Temporal trends in acute renal dysfunction among critically ill patients according to I/D and -262A > T ACE polymorphisms

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Abstract

Multiple organ failure syndrome and acute renal dysfunction share many of physiologic factors involved in their development. Recent studies correlate the susceptibility to organ dysfunction in critically ill patients with genetic inheritance. Many of them consider ACE gene could be a possible candidate to elucidate a genetic risk predisposition or a genetic factor. We aimed to examine the effects of I/D and -262A > T ACE polymorphisms in the renal function in ill southern Brazilians patients. A multi-organic worldwide known failure score, the SOFA (sequential organ failure assessment), was used to determine the basal health state at first day (ICU admission). Considering admission SOFA score and trend of renal function (measured by daily renal SOFA scores, with daily measure of serum creatinine and diuresis), we hypothesize that ACE polymorphisms could influence in the trend of renal function in ICU patients. A total of 153 critically ill adult patients (79 men) were included in this study. We monitored the patients daily during their entire ICU and post-ICU (hospital) stay (measured from the ICU admission day to a maximum of 224 days). We observed progression to renal failure (SOFA scores 3 and 4) in first seven days of ICU stay and need for dialysis. The general genotypic frequencies in our sample were II = 0.17; ID = 0.46; DD = 0.37 and AA = 0.30; AT = 0.55; TT = 0.15, and the allelic frequencies were I = 0.40; D = 0.60 and A = 0.56; T = 0.44. This is the first study to verify the influence of I/D and -262A > T ACE polymorphisms in acute renal dysfunction among critically ill patients. No significant association was found between genotypes or allele frequencies and the trend of the renal function. The I/D and -262A > T ACE polymorphisms have no significant impact on the trend of renal function during the first week of ICU stay, neither any influence in mortality in critically ill patients.

Keywords: genetics medical, kidney failure acute, renin-angiotensin system.

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Introduction

Acute kidney injury is often present among the critically ill patients in the intensive care unit (ICU). It is characterized by an abrupt reduction in renal function, developing in these subjects due to conditions associated with high mortality.1 Nowadays, renal failure in multiorganic dysfunction is considered as a causal pathway for mortality and not simply an epiphenomenon; it is an independent risk factor for mortality.[1,2] Acid-base disequilibrium and inflammatory response effects are associated with prolonged hospital and ICU length of stay.[3] As a part of multiple organ failure syndrome (MOFS), acute renal dysfunction has the same physiologic factors involved in its development.[4] Renal vasoconstriction is caused by a disequilibrium among systemic and local vasoactive substances, with changes in glomerular hemodynamic. Renal vasoconstriction can lead to merely hemodynamic acute renal failure, with reduction of glomerular filtration rate, or to acute tubular necrosis, probably caused by hemodynamic systemic failure and inflammatory mediators.[5] Besides better understanding in physiopathological mechanisms, the mortality rates

persist elevated.[4] During sepsis, there is a severe production of pro-inflammatory cytokines, involved in MOFS. Considering the clinical repercussion of microvascular control, factors that modify the adequate performance of the vascular and endothelial function can lead to hemodynamic or vascular alterations that intervene with the recovery of septic patients.[6] The renin-angiotensin-aldosterone system and the sympatic nervous system are activated during endotoxic (septic) shock, with an increase in plasma renin activity and renal vasoconstriction.[5] Individual susceptibility to organ dysfunction in critically ill patients can be related with genetic inheritance.[7-10] Polymorphic variation in human genes can result in structural or regulatory modifications that can alter many metabolic functions. Angiotensin-converting enzyme has a potential role in homeostasis, including effects in vascular tone, permeability, epithelial cell survival and fibroblast activation.[11] It has been demonstrated that an insertion/deletion (I/D) polymorphism in the ACE gene (locus 17q23) affects renal prognosis in some pathologic conditions.[12] ACE activity can be genetically modulated by a 287-bp insertion/deletion polymorphism in intron 16 of ACE gene.[12,13] Studies using the ACE I/D polymorphism have shown a dose effect of the D allele as a genetic risk factor for circulatory dysfunction and vasoconstriction,[14-18] but other authors present controversial results or do not describe any significant association.[19-25] It is believed that this intronic (noncoding) polymorphism may be a marker for another genetic locus (or loci) with more functional significance. Extensive haplotyping of the region has evaluated the promoter region and a number of exons.[11,26] A transversion A > T (-262A > T) located inside 5UTR region (which modulates the translation capability in eukaryotes) is another polymorphism studied in the ACE gene, with some published reports about its influence in human diseases but no one previously published about influence in renal function.[27-36] Serum creatinine, diuresis and use of vasopressors in an ICU context are important indicators of acute renal failure or predisposition to dysfunction.[2] These variables can be measured by a multiorganic failure score, the SOFA (sequential organ failure assessment) score.[37] Considering the admission SOFA score and the trend of renal function (measured by daily renal SOFA scores), we hypothesize that ACE polymorphisms could influence in the trend of renal function in ICU patients. We aimed to examine the effects of I/D and -262A > T ACE polymorphisms on the evolution of renal dysfunction in critically ill patients.

