UNIVERSIDADE VILA VELHA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

CISTATINA C COMO PREDITOR DE SEVERIDADE EM ADULTOS INTERNADOS EM UMA UNIDADE DE TERAPIA INTENSIVA MISTA

DYANNE MOYSÉS DALCOMUNE

VILA VELHA - ES

AGOSTO/2015

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Dissertação apresentada à Universidade Vila Velha, como pré-requisito do Programa de Pós-graduação em Ciências Farmacêuticas para obtenção do título de Mestre em Ciências Farmacêuticas.

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Banca Examinadora:

Profa. Dra. Ana Raquel Santos de Medeiros Garcia – IFES

Profa. Dra. Bianca Prandi Campagnaro – UVV

Prof. Dr. Thiago de Melo Costa Pereira – UVV
(orientador)

Dedico esta Dissertação aos meus alunos. Mola propulsora de toda sede de conhecimento e busca interminável por excelência. Surpreenderam-me com um mundo novo de desafios e paixão pela sala de aula.

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“Todo caminho da gente é resvaloso. Mas também, cair não prejudica demais. A gente levanta, a gente sobe, a gente volta!... O correr da vida embrulha tudo, a vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem. Ser capaz de ficar alegre e mais alegre no meio da alegria, e ainda mais alegre no meio da tristeza...”

João Guimarães Rosa

SUMÁRIO

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LISTA DE ABREVIATURAS E SIGLAS

°C – graus Celsius

APACHE II – Acute Phisiology and Chronic Health disease Classification System II

AURC – área sob a curva ROC

BMI – Índice de Massa Corporal

Cys C – cistatina C

GFR –Taxa de Filtração Glomerular

ICU – Unidade de Terapia Intensiva

KDa – kilodalton

MDRD – Modification of Diet in Renal Disease

ROC – Receiver Operating Characteristic

Rpm – rotações por minuto

SD – desvio-padrão

RESUMO

DALCOMUNE, Dyanne Moysés, M. Sc., Universidade Viva Velha – ES, 2015. **Cistatina C como preditor de severidade em adultos internados em uma unidade de terapia intensiva mista.** Orientador: Thiago de Melo Costa Pereira.

INTRODUÇÃO: Apesar da cistatina C ter sido amplamente investigada pela sua precocidade no diagnóstico de lesão renal aguda, recentemente tem sido proposta uma relação entre os níveis séricos de cistatina C e a gravidade da doença. Assim como os níveis séricos de cistatina C estão associados a eventos adversos e mortalidade independente da função renal. Sendo assim, nós pretendemos comparar o valor preditivo da cistatina C com biomarcadores convencionais de função renal em predizer gravidade de doença em uma Unidade de Terapia Intensiva (UTI) mista. Nós hipotetizamos que níveis elevados de cistatina C podem predizer severidade em condições clínicas diferentes, em pacientes adultos admitidos numa UTI mista.

RESULTADOS: Comparamos a atuação da creatinina sérica, ureia e cistatina C, assim como a taxa de filtração glomerular estimada pelos métodos de Cockroft-Gault, MDRD e Larsson em 61 pacientes criticamente enfermos. Os pacientes adultos admitidos no hospital foram avaliados e selecionados para este estudo prospectivo e observacional. A idade média foi 52±19 anos. O APACHE II médio foi 9.5±6 para toda a amostra. Os pacientes foram separados em dois graus de severidade e o ponto de corte escolhido foi o escore APACHE II < 10 ou ≥ 10. Houve uma correlação entre a cistatina C sérica e ureia com o APACHE II, mesmo quando ajustado para idade. Ureia e cistatina C sérica permaneceram significativamente elevadas nos pacientes com APACHE II ≥ 10, assim como a TFG estimada pelo método de Larsson e Cockroft-Gault. A análise da curva ROC mostrou que tanto a ureia como a cistatina C tiveram altas especificidade e sensibilidade em prever severidade quando comparadas com o APACHE II como padrão-ouro. Houve também uma superioridade significativa de ambos os métodos Larsson e Cockroft-Gault sobre MDRD. Contudo, diferentemente da ureia, a cistatina C foi um bom preditor em pacientes jovens e idosos.

CONCLUSÕES: No presente estudo, nossos dados sugerem que a cistatina C sérica e seu método de estimativa da TFG (Larsson) são bons preditores de severidade em pacientes adultos hospitalizados em uma UTI.

Palavras-chave: Cistatina C. Unidade de Terapia Intensiva. APACHE II. Creatinina. TFG.

ABSTRACT

DALCOMUNE, Dyanne Moysés, M. Sc., Universidade Viva Velha – ES, 2015. **Cystatin C as a predictor of severity in adults admitted to a unit of mixed intensive care.** Adviser: Thiago de Melo Costa Pereira.

