Original Articles

Cystatin C, mortality risk and clinical triage in US adults: threshold values and hierarchical importance

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Abstract

Background. It has been suggested that cystatin C may be a superior measure of estimated glomerular filtration rate (eGFR) than creatinine-based methods. We aimed to assess the utility of cystatin C for clinical triage in community-based settings.
Methods. We identified cystatin C thresholds that maximize sensitivity and specificity ($Max_{Sn + Sp}$) for predicting death and subsequently applied classification tree methodology considering serum creatinine, creatinine-based eGFR, urinary albumin–creatinine ratio and conventional modifiable risk factors to define subgroups, interactions and hierarchical ranks in fasting US adults (National Health and Nutrition Examination Survey 1988–94, followed through 2006).
Results. A threshold cystatin C value of 0.94 mg/L exhibited the best maximum combined value of sensitivity and specificity for predicting death ($Max_{Sn + Sp}$, Sn 0.64/Sp 0.78). When all variables were considered jointly in a classification tree, cystatin C and albumin–creatinine ratio were the primary mortality discriminators in subgroups that added up to 41 and 14% of the study population, respectively; serum creatinine and creatinine-based eGFR were non-discriminatory.
Conclusion. Cystatin C may be useful for risk-based clinical triage in public health settings.

Keywords: albumin–creatinine ratio; creatinine; cystatin C; estimated glomerular filtration rate; mortality

Introduction

As chronic kidney disease is common and associated with adverse outcomes such as cardiovascular disease, end-stage kidney disease and death [1–4], there is increasing interest in routine measurement of kidney function in community-dwelling adults, in much the same way that blood pressure, lipid and body mass measures are recommended periodically [5–20]. Before considering screening with a biological measure, it would be helpful to know the efficacy of different threshold levels for predicting major health outcomes. For death within a finite time interval, for example, a threshold at which individuals classified as 'normal' show low mortality rates (a high proportion of true negatives) and those classified as 'abnormal' show high mortality rates (a high proportion of true positives) might be useful for defining subgroups in which intensive follow-up and treatment may be appropriate.

The low-molecular weight protein cystatin C has several potentially attractive features as a measure of estimated glomerular filtration rate (eGFR), including stable production rates, free filtration by the glomerulus, no overall renal tubular effect on serum levels and serum levels that are not heavily influenced by race, sex or lean body mass proportions [21,22]. Regarding the issues of cystatin C levels, mortality and clinical triage, several questions have yet to be addressed: should serum cystatin C, serum creatinine, $eGFR_{Creatinine}$ or urinary albumin–creatinine ratio (ACR) be used? When clinical triage with discrete thresholds is being considered, it would be useful to define optimal thresholds for the entire population and, in addition, to understand whether subpopulations exist in which different thresholds are needed. Finally, as kidney function correlates with many other classical mortality risk factors, is it more efficient to screen for factors like body mass index, cholesterol levels, blood pressure and blood glucose? In this nationally representative study, we used diagnostic test and classification tree methodology to assess the efficacy of cystatin C as a mortality discriminator among community-dwelling adults.

Materials and methods

Objectives

Among adult participants in the Third National Health and Nutrition Examination Survey (NHANES III, 1988–94), the main objectives of this study were the following:

1. To identify the cystatin C threshold with maximum combined sensitivity and specificity ($Max_{Sn + Sp}$) predictions for death through 31 December 2006;

2. Based on $Max_{Sn + Sp}$ for death, to use a classification tree analysis to rank cystatin C thresholds in a framework that also considered creatinine-based estimated GFR, urinary ACR and other major mortality risk factors, particularly those recommended for screening in community-dwelling adults.

Study population and measurements

NHANES III was a cross-sectional, multistage, stratified, clustered probability sampling of the non-institutionalized US civilian population that was undertaken in two phases (1988–91 and 1991–94 [23]); as recommended by the National Center for Health Statistics [24,25], the 1988–91 and 1991–94 subpopulations were examined in combination. A strategy of systematic oversampling was employed among elderly, Mexican American and non-Hispanic African American participants. Interviews were performed at participants' homes, and physical examinations and blood and urine collections were performed at mobile examination centers. For cystatin C, a systematic sampling strategy was employed; specifically, cystatin C was assayed in stored serum samples from all participants with the following characteristics: women and men with standardized serum creatinine levels >1.0 and 1.2 mg/dL, respectively, age ≥ 60 years. In addition, cystatin C was measured in a 25% random sample of participants without these characteristics. For this study, we limited the study population to participants examined in a mobile examination center, aged ≥20 years, with serum cystatin C and creatinine and urinary albumin–creatinine measurements.

A particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, IL) was used to measure cystatin C. The range of this assay is from 0.23 to 7.25 mg/L, and interassay coefficients are 5.05 and 4.87%, respectively, at cystatin C levels of 0.97 and 1.90 mg/L [26]. The kinetic alkaline picrate method was used to measure serum creatinine; levels were then aligned to standardized creatinine measured at the Cleveland Clinic Research Laboratory (Cleveland, OH) according to the following relationship: standardized creatinine = $0.960 \times$ actual creatinine $- 0.184$ [27]. We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [28] formula for creatinine-based GFR estimation (mL/min/1.73 m²), calculated from the following functions of race, sex, age (years) and serum creatinine (Scr, mg/dL):

Women:

$$Scr \leq 0.7 : GFR = 144 \times (Scr/0.7)^{-0.329} \times (0.993)^{age}$$
$$\times 1.15 \text{ if African American}$$

$$Scr > 0.7 : GFR = 144 \times (Scr/0.7)^{-1.209} \times (0.993)^{age}$$
$$\times 1.15 \text{ if African American}$$

Men:

$$Scr \leq 0.9 : GFR = 141 \times (Scr/0.9)^{-0.411} \times (0.993)^{age}$$
$$\times 1.16 \text{ if African American}$$

$$Scr > 0.9 : GFR = 141 \times (Scr/0.9)^{-1.209} \times (0.993)^{age}$$
$$\times 1.16 \text{ if African American}$$

We used five formulas relating GFR to cystatin C measured with the same technique used in this study, those of Stevens (GFR, mL/min/1.73 m² = $76.7 \times$ cystatin $C^{-1.19}$), Hoek ($-4.32 + 80.35 \times 1$ / cystatin C), Filler ($10^{1.962 + 1.123\log[1 / \text{cystatin C}]}$), Larsson ($77.239 \times$ cystatin $C^{-1.2623}$) and Rule and colleagues ($66 \times$ cystatin $C^{-1.30}$) [29–33]. As substantive insights were formula-independent, GFR levels using the formula of Stevens and colleagues are presented throughout.

Urinary albumin and creatinine concentrations were measured at the University of Minnesota, Minneapolis, MN, from random spot urine samples, using the modified kinetic Jaffe method and a Synchron AS/Astra Analyzer (Beckman Coulter, Fullerton, CA). Current smokers were defined by affirmative answers to the questions 'Do you now smoke cigarettes?' and 'Have you smoked at least 100 cigarettes in your life?'

Diabetes, hypertension and cardiovascular disease (prior history of myocardial infarction, congestive heart failure or stroke) were self-reported.

Outcomes

Vital status for NHANES III participants was established through 31 December 2006, through linkage with death certificate data in the National Death Index. To reduce the risk of participant identification, data perturbation techniques that introduce statistical noise were applied to the public-use vital status dataset, with synthetic dates substituted for real death dates for participants who died. Mortality hazards ratios from the perturbed dataset have been shown to correspond closely with those from unperturbed datasets [34].

To identify mortality $Max_{Sn + Sp}$ levels for cystatin C, true positive (exposure among subjects who died) and true negative (non-exposure among subjects who survived) values were computed separately for cystatin C thresholds that varied in 0.01 mg/L increments between 0.6 and 2.0 mg/L. A similar procedure was used for serum creatinine, in the range 0.5–2.0 mg/L. For all other continuous variables, thresholds were moved in 1-unit increments, in the following ranges: ACR, 1–100 mg/g; age, 20–89 years; body mass index, 18–40 kg/m²; high-density lipoprotein (HDL) cholesterol, 30–80 mg/dL; eGFR, 30–120 mL/min/1.73 m²; systolic blood pressure, 90–150 mm Hg; total cholesterol, 100–300 mg/dL. Because the discriminatory power of many variables might (i) reflect correlations with other variables such as age and (ii) differ substantially in major population subsets, we constructed classification trees for death based on the highest $Max_{Sn + Sp}$ value when all variables were considered simultaneously, with the proviso that at least 100 deaths were available. At any given node, the next cluster of branches was defined by $Max_{Sn + Sp}$, provided that P-values for mortality association were <0.05 with logistic regression. This process was repeated within subgroups until four orders of dichotomization had been completed and up to 16 terminal subgroups had been identified; nodes at which <100 deaths occurred were considered terminal. Thereafter, terminal subgroups were used to classify the entire population, and logistic regression was used to compute mortality odds ratios and overall model C-statistics.

