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HOT TOPIC

p16^{INK4a} immunostaining in cytological and histological specimens from the uterine cervix: A systematic review and meta-analysis

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SUMMARY

Background: P16^{INK4a} is a biomarker for transforming HPV infections that could act as an adjunct to current cytological and histological assessment of cervical smears and biopsies, allowing the identification of those women with ambiguous results that require referral to colposcopy and potentially treatment.
Material and methods: We conducted a systematic review of all studies that evaluated the use of p16^{INK4a} in cytological or histological specimens from the uterine cervix. We also estimated the mean proportion of samples that were positive for p16^{INK4a} in cytology and histology, stratified by the grade of the lesion.
Results: Sixty-one studies were included. The proportion of cervical smears overexpressing p16^{INK4a} increased with the severity of cytological abnormality. Among normal smears, only 12% (95% CI: 7–17%) were positive for the biomarker compared to 45% of ASCUS and LSIL (95% CI: 35–54% and 37–57%, respectively) and 89% of HSIL smears (95% CI: 84–95%). Similarly, in histology only 2% of normal biopsies (95% CI: 0.4–30%) and 38% of CIN1 (95% CI: 23–53%) showed diffuse staining for p16^{INK4a} compared to 68% of CIN2 (95% CI: 44–92%) and 82% of CIN3 (95% CI: 72–92%).
Conclusion: Although there is good evidence that p16^{INK4a} immunostaining correlates with the severity of cytological/histological abnormalities, the reproducibility is limited due to insufficiently standardized interpretation of the immunostaining. Therefore, a consensus needs to be reached regarding the evaluation of p16^{INK4a} staining and the biomarker needs to be assessed in various clinical settings addressing specific clinical questions.

Introduction

Since the Papanicolaou (Pap test) cytological screening for cervical precancerous lesions was introduced in the 1940s, there has been a significant reduction in the incidence and mortality from cervical cancer.[1] However, the efficacy of the Pap test is hampered by high interobserver variability and high false negative and false positive rates that range between 20–30%[2] and 5–70%[3], respectively. Technical improvements of the Pap test such as the liquid based cytology (LBC) have not been shown to improve sensitivity or specificity for detection of high-grade cervical intraepithelial neoplasia (CIN) compared to the conventional cytology.[4]

The introduction of human papillomavirus (HPV) DNA testing in clinical practice raised hopes for further improvements in the efficacy of the primary screening, triage and post-treatment surveillance. Randomized clinical trials published recently have demonstrated that HPV testing can be efficiently integrated into primary screening, either as an adjunct to cytology or as a sole primary test.[5,6] It has also been shown that HPV DNA testing can be used to triage women with equivocal cytological abnormalities[7] and that it has a potential role in identifying women at risk of residual or recurrent disease after treatment for CIN.[8,9] However, it fails in the triage of low-grade lesions[9] and even if implemented as a primary screening test, it would be necessary to have a more disease specific triage marker to identify women that would need to undergo colposcopy. Furthermore, a single HPV DNA test although it could confirm infection by the virus, present in 99% of all cervical cancers[10], it does not discriminate between transient and chronic infection. The discrimination between the two types of infection is crucial as it is the persistent infection that predisposes to progression to cervical neoplasia and not the transient one.[11]

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Even the histological assessment of cervical biopsies that is often considered as the "gold standard", can be significantly hampered by intra- and inter-observer variability.[12] Novel markers applied on histological specimens could improve the identification of women with ambiguous results that require treatment.

Research nowadays is focused on the development of objective biomarkers that can distinguish transforming from productive HPV infections and predict disease severity. The cellular tumor suppressor protein $p16^{INK4a}$ (p16) has been identified as a biomarker for transforming HPV infections. Physiologically, p16 blocks the activity of cyclin-dependent kinases CDK4/6. In a transforming HPV infection the viral oncogenes E6 and E7 interfere substantially with apoptosis and cell cycle regulation. Most importantly, E7 disrupts the protein of retinoblastoma (pRb) from its binding to E2F transcription factor and thereby promotes cell cycle progression, a molecular switch that is usually activated by CDK4/6. Affected cells strongly express p16 to counteract the irregular cell cycle activation; however, since E2F is not released through CDK4/6 action, but by E7, p16 expression has no effect on cell cycle activation. Over time, p16 accumulates in the nucleus and cytoplasm of affected cells and can be detected by immunostaining.[13]

This review represents an attempt to collect, systematically present and analyse the existing evidence on possible clinical applications of p16 in cytological and histological samples from the uterine cervix.