Background: Although serum cystatin C (Cys C) has been widely investigated for its precocity in diagnosis of acute kidney diseases, recently it has been proposed a relationship between serum Cys C levels and several diseases. Also, Cys C levels are associated to adverse events and mortality independent of renal function. Thus, we intended to compare the predictive value of serum Cys C with conventional biomarkers of renal function in predicting severity of illness of patients in a mixed intensive care unit (ICU). We hypothesized that elevated Cys C levels could predict severity in different clinical conditions in adult patients admitted in a mixed ICU.
Results: We compared the performance of serum creatinine, urea and Cys C as well as GFR estimates (Cockcroft-Gault, MDRD and Larsson) in 61 critically ill patients. Adult patients admitted in a Hospital were screened for eligibility in this prospective and observational study. The mean age was 52 ± 19 years. The average APACHE II score was 9.5 ± 6 for the whole sample. Patients were assigned into two different degrees of severity and the internal derived cut off value was chosen as APACHE II score < 10 or ≥ 10. There was a significant correlation between serum Cys C and urea with APACHE II, even after adjusting for age. Urea and serum Cys C remained significantly elevated in patients APACHE II ≥10 group as well as the GFR estimated by the method of Larsson and Cockcroft-Gault. The ROC curve analyses showed that both urea and Cys C have high sensitivity and specificity in predicting severity of illness by using APACHE II score as a gold standard. Also, there was a significant superiority of both Larsson and Cockcroft-Gault over the MDRD method. However, unlikely urea, Cys C was found a good predictor in both young and old patients.
Conclusions: In the present study, our data suggest that serum Cys C and its GFR estimate (Larsson's method) are good predictors of severity in adult patients hospitalized in ICU.

Keywords: Cystatin C. Intensive Care Unit. APACHE II. Creatinine. GFR.

CYSTATIN C AS A PREDICTOR OF SEVERITY IN ADULTS ADMITTED TO A UNIT OF MIXED INTENSIVE CARE

Dyanne Moysés Dalcomune[1,2], Jorge Terrão[2], Marcella Leite Porto[3], Elisardo Corral Vasquez[1,3], Marcelo Perim Baldo[4], Thiago de Melo Costa Pereira[1,5§]

[1]Pharmaceutical Sciences Graduate Program, Vila Velha University (UVV), Vila Velha, ES, Brazil

[2] Meridional Hospital, Cariacica, ES, Brazil

[3]Laboratory of Translational Physiology, Health Sciences Center, Federal University of Espírito Santo, Vitória, Brazil

[4]Laboratory of Cardiovascular Pathophysiology, Department of Physiological Sciences, Federal University of Espírito Santo, Vitória, Brazil

[5]Federal Institute of Education, Science and Technology (IFES), Vila Velha, ES, Brazil

[§]Corresponding author

Email addresses:

DMD: dydalcomune@gmail.com

JT: jterrao@tommasi.com.br

MLP: cella.porto@gmail.com

ECV: evasquez@pq.cnpq.br

MPB: marcelobaldo@ymail.com

TMCP: pereiratmc@gmail.com

§ Corresponding author: Pharmaceutical Sciences Graduate Program, Vila Velha University (UVV), Av. Comissário José Dantas de Melo, n°21, 29102-920 Boa Vista, Vila Velha, ES, Brazil

E-mail address: pereiratmc@gmail.com

ABSTRACT

Background: Although serum cystatin C (Cys C) has been widely investigated for its precocity in diagnosis of acute kidney diseases, recently it has been proposed a relationship between serum Cys C levels and several diseases. Also, Cys C levels are associated to adverse events and mortality independent of renal function. Thus, we intended to compare the predictive value of serum Cys C with conventional biomarkers of renal function in predicting severity of illness of patients in a mixed intensive care unit (ICU). We hypothesized that elevated Cys C levels could predict severity in different clinical conditions in adult patients admitted in a mixed ICU.

Results: We compared the performance of serum creatinine, urea and Cys C as well as GFR estimates (Cockcroft-Gault, MDRD and Larsson) in 61 critically ill patients. Adult patients admitted in a Hospital were screened for eligibility in this prospective and observational study. The mean age was 52 ± 19 years. The average APACHE II score was 9.5 ± 6 for the whole sample. Patients were assigned into two different degrees of severity and the internal derived cut off value was chosen as APACHE II score < 10 or ≥ 10.

There was a significant correlation between serum Cys C and urea with APACHE II, even after adjusting for age. Urea and serum Cys C remained significantly elevated in patients APACHE II ≥10 group as well as the GFR estimated by the method of Larsson and Cockcroft-Gault. The ROC curve analyses showed that both urea and Cys C have high sensitivity and specificity in predicting severity of illness by using APACHE II score as a gold standard. Also, there was a significant superiority of both Larsson and Cockcroft-Gault over the MDRD method. However, unlikely urea, Cys C was found a good predictor in both young and old patients.

Conclusions: In the present study, our data suggest that serum Cys C and its GFR estimate (Larsson's method) are good predictors of severity in adult patients hospitalized in ICU.

Keywords: Cystatin C. Intensive Care Unit. APACHE II. Creatinine. GFR.

1 Background

Nowadays, illness severity scoring systems have become central tools for risk stratification and follow-up of patient outcomes in intensive care unit (ICU) (POLITA et al., 2014). In this regard, APACHE II (Acute Physiology and Chronic Health Evaluation II) score has been used as a satisfactory marker for prognosis in adult patients in the ICU (DUSEJA et al., 2013). In clinical practice, serum creatinine remains as the main kidney biomarker used in several ICU risk scoring systems worldwide, even though it is influenced by a variety of factors related to nitrogen metabolism (LAGOS-AREVALO et al., 2016; SHLIPAK et al., 2006; SUNDER et al., 2014; WU et al., 2010;). In this context, new renal biomarkers such as the cystatin C (Cys C) are emerging and may have novel implications in ICU routine, to improve the management and avoid poor outcomes.

