Letters to the Editor

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: 30% Versus 10% Cutoff for Immunohistochemistry

To the Editor.—We read with interest the letter to the editor by Nielsen and colleagues regarding the recently reported new guidelines for human epidermal growth factor receptor 2 (HER-2) testing in breast cancer as developed by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) expert panel, and we agree that the recommendations are very important to ensure test accuracy and standardization among different laboratories.

The main issue Nielsen et al appear to have with these guidelines is that 30% of tumor cells (rather than 10%) are now required to show uniform strong membranous staining by immunohistochemistry (IHC) for a tumor to be considered positive for HER-2 overexpression (3+). With their main argument being that “it is not clear whether there are data that support this new cutoff.”

The ASCO/CAP panel argument makes a strong case for using this higher cutoff; however, we have to agree with Nielsen et al that up until now, there were no studies to our knowledge that directly compared results of HER-2 IHC using different cutoffs. Using a series of 98 cases of breast cancer, we have shown that using a 30% cutoff increased both the specificity of HER2 immunohistochemistry in breast cancer.

The authors have no relevant financial interest in the products or companies described in this article.

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Lesions of Ductal Morphology in the Prostate

To the Editor.—During the last few decades, lesions with ductal morphology in the prostate have raised considerable controversy. This includes the distinction of a subset of lumens-spanning neoplastic proliferations within distended prostatic ducts from the more common forms of high-grade prostatic intraepithelial neoplasia (eg, those with tufted, micropapillary, and flat architecture) and the separate categorization of ductal adenocarcinoma of the prostate. In their recent authoritative review of the subject, 4 eminent urogenital pathologists make a proposal on the reporting of these lesions in pathologic practice. Strikingly, the outcome of their analysis is largely in line with another review on the same topic. Both reviews recommend reporting intraductal carcinoma as a separate entity based on (1) the feasibility of defining histopathologic criteria that allow its distinction from high-grade prostatic intraepithelial neoplasia and adenocarcinoma and (2) its unfavorable clinical impact as compared to high-grade prostatic intraepithelial neoplasia. Furthermore, both reviews propose to include intraductal carcinoma identified in a prostate biopsy specimen in the Gleason score (as grade 4 or 5) in the rare event that prostate core biopsy specimens contain grade 3 carcinoma associated with intraductal carcinoma, resulting in at least a Gleason score 7. Although Gleason himself failed to separate intraductal carcinoma (cribriform or comedo-type) from invasive carcinoma, the recognition of intraductal carcinoma would not impact the Gleason grading system.

The review by Cohen et al is somewhat contradictory with regard to their recommendation of repeat biopsies in the rare instance of an isolated intraductal carcinoma identified in prostate biopsy specimens. At one point the authors suggest that this finding should immediately lead to a radical prostatectomy without further exploration for the presence of invasive carcinoma. In their final recommendations, however, an early repeat biopsy is recommended. In our opinion the latter would be advised, as it seems that occasionally intraductal carcinoma may not be associated with invasive disease. It may otherwise be difficult to explain to the urologist and patient after radical prostatectomy that major surgery was performed for noninvasive disease.

A difference in opinion seems to emerge from the point of view of Cohen et al that ductal adenocarcinoma can be superseded by the unifying
In Reply.—Pickup and Van der Kwast propose that ductal adenocarcinoma of the prostate gland should be considered a variant of prostatic adenocarcinoma, rather than incorporated within the unifying classification of intraductal carcinomas as they believe we have suggested in our review article.1 We have carefully considered this important issue but stand by our position that any lesions with so-called ductal morphology (ie, lumenspanning neoplastic cells with papillary/trabecular, cribriform, or solid architecture) should be classified as: (1) intraductal carcinoma of the prostate (IDC-P) if surrounded by a complete or partial basal cell layer or (2) invasive adenocarcinoma if a basal cell layer is not detectable by immunostaining, assigned Gleason grade 3, 4, or 5 if comedonecrosis is present. Under our proposed system, invasive ductal adenocarcinoma, as defined by lesions with “ductal morphology” but no surrounding basal cell layer, would be simply classified as invasive adenocarcinoma, not IDC-P. Such cases may be recognized by some pathologists as a morphologic variant of prostatic adenocarcinoma, as with mucinous or clear-cell variants. However this “ductal” morphologic variant has not been associated with any prognostic implications independent of tumor Gleason grade or pathologic stage,2 and there is evidence that this term should no longer be used.3 We therefore see no reason to discuss it separately from other types of invasive prostatic adenocarcinoma. The focus of our article was on the identification, histologic reporting, and clinical implications of IDC-P, and the importance of distinguishing this lesion from high-grade prostatic intraepithelial neoplasia.

