УДК: 616.36-004-06:616.61-02]-008.6-07-08-035

CYSTATIN C AS AN EARLY MARKER OF HEPATORENAL SYNDROME

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Summary

The research was aimed to investigate the use of cystatin C (CysC) for early detection of hepatorenal syndrome (HRS) in cirrhotics. CysC, a low-molecular-weight cysteine proteinase inhibitor, is a potentially more accurate marker of glomerular filtration. We conducted a prospective multicenter study in patients with alcoholic liver cirrhosis, comparing changes in CysC and serum creatinine (Scr) immediately following onset of HRS as predictors of a composite endpoint of dialysis or mortality. The results of our study confirmed, that CysC has demonstrated less variability between samples than Scr. Patients were stratified into four groups reflecting changes in Scr and cystatin: both unchanged or decreased 38 (36%) (Scr−/CysC−); only cystatin increased 25 (24%) (Scr−/CysC+); only Scr increased 15 (14%) (Scr+/CysC−); and both increased 28 (26%) (Scr+/CysC+). With Scr−/CysC− as the reference, in both instances where cystatin rose, Scr−/CysC+ and Scr+/CysC+, the primary outcome was significantly more frequent in multivariate analysis, and, respectively. However, when only Scr rose, outcomes were similar to the reference group. Summarizing all above, we can conclude, that changes in CysC levels early in HRS are more closely associated with eventual dialysis or mortality, than Scr and may allow more rapid identification of patients at risk for adverse outcomes.

Keywords: hepatorenal syndrome, cystatin C, alcoholic liver cirrhosis

Introduction

Hepatorenal syndrome (HRS) is a common complication in patients with alcohol liver cirrhosis (ALC) and associates with higher mortality in proportion to progressive HRS severity [1, 2]. However, the most common indicator of renal function, serum creatinine (Scr), may be an unreliable surrogate for glomerular filtration rate (GFR) due to the impact of nonrenal determinants such as sex, race, age, body composition, and medications. In the setting of an acute drop in GFR, Scr is insensitive to small decrements in function, and its rise can lag actual kidney injury by several days. These shortcomings of Scr are magnified in patients with ALC, as they have an enlarged volume of fluid distribution and decreased Scr production secondary to muscle atrophy and liver dysfunction, further dissociating Scr

from GFR [3]. The accuracy of Scr in reflecting GFR declines with worsening stages of cirrhosis [4] and can be further compromised by elevated bilirubin interfering with Scr assays [5]. The literature data have previously shown that progression of HRS associates with mortality [6]. However, progression of HRS to a higher Scr defined stage may be delayed in the setting of ALC due to early fluctuation in Scr levels unrelated to renal function and potentially beneficial treatments may resultantly be deferred. A more accurate means of rapidly and accurately detecting changes in renal function early in the course of HRS that associate with outcomes may allow for more prompt initiation of therapy and improved outcomes.

Cystatin C (CysC) is a low-molecular-weight cysteine proteinase inhibitor synthesized at a constant rate by all nucleated cells. CysC is freely filtered by the glomerulus, nearly completely reabsorbed and catabolized by the proximal tubule, and does not undergo secretion. CysC levels are less influenced by nonrenal factors than Scr and it has thus been proposed as a superior marker of glomerular filtration. In HRS, CysC rises more rapidly than Scr in some settings and has been shown to associate more strongly with outcomes. CysC performs better than Scr in early detection of HRS [7, 8, 9, 10]. CysC associates with duration of HRS [11], need for renal replacement therapy [8, 12], and short and long term mortality in HRS [12, 13]. Patients who experience increases in both CysC and Scr experience worse outcomes than those with an increase in either marker alone [14, 15]. In patients with ALC, CysC has been shown to more accurately correlate with measured GFR than Scr or Scr based estimation equations [16]. CysC is also more sensitive than Scr in cirrhotics for detecting mild decreases in baseline GFR [17, 18] and superior in predicting HRS or 3-month mortality [19]. Despite these attributes, CysC has been challenging to study in patients with cirrhosis and HRS due to the typical lack of a documented baseline value. The absence of a baseline renders CysC ineffectual in practice for diagnosing HRS prior to Scr, as there is no value to compare to for assessment of absolute or relative changes. However, due to its lesser dependence on nonrenal determinants, small changes in CysC levels early in the course of HRS may be more reflective of true trends in renal function than those of Scr, which might continue to oscillate for several days before displaying a clear trend towards renal worsening or recovery. An alternative study design therefore is comparing trends in CysC and Scr levels immediately, following the onset of clinical apparent HRS to evaluate the relative utility of early fluctuations in each marker in predicting outcomes following HRS. We conducted a prospective multicenter study in patients with ALC comparing changes in CysC and Scr immediately following onset of HRS as predictors of dialysis and mortality during this early time period.