Cys C is a cysteine proteinase that exhibits some physiological advantages over creatinine (DHARNIDHARKA, KWON, STEVENS, 2002; O'SEAGHDHA et al., 2014). This nonglycosylated protein can be expressed at a stable rate by all nucleated human cells in organs/tissues (GRUB, 2000; LASSUS; HARJOLA, 2012; SUNDER et al., 2014) being practically unaffected by non-renal morbidities (CHEN, 2010; Delanaye, 2014; ONOPIUK, TOKARZEWICZ, GORODKIEWICZ, 2015). Due to its non-significant protein binding, positive charge at physiological pH and low molecular weight (~13 kDa), Cys C can be freely filtered by the glomerulus and fully reabsorbed by the proximal tubules, becoming a suitable biomarker for renal function evaluation (ANGELIDIS et al., 2013; FILLER et al., 2005; JIANG et al., 2014; SCHIFFRIN, LIPMAN, MANN, 2007; ZHANG, 2012). Recently, many researchers have demonstrated a strong relationship between Cys C levels and several diseases and has been tightly associated to adverse events and mortality independent of renal function (ANGELIDIS et al, 2013; BEEL, et al., 2009; CARRASCO-SÁNCHEZ, et al., 2011; LEELAHAVANICHKUL et al., 2004; ZENG, et al., 2015), thus considered a marker of poor prognosis in elderly patients (DALBONI et al., 2013; SHLIPAK et al., 2006; WU ET AL., 2010.

However, there are still insufficient studies to validate the incorporation of Cys C measurement into the protocols of patients admitted in a mixed ICU routine.

Thus, we aimed to determine whether Cys C levels could predict different clinical conditions in adult patients hospitalized in a mixed ICU. Moreover, we

intended to compare serum Cys C with conventional biomarkers of renal function, thus optimizing their management of diagnostic and clinical treatment in ICU.

2 Methods

2.1 Patients

This prospective and observational study was conducted with 61 consecutive adult patients (> 18 years old) admitted to the ICU in Meridional Hospital, Cariacica-ES, Brazil. The study followed the rules recommended by Meridional Hospital Clinical Trials Committee. For the first 24 h of ICU stay, all patients were classified according to the criteria of "Acute Physiology and Chronic Health Evaluation II" (APACHE II). This study was previously approved by the Brazilian Ethical Committee for human research 'Plataforma Brasil' (#648.184) and informed written consent was achieved on the admission. The exclusion criteria were: length of stay for less than 48 hours, life expectancy less than 72 hours, morbid obesity (BMI > 40kg/m^2), under chemotherapy, with acute or chronic kidney injury or non-informed consent.

2.2 Data Collection

Clinical follow-ups and daily laboratory tests were performed (blood urea, creatinine, C-reactive protein), which included Cys C measurements for all patients during their stay in the ICU for 4 days. The main measurements were those related to the patient's gender, age, BMI, diagnostic, mechanical ventilation, use of vasopressor drugs, urine output and noninvasive estimated glomerular filtration rate based on Cockcroft–Gault, Modification of Diet in Renal Disease (MDRD) (HERGET-ROSENTHAL, BÖKENKAMP, HOFMANN, 2007; KOETJE, 2011) and Larsson (LIMA et al., 2014; RISCH, DREXEL, HUBER, 2005) formulas.

2.3 Blood samples

Serum biomarkers were obtained from blood withdrawn for the scheduled daily examinations in admitted patients and during 3 days following admission. For this purpose, EDTA blood samples were collected in sterile vials, centrifuged at 3,500 rpm for 10 min and the supernatant stored at –20 °C. After, the blood levels of creatinine and urea nitrogen in the blood samples were determined by Jaffe method (HERVEY, 1953) and urease-indophenol method (FAWCETT, SCOTT, 1960). The

serum concentration of Cys C was measured using turbidimetric method (ANGELIDIS et al., 2013). All the measurements were obtained by an automatic biochemical analyzer (AU 400 or 680, Olympus/Beckman Coulter, Munich, Germany).

2.4 Statistical analysis

All data are expressed as mean ± SD or as percentages. Comparisons between groups were made by the chi-square test (X^2), Student´s test and by two-way analysis of variance (ANOVA), as appropriate. When the ANOVA showed significant differences, the Tukey's test was applied as a *post hoc* analysis. Total and partial correlation analyses (Pearson) were performed to estimate associations between biomarkers and APACHE II score severity. The model was adjusted for age. The prediction ability of Cys C, urea and creatinine measurements and the sensitivities and specificities of the indexes were identified by ROC curve analysis. Differences in the areas under the ROC curve (AURC) were compared and the optimal cutoff points were registered as the measures representing the largest concomitant sensitivity and specificity. The statistical analyses were performed using Prism software (Prism 6, GraphPad Software, Inc., San Diego, CA, USA) and MedCalc 12.1.4.0 statistical software (MedCalc Software, Mariakerke, Belgium). All the differences were considered significant when $p < 0.05$.

