Prognostic Value of Isolated High Serum Cystatin C Levels Without Glomerular Filtration Rate Reduction

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Research Article

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Abstract

OBJECTIVES: Cystatin C is increasingly used as a marker of renal function as a complement or alternative to serum creatinine and GFR calculated from it. However, cystatin C is also a marker of inflammation. We have assessed its efficacy as a predictor of mortality in a group of patients with increased cystatin C and GFR > 60 ml/min.

DESIGN AND METHODS: We included 608 patients seen at our hospital, 65.9% of whom were male and 34.6% had diabetes mellitus. The mean age was 58.5±14.5 years and a mean GFR of 64.1±33.5 ml/min. Patients were divided into 3 groups: CONTROL (normal cystatin C and GFR > 60 ml/min, age 53.3±12.8 years, GFR 96.6±22.4 ml/min, n=193), INCREASED CYSTATIN (increased cystatin C and GFR >60 ml/min, age 58.9±13, 1 years, GFR 72.2± 10.4 ml/min, n = 40 ) and CKD (chronic kidney disease, increased cystatin C and GFR <60 ml/min, age 61.4±14.8 years, GFR 36.0±12.7 ml/min, n = 160). The relationship with overall mortality was analyzed using the survival curve by Kaplan-Meier method.

RESULTS. Mean cystatin C was 0.75±0.13 mg/l, versus 1.79±0.54 in the CKD group and 1.14±0.14 mg/l, p<0.001 ANOVA). In the CONTROL group survival was 93.9% at five years, compared to 78.8% in the ERC group and 82.3% in the INCREASED CYSTATIN group (p < 0.001 Log Rank). Five-year survival before renal replacement therapy was also different for the ERC group (73%, p < 0.001 Log Rank) but not between the other two groups (CONTROL 99.0%, INCREASED CYSTATIN 94.3% p = 0.08 Log Rank).

CONCLUSIONS. The presence of increased plasmatic levels of cystatin C in patients with GFR > 60 ml/min was a predictor of increased mortality but not of progression to end-stage renal failure. These results confirm the interest of routinely measuring cystatin C in our patients.

INTRODUCTION

Chronic Kidney Disease (CKD) affects 10% of the world's populatio and ranks in the top ten noncommunicable diseases contributing to disease and disability. Its incidence is increasing worldwide, and mortality owing to CKD rose between 2005 and 2017 from 0.9 million to 1.2 million deaths annually.

Serum creatinine and creatinine clearance were first used to evaluate renal function by the Danish physiologists, Rehberg and Holten in the mid-1920s. Serum creatinine is the only renal plasma biomarker currently used in daily clinical practice to estimate GFR. However, a proper interpretation of the serum creatinine result remains sometimes problematic. Creatinine clearance is a relatively easy method to estimate GFR but it has some important limitation of this measurement (i.e. creatinine tubular secretion which is variable from one subject to another). Creatinine clearance systematically overestimates measured GFR and this overestimation is higher at low GFR levels.Nowadays, the creatinine based equations, especially the Modification of Diet in Renal Disease study (MDRD) equation and the CKD-EPI one, are used all over the world to estimate GFR.
Cystatin C (CysC) is an interesting new marker for the estimation of GFR. It does offer several advantages over creatinine or other similar molecular weight proteins. CysC is produced by all nucleated cells in the human body and because the protein is coded by a housekeeping gene, (i.e. a gene expressed both constitutively and in an unregulated manner), CysC is considered to be constantly produced. After being filtered without restriction by the glomeruli because of its low molecular mass and absence of protein binding, CysC is entirely reabsorbed by the proximal tubules, where it is almost entirely catabolized. Current KDIGO Guidelines for CKD suggests measuring CysC in adults with GFR 45–59 ml/min/1.73 m2 who do not have markers of kidney damage if confirmation of CKD is required.

The proportion of CKD patients defined by GFR who progress to end-stage renal disease is extremely small. The KDIGO Guidelines recognized the importance of albuminuria, and the underlying diagnosis as well as GFR. A table was designed to categorized risk in populations with CKD using group eGFR and albuminuria severity, although the evidence was not graded. We have tried to evaluate the value of isolated high plasma CysC levels in patients with normal plasma creatinine level and estimated GFR higher than 60 ml/min as renal risk marker.