Material and Methods

This prospective, multicenter observational cohort study was conducted between 2014 and 2016 at the Chernivtsi Regional Clinical Hospital and Chernivtsi Regional Narcological Dispensary, Ukraine. Eligible patients were admitted with HRS or developed it during the course of hospitalization. Inclusion criteria included a known diagnosis of cirrhosis and availability of documented serum Scr within 1 year prior to HRS. Exclusion criteria included prior kidney or liver transplant, advanced chronic kidney disease (CKD) (baseline Scr 4.0 mg/dL), acute or chronic renal replacement therapy at enrollment, estimated life expectancy 3 days, confirmed pregnancy, and other known causes of renal insufficiency such as glomerulonephritis or urinary obstruction. Informed consent was obtained from all participants or, if patients were unable to provide consent, from designated surrogates. All consecutive eligible patients identified during screening were approached for enrollment and all

participants were enrolled within 5 days of meeting HRS criteria. The study was approved by the institutional review board at each institution.

A fresh 10 mL blood sample was collected daily for three days following the onset of HRS. Samples were immediately refrigerated and then centrifuged at 5000 g for 10 minutes at −4° C for CysC measurement. CysC was measured using a BN II nephelometer (Siemens), which has an approximate coefficient of variation of 2% [20]. Scr was measured from samples collected as part of routine clinical care via the modified Jaffe method. Laboratory measurements were performed by personnel blinded to patient information.

Patients who were eligible carried an existing documented diagnosis of ALC based on liver biopsy, when available, or a combination of clinical, biochemical, ultrasonographic, and endoscopic findings. The acute kidney injury network (AKIN) criteria were applied for diagnosis of HRS as recommended by a working group composed of members of the International Ascites Club (IAC) and the Acute Dialysis Quality Initiative (ADQI) [21]. AKIN quantifies the severity of HRS based on degree of increase in serum Scr relative to baseline and is defined as follows: stage 1, increase in Scr by 0.3 mg/dL or 50%; stage 2, 2- to 3-fold increase; stage 3, 3-fold increase, or Scr 4.0 mg/dL after a rise of at least 0.5 mg/dL or acute dialysis requirement. As urine collection and output documentation can be inconsistent, only the serum Scr component of the AKIN criteria was utilized.

Baseline serum Scr was defined as the most recent stable measurement within a year prior to admission for the index hospitalization. When possible, outpatient measurements were utilized though values were also used from previous admissions not complicated by HRS. In rare cases, patients without an outpatient measurement were included in the analytic cohort if, prior to onset of HRS, they manifested at least 5 initial days from admission of stable values within the normal Scr range. In these instances, the Scr at admission was considered the baseline.

When calculating between-sample percent change in Scr and cystatin C, the first and last available samples were utilized. GFR was estimated via the CKD-EPI equation using the baseline Scr value [22]. CKD was defined by as GFR 60 mL/min. MELD and Child-Pugh scores were calculated on the day of first sample collection.

Our primary outcome was a composite of dialysis and in-hospital mortality during the index hospitalization.