3 Results

3.1 General characteristics

After exclusion of patients not suitable for this study (18% or 13/74), a total of 61 patients were identified as meeting the inclusion criteria. The clinical and demographic characteristics of the whole sample are showed in Table 1. The mean age was 52 ± 19 years, and males representing 57% of the whole sample. In parallel, the average APACHE II score was 9.5 ± 6. In order to assign patients into different degrees of severity, patients were allocated into one of two different groups based on their APACHE II classification. For that purpose, the internal derived cut off value was chosen as APACHE II score <10 or ≥10. Patients with APACHE II score ≥10 were significantly older than those with better prognosis. Moreover, blood levels of urea and serum Cys C were significantly higher in those patients (Table 1). Four patients with APACHE II score ≥10 died at the ICU after admission. On the other hand, there were no deaths in those patients at APACHE II score <10 at admission.

3.2 Kinetics of kidney biomarkers and GFR and its relationship with APACHE II score

As some of the serum kidney biomarkers (e.g., Cys C and urea) were increased in those patients with poor prognosis based on their APACHE II score, we hypothesized that it would indicate that they might be related. Firstly, we conducted a Pearson correlation analysis in order to test the influence of age on kidney biomarkers and GFR estimate methods. As showed in Table 2, except for serum creatinine, all other parameters were significantly affected by age. Thus, we next performed total and partial Pearson correlation analysis among APACHE II score and kidney biomarkers (Cys C, urea and creatinine) or different GFR estimate methods. As observed in Table 3, a significant correlation was found between serum Cys C (r: 0.529, $p<0.05$) or urea (r: 0.417, $p<0.05$) and APACHE II score at admission. Also, we detected a significant inverted correlation between GFR measured by Larsson (r: -0.527, $p<0.05$) and Cockcroft-Gault (r: -0.428, $p<0.05$) methods (Table 3). However, after adjustment for age, only urea, Cys C and the GFR estimated by Larsson´s method remained significantly associated with APACHE II score.

Additionally, the three main kidney biomarkers and estimated GFR were evaluated daily over 72h after admission at the ICU. As observed in Figure 1, both biomarkers and all GFR estimates presented a good temporal steadiness during these 3 days at ICU. Likewise observed at admission, urea and Cys C were kept significantly higher in those patients with APACHE II score ≥10 (Figure 1A), as well as the GFR estimated by Cockcroft-Gault and Larsson methods (Figure 1B).

3.3 The predictive value of kidney biomarkers and GFR as an APACHE II score surrogate

In order to evaluate the ability of different kidney biomarkers and GFR estimate methods in predict the severity of patients stratified by APACHE II score, we performed a ROC (Receiver Operating Characteristic) curve analysis. Figure 2A shows the ROC curves for creatinine, urea and Cys C with regard to the ability to identify APACHE II score severity. As observed in Figure 2A, both urea and Cys C demonstrated a high significant specificity and sensitivity in predicting APACHE II score stratification, even though a non-significant difference (p=0.143) was found in favor of Cys C. Table 4 shows the AURC curves and the optimal kidney index cutoff (according to the highest sensitivity and specificity) to identify APACHE II score severity. In relation to the ability to identify APACHE II severity, Figure 2B shows ROC curves for different GFR estimates methods. There was a significant Larsson and Cockcroft-Gault superiority in relation to MDRD. Despite a non-significant difference (p=0.247), GFR measured by Larsson´s method showed a higher sensitivity and specificity as compared to Cockcroft-Gault method. Thus, GFR estimated by Cockcroft-Gault and Larsson methods are suitable prognostic markers for severity associated to APACHE II score.

4 Discussion

In the last years, Cys C and its association with morbi-mortality in ICU started to be investigated (DALBONI, et al., 2013; MÅRTENSSON, MARTLING, BELL, 2012; STEWART et al., 2015). In our study, we extended the idea that serum Cys C can predicts severity of illness in adult patients admitted and maintained to a mixed ICU, showing superior sensitivity and specificity when compared to creatinine, the conventional renal biomarker used in APACHE II scoring (KNAUS et al., 1985; WHEELER, 2009). Additionally, we demonstrated that estimated GFR based on Cys C is more accurate than common GFR equations based on serum creatinine.

It is known that a desirable renal biomarker should be characterized by high accurate and rapid measurement, unaffected by typical confounders and applicable across a wide range of illness phases (NEJAT et al., 2010). In this context, Cys C seems to be a good candidate as it gathers all these features (ALGELIDIS et al., 2013; FILLER et al., 2005; KÖTTGEN, 2008; RULE et al., 2006; WOO, et al., 2014; ZHANG et al., 2012). However, the profile involved in the relation between severity in ICU routine and elevation in Cys C began to be studied a few years ago (DALBONI et al., 2013; DELANAYE et al., 2014). Thus, an increase of serum Cys C levels could identify initial abnormalities undetectable by conventional renal biomarkers, being a sensitive prognostic indicator for evaluation which may thus be associated with unfavorable outcome or death (DALBONI et al., 2013; SHLIPAK et al., 2011; WU et al., 2010).

