

LABletter

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

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eGFR and the Diagnosis of Early Renal Failure

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Asking About Antibodies: New Format for Serology Testing

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eGFR and the Diagnosis of Early Renal Failure

The National Kidney Disease Education Program (NKDEP), part of the National Institutes of Health, and many nephrology societies have been urging laboratories to report plasma creatinine as **eGFR (estimated Glomerular Filtration Rate)**. Beginning next month, with certain exceptions (see below), Stanford Clinical Laboratory will begin to do so.

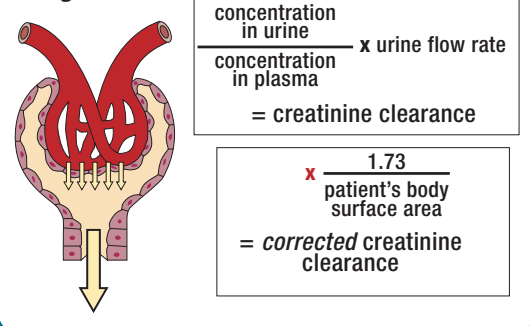
Glomerular filtration rate (GFR) is considered the best overall indicator of renal function. The gold standard involves continuous intravenous infusion of an inert substance not normally present in blood. The substance needs to be freely filtered by the glomerulus and neither reabsorbed nor secreted by the renal tubules. The amount excreted in the urine is therefore proportional to the amount filtered in the glomerulus. It is cumbersome to perform this maneuver using inulin or radiolabeled agents routinely. Measuring the clearance of endogenous creatinine is a good substitute.

Creatinine Clearance

A small amount of creatine, which serves as a storage form of high energy in muscle, degrades each day, forming creatinine. The rate of creatinine formation varies from person to person (and depends on many factors, especially the amount of muscle) but, for each person, it is relatively constant. Creatinine is freely filtered and not reabsorbed. It is secreted to some degree (which causes creatinine clearance to slightly overestimate GFR). Results are normalized to account for differences in muscle mass from one

patient to another. Normalization is done by estimating the patient's body mass and standardizing the creatinine clearance to that of a 70 kg man; this is designated as the "**corrected**" creatinine clearance. (Figure 1).

Figure 1:



Nonetheless, even using endogenous creatinine, clearance determinations are difficult and inconvenient to perform, especially in an out-patient setting. For this reason, plasma creatinine alone has been used for decades to test for renal disease.

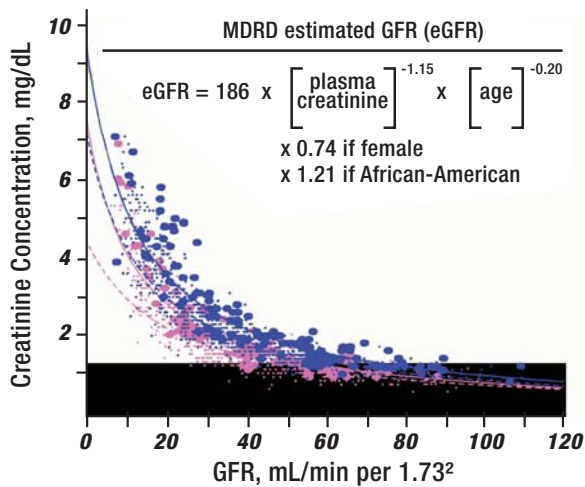
Creatinine and the eGFR

Plasma creatinine is a good way to monitor patients with known renal impairment but it is not a particularly good way to detect early renal disease. GFR may decline by as much as 50% before the plasma creatinine becomes abnormal, and patients with results in the "high normal" range may already have decreases in GFR that will go unnoticed.

New eGFR Reporting

The Modification of Diet in Renal Disease (MDRD) study was a multi-center controlled trial that looked at the effect of restricting dietary protein on the progression of renal disease. Nearly 2000 patients with varying degrees of chronic renal failure were enrolled. In addition to plasma creatinine, GFR was measured using renal clearance of ^{125}I -iothalamate. Using the observed relationship between plasma creatinine and GFR from this study, an equation was derived that allows estimation of GFR based on the patient's age, gender and, to some degree, race (**Figure 2**).