Several sensitivity analyses were performed. Because abnormal kidney function at the baseline assessment could reflect concurrent illness, mortality classification trees were repeated for the subgroup who survived the first year of follow-up. As baseline assessments took place over a 6-year period, available follow-up time was shorter for later participants; thus, mortality classification trees were repeated with follow-up truncated at 12 years for all participants. Mortality risk ratios were also estimated with proportional hazards regression. As findings were similar with all strategies, only findings using all the available follow-up and logistic regression models are reported.

Analytical procedures recommended by NHANES were used, and sampling weights for complex survey designs were incorporated in all analysis [10,35]; WTCYPEX6, WTPFEX6, SDPPSU6 and SDPSTRA6 were used as cystatin C-population weight, overall-population weight, cluster and stratum and variables, respectively. SUDAAN, v10 (Research Triangle Institute, Research Triangle Park, NC) and SAS, v9.1.3 (Cary, NC) were used for data analysis.

Results

Table 1 shows the baseline characteristics of the study population. Mean age was 44.43 years. Mean cystatin C level was 0.90 mg/L, standardized serum creatinine 0.84 mg/dL, eGFR$_{Cystatin\,C}$ 93.42 mL/min/1.73 m² and eGFR$_{Creatinine}$ 99.54 mL/min/1.73 m². While older age was associated with higher cystatin C ($r = 0.49$), higher serum creatinine ($r = 0.21$), higher ACR ($r = 0.09$) and lower eGFR$_{Creatinine}$ levels ($r = -0.75$), no linear correlation between age and eGFR$_{Cystatin\,C}$ was observed. Other correlations of older age included systolic blood pressure, diastolic blood pressure, body mass index, total cholesterol, female sex, white race/ethnicity and self-reported hypertension, diabetes and cardiovascular disease; correla-

Table 1. Characteristics of US Adults, 1988–94

| Characteristics	Overall (n = 15,124) Mean or % (SE) Median [IQR]	With measured cystatin C levels (n = 6656)		
		Mean or % (SE) Median [IQR]	Correlation with Age	
			r	P
Cystatin C, mg/L	–	0.90 (0.01)	0.49	<0.001
eGFR$_{Cystatin C}$, mL/min/1.73 m^2	–	93.42 (0.83)	−0.26	0.2
Creatinine, mg/dL	0.84 (0)	0.84 (<0.01)	0.21	<0.001
eGFR$_{Creatinine}$, mL/min/1.73 m^2	99.14 (0.51)	99.54 (0.77)	−0.75	<0.001
Urinary albumin–creatinine ratio, mg/g	5.74 [3.60–10.34]	5.8 [3.5–10.6]	0.09	<0.001
Age, years	44.63 (0.46)	44.43 (0.75)	–	–
Systolic blood pressure, mm Hg	122.43 (0.4)	122.5 (0.53)	0.57	<0.001
Diastolic blood pressure, mm Hg	74.19 (0.18)	74.39 (0.3)	0.14	<0.001
Body mass index, mm Hg	26.56 (0.11)	26.62 (0.17)	0.11	<0.001
Total cholesterol, mg/dL	208.72 (0.87)	208.36 (1.3)	0.36	<0.001
HDL cholesterol, mg/dL	50.71 (0.35)	50.24 (0.38)	0.03	0.06
Women, %	52.13 (0.48)	52.3 (1.53)	0.05	0.007
White, %	76.75 (1.27)	76.46 (1.89)	0.13	<0.001
African American, %	10.37 (0.59)	10.94 (0.92)	−0.06	<0.001
Hispanic, %	5.1 (0.42)	5.1 (0.54)	−0.09	<0.001
Other, %	7.78 (0.84)	7.51 (1.21)	−0.06	0.01
Hypertension, %	23.65 (0.66)	23.89 (0.91)	0.30	<0.001
Diabetes, %	5.32 (0.26)	4.98 (0.45)	0.17	<0.001
Cardiovascular disease, %	5.53 (0.35)	4.79 (0.52)	0.30	<0.001
Current smoker, %	28.36 (0.84)	28.87 (1.24)	−0.19	<0.001

eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; SE, standard error. Missing data, overall population/ with cystatin C levels: systolic blood pressure, n = 19/6; diastolic blood pressure, n = 21/7; body mass index, n = 24/12; HDL cholesterol, n = 139/54.

tions of younger age included African American and Hispanic race-ethnicity and smoking.