Results

A total of 192 patients were enrolled in our cohort with cirrhosis and HRS. Of these, 106 had at least 2 blood samples collected and were included in this study. Samples were not collected in the remaining 86 patients either due to failure to consent to blood collection or initiation of dialysis prior to obtaining consent. There were no significant differences in any demographic variables or in those relating to the patients' liver disease between those patients who did and did not have serum samples collected. The mean patient age was 56.3 and 66% were male. Thirty-seven (35%) patients met the primary composite endpoint during their hospitalization. Of these, 28 patients died and 22 required dialysis, with 13 of these experiencing both dialysis and death. On sensitivity analysis, there was no difference in death, 28/106 (26%) versus 22/86 (26%), or the composite of death or dialysis, 37/106 (35%) versus 30/86 (35%), between those patients with and without blood samples obtained. The majority of patients had advanced cirrhosis evidenced by previously suffered complications including ascites, 76%, hepatic encephalopathy, 63%, variceal bleeding, 23%, and spontaneous bacterial peritonitis, 12%. Reasons for

admission were similar between the four groups. The median Child-Pugh score was 10 and MELD 26.4 at the time of enrollment. There was no difference in Child-Pugh and MELD scores across groups nor were serum sodium levels or the presence of hyponatremia at enrollment significantly different. Three blood samples were collected in 77 (73%) patients, and two were collected in the remainder, 29 (27%). The first sample was collected at a median of 2 (IQR 2–4) days after first meeting AKIN criteria. While Scr and CysC levels from the first sample were moderately correlated, the relative changes in Scr and CysC values between the first and last sample were less, so. We revealed strong correlations between between Scr and CysC levels in the initial samples and between relative and absolute changes in each filtration marker between samples ($p<0.05$). CysC exhibited less variability between samples than seen with Scr with the interquartile range for percent change in Scr ranging from −17 to +11% compared with CysC ranging from −9 to +12%. A change of 10% was observed in 35/106 (33%) patients by Scr and 53/106 (50%) patients based on CysC. The median change in CysC values differed significantly between those patients with the primary outcome, +6% (95% CI −2 to +14%), and those without, −3% (−9 to +9%). The difference in changes in Scr for those with and without the primary outcome trended in the same direction but did not reach statistical significance, 0% (−12 to +17%) versus −5% (−21 to +8%). Patients experiencing an increase in CysC levels between samples were significantly more likely to meet the primary endpoint, 47%, than those without such an increase, 23%. However, there was no significant difference in the incidence of dialysis or mortality among those whose Scr increased, 40%, than among those where it did not, 32%. Neither the CysC nor Scr values from the first sample collected showed any association with the primary outcome.
Patients were stratified into four mutually exclusive groups based on changes in Scr and CysC: both unchanged or decreased 38 (36%) (Scr−/CysC−); only CysC increased 25 (24%) (Scr−/CysC+); only Scr increased 15 (14%) (Scr+/CysC−); and both Scr and CysC increased 28 (26%) (Scr+/CysC+). Taking the Scr−/CysC− group as the reference, in both instances where CysC rose, Scr−/CysC+ and Scr+/CysC+, the occurrence of the primary outcome was significantly higher, and 0.03, respectively. However, in the group where only Scr rose, outcomes were similar to the reference group. Both the Scr−/CysC+ and Scr+/CysC+ but not Scr+/CysC− groups were associated with a significantly increased relative risk for the primary outcome in unadjusted analysis as well as after adjustment for age, race, and sex.

Discussion

HRS in patients with ALC is often severe and associated with significant mortality risk. Potentially efficacious therapies exist but must be appropriately applied to patients at highest risk for adverse outcomes [23]. We have demonstrated that progression to a more advanced stage of HRS is independently associated with mortality but the likelihood of progression can be difficult to predict early in the course of HRS. Scr levels are dependent on multiple demographic and clinical factors beyond renal function and thus may be susceptible to short term fluctuations early in the course of HRS unrelated to changing GFR [3, 24]. Cirrhosis potentiates these shortcomings of Scr due to associated low protein intake, reduced muscle mass, defective Scr production, and frequent large fluid shifts. In patients with cirrhosis, Scr based estimation of GFR is within 50% of measured values in only 9% of patients [25]. CysC has been proposed as a biomarker of glomerular filtration less susceptible to extrarenal variation. In patients with cirrhosis, GFR estimates are less biased and more precise with CysC than Scr [25, 26]. CysC levels, but not Scr, are associated in cirrhotic patients with development

of HRS and mortality over a 3–6-month period [19] and the onset of HRS and mortality at one year [27]. The purpose of this study was to compare the association of changes in CysC and Scr early in the course of HRS in patients with ALC with a composite outcome of dialysis or death.