Pickup and Van der Kwast indicate that our original review article1 is contradictory with regard to our recommendations in rare cases where isolated IDC-P is detected in prostate biopsy specimens without associated invasive carcinoma. In our article we clearly explained that in such a case, we would recommend radical intervention (surgery or other) if IDC-P is recognized in biopsy material that demonstrates solid or cribriform tumor masses with comedonecrosis. However, if the IDC-P involved thin trabecular morphology, which can be difficult to distinguish from micro-papillary prostatic intraepithelial neoplasia, especially on limited biopsy material, then we recommended immediate repeat biopsy. However, in the conclusions of our article, it is true that we only summarized our recommendations as immediate repeat biopsy. We neglected to specify that this recommendation was only for trabecular/papillary IDC-P, while for IDC-P with cribriform or solid central masses and comedonecrosis this is unnecessary, and progression to radical therapy is recommended.

Finally, the authors of the letter also refer to the risk of performing radical surgery and finding no invasive elements if only IDC-P is seen in the material from the initial biopsy. They further refer to a published case report,4 for which one of us (R.J.C.) was first author, in support of their argument. This case4 documents a patient presenting with pure IDC-P on repeat biopsy without invasive elements who was then subjected to a radical prostatectomy. This represents the first such published case in the literature, confirming the rarity of such an event. Yet, despite the absence of adjacent stromal invasive elements, IDC-P had already spread transmucosally to the seminal vesicle, which is consistent with its aggressive behavior. In our opinion, further repeat biopsies and the subsequent delay in definitive therapy would not have been in the interest of this patient, and likewise for any patient with IDC-P.

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Anemia is typically characterized by a deficiency of red blood cells and/or hemoglobin. For adult men, a hemoglobin level less than 130 g/L (8.07 mmol/L) is diagnostic of anemia, whereas for adult women the diagnostic threshold is commonly less than 120 g/L (7.45 mmol/L). Thyroid hormones have a significant influence on erythropoiesis, in that various forms of anemia (normocytic, hypochromic-microcytic, or macrocytic) have been associated with declines in thyroid function. Anemia might also be encountered in hyperthyroidism and, when present, may be morphologically similar to that observed in hypothyroidism. Since it is now widely recognized that thyroid stimulating hormone (TSH) measurement is a sensitive test for detecting both hypothyroidism and hyperthyroidism, and this measurement is recommended as the first test for diagnosing thyroid dysfunction in ambulatory patients,4 we have evaluated the potential association between thyroid dysfunction and anemia, retrospectively analyzing the results of serum TSH and complete blood counts performed on consecutive female outpatients referred by general practitioners to our clinical laboratory for routine blood testing during the past year (June 2006 to June 2007).

Venous blood from outpatients was routinely collected in the morning on fasting subjects. Serum TSH was quantified by a third-generation assay (functional sensitivity of 0.01–0.02 mIU/L with an interassay imprecision of 20%) on the Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc, Deerfield, Ill). The reference range (0.2–2.5 mIU/L) was established in accordance with the practice guidelines issued by the Guidelines Committee of the National Academy of Clinical Biochemistry (NACB).4 The complete blood count, including hemoglobin and mean corpuscular volume (MCV) measurements, was performed on the ADVIA 120 (Bayer Diagnostics, Newbury, United Kingdom). Anemia was defined as a total hemoglobin concentration less than 120 g/L and was further classified as microcytic, normocytic, or macrocytic in the presence of MCV values of less than or equal to 80 fL, 80 to 100 fL, and greater than or equal to 100 fL, respectively.2 The significance of differences between groups was assessed by the Mann-Whitney U test (for continuous variables) or the chi-squared test (for categorical variables). Skewed variables were logarithmically transformed to improve normality prior to analysis. Statistical analyses were performed using the statistical package SPSS version 12.0 (SPSS, Chicago, Ill), and the level of statistical significance was always set at \( P < .05 \). Data are presented as means and 95% confidence intervals (CIs) or percentages.