**DESIGN AND METHODS**

A group of 608 patients were studied: 401 males and 207 females; mean age was 53.3 ± 12.8 years; 34.6% have diabetes mellitus. Serum cystatin C was measured using a BNII nephelometer (Dade Behring Inc., Deerfield, IL, USA) that used a particle-enhanced immunonephelometric assay (N Latex Cystatin-C). The assay range is 0.195–7.330 mg/L, with the reference range for young healthy individuals reported as 0.53–0.95 mg/L. Microalbuminuria was measured in 24 h urine collection and 18.5% showed increased urine albumin excretion (≥ 30 mg/day). GFR was estimated from serum creatinine using the CKD-EPI equation for every sex. Only Caucasian patients were included in the study, so that race was not included in calculation. Albuminuria was analyzed in 24h urine collection.

Patients were classified according to KDIGO stages of chronic renal disease: 13.6% were in stage IV or V, 30.9% were in stage III, and the remaining patients had GFR higher than 60 mL/min (39.1%). The cut-off point for the highest quartile of serum cystatin C distribution was 1.03 mg/L. GFR was estimated from cystatin C using the CKD-EPI formulation for cystatin C. Microalbuminuria was defined as an urinary albumin excretion ≥ 30 and < 300 mg/day; macroalbuminuria was diagnosed when albuminuria was equal or higher than 300 mg/day.

Patients were split into three groups: CONTROL (normal cystatin C levels and GFR > 60 ml/min, n = 193), HIGH CYSTATIN (HCy), (cystatin C higher than 1.03 mg/l but GFR > 60 ml/min, n = 40 ) and CKD (Chronic Kidney Disease, cystatin C above 1.03 mg/g and GFR < 60 ml/min, n = 160). Table 1 show the characteristics of each group.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration...
and its later amendments or comparable ethical standards. The ethical approval was supplied by the University of Extremadura Ethics Committee (#21/2014). Informed consent was obtained from all individual participants included in the study.

Statistics

Results are expressed as mean ± 1 standard deviation. All statistical tests were two-sided. P values lower than 0.05 were considered as significant. For comparisons between groups, Anova test and Bonferroni post-hoc analysis was used for continuous variables and Chi-square test for categorical variables. Since Kolmogorov-Smirnov Z test found that albuminuria did not follow a normal distribution, Kruskall-Wallis test was used to compare values. These parameters have been expressed as median (IR, interquartilic range). The statistical analysis was developed with the package SPSS 21.0.

Kaplan-Meier survival analysis was used to calculate survival before death, reaching stage V KDIGO CKD or renal replacement therapy. Associations between the three subgroups of patients and risk of death or reaching renal replacement therapy were assessed using Cox proportional hazards survivorship model. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were calculated, and a p value < 0.05 was considered to be statistically significant. All variables achieving a significance level of p < 0.1 in univariate analysis were considered for inclusion in the construction of the Cox model. The data were analyzed using the IBM® statistical program SPSS ® Statistics V.21 (IBM Corporation, Armonk, NY, USA).

RESULTS

Median follow-up time was 72 months (IQR 60–76). Mean plasma cystatin in the CONTROL group was 0.74 ± 0.14 mg/l, vs. a 1.80 ± 0.54 (CKD group) and 1.14 ± 0.14 mg/l (HCy group), (p < 0.001 ANOVA). The control group was younger than the other groups (p < 0.001, ANOVA). Urinary albumin excretion was higher in the CKD group compared with the others (see values and significances in Table 3). Mean GFR from cystatin C was 67.0 ± 7.8 ml/min for HCy group, 107.1 ± 22.6 for control group (p < 0.001 vs. other groups, ANOVA) and 44.1 ± 13.2 for the CKD one (p < 0.001 vs. other groups, ANOVA).

In the CKD group 63.1 (95%CI 55.4–70.2) of patients were in stage 3 KDIGO, 35.6% (95%CI 28.6–43.3) subjects were in stage 4, and only 2 ones (1.25, 95%CI 0.03–4.44). In the CONTROL group 21.8% (95%CI 16.5–28.4) of patients have microalbuminuria and 12.8 (95%CI 8.7–18.3) macroalbuminuria; in the CKD one 36.2% (95%CI 29.0-44.1) of patients have microalbuminuria and 40.8 (95%CI 33.3–48.7) macroalbuminuria; and in the HCy group 42.1. % (95%CI 27.9–57.8) of patients have microalbuminuria and 18.4 (95%CI 9.2–33.4) macroalbuminuria (p < 0.001, square Chi test).

Kaplan-Meier survival for general mortality at 5 years was 93.9% for CONTROL group, 78.8% for CKD group and 82.3% in the HCy group (p < 0.001, Log Rank test for the difference between CONTROL group and HCy one) (Fig. 1). Regarding survival before starting renal replacement therapy or reaching stage V KDIGO, the Kaplan-Meier method shows a survival at 5 years 99.0% for CONTROL group, 73.0% for CKD
group and 94.3% in the HCy group (p < 0.001 Log Rank for the CKD group, there is no difference between HCy and Control ones, p = 0.08) (Fig. 2).

In the global sample, after adjusting for possible confounding factors, Cox analysis showed a significant relationship of high cystatin C levels with mortality (p < 0.001), only age and diabetes mellitus showed a significant relationship (see Table 2). Contrariwise, after adjusting for possible confounding factors, survival before reaching renal replacement therapy was significantly associated with cystatin C levels (p = 0.008), GFR measured by CKD-EPI equation and urinary albumin excretion (see significances in Table 3).

**DISCUSSION**

We have found that in a group of patients with high plasma cystatin C levels and GFR below 60 ml/min mortality was higher than in those patients with normal cystatin C levels and GFR > 60 ml/min. Contrariwise, the risk of progression of chronic kidney disease was not higher in the HCy group. Those patients with high cystatin levels and reduced GFR both mortality and CKD have the highest risk of mortality and progression to stage V KDIGO of CKD or renal replacement therapy.

The renal system carries several physiologic roles but GFR is considered the best surrogate of overall kidney function and, for this reason, its assessment has become an important tool in clinical practice. GFR cannot be measured directly, but instead can be estimated by the clearance of filtration markers. Regardless, the clinical assessment of GFR can aid the clinician in estimating the degree of renal dysfunction and/or progression of established kidney disease. Various creatinine-based equations have been developed in an attempt to improve the estimation of GFR from serum creatinine. Current KDIGO Guidelines recommend CKD-EPI Eq. 7 and that is the one that we have selected. In this regard, the current definition for chronic kidney disease is a GFR < 60 ml/min for more than three months and this is the threshold that was selected to split the study group.

Patients with CKD exhibit a pronounced risk for cardiovascular events: 50% of all patients with CKD stage 4 to 5 have CVD, and cardiovascular mortality accounts for ≈ 40–50% of all deaths in patients with advanced CKD (stage 4) as well as end-stage kidney disease (stage 5), compared with 26% in controls with normal kidney function. The proportions of deaths from heart failure and valvular disease specifically increased with declining eGFR along with the proportions of deaths from infectious and other causes, whereas the proportion of deaths from cancer decreased. In the same way, lower eGFR is associated with an increased mortality risk caused by infection. The cut point used was, as in our study, a GFR < 60 ml/min but mortality it is specially increased when GFR get down of 45 ml/min. Therefore, it could explain why mortality is higher in the CKD group but cannot give light about the relationship of higher plasma cystatin levels with a GFR above 60 ml/min and mortality.

Cystatin C is an interesting marker for the estimation of GFR. It does offer several advantages over creatinine or other similar molecular weight proteins. It is produced by all nucleated cells in the human body and is considered to be constantly produced. After being filtered without restriction by the glomeruli
because of its low molecular mass and absence of protein binding, cystatin C is entirely reabsorbed by
the proximal tubules, where it is almost entirely catabolized. Current KDIGO Guidelines for CKD suggests
measuring cystatin C in adults with GFR 45–59 ml/min/1.73 m2 who do not have markers of kidney
damage if confirmation of CKD is required. Cystatin C is also a correlate of cardiovascular risk. These
associations persisted with the additional exclusion of persons with CKD or with microalbuminuria.23
Nevertheless, these two abilities of cystatin C seem not to be correlated in our study. In fact, mortality was
independently associated with cystatin C levels but this relationship was not found with eGFR or
albuminuria. These findings can be interpreted in various ways. First of all, persons with high cystatin C
levels but without CKD may have preclinical CKD and an associated elevated risk factor burden that is
similar to persons with CKD. Because of measurement error involved in the quantification of eGFR, it is
also possible that persons with high cystatin C but GFR that is not in the CKD range are more accurately
classified as renal patients by using cystatin C levels. Nevertheless, this hypothesis seems unlikely since
GFR calculated from cystatin C or creatinine rendered very close results. Moreover, cystatin C -without
diminished GFR- is not associated to CKD progression. Alternatively, high cystatin C in persons without
CKD may be the result of extra-renal sources of cystatin C variability and so that they were not related to
kidney function. There is some evidence that serum cystatin C level is heritable and high serum cystatin C
levels were associated with most major CVD risk factors.23

