

CAP-ASCCP LAST Work Group 4: Molecular Markers for Histopathology

Background

WG4 was tasked with assessing the use of molecular markers in conjunction with morphology in the assessment of specimens from the lower anogenital tract. In doing so, over 2000 articles were identified from the literature search. Using pre-specified criteria and following a systematic title/abstract and full text review process, this number was culled to 50 from which complete data extraction was performed. The vast majority of the articles dealt with cervical disease, however some articles did address disease of vulvar/penile and anal sites. Of the selected literature, prospective studies and those having histologic adjudication as a gold standard were given more weight. A formal and more rigorous evidence grading process is currently in progress, and will be finalized prior to the March 2012 conference.

Clinical Validity

The literature and expert review process was directed toward evaluating and selecting the best science for the best possible patient care, regardless of costs. In this regard, the committee is highly cognizant of the interplay between medicine and industry in the published literature. Just as historically, the utility of HPV testing in the context of cytology screening and triage is critically tied to the concept of the performance characteristics of what is or is not a clinically valid HPV test, in the WG's opinion, similar concepts absolutely apply for the biomarker based tests evaluated. Based on these considerations, the clinical utility of p16 immunohistochemistry as proposed by WG4 is directly related to the literature performance characteristics of a particular clone and in some cases IHC kits, that yield defined characteristic staining patterns in consensus adjudicated diagnostic categories. For example, >99% of histologic CIN 3 are p16 positive. In contrast, <5% of histologically NILM biopsies are p16 positive, and many of such positive "NILM" cases, in retrospect, contain small missed lesional areas. Use of alternative clones, kits, etc. mandates analytical and clinical validation to insure similar clinical performance. Similar clinical validation concepts would apply to any other potential biomarker (e.g. ProEx C, ki-67, etc.) with similarly developed, albeit somewhat different, marker-specific criteria.

Key Questions for WG4

KQ1 What (if any) are the molecular markers reported on in the lower anogenital tract literature, and when should they be used for lower anogenital lesions? The specific focus of the WG for this over-arching question, was on utility for use in histologic specimens as an aid to differential diagnosis or with potential consideration as being definitional of the patient's biologic state as clarified in the subsequent key questions

- **The potential biomarkers evaluated based on the broadest possible literature selection process were the following: p16, Ki67, ProEx C, L1, HPV 16/18 mRNA, telomerase/TERC and HPV genotyping**

KQ2 Is any marker ready for primetime use, meaning it should be used commonly and reliably to refine diagnostic issues?

- **p16 is the only marker with sufficient data available to allow an analysis for use in lower anogenital tract specimens. ProEx C and Ki67 have similar trending data, but their accumulated data are insufficient to make an independent recommendation for use, alone or in combination (see below).**
- **Although only a few studies are available that focus on body sites other than cervix, all of these studies show results similar to cervix, and hence, given the underlying similarities in HPV-associated biology in all lower anogenital tract sites, WG4 concludes that the recommendations and summary below are applicable across all lower anogenital tract sites.**

Note: These data and recommendations do not apply to non HPV-related precancerous lesions (e.g. simplex VIN)

Recommendations

Recommendation #1: p16 IHC is recommended when the hematoxylin and eosin (H&E) morphologic differential diagnosis is between precancer (CIN 2 or CIN 3) and a mimic of precancer (e.g., processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting, etc.). Block positive* p16 results support a categorization of precancerous disease.

Recommendation #2: If the pathologist is entertaining an H&E morphologic interpretation of CIN 2 (under the old terminology; which is a

biologically equivocal lesion falling between the morphologic changes of HPV infection (low grade lesion) and precancer, p16 IHC is recommended to help clarify the interpretation. Block positive p16 results support a categorization of precancerous disease. Negative or non-block positive staining strongly favors an interpretation of low-grade disease or a non-HPV associated pathology.

Note: p16 should not be used if the H&E morphologic differential diagnosis is between low grade disease (CIN 1) and negative, as p16 positivity is not definitional for CIN (of any level). Further, for histologic uncertainty between CIN 1 & CIN 2 see below.

Note: If the pathologist's histologic diagnosis is "obvious" CIN 1, the working group does not recommend further immunohistochemistry. As noted below in KQ6, there is insufficient evidence to determine whether there is a difference in the natural history between p16 positive and p16 negative CIN 1. Hence, at the present time, it is recommended that clinical management of CIN1 be based on the histologic diagnosis alone.

Rationale/Evidence: In the largest prospective, adjudicated study using p16, Galgano et al (2111) showed that strong block staining with p16 showed similar accuracy for high-grade disease when compared to an adjudicated histology result. Given that CIN 2 has been repetitively proven to be a poorly reproducible category, p16 immunostaining would improve the accuracy of single pathologist interpretations of high grade vs. low grade disease relative to adjudicated pathology panel interpretations which are the best surrogate we have for biologic accuracy (again, with the caveat that the pathologist was already entertaining an interpretation of CIN 2). Hence, adding a p16 result to the H&E morphologic assessment leads to a more accurate prediction of the risk of the patient for having a precancerous lesion. Additional studies have demonstrated a strong positive correlation between p16 block staining and precancerous disease (1528, 1994, 1425, 1276, 1183, 112) and p16 staining is found to substantially reduce interobserver variability in diagnosis of precancerous disease, see below (890, 1425, 663, 594, 2111). Studies also show that p16 block staining is highly associated with a positive test for HPV 16 (or other hrHPVs)(1229, 589, 189).

REFIDS: 2111, 1528, 1994, 1425, 1183, 1276, 1229, 890, 663, 594, 589, 189, 112

***Block staining for p16 = p16 positive:** *In squamous epithelia, this is defined as strong continuous nuclear or more usually nuclear plus cytoplasmic*

*staining from the basal cell layer and extending upwards at least 1/3 of the epithelial thickness. The latter is admittedly somewhat arbitrary but adds specificity. Note that extension up to the surface or upper third or upper half is specifically **not** required to call a specimen positive.*

All other staining patterns, described as focal, patchy, blob-like, puddled, scattered, single cells, etc. are defined as negative.

*Clearly the concept of continuous block staining requires "adequate" tissue size and orientation and should correlate with the area of morphologic concern. Small fragments, tangential cuts, free-floating single cells, etc. may lead to more subjective and variable interpretations, but in such cases the minimum would be that all cells in question are strongly stained **and** morphologically are already under consideration in the differential diagnosis of a precancerous lesion.*

KQ3 What can be done to reduce interobserver variability in the interpretation of lower anogenital lesions based on molecular markers?

Recommendation #3: p16 IHC is recommended for use as an adjudication tool for cases in which there is a professional disagreement in histologic specimen interpretation, with the caveat that the differential diagnosis includes a precancerous lesion (CIN 2 or 3).

Rationale/Evidence: A number of studies address the issue of interobserver variability in interpretation of lower anogenital tract squamous lesions (890, 1425, 663, 594, 2111). These studies all show that there is substantial improvement in correlation between observers when p16 immunostaining is utilized. Therefore, in association with recommendation #1 above, the addition of p16 provides a more objective adjudication of the differential diagnosis than does H&E histologic assessment alone:

REFIDS: 890, 1425, 663, 594, 2111

KQ4 Regarding the problem of missing important lesional pathology during slide screening, does the weight of evidence support marker utility, particularly for increased lesion finding sensitivity, and if so should it be used on all specimens or just those where the pathologist is struggling with a differential diagnosis?

Recommendation #4: p16 IHC is recommended as an adjunct to histologic assessment of specimens at significant risk for actually having a precancerous lesion. Examples include a biopsy(s) interpreted as \leq CIN1

following a Pap test of HSIL or ASC-H , or similarly after a predictive screening test marker such as a positive HPV 16/18 genotyping result.

Rationale: Based on the high sensitivity of p16 for precancerous lesions, areas of small or equivocal high-grade disease have been identified on histologic specimens using p16, which were not readily identifiable on H&E sections alone. In a "high risk" situation (i.e. prior high grade PAP (HSIL, ASC-H) or prior biopsy (CIN 2 or 3) or potentially individuals with HPV 16 who have undergone colposcopy), p16 positive areas are most likely to represent precancerous disease.

REFIDS: 2111, 798

KQ5 What are the recommendations to clarify the histologic terminology, based on molecular marker input (in conjunction with WG 2 and WG 3)?

- **Based on the evidence reviewed, WG4 cannot make a recommendation for or against a 2-tier or 3-tier nomenclature system based on histologic evaluation alone. However, the work group does note that while all the marker studies examined were neutral or supportive of a two tier system/biology, we could find no positive marker based studies to support a distinct 3 tier biology.**

Note: Because of the lack of evidence for a biologically defined intermediate category, p16, as noted above, is recommended to clarify any considered intermediate category (CIN 2) into either a low grade or precancerous lesion (Recommendation #2). Therefore, use of p16 may effectively support the use of a 2-tier classification system in this particular circumstance.

KQ6 For low grade versus precancerous disease (CIN1 vs. CIN 2/3), will any marker positivity be definitional for precancer?

- **Based on the available data, WG4 concludes that there is insufficient evidence to prospectively make a determination of high vs. low-grade disease based solely on a p16 result. In particular, the natural history of CIN1 adjudicated by p16 is uncertain and critically needs further study. Hence, at present, no recommendation can be made for or against the use of p16 for this purpose.**

Note: As noted above, p16 has utility in making this distinction only in the circumstance in which a precancer (CIN 2 or 3) is under consideration based on the H&E morphology.

Note: If the pathologist's histologic diagnosis is "obvious" CIN 1, the working group does not recommend further immunohistochemistry.

KQ7 In making a determination of CIN 1 vs. no CIN, does p16 perform in supporting a diagnosis of any CIN?

- **WG4 concludes that there is insufficient evidence to prospectively make a determination of CIN 1 vs. no CIN based solely on the use of p16. While block positive p16 staining in the appropriate morphologic context strongly favors CIN, a negative stain does not exclude a majority of CIN 1 or a minority of CIN 2/3. Thus, at present, no recommendation can be made for or against the use of p16 for this purpose. Hence, p16 should not be used to screen morphologically negative or CIN 1 H&E histology specimens to determine the presence of absence of CIN.**

KQ8 Are there any prognostic markers of value, and if so, what are they?

a) Does low-grade disease (CIN 1) with p16 staining (positive or negative) need to be managed differently from current practice?

- **WG4 concluded that no recommendation regarding any differences in CIN1 management (based on the addition of a p16 stain) could be made at this time. There are 2 articles that provide data regarding this question (1250, 723). In both studies, the presence of block positive p16 immunostaining in CIN 1 lesions was associated with increased "progression" or precancer outcomes on followup. Conversely, those cases testing negative for p16 were far more likely to regress on followup. However, this association is not absolute as there were cases that progressed which were p16 negative and visa versa. Therefore at this time, although p16 positive CIN 1 lesions may represent a subgroup of cases which are at higher risk of progression, no management recommendation can be made based solely on a p16 result.**

REFIDS: 1250, 723

b) Should lesions classified morphologically as CIN 3 that are marker negative be managed differently?

- **WG4 can make no recommendation regarding any management differences in morphologically determined high-grade dysplasia (CIN 3) based solely on the addition of a p16 result. However we note that the vast majority of adjudicated CIN 3 lesions are p16 positive (>99%).**

Note: p16 staining is not recommended for use in cases interpreted as CIN 3 on H&E morphologic evaluation

REFIDS: 2111

KQ9 For those studies involving multiple markers, is a combination of markers equivalent or better than a single marker?

- **WG4 concludes that the evidence does not support any combination of markers to substantially improve performance when compared to the use of p16 alone.**

KQ10 Based on the recommendations, what are the differences that affect clinical management that the clinicians need to understand?

- **The addition of p16 staining provides a more accurate (but not perfect) assessment of the risk of having a precancerous lesion, when compared to an equivocal and therefore only possibly precancerous lesion (CIN 2 or precancer mimic) as determined by single pathologist H&E histologic examination alone. In this specific histologic/clinical scenario, management will be driven by the p16 result as p16 block positive cases will be categorized as precancerous lesions (see WG2 recommendations).**

Final Note: WG4 did not conduct a true cost effective benefit analysis of biomarkers usage in histologic specimens.

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