The correlation between eGFR$_{Cystatin C}$ and eGFR$_{Creatinine}$ was modest (r^2 = 0.11). On pairwise comparison, eGFR$_{Creatinine}$ levels were higher than eGFR$_{Cystatin C}$ levels; the median difference was 7.20 and corresponding 5th, 25th, 75th and 95th percentiles were −23.3, −4.68, 18.98 and 34.53 mL/min/1.73 m^2, respectively.

A death rate of 1.33% per year was observed over the follow-up interval of 12.20 years. Figure 1 shows sensitivity and specificity values for predicting death at different cystatin C thresholds. A threshold value of 0.94 mg/L exhibited the highest maximum combined value of sensitivity (Sn) and specificity (Sp) for predicting death (Max$_{Sn + Sp}$, Sn 0.64/Sp 0.78).

Max$_{Sn + Sp}$ thresholds for other intrinsically continuous variables are shown in Table 2, as are mortality odds ratios. Ranked by Max$_{Sn + Sp}$, age >58 years was the best discriminator between survival and death, followed by eGFR$_{Creatinine}$ ≤92 mL/min/1.73 m^2, systolic blood pressure >127 mm Hg, cystatin C >0.94 mg/L, ACR >11 mg/g, self-reported hypertension, total cholesterol >204 mg/dL, self-reported cardiovascular disease, standardized serum creatinine >0.97 mg/ dL, diabetes, body mass index >27 kg/m^2 and HDL cholesterol ≤40 mg/dL. While summations of sensitivity and specificity values were similar for eGFR$_{Creatinine}$ ≤92 mL/min/ 1.73 m^2 and cystatin C >0.94 mg/L, the former was comparatively more sensitive and less specific than the latter; in addition, adjustment for age negated the statistical significance of eGFR$_{Creatinine}$ ≤92 mL/min/1.73 m^2.
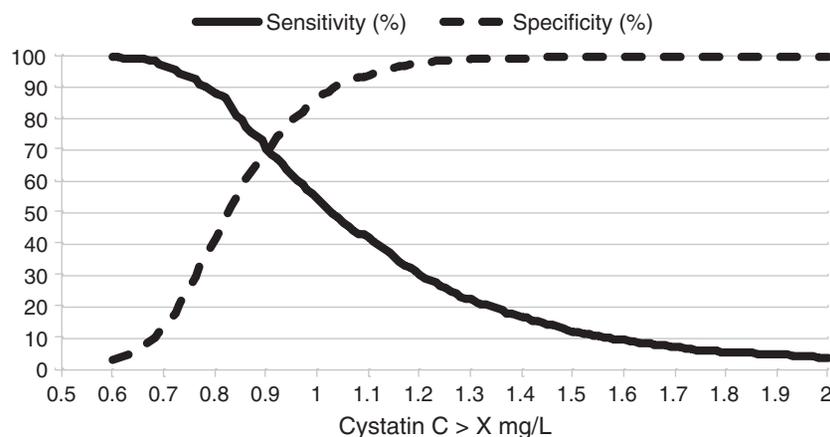
Fig. 1. Sensitivity (cystatin C > X among subjects who died) and specificity (cystatin C ≤ X subjects who died) for predicting death at different cystatin C thresholds. A threshold value of 0.94 mg/L exhibited Max$_{Sn + Sp}$ (Sn 0.64/Sp 0.78).

Table 2. Threshold values for mortality discrimination, ranked by maximum true positive and true negative values[a]