In our study, changes in CysC, but not Scr, over the period of sample collection differed significantly for those with and without the primary outcome. Participants experiencing a rise in CysC alone (Scr−/CysC+) between samples progressed to the need for dialysis or death at the same rate as those with a rise in both biomarkers of filtration (Scr+/CysC+). However, those with a rise in Scr alone (Scr+/CysC−) experienced the primary outcome with no greater frequency than those in whom both biomarkers fell (Scr−/CysC−). Relative to the group in which both markers fell, both groups with rising cystatin were independently associated with the primary outcome. The lack of association between rising Scr and our primary endpoint stands in contrast to our previous demonstration of a strong association between progression of HRS to a higher Scr defined stage and mortality [6]. This discrepancy is again evidence of the poor sensitivity of Scr for detecting acute falls in renal filtration function. Given its extrarenal influences and the extent to which changes in levels lag falling GFR, Scr rising over the entire duration of an HRS episode sufficient to qualify for a higher HRS stage is indeed specific for a significant fall in renal function and resultantly associates with poor outcomes. Over the short term, however, early in the course of HRS, Scr changes need not reflect trends in renal function and thus show poor association with outcomes when not coupled with similar changes in CysC levels. CysC strongly associates with outcomes in multiple settings of HRS including intensive care unit and emergency room [7, 28, 29]. Intriguingly, changes in CysC may be more specific to outcomes than Scr. The mortality in patients with acute elevation in CysC but without Scr based HRS (28.6%) was similar to patients with AKIN stage 1 HRS (33.3%) and far outstripped that of patients with no elevations in either biomarker (5.7%). The apparent prognostic advantage of CysC may be due to its ability to more accurately reflect early/small changes in GFR due to fewer nonrenal influences. Early in HRS, before GFR has undergone a truly dramatic fall, Scr may be subject to greater fluctuations than CysC, fluctuations untethered from changes in GFR. In our study, Scr and CysC levels exhibited good correlation at time of first sample collection. However, the correlation between changes in these markers over the course of sample collection was significantly lower. Changes in CysC levels during the period of sample collection were more tightly bunched than those of Scr. CysC demonstrated less variability with a smaller interquartile range for changes and a significantly higher number of patients with a change of 10%.

In addition to being more specific for early changes in GFR than Scr, CysC may also be more sensitive. The superiority of CysC over Scr for detecting early acute changes in renal function has been noted in multiple settings.

Our study has several significant strengths. Data were collected prospectively for what is, in this challenging study population, a large cohort of patients. Unlike many studies of ALC and HRS, ours was multicenter and contained patients from both general medical floors and the ICU, enhancing its generalizability. However, the study is not without limitations. CysC can be influenced by several nonrenal factors including steroids and thyroid function. While we do not have data for these variables, it is reassuring that none of the baseline and demographic variables predicted which of the four groups patients would assort into. This is especially true for cirrhosis etiology, where the potential use of steroids to treat acute hepatitis in ALC did not dictate the pattern of changes in CysC. However, we

cannot definitively rule out that changes in CysC may be reflecting some other physiologic processes in addition to renal functions that may be contributing to the primary outcome. We did not have data on baseline CysC levels and patients were enrolled based on Scr defined HRS. This raises a concern that the results could be biased for patients whose Scr fell due to regression to the mean. However, the lack of association between enrollment CysC and Scr values and the primary outcome assuages this concern.

In conclusion, changes in serum CysC early in the course of HRS in patients with ALC associate more strongly with the need for dialysis and mortality, than do changes in serum Scr. Prospective trials indexing interventions to changes in CysC are required to determine if routine monitoring of CysC in patients with ALC and HRS may lead to improved outcomes.

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