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Calibration of Cystatin C in the National Health and Nutrition Examination Surveys

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The National Health and Nutrition Examination Surveys (NHANES) are one of the most important sources of data for evaluating the burden and trends in chronic kidney disease in the U.S. Cystatin C is a useful marker of kidney function and was recently proposed as a confirmatory test for chronic kidney disease (1). Cystatin C data are available from the NHANES, but evidence of drift in the assay over time has limited the utility of these data. Cystatin C measurements were conducted in stored surplus serum samples obtained from participants in the NHANES III (1988–1994) and 1999–2002 surveys (2–4). Due to concerns regarding the comparability of these measurements conducted during different time periods (drift), we performed a formal comparison to harmonize the cystatin C values in NHANES.

In 2006, cystatin C was measured in stored samples collected during the NHANES III and 1999–2002 cycles at the Cleveland Clinic Research Laboratory (CCRL) using the Dade Behring N Latex Cystatin C assay, an automated particle-enhanced nephelometric assay (PENIA) run on the Dade Behring BNII Nephelometer. Inter-assay CVs were 5.1% and 4.9% at mean concentrations of 0.97 and 1.90 mg/L, respectively. In 2009, we repeated cystatin C measurements at the University of Minnesota (UMN) in a random sample of 200 stored serum aliquots from each of the NHANES cycles that had not been previously thawed. Measurements at the UMN were conducted using the same PENIA reagents run on the Siemens ProSpec analyzer (Siemens Healthcare Diagnostics acquired Dade-Behring). The inter-assay CV was 4.0% at a mean cystatin C concentration of 0.689 mg/L. As an additional check, we also measured serum creatinine using the Roche enzymatic method in the NHANES III samples to compare to original NHANES III standardized (IDMS-traceable) creatinine values (5). We used Deming regressions ($Y=UMN$, $X=CCRL$) to address the comparability of serum cystatin C across surveys (laboratories) in the calibration samples. Extreme outliers (>3 standard deviations from the mean) were removed.

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There were 178 paired cystatin C values samples in NHANES III after exclusion of samples with insufficient volume and removing 6 outliers. (Results were similar before and after excluding outliers.) The mean cystatin C was significantly lower at UMN during 2009 (0.89 mg/dL) as compared to CCRL during early 2006 (1.08 mg/dL, p -value<0.001) but the values were highly correlated ($r=0.98$) (Table and Figure, Panel A). The Deming calibration equation for NHANES III cystatin C was: $Y=0.022+0.800\times[CCRL\ 2006\ cystatin\ C]$ (Table).

There were 184 paired cystatin C values available for calibration in NHANES 1999–2002 after exclusion of samples with insufficient volume and removing 2 outliers. The mean cystatin C was also significantly lower at UMN in 2009 (0.88 mg/dL) as compared to CCRL in late 2006 (1.00 mg/dL, p -value<0.001) ($r=0.99$) (Table and Figure, Panel B). However, because the slope was not statistically significantly different from 1.0 (constant) in the Deming regression, the calibration for cystatin C in NHANES 1999–2002 can be conducted simply using the difference only: $Y=[CCRL\ 2006\ cystatin\ C]-0.12$ (Table).

All NHANES III and 1999–2002 cystatin C values provided on the NHANES website should thus be corrected using the above-listed equations and then converted to standardized ERM471/IFCC traceable cystatin C values by multiplying by 1.12 based on previous work relating cystatin C values obtained at the UMN using this same method to the IFCC cystatin C standard (6). After re-calibration, the mean (SD) IFCC standard cystatin C mean (SD) were 0.99 (0.23) and 0.98 (0.57) in the NHANES III and 1999–2002 calibration samples, respectively.

Serum creatinine re-measured at UMN in the NHANES III samples for our calibration study was well aligned to standardized creatinine values and did not need re-calibration (Figure, Panel C). The slope of the Deming regression was not statistically different from 1.0 and the intercept was not statistically different from 0 ($r=0.97$, $n=184$ after excluding outliers).

Recent efforts towards global standardization of serum creatinine assays have been successful (7), but cystatin C calibration has differed markedly across assays and time (6, 8–10). We found that within our research laboratories, the creatinine assay remained stable (no drift) while cystatin C changed by as much as 20%, consistent with previous studies (6, 10). Our results for serum creatinine suggest that efforts to standardize serum creatinine can be successful as our measurements in long-term stored samples were well aligned with previously standardized creatinine measurements in NHANES III (5).

In summary, these data provide a basis for standardization of cystatin C in existing data from NHANES and provide the equations needed for data users to ensure harmonization. The recommended equations eliminate the bias in the originally reported measurements that resulted from drift in the calibration of the measurement procedures. In future studies using NHANES III and 1999–2002 data, cystatin C measurements can be directly adjusted using the equations presented here and then converted to ERM471/IFCC-traceable cystatin C values (6).

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Figure.

Scatterplots of cystatin C and serum creatinine in calibration subsamples comparing measurements at the two laboratories, Cleveland Clinic Research Laboratory (CCRL) in 2006 and University of Minnesota (UMN) in 2009

Table

Cystatin C Calibration Summary Statistics, National Health and Nutrition Examination Surveys III and 1999–2002

	NHANES III (1988–1994) N=178	NHANES 1999–2002 N=184
% Male	48%	57%
Mean age (years) (SD)	69.5 (7.0)	53.5 (24.8)
Mean serum cystatin C at CCRL in 2006 (mg/L) (SD)	1.08 (0.26)	1.00 (0.51)
Mean serum cystatin C at UMN in 2009 (mg/L) (SD)	0.89 (0.21)	0.88 (0.52)
Mean difference in serum cystatin C (UMN - CCRL) (mg/L) (SD)	−0.19 (0.07)	−0.12 (0.07)
<i>P-value</i> *	<0.001	<0.001
Deming regression of UMN in 2009 (Y) on 2006 CCRL (X) serum cystatin C (mg/L)		
Intercept (SE)	0.022 (0.01)	−0.125 (0.10)
Slope (SE)	0.80 (0.02)	1.01 (0.11)
Recommended final calibration equations:		
	IFCC standard cystatin C = 1.12× [0.022+0.80×(NHANES III reported [†] cystatin C)]	IFCC standard cystatin C = 1.12×[(NHANES 1999–2002 reported [†] cystatin C)−0.12]

* Paired t-test of the hypothesis that the means are equal.

[†] Reported cystatin C is the un-calibrated value (NHANES website public use data).