| Rank	Risk factor	Prevalence (%)	Sensitivity/ specificity	OR, death, unadjusted	P	OR, death, age adjusted	P
Overall population							
1	Age >58	22.9	0.73/0.87	18.2 (13.9–23.9)	<0.001	–	–
2	eGFR$_{Creatinine}$ ≤92	32.3	0.71/0.75	7.4 (5.7–9.7)	<0.001	0.9 (0.7–1.2)	0.5
3	Systolic BP >127	31.0	0.66/0.76	6.2 (4.9–8.0)	<0.001	1.5 (1.1–2.1)	0.007
4	Cystatin C >0.94	28.5	0.64/0.78	6.3 (5.2–7.5)	<0.001	1.6 (1.4–1.9)	<0.001
5	ACR >11	24.0	0.51/0.81	4.4 (3.5–5.6)	<0.001	2.5 (2.0–3.1)	<0.001
6	Hypertension	23.9	0.44/0.80	3.2 (2.5–4.2)	<0.001	1.5 (1.1–2.1)	0.02
7	Cholesterol >204	47.1	0.62/0.56	2.0 (1.7–2.4)	<0.001	0.9 (0.7–1.1)	0.4
8	CVD	4.8	0.19/0.98	11.5 (8.7–15.3)	<0.001	3.0 (2.2–4.1)	<0.001
9	Creatinine >0.97	14.7	0.28/0.88	2.8 (2.3–3.5)	<0.001	1.4 (1.2–1.7)	<0.001
10	Diabetes	5.0	0.14/0.97	4.9 (3.5–6.7)	<0.001	2.7 (1.8–4.1)	<0.001
11	BMI >27	39.4	0.46/0.62	1.4 (1.2–1.6)	<0.001	1.2 (1.0–1.5)	0.1
12	HDL ≤40	26.4	0.30/0.74	1.3 (1.1–1.5)	0.01	1.3 (1.1–1.7)	0.02
Age ≤58							
1	Age >42	29.9	0.62/0.72	4.2 (2.5–7.1)	<0.001	–	–
2	Systolic BP >125	23.4	0.43/0.78	2.7 (1.4–5.1)	0.002	1.8 (1–3.3)	0.064
3	Cystatin C >0.82	48.1	0.68/0.53	2.4 (1.3–4.3)	0.005	1.9 (1.1–3.4)	0.028
4	BMI >27	37.8	0.56/0.63	2.2 (1.5–3.4)	<0.001	1.8 (1.1–2.8)	0.011
5	ACR >11	18.4	0.36/0.83	2.7 (1.6–4.4)	<0.001	2.4 (1.5–3.9)	0.000
6	Smoking	32.9	0.49/0.68	2 (1.3–3.2)	0.002	2.4 (1.5–3.9)	0.000
7	Cholesterol >239	14.4	0.29/0.86	2.6 (1.9–3.6)	<0.001	1.8 (1.2–2.6)	0.002
8	Hypertension	17.8	0.32/0.83	2.3 (1.3–4.2)	0.007	1.7 (0.9–3.2)	0.080
9	Diabetes	3.3	0.11/0.97	4.0 (2.0–8.0)	<0.001	2.7 (1.2–6)	0.015
10	CVD	1.4	0.07/0.99	7.5 (3.2–17.7)	<0.001	4.4 (1.7–11.6)	0.003
11	African American	11.6	0.17/0.89	1.6 (1.1–2.2)	0.012	1.7 (1.2–2.4)	0.005
12	Creatinine >1.17	1.5	0.05/0.99	4.4 (2.4–8.2)	<0.001	3.6 (1.9–6.7)	0.000
Age >58							
1	Age >71	39.2	0.58/0.81	5.8 (4.6–7.4)	<0.001	–	–
2	Cystatin C >1.15	32.2	0.47/0.84	4.6 (3.7–5.6)	<0.001	2.9 (2.3–3.7)	<0.001
3	ACR >12	40.1	0.54/0.74	3.4 (2.9–4)	<0.001	2.7 (2.3–3.2)	<0.001
4	eGFR$_{Creatinine}$ ≤68	30.9	0.42/0.81	3.1 (2.5–3.7)	<0.001	1.7 (1.4–2.1)	<0.001
5	Systolic BP >135	52.8	0.62/0.57	2.1 (1.8–2.6)	<0.001	1.5 (1.2–1.8)	<0.001
6	CVD	16.3	0.23/0.91	3.3 (2.6–4.2)	<0.001	2.8 (2.1–3.6)	<0.001
7	Creatinine >0.88	42.7	0.5/0.65	1.8 (1.5–2.1)	<0.001	1.5 (1.2–1.9)	<0.001
7	Hypertension	44.3	0.49/0.61	1.5 (1.2–1.8)	<0.001	1.5 (1.2–1.8)	<0.001
8	Diabetes	10.6	0.15/0.94	3 (2.2–4.2)	<0.001	3.4 (2.4–4.9)	<0.001
9	HDL ≤44	38.2	0.42/0.66	1.4 (1.2–1.7)	<0.001	1.7 (1.4–2.2)	<0.001
10	Men	43.1	0.47/0.61	1.3 (1.1–1.7)	<0.001	1.6 (1.2–2.1)	<0.001
11	Smoking	15.2	0.18/0.88	1.7 (1.3–2.2)	<0.001	3.3 (2.4–4.4)	<0.001
12	African American	8.6	0.1/0.93	1.4 (1.1–1.7)	<0.001	1.6 (1.3–1.9)	<0.001