Although several reports have not shown association between the non-renal factors and Cys C (CHEW et al., 2008), recent findings reported that several variables, such as tissue adipose mass, gender or inflammatory status could interfere with Cys C levels, as well as with other traditional renal biomarkers (ANGELIDIS et al., 2013; KNIGHT et al., 2004; MUNTNER et al., 2008). In our study, we found a huge difference in age between groups of severity. It has been showed that older patients may present a positive association with Cys C levels (FINNEY, NEWMAN, PRICE, 2000; GALTEAU et al., 2001; GROESBECK et al, 2008; KNIGHT et al., 2004; NEJAT et al., 2010) and with urea/creatinine in clinical routine (FEHRMAN-EKHOLM, SKEPPHOLM, 2004; MUSCH, VERFAILLIE, DECAUX, 2006; TIAO et al., 2002). Our correlation analysis, in accordance with previous studies, showed that all those tested variables were influenced by age, excepting serum creatinine.

We found significant correlation between renal parameters and APACHE II score, except for creatinine and MDRD estimate. However, due to the age-dependent influence aforementioned, we decided to perform partial correlations adjusted for age. This investigation was necessary to elucidate whether these parameters, regardless of age, could be correlated to the APACHE II score. Then, our results demonstrated that Cys C and their respective GFR estimation maintained positive correlation, being important predictors of severity in ICU patients independent of renal function, as recently observed by others in several clinical situations (CHEN, 2010; SHLIPAK et al., 2006; STEVENS, LEVEY, 2005; WU et al., 2010). In relation to serum urea, our results reinforced previous data reported by Beier et al. (2011) that also showed strong association at ICU admission with the risk of death in critical illness, independent of basal levels of creatinine. Interestingly, due to a good temporal steadiness during these 3 days of renal biomarkers and estimated GFR, we affirm that these observations presented in this study are not a transient epiphenomenon but a relevant association of prognostic markers for severity associated to APACHE II score.

Our data overcome a relative limitation of other recent studies regarding Cys C (CHEN, 2010; DELANAYE et al., 2014; KNIGHT et al., 2004; NEJAT et al., 2010) because we measured the renal function by other parameters, being possible to determine the extent in which Cys C levels reflects in kidney function. It should be emphasized that the GFR estimates that have been applied in clinical practice using the recommended creatinine based formulas Cockcroft-Gault and MDRD (BOTEV et al., 2009; FROISSART et al., 2005) showed some limitations (HERGET-ROSENTHAL, BÖKENKAMP, 2007; RULE et al., 2004; SEBASKY et al., 2009). Several reports have shown bias in subgroups of the population variations in serum creatinine such as age, muscle mass, diet, gender or liver disease (FAN et al., 2014; PÖGE et al., 2006), or poor concordance between Cockcroft-Gault and MDRD results (PEDONE et al., 2006; SHARA et al., 2009). In our study, we confirmed this discrepancy justified by the greater accuracy of the Cockcroft-Gault when compared for the MDRD equation both at admission and followed by three days. More interestingly, we showed that Larsson´s test was superior to Cockcroft-Gault (also confirmed by ROC analysis), therefore suggesting preferential use when serum Cys C is measured, as observed by others with different subpopulations (FAN et al., 2014; PÖGE et al., 2006; QUTB et al., 2009). Thus, our data are in accordance with the general idea that Cys C may be a relevant alternative to serum creatinine for

estimating GFR (ANGELIDIS et al., 2013; FILLER et al., 2005; ONOPIUK, TOKARZEWICZ, GORODKIEWICZ, 2015), even if the traditional reliance of management combined with cost-minimization analysis are factors that explain the maintenance of creatinine in ICU routine (CHEW et al., 2008).

Based on the results obtained, we evaluated whether this linear association between the parameters and the APACHE II score could be used as predictors. Then, through comparison with AURC, our study confirmed the superiority of Cys C and urea over creatinine, and also Larsson and Cockcroft–Gault over MDRD with respect to APACHE II classification. Thus, firstly, our data emphasize that serum Cys C is a suitable prognostic marker for severity associated to APACHE II score. Also, we suggest that in ICU routine, the evaluation through creatinine (unique renal biomarker used as a variable of APACHE II) is adjusted by Cockcroft-Gault but not by MDRD equation. Thirdly, we recommend that, by unstable endogenous nitrogen sources (BEIER et al., 2011), the urea parameter needs to be analyzed more carefully in comparison to Cys C.

In order to exclude the influence of age in the ROC curve, we performed the ROC analyses for all parameters after divide the 61 patients in two different groups based on average age of the whole sample (Group 1: <50, and Group 2: ≥50). Interestingly, Cys C showed a significant prediction power in both groups, showing that it can be used as suitable surrogates for APACHE II score. However, blood urea showed a significant predictive power only in patients below 50 years age, showing that blood urea may not be an adequate surrogate for the general population.

Once this is an observational study, some residual confounding of the present study should be noted. First, although the use of vasopressor drugs in ICU with hypotension and evidence of impairment renal function is still controversial (BELLOMO, GIANTOMASSO, 2001; MIZOI, DEZOTI, VATTIMO, 2008), the possible hypoperfusion could also increase serum Cys C concentration. Second, we did not associate the thyroid hormones or liver diseases with levels of Cys C, which are known enhancers of this biomarker (ANGELIDIS et al., 2013; CHEN, 2010; CHU et al., 2004; WANG et al., 2014). Finally, as our results were obtained with small sample size, further investigations in larger samples will be warranted.