Subjects and Methods

Study design and inclusion/exclusion criteria

This was a cross sectional study of random, critically ill patients admitted to the general Intensive Care Unit (ICU) of the São Lucas Hospital (HSL) of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil, between May 1st, and November 28th, 2002, and between January 1st, and June 30th, 2005. Each patient was individually invited to participate in the study and gave its informed consent. One member of the family signed the form consent when the patient was unconscious or in no medical conditions to give him/herself the consent. The PUCRS Research Ethics Committee approved this study (protocols # 05-02357, and # 03-01732).

Patient selection

All the patients were southern Brazilians. All the personnel involved in patient care were blind to the selection process and genotyping results. Patients were not eligible if they were under 18 years old or diagnosed with HIV-infection, pregnant or breastfeeding or taking immunosupressive drugs.

Selected polymorphisms

The ACE gene is responsible by synthesis of the Angiotensin Converting Enzyme (ACE). It is located in locus 17q23 and interferes in modulation of systemic vascular function, with organic repercussion of some diseases.[38,39] Many polymorphisms of ACE gene have been described, but the most frequently described in studies is the Insertion/Deletion (I/D) polymorphism.[13] It consists in the presence (allele I) or absence (allele D) of a 287 bp Alu fragment inside of the intron 16 of the ACE gene. Although I/D represent an intronic polymorphism, there have been many studies describing a correlation with their isolated or in-haplotype heritance that could explain part of the total variability of the ACE enzyme activity and some clinical conditions.[26,28,40] The possible genotypes to the I/D ACE polymorphism are II, ID or DD. The -262A > T ACE is a single nucleotide polymorphism (SNP) first identified by Villard *et al*. in 1996,40 originally described as -240A > T. Zhu *et al*.[28] changed the nomenclature to -262A > T and suggested a new name, the ACE4 polymorphism, for the sake of clarity among other polymorphisms focused in their paper. Some papers refer to the nomenclature A-239T41. The possible genotypes to the -262A > T ACE SNP are AA, AT or TT.

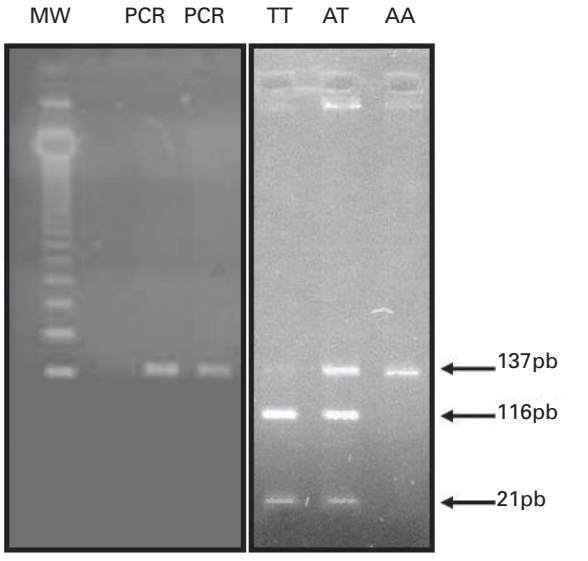
DNA collection

A 5mL blood sample was collected in a sterile system with EDTA from each patient at ICU admission and maintained refrigerated at 4° C or frozen at -20° C until DNA extraction. Genomic DNA was isolated from leukocytes by standard procedures and maintained in freezer (-20° C) as described by Lahiri & Nurnberger.[42]