ACR, albumin–creatinine ratio; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; OR, odds ratio.Units: Age, years; albumin–creatinine ratio, mg/g; body mass index, kg/m^2; cholesterol (HDL, LDL, total), mg/dL; creatinine, mg/dL; estimated glomerular filtration rate, mL/min/1.73 m^2; glucose, mg/dL; systolic blood pressure, mm Hg.

[a]Thresholds for intrinsically continuous variables (those showing maximum combined true positive and true negative values for predicting death) were determined by applying 1-unit increments in the following ranges: ACR, 1–100 mg/g; age, 20–90 years; eGFR, 30–120 mL/min/1.73 m^2; systolic blood pressure, 90–150 mm Hg; LDL cholesterol, 70–200 mg/dL; glucose, 7–140 mg/dL. Increments of 0.01 units were used for creatinine (between 0.50 and 2.00 mg/dL) and cystatin C (between 0.60 and 2.00 mg/L). Odds ratios (with 95% confidence intervals in parentheses) were calculated from logistic regression models with death (no/yes) as outcome variable. Sensitivity, the proportion with the risk factor among participants who died; specificity, the proportion without the risk factor among participants who survived. The comparator for all variables was absence of the indicated condition.

Because age exhibited the highest mortality discrimination, age ≤58 and >58 years were the first two branches of the classification tree. Table 2 ranks candidate variables by thresholds of Max$_{Sn + Sp}$ in the subgroup aged ≤58 years. Age >42 years was the best discriminator, followed by systolic blood pressure >125 mm Hg, cystatin C >0.82 mg/L, body mass index >27 kg/m^2, ACR >11 mg/g, smoking, total cholesterol >239 mg/dL, hypertension, diabetes, cardiovascular disease, African American race/ethnicity and standardized serum creatinine >1.17 mg/dL. Table 2 also ranks candidate variables in the subgroup aged >58 years. Age >71 years was the first-ranked discriminator, followed by cystatin C >1.15 mg/L, ACR >12 mg/g, eGFR$_{Creatinine}$ ≤68 mL/min/1.73 m^2, systolic blood pressure >135 mm Hg, cardiovascular disease, standardized serum creatinine >0.88 mg/dL, hypertension, diabetes, HDL cholesterol ≤44 mg/dL, male sex, smoking and African American race/ethnicity.

Figure 2 shows first-ranked discriminators of death or survival in subgroups defined by higher order Max$_{Sn + Sp}$ values, with all variables considered jointly. Among continuous variables, only cystatin C and ACR were represented in the classification tree. Cystatin C was a primary discriminator in subgroups that added up to 41% of the
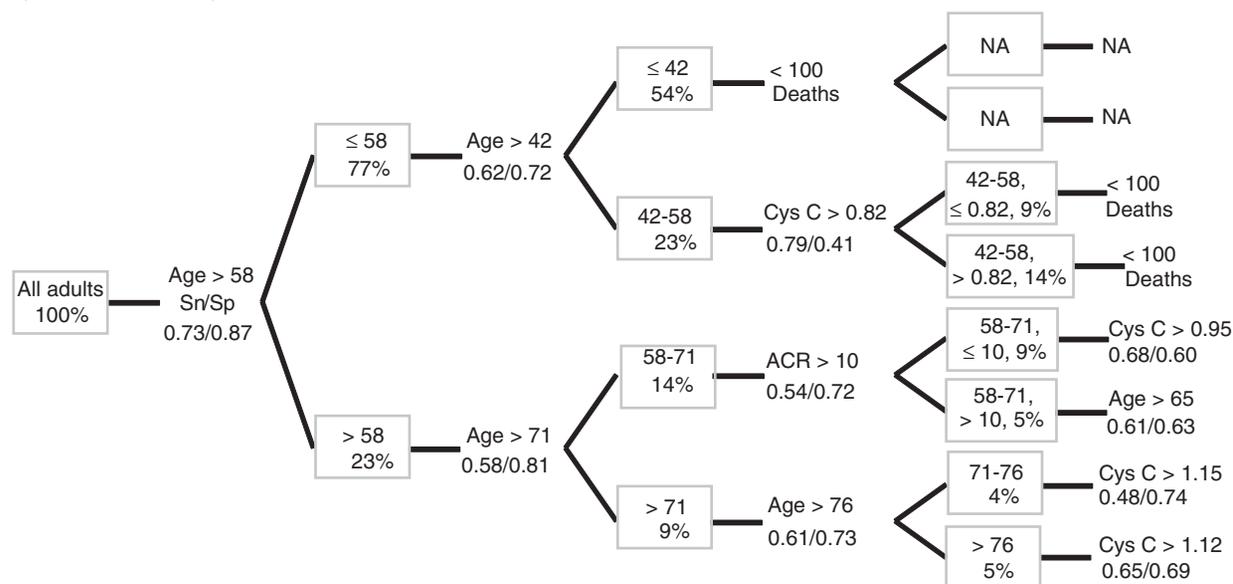
Fig. 2. Mortality classification tree, based on maximum combined value of sensitivity and specificity (see Table 3). ACR, urinary albumin–creatinine ratio; Cys C, cystatin C; NA, not analysed (because fewer than 100 deaths available); Sn, sensitivity for predicting death; Sp, specifity for predicting death. Units: age, years; albumin–creatinine ratio, mg/g; cystatin C, mg/L.

