant medical history, thereby reducing unnecessary procedures while maintaining safe cancer detection.

1. INTRODUCTION AND RATIONALE

Cervical cancer is the fourth most common cancer among women worldwide and remains a leading cause of cancer-related mortality.[1] Prevention is largely possible through organized screening programs. These typically involve the collection of cervical samples to test for the presence of human papillomavirus HPV and to detect cytological abnormalities suggestive of (pre)cancerous lesions.[2,3] Implementation of such screening programs has been associated with a reduction in the incidence of cervical cancer.[4,5]

In the Netherlands, the national cervical cancer screening program invites women aged 30 to 60 every five years.[6] Since 2017, the Dutch program has shifted from a cytology-based to primary HPV based screening, according to international guidelines.[6–8] This shift aims to improve early detection of high-grade lesions and reduce cervical cancer incidence, as over 90% of women with cervical cancer are HPV-positive.[9] Women who test HPV-positive undergo follow-up cytology, and those with abnormal cytology—regardless of grade— are referred for colposcopy.

However, a considerable proportion of HPV-positive women present with low-grade cytological abnormalities, which often regress spontaneously without progressing to cervical cancer [10–12] Previous studies have shown that the long-term risk of cervical cancer in these women is relatively low. Nonetheless, referring them for colposcopy places a burden on healthcare resources, contributes to patient anxiety, and may lead to overtreatment.[13] In the context of rising healthcare costs and increasing pressure on the medical system, it is increasingly relevant to assess whether follow-up intensity for this subgroup can be safely reduced.

To date, the long-term risk of cervical cancer in HPV-positive women with low-grade cytological abnormalities has not been thoroughly evaluated. Given that eligible women are invited for screening every five years, it is crucial to assess whether these baseline abnormalities may progress to cervical cancer within that interval. Therefore, this study investigates the five-year incidence of cervical cancer among HPV-positive women with low-grade cervical abnormalities participating in the Dutch cervical cancer screening program.

2. OBJECTIVES

Primary Objective:

To determine the five-year incidence of histologically confirmed cervical cancer in HPV-positive women with low-grade cytological abnormalities detected through the Dutch cervical cancer screening program.

Secondary Objectives:

- To assess time to cervical cancer diagnosis, all-cause mortality, type of examination at which the diagnosis was established and the histological characteristics of the carcinoma (subtype) in this population , within five years after enrollment.
- To assess cervical cancer risk stratified by histological diagnosis within three months of baseline abnormal cytology (≤ CIN I vs CIN 2+) and, for the ≤ CIN I group, further stratified by cytology at 12-month follow-up (no or nondiagnostic cytology, ≤ LSIL, HSIL +), with a follow-up period of five years and three months after enrollment.
- To perform a sub-analysis of women with a history of abnormal cytology or histology and assess their five-year cervical cancer risk.
- To evaluate the incidence of cervical cancer in women with stable NILM cytology after one year and compare it to the five-year incidence in women with low-grade cytological abnormalities.

3. STUDY DESIGN

This retrospective cohort study includes women who participated in the Dutch national cervical cancer screening program between January 2017 and December 2018, with a follow-up period of five years.

4. STUDY POPULATION

4.1 Population (base)

The study population consists of women who participated in the Dutch national cervical cancer screening program between January 2017 and December 2018. Eligible women were HPV-positive at baseline with either low-grade cytological abnormalities (ASC-US, AGC, LSIL) or stable NILM cytology one year after initial screening.

4.2 Inclusion criteria

To be eligible to participate in this study, a participant must meet the following criteria:

- Women participating in the Dutch national cervical cancer screening program between January 2017 and December 2018.
- HPV-positive at baseline screening.
- One of the following cytology results:
    - ASC-US, AGC, or LSIL cytology according to the Bethesda system

      or

    - NILM cytology at one-year follow-up.

4.3 Exclusion criteria

There are no exclusion criteria.

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

- Incidence of histologically confirmed cervical cancer within the five-year screening interval in HPV-positive women with low-grade cytological abnormalities.

5.1.2 Secondary study parameters/endpoints

- Time to diagnosis of cervical cancer.
- All-cause mortality.
- Type of examination at which cervical cancer diagnosis was established.
- Histological characteristics of carcinoma (subtype).
- Cervical cancer incidence in women with stable NILM cytology after one year.
- Cervical cancer incidence in women with a history of abnormal cytology or histology.
- Cervical cancer risk stratified by histological diagnosis within three months of baseline abnormal cytology (≤ CIN I vs > CIN I) and, for the ≤ CIN I group, further stratified by cytology at 12-month follow-up (no or nondiagnostic cytology, ≤ LSIL, > LSIL).

5.1.3 Other study parameters

- Baseline characteristics such as age at inclusion.
- Cytology according to the Bethesda system and histology results at baseline and follow-up.
- HPV test results at baseline and follow-up.
- Relevant medical history, including prior abnormal cytology or histology.

6. STATISTICAL ANALYSIS

6.1 Primary study parameter(s)

The primary study parameter is the incidence of histologically confirmed cervical cancer within the five-year follow-up period. The cumulative incidence will be calculated as the number of new cases diagnosed divided by the total number of women included in the cohort. The incidence rate will also be calculated as the number of cervical cancer cases divided by the total follow-up time in person-years and expressed per 1,000 person-years.