Material and methods

Search strategy

We searched two electronic databases – MEDLINE and EMBASE – targeting reports published between January 1998 and September 2007. The search strategy used terms such as "cancer", "dysplasia", "SIL", "CIN", "cervix", "p16" and "cyclin-dependent kinase". The references of retrieved articles together with the proceedings of relevant conferences were hand-searched in order to identify other potentially eligible studies for inclusion in the analysis missed by the initial search or any unpublished data. Additional cross-searches were performed in MEDLINE using the names of investigators who were the lead authors of at least one eligible study.

The literature search, assessment of inclusion and exclusion criteria, quality of studies and extraction of data were independently undertaken and verified by two investigators (IT, MK). The results were then compared and, in case of discrepancies, a consensus was reached with the involvement of a third investigator (MA). There was no language restriction.

Type of studies, inclusion and exclusion criteria

All retrospective or prospective studies that assessed p16 immunostaining in cervical cytological samples, in conventional cytology or in LBC, as well as in histological specimens from the uterine cervix were included in this review. We evaluated all methods and interpretation of p16 immunostaining.

We excluded studies that assessed the expression of the biomarker in glandular or invasive cervical lesions. In cases of overlap or duplicate studies, we retained only the most comprehensive one.

Types of outcome measures

All outcomes were defined prior to the literature search. The primary outcome was the correlation between cytological or histological degree of cervical abnormality and overexpression of p16 identified by immunochemistry. Other parameters assessed were the role of the biomarker in the cervical cancer screening, its role in the triage of equivocal or low-grade cytological abnormalities compared to HPV testing and its efficacy as a marker of progression risk in low grade cervical lesions.

Data extraction and statistical analyses

For all included studies we generated descriptive tables for population and study characteristics. We recorded the first author, publication year, country of the investigators, sample size and interventions. Furthermore, we described the method of p16 immunostaining, the type of antibody and medium used and the various interpretations of p16 immunoreactivity as adopted by each author.

We applied the 1991 Bethesda reporting system (TBS91) for the cytological classification[14] as this was the system adopted by the majority of the studies (18 versus 9 studies). Three cytological groups were considered, i.e. atypical squamous cells of undetermined significance (ASCUS), low-grade (LSIL) and high-grade squamous intra-epithelial lesions (HSIL). If the cytological abnormalities were presented in different reporting formats, they were converted into TBS91 using published standard translation tables.[15] The CIN nomenclature was applied in order to describe histological outcomes.[16]

Statistical analysis was performed using STATA (Stata Corp., College Station, Texas, US). Random effects models were used to pool proportions for pooling[17] and analysis and interstudy heterogeneity was assessed with the Cochran's Q test.[18] In the meta-analysis of cytological studies we adopted the cut-off of p16 positivity proposed by each author. For the analysis of histological studies we included in the meta-analytic pool only those that either adopted the distribution of staining proposed by Klaes and colleagues[19] or could be converted to the above classification system. According to the Klaes classification the diffuse staining (>25% of cells stained for p16) was considered as the cut-off of positivity.

Results

The electronic search yielded 584 studies that were assessed for inclusion in the review. Of those, 97 were potentially eligible and subsequently scrutinized in full text (Fig. 1).

Excluded studies

Amongst the relevant studies, thirty-six failed to meet the inclusion criteria and were excluded from this overview. Nine of the studies assessed p16 immunostaining in glandular[20–28] and twenty-one in invasive cervical lesions only.[29–49] Another six studies represented duplicate reports and were subsequently excluded[50–55] (Fig. 1).

Included studies

Sixty-one studies qualified for the overview. Twenty-seven assessed the p16 immunoreactivity in cytological specimens[56–81] and in six amongst them[56,58,59,66,70,72] the biomarker was assessed in histological specimens as well, whereas 34 studies assessed staining only in histological samples.[19,83–115] The characteristics of included studies are presented in Tables 1 and 2 respectively.

In cytology, the immunostaining for p16 was performed in selected series of smears of different degrees of cytological abnormality. Cervical smears were selected at random from a screening population in only one study[64]; however only those