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ACCURACY OF SEPT9 GENE HYPERMETHYLATION IN THE DIAGNOSIS OF PRECANCEROUS CERVICAL LESIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ADMINISTRATIVE INFORMATION

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Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 21 October 2025 and was last updated on 21 October 2025.

INTRODUCTION

Review question / Objective Is SEPT9 gene hypermethylation a biomarker for the diagnosis of precancerous cervical lesions?

Rationale Currently, although screening tests for cervical cancer (CC) generally show satisfactory results, there are important limitations. Among them are low reproducibility, sensitivity, and specificity, which may vary depending on the test, even when properly performed. Furthermore, current methods are not able to predict the real likelihood of HPV infection progressing to CC, resulting in unnecessary invasive procedures that can directly impact patients' future gynecological health.

In this context, studies on specific biomarkers are needed to enable the early diagnosis of lesions whose final outcome would be CC. The present study is a systematic review that will assess the accuracy of SEPT9 gene hypermethylation as a biomarker for the diagnosis of precancerous cervical lesions. It is expected that hypermethylation of this gene will prove to be more effective in detecting lesions that may actually progress to CC compared to current screening tests, with greater specificity and sensitivity.

Condition being studied Cervical cancer (CC) is one of the most prevalent malignant neoplasms among women worldwide and represents a major public health concern, particularly in developing countries. The primary cause of CC is persistent infection with the human papillomavirus (HPV), a sexually transmitted virus capable of inducing genetic and epigenetic alterations in cervical epithelial cells. Although most HPV infections are transient and eliminated by the immune system, a subset of women develop precursor lesions, known as cervical intraepithelial neoplasia (CIN), which can progress to invasive cancer if not detected and treated early.

Currently, screening methods such as cytology (Pap smear) and HPV DNA testing have contributed to reducing CC mortality. However,

they present limitations related to sensitivity, specificity, and their inability to predict the true risk of HPV infection progressing to carcinoma. In this context, SEPT9 gene hypermethylation has emerged as a potential epigenetic biomarker for the diagnosis of precancerous cervical lesions, as it alters gene expression and is involved in carcinogenic mechanisms. Investigating the diagnostic accuracy of this marker may contribute to improving screening and early detection programs, reducing unnecessary invasive procedures, and optimizing the identification of clinically significant lesions.

METHODS

Search strategy Systematic searches will be conducted in PubMed, Embase, LILACS, and the Cochrane Central Register of Controlled Trials for studies carried out in any country, limited to human studies and with no restrictions on language or year of publication. The following search strategy will be applied based on the Medical Subject Headings (MeSH) terms:
(SEPT9) OR (SEPT9 protein, human) OR (MLL septin-like fusion protein, human) OR (MSF protein, human) OR (MSF septin-like protein, human) OR (MSF-A protein, human) OR (Ov-Br septin protein, human) OR (septin 9 protein, human) OR (septin D1 protein, human) OR (SEPT-9) AND (Cervical Neoplasm, Uterine) OR (Neoplasm, Uterine Cervical) OR (Uterine Cervical Neoplasm) OR (Neoplasms, Cervix) OR (Cervix Neoplasm) OR (Neoplasm, Cervix) OR (Cervix Neoplasms) OR (Cervical Neoplasms) OR (Cervical Neoplasm) OR (Neoplasms, Cervical) OR (Cancer of the Uterine Cervix) OR (Cancer of Cervix) OR (Cancer of the Cervix) OR (Cervix Cancer) OR (Cancer, Cervix) OR (Uterine Cervical Cancer) OR (Cancer, Uterine Cervical) OR (Cervical Cancer, Uterine) OR (Uterine Cervical Cancers) OR (Cervical Cancer) OR (Cancer, Cervical) OR (Cervical Cancers).

Data extraction will be performed by the authors, with the primary outcome of interest being the association between SEPT9 gene hypermethylation and cervical cancer in women. In addition, it will be verified whether systematic reviews on this topic already exist and their respective publication dates.

Participant or population This review will include studies involving women aged 18 years or older who have been evaluated for cervical intraepithelial neoplasia (CIN) or cervical cancer, regardless of geographic location, ethnicity, or clinical setting. Eligible participants may include those with histologically confirmed cervical lesions, classified as low-grade (CIN 1) or high-grade (CIN 2/3), as well as women diagnosed with invasive cervical carcinoma.

Control groups will consist of women aged 18 years or older without cervical lesions or with normal cytology/histology results. Studies including samples from cervical tissue, cervical scrapings, or exfoliated cells will be considered, provided that the analysis of SEPT9 gene methylation status was performed using validated molecular techniques such as methylation-specific PCR (MSP), quantitative MSP (qMSP), or next-generation sequencing (NGS).