Genotyping

Genotyping protocols for the determination of intron 16 I/D ACE polymorphism gene was previously described by Rigat *et al*.[13] The biallelic I/D ACE polymorphism was determined according to the PCR–AFLP method. Polymerase Chain Reaction was carried out with a total volume of 25 µL with about 10-100 ng of genomic DNA, 2.5 U Taq DNA Polymerase in Taq Buffer (Invitrogen-Life Technologies, São Paulo, SP, Brazil), the final concentration of each dNTP was 0.2 mmol/L, and 2 mmol/L $MgCl_2$. The I/D ACE polymorphism was amplified using 0.4 pmol of each primer sense, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'; and antisense, 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' (synthesized by Invitrogen-Life Technologies, São Paulo, SP, Brazil) in a PTC-100 thermocycler (MJ Research, Watertown, MA, USA) as follows: an initial denaturation at 94° C for 10 minutes, followed by 35 cycles at 94° C for 1 minute, 60° C for 1 minute, 72° C for 1 minute, and final extension step for 5 minutes. The genotype was determined by electrophoretic analysis of the amplified DNA segments on a 1.5% agarose gel, on the assumption that the I allele amplified one segment of 480 bp, and the D allele one of 190 bp. The ACE intron 16 gene sequence, and both I/D alleles are registered in the EMBL data base as GI 28921 (GenBank accession number X62855). The -262A > T ACE polymorphism was amplified using 0.3 pmol each primer forward, 5'-TCG GGC TGG GAA GAT CGA GC -3' and reverse, 5'-GAG AAA GGG CCT CCT CTC TCT-3' (synthesized by Invitrogen-Life Technologies, São Paulo, SP, Brazil), in which the underlined nucleotide represents the deliberated primer mismatch designed to introduce an artificial restriction site. Polymerase chain reaction was performed in a total volume of 30 µL with 10-100 ng of genomic DNA, 1.7U Taq DNA Polymerase in Taq Buffer (Life Technologies-Brazil. Invitrogen. São Paulo, SP, Brazil), the final concentration of each dNTP was 0.2 mmol/L, and 2 mmol/L MgCl2. Amplification was done using the same thermocycler mentioned before, as follows: initial denaturation at 94° C for 5 minutes, followed by 40 cycles at 94° C for 1 minute, 63° C for 25 seconds, and 72° C for 10 seconds. The final extension step was prolonged to 5 minutes at 72° C. The 137bp PCR amplified product (20 µL) was cleaved in an appropriate buffer with 10U of the XbaI (5`-T/CTAGA-3`; GibcoBRL®-LifeTechnologies™, Rockville, MD, USA) in a total volume of 25 µL at 37° C for 3 hours. Afterwards, the restriction digests were electrophoresed on a 3% agarose gel, stained with ethidium bromide, and visualized over a UV transilluminator to determine the genotypes AA (137bp fragment); AT (137pb, 116pb, and 21pb fragments); TT (116pb and 21pb fragments) (Figure 1). In order to confirm that the 137bp PCR amplified product really represents the targeted product, we performed a sequence analysis in MegaBase 1,000 capillary DNA sequencer (Amersham Biosciences UK Ltd, Chalfont St Giles, Bucks, UK), also using the designed primers (forward and reverse). The sequence obtained was submitted to a nucleotide-nucleotide BLAST on line alignment (blast, at http://www.ncbi.nlm.nih.gov/BLAST/) with the databases, and we found consensus with the Homo sapiens angiotensin-converting enzyme gene, promoter region (GenBank accession number AF229986) and the sequence exported from chromatogram file. The alignment view

Figure 1. Electrophoresis image from a representative sample in 3% agarose gel.



MW: banding pattern from a 123bp molecular weight marker (GibcoBRL®-Life Technologies™, Rockville, MD, USA); PCR, amplified product with 137bp; TT, banding pattern for TT homozygous (116bp and 21bp fragments); AT, banding pattern for heterozygous (137bp, 116bp, and 21bp fragments); AA, banding pattern for AA homozygous (137bp fragment). Scale of the fragments is on the right.

was performed in ClustalX program (version 1.8, as described in Thompson *et al*.,[43] at ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) in multiple alignment mode, with sequences loaded in FASTA format.

Study subjects

A total of 153 critically ill adult patients (79 men and 77 women) admitted to the Intensive Care Unit (ICU) at São Lucas Hospital – Pontifícia Universidade Católica do Rio Grande do Sul (HSL-PUCRS) were included in this study. We monitored the patients daily during their entire ICU and post-ICU stay which resulted in measurements from the ICU admission day to a maximum of 224 days. This group of critically ill adult patients was previously described partially by D'Avila *et al*. (2006).[44]

Clinical data

The dysfunction was evaluated using the SOFA (Sequential Organ Failure Assessment) score obtained during the first seven days from the ICU admission.[37,45] The SOFA is a worldwide applied ICU-risk prediction score that has undergone significant development, validation and refinement during the last decade, and have been employed to risk-adjust patients with longer, more severe illnesses (e.g., sepsis or acute respiratory failure). The score describes organ dysfunction or failure. We list the six evaluated systems and its respective interest clinical conditions, obtained to determine this score: cardiovascular function (systolic and diastolic blood pressure, use of vasopressors), liver function (serum bilirrubin levels), respiratory (PaO_2, FiO_2), neurologic function (Glasgow coma score), coagulation function (platelet count) and renal function (serum creatinine levels, urine output). The total SOFA score for a patient is obtained by the sum of the six systems scores (each one with values ranging from 0 to 4), what results in a number varying from 0 to 24. For all systems, the higher the score the worst the specific organ function, and a higher total SOFA scores predicts higher ICU mortality tax. To compare renal dysfunction, we used the renal SOFA scores. Score 0 corresponds to Creatinine serum levels under 1.2 mg/dL (110 µmol/L); score 1 corresponds to Creatinine serum levels of 1.2-1.9 mg/dL (110-170 µmol/L); score 2 corresponds to Creatinine serum levels of 2.0-3.4 mg/dL (171-299 µmol/L); score 3 corresponds to Creatinine serum levels of 3.5-4.9 mg/dL (300-440 µmol/L) or oliguria (24-hour diuresis < 500 mL); and score 4 corresponds to Creatinine serum levels > 5.0 mg/dL (300-440 µmol/L) or anuria (24-hour diuresis < 200 mL). We chose SOFA scores in order to establish comparisons of renal dysfunction between groups instead of the use of a laboratorial index (as creatinine or urea, for example) because in some cases, the mere serum measures of these biochemical analytes do not reflect the real state of renal dysfunction. This is the case of patients submitted to dialysis treatment, in which isolated measures of serum creatinine or urea reflects the effect of the machine clearance plus the renal clearance, but do not reflect directly the impairment condition of renal function. We used SOFA to measure the renal dysfunction because we consider it more precise and informative.