In conclusion, our data suggest that Cys C might potentially serve as indicator of severity base on APACHE II score. In addition, this study from a mixed ICU population has revealed that Cys C was an effective and earlier prognostic

marker of severity than serum creatinine or their respective GFR estimates, demonstrating the potential clinical role in the management of patients admitted to intensive care units.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

DMD carried out acquisition of data and interpretation of the data. JT and MLP contributed to the study´s design and the critical revision of the manuscript. ECV, MPB and TMCP contributed to the concept, design, supervision of the study and interpretation of data. All authors read and approved the final version of the manuscript.

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APÊNDICE 1:

FIGURAS E TABELAS

Table 1. Demographic and clinical characteristics of all patients in a mixed ICU according to APACHE II <10 and ≥10 classification (n=61)

Parameters	All (n=61)	APACHE II <10 (n=34)	APACHE II >10 (n=27)	P value
APACHE II mean	9.5 ± 6	5 ± 2	15 ± 4#	<0.0001
Age	52 ± 19	42 ± 13	66 ± 18#	<0.0001
Gender (% males)	57	56	59	0.7911
BMI (Kg/m^2)	25 ± 4	25 ± 4	24 ± 4	0.573
ICU hospitalization (days)	10 ± 13	6 ± 4	15 ± 17#	0.0044
Diagnostic				
Sepsis	8 (13%)	2 (6%)	6 (22%)	0.060
Trauma	4 (7%)	2 (6%)	2 (7%)	0.811
Major Surgery	18 (29%)	12 (35%)	6 (22%)	0.266
Neurologic	13 (21%)	6 (18%)	7 (26%)	0.433
Shock	3 (5%)	1 (3%)	2 (7%)	0.423
Others	15 (25%)	11 (32%)	4 (16%)	0.114
Mechanical ventilation *	19(31%)	8(24%)	11 (40%)	0.149
Use of vasopressor drugs	10(16%)	2(6%)	8 (29%)#	0.012
C-reactive protein (mg/dL)*	65±80	49±61	85±97	0.079
Urine Output *	1280 ± 1189	1303 ± 1273	1250 ± 1097	0.864
Serum creatinine (mg/dL)*	0.91±0.26	0.90±0.23	0.94±0.29	0.573
Cockcroft-Gault (mL/min per 1.73m^2)	93 ± 36	107 ± 34	74 ± 28#	0.0001
MDRD (mL/min per 1.73m^2)	89 ± 26	92 ± 23	86 ± 29	0.3355
Blood urea (mg/dL)*	32 ± 17	26 ± 10	40 ± 21#	0.0006
Serum cystatin C (mg/dL)*	0.92±0.50	0.68±0.24	1.21±0.59#	<0.0001
Larsson (mL/min per 1.73m^2)	116 ± 59	145 ± 56	78 ± 40#	<0.0001
Death	4 (7%)	0	4 (15%)#	0.033

Student´s test and chi-square test (X^2) when appropriate. All data are expressed as mean values ± SD. # p<0.05 vs APACHE < 10 subgroup

* At admission in ICU.

BMI: body mass index; ICU: intensive care unit.

Table 2. Pearson correlation coefficients (r) between age and different kidney biomarkers and GFR estimate methods

	r	*P*-value
Creatinine	0.054	0.681
Urea	0.279	0.029
Cystatin C	0.470	0.000
Cockroft-Gault	-0.632	0.000
MDRD	-0.275	0.032
Larsson	-0.573	0.000

Tabela 3. Pearson correlation coefficients (r) between APACHE II score and different kidney biomarkers and GFR estimate methods

	Total correlation		Partial correlation*	
	r	*P*-value	R	*P*-value
Creatinine	0.027	0.834	-0.003	0.981
Urea	0.417	0.001	0.328	0.011
Cystatin C	0.529	0.000	0.364	0.004
Cockcroft-Gault	-0.428	0.000	-0.117	0.375
MDRD	-0.030	0.815	0.154	0.239
Larsson	-0.527	0.000	-0.305	0.018

* Partial correlations were adjusted for age.

Table 4. Area under the receiver operating characteristic plus curve and 95 % sensitivity and specificity thresholds for biomarkers to discriminate for APACHE II classification (<10 and ≥ 10)

Parameters	AUC	95% CI	Cut off	Sensitivity	Specificity	P value
Serum values						
*Serum creatinine (mg/dL)**	0.472	0.342-0.604	≤0.6	18.5	94.1	0.706
*Blood urea (mg/dL)**	0.745*	0.617-0.848	>27	74.1	64.7	0.0002
*Serum cystatin C (mg/dL)**	0.848*	0.733-0.927	>0.74	81.5	76.5	0.0001
GFR estimates						
Cockroft-Gault (mL/min per 1.73 m^2)	0.770 #	0.645-0.868	≤ 75.1	59.3	85.3	0.0001
MDRD (mL/min per 1.73 m^2)	0.573	0.440-0.699	≤ 64	33.3	94.1	0.321
Larsson (mL/min per 1.73 m^2)	0.848 #	0.733-0.927	≤ 107.4	81.5	76.5	0.0001

ANOVA (one way) followed by Tukey's test. * p< 0.05 vs. serum creatinine; # p< 0.05 vs. MDRD.

