Question 6

For women with biopsy confirmed CIN2 what is the safety and effectiveness of p16 immunohistochemistry and treating only p16 positive CIN2 cases while conservatively managing p16 negative CIN2 cases when compared with treating all CIN2 cases?

Search terms: cervical intraepithelial neoplasia, CIN2, grade 2, high grade neoplasia, HG neoplasia, p16, cyclin-dependent kinase inhibitor p16, CDK, CDKI. Search conducted from 2011-current.

Results: No articles were found that could directly address the question. The following articles may provide some relevant information.

Author	Country	Methods		Findings
p16 to predict CIN2 progression				
Miralpeix et al, 2015	Spain	Prospective cohort	102 patients newly diagnosed with CIN2 by cervical biopsy, mean age 30y (range 18-56y), followed-up every 4m for 2y. p16 analysed in all biopsies. HPV status was reported at baseline.	The rate of spontaneous regression at 12 months was 65.7%, while 7.8% progressed and 26.5% had a persistent disease. Regression was observed in all cases that were p16-negative and 56.8% that were p16-positive (P=0.001). The authors concluded that use of p16 to predict CIN2 regression would have a great clinical value and could reduce unnecessary cone excision. (**Limited information – conference abstract**).
Omori et al, 2007	Japan	Retrospective cohort	52 cases histologically diagnosed with CIN2 from colposcopic biopsies were retrospectively analysed by p16 immunohistochemistry and hr-HPV in situ hybridization signal types Followed up with cytology and colposcopy every 3-4 months for a mean of 45.5 months. CIN2 considered regressed if dysplastic cells not found on at least 2 consecutive follow-up visits	Progression to CIN3 6 0f 6 cases with strong p16 staining 4 of 8 cases with moderate p16 staining 2 of 31 cases with weak p16 staining 1 of 7 cases with negative p16 staining In 10/11 cases of CIN2 progressed to CIN3 when both a moderate to strong immunoexpression of p16 and a punctate hr-HPV signal were observed at the same time. When strong immunoexpression of p16 was observed or only a punctate hr-HPV signal was detected 9/12 cases progressed and 3/12 persisted but none regressed. The authors stated that combining p16 overexpression with hr-HPV punctate signal at initial diagnosis may enable prediction of progression of CIN2 cases although due to small numbers, findings need to be confirmed by larger studies.
p16 to detect/distinguish CIN2				
Bergeron et al, 2010	Germany and France	12 community based pathologists provided independent diagnoses on a set of 500 H&E stained slides (254 cervical punch and 246 cone biopsies). The community-based		When p16 stained slides were added and conjunctively interpreted with the H&E-stained slides, a significant increase in diagnostic accuracy for the detection of high-grade CIN was seen (P =0.0004). Sensitivity for high-grade

		diagnoses of these biopsies were chosen from a total of 550 samples constrained to include ≥200 negative, ≥100 CIN1, ≥100 CIN2 and ≥100 CIN3. Results were compared with a dichotomized "gold standard" established by 3 expert gynaecopathologists. Immunostaining for p16 was performed on the same specimens and the resulting slides were provided in addition to the H&E stained slides to the community based pathologists for a 2nd review. They were blinded to both their original diagnoses and the diagnoses of the expert gynaecopathologists.	CIN increased by 13%, cutting the rate of false-negative results by half. Agreement of community-based pathologists in diagnosing high-grade CIN was significantly improved (mean κ values advanced from 0.566 to 0.749; P <.0001). The highest level of improvement in diagnoses was achieved in the CIN2 category. Based on H&E slides alone, community-based pathologists identified only 340 cases. When p16 slides were reviewed together with the H&E slides, community-based pathologists' diagnoses of CIN2 cases increased to 447. The majority of the re-diagnosed 107 cases of CIN2 were previously diagnosed cases of CIN1 and negatives.
Galgano et al, 2010	US	A community- and population-based evaluation was conducted on consecutive cervical biopsies submitted to Pathology at the University of Virginia during a period of 14 months. Thin-sections of each biopsy from 1451 biopsies (755 negative, 451 CIN1, 147 CIN2, 92 CIN3/AIS and 6 cancer according to community diagnosis) were evaluated by immunohistochemical stains for three biomarkers, including p16. Original diagnosis was masked, and results were compared to an adjudicated, consensus diagnosis by 3 pathologists. All biopsies were fixed in formalin.	The 147 histology samples originally classified as CIN 2 based on the community diagnosis were classified as 6 negative, 23 CIN1, 70 CIN2, and 48 CIN3/AIS (none as cancer) by the consensus panel review. Data were not available in sufficient detail from the paper to determine how p16 results from the 147 community CIN2 histology diagnoses corresponded to those which were considered as <CIN2, CIN2, or >CIN2 according to the consensus panel. Therefore it was not possible to ascertain whether p16 immunostaining would have helped to identify those samples originally classified as CIN 2 which were determined by consensus to be <CIN2 (N=29), CIN 2 (N=70) or CIN3+ (N=48). While not directly relevant to this question, results were reported for the sensitivity and specificity of p16 compared to a consensus panel gold standard across the full set of biopsies (ie including those classified as both <CIN2 and >CIN2 according to the community diagnosis). Across the full set of biopsies, p16 immunostaining, using the strongest staining as the cutpoint, was 86.7% sensitive and 82.8% specific for CIN2/CIN2+ diagnoses but not useful for distinguishing CIN1 from non-CIN. 77% of CIN2 and 99.2% of CIN3/AIS specimens scored the highest staining for p16. The p16 performance was more sensitive ($p < 0.001$), less specific ($p < 0.001$), and of similar overall accuracy for CIN2+ compared to the combined performance of all pathologist reviews in routine clinical diagnostic service (sensitivity = 68.9%, specificity = 97.2%). A second review on a random subset of immunohistochemical stained slides (across the full set of biopsies) was undertaken to assess the reproducibility of grading using the p16 immunohistochemical staining score of 3 as the positive cutpoint, the raw agreement was 95.1% and kappa was 0.87. The authors found immunohistochemical staining for p16 to be a useful and reliable diagnostic adjunct for distinguishing biopsies with and without

			CIN2+. Note that estimates of specificity across the full set should be interpreted with caution as they may not be directly applicable to potential use specifically in the context of resolving CIN2 histology.
Dray et al, 2005	Australia	188 consecutive and unselected cervical biopsies collected prospectively were sectioned and examined by H&E and immunostained for p16. The clinical context, results of concurrent Papanicolaou smears/ ThinPrep slides and Digene hybrid capture tests for high-risk HPV subtypes, as well as follow-up cervical smears/ThinPrep, biopsies and loop excisions of transformation zones or cone biopsies were all correlated with the morphological and immunohistochemical findings.	Diffuse strong parabasal immunostaining for p16, suggestive of integrated high-risk HPV DNA into the host genome, was observed in 81 biopsies and correlated (>90%) with HGSIL in the H&E sections. 56/81 had been initially regarded as exhibiting features consistent with a HGSIL, 15 as displaying a LGSIL and the remaining 10 showing a range of 'nondysplastic' or reactive changes. On review of 25 cases where discordant results were noted between the H&E appearances and expected p16 immunostaining, 20 cases were considered to display cellular changes justifying an upgraded diagnosis. Thus finally 73/81 biopsies with intense p16 staining showed a HGSIL. The remaining 7 showed a LGSIL and 1 failed to show any convincing evidence of dysplasia. Focal and weaker superficial p16 immunostaining, suggestive of episomal HPV infection, was noted in 19 biopsies (10%) and these biopsies exhibited a range of histological changes but predominantly LGSIL. 1/19 was a HGSIL. 89/189 biopsy specimens had a negative immunostaining pattern for p16. Of these, 5 had been initially regarded as having HGSIL features, 8 as displaying a LGSIL and the remaining 76 showing a range of 'non-dysplastic' or reactive changes. The 13 cases in which the H&E features and immunostains were 'discordant' were reviewed in light of the p16 findings. 2/5 HGSIL cases and 1/8 LGSIL cases were considered to display cellular changes justifying a downgrade of diagnosis to non-dysplastic. On the basis of this study, the authors stated that strong high-risk pattern of p16 immunostaining is a reliable surrogate marker for HGSIL and potentially progressive disease. It can be used to confirm a HGSIL and to identify histologically obscure or focal severe dysplasia. Conversely, they found the test sufficiently robust that a complete absence of staining can be used to eliminate an associated HGSIL.
p16 to improve inter-observer agreement			
Horn et al, 2008	Germany	Cervical punch biopsies were retrieved from 250 consecutive archived cases from 2003 from Pathology/University of Leipzig and 249 consecutive cone biopsies from the Institute of Pathology/ Manheim. Sections were taken from paraffin blocks. Slides were stained by H&E and separately for p16. 3 expert	Based on consensus diagnosis by the 3 expert gynecopathologists, 247 punch biopsies were categorised as: 147 nondysplastic; 43 as CIN1, 17 as CIN2, 35 as CIN3 and 5 and invasive carcinomas. 249 cone biopsies comprised of 84 nondysplastic tissues, 14 CIN1 lesions, 21 CIN2 lesions, 123 CIN3 lesions and 7 invasive carcinomas. Separate results were not presented for each type of CIN. In general, when using a p16-stained slide in conjunction to the H&E-stained slide, inter-observer agreement between 6 pathologists improved

		pathologists established a consensus diagnosis for each H&E stained slide. 6 certified pathologists performed independent diagnostic interpretation of the H&E slides in 1 review and after a washout period the same pathologists reviewed the same slides together with p16 stained slides and negative reagent slides.	significantly for both cervical punch and cone biopsies (P<0.001). For punch biopsies, k value increased from 0.49 (moderate agreement) to 0.64 indicating substantial agreement, and inter-observer agreement for cone biopsies improved from 0.63 (conventional H&E slide reading) to 0.70 when H&E-stained slides when read conjunctively with p16-stained slides. In comparison to a common consensus diagnosis established by 3 independent experts, 4 pathologists reached an improvement with the conjunctive p16 test, 2 of them showing significantly better agreement. The authors concluded that p16 staining as an adjunct to H&E-stained specimens contributes to a more reproducible diagnosis of CIN.

Abbreviations: CIN: cervical intraepithelial neoplasia; H&E- hematoxylin and eosin; FU: follow-up

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