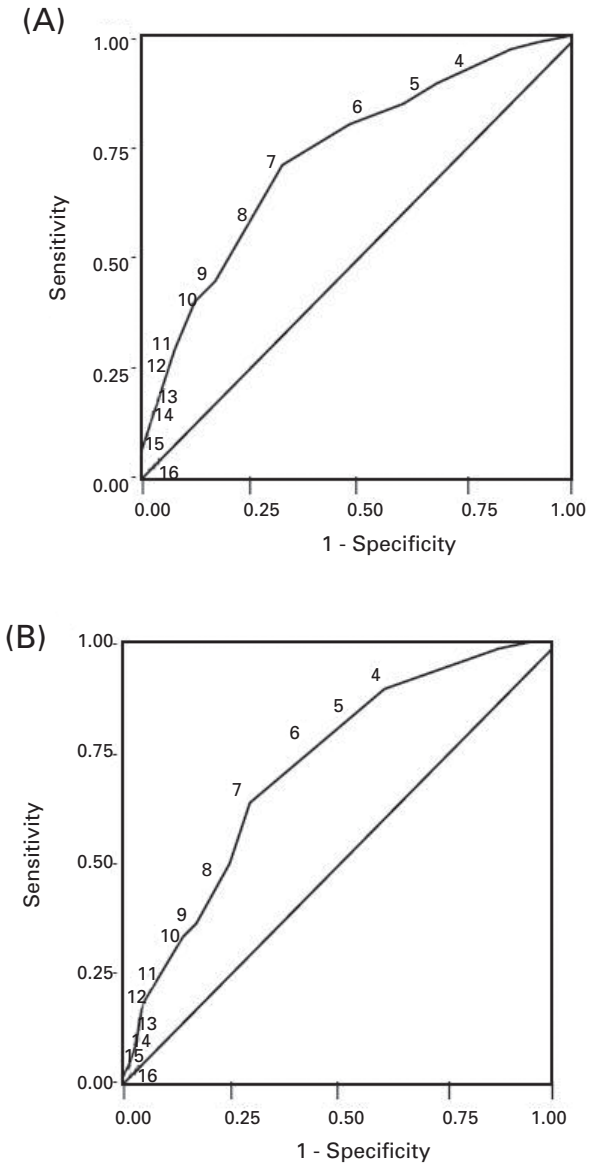
Mathematic representation of the renal SOFA trend in one week

For each patient, the temporal trend of renal function was obtained considering evolution of Renal SOFA scores from the 1st to 7th observation day (or earlier, if the length of ICU stays was shorter than 7 days). We plotted the results of the scores (y) *versus* time (x) and obtained the trend line for each patient using the Microsoft ® Excel software. The mathematical equation for the straight line is $y = Ax + B$, in which A is the declivity and B is a constant representing the elevation. We used the A coefficient as an indicator of renal function trend along time. Positive A values indicate average progressive crescent renal SOFA scores along the seven days of observation, which means non-favorable evolution. In the other hand, negative A coefficients represent a descending line, which means a trend of recovery of the renal function (favorable evolution) along time. The absence of modifications in the renal SOFA scores along the week prompts to an A value of zero.

Groups for comparison. As a prognostic score, the total SOFA score in the first day consensually can predict mortality, but there is no consensus in which one is the score number (from 0 to 24) that represents the ideal cutoff point. As different populations are considered in the studies reported in the literature,[46] a selection bias could lead to different cutoff points in different studies. Therefore, in our study we determined the ideal threshold based on higher sensitivity and specificity criteria. We performed the determination of mortality prediction with the area under the receiver operating characteristic curve (ROC curve) and observed a consistent ICU or hospital death discrimination by total SOFA score in first day, with nearest the same area under the curve for both outcomes (mortality in ICU or in hospital, 0,73 ± 0,04, $p < 0.001$). The better combination of the sensitivity and specificity for predicting death outcomes was

obtained using the score 7 in total SOFA in the first day. Lower frequency of deaths was observed in the group with scores under 7 and worst prognosis when the total SOFA score in admission day was equal or above 7 (Figure 2). The groups for comparison where chosen considering: (i) the total Renal SOFA score of the first (admission) day; and (ii) the trend of renal SOFA along the seven first days. Four distinct groups representing four evolution tendencies of renal function in the first week of ICU stay resulted: (i) Group 1: total SOFA in admission day < 7 (good survival prognosis), with similar tendency to renal function in first week (stability or improvement of renal function; A coefficient = 0); (ii) Group 2: total SOFA in admission day < 7 (good survival prognosis), with opposite tendency of renal function in first week (worsening of renal function; A coefficient > 0) (iii) Group 3: total SOFA in admission day = 7 (bad survival prognosis), with opposite tendency of renal function in first week (improvement of renal function, A coefficient < 0); (iv) Group 4: total SOFA in admission day = 7 (bad survival prognosis), with similar tendency of renal function in first week (stability or worsening; A coefficient = 0).