Figure 2:



From *Ann Intern Med* 130:461-470, 1999. Blue points are males; pink points are females. Solid lines are African-Americans; dashed lines are Caucasians. Reference range for plasma creatinine shown in black.

eGFR will appear as a new result in all of the panels currently in use that contain plasma creatinine, such as the Basic and Comprehensive Metabolic Panels, except as discussed below. The units are the same as those of the corrected creatinine clearance: ml/min/m².

The eGFR result will **not** be reported under the following circumstances:

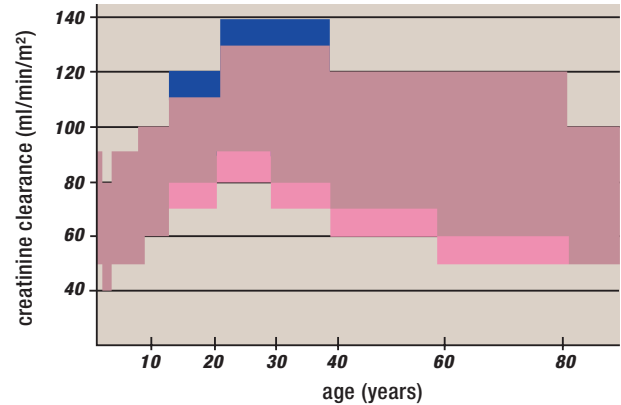
- **if the eGFR is >60 ml/min/m²**

Reference ranges for creatinine clearance change with age (see Fig. 3) and, presumably, so do ranges for eGFR. The relatively conservative cut-off of 60 was chosen for two reasons: the MDRD study did not include "normals"; and calibration bias is greatest in the "high normal" creatinine range. An effort (in which we are participating) is underway to standardize creatinine measurements but, in the meantime, we will only flag eGFR results <60 ml/min/m² (high probability of early renal failure).

- **if creatinine is >2.0 mg/dl**

Similarly, once a patient is known to have renal failure, plasma creatinine becomes a tool for monitoring their disease, not a

Figure 3: reference ranges for creatinine clearance by age and gender



Blue is range for males; pink is range for females. eGFR may be especially useful when screening patients between 20-60 years of age.

screening test. eGFR will not be reported if creatinine is >2.0 mg/dl (consistent with an eGFR of <30, defined by NKDEP as severe renal failure). Also, note that individual orders for creatinine alone (presumably to specifically monitor renal function) will not have eGFR reported.

- **if the patient is hospitalized**

Another limitation of the equation is that the patient should be at baseline. We are restricting eGFR reporting to out-patients. Panels ordered during patients' in-hospital stays (during which renal function may be temporarily affected by critical illness) will not have eGFR reported.

- **if the patient is <18 years of age**

The MDRD did not include any pediatric patients and the equation derived from the study is not believed to be reliable for children and teenagers. Other options exist to estimate GFR using plasma creatinine in pediatric patients, including the Schwartz equation and cystatin C (see below).

What should you do if a BMP or CMP report includes an alert that the eGFR is <60? First, consider the patient's risk factors. The most common etiologies of early renal failure include hypertension and diabetes mellitus. Second, consider the patient's race. The MDRD equation is adjusted for age and gender but we do not capture race as a demographic field and have no way to adjust the eGFR for African Americans; how patients of other races are affected is unknown.

Finally, consider additional testing to verify whether early renal failure is present or not. Another early sign of renal failure is an increase in urinary albumin excretion, easily tested by ordering albumin/creatinine ratio ("microalbumin") on a random urine specimen. Cystatin C (which we are also implementing this month in connection with the roll-out of eGFR) may be an ideal way to follow-up an abnormal eGFR (see Laboratory Update).