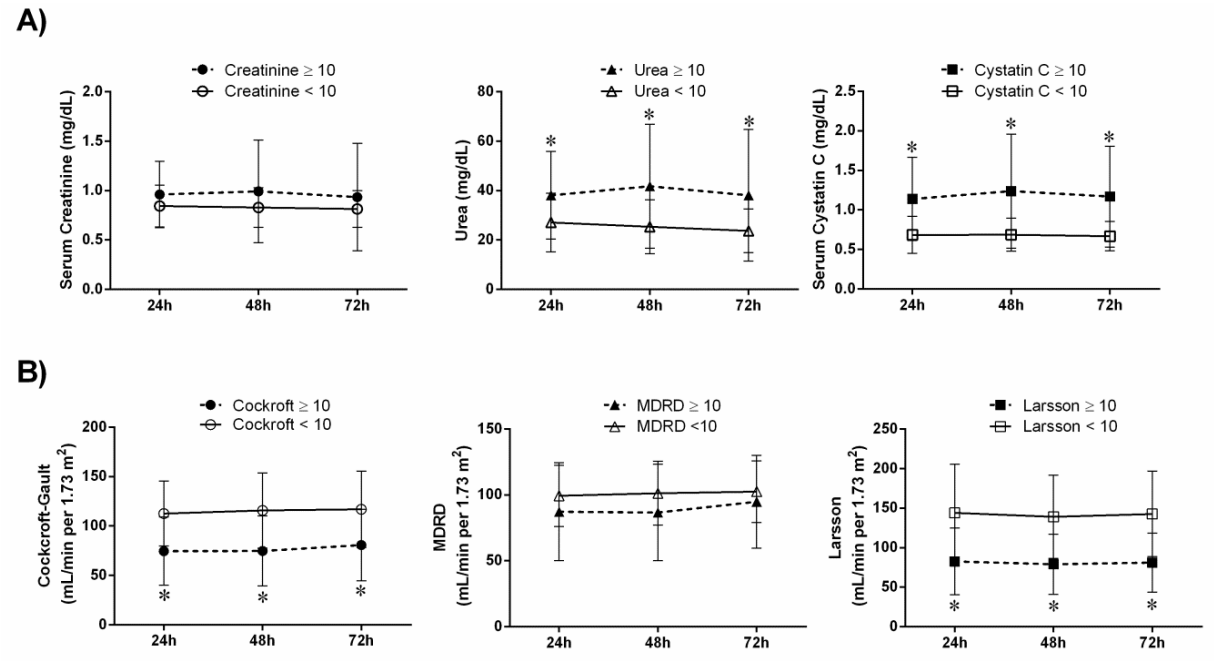

Figure 1. Follow-up of kidney function in patients admitted in ICU 24, 48 and 72 hours after classification in APACHE II < 10 or ≥10 groups. A) Serum levels of renal biomarkers: the left and right panel "A" shows maintenance of higher serum cystatin C and urea levels for 3 days, respectively in APACHE ≥10 group.The middle panel "A" demonstrated no difference in serum creatinine levels between groups. B) GFR estimates: The left panel "B" shows impairment of clearance of cystatin C in APACHE ≥10 group. The middle panel "B" indicates an impairment of clearance of creatinine in APACHE ≥10 group by Cockcroft-Gault method. In contrast, in the right panel "B", the MDRD was not modified between groups during follow-up. All data are expressed as mean values ± SD. * p<0.05 vs. APACHE <10 group (ANOVA, 2 way).

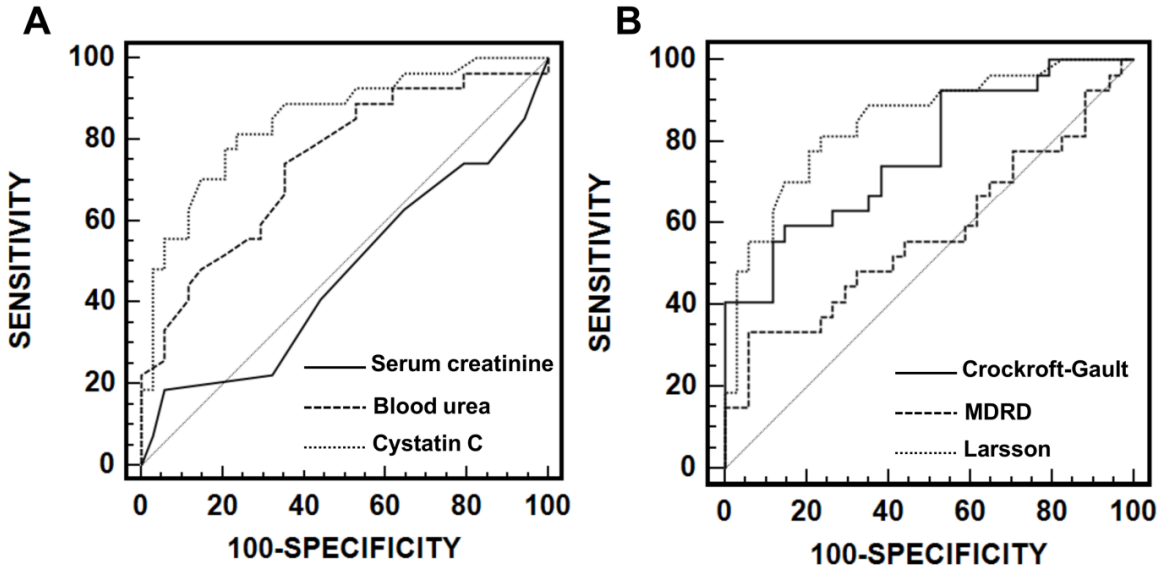

Figure 2. ROC (Receiver Operating Characteristic) Curves of: A) Serum Creatinine, Urea and Cystatin C; B) GFR estimated by Cockcroft-Gault, MDRD and Larsson equations.

