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Identification of the value of a novel test for the surveillance of women with precancerous lesions of the cervix who wish to avoid surgical treatment

Submission date
27/10/2023

Recruitment status
No longer recruiting

Registration date
30/10/2023

Overall study status
Ongoing

Last Edited
13/11/2025

Condition category
Urological and Genital Diseases

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[_] Results
[_] Individual participant data
[_] Record updated in last year

Plain English summary of protocol

Background and study aims
Cervical cancer is one of the common cancers in women worldwide. It's the fourth most common cancer in women globally and the sixth most common in Europe. In lower-income countries, it's the second most common gynecological cancer for all ages and the third most common for women aged 15 to 44 in wealthier countries.

Scientists have found that certain types of HPV (human papillomavirus) are a major cause of cervical cancer. This discovery has led to a rethinking of how we screen for cervical cancer. We're now using HPV testing and genotyping more often for initial screening because they're better at detecting cervical (pre)cancer in its early stages compared to traditional cytology tests.

The main goal of cervical cancer screening is to find precancerous changes in the cervix early so we can treat them and prevent them from turning into full-blown cervical cancer. The earliest form of this precancer is called cervical intraepithelial neoplasia (CIN) grade 3. However, CIN grade 2 is also seen as a serious issue. Women with high-grade CIN often need treatment, but this decision depends on factors like their age and plans for future pregnancies.

We tend to avoid excisional treatments, like removing a part of the cervix, for young women who might want to have kids later. That's because these treatments have been linked to an increased risk of having a premature baby in future pregnancies. So, some women, especially those with CIN2, choose to avoid these treatments because they're more worried about the risks of pregnancy complications than getting cervical cancer. These women are monitored closely with cytology (a cell study) and colposcopy (an exam to look at the cervix).

Now, we're proposing a research plan to test a new method called p16/Ki-67 dual immunocytochemistry. We want to see if it's a good way to keep an eye on women with CIN2 who don't want excisional treatment. In this study, women with CIN2 will get tested with this method when they're first diagnosed and then every 6 months for two years. We're doing this to

figure out how well this new test can help us watch over these women without the need for surgery.

Who can participate?
Women aged 21 - 65 years with CIN2 who don't want excisional treatment.

What does the study involve?
1. At the beginning (t=0), women with CIN2 are tested with p16/Ki-67 immunocytochemistry (ICC) and high-risk HPV DNA testing. We select these women based on specific criteria.
2. After six months (t=1), the same women undergo colposcopy, and before that, we collect cervicovaginal samples for cytology and p16/Ki-67 ICC.
3. At the 12-month mark (t=2), the women with CIN2 are again examined with colposcopy, and before that, we collect cervicovaginal samples for high-risk HPV testing, cytology, and p16/Ki-67 ICC.
4. The process at t=3 is similar to t=1, and the one at t=2 is similar to t=4.
5. Women exit the study after visit t=4 and are referred for treatment as per local guidelines.

For p16/Ki-67 dual-staining, we use a special kit designed for detecting p16 and Ki-67 in cervical samples. The test is conducted according to the manufacturer's instructions, and cells are considered positive when both p16 and Ki-67 are present in the same cell.

HPV DNA testing is done using the cobas 4800 HPV PCR master mix, which identifies 14 high-risk HPV types. The test allows us to identify HPV 16 and HPV 18 separately from the other high-risk types.

If women are referred to the Unit of Colposcopy and Cervical Pathology, doctors perform colposcopies. Biopsies are taken from the cervix based on colposcopy findings. Histopathologic examinations are conducted by two specialized pathologists who assess the biopsy results. If there's a diagnosis of CIN3, women are informed about the need for a cervical procedure, and if it's cancer, they're referred for further management and leave the study.

