Q & A, 3/13

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Q. What is the recommended use of p16 immunostaining as an adjunct diagnostic biomarker in HPV-associated lesions of the lower anogenital tract?

A. Recommendations for the use of p16 were published recently by the LAST Project, cosponsored by the CAP and the American Society for Colposcopy and Cervical Pathology (ASCCP).[1] The LAST Project recommended standardized terminology for all lower anogenital tract biopsies with noninvasive squamous pathology to follow the Bethesda System abbreviations of LSIL (low-grade squamous intraepithelial lesion) and HSIL (high-grade squamous intraepithelial lesion) in place of previously used mild-moderate-severe dysplasia or intraepithelial neoplasia grade 1, 2, or 3 (–IN 1–3). The lower anogenital tract (LAT) includes cervix, vagina, vulva, anus, perianal area, penis, and scrotum. The LAST Project also recognized p16 as a biomarker for E6/E7 oncogene activation in all HPV-related precancerous squamous lesions of the LAT. Overexpression of p16, as detected by immunohistochemistry, serves as a surrogate marker for cell-cycle dysregulation, a key step in HPV-mediated carcinogenesis.[1,2] Additionally, Work Group 4 of the project issued specific recommendations for p16 to be used as an adjunct to standard morphology. When combined with the two-tiered grading system, this approach significantly improves diagnostic accuracy of the pathologic diagnosis of precancer[1,3,4] and optimizes therapeutic management of patients for better clinical outcomes.[1,5]

The summary of the LAST Project consensus recommendations, including p16 biomarker recommendations, is available at the CAP Web site: www.cap.org/apps/docs/membership/transformation/new/asccp_sum_last_recom.pdf.

Briefly, p16 staining is recommended whenever there is:

- Differential diagnosis between precancer (HSIL) and precancer mimics.
- Disagreement in interpretation of precancer.
- High risk for missing precancer (high-risk cytology with negative/LSIL biopsy findings).
- H&E morphologic pattern of –IN 2. This recommendation has proved to reduce the equivocal and poorly reproducible –IN 2 diagnostic category.[3,4] Positive p16 staining supports classifying –IN 2 as definitive HSIL.
- Staining for p16 is not recommended for biopsies that are negative or show unequivocal LSIL or HSIL (–IN 3).

Positive p16 staining is defined as strong and diffuse (continuous nuclear or nuclear and cytoplasmic) staining of the basal cell layer that involves at least the lower third of the epithelial thickness with or without full-thickness extension.[1-4] p16 should be used in conjunction with standard morphologic diagnosis and not as a replacement.

Other biomarkers of viral transformation (for example, ProEx C, Ki-67) may be helpful in especially challenging cases, but they add no additional value to p16 staining.[1]

Application of p16 immunocytochemistry in triage of squamous atypia in cervical cytology remains controversial. Procedural and interpretation differences as well as a lack of standardized protocols make the analysis challenging.[6,7] Recent meta-analysis from 17 studies in which p16 was used to predict HSIL (CIN 2+) showed higher specificities in ASC-US and LSIL groups with loss of sensitivity in LSIL category when compared with HPV testing.[7] However, no consensus exists for cytologic application of p16 in standard clinical practice, pending further studies.

The use of p16 immunohistochemical staining when combined with other markers may help to distinguish endocervical from endometrial adenocarcinoma, though serous carcinomas can also be positive.[8-10] Strong diffuse p16 staining is usually observed in HPV-associated preinvasive lesions of the endocervix (endocervical dysplasia and adenocarcinoma in situ) but not in benign mimics (tubal and squamous metaplasia), which may show focal or patchy staining.[10]

A p16 immunostain should always be interpreted in conjunction with morphologic findings, using previously validated antibodies and methods.

References

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Q. We have validated that our centrifuge produces platelet-poor plasma (<10 × 109/L) for coagulation samples at a time of 10 minutes. The Clinical and Laboratory Standards Institute (CLSI) recommendation is 1,500g for no less than 15 minutes. Is the time and speed centrifuge-specific? Have studies been performed on StatSpins? Can StatSpin-type centrifuges be used to spin coagulation samples?

A. CLSI document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline—5th edition," as correctly pointed out, provides a recommendation for the relative centrifugal force (RCF) and time of centrifugation necessary to obtain sodium citrate platelet-poor plasma (PPP) samples.1 PPP is generally defined as post-centrifugation plasma that contains less than 10 × 109/L platelets. Specifically, H21-A5 states that the most common condition under which to obtain PPP is 1,500g for no less than 15 minutes at room temperature, but that centrifugal speed and duration must be established by each laboratory. The RCF and duration required to consistently produce PPP will vary depending on the brand and model of centrifuge used. This is because RCF is dependent on the speed (revolutions per minute, or RPM) and distance of the specimen from the axis, or the rotating radius. Furthermore, to prevent remixing of plasma and reintroduction of cellular elements, it is recommended that a swing-out bucket (angle) rotor be used and that the brake not be applied at the end of centrifugation.

It has been documented, however, that routine coagulation assays, such as APTT, PT/INR, and thrombin time, are not affected by platelet counts up to 200 × 109/L (200,000/µL) when testing is performed on fresh samples.[2,3] Shorter centrifuge times at 1,500g therefore are acceptable for routine coagulation assays, if testing is performed on fresh samples immediately post-centrifugation and only when there are no subsequent test requirements, thereby ensuring that plasma will not be frozen or processed for additional assays.[4]

Another means to reduce the time needed for centrifugation, but still achieve PPP, is to increase the RCF. Using centrifugal forces greater than 1,500g is generally discouraged as this may induce platelet activation and lysis of red blood cells.[4] To the contrary, a number of studies have reported no adverse effect on routine coagulation testing, such as APTT, PT, and fibrinogen, if centrifuged at high speed (for example, 11,000g) for short (for example, two-minute) durations.[5,6] H21-A5 also states that higher-speed and shorter-duration centrifuges (also known as "Statfuge") can be used as long as speed and duration of centrifugation are tested to determine optimum conditions for producing PPP.1 It has been cautioned, however, that samples spun in this manner should be tested within about 10 minutes if sampled from the primary tube or promptly aliquoted to a secondary tube, to prevent the drift of platelets, which cling to the side of the tube at high RCF, back into the plasma.[7] Also of potential relevance here is the recommendation from the International Society on Thrombosis and Haemostasis Scientific Standardisation Committee on Lupus Anticoagulants (LA) that samples destined for LA testing after freezing be first processed by double centrifugation, with recommended speeds of 2,000g and ">2,500g," respectively.[8]

In summary, the most important considerations are that any deviation from recommended practice such as CLSI1 should be validated by the individual laboratory and that PPP should contain less than 10 × 10^9/L platelets if the

sample is not tested immediately and instead needs to be frozen for subsequent testing (for routine coagulation and specialized hemostasis tests).

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