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PAX1 methylation as an auxiliary biomarker for cervical cancer screening: A meta-analysis

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ABSTRACT

Objective: Several studies have implicated PAX1 epigenetic regulation in cervical neoplasia. The aim of this meta-analysis was to assess PAX1 gene methylation as a potential biomarker in cervical cancer screening.

Methods: A systematical search of all major databases was performed, in order to include all relevant publications in English until December 31st 2014. Studies with insufficient data, conducted in experimental models or associated with other comorbidities were excluded from the meta-analysis. Summary receiver operating characteristics (SROC) for Cervical Intraepithelial Neoplasia grade 2 or worse ($CIN2^+$) versus normal, and CIN grade 3 or worse ($CIN3^+$) versus normal, were estimated using the bivariate model.

Results: Out of the 20 initially included studies, finally 7 (comprising of 1385 subjects with various stages of CIN and normal cervical pathology) met the inclusion criteria. The sensitivity of $CIN2^+$ versus normal was estimated to be 0.66 (CI 95%, 0.46-0.81) and the specificity 0.92 (CI 95%, 0.88-0.95). On the other hand, the sensitivity of $CIN3^+$ versus normal was 0.77 (CI 95%, 0.58-0.89) and the specificity 0.92 (CI 95%, 0.88-0.94). Moreover, the area under the curve (AUC) in the former case was 0.923, and in the latter 0.931.

Conclusion: The results of this meta-analysis support the utility of PAX1 methylation as an auxiliary biomarker in cervical cancer screening. PAX1 could be used effectively to increase the specificity of HPV DNA by detecting women with more advanced cervical abnormalities.

1. Introduction

Despite the significant reduction in cervical cancer incidence and mortality, this gynecological malignancy is responsible for approximately 528.000 new cases and 266.000 global deaths, on an annual basis [1]. The burden is more pronounced in developing countries, due to lacking healthcare infrastructure, inequitable medical costs and secondary prevention failures [2–4]. The introduction of the Pap-test as a screening method has helped save millions of women's lives over the course of years [5]. More recently, the discovery of Human Papilloma Virus (HPV) as the primary risk factor for cervical intraepithelial neoplasia, has not only enabled the development of a more accurate diagnostic tool based on the molecular detection of HPV-DNA, but also led to the elaboration of the HPV vaccine as a means of immunological protection against the most oncogenic forms of this virus [6–9].

HPV-DNA testing may be preferred as a primary cervical cancer screening tool over traditional cytology, due to higher sensitivity (especially for cervical adenocarcinoma), rapid result generation and automatization [10,11]. Nevertheless, the transient nature of age-dependent prevalence of HPV infection suggests that the overall specificity and/or positive predictive value can be compromised [12–14]. Only a minute fraction of HPV infections actually progress to cervical neoplasia. This may lead to overwhelming concern, particularly among younger women, and warrant excess referrals for colposcopy, further increasing medical and healthcare costs [15].

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Despite the fact that HPV is a pre-requisite for the development of cervical cancer, a battery of yet unidentified cellular factors and molecular pathways are involved in disease propagation and clinical manifestation [16]. On-going research is investigating these pathophysiological mechanisms, in order to reveal potential new targets for pharmacological intervention and/or prognostic biomarkers for implementation in everyday clinical practice.

An interesting possibility arises from the field of epigenetics. Aberrant DNA methylation and histone modifications constitute hallmarks of neoplastic disease [17,18]. Hypermethylation of CpG island promoters can silence genes whose normal physiological role is to suppress tumour growth by controlling DNA repair, survival and apoptosis, and hence assist tissue hyperplasia and metastasis. Understanding how these processes work at the molecular level and unraveling the role of complex regulatory networks that promote epigenetic carcinogenesis is a fundamental aspect of modern translational research.

Several candidates genes have been studied in cervical cancer pathophysiology, including paired boxed gene 1 (PAX1), sex-determining region Y (SRY)-box 1 (SOX1), LIM homeobox transcription factor 1α (LMX1A) and death-associated protein kinase (DAPK1) [19–21]. The methylation of PAX1, in particular, has been extensively documented as a possible target for the detection of cervical intraepithelial neoplasia (CIN) at grade 3 or worse (CIN3$^+$), with variable outcomes and endpoints [22–26].

Here, we present a meta-analysis of the diagnostic test accuracy of PAX1 methylation and provide conclusive data from eligible studies. The meta-analytical sensitivity and specificity was estimated from these studies, along with the associated test performance, concerning the CIN2$^+$ versus normal and CIN3$^+$ versus normal diagnostic capacity. The potential of using PAX1 methylation as part of a triage protocol to improve cervical cancer screening is also discussed.

2. Materials and methods

2.1. Study selection

The PubMed, Web of Science, Cochrane Library, Science Direct and Embade were systematically searched by two authors (CN and EN), using the following keywords: [“methylation” or “DNA methylation” or “hypermethylation” or “hypomethylation” or “demethylation”] and [“cancer of the cervix” or “cervical cancer” or “cervical dysplasia”] and [“Paired box PAX1” or PAX1”] to identify appropriate studies published in English, before December 31st 2014. In addition, the reference lists of all identified studies were manually searched to identify any additional studies.

2.2. Inclusion/exclusion criteria

To be eligible for inclusion, studies had to utilize PAX1 methylation as a biomarker for the detection of Cervical Intraepithelial Neoplasia (CIN). Studies were excluded if they were not conducted in human subjects; were associated with other types of malignancies and/or morbidities; and did not provide sufficient data to perform calculations for true positive (TP), false positive (FP), true negative (TN) and false negative (FN) results.

2.3. Data extraction and methodological assessment

Two reviewers (CN and EN) independently extracted the following data from each study: first author, year of publication, study population characteristics (number of patients included and tumour stage), and positive or negative result for PAX1 gene methylation status. Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) guidelines were used to assess the methodological quality of each study. This tool comprises of four domains, namely patient selection, index test, reference standard, and flow and timing. Each domain is assessed in terms of risk of bias and concerns regarding applicability [27].

2.4. Statistical analysis

The meta-analysis was performed in line with recommendations from the Cochrane Collaboration [28]. The bivariate model was used to calculate the sensitivity and specificity of pooled data [29]. This approach allows estimation of the correlation between sensitivity and specificity. Confidence and prediction regions were computed using standard error calculations and logistic transformations. Summary receiver operating characteristic (SROC) plots were used to display the results of individual studies in ROC space, where each study represents a single sensitivity/specificity reference point. A Monte Carlo Markov Chain (MCMC) was used to generate positive and negative likelihood ratio (PLR, NLR) and diagnostic odds ratio (DOR) from the bivariate model [30]. All statistical analyses where conducted in R, using the “metafor” and “mada” software packages.

3. Results

3.1. Characteristics of included studies

A systematic literature search based on pre-defined criteria, initially yielded a total of 20 studies (Fig. 1). One duplicate study was removed, and nine studies were excluded due to irrelevant content (i.e. they were associated with other type of malignancies and/or morbidities, such as oral squamous cell carcinoma, head and neck cancer, ovarian cancer and primary hyperthyroidism). Moreover, three studies were excluded due to study design inconsistencies that did not allow calculation of PAX1 methylation status per CIN stage [19,22,25]. The baseline characteristics of included studies, corresponding to a total population of 1385 individuals, are presented in Table 1 [23,24,26,31–34]. Normal cervix morphology was reported in 521 (37.6%) women and cervical dysplasia at various stages (CIN 1,2 or 3) in 864 (62.4%). The sample size of eligible studies ranged from 73 to 346 individuals. As

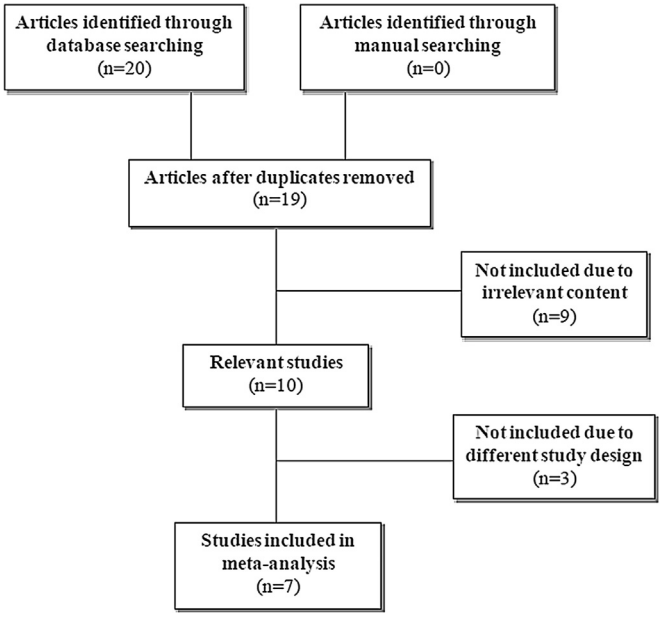

Fig. 1. Meta-analysis flow chart.

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