Q&A column

Editor: Frederick L. Kiechle, MD, PhD

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.

Submit a Question

Q. What is the role of total testosterone and free testosterone in gauging the effectiveness of androgen deprivation therapy?

A. Before answering this specific question regarding androgen deprivation therapy, we thought it wise to first discuss the clinical use of ADT more generally. Androgens are male sex hormones, with testosterone being the major one in healthy males. ADT has been the initial approach for therapy in men with prostate cancer with evidence of disseminated disease for more than 50 years. More recently, ADT in combination with chemotherapy has been found to increase survival compared with ADT alone or ADT followed by chemotherapy.1 ADT can be accomplished with either bilateral orchiectomy (i.e. surgical castration) or medical orchiectomy (i.e. medical castration). The most commonly used medical castration drug is a long-acting gonadotropin-releasing hormone agonist (e.g. Lupron) administered in a depot form. This drug dramatically decreases testicular production of testosterone through its effects on the hypothalamic-pituitary axis. The general goal of medical castration is to have the total testosterone concentration fall to values equivalent to those found after surgical bilateral orchiectomy, and it is important to ensure that very low testosterone concentrations are reached and maintained at surgical castration levels.

Prior to about 2000, target serum total testosterone concentrations of <50 ng/dL (<1.7 nmol/L) were recommended, based on the immunoassay measurement procedures available until that time. With the improvement in analytical specificity and lower limits of detection for serum testosterone in the clinically available testosterone measurement procedures, mainly via introduction of very high analytical specificity liquid chromatography mass spectrometry (LC-MS/MS) measurement procedures, it was suggested that the target values for what is considered to be adequate testosterone suppression be reduced to <20 ng/dL (<0.7 nmol/L). These are the values usually achieved after bilateral surgical castration.[1,2] However, there remains some debate on what values are achieved after bilateral surgical castration and what the target values for adequate testosterone suppression with medical castration should be.[3] It is likely that some of the ambiguity on postsurgical castration testosterone concentrations and appropriate target concentrations for medical castration revolves around the analytical specificity of the testosterone measurement procedures used in the various published studies, with many measurement procedures showing cross-reactivity with other related androgen hormones.[4] Interestingly, a total testosterone target value of <50 ng/dL (<1.7 nmol/L) with medical castration is still listed in the National Comprehensive Cancer Network's most recent 2018 prostate cancer guidelines.[5] When there is failure to reach a low enough serum total testosterone concentration, additional hormonal manipulations (with estrogen, antiandrogens, other LHRH antagonists, or steroids) are considered, although the clinical benefit of these additional therapies remains uncertain.[1,3,5] Rechecking total testosterone values using high analytical specificity measurement procedures with low limits of quantitation (e.g. LC-MS/MS) is important to consider when anticipated values are not observed during ADT, due to potential cross-reactivity of closely related androgens when using certain immunoassays.

To our knowledge, free testosterone concentrations have not been incorporated into any of the guidelines for gauging effectiveness of ADT. The majority of circulating testosterone in the blood is bound tightly to sex hormone binding globulin and loosely to albumin, with only two to three percent remaining free and unbound. There is

uncertainty on the degree of bioactivity of albumin-bound testosterone relative to free (unbound) testosterone. Clinically available free testosterone values are often calculated from total testosterone along with sex hormone binding globulin measurements (sometimes albumin measurements), rather than directly measured by techniques such as equilibrium dialysis. Both approaches have been described to have shortcomings and laboratory-to-laboratory variability.[6] Several small studies have suggested free testosterone may be useful in stratifying early prostate cancer risk for progression,[7] but we believe these do not relate to assessing adequacy of ADT, which was the question posed.

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Q. We are planning to validate the mismatch repair panel in our immunohistochemistry laboratory. Do we use the CAP guidelines for antibody validation for a nonpredictive marker or a predictive marker?

A. Universal screening for Lynch syndrome is recommended for colorectal carcinomas and, although currently being sorted out in the gynecological community, a hybrid approach has been suggested for endometrial carcinomas (incorporating clinical, morphology, and MSH6). Immunohistochemical analysis of the MMR proteins (MLH1, PMS2, MSH6, MSH2) is a cost-effective, sensitive, and specific method for determining MMR status. When planning an IHC validation of this panel, one must take into consideration that the clinically significant result is a loss of expression of these proteins in tumors (MMR deficient [dMMR]); therefore, one cannot use only "normal" tissues in the validation set. We recommend using at least colorectal and endometrial carcinomas that are dMMR as the positives in your validation cohort given that these are typically the most commonly requested and available tumor types for MMR testing. Other tumors that might be reasonably available to potentially include in your validation include sebaceous neoplasms or glioblastoma multiforme, depending on one's practice.

The FDA approved in 2017 pembrolizumab, a programmed death receptor-1 blocking antibody, as a treatment for patients with microsatellite instability-high or dMMR colorectal carcinoma who have progressed following chemotherapy. With regard to nonpredictive versus predictive type validation of the MMR panel, if your clinicians will be using the MMR status of these patients' tumors for treatment with pembrolizumab, then your laboratory would be required to validate this panel as a predictive marker. This requirement could be met by including a minimum of 20 positive and 20 negative cases (optimally processed using the same methods as future clinical specimens). The medical director has the discretion to determine whether fewer validation cases can be used if the antigen is considered rare. Having said that, approximately 15 percent of colorectal and 30 percent of endometrial carcinomas will exhibit dMMR phenotype. Other means of validation include comparing the results of your panel with the results of a validated MMR panel from another laboratory by testing the same tissue or correlating with molecular studies (MSI and NGS testing). Ultimately these validation means would require 90 percent concordance between your laboratory's newly validated MMR IHC panel and other molecular methods or that of the comparison laboratory.

If an IHC laboratory has previously validated the MMR panel using the nonpredictive marker guidelines but the intention is to expand the use of the MMR status as a predictive marker (in this case determining eligibility for treatment with pembrolizumab), then achieving compliance with predictive marker validation requirements can be reached retroactively by supplementation with a larger validation cohort. This may include combinations of the following: documentation of cases with dual testing (agreement of IHC versus other methods); documentation of agreement of your prior IHC versus IHC performed in another laboratory (additional cases may need to be sent out); and review with documentation of previous MMR proficiency testing results (for example, the CAP DNA Mismatch Repair Survey).

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