study population: age 42–58 years (23%), age 58–71 years with ACR ≤10 mg/g (9%), age 58–71 years with ACR > 10 mg/g (5%) and age 71–76 years (4%). ACR was a primary discriminator in the subgroup aged 58–71 years (14%).

Table 3 shows mean ages, death rates and mortality odds ratios when the terminal nodes of the classification tree shown in Figure 2 were used to classify the study population. Overall, this classification system appeared to graduate mortality risk relatively efficiently, whether or not age adjustment was used.

Discussion

We attempted to identify threshold values for cystatin C that maximally discriminate death from survival and to determine their hierarchical importance when two themes

were explored: performance in relation to routinely measured renal (e.g. serum creatinine, $eGFR_{Creatinine}$ and urinary ACR) and periodic health screening (e.g. body mass index, cholesterol and blood pressure) parameters and a hierarchical analytical approach that incorporated the possibility that association estimates might differ in large segments of the overall population. In the overall population, and using analyses that did not consider the possibility of association modification, optimal cystatin C, $eGFR_{Creatinine}$ and ACR thresholds demonstrated similar prognostic discrimination, close to, or exceeding, that exhibited by other commonly recommended periodic health screening measures. In contrast, serum creatinine appeared to be a less useful discriminator than cystatin C, $eGFR_{Creatinine}$ or ACR. Finally, the classification tree analysis suggested that cystatin C and urinary ACR might be useful for clinical triage in meaningful segments of the overall population.

Table 3. Mortality risk estimates from categories derived from classification tree analysis[a]

Categories	Prevalence	Mean age	Death rate	OR, death, unadjusted	OR, death, age adjusted
Age ≤42	54.1	31.2	2.0	1 (Referent)	1 (Referent)
Age >42, ≤58, cystatin C ≤0.82	8.9	49.1	4.4	2.2 (1–4.5)	1.3 (0.7–2.7)
Age >42, ≤58, cystatin C >0.82	14.1	50.5	10.6	5.7 (3.3–9.8)	3.4 (1.9–6.2)
Age >58, ≤71, ACR ≤10, cystatin C ≤0.95	4.6	64.2	11.7	6.3 (3.8–10.3)	2.6 (1.3–5.4)
Age >58, ≤71, ACR ≤10, cystatin C >0.95	4.1	65.1	29.7	19.5 (12–31.8)	7.9 (4.1–15.2)
Age >58, ≤65, ACR >10	2.6	62.4	32.5	21.6 (13.2–35.4)	9.5 (5.1–17.7)
Age >65, ≤71, ACR >10	2.6	68.5	61.2	56.7 (35.1–91.6)	21.1 (9.3–47.9)
Age 71–76, cystatin C ≤1.15	2.6	73.6	48.3	39.7 (23.5–67.1)	12.9 (4.8–34.2)
Age 71–76, cystatin C >1.15	1.7	74.1	81.1	103.9 (60.6–178.1)	33.2 (13.7–80.4)
Age >76, cystatin C ≤1.12	1.8	80.7	86.2	120.9 (71.4–204.6)	32.4 (10.7–98)
Age >76, cystatin C >1.12	2.9	82.4	140.1	508.5 (284.7–908.1)	130.5 (43.9–387.6)
				Model P < 0.0001	Model P < 0.0001

ACR, albumin–creatinine ratio; OR, odds ratio. Units: age, years; albumin–creatinine ratio, mg/g; cystatin C, mg/L.
[a]Death rates are per 1000 subject-years. Logistic regression was used to calculate odds ratios for death. Values in parentheses are 95% confidence intervals.

