PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Utility of extended HPV genotyping for the triage of self-sampled HPV-positive women in a screen-and-treat strategy for cervical cancer prevention in Cameroon: a prospective study of diagnostic accuracy.
AUTHORS	Broquet, Celine; Vassilakos, Pierre; Ndam Nsangou, François Marcel; Kenfack, Bruno; Noubom, Michel; Tincho, Evelyn; Jeannot, Emilien; Wisniak, Ania; Petignat, Patrick

VERSION 1 – REVIEW

REVIEWER	Sanchez, Gloria Universidad de Antioquia, School of Medicine
REVIEW RETURNED	30-Oct-2021

GENERAL COMMENTS	Is the abstract accurate, balanced and complete? The abstract does not give the numbers of the outcomes CIN2+, CIN3+ positive for each test. This is very important because the sample size is very small Is the study design appropriate to answer the research question? Thera are many missing information that does not allow to evaluate the appropriateness of the methodology. Were the women recruited in a primary care facility? How the eligibility was checked? How was it checked that these women have not been treated because cervical lesions before entering the study? In a hospital? How were they recruited? Are they representative of a population eligible for screening? How long were the women HPV+ but not lesions followed to confirmed that were disease free? Are the methods described sufficiently to allow the study to be repeated? How was the order of the procedures conducted? It is known that visual inspection is affected by the cervical brushing to obtain the samples for HPV tests and cytology. Who performed the cytology? Who performed the colposcopy? Were the colposcopists re-trained before the study? All the women negative in the HPV test but positive in the VIA have the chance to receive the gold standard diagnosis (colposocopy?). Were colposcopists blind to the result of the HPV test and VIA? Authors references of previous publications do not clearly describe these main points of the methodology of the study. If statistics are used, are they appropriate and described fully?

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	Authors do not state the power of the study. In total there were 44 cases CIN2+ and 30 cin3+ which are very limited numbers to compare the proportions of cases that will shift from positive to negative result or the proportion of cases that will shift from negative to positive results with both tests, especially in HPV+ women, since the performance of the tests do not differ widely. Are the discussion and conclusions justified by the results? The sample size limitation does not allow to conclude if the difference between the tests is real or by chance. Are the study limitations discussed adequately? Authors do not discuss about the limitation of the sample size and the limitation that not all women were equally verified by the gold standard 13. Is the supplementary reporting complete (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)? Yes, it is complete but not necessarily in agreement with the information that needs to be fulfilled. Ex, authors do not mention Intended sample size and how it was determined although they referred that this information was on page 6 where the statistical analysis was described.

REVIEWER	Helenius, Gisela Örebro University Örebro Life Science Center, Medical Sciences
REVIEW RETURNED	27-Dec-2021

GENERAL COMMENTS	In this study, the authors aim to investigate if self-sampling and HPV detection, followed by triaging using genotyping is clinically useful for cervical screening in a low-income country. HPV genotyping as a triage method was compared to VIA and cytology. In a screen-and-treat setting, genotyping was compared to VIA for evaluation of the risk for overtreatment. This is a very well designed study with highly relevant objectives and aims. The manuscript is very well written, clear and easy to follow and understand. The real-life setting of the study strengthen the results and the results on vaginal self-sampling followed by HPV detection and genotyping for triage are relevant, not only for low-income countries. In the part where the study procedure is described (page 6), I wonder what kind of swab that was used, please add that information. In the HPV testing text, it is unclear if it was the swab or the saline solutions that is transferred to the cartridge. I guess it was the saline solution, but was it the complete volume of 20 ml? Also, is it a human control gene included in the GeneXpert assay? Did you run any internal control samples in order to verify the assay? In table 3, under ≥CIN3, letters have been switched and it is written VPP instead of PPV.

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	It was a pleasure to read this manuscript and I look forward to follow the 3T-study.

REVIEWER	Giorgi Rossi, Paolo AUSL Reggio Emilia, Servizio Interaziendale Epidemiologia
REVIEW RETURNED	28-Feb-2022

GENERAL COMMENTS	Title Self sampling should be mentioned in the title. Abstract I suggest reporting results in more detail: which is the sensitivity of pool 1? Which is the sensitivity of VIA? And specificity or positivity rate? On the other hand, results for CIN2+ and CIN3+ are very similar and it is not worth to report both. It would be important to understand how many women are HPV+/triage-negative for each strategy (this information is directly related to specificity, but in this context gives immediately the needed performance indicator). Furthermore cytology is mentioned in the main findings and not in the abstract. Finally, what would be the number need to treat if all HPV+ women would be treated? Strength and limitations Please define ECB Methods How could you assess the outcome in VIA-positive women treated with thermal ablation? Description of the intervention is not clear, particularly it is not clear why the treatment procedure are described in the VIA paragraph and not in the paragraph describing the assessment procedures. Statistical analyses Please, define which group you compare for their sociodemographic characteristics. I miss a clear list of the comparisons that you are going to present. Public involvement This study is a typical example needing women involvement to better value the outcomes. I think the statement should be revised. Results Please check percentages (99.7% should be 99.5%). Table 4 could report also the performance of referring only pool-1 and all HPV-pos. Discussion The first paragraph repeats something already stated in the background. In the second paragraph: extended genotyping is available with several commercial tests, not only with Xpert. Third paragraph: please check the specificity values. Fourth paragraph: the sentence stating that specificity may be affected by prevalence of disease is not correct. In fact, in the case of typing, if we assume other characteristics, mainly previous screening history and the duration of the infections, not changing, type-specific positive predictive value is almost constant and specificity of a pool of types is completely determined by the mix of types in that population. For a demonstration of the constant PPV and variable specificity, and consequently the inverse application

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	of the Bayes theorem in the case HPV, see Giorgi Rossi et al, Int J Cancer 2012. Page 9, fourth paragraph: authors state that VIA should exclude the presence of cancer and eligibility for thermal ablation, but in this study, VIA missed 2 out of 3 cancers. The authors should highlight this point. Conclusions It is worth mentioning that the study has been performed on self-sampling.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:
Dr. Gloria Sanchez, Universidad de Antioquia

Comments to the Author:

This a very a tremendous effort to try to implement the recommended guidelines for early detection and treatment of cervical precursors lesions in low resource settings. The study was conducted in Cameron. It seems that the authors aimed to do a 10.000 study but are presenting preliminary results of 2014 women. Because they have only included these number of women, the number of CIN2+, CIN3+ outcomes are not yet adequate to infer which of the tests evaluated works better for triaging HPV positive women. If the study gets to the targeted number, there is not doubt this study can contribute a lot to this great need worldwide.

Dear Dr Sanchez,
It is true that the results of the study need to be confirmed on a larger scale. A RCT is currently underway with the objective of supporting these initial results (ClinicalTrials.gov ID NCT05385406). However, we would like to highlight the fact that most of the results obtained in the study are statistically significant at a $p<0.05$ level despite a smaller sample size than initially expected.

Is the abstract accurate, balanced and complete?
The abstract does not give the numbers of the outcomes CIN2+, CIN3+ positive for each test. This is very important because the sample size is very small.

Thank you for this comment. According to your proposal, the requested numbers have been added in the abstract.
Abstract (Page 2), Line 70-71: 382 (18.2%) women were HPV-positive among which 11.5% (n=44) were CIN2+. Of those, 41 were triaged positive by extended genotyping, versus 35 by VIA and 33 by cytology.

Is the study design appropriate to answer the research question?
Thera are many missing information that does not allow to evaluate the appropriateness of the methodology:

- Were the women recruited in a primary care facility? In a hospital? How were they recruited? Women were recruited by the Dschang District Hospital through a poster campaign, through door-to-door recruitment by community health workers and via radio announcements. This information was specified in the « Design » section of the study, Materials and Methods: Lines 185-187.

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- How the eligibility was checked? Eligibility was checked through a questionnaire completed with a midwife specifically trained for the study. This has been added in the Methods section: Lines 192-194.

- How was it checked that these women have not been treated for cervical lesions before entering the study? As stated above, this was assessed through a study questionnaire completed with a midwife specifically trained for the study. The Methods section has been clarified as follows: Lines 187-189 and 192-195.

- Are they representative of a population eligible for screening? As all women between 30 and 49 years of the general population of the health district were eligible for the study, no random sampling was performed. Therefore, the study sample is likely not completely representative of the general population in terms of sociodemographic characteristics such as educational level and professional status. However, efforts were made to recruit patients living in outlying areas with less access to care by deploying recruitment through community health workers and covering travel costs to the screening center.

Page 10, Discussion, Lines 432-438.

- How long were the women HPV+ but not lesions followed to confirmed that were disease free? Patients were recruited, screened and treated on the same day when cervical lesions were identified upon visual assessment. When no lesions were detected (negative VIA), HPV-positive women were followed up every 12 months, until clearance of the HPV infection.

Page 5, Study Procedures, Line 201-204.

- How was the order of the procedures conducted? We acknowledge that in the submitted version of our article, the stages of screening and their temporal sequence was not clearly specified and we thank you for highlighting this. We have made the following clarifications:

Study Procedures, Page 5, Lines 192-204: Sociodemographic characteristics, eligibility and medical history of the participants (including self-reported HIV status) were obtained through a questionnaire completed with a midwife specifically trained for the study; and later electronically transcribed using the SecuTrial© software (Berlin, Germany). Women were asked to provide a self-collected vaginal sample for HPV testing. Patients with a negative HPV test were discharged home without further investigations. In case of a positive result, participants were triaged by PAP smear and VIA/VILI. Endocervical brushing (ECB) and cervical biopsies were performed at the end of the procedure as the gold-standard. Identified lesions were treated by thermal ablation or large loop excision of the transformation zone (LLETZ) and the patient was discharged home after 30 minutes surveillance. When no lesions were detected (negative VIA), HPV-positive women were followed up every 12 months until clearance of the HPV infection. VIA-positive women treated with thermal ablation or LLETZ were followed up at 6 months and 1 year by self-HPV/VIA/cytology/biopsies and ECB. More details on the *3T-study* have been previously published.[18–20]

- It is known that visual inspection is affected by the cervical brushing to obtain the samples for HPV tests and cytology. Cervical Brushing was made after visual inspection with Acetic Acid, as mentioned above.

- Who performed the cytology? Who performed the colposcopy? The Pap smear and VIA were performed by a trained midwife, as specified in the paragraph above. No formal colposcopy was conducted, but VIA was made by naked eye and assisted by Smartphone images. Page 6, Line 223-230.

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- Were the colposcopists re-trained before the study? Midwifes were trained to perform VIA before the launch of the study and received ongoing training from a gynecologist on a bi-monthly basis. In addition, all cervical images captured with a Smartphone were reviewed by a gynecologist. This has been added to the Methods section.

Lines 225-226 and 230.

- All the women negative in the HPV test but positive in the VIA have the chance to receive the gold standard diagnosis (colposocopy?). Patients with a negative HPV test were discharged home without further investigations. In our study, the gold-standard was defined by histological results (biopsies and/or ECB). This has been clarified in the Methods section.

Lines 197-199.

- Were colposcopists blind to the result of the HPV test and VIA? Because only HPV-positive patients received VIA, and as it was performed the same day as the self-sampling, it was not possible to blind the midwives performing VIA to the results of HPV tests. No further colposcopy was performed within this study following the smartphone-assisted VIA.

Authors references of previous publications do not clearly describe these main points of the methodology of the study. It is true that these points have not been clarified by the previous authors or by us. They have now been added to the article, following your suggestions.

If statistics are used, are they appropriate and described fully?
Authors do not state the power of the study. In total there were 44 cases CIN2+ and 30 cin3+ which are very limited numbers to compare the proportions of cases that will shift from positive to negative result or the proportion of cases that will shift from negative to positive results with both tests, especially in HPV+ women, since the performance of the tests do not differ widely.
Thank you for this comment. Using McNemar's test for paired samples, the final sample size (including 44 CIN2+) was powered at 57% to detect a difference at the alpha=0.05 level between the obtained sensitivities for VIA and extended genotyping, and at 83% for the difference in sensitivity between cytology and extended genotyping. For the difference in specificity, the power of our sample size was of 57% for the comparison of VIA to extended genotyping, and of 100% for the comparison of cytology to extended genotyping. This has been added to the results section Lines 347-352.

Are the discussion and conclusions justified by the results? The sample size limitation does not allow to conclude if the difference between the tests is real or by chance. Are the study limitations discussed adequately?
As stated before, it is true that the results of the study need to be confirmed on a larger scale. However, most of the results obtained in the study are statistically significant despite a smaller sample size than initially expected (p value < 0.05 for every result except PPV and NPV of different triage tests for CIN2 and CIN3 detection). These issues have been added to the Discussion section of the article, Lines 427-438.

Authors do not discuss about the limitation of the sample size and the limitation that not all women were equally verified by the gold standard.
All the cases (HPV-positive women) were equally verified by the gold-standard (histopathology). Line 197-199; 306-308.
The limited sample size has been added to the discussion as stated above, Line 427-438.

13. Is the supplementary reporting complete (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)?
Yes, it is complete but not necessarily in agreement with the information that needs to be fulfilled. Ex, authors do not mention Intended sample size and how it was determined

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although they referred that this information was on page 6 where the statistical analysis was described.

Thank you for this comment. Explanations on the selected sample size have been provided in the Methods section.
Lines 278-286: The initial sample size planned for this study was of 6000 women. Considering an HPV prevalence of approximately 20% in the target population among which approximately 10% have CIN2+, we expected to obtain a total of 120 women with CIN2+ in our sample. Anticipating a sensitivity to detect CIN2+ of 75% for VIA and 90% for extended genotyping, based on previous experience and preliminary analyses, we calculated that 100 women with CIN2+ would be necessary to detect a difference in sensitivity with a confidence level of 95% and 80% power; thus the sample size of 6000 women would be sufficient for this purpose. However, the study had to be temporarily stopped due to the COVID-19 pandemic in March 2020, which is why this intermediate analysis was conducted with the data collected up to that time.

Reviewer #2:
Dr. Gisela Helenius, Örebro University Örebro Life Science Center

Comments to the Author:
In this study, the authors aim to investigate if self-sampling and HPV detection, followed by triaging using genotyping is clinically useful for cervical screening in a low-income country. HPV genotyping as a triage method was compared to VIA and cytology. In a screen-and-treat setting, genotyping was compared to VIA for evaluation of the risk for overtreatment. This is a very well designed study with highly relevant objectives and aims. The manuscript is very well written, clear and easy to follow and understand.
The real-life setting of the study strengthen the results and the results on vaginal self-sampling followed by HPV detection and genotyping for triage are relevant, not only for low-income countries.

In the part where the study procedure is described (page 6), I wonder what kind of swab that was used, please add that information. In the HPV testing text, it is unclear if it was the swab or the saline solutions that is transferred to the cartridge. I guess it was the saline solution, but was it the complete volume of 20 ml? Also, is it a human control gene included in the GeneXpert assay? Did you run any internal control samples in order to verify the assay?
In table 3, under ≥CIN3, letters have been switched and it is written VPP instead of PPV.
It was a pleasure to read this manuscript and I look forward to follow the 3T-study.

Response:
What kind of swab was used? We used the FloqSwab. This information has been added in the manuscript, Page 5, Study Procedures, Line 206.

The saline solution is transferred to the cartridge, complete volume or 20 ml? Only 1 ml of the solution was transferred to the cartridge. This information has been added in the manuscript, Page 5, Study Procedures, Line 207-208.

Is a human control gene included in the GeneXpert assay? Yes, the GeneXpert assay contains a 'Sample Adequacy Control' system with reagents that amplify and detect an endogenous single-copy human gene, thus determining if it contains sufficient patient cells for reliable performance. This has been added to the Methods section, lines 209-212.

Internal control samples runned in order to verify the assay? Yes, a sample processing internal control in the form of an exogenous nucleic acid pre-loaded in the cartridge is included in the

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GeneXpert assay to verify adequate functioning of the assay. This has been added to the Methods section, Lines 212-214.

In table 3, under ≥CIN3, letters have been switched and it is written VPP instead of PPV.
The necessary corrections have been made in Table 3.

Reviewer #3:

Dr. Paolo Giorgi Rossi, AUSL Reggio Emilia

Comments to the Author:
Title
Self sampling should be mentioned in the title.
We have changed the title of the manuscript as follows: "Utility of extended HPV genotyping for the triage of self-sampled HPV-positive women in a screen-and-treat strategy for cervical cancer prevention in Cameroon: A prospective study of diagnostic accuracy".

Abstract
I suggest reporting results in more detail: which is the sensitivity of pool 1? Which is the sensitivity of VIA? And specificity or positivity rate? On the other hand, results for CIN2+ and CIN3+ are very similar and it is not worth to report both.
In accordance with your requests, the result section of the abstract has been modified, Line 70-71.

It would be important to understand how many women are HPV+/triage-negative for each strategy (this information is directly related to specificity, but in this context gives immediately the needed performance indicator). Furthermore cytology is mentioned in the main findings and not in the abstract.
In accordance with your request, we have now added the required statistics in the results section of the abstract, as well as mentioned cytology.
Line 70-71.

Finally, what would be the number need to treat if all HPV+ women would be treated? As the abstract offers limited space and this was not considered one of our main results, we were not able to include this information in the abstract.

Strength and limitations
Please define ECB The abbreviation for Endocervical Brushing is now defined in the text, in Page 5, Study Procedures, Line 197.

Methods
How could you assess the outcome in VIA-positive women treated with thermal ablation?
VIA-positive women treated with thermal ablation were followed-up at 6 months and 1 year by self-HPV/VIA/cytology/biopsies and ECB. This information was added to the manuscript. Study Procedures, Line 202-204.

Description of the intervention is not clear, particularly it is not clear why the treatment procedure are described in the VIA paragraph and not in the paragraph describing the assessment procedures.
Thermal ablation is described in the VIA paragraph because VIA interpretation determines further management of the patient. Further, the intervention sequence has been clarified in the Methods section.

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Lines 192-204: Sociodemographic characteristics, eligibility and medical history of the participants (including self-reported HIV status) were obtained through a questionnaire completed with a midwife specifically trained for the study; and later electronically transcribed using the SecuTrial© software (Berlin, Germany). Women were then asked to provide a self-collected vaginal sample for HPV testing. In case of a positive result, participants were triaged by VIA/VILI. A trained midwife performed a Pap smear before application of Acetic Acid. In the end, endocervical brushing (ECB) and cervical biopsies were realized. Patients with a negative HPV test were discharged home without further investigations. All the cases (HPV positive women) were equally verified by the gold-standard (biopsies, ECB). Identified lesions were then treated by thermal ablation or large loop excision of the transformation zone (LLETZ) and the patient was discharged home after 30 minutes surveillance. More details on the *3T-study* have been previously published. [18-20] When no lesions were detected (negative VIA), HPV-positive women were followed up every 12 months, until clearance of the HPV infection.

Statistical analyses

Please, define which group you compare for their socio-demographic characteristics. I miss a clear list of the comparisons that you are going to present.

According to your proposal, a more detailed list of socio-demographic characteristics described in both groups has been added in the statistical analysis section, Line 308-312.

Public involvement

This study is a typical example needing women involvement to better value the outcomes. I think the statement should be revised.

We didn't involve patients or the public in the design, or conduct, or reporting, or dissemination plans of the research. However our study design and procedures were based on a previous pilot study conducted in the same setting which was well accepted by patients and health-care providers (Kunckler M et al. Cervical cancer screening in a low-resource setting: a pilot study on an HPV-based screen-and-treat approach. Cancer Med. 2017 Jul;6(7):1752-1761. doi: 10.1002/cam4.1089).
Page 6, Line 263-266.

Results

Please check percentages (99.7% should be 99.5%).

Thank you for drawing our attention to this mistake, which has now been corrected Page 7, Line 306.

Table 4 could report also the performance of referring only pool-1 and all HPV-pos.

The requested statistics have been added to Table 4. Also, the table has been modified to include only cases with valid histological results to ease interpretation.

Discussion

The first paragraph repeats something already stated in the background.

According to your suggestion, the first paragraph of the discussion has been removed (redundant).

In the second paragraph: extended genotyping is available with several commercial tests, not only with Xpert.

We mentioned in our article that there are other commercial tests that can genotype HPV (Line 361, Page 9). However, GeneXpert is particularly suitable for Africa because the results are available quickly, and the machine that performs it can easily be set up in low-resource settings allowing treatment on the same day as the first consultation.

Third paragraph: please check the specificity values.

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Thank you for your comment. Indeed, the specificity values in the 3rd paragraph of the results section did not agree with those in Table 3. The necessary changes have been made.

Fourth paragraph: the sentence stating that specificity may be affected by prevalence of disease is not correct. In fact, in the case of typing, if we assume other characteristics, mainly previous screening history and the duration of the infections, not changing, type-specific positive predictive value is almost constant and specificity of a pool of types is completely determined by the mix of types in that population. For a demonstration of the constant PPV and variable specificity, and consequently the inverse application of the Bayes theorem in the case HPV, see Giorgi Rossi et al, Int J Cancer 2012.
Dear Dr. Rossi, thank you for this comment. The reference did indeed clarify the way the factors which influence the specificity of a test. However, we do not understand the changes you want us to make in the manuscript.

Page 9, fourth paragraph: authors state that VIA should exclude the presence of cancer and eligibility for thermal ablation, but in this study, VIA missed 2 out of 3 cancers. The authors should highlight this point.
Page 9, fourth paragraph: we have specified in the text that VIA has missed 2 cancer diagnoses. Line 389-391.

Conclusions
It is worth mentioning that the study has been performed on self-sampling.
This clarification has been added in our conclusion, Line 447-449.

VERSION 2 – REVIEW

REVIEWER	Helenius, Gisela Örebro University Örebro Life Science Center, Medical Sciences
REVIEW RETURNED	05-Jul-2022

GENERAL COMMENTS	Page 7 row 296: avoid to use a subjective pronoun In the discussion, page 11, row 459, you're referring to figure 1. I can't find the figure.

REVIEWER	Giorgi Rossi, Paolo AUSL Reggio Emilia, Servizio Interaziendale Epidemiologia
REVIEW RETURNED	16-Jul-2022

GENERAL COMMENTS	The paper presents interesting data useful to define the best Test Triage and Treat strategy in Cameroon. Abstract I suggest also including the detection rate and false positive rate using the total screened women or the total HPV-positive women as a denominator. The ratio CIN2+/treatment is a good measure, but the proportion of false positive treatment out of positive (1-PPV) is not intuitive and uncommon. Summary, third bullet point: see the comment to the abstract: proportion of overtreatment among positive to the triage test is not a good measure of harm of screening. Methods

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	I did not understand where the women collected the self-sample and when and who vortexed the vials, could you better explain? Results The text describing table 2 and table 3 is redundant. All the information is simply determined by the 2X2 accuracy table. Table 4. If you want to measure the undesirable effect of the screening algorithm, the measure of overtreatment should have HPV-positive women or all the screened women, using the triage-positive women, the measure becomes a measure of efficiency. In fact, in the discussion, you use the rate of treatment and over-treatment on the whole screened population. Discussion Pag 11 first line: I suggest avoiding the term “real life data” since this is an experimental study, furthermore, distinguishing real life and non-real life in pragmatic trials is not useful, just describe what is “as would be in routine practice” and what was different due to the study (i.e. informed consent, biopsy for all, etc.)

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Dr. Gisela Helenius, Örebro University Örebro Life Science Center

Comments to the Author:

Page 7 row 296: avoid to use a subjective pronoun

The corrections have been made in Page 7.

In the discussion, page 11, row 459, you're referring to figure 1. I can't find the figure.

Figure 1 was uploaded as a separate file, as requested in the author submission guidelines.

Reviewer: 3

Dr. Paolo Giorgi Rossi, AUSL Reggio Emilia

Comments to the Author:

The paper presents interesting data useful to define the best 3T-strategy in Cameroon.

Abstract

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I suggest also including the detection rate and false positive rate using the total screened women or the total HPV-positive women as a denominator. The ratio CIN2+/treatment is a good measure, but the proportion of false positive treatment out of positive (1-PPV) is not intuitive and uncommon.

We have added a column in Table 4 with the overtreatment rate calculated as recommended, using all the screened women (with valid triage and histological results for HPV-positive women) as a denominator. We also have renamed the original column « Proportion of false positive triage ».

The corresponding changes have been made in the Abstract.

Summary, third bullet point: see the comment to the abstract: proportion of overtreatment among positive to the triage test is not a good measure of harm of screening.

The third bullet point has been modified as well in accordance with the changes in Table 4 described above.

Methods

I did not understand where the women collected the self-sample and when, and who vortexed the vials, could you better explain?

Women were asked to provide a self-collected vaginal sample for HPV testing (Lines 195-196, Page 5). A technician then rinsed the swab in a vial with 20 ml of saline solution (sodium chloride 0.9%) and vortexed for 30 seconds (Lines 207-209, Page 5).

Results

The text describing table 2 and table 3 is redundant. All the information is simply determined by the 2X2 accuracy table.

We would prefer to keep the text describing the results presented in Tables 2 and 3, as it highlights the results that we consider most relevant.

Table 4. If you want to measure the undesirable effect of the screening algorithm, the measure of overtreatment should have HPV-positive women or all the screened women, using the triage-positive women, the measure becomes a measure of efficiency. In fact, in the discussion, you use the rate of treatment and over-treatment on the whole screened population.

We have added a column in Table 4 with the overtreatment rate calculated as requested, using all the screened women (with valid triage and histological results for HPV-positive women) as a denominator. We have also renamed the original column « Proportion of false positive triage ».

Discussion

Pag 11 first line: I suggest avoiding the term “real life data” since this is an experimental study. Furthermore, distinguishing real life and non-real life in pragmatic trials is not useful.

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“Real-life data” was removed from the text, as suggested (Page 3 Bullet points and Page 9, Line 360).

Just describe what is “as would be in routine practice” and what was different due to the study (i.e. informed consent, biopsy for all, etc.)

The same modification was made on Page 10, Line 427.

VERSION 3 – REVIEW

REVIEWER	Giorgi Rossi, Paolo AUSL Reggio Emilia, Servizio Interaziendale Epidemiologia
REVIEW RETURNED	29-Sep-2022

GENERAL COMMENTS	Abstract Clear and informative. Some minor points: lines 70 and 71: “of those” it is not clear if of the HPV positive or of the CIN2+. Specificity is a universally used parameter, but in triage it does not help too much, better to report the overall positivity to each triage test, then you give also the false positive, specificity is fully determined if you have total positive, true positives, and false positives (actually this is even redundant). Bullet points Lines 96-97: there is something unclear: what are the percentages in brackets? They are not detection rates (detected cases/screened) if this is a sensitivity estimate, it does not present any comparison with the VIA, but the sentence states “improves…”. The sentence should be re-worded. Background Well written and clear. Methods Ascertainment bias? It is not clear to me if histology on LEETZ has been used to assess the outcome or only ECB were used. If also histological results from the LEETZ are used, cases VIA-positive have a different (more accurate) assessment of the outcome. Please be explicit about which histological results have been used to assess the outcome. Results The subheading “histology results by triage…” is redundant and not easy to follow. The table is self-explaining. You can briefly report the positivity for each test. The post hoc power analysis is questionable. In any case I think you should report the minimal clinically interesting difference for each outcome, otherwise I do not understand what the power is. Discussion Line 348: please drop “such as” actually you compare only to VIA and cytology (not applicable in a one-step strategy), not other traditional methods.

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	Lines 369-372: HIV status and CD4 also change the relative proportion of HPV types in high grade lesions with more types at low oncogenic potential observed in HIV-positive women, this point is more relevant for your findings than the generic observation of decreased specificity of HPV in HIV-positive women that is simply o consequence of the higher prevalence of HPV infections in HIV-positive women, in fact, HPV specificity is inversely associated (almost perfectly) with HPV prevalence (Giorgi Rossi et al Int J Cancer 2012). Conclusions are balanced and well linked to results.

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