ANEXO 1:

TERMO DE CONSENTIMENTO INFORMADO

TERMO DE ESCLARECIMENTOS, CIÊNCIA E CONSENTIMENTO ("CONSENTIMENTO INFORMADO")
PROCEDIMENTO: INTERNAÇÃO E PROCEDIMENTO EM UNIDADE DE TRATAMENTO INTENSIVO

Eu_____
_____, nº de identidade _____, paciente / responsável (Grau de Parentesco _____), declaro que:

1º) estou ciente de que a internação no CTI (Centro de Tratamento Intensivo Adulto / Centro de Tratamento Intensivo Cirúrgico / Centro de Tratamento Intensivo Pediátrico / Centro de Tratamento Intensivo Neonatal) justifica-se pela necessidade de serem mantidos cuidados médico-assistenciais intensivos;

2º) estou ciente de que podem ser necessários procedimentos habitualmente realizados em CTI tais como:

•Monitorização dos sinais vitais (pressões, temperatura, freqüência respiratória, função cardíaca e respiratória, saturação de oxigênio e outros gases);

•Instalação de equipamentos de suporte da função respiratória que auxiliam a respiração (máscaras e tubos na traqueia ligados a aparelhos ou a fontes de gases);

•Procedimentos invasivos, como colocação de cateteres ou sondas em vasos sanguíneos, no aparelho urinário, no sistema nervoso central, no tórax ou no abdômen, ou outras intervenções cirúrgicas emergenciais;

•Medicamentos e nutrientes para a manutenção do estado nutricional e metabólico;

•Atendimento por equipe multiprofissional (médicos e enfermeiras especialistas, fisioterapeutas, nutricionistas, etc);

•Instalação de equipamentos que auxiliam ou substituem a função dos rins (diálise);

•Realização de exames de imagem, eventualmente com administração de contraste radiológico e/ou com anestesia geral;

•Sedação;

3º) estou ciente de que, nos exames e/ou procedimentos acima especificados, poderão ocorrer transtornos ou complicações inerentes aos mesmos, durante e após a intervenção, como os seguintes: infecções; sangramentos; lesões viscerais, mucosas e cutâneas; alterações do estado de consciência e coma; necessidade de transfusão de sangue e/ou derivados, parada cardiorrespiratória; perda ou piora da função orgânica; reações alérgicas e outros;

4º) estou ciente de que, para realizar os procedimentos acima especificados, poderá ser necessário o emprego de anestesia, cujos métodos, técnicas e fármacos anestésicos serão de indicação exclusiva do médico anestesista;

5º) por livre iniciativa, autorizo a equipe assistencial do Centro de Tratamento Intensivo do Hospital Meridional (Centro de Tratamento Intensivo Adulto / Centro de Tratamento Intensivo Cirúrgico / Centro de Tratamento Intensivo Pediátrico / Centro de Tratamento Intensivo Neonatal) e aos médicos responsáveis, a realizarem os procedimentos na forma como foi exposto no presente termo, assim como os procedimentos necessários para tentar solucionar as situações imprevisíveis ou emergenciais. Permito que esta equipe utilize seu julgamento técnico para que sejam alcançados os melhores resultados possíveis, através dos recursos conhecidos no Hospital Meridional.

_____, ___ de _____ de _____

Assinatura
_____
_____
TESTEMUNHAS
1) Nome completo
_____
Nº de Identidade _____ Assinatura
_____
2) Nome completo
_____
Nº de Identidade _____ Assinatura
_____

DEVE SER PREENCHIDO PELO MÉDICO
Confirmo que expliquei todo o procedimento ao paciente/responsável acima identificado, expliquei ainda sobre os benefícios, riscos e alternativas, tendo respondido às perguntas formuladas pelo(s) mesmo(s). De acordo com o meu entendimento, o paciente e/ou seu responsável, está em condições de compreender o que lhe(s) foi informado.
_____, ___ de _____ de _____
Nome completo
_____
Assinatura _____ CRM
_____
1ª Via: Médico 2ª Via: Paciente 3ª Via: Prontuário.

ANEXO 2:

NORMAS TÉCNICAS PARA PUBLICAÇÃO NO JOURNAL OF BIOMEDICAL SCIENCE

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Conclusions
List of abbreviations used (if any)
Competing interests
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Authors' information
Acknowledgements
Endnotes
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Tables and captions
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Thiago de Melo Costa Pereira, Ph.D.; Dyanne Moyses Dalcomune; Jorge Terrão; Marcella Leite Porto; Elisardo Corral Vasquez; Marcelo Perim Baldo
Journal of Biomedical Science

Dear Dr. Pereira,

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