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Estrogen and Progesterone Receptor Testing

Estrogen and progesterone receptor analysis of breast tumors will now be performed in-house at Valley View Hospital using the DakoCytomation ER/PR pharmDx assay. This testing is used to identify patients eligible for treatment with anti-hormonal or aromatase inhibitor therapies and to provide prognostic information. Testing is performed on formalin-fixed, paraffin-embedded tumor sections run concurrently with positive and negative human cell line controls to ensure kit reagent performance. Non-neoplastic breast epithelium located on the same slide as the tumor also provides a perfect internal positive control for tumors that do not express estrogen or progesterone receptors.

Before testing newly diagnosed breast cancers, an in-house concordance study was performed on twenty previously diagnosed breast cancers with known ER/PR results, and concordance was established. Ongoing quality control measures will include comparing cumulative patient results with published benchmarks (Table 1) and evaluating inter-observer variability among the pathologists on test scoring. If a negative ER or PR result on a core biopsy specimen is obtained, the test will also be repeated on the excisional lumpectomy specimen.

Test slides are examined by light microscopy and scored using the Allred method, which has been validated in studies linked to clinical outcome for breast cancer patients¹⁻³. This scoring system incorporates not only the proportion of cells staining but also the intensity of the staining (Table 2, Figure 1 and 2).

Tumor types that tend to be ER+ include lobular, tubular, mucinous, and papillary carcinomas, along with low grade ductal carcinomas. It is interesting to note that one of the effects of estrogen is to induce the progesterone receptor, such that most PR+ tumors are also ER+, and less than 5% of PR+ tumors are ER-. Patients with positive PRs have a significantly longer disease-free survival than patients who are PR-.

References:

1. Allred DC, et al. *Mod Pathol* 1998; 11(2):155
2. Elledge RM, et al. *Int J Cancer* 2000; 89(2):111
3. Mohsin SK, et al. *Mod Pathol* 2004; 17, 1545

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Table 1: Incidence of Estrogen Receptor/ Progesterone Expression Phenotypes

Phenotype	Incidence (%)
ER+/PR+	58
ER+/PR-	23
ER-/PR+	4
ER-/PR-	15

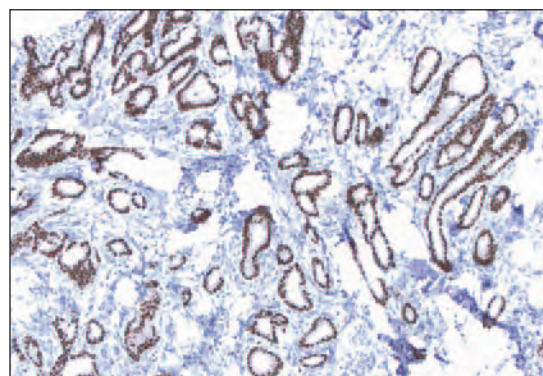


Figure 1: Breast Carcinoma stained for ER (PS 5) + (IS 2) = TS 7 (POSITIVE)

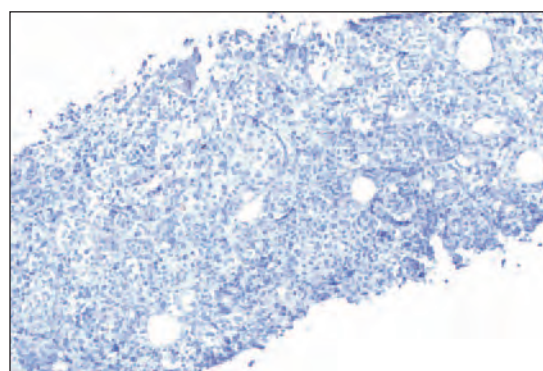


Figure 2: Breast Carcinoma stained for ER (PS 0) + (IS 0) = TS 0 (NEGATIVE)

“Tumor types that tend to be ER+ include lobular, tubular, mucinous, and papillary carcinomas, along with low grade ductal carcinomas.”

Table 2: Scoring Guidelines

<u>Proportion Score (PS)</u>	<u>PS Observation</u>	<u>Proportion Score (PS):</u> Proportion of tumor cells with positive nuclear staining.
0	None	
1	> 0 to 1/100	
2	>1/100 to 1/10	
3	>1/10 to 1/3	
4	>1/3 to 2/3	<u>Intensity Score (IS):</u> Average intensity of all positive tumor cells.
5	>2/3 to 1	
<u>Intensity Score (IS)</u>	<u>IS Observation</u>	
0	None	<u>Total Score:</u> Sum of Proportion Score (PS) and Intensity Score (IS).
1	Weak	
2	Intermediate	
3	Strong	
<u>Total Score (TS)</u>	<u>Interpretation</u>	
0-2	Negative	
>3	Positive	

Estimated GFR: A Primer

"All regional hospital laboratories will report the estimated GFR with every serum creatinine ordered."

Hospital laboratories in our area (Aspen Valley, Grand River, Vail Valley, and Valley View) are all beginning to routinely report the estimated GFR (glomerular filtration rate). This has recently been recommended by the National Kidney Disease Education program in an attempt to aid in the detection of chronic renal disease, and improve clinical outcomes by delaying or preventing renal failure. This discussion is meant to introduce the concept of estimated GFR, and aid in its interpretation.

1.) What is the estimated GFR?

It is a mathematical calculation based on the patient's serum creatinine, their age, sex, and race, which is designed to screen for chronic kidney disease by offering an estimation of the glomerular filtration rate of the kidneys.

2.) Why is the estimated GFR currently being promoted?

While chronic renal disease is increasing in our country, it is generally under diagnosed and treated. Our traditional biochemical screen of kidney function, the serum creatinine, is elevated to abnormal levels relatively late in the progression of chronic kidney disease, often not being abnormal until approximately 50% of renal function is compromised. In fact a 65, year old white woman is at the beginning stage of chronic kidney disease at a serum creatinine of 0.94 mg/dL, a value within the stated normal range of serum creatinine. It is hoped that by routinely reporting the estimated GFR, renal disease will be detected earlier in its course.

3.) How will the estimated GFR be reported?

All regional hospital laboratories will report the estimated GFR with every serum creatinine ordered. It will only be reported in patients greater than 18 years of age, having not been validated in children. The units of reporting are ml/min/1.73m², representing renal blood flow per minute for an average size patient.

4.) What is the charge for estimated GFR?

There will be no charge for these data.

5.) What is the currently accepted definition of chronic kidney disease?

Currently, this is defined as the presence of kidney damage, from any cause, for 3 months or more. Chronic kidney disease is frequently identified by abnormal laboratory tests, on at least 2 occasions, over three months. This definition dictates that when an abnormal estimated GFR is obtained, it be repeated within 3 months, possibly combined with urinary microalbumin to creatinine ratios. (When any urinary constituent is measured and expressed as a ratio of urine creatinine it represents a normalized value and, as such, obviates the need for a 24-hour urine collection.)

6.) What are the limitations of the estimated GFR?

Because of the focused nature of the original studies, the estimated GFR cannot be applied to patients less than 18 years of age, or over 70 years of age, nor to pregnant women. For black patients, results must be adjusted for race as directed. Results are somewhat less reliable in hospitalized patients because of non steady state nutritional status, and potential drug interferences.

7.) Why are normal results of the estimated GFR simply reported as > 60 ml/min/1.73m² ?

Currently, generally utilized creatinine methods are not well calibrated in the patient normal range. This imprecision of low serum creatinines causes too much variability in high GFR values to warrant reporting a specific number.

Reference: NKDEP Laboratory Working Group Recommendations. Clinincal Chem 2006; 52: 5-18.

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C-Reactive Protein

"...patients with elevated baseline levels of CRP are at an increased risk of cardiovascular disease."

C- Reactive Protein (CRP) was initially discovered in associated with pneumococcal pneumonia, and first recognized as the original "acute phase reactant", being elevated in a variety of inflammatory disorders, including most bacterial (but not usually viral) infections. In normal individuals the median CRP concentration is about 1mg/L, and the normal range is generally listed as up to 5mg/L. In individuals with acute inflammatory illnesses, the hepatic production of CRP is stimulated and plasma levels reach 300mg/L or more.

In recent years, numerous studies have demonstrated that patients with elevated baseline levels of CRP are at an increased risk of cardiovascular disease. Importantly, for the accurate laboratory measurement of CRP, the elevation of plasma CRP which confers an increased risk of coronary disease and stroke is minute compared to the 100-1000 fold increase that occurs with inflammatory conditions. This fact led to the introduction of "high sensitivity" CRP methods several years ago, which could accurately measure the low levels of CRP required for cardiac risk stratification.

Most recently, two separate CRP procedures are being offered by our laboratories, "cardio CRP" which is standardized to offer sufficient low level precision for use in cardiac risk stratification, and "inflam CRP" which allows accurate measurement of the 100 fold increases sometimes seen with inflammatory conditions. The notation of "hs CRP" (for high sensitivity) will no longer be used. Our laboratory computer systems are set up to require specification of which CRP is to be done; if a definitive order is not received we will default to the "inflam CRP" as this will offer the high level precision required in inflammatory conditions, as well as provide distinction of high cardiac risk from moderate (but not from low) risk. Thus, the "cardio CRP" should be ordered when prospective cardiac risk stratification is required. Alternatively, "inflam CRP" is indicated for the diagnosis and follow up of infectious conditions, such as appendicitis.

Recently a joint committee of the American Heart Association and CDC issued CRP guidelines. These included CRP testing on 2 samples, drawn about 2 weeks apart. If one level is > 10mg/L a search should be made for an obvious case of infection or inflammation, the condition treated, the elevated value discarded and an additional CRP value be obtained two weeks later. The two values are then averaged for cardiac risk stratification. If one of the values continues to be elevated over 10mg/L, cardiac risk stratification cannot be done.

Reference: Pearson, TA et al. Circulation 2003, 107: 499-511.

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