6.2 Secondary study parameter(s)

Secondary study parameters include:

- Time-to-diagnosis of cervical cancer, which will be displayed in a line diagram with time on the y-axis and number of cases on the x-axis.
- All-cause mortality, type of examination at which the diagnosis was established, and histological subtype of cervical cancer, which will be summarized as frequencies and percentages for each relevant group.
- Cervical cancer incidence in women withstable NILM cytology after one-year follow-up, cervical cancer incidence in women with a history of abnormal cytology or histology, and risk stratification by histology within three months of baseline cytology and follow-up cytology at 12 months. For these parameters, cumulative incidence and incidence rates will be calculated.

6.3 Other study parameters

Other study parameters include baseline characteristics such as age, which will be assessed for normality using the Shapiro-Wilk test. If normally distributed, this variable will be reported as means with standard deviation (SD). If non-normally distributed, it will be reported as medians with interquartile range (IQR). Cytology and histology results at baseline and follow-up, HPV test results, and relevant medical history will be summarized as frequencies and percentages for each relevant group. For the relevant medical history, the highest histology results will be presented. In case a patient has both cytological and histological results, histology will be prioritized. Pap scores will be converted to Bethesda system classifications using the KOPAC scores.[14] Missing data will be described, and analyses will include all available data.

All statistical analyses will be conducted using SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA, 2025).

7. ETHICAL CONSIDERATIONS

7.1 Regulation statement

This study will be conducted in accordance with the principles of the Declaration of Helsinki (2013 version) and in compliance with applicable Dutch laws and regulations. The study was approved by the scientific committee of Palga, the Dutch nationwide pathology databank in the Netherlands (approval number 2025-3). Ethical approval by a medical ethical committee was not required under Dutch law, as no patients were directly involved in the conduct of the research and only non-identifiable data were used.

7.2 Recruitment and consent

No recruitment or informed consent procedures were applicable, as the study uses retrospectively collected, fully anonymized data from Palga.

8. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

8.1 Handling and storage of data and documents

Data for this study are obtained from Palga (the Dutch nationwide pathology databank). All data and results will be used solely for the purposes described in this study and will not be used for any other purpose or published beyond this scope. The data will not be shared with third parties not involved in the research. Data will be stored securely and will be destroyed five years after publication.

All data are fully anonymized. No procedures will be performed that could reveal the identity of participants if indirectly identifying information is inadvertently included. Access to the data is restricted to the research team. Procedures are in place to ensure data security and participant privacy.

8.2 Amendments

Amendments are changes made to the research after a favourable opinion by the review committee has been given. All amendments will be notified to the review committee that gave a favourable opinion. Non-substantial amendments will not be notified to the review committee, but will be recorded and filed by the sponsor.

8.3 Public disclosure and publication policy

The results of the study will be published in peer-reviewed scientific journals and presented at scientific meetings.

9. REFERENCES

[1] Arbyn M, Weiderpass E, Bruni L, Sanjosé S de, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. The Lancet Global Health 2020;8:e191–203. https://doi.org/10.1016/S2214-109X(19)30482-6.

[2] Bruni L, Serrano B, Roura E, Alemany L, Cowan M, Herrero R, et al. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. The Lancet Global Health 2022;10:e1115–27. https://doi.org/10.1016/S2214-109X(22)00241-8.

[3] US Preventive Services Task Force. Screening for Cervical Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2018;320:674–86. https://doi.org/10.1001/jama.2018.10897.

[4] Melnikow J, Henderson JT, Burda BU, Senger CA, Durbin S, Soulsby MA. Screening for Cervical Cancer With High-Risk Human Papillomavirus Testing: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Rockville (MD): Agency for Healthcare Research and Quality (US); 2018.

[5] Jansen EEL, Zielonke N, Gini A, Anttila A, Segnan N, Vokó Z, et al. Effect of organised cervical cancer screening on cervical cancer mortality in Europe: a systematic review. Eur J Cancer 2020;127:207–23. https://doi.org/10.1016/j.ejca.2019.12.013.

[6] Cervical cancer screening programme. RIVM n.d. https://www.rivm.nl/en/cervical-cancer-screening-programme (accessed June 1, 2025).

[7] WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention n.d. https://www.who.int/publications/i/item/9789240030824 (accessed June 1, 2025).

[8] Eramus MC, Palga. Bevolkingsonderzoek Baarmoederhalskanker - Monitor 2017. RIVM 2017.

[9] Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796–802. https://doi.org/10.1093/jnci/87.11.796.

[10] Ciavattini A, Serri M, Di Giuseppe J, Liverani CA, Gardella B, Papiccio M, et al. Long-term observational approach in women with histological diagnosis of cervical low-grade squamous intraepithelial lesion: an Italian multicentric retrospective cohort study. BMJ Open 2019;9:e024920. https://doi.org/10.1136/bmjopen-2018-024920.

[11] Katki HA, Gage JC, Schiffman M, Castle PE, Fetterman B, Poitras NE, et al. Follow-up testing after colposcopy: five-year risk of CIN 2+ after a colposcopic diagnosis of CIN 1 or less. J Low Genit Tract Dis 2013;17:S69-77. https://doi.org/10.1097/LGT.0b013e31828543b1.

[12] The Netherlands: Integraal Kankercentrum Nederland. National Guidelines: CIN, AIS en VAIN (Dutch). Richtlijnendatabase Federatie Medisch Specialisten;Accessed June 2025.

[13] Sharp L, Cotton S, Cruickshank M, Gray NM, Harrild K, Smart L, et al. The unintended consequences of cervical screening: distress in women undergoing cytologic surveillance. J Low Genit Tract Dis 2014;18:142–50. https://doi.org/10.1097/LGT.0b013e31829c97d8.

[14] The Netherlands: Integraal Kankercentrum Nederland. National Guidelines: Cervixcytologie- Codering van de uitslag (Dutch). Richtlijnendatabase Federatie Medisch Specialisten; Accessed June 2025.