No restrictions will be applied regarding HPV status, menopausal status, or comorbidities, as long as the data allow the assessment of the relationship between SEPT9 hypermethylation and the presence of precancerous or cancerous cervical lesions.

Intervention The intervention (or exposure) of interest in this review is the detection of SEPT9 gene hypermethylation in biological samples obtained from women aged 18 years or older who have been screened for cervical lesions or cervical cancer. The studies must have performed epigenetic analysis of the SEPT9 gene using validated molecular techniques such as methylation-specific PCR (MSP), quantitative methylation-specific PCR (qMSP), bisulfite sequencing, or next-generation sequencing (NGS).

The focus of the review is to evaluate the diagnostic accuracy of SEPT9 hypermethylation in identifying precancerous cervical lesions (CIN 1–3) and invasive cervical carcinoma. The review will consider studies that compare the methylation status of SEPT9 between women with histologically confirmed cervical lesions and control groups without lesions, in order to determine its potential as a biomarker for early detection and screening of cervical cancer.

Comparator The comparator group will consist of women aged 18 years or older without cervical lesions, confirmed by normal cytology or histopathology results. These participants will serve as the control group for comparison with women presenting cervical intraepithelial neoplasia (CIN 1–3) or invasive cervical cancer.

Studies comparing SEPT9 gene methylation levels between women with and without cervical lesions will be included. In some studies, other diagnostic or screening methods, such as HPV DNA testing, Pap smear cytology, or colposcopy, may also

serve as secondary comparators to evaluate the relative diagnostic performance of SEPT9 hypermethylation in detecting precancerous or cancerous cervical lesions.

Study designs to be included We will include diagnostic accuracy studies evaluating SEPT9 hypermethylation: cross-sectional, case-control, cohort (including screening-based), and prospective studies reporting sensitivity/specificity against a histopathological gold standard (or confirmed cytology/colposcopy). Case reports, case series, reviews, and editorials will be excluded.

Eligibility criteria Based on the studies identified during the literature search, a screening process will be conducted independently by two reviewers (GSC and NSB) using the software Rayyan (https://rayyan.qcri.org/). These reviewers will read the titles and abstracts of all studies retrieved from each database and, in a blinded manner, select those that fit the scope of the review for full-text assessment. A third reviewer (GSP) will be consulted to resolve any conflicts regarding study inclusion.

After the initial screening, the selected articles will be read in full by the two reviewers (GSC and NSB). This stage will determine the final inclusion or exclusion of each study in the systematic review, based on the pre-established inclusion criteria defined by the PICO framework. In cases of disagreement, the third reviewer (GSP) will again be responsible for resolving conflicts.

Thus, at this stage, studies evaluating SEPT9 hypermethylation in cervical cancer, using histopathology as the reference standard, will be included. Finally, studies such as review articles, letters to the editor, or those involving interventions that may influence the analysis of this biomarker's hypermethylation will be excluded.

Information sources The information sources for this systematic review will include electronic databases such as PubMed, Embase, LILACS, and the Cochrane Central Register of Controlled Trials (CENTRAL). Additional sources will include the reference lists of relevant articles, as well as manual searches of review articles and meta-analyses related to cervical cancer and epigenetic biomarkers.

The review will also consider gray literature, including conference abstracts, theses, and dissertations, to minimize publication bias. When necessary, authors of the primary studies will be contacted to obtain missing or unpublished data.

All searches will be performed without restrictions on language or publication year, and the complete search strategy will be based on the Medical Subject Headings (MeSH) and Emtree terms for the SEPT9 gene and cervical neoplasms.

Main outcome(s) The primary outcome of this systematic review will be to evaluate the diagnostic accuracy of SEPT9 gene hypermethylation for detecting precancerous cervical lesions (CIN 1–3) and invasive cervical cancer in women aged 18 years or older.

The main measures of effect will include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic odds ratio (DOR), and area under the receiver operating characteristic curve (AUC). When possible, 95% confidence intervals (CIs) will be calculated for all effect measures.

Data will be extracted from eligible studies and synthesized quantitatively through meta-analysis using random- or fixed-effects models, depending on the degree of heterogeneity (I^2 statistic). Subgroup analyses may be conducted according to type of lesion (CIN vs. invasive cancer), population characteristics, and molecular detection technique (e.g., MSP, qMSP, NGS).

The overall goal is to determine whether SEPT9 hypermethylation can serve as a reliable biomarker for early detection and screening of cervical cancer, potentially improving diagnostic accuracy compared to conventional screening methods such as Pap smear and HPV DNA testing.

Quality assessment / Risk of bias analysis All articles selected for the study based on the eligibility criteria will be assessed for their methodological quality using QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies). This evaluation involves analyzing the clinical design of the study and relevant data regarding the study population, the diagnostic test, and the reference standard.

Strategy of data synthesis Data analysis will be performed using quantitative synthesis (meta-analysis) when sufficient homogeneous data are available. The main effect measures will include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic odds ratio (DOR), and area under the ROC curve

(AUC), each reported with 95% confidence intervals (CI).

A 2×2 contingency table (true positives, false positives, true negatives, false negatives) will be constructed for each study to calculate the diagnostic accuracy metrics. Heterogeneity among studies will be assessed using the I^2 statistic and the Chi-square test. Depending on the level of heterogeneity, either fixed-effects or random-effects models will be applied. If meta-analysis is not feasible, a narrative synthesis will be presented, summarizing key findings, methodological characteristics, and sources of bias.

Subgroup analyses may be conducted based on lesion grade (CIN 1–3 vs. invasive cancer), detection technique (MSP, qMSP, NGS), or population characteristics. Sensitivity analyses will assess the influence of individual studies on overall results.

Publication bias will be examined through Deeks' funnel plot asymmetry test and visual inspection of funnel plots when at least 10 studies are included.

All analyses will be conducted using Review Manager (RevMan) and MetaDTA STATA software to generate forest plots and summary ROC curves, ensuring transparency and reproducibility of the synthesis process.

Subgroup analysis Subgroup analyses will be conducted to explore potential sources of heterogeneity and to assess whether diagnostic performance varies across different study characteristics. The following subgroups are planned:

Type of cervical lesion: comparison between studies evaluating precancerous lesions (CIN 1–3) and those assessing invasive cervical cancer.

Detection method: studies using different molecular techniques, such as methylation-specific PCR (MSP), quantitative MSP (qMSP), bisulfite sequencing, or next-generation sequencing (NGS), will be compared to determine whether the diagnostic accuracy of SEPT9 hypermethylation differs by method.

Sample type: comparison between studies analyzing cervical tissue biopsies, cervical scrapings, or exfoliated cells to identify potential differences in methylation detection depending on the biological material used.

Geographic region: subgroup analysis by continent or country (e.g., Asia, Europe, Latin America) to evaluate possible regional variations related to population characteristics, HPV prevalence, and laboratory practices.

Study design: differentiation between case-control, cross-sectional, and prospective cohort studies to assess their influence on diagnostic accuracy estimates.

If sufficient data are available, additional subgroup analyses will be conducted to evaluate the impact of HPV status, age group, or menopausal status on the relationship between SEPT9 hypermethylation and cervical lesions.

The statistical significance of differences between subgroups will be tested using meta-regression analysis or interaction tests, depending on the available data.

Sensitivity analysis A sensitivity analysis will be conducted to assess the robustness and stability of the meta-analysis results, verifying whether the overall conclusions remain consistent after excluding specific studies or varying the inclusion criteria.

Initially, sensitivity analyses will be performed through sequential removal of each individual study (leave-one-out analysis) to observe the impact on the pooled estimates of diagnostic accuracy (sensitivity, specificity, and AUC). This approach will help identify influential studies that may distort overall results.

Additional analyses will include:

Methodological quality of studies, based on the QUADAS-2 assessment, comparing results with and without inclusion of studies classified as having a high risk of bias;

Sample size, to determine whether studies with small populations significantly affect pooled estimates;

Molecular technique used (MSP, qMSP, NGS), analyzing the influence of methodology on result stability;

Statistical model applied, comparing fixed-effects and random-effects models to evaluate the consistency of the estimates.

If significant differences are detected between analyses, the results will be discussed in detail,

highlighting potential sources of heterogeneity and limitations of the included studies.

Sensitivity analyses will be performed using RevMan and MetaDTA software, ensuring full traceability and reproducibility of all stages of the statistical synthesis.

Language restriction There will be no language restriction.

Country(ies) involved The study is being conducted in Brazil, with all authors affiliated with the University of the Extreme South of Santa Catarina (UNESC), Criciúma, Santa Catarina, Brazil.

Keywords Septin 9; Cervical neoplasms; Cervical cancer; Tumor biomarkers; Screening.

Contributions of each author

Author 1 - Gabriele Coelho - Author 1 contributed to the conceptualization and design of the project, development of the search strategy, and drafting of the initial version of the protocol. The author also participated in defining the inclusion criteria and initiating the literature search.
Email: gabrielescoelho@unesc.net
Author 2 - Nayara Batista - Author 2 contributed to the refinement of the research question, review of the search strategy, and drafting of the methodological section of the protocol. The author also collaborated in defining the eligibility criteria and selecting the databases for the literature search.
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Author 3 - Gabriele Prestes - Author 3 coordinated the development of the project, supervised all stages of protocol preparation, and provided critical review and guidance throughout the methodological design. The author also approved the final version of the protocol and will oversee the subsequent stages of the systematic review.
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