Increased urinary albumin excretion in diabetic patients has been found to be a predictor of progression
of diabetic nephropathy and also as a powerful independent risk factor for cardiovascular morbidity and
mortality. In nondiabetic hypertensive patients urinary albumin excretion has been shown to predict
cardiovascular events, and a continuous relation between urinary albumin excretion and cardiovascular,
as well as general, mortality has been demonstrated in a general population study. Therefore, searching
for microalbuminuria is currently recommended, because of the evidence that it may be a sensitive
marker of target organ damage, not only in diabetes but also in hypertension. Therefore, current KDIGO
Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease state to assess
GFR and albuminuria at least annually in people with CKD. Moreover, GFR and albuminuria should be
assessed more often for individuals at higher risk of progression, and/ or where measurement will impact
therapeutic decisions. Although a significant proportion of subjects in the CONTROL and HCy had
increased urinary albumin excretion, it was not associated to mortality risk, but it was significantly related
to CKD progression.

**Strengths and limitations**

The mean limitation of this study is the small size of the sample with high cystatin C values and GFR
below 60 ml/min. Nevertheless, this kind of patients are uncommon in the clinical practice and, therefore,
the information resulting for our results become more important. As a matter of fact, few data have been
reported on this issue. Shlipak et al.14 used data from de Cardiovascular Health Study where, at baseline,
78% of participants have estimated GFR > or = 60 mL/min. Cystatin C concentrations had strong
associations with death, cardiovascular death, and major cardiovascular events among these
participants. Serum creatinine concentrations had much weaker associations with each outcome and only predicted cardiovascular death. This study did not measure albuminuric and, so that, our study, that included this parameter offers valuable data on the relationship of cystatin C and mortality.

Conclusions

Cystatin C is a good marker of mortality in patients without renal disease defined as a GFR below 60 ml/min. In this group of patients neither GFR nor albuminuria showed independent effect. Contrariwise, albuminuria and baseline GFR, as well as, cystatin C, were prognostic markers of kidney disease progression in patients with CKD. Isolated cystatin C plasma levels are a good marker of mortality but are not associated to higher risk of chronic kidney disease.

Declarations

The authors declare no conflict of interest.

References


Tables
Table 1

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<thead>
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<th></th>
<th>CONTROL</th>
<th>HCY</th>
<th>CKD</th>
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<tbody>
<tr>
<td>n</td>
<td>193</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>AGE (years)</td>
<td>53.3 ± 12.8</td>
<td>58.9 ± 13.1*</td>
<td>61.4 ± 14.8*</td>
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<tr>
<td>MALE GENDER (%)</td>
<td>47.2</td>
<td>40.0</td>
<td>55.9</td>
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<tr>
<td>DIABETES MELLITUS (%)</td>
<td>24.4</td>
<td>17.5</td>
<td>39.8$</td>
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<td>CYSTATIN C</td>
<td>0.74 ± 0.14</td>
<td>1.14 ± 0.14*</td>
<td>1.80 ± 0.54* &amp;</td>
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<td>ALBUMINURIA</td>
<td>12.1 (5.3–51.0)</td>
<td>49.0 (12.4–213)*</td>
<td>223.5 (38.9–720)* &amp;</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>96.6 ± 22.4</td>
<td>72.2 ± 10.4*</td>
<td>36.0 ± 12.7* &amp;</td>
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</table>

*p < 0.001 vs. CONTROL; & p < 0.001 vs. HCY; $ p < 0.05 vs CONTROL and HCY # p = 0.035 vs. HCY

Table 2
COX ANALYSIS FOR LIFE SURVIVAL

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Confidence</th>
<th>Interval</th>
<th>p</th>
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<tr>
<td>AGE</td>
<td>0.065</td>
<td>0.050</td>
<td>0.085</td>
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<td>GENDER</td>
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<td>0.249</td>
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<td>0.003</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.431</td>
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<tr>
<td>GFR</td>
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<td>-0.004</td>
<td>-0.016</td>
<td>0.583</td>
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Table 3
COX ANALYSIS FOR SURVIVAL BEFORE RENAL REPLACEMENT THERAPY

<table>
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<th>Confidence</th>
<th>Interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>GENDER</td>
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<td>0.137</td>
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<td>&lt; 0.001</td>
</tr>
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<td>GFR</td>
<td>0.043</td>
<td>-0.058</td>
<td>-0.028</td>
<td>0.005</td>
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Figure 1

Comparative life survival using Kaplan-Meier method. The differences among the three groups are significant.
Figure 2

Comparative survival before reaching CKD stage 5 KDIGO or renal replacement therapy. The CKD group has a statistically significant worse survival (see text).