Cumulative results for hemoglobin, MCV, and serum TSH levels were retrieved for 6534 female outpatients older than 15 years (mean age, 50 years; 95% CI, 19–85 years). The mean values (95% CI) of serum TSH, hemoglobin, and MCV were 1.46 mIU/L (0.03–11.4 mIU/L), 133 g/L (99–156 g/L), and 88 fL (69–101 fL), respectively. As compared with women with TSH values within the reference range, the mean hemoglobin value was significantly lower in subjects with abnormal TSH values (either decreased or increased), while the mean MCV value was significantly lower in women with TSH less than 0.2 mIU/L, but not in those with TSH greater than or equal to 2.5 mIU/L (see Table). Although the

| Baseline Characteristics, Hemoglobin, and Mean Corpuscular Volume (MCV) Values of the Study Participants (N = 6534), Grouped According to Thyroid Stimulating Hormone (TSH) Serum Levels |
|---|---|---|---|
| TSH (mIU/L) | 0.20–2.5 | <0.20 | >2.5 |
| n | 4426 | 120 | 1988 |
| Age, mean (95% CI), y | 50 (19–85) | 52 (26–86) | 50 (19–84) |
| Hemoglobin, mean (95% CI), g/L | 133 (98–156) | 131 (104–155) | .04 | 132 (99–155) | .03 |
| MCV, mean (95% CI), fL | 89 (71–100) | 86 (64–97) | .<.001 | 87 (67–102) | .27 |
| Anemia, No. (%) | 612 (13.8) | 19 (15.8) | .58 | 308 (15.5) | .64 |
| Microcytic | 268 (6.1) | 8 (6.6) | 135 (6.8) |
| Normocytic | 294 (6.6) | 11 (9.2) | .<.001 | 139 (7.0) | .95 |
| Macrocytic | 50 (1.1) | 0 (0) | 34 (1.7) |

* \( P \) values versus subjects with TSH values between 0.20 and 2.50 mIU/L.
overall prevalence of anemia (hemoglobin < 120 g/L) did not significantly differ throughout the spectrum of TSH thresholds, women with TSH less than 0.2 mIU/L had an increased prevalence of normocytic anemia and a decreased prevalence of macrocytic anemia, as compared to euthyroid women.

A growing pressure is being placed on healthcare systems and clinical laboratories to improve the appropriateness of diagnostic testing, which would ultimately decrease avoidable expenditures and reduce the potential adverse consequences on patients’ health from unnecessary testing. Pernicious anemia is currently included within the risk factors for developing thyroid dysfunction, which suggests that TSH screening might be worthwhile in these patients. On the other hand, although it has been reported that thyroid dysfunction might be associated with some forms of anemia, especially in childhood, the prevalence of this association in adults varies widely, which suggests that TSH screening laboratories can expect to receive more DSEK specimens. This letter describes 1 technique for preparing these thin, translucent specimens for sectioning.

DSEK, initially described for treatment of Fuchs endothelial corneal dystrophy and pseudophakic or aphakic bullous keratopathy, is rapidly increasing in popularity for the treatment of corneal endothelial dysfunctions. In this technique, a central, circular area of Descemet membrane is carefully loosened and stripped off the posterior corneal stroma with little or no attached stromal elements. This membrane may then be placed in fixative and submitted for histopathologic analysis. Specimens obtained in this manner are translucent, thin (20 ± 5 µm in 1 study), and often folded, leading to paraffin sections that show closely apposed, folded, and redundant tissue. Such specimens may appear invisible to the pathologist or technician in grossing, embedding, or sectioning. A folded specimen may preclude evaluation of whether the guttata, the diagnostic criterion, are central, suggesting Fuchs endothelial dystrophy, or peripheral, suggesting Hassel-Henle bodies (Figure 1).

To better visualize and process DSEK specimens, we instill 1 drop of Mercurochrome or eosin, added to the 10% formalin, and incubate the DSEK specimen for several hours. The specimen will appear orange (Figure 2) and can be easily visualized in water for gross examination. The thin membrane is floated onto an embedding paper such that it lays flat and unfolded. The orange dye remains after paraffin infiltration for visualization during embedding. The specimen can be easily bisected, embedded on the cut edge such that a cross section of the specimen can be seen on stained slides. In this manner, unfolded sections of DSEK specimens can be obtained for histopathologic evaluation (Figure 3).

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**Illuminating the Invisible Specimen: Descemet Membrane Endothelial Keratoplasty**

To the Editor.—With an increasing number of Descemet stripping endothelial keratoplasty (DSEK) procedures being performed, pathology laboratories can expect to receive more DSEK specimens. This letter describes 1 technique for preparing these thin, translucent specimens for sectioning.

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