What are the possible benefits and risks of participating?
Possible benefits of the study will be the characterization of a novel test (P16/Ki-67 immunocytochemistry testing) as suitable for the surveillance of women with CIN2 who wish to avoid surgical treatment. The test could be identified as having better diagnostic accuracy than the current standard which is cytology testing. Women who wish to preserve fertility and who avoid surgical treatment for cervical precancer will have a lower risk for preterm delivery in a subsequent prevnancy.
A possible risk would be delay in the treatment of a cervical precancerous lesion and the occurence of infiltrative cervical cancer at the end of the study, however this risk is being mitigated by the close follow up of the participants with colposcopy and biopsy.

Where is the study run from?
Papageorgiou General Hospital (Greece)

When is the study starting and how long is it expected to run for?
August 2022 to April 2028

Who is funding the study?
Roche Diagnostics (USA)

Who is the main contact?
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Public, Scientific, Principal investigator

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Additional identifiers

Protocol serial number
2023-B2015-253

Study information

Scientific Title
Surveillance of Cervical Intraepithelial Neoplasia (CIN) grade 2 with Dual P16/Ki-67 Immunocytochemistry staining

Acronym
CIN-2-DIN

Study objectives
P16/Ki-67 dual staining can accurately detect progression to CIN3 in women with CIN2 who have not been subjected to excisional treatment.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 18/04/2023, Scientific Council of Papagerogiou (Ring road, Municipality of Pavlou Mela, Area N. Evkarpia, Thessaloniki, 56403, Greece; +30 2313323000; epist@papageorgiou-hospital.gr), ref: 12035/18-04-2023

Primary study design
Observational

Study design
Prospective observational diagnostic accuracy study

Study type(s)
Diagnostic

Health condition(s) or problem(s) studied
Timely diagnosis of Cervical Intraepithelial Neoplasia (CIN) grade 3 or worse in women with CIN2 who opt for no treatment.

Interventions
According to the study design, women with Cervical Intraepithelial Neoplasia (CIN) grade 2 at t=0 will be tested for p16/Ki-67 immunocytochemistry (ICC) with the CINtec Plus Cytology test and high-risk HPV DNA testing with the Cobas HPV test. Those women will be recruited from the pool of women as described in the inclusion criteria.

After six months (t=1), women with CIN2 at t=0 will be subjected to colposcopy, and, prior to colposcopy, to cervicovaginal sampling for cytology and p16/Ki-67 ICC.

After 12 months (t=2) women with CIN2 at t=0 will be subjected to colposcopy, and, prior to colposcopy, to cervicovaginal sampling for hrHPV testing with the Cobas HPV test, cytology and p16/Ki-67 ICC.

The process followed at t=3 will be similar to the one followed at t=1, and the process followed at t=2 will be similar to the one at t=4.

All women after visit t=4 exit the study. After exiting the study all women are referred to treatment according to the local guidelines.

For the p16/Ki-67 dual-staining, a commercial kit specifically designed for the simultaneous detection of p16 and Ki-67 in cervical cytology preparations is used (CINtec PLUS, Roche laboratories AG, Mannheim, Germany) with the Ventana Medical System (Ventana Medical System Inc, Tucson, AZ) on BenchMark GX automated instruments according to the

manufacturer's instructions. Slides are subjected to p16/Ki-67 dual-staining according to the instructions of the manufacturer. Cells are considered positive when immunoreactivity for both p16 and Ki-67 is detected within the same cell (i.e., a cytoplasmic brown staining for p16, together with a nuclear red staining for Ki-67). The presence of at least one dual-stained cell is used as a cut-off to rate the sample as positive for the CINtec PLUS test. Interpretation of p16/Ki-67 dual-stain slides will be performed by a trained cytologist after quality control of staining from lab technicians.

HPV DNA testing will be performed using the cobas 4800 HPV PCR master mix includes primers and fluorescently labeled probes to detect the DNA of 14 high-risk (HR) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and human beta-globin, which acts as a measure of human cellularity in the specimen. Specifically, the cobas HPV test employs primers that amplify a region of approximately 200 base pairs within the L1 polymorphic region of the HPV genome. The fluorescent signal from twelve HR types of HPV (12HR HPV) (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) is detected using the same fluorescent label, while the HPV 16, HPV 18 and beta-globin signals are detected with three separate spectrally unique fluorescent labels, respectively. The distinct individual wavelengths characterizing each label allow for simultaneous genotyping of HPV 16 and HPV 18 amplicon separately from the twelve other HR types.

Women referred to the Unit of Colposcopy and Cervical Pathology, Department of Gynaecological Oncology, 1st Department of Obstetrics and Gynaecology, are subjected to colposcopy. All colposcopies are performed by medical doctors, specialized in colposcopy and cervical pathology. If the colposcopic findings are within normal limits (WNL; no signs of CIN), random biopsies are taken from the four quadrants of the cervix. In case of abnormal or suspicious for invasion colposcopic findings, multiple focal colposcopy-guided biopsies, and/or endocervical curettage (ECC), are taken from the abnormal area of the cervix. ECC is performed in cases with a transformation zone type III.

Histopathologic examinations are conducted by two expert pathologists specialized in cervical pathology. These specialists are aware of the colposcopy result, but not of the hrHPV DNA genotyping result, and the p16/Ki-67 dual-staining result. The biopsy/ECC results can be within normal limits (WNL) (no signs of CIN), low-grade CIN (CIN1) lesion, high-grade CIN lesions (CIN2 or 3) or cancer. Abnormal findings (CIN of any degree) are independently reassessed by a second specialized pathologist, and in case of disagreement between the two experts a third expert examines the specimen in question, so that at the end a two out of three decision can be made. In case of a diagnosis of CIN3, women are informed about the necessity of an excisional cervical procedure and exit the study. In case of a diagnosis of cancer women are referred to the Department of Gynaecological oncology for further management and exit the study.

Intervention Type
Not Specified

Primary outcome(s)
Diagnostic accuracy of CINtec Plus Cytology is measured using sensitivity and positive predictive value of the test for the diagnosis of CIN2+ and CIN3+ at 6, 12, 18, and 24 months after recruitment.

Key secondary outcome(s)

Diagnostic accuracy of Liquid-based Cytology is measured using sensitivity and positive predictive value of the test for the diagnosis of CIN2+ and CIN3+ at 6, 12, 18, and 24 months after recruitment.

Completion date
30/04/2028

Eligibility

Key inclusion criteria
1. Women referred to the Unit of Colposcopy and Cervical Pathology, Department of Gynaecological Oncology, 1st Department of Obstetrics and Gynaecology, “Papageorgiou” General Hospital, Thessaloniki, Greece.
2. Reason for referral:
2.1. Abnormal pap test (ASCUS or worse)
2.2. hrHPV positivity in cervical samples
2.3. Histologic diagnosis of CIN 1 or 2
3. Of these women those with a final diagnosis of CIN2, who do not wish to be subjected to excision cervical procedure, are eligible to be included in the analysis.
4. Age range: 21-65 years old

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
21 Years

Upper age limit
65 Years

Sex
Female

Total final enrolment
0

Key exclusion criteria
1. Pregnancy
2. Inability to adequately communicate
3. Inability to comply with the required follow-up during the study
4. Recurrent or acute vaginitis
5. Taking systemic immunosuppressive or immunomodulatory treatment (methotrexate, glucocorticoids, non-steroidal anti-inflammatory drugs, etc.)

6. CIN3
7. Cancer
8. Chemotherapy
9. Radiotherapy

Date of first enrolment
30/04/2023

Date of final enrolment
30/10/2024

Locations

Countries of recruitment
Greece

Study participating centre
1st Department of Obstetrics and Gynaecology, Papageorgiou General Hospital, Aristotle University of Thessaloniki
Ring Road, Monicipality of Pavlos Melas (area of Nea Efkarpia)
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Sponsor information

Organisation
Papageorgiou General Hospital

ROR
https://ror.org/01663qy58

Funder(s)

Funder type
Industry

Funder Name
Roche Diagnostics

Alternative Name(s)
Roche Diagnostics Corporation

Funding Body Type
Private sector organisation

Funding Body Subtype
For-profit companies (industry)

Location
United States of America

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Data sharing statement to be made available at a later date