The classification tree methodology used here differs substantially from more traditional multivariate regression-based methods. In particular, because the decision to nominate a 'winner' was based on threshold values of maximum combined sensitivity and specificity, risk factor prevalence is a substantial component of the decision-making process. Another potential advantage of the newer approach is that it systematically unmasks interactions and subgroups in which mortality risk estimates differ substantially. Finally, this approach is intrinsically hierarchical and allows different threshold values to be compared, both within and across risk factors. These features (incorporation of risk factor prevalence, a systematic approach to subgroup formation, and a natural tendency to form hierarchies and thresholds) could be viewed as very useful for public health initiatives such as screening.

Associations between kidney function and mortality have been studied extensively. For example, a PubMed search of human studies carried out in April 2010 with the search terms 'mortality or survival', 'glomerular filtration rate or cystatin C or creatinine or albuminuria or proteinuria or chronic kidney disease' and 'community or general population' yielded 1555 citations, a value that fell to 106 and 0, respectively, with sequential addition of the terms 'sensitivity and specificity' and 'threshold'. Hence, while many studies have examined associations between kidney function and mortality, it appears that few studies have attempted to define maximally discriminatory threshold values regarding death or survival. One exception was a Swedish study of 50-year-old men followed for 20 years, which examined optimal creatinine clearance thresholds for future occurrence of myocardial infarction and cardiovascular death [36]. Thresholds of 98 mL/min/1.73 m^2 for myocardial infarction and 92 mL/min/1.73 m^2 for cardiovascular death were identified.

While it remains an area of active research and findings are not completely homogenous, the preponderance of available evidence suggests that cystatin C level may be a better measure of GFR than a measure based on serum creatinine. When one further considers the superior prediction of adverse events such as cardiovascular disease and death [37–43], it is tempting to speculate that cystatin C may supplant creatinine as a 'routine' measure of GFR in clinical practice.

In public health, threshold values of essentially continuous risk markers are often used to identify at-risk individuals in whom more rigorous follow-up and treatment are indicated. For outcomes like death, a traditional method for defining appropriate thresholds involves identification of a notional point at which risk ratios change rapidly. This approach fails to naturally incorporate the influence of risk-factor prevalence. In addition, what constitutes a sudden acceleration of risk appears to be a largely subjective construct. Finally, this traditional approach does not lend itself naturally to production of hierarchies among diverse risk factors. In public health, where lengthening survival is the prime objective, it seems intuitively obvious to advance the case for a threshold that maximizes the chances of 'normal' levels of the risk factor predicting survival and 'abnormal' levels predicting death. Similarly, methodologies that naturally tend to identify major subgroups, interactions and hierarchies among risk factors may be attractive, from a public health standpoint. An approach that uses death analogously to a diagnostic test, combined with classification tree methodology, seems to address many of these issues.

This study has limitations. Findings were generated from the US population between 1988 and 1994, and generalizability to other populations and eras is difficult to quantify. In the NHANES III, the precision of cause-specific mortality determinations is unknown, and we did not attempt to identify separate cystatin C, creatinine, eGFR and ACR thresholds for renal and cardiovascular death. The study protocol did not include state-of-the-art measurement techniques for renal parameters, such as inulin and radioisotope clearance to measure GFR and timed urine collection to measure urinary albumin excretion. Renal parameters were measured only once, precluding identification of individuals with rapidly progressive loss of kidney function. Cystatin C levels likely reflect things other than GFR, such as inflammation. If this is true, some or all of the mortality discrimination afforded by cystatin C could reflect an association between inflammation and mortality. Finally, there is heterogeneity in the methods used to measure cystatin C within different laboratories; when cystatin C methodology becomes fully standardized, it is possible that the threshold values identified here may shift.

Limitations notwithstanding, this study has some attractive features. By design, the study is representative of the US population as a whole, at least between 1988 and 1994. Several commonly measured risk-stratification measures, such as body mass index, blood pressure and cholesterol were routinely available. While further clarification is needed, this study suggests that measuring cystatin C may be useful for clinical triage in public health settings.

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