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PRIVATE & CONFIDENTIAL

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Mr. Peter Dawe
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Dear Peter:

I am writing in follow up to discussions we've had in the past and our most recent discussion of April 27, 2006.

I certainly strongly support your endeavours to get the issue of hormone receptor testing on the national agenda with a view to improving the quality and reproducibility of these tests across the country. The results of these tests are being relied on as important factors in determining treatment for breast cancer patients.

I've attached a dossier of the literature that we have reviewed here in Eastern Health with respect to methodologies and outcomes with respect to estrogen and progesterone receptor testing. As you can see from other jurisdictions, the accuracy/reproducibility/reliability has been called into question. It would seem reasonable that there be some kind of mechanism put in place nationally to monitor labs from a quality perspective who provide this testing. I know there has been discussion within the ranks of the Canadian Association of Pathologists with respect to this matter, and further follow up, I hope, will take place at their national meetings which are coming up this summer. They are a national voluntary organization who are not in the position to mount any service with respect to quality review of these testing procedures, but I am sure would be willing to participate in discussions with a view to furthering the quality agenda in this respect.

I would also hope that discussions could take place in the Oncology community on this issue, as Oncologists need reliable testing to offer optimal treatment to patients with breast cancer. Also, the literature indicates that there are variable cut-off points with respect to when hormonal therapy will be offered depending on the results of ER and PR testing. I think this is another issue that needs national discussion to achieve consensus.

Any efforts your national association could offer as you move forward with a national cancer strategy to encompass enhancing quality in this important area should be pursued.

As I advised you, our two consultants have revisited the Province to review our efforts here, and once we receive their reports, we will be making a decision concerning reinstituting immunohistochemical testing within the Laboratory services here in St. John's. Before we reinstitute testing, we will be doing an update and briefing session, and I would like to extend to you an opportunity to be involved in that process.

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Again, thank you for your interest to date. You can be assured of Eastern Health's support in any further attempts you make in moving the hormone receptor testing forward in terms of quality and as part of any national cancer strategy for our country.

Yours sincerely,

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/dd

Enclosure

c Mr. George Tilley

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COMMENTS AND CONTROVERSIES

Estrogen Receptor Testing of Breast Cancer in Current Clinical Practice: What's the Question?

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Assessment of estrogen receptor (ER) status is an essential component of the evaluation of breast cancers. Although ER status provides prognostic information, currently the major clinical value of determining ER status is to assess the likelihood that a patient will respond to endocrine therapy.[1-3] The first ER assays used routinely in the clinical setting were radiolabeled ligand binding assays (LBAs), such as the dextran-coated charcoal method. Assays of this type had the disadvantage of requiring prospective collection of fresh tumor tissue, among other drawbacks,[3] but resulted in quantitative determination of ER content of the tumor, expressed as femtomoles of ER protein per milligram of cytosol protein. When determined using LBA, breast cancers were found to exhibit a broad range of values for ER, and the magnitude of the benefit from endocrine therapy was shown to be related to the quantity of ER protein in the tumor.[3-5]

During the last two decades, mammographic screening resulted in such a dramatic decrease in the size of the average breast cancer that it became impossible to collect tissue prospectively for ER determination by LBA in many cases because of the small tumor size. The availability of antibodies that recognize ER in formalin-fixed, paraffin-embedded (FFPE) tissue permitted the development of immunohistochemical assays to detect ER retrospectively in such specimens, and immunohistochemistry (IHC) in FFPE tissue gradually replaced LBA as the most common method for determination of ER status of breast cancers. Several studies have indicated that ER status as determined by IHC is not only predictive of response to endocrine therapy, but that the ability of ER status as determined by IHC to predict such responses is superior to that of ER status as determined by LBA.[6-8] Thus, the use of IHC to assess the ER status of breast cancers in paraffin sections is now a routine part of pathology practice worldwide.[2,9]

As a consequence of the experience with LBA, there had been (and still remains) the expectation that IHC assays for ER should result in a broad range of values among ER-positive patients, similar to that observed with LBA. However, this presupposes that there is a direct, linear relationship between the amount of ER protein present in the tumor cells and the amount of ER antigen detected by IHC. Although some studies have certainly suggested such a relationship,[8,10-13] others have not. For example, two recent studies that together included the analysis of almost 7,000 breast cancers found that the distribution of ER values using contemporary IHC methodology was essentially bimodal, with more than 90% of tumors being either completely ER negative or unequivocally and strongly ER positive.[14,15] These data clearly are at odds with the continuum of values observed when ER status was determined by LBA.[16,17] However, this may simply reflect the fact that IHC is not an intrinsically quantitative method, and that there is not a direct, linear relationship between the intensity and distribution of the chromogenic reaction product as determined by IHC and the amount of ER protein in breast cancer cell nuclei when highly sensitive anti-ER antibodies and detection systems are used on adequately fixed tissue samples.

In fact, the relationship between the actual quantity of ER protein in the tumor cell nuclei and the apparent amount of ER antigen demonstrated by IHC assays is highly complex and may be as much a function of preanalytic factors (such as details of tissue fixation and processing) and assay sensitivity as of the actual amount of antigen present in the tumor cells.[2,18-24] A number of recent studies that support this contention are particularly noteworthy. Rhodes et al,[21] in an analysis of data from 66 laboratories participating in a United Kingdom external quality assurance program, found that ER IHC staining results were highly affected by the efficiency of the antigen retrieval step, and that this was, in fact, the single most important factor contributing to interlaboratory reproducibility. Goldstein et al[22] noted that IHC staining results for ER were highly dependent on the time of tissue fixation. Using the assay employed in their laboratory, the minimum fixation time for optimal ER IHC staining results was 6 to 8 hours, regardless of specimen type or size. Of note in that study, underfixation had more of a detrimental effect on IHC staining results than did overfixation. Vassallo et al[24] performed IHC assays for ER on 20 invasive ductal carcinomas using two different anti-ER antibodies (1D5 and 6F11), two different antigen retrieval methods, and three different detection systems. Thus, for each patient, 12 different technical variations were studied. ER IHC results were scored semiquantitatively on a scale of 0 to 4. In five of these 20 patients, the ER scores varied from 0 to 4, and in three patients, the scores ranged from 1 to 4, depending on the assay conditions used. Moreover, there was not a single patient in which all 12 assay variations resulted in the same IHC staining score. Umemura et al[23] studied 44 breast cancers with a biochemical assay and with two different IHC assays, one considered by the authors to be "highly sensitive" and the other "nonhighly sensitive." The same anti-ER antibody (1D5) was used in both IHC procedures. These

authors found that the nonhighly sensitive procedure resulted in a broad distribution of ER staining, and that the correlation with the biochemical assay was linear. In contrast, the highly sensitive procedure resulted in an increase in both the proportion of positive tumor cells and staining intensity compared with the nonhighly sensitive procedure. This resulted in a shift of patients toward the higher end of ER positivity, and this in turn resulted in a nonlinear correlation with the biochemical assay.[23] Taken together, the results of these studies highlight the critical role of preanalytic factors and assay details in determining ER IHC results in any given population, and underscore the problems inherent in attempting to quantify ER by IHC.

So, what type of information is required from ER assays in current clinical practice? A consensus development panel of the US National Institutes of Health recommended in 2001 that any ER staining in breast cancers should be sufficient to consider a tumor ER positive and the patient a suitable candidate for endocrine therapy.[25] This view supported the notion that clinicians required ER results reported either as positive or negative to formulate a therapeutic recommendation, and that quantification of ER IHC results beyond this was unnecessary. In contrast, Ellis et al[26] have reported a linear relationship between the level of ER expression as determined by the semiquantitative Allred IHC score and response to both tamoxifen and letrozole in the neoadjuvant setting. More recently, Albain et al[27] suggested that quantification of ER by IHC may be of value in helping to identify patients with ER-positive tumors who may benefit from adjuvant chemotherapy. In that study of patients enrolled in the phase III intergroup trial 0100 (Southwest Oncology Group 8814), those whose tumors showed low to intermediate levels of ER expression as determined by the Allred score had an improved outcome when chemotherapy was added to hormonal therapy, but there was no additional benefit with the addition of chemotherapy among those whose cancers had high levels of ER. It remains to be seen if results such as these can be extrapolated to other patient populations in which ER IHC assays were performed using methods that differ from those used in these studies. However, validation of these findings would provide a strong argument in favor of returning to quantification of ER results in at least some clinical settings.

Ultimately, how best to assess and report ER status in breast cancers will depend on the clinical question being asked. If the question were simply whether or not a tumor expresses *any* ER, then the use of highly sensitive IHC methods that tend to dichotomize ER results and minimize the likelihood of false-negative results in tumors with low levels of ER expression would be adequate. If, on the other hand, the question is *how much* ER is present in ER-positive tumors, an assay that is capable of permitting accurate quantitation of ER in the tumor cells is required. In our view, the weight of evidence suggests that IHC for ER on FFPE tissue employing the highly sensitive antibodies and detection systems that are currently in clinical use may not be well suited for this role because of their relatively limited dynamic range and the lack of a linear relationship between IHC assay results and actual ER tumor cell content in this setting. An intrinsically quantitative method that can be performed in FFPE tissue may be much better suited for this purpose. The use of a quantitative reverse transcriptase polymerase chain reaction assay to detect the level of ER mRNA expression may be one such option,[28] but additional studies will be needed to address this important issue.

Moreover, analyzing other biomarkers in addition to ER that might be associated with sensitivity or resistance to various forms of endocrine therapy may ultimately be of greater value than the analysis of ER alone, at least in some clinical settings. For example, recent data have suggested that among women with ER-positive breast cancers, the level of progesterone receptor expression further helps to predict the likelihood of response to letrozole.[29] In addition, several estrogen receptor coregulator proteins have been described, and measuring their levels of expression may represent yet another means to refine the predictive value of ER.[30,31] Of course, the introduction of assays for additional biomarkers such as these would raise concerns regarding testing methodology and interpretation similar to those that have been raised for ER testing.

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