Laboratory Update:

Cystatin C & Cardiac Risk Assured

Cystatin C:

Test code: *CYSTC*

Specimen Type:	Plasma or Serum
Collect:	1.3 mL Mint-top gel tube or 1.3 mL Gold-top gel tube
Specimen Volume:	1 mL plasma or serum
Special Handling:	None
Methodology:	Nephelometry
CPT Code:	83883
TAT:	2-3 days
Report:	mg/L

Cystatin C is a protease inhibitor produced by all nucleated cells at a constant rate. Like creatinine, it is freely filtered and not reabsorbed. (It is actually reabsorbed by the renal tubular cells but, because they destroy it, it is not reabsorbed into the bloodstream.) Unlike creatinine, however, cystatin C is not secreted at all and it is not affected by muscle mass.

For these reasons, measuring plasma cystatin C may be a better way to estimate glomerular filtration rate (GFR). However, it is not as easy to perform as creatinine (and is more expensive). The NKDEP does not recommend performing creatinine clearance to verify abnormal eGFR results. Because it does not show the racial effects of the MDRD equation, and because the equation may not be valid if the patient is at an extreme of body size, muscle mass or nutritional status, ordering cystatin C may be a good way to confirm the presence of early renal failure.

Since we cannot report the eGFR if the patient is <18 years of age, cystatin C may also be used to screen for early renal failure in this population. Unlike creatinine, it reaches adult levels by one year of age.

Cardiac Risk Assured:

Test code: *LPDRAC*

Specimen Type:	Plasma/Serum
Collect:	1.2 mL Lavender-top tube (EDTA), 1 (4.5 mL) Mint-top gel tube (lithium heparin), 1.3 mL Gold-top gel tube
Specimen Volume:	1 mL EDTA Plasma, 2 mL Lithium heparin plasma, 1 mL serum
Special Handling:	Transport Refrigerated; Fasting specimen
Methodology:	Spectrophotometry, Immunoassay, and Polyacrylamide Gel Electrophoresis
Components:	Triglycerides, Total cholesterol, HDL cholesterol, LDL Cholesterol (calculated), Non-HDL Cholesterol, hs-CRP, Lp-PLA ₂ (PLAC test), Lipoprotein (a), Homocysteine, LDL phenotype
CPT Code:	80061, 83090, 83695, 83698, 83701, 86141
TAT:	2-3 days
Report:	Custom Report with Lipoprint densitometry image and Risk Interpretation

Cardiac Risk Assured is a new panel that features the traditional NCEP lipid targets, high-sensitivity CRP and procoagulant markers but adds Lp-PLA₂ (the PLAC test, a more specific inflammatory marker) as well as LDL subfractionation. It includes a color report with risk interpretation.

Stanford's CARDIAC RISK Assured

- Traditional NCEP Lipid targets
- Additional Risk Markers:
 - High Sensitivity CRP (hsCRP)
 - Lp-PLA₂ - New more sensitive inflammatory marker
 - Procoagulant Markers
 - Homocysteine
 - Lipoprotein (a)
 - LDL Phenotyping

- Color chromatogram of the patient's assay

* Easy-to-read custom report with risk interpretation

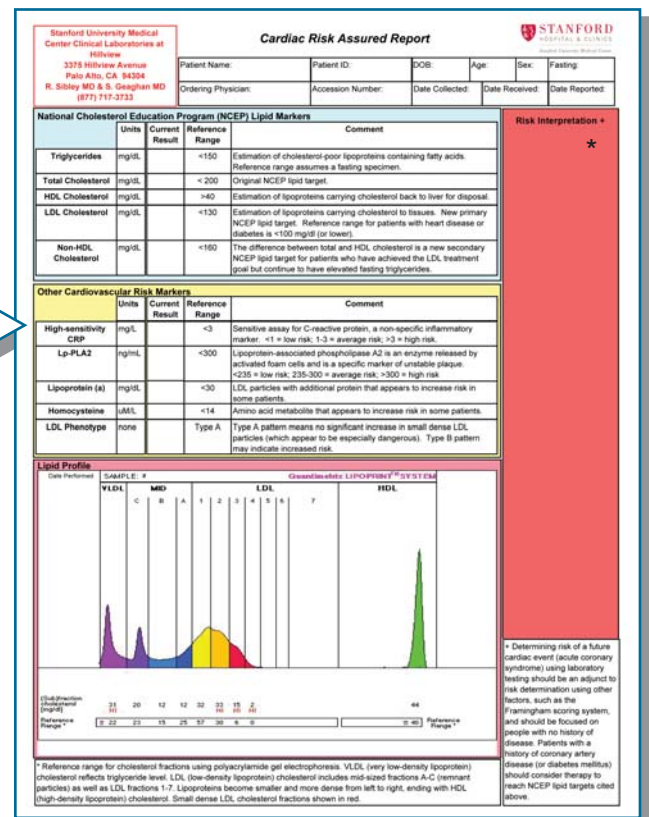
GREEN indicates laboratory support for low or average risk

RED indicates potential for increased risk

Contact your sales representative to:

- Add this panel to your custom requisition

- Request the Cardiac Risk Assured informative brochure



Q&A: Submit Q&A topics of interest to: labmarketing@stanfordmed.org

An opportunity for the Bay Area Medical Community to request specific Q&A topics to our medical directors that are relevant to patient's clinical needs.



James Faix, M.D.

Asking about Antibodies: New Formats for Serology Testing

Q&A with James Faix, M.D., Director of Clinical Chemistry and Immunology at Stanford Clinical Laboratory and Associate Professor of Pathology at Stanford University School of Medicine.

Stanford Clinical Laboratory will revise the way that antibody testing is reported (and, in many cases, the way that the testing is performed). This will affect both autoantibodies as well as infectious disease serology testing. We are starting off this month with one of the most frequently ordered autoantibody tests: anti-nuclear antibody (ANA).

Q1: How has testing for ANA changed?

A1: For many years, we used the traditional approach (indirect immunofluorescence) as our initial screen for ANA. Serum is added to cells fixed on a microscopic slide which is washed after a period of incubation. Fluorescent-labeled anti-human immunoglobulin antibody is then added to the slide, binding to any ANA on the cells. After a final wash, the cells are examined in a fluorescent microscope. If ANA is present in the patient's specimen, the nuclei are fluorescent green – with patterns associated with different specific anti-nuclear antibodies and disorders. We have recently switched to ELISA for screening.

Q2: What are the advantages of ELISA screening?

A2: Enzyme-Linked ImmunoSorbent Assay, or ELISA, is a different way to detect the presence of an antibody. Antigens are coated onto the wells of microtiter plates to which serum is added. After a period of incubation, the wells are washed and enzyme-labeled anti-human immunoglobulin antibody is then added. After a final wash, the substrate of the enzyme is added. So, it's similar to immunofluorescence, except that the final read-out is enzymatic. This makes it much less subjective and easily automated.

Q3: Are there disadvantages with ELISA screening?

A3: Traditionally, some anti-nuclear antibodies could only be detected using immunofluorescence. One reason for this is the fact that synthetic or recombinant antigens on a microtiter well do not always "look" exactly the way that they do in nuclei. This is why we made sure that the ELISA we chose used purified antigens from nuclear extracts. We also increased the sensitivity of the assay by lowering our cut-off for calling the screen "positive". Because this may result in "false positives", we will confirm positive ELISA screens for both ANA and anti-dsDNA by immunofluorescence. This allows us to be sure that the result is positive. It also allows us to continue to report the traditional "titer" (rather than a ratio).

Q4: How will the ANA and anti-dsDNA reporting change?

A4: We are using this opportunity to roll out a new paradigm for serology test reporting. Both ANA and anti-dsDNA will be test "panels" containing the qualitative result ("Negative" or "Positive"); the quantitative result ("Titer") and a brief interpretation. This new format will work much more easily with the variety of electronic medical records. The interpretation will explain "what the result means" and, for ANA, will also include the information about the pattern observed. We plan to use this same format as we revamp additional serology tests.

Q5: What is the next test to change to ELISA?

A5: The serologic screen for syphilis will change from the current rapid plasma reagin (RPR) test to an anti-treponemal ELISA test. We will confirm all positive anti-treponemal ELISA screens with the RPR. (More about this new change in the next Lab Letter). Now that we have the new resources of the Stanford Clinical Laboratory at Hillview, we will be expanding our in-house serology test menu by the end of the year.



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