Figure 2. ROC Curve for day 1 total SOFA score.



Sensitivity and specificity of ICU (A) and hospital (B) mortality (diagonal segments are produced by point ties).

Other clinical data

The follow-up of our patients was extended up to the total time that patients stayed in the hospital (up to 224 days). We monitored the patients daily during their entire ICU and hospital stay, measuring their clinical data from the admission day to a discharge of the hospital or death. For diagnosis of sepsis and septic shock we used the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference criteria:[47] at least two of the following criteria: Fever or hypotermia (temperature in the core of the body > 38° C or < 36° C); Tachycardia (ventricular rate > 90 bpm); Tachypnea or Hyperventilation (> 20 breaths/min or $PaCO_2$ < 32 mmHg); Leucocytosis or Leucopenia. For illness severity evaluation we used the APACHE-II (Acute Physiology And Chronic Health Evaluation II)[48] score obtained on ICU admission day. Clinical endpoints of the study were discharge from the hospital ("survivors") or death ("non-survivors"). Mortality was measured in days until death. For those patients with multiple ICU admission during the study period, only data from the first entrance was considered. The Delta Renal SOFA is obtained by the simply difference of the Renal SOFA Score number of the 7th day of ICU stay (or the last ICU day of stay, if exit was before the 7th day), minus the score for renal SOFA in the first day. The Delta Renal SOFA summarizes the evolution of renal SOFA scores, but not adequately reflects reversible alterations occurred inside the considered period.

Statistical analysis

Statistical calculations, including multivariate analysis, were performed using the statistical package SPSS 13 (SPSS, Chicago, Illinois, USA). Unless otherwise stated, continuous variable results are expressed as a

mean ± standard deviation (SD), and the categorical variables as percentage and frequencies. Means were compared using one-way analysis of variance or the Kruskal-Wallis test for non normal distributed variables. For the categorical data we used Pearson chi-square test or Fisher exact test. Pearson chi-square test was also used to test for Hardy-Weinberg equilibrium. The Kaplan-Meier method and Cox regression were used in the survival analysis.[49] All reported P values are two-tailed and considered statistically significant when equal to 0.05 or less. ROC Curves were employed during the step of methods definitions and its use was described previously.

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Results

Clinical data

Groups were essentially different by its admission severity (measured by Total SOFA Score), and tendency of renal function. Agreeing with our expectative to be distinct, Group 1 showed the youngest patients (p=0.018), lower APACHE-II scores, (p = 0.001) and lower prevalence of sepsis (42.5%, *versus* 84% in group 4, p = 0.001) or septic shock (17.8%, p = 0.001; see Table 1). ICU length of stay was higher in Group 4 (p = 0.018). During whole observation period, the lowest prevalence was in group 1 and highest in group 4 (p = 0.001), and when considering the first week of ICU stay, only Groups 3 and 4 showed deaths (Table 1).

Need for renal replacement therapy in first week was higher in Group 4 (p = 0.012), with 7 of 9 interventions occurring in this group (14%). Nine patients (5.8%) were submitted to a renal substitutive therapy during ICU care stay. The method of choice to the critically ill unstable patients was the continuous veno-venous hemodialysis (CVVHD). Only one patient without sepsis was submitted to conventional intermittent hemodialysis, recovering function in the

Table 1 Clinical characteristics of the patients among groups

	Group 1(i) (n = 73)	Group 2(i) (n = 9)	Group 3(i) (n = 21)	Group 4(i) (n = 50)	p Value
Sex (male/female)[3]	35/38	7/2	14/07	21/29	NS
Age [years; mean ±SD] *[1]	51 ± 20.2	64.4 ± 19	62.4 ± 15.8	59 ± 18.3	0.018*
APACHE II score [mean ±SD]*[1]	16.1 ± 6.3	22.2 ± 6.1	25.8 ± 6.1	23.9 ± 7.5	0.001*
Septic Patients [n (%)][2]	31 (42.5%)	6 (66.7%)	17 (81%)	43 (86%)	0.001*
Septic Shock Patients [n (%)][2]	13 (17.8%)	3 (33.3%)	15(71.4%)	35 (70%)	0.001*
ICU LOS [days; mean ± SD][1]	14.6 ± 11.2	14.7 ± 11.2	16.4 ± 11.6	22.1 ± 16.3	0.018*
Post-ICU LOS [days; mean ± SD][1]	15.2 ± 21.6	18.9 ± 25.7	11.1 ± 21.5	11.6 ± 22.5	NS
Hospital LOS [days; mean ± SD][1]	37.6 ± 26.7	34.6 ± 25.3	42.3 ± 26.5	45.2 ± 28.8	NS
ICU Outcome in first week [death; n (%)][2]	0	0	2 (9.5%)	4 (8%)	NS
ICU Outcome [death; n (%)][2]	13 (17.8%)	3 (33.3%)	11(54.2%)	28(56%)	0.001*
Hospital Outcome [death; n (%)][2]	23 (31.5%)	5 (55.6%)	12(57.1%)	37(74%)	0.001*
Day 1 Total SOFA score (0-24) [mean ± SD] *[1]	3.8 ± 1.7	4.9 ± 1.3	10.1 ± 2	9.3 ± 2.2	0.001*
Day 1 Renal SOFA (0-4) [mean ± SD] *[1]	0.1 ± 0.6	2 ± 1.3	1.2 ± 1.2	1.4 ± 1.7	0.001*
Need for Dialysis during first week	0	1 (11.1%)	1(4.8%)	7 (14%)	0.012*

n = Count Number; SD = Standard Deviation of the mean; APACHE-II = Acute Physiology And Chronic Health Evaluation II; SOFA = Sequential Organ Failure Assessment; ICU = Intensive Care Unit; Hospital LOS: Total Hospital Stay, including period before, during and after ICU stay; LOS = Length of Stay; 1: One-way ANOVA; 2: Chi-Square Test * Significance to p<0.05 (i) see material and methods for Groups description.

first week (Group 4). There was only one death in the first week among patients with renal dysfunction. It was observed in a septic patient with abdominal sepsis who had partial recovery of renal function after CVVHD (Group 1), but died in the first week because of other complications (cardiovascular and hematologic). Eight of nine patients submitted to hemodialysis were septic and undergone CVVHD (1 from Group 2; 1 from Group 3; 6 for Group 4). We noticed one death in first week (Group 4), seven of which survived the first week, but gone to death during ICU stay. Two of nine submitted to renal substitutive therapies survived during whole ICU stay (both from group 4).

Genotypic data

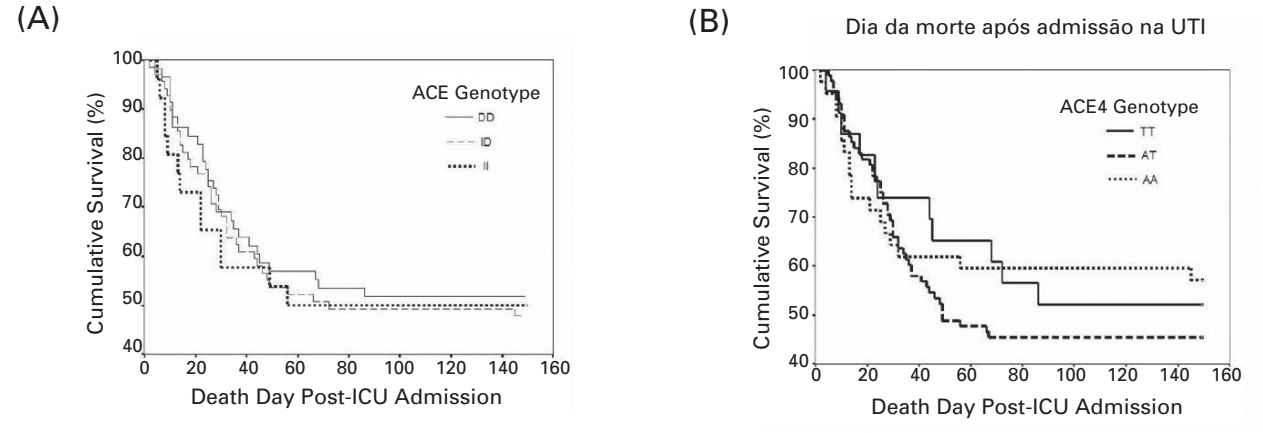
The frequencies of alleles and genotypes did not differ from the values expected by the Hardy-Weinberg model for both ACE polymorphisms (Table 2). Although significant difference in clinical characteristics had been observed among the four groups, there was not significant association between genotypic or allelic ECA frequencies and the groups, that is, there were no statistically significant differences in the frequencies for I/D genotypes (II, ID or DD) or -262A > T ACE genotypes (AA, AT or TT) in the four specific prognostic groups (Table 2). In the same way, there was not significant association between the groups and the genotypes when the two polymorphisms were studied together (Table 2). Considering the observed I/D and -262A > T ACE allele frequencies (I = 0.40; D = 0.60; A = 0.56; T = 0.44), the expected frequencies for the haplotypes would be I/A = 0.224; I/T = 0.176; D/A = 0.336; D/T = 0.264, and these four haplotypes can generate nine possible double genotypes. There was no significant difference between the expected and observed number of patients for each one of the double genotypes.

Table 2 Genotypic, allele, and double genotypes frequencies of the I/D and - 262A>T ACE polymorphisms studied Groups 1, 2, 3, and 4

	Group 1 (n = 73)	Group 2 (n =9)	Group 3 (n = 21)	Group 4 (n = 50)	Total (n = 153)	p Value*
Genotypes						
II	0.178	0.111	0.190	0.160	0.167	NS
ID	0.452	0.333	0.571	0.420	0.462	
DD	0.370	0.556	0.238	0.420	0.372	
AA	0.301	0.333	0.333	0.200	0.275	NS
AT	0.548	0.222	0.619	0.600	0.575	
TT	0.151	0.444	0.048	0.140	0.150	
Alleles						
I	0.405	0.277	0.476	0.370	0.396	NS
D	0.595	0.723	0.524	0.630	0.604	
A	0.575	0.440	0.640	0.530	0.562	NS
T	0.425	0.560	0.360	0.470	0.438	
Double Genotypes						
II/AA	0.068	0.111	0.048	0.020	0.052	NS
II/AT	0.096	0	0.143	0.140	0.111	
II/TT	0.014	0	0	0	0.006	
ID/AA	0.178	0.111	0.238	0.140	0.170	
ID/AT	0.233	0	0.333	0.280	0.248	
ID/TT	0.041	0.222	0	0	0.033	
DD/AA	0.055	0.111	0	0.040	0.046	
DD/AT	0.219	0.222	0.190	0.240	0.222	
DD/TT	0.096	0.222	0.048	0.140	0.111	

NS: Not Significant; * Chi-Square Test, comparing frequency of a same genotype among different groups.

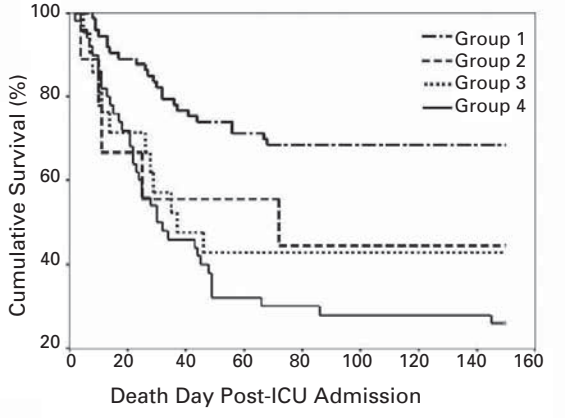
Figure 3. Kaplan-Meier survival curves for I/D (A) and -262A>T (B) ACE polymorphisms.



Relations between clinical findings and genotypic data

Considering the whole studied population (n = 153), we proceeded ANOVA in order to compare means of total SOFA in day 1, Renal SOFA in day 1, Renal SOFA in day 7 or in the last measured day, and Renal Delta SOFA with genotypes, alleles or double genotypes for I/D and -262A > T ACE polymorphisms, we did not observed any difference statistically significant. At the end of observation period, ICU deaths observed for II, ID and DD genotypes were respectively 10 (38.5%), 27 (39.1%) and 18 (31%) cases (Chi-square test, non-significant p). The same was noted among AA, AT and TT genotypes that had 16 (38.1%), 33 (37.5%) and 6 (26.1%) cases (Chi-square test, non-significant p). Hospital deaths showed similar results for II, ID and DD, respectively 13 (50%), 36 (52.2%) and 28 (48.3%) cases (Chi-square test; non-significant p). For genotypes AA, AT and TT, similar non-significant results for the same statistical study was found, with 18 (42.9%), 48 (54.5%) and 11 (47.8%) cases for each genotype. We performed survival analysis, considering all the deaths. In Kaplan-Meier survival analysis, according to its ACE I/D genotypes, there was a similar trend to I/D and -262A > T ACE genotypes (non-significant p; see Figure 3). Figure 4 shows Kaplan-Meier survival curves for considered Groups 1, 2, 3 and 4. As expected by results described in table 1, death rates increase from Group 1 to 4, with this last one presenting the higher death rates ($p < 0.05$). A Cox proportional hazards regression model for survival time since initiation of ICU stay was constructed, incorporating the two-loci genotypes in an overall

Figure 4. Kaplan-Meier survival curves for Groups 1, 2, 3 and 4.



model, as illustrated in table 3. Gender, age, occurrence of sepsis or septic shock, need of dialysis treatment and groups (1 to 4) were examined. Models satisfied proportional hazards assumptions ($p = 0.0001$). We observed that only age and occurrence of septic shock had statistical association with deaths. No correlation was found with ACE genotypes for considered polymorphisms. The same analysis was also performed excluding the Groups 1 to 4 but considering other variables (some of them were direct contributors to the delineation of those groups). We noticed a statistically significant association with increased risk of mortality by total SOFA score in the first day and with higher Delta SOFA scores (Table 3) and again, no correlation was found with genotypes for the two focused ACE polymorphisms (data not shown).

Table 3 Cox proportional hazards regression model for mortality incorporating I/D and - 262A > T ACE polymorphisms

Variable	Hazard Ratio	Confidence Interval	Overall p value
Considering Groups 1 to 4			0,41
I/D ACE genotype (II)*			0,47
ID	1.10	0.53-2.27	
DD	1.39	0.81-2.39	
- 262A > T ACE genotype (AA)*			0.69
AT	0.77	0.33-1.75	
TT	0.98	0.47-2.05	
Age	1.02	1.01-1.04	0.0001
Gender (male)*	1.19	0.71-1.98	0.496
Sepsis (negative)*	0.71	0.33-1.49	0.37
Septic shock (negative)*	0.40	0.19-0.84	0.01
Dialysis (negative)*			0.52
Continuous VVHD	0.31	0.03-2.72	
Intermitent VVHD	0.38	0.03-3.91	
Without considering Groups 1 to 4			
Pre-ICU LOS (days)	1.02	0.99-1.05	0.051
ICU LOS (days)	0.98	0.96-1.01	0.15
Hospital LOS (days)	0.98	0.95-1.01	0.22
Total SOFA (Day 1)	1.18	1.06-1.31	0.0016
Renal SOFA (Day 1)	0.99	0.79-1.24	0.97
Delta SOFA (7th – 1st)	1.36	1.03-1.80	0.03

*Risk factor listed in parentheses; a Overall p value is for the term in the general model, not individual categories. byears; LOS: Length of Stay.

Discussion

The analysis of human genome has suggested that genetic information could be used as a complementary prognostic tool for many severe diseases.[7,8,50,51] However, some specific genes have a higher and more direct influence on this setting. Otherwise genomic studies designed to identify genes, genetic markers or group of genes that confers susceptibility or resistance to pathologies, specific genotypes cannot yet predict the final phenotype, but only detect the micro-predisposition to a phenotype. Even in this case, the studies try to identify susceptibilities due to genetic inheritance or trait.[52-58] Other conclusive studies exhibit several limits common to the "candidate-gene" approach. Despite of large study population size in some papers, a high number of statistical comparisons is necessary to take into account all possible interactions, increasing the probability of a significant association.[59-61] ACE activity is a rate-limiting step for angiotensin II formation. ACE inhibitors have been shown to reduce blood pressure and slow the progression of renal diseases, and a substantial interindividual variability in treatment response has also been noted.[62] Many studies demonstrate importance of renin-angiotensin system in renal disorders, with clinical repercussion associated with genetically modulated ACE serum levels or due to implications in pharmacological system modulation.[12,41] Previous studies have evaluated association between renin-angiotensin system (RAS) polymorphisms and progression of renal

failure secondary to a variety of diseases, but there are still several reasons for discrepancies in genetic studies of progression. Genetic basis of renal failure can be genetically complex, being likely that several genes contribute in conjunction, with individual genes showing quantitative small effects that are difficult to detect or confirm.[63] The diversity of the study population can affect the results of genetic associations. The relevance of newer studies is to evaluate clinical concomitantly with genetic risk factors.[63] The most frequently studied is I/D polymorphism. It may modulate renal response of ACE inhibitors in terms of kidney function in patients with type 1 diabetes[12] and also has proven effects in type 2 diabetic nephropathy.[64] Other works describes influence of ACE polymorphisms as a risk factor for cardiovascular complications in long-term hemodialysis patients,[65] or effects on inflammatory cytokine levels, modulating its production and hence chronic inflammation in hemodialysis patients.[66] Few studies describes the -262A > T ACE polymorphism in relation to renal dysfunction. Wetmore *et al*. (2006) describe that a specific ACE haplotype predicts survival in patients with end stage renal disease, and the majority of this association was captured by this specific promoter polymorphism.[41]

Critically ill patients admitted in an ICU are individuals that require sophisticated monitoring procedures which include frequent clinical, physiological and biochemical data collection. Any study involving complex clinical features as ICU patients has the possibility of confounding bias. A strategy to minimize this and standardize comparisons over the different countries is the use of predictor scores. They are extensively validating processes of collecting data about the state of health in ICU with prognostic implications. Ceriani *et al*.[46] show results similar to ours, demonstrating that the derived SOFA variables were predictive of mortality. They suggest that the total score of the first day is representative of the patient conditions at admission to ICU and that Delta SOFA could be used as a measure of the development of dysfunction, the degree of improvement or its lack, during ICU care. SOFA has the advantage over APACHE II that can be repeatedly measured along the ICU stay, with direct correlations with significant outcomes. We corroborate the findings of other studies that related higher scores of SOFA in day 1 with higher mortality rates. In ICU patients, the most important risk factors for acute renal failure or mortality from acute renal failure are often present on admission.[67] Our aim was to correlate the trend of renal function along the first week of ICU stay with specific ACE genotypes. In order to make comparisons, we decided to adopt a strategy to identify groups of patients according to two factors: the health state in admission day measured by SOFA, and its tendency of renal function along the first week of ICU stay. We were interested in this short period of observation because it is closely related with the original cause of ICU admission. After this period, and sometimes before, many other factors can be superimposed in a patient demanding intensive care, as need for surgeries and their own complications, diagnostic procedures, nosocomial infections due to need to main drains, tubes or catheters or use of large-spectrum antibiotics. Changes in organ function in acute renal failure patients during renal replacement therapy and its relation outcome in ICU have been previously studied. A recent work analyzed changes in SