ADJUSTING MEASURED CREATININE FOR LOW SERUM GLUCOSE TO IMPROVE MORTALITY PREDICTION

Michael Fulks, MD
fulksmd@volcano.net

Vera F. Dolan, MSPH, FALU
dolanvp@consultancy.com

Robert L. Stout, PhD
stoutr@crlcorp.com

Clinical Reference Laboratory
Lenexa, KS

Executive Summary *A delay in centrifugation of blood samples can decrease the glucose level through glycolysis while increasing the measured level of creatinine. Adjustment of the creatinine value for glucose levels below 40 mg/dL substantially improved the specificity (fewer false positives) of eGFR for mortality with little or no sacrifice in sensitivity. The need for such an adjustment is eliminated if an enzymatic creatinine test (which is insensitive to the pseudo-creatinines leaking from red and white blood cells before centrifugation) is substituted for the commonly used rapid Jaffe method on the laboratory analyzer but testing cost is increased.*

Both a correction formula based on measured serum glucose and a suggested approach to enzymatic creatinine testing to maximize benefit and minimize cost are presented.

Introduction

The insurance industry and clinical medicine have traditionally used serum creatinine as the surrogate measure of glomerular filtration rate (GFR). Recently, interest has increased in substituting an estimated GFR (eGFR) based on a calculation utilizing serum creatinine, age and sex as a way to more accurately assess kidney function.

Creatinine is a waste product of muscle (creatine) metabolism excreted by the kidney. The serum level is affected by muscle mass (related to age and sex) and sample handling issues as well as by GFR. Calculating an eGFR adjusts for muscle mass by using age and sex, but not for handling issues.[1]

The eGFR value, indicating the amount of fluid filtered by the kidneys, is usually expressed as mL/min/1.73 m^2. The last term (most often not shown) is the surface area of an average person and that correction (estimated by age and sex in commonly used eGFR calculations) allows us to compare the kidney function among people of different sizes.

An insurance handling issue with both serum creatinine and any eGFR calculation based on it is the variable time delay and holding temperature before centrifugation (separating the serum from red and white blood cells) of the blood sample. This can sometimes be hours at room temperature, or higher, resulting in creatinine-like substances ("pseudo-creatinines") leaking from cells into the serum. These substances mimic creatinine during serum testing performed by many automated blood analyzers (rapid Jaffe method) resulting in an artifactual increase in measured serum creatinine (and decreasing calculated eGFR).[2,3,4] These pseudo-creatinines are not thought to impact the alternative (but more expensive) enzymatic method of creatinine measurement.

Time to centrifugation may not be reliably available from examiners (any reported delay may be penalized), but the combination of time and temperature can be estimated by observing the reduction in blood sugar as a result of red and white blood cells metabolizing the available blood glucose (glycolysis). Blood sugar decreases by about 5-10 mg/dL each hour in unspun blood at room or higher temperature.[2,4,5,6] Roughly 22% of CRL samples have blood sugar <60 mg/dL (usual physiologic lower limit) with 12% being ≤40 and 7.6% being ≤20 mg/dL, indicating many insurance samples have centrifugation delayed by several hours.

We performed a study comparing enzymatic creatinine with standard rapid Jaffe creatinine determination to assess the degree to which this artifactual elevation of creatinine impairs risk assessment, and if any systematic adjustment to creatinine values could assist in limiting the impact of false elevations of creatinine associated with glycolysis.

How the Study Was Done

The samples studied were obtained from 2001 to 2007 with mortality follow-up of the applicants associated with the samples in late 2011 by the Social Security Death Master File (last DMF available before all state-provided deaths were removed). Because our goal was to compare the independent impact of adjusting creatinine on all-cause mortality, those applicants with urine protein/creatinine ratios of ≥0.21 g/g or HbA1c ≥7.0% were excluded. This resulted in 4.9 million applicants with 54,489 deaths.

Measurements of serum creatinine and serum glucose were performed on Roche Hitachi Cobas analyzers with FDA-approved reagents. A separate analysis was done for 10,622 recent consecutive insurance applicant blood samples with serum glucose and creatinine results, with creatinine evaluated using both enzymatic and standard rapid Jaffe test methodology. The methodology for all testing was performed according to Roche's instructions with FDA-approved reagents.

Based on the samples tested using both creatinine test methods, an adjustment formula was developed and applied to create adjusted creatinine values for those with low serum glucose. The ability of these adjusted values converted to eGFR to predict mortality was compared to use of the unadjusted values by comparing receiver operator characteristic (ROC) curves.

What the Study Found

Figure 1 is a scatter plot showing the ratio between the results from rapid Jaffe (higher) and enzymatic creatinine testing for 1,769 samples with glucose values between 1 and 60 mg/dL. Also shown is the trendline based on a quadratic equation, which had the best fit compared to linear or logarithmic alternatives (ratio = 0.00009 x glucose^2 - 0.0115 x glucose + 1.3337). This trendline is based on the average (mean) value of the ratio of the rapid Jaffe and the enzymatic method creatinine results; individual sample results may fall above or below that trendline.

For serum glucose values <5 mg/dL, the range of differences in results by each method varies more substantially. This is likely related to the large variation in time and temperature before centrifugation reflected in these very low glucose values. For glucose of 0 mg/dL (not shown) the ratio was very high for a few samples; this point was excluded to avoid creating an algorithm which might substantially underestimate the true creatinine level for some samples at very low glucose values. The difference in creatinine levels is approximately 4.5%, 10%, 19%, 25% and 32% higher by the standard rapid Jaffe testing for glucose ranges 30-39, 20-29, 10-19, 5-9 and 0-4 mg/dL, respectively.

Both rapid Jaffe and enzymatic results were also plotted independently against glucose level as shown by the quadratic (polynomial) trendlines in Figure 2 [next page]. We confirmed there was an inverse relationship for creatinine values by the rapid Jaffe test and glucose when glucose values fell below 40 mg/dL, but no variation by the enzymatic method.

Figure 1. Scatter plot of ratio of creatinine results for rapid Jaffe to enzymatic by glucose level, with fitted quadratic trendline and equation

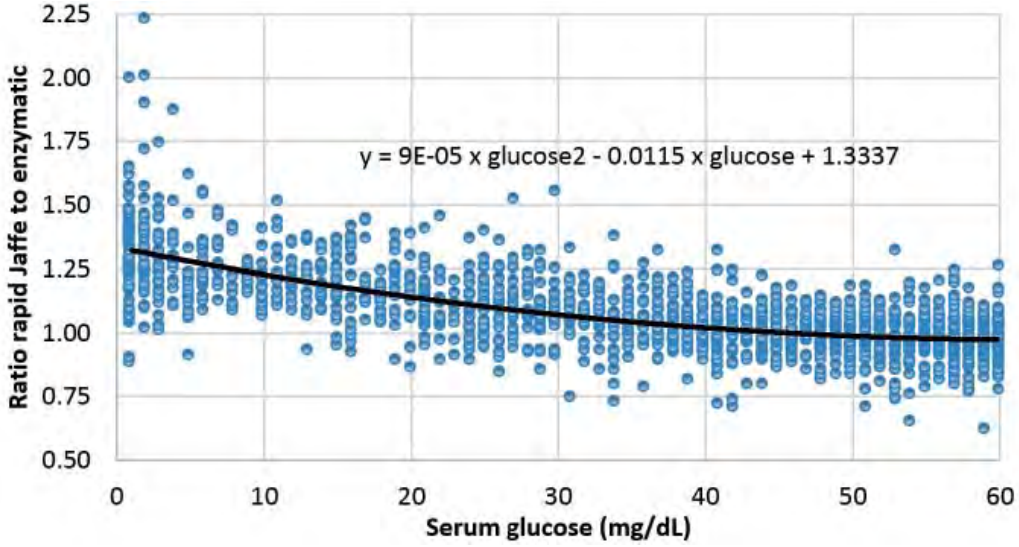
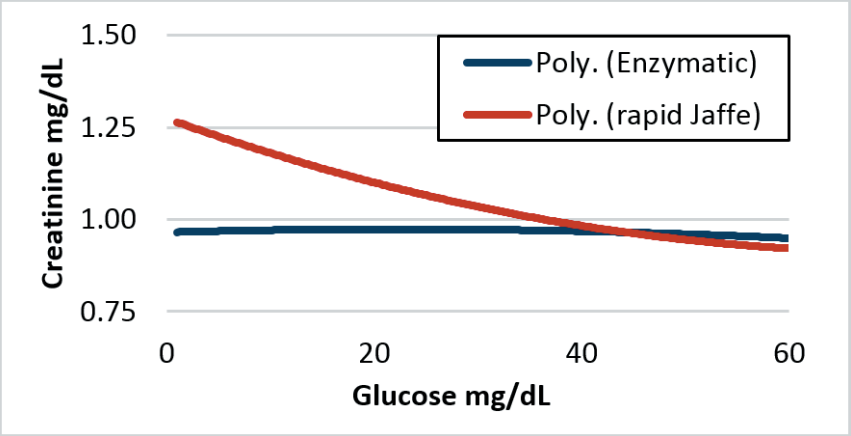


Figure 2. Trendlines (polynomial) for creatinine by enzymatic and rapid Jaffe methods by glucose level



Using this creatinine adjustment formula for glucose values <40 mg/dL, the difference in the ability of the raw and adjusted creatinine values to predict mortality was examined by converting the creatinine to an eGFR based on the Rule (Mayo Clinic) quadratic method.[7] The tool used for this analysis is the ROC curve commonly employed to compare the effectiveness of tests in predicting an outcome. It generates an area under the curve (AUC) which ranges from 0.5 (no prediction) to 1 (perfect prediction); the higher the AUC, the more effective the test.

Because very low creatinine values (very high eGFR) are also associated with increased risk (related to low muscle mass) and impact the AUC, eGFR values >90 mL/min were excluded from this AUC analysis (but not the other measures of impact) to focus on normal to low eGFR as a predictor of mortality associated with renal dysfunction.

As seen in Table 1 [next page], this adjustment improved the ability of eGFR to predict mortality for each age and sex group. The differences in AUC shown translate into meaningful improvements in mortality risk prediction. Perhaps more importantly, adjustment resulted in a 36% reduction in the percentage of applicants with measured eGFR <80 mL/min by the Rule equation (and identified as potentially higher risk requiring underwriter review).

The overall mortality rate for those with the lowest risk eGFR of ≥80 mL/min was little changed (-0.37% to +0.59% depending on age and sex) after the adjustment of creatinine for applicants with artificially low glucose test results moved many applicants back into this lowest risk group.

What Do the Study Results Contribute to Risk Assessment?

Use of serum creatinine as a marker of kidney function and predictor of mortality risk during insurance screening has been complicated by: (1) varying muscle mass largely dependent on age and sex, (2) the fact that expected GFR decreases with age, and (3) the variable delay to separation of serum from cells resulting in the release of pseudo-creatinines. Conversion to eGFR helps substantially with issue (1), using age-specific eGFR ranges helps with issue (2), and now we have a systematic adjustment for time delay to centrifugation using the serum glucose for issue (3) (or use enzymatic creatinine).

The adjustment to creatinine based on low serum glucose level provides a 36% reduction in the number of low eGFR values, which reduces unnecessary underwriting evaluation and potential adverse action when the renal function is "normal." It does this without increasing the risk (mortality rate) of the enlarged group with eGFR ≥80 mL/min and with demonstrated improvement in mortality risk assessment. Although we focused on eGFR for our assessment, this adjustment is likely to have a similar impact on use of the creatinine value for initial screening.

Because low glucose values on insurance laboratory reports are often reported simply as "60 mg/dL," our suggested adjustment is impossible for the underwriter to perform unless the actual glucose values are included. However, the laboratory would have that value and could perform the adjustment. Another option is to routinely report all glucose values, even those below the physiologically expected range, even if that value is an artifact of collection.

A second approach is for the laboratory to use a different methodology to avoid the effect of glycolysis on the creatinine value. The enzymatic method achieves this but at much higher test cost as compared to the commonly used rapid Jaffe method. Based on this study, CRL plans to switch to enzymatic creatinine testing for those samples with glucose sufficiently low and creatinine sufficiently high that underwriting ac-

Table 1. AUC for mortality outcome, percentage with eGFR <80 mL/min, and percentage difference in mortality rate for eGFR ≥80, with no adjustment and after adjustment for low glucose

	Age-sex group					
	F 20-49	F 50-69	F 70-89	M 20-49	M 50-69	M 70-89
AUC no adjustment	.605	.521	.593	.522	.550	.568
AUC after adjustment	.609	.560	.600	.557	.565	.570
% <80 mL/min no adj.	1.3	7.9	35.1	4.2	12.7	35.7
% <80 mL/min after adj.	0.6	5.3	31.7	2.0	9.1	32.0
% mortality rate difference for eGFR ≥80 mL/min	0.34	0.59	0.08	-0.23	-0.37	-0.07

tion might be impacted. This captures the creatinine values of concern while limiting this more expensive testing to a small percentage of samples.

For blood specimens with low glucose not using the enzymatic testing, the glucose adjustment to estimate the likely creatinine level can be done if the actual glucose level is known. But, this is an average adjusted value for creatinine; the actual value may be higher or lower than the adjusted value for a particular applicant.

A third potential approach is to add cystatin C when glucose is low and/or creatinine is high. However, in a CRL comparison of cystatin C and enzymatic creatinine for samples with initial Jaffe creatinine results greater than 1.5 mg/dL, 22% and 49% respectively were reclassified as "normal" based on cystatin C ≤1.0 mg/L and enzymatic creatinine ≤1.5 mg/dL (data not shown). Other cystatin C cut-offs (including age-specific) or eGFR based on cystatin C might give better performance, but it is not a panacea.

Conclusion

When centrifugation for blood samples is delayed, creatinine levels are commonly overstated and associated eGFR underestimated. Approximately 36% of abnormal eGFR values are caused by this. Adjusting the measured creatinine value, guided by the specific glucose level to account for pseudo-creatinines released into the serum when sample centrifugation is delayed, results in substantially improved specificity (fewer false-positives) with little or no sacrifice in sensitivity for the prediction of mortality.

The alternate or complementary approach is for laboratories to utilize enzymatic creatinine testing, but the high cost when used for screening is a potential issue which can be mitigated if only those samples with low glucose and/or higher creatinine values associated with eGFR of potential concern are tested using the enzymatic creatinine method.

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About the Authors

Michael Fulks, MD, Consulting Medical Director, is board certified in internal and insurance medicine. After leaving practice, he served as a medical director, creating or editing several underwriting manuals and preferred programs. For the past 8 years, Dr. Fulks has consulted for CRL, participating in its mortality research on individual tests and all laboratory test results, BP and build in combination. Mike is also involved in the development and implementation of automated screening tools for non-laboratory data.

Vera F. Dolan, MSPH, FALU, Senior Research Scientist at Clinical Reference Laboratory, is a consultant specializing in underwriting research and product development. At CRL, Vera assists with the analysis and publication of CRL's mortality study data. In her consulting practice, Vera develops risk assessment tools for underwriters, including underwriting manuals, automated risk assessment systems and underwriter training. Vera provides litigation support for misrepresentation and other underwriting issues, as well as life expectancy calculations for use during litigation.

Robert L. Stout, PhD, is Chief Science Officer, Associate Laboratory Director and board member of the Clinical Reference Laboratory based in Lenexa, KS. He completed undergraduate studies at California State University (Fullerton) and obtained a PhD in Biological Chemistry from UCLA School of Medicine. Since 1978 he has been directly responsible for introducing many of the new tests and procedures used in risk assessment such as urine and saliva HIV. Dr. Stout has produced nine US patents and numerous papers on the relationship between laboratory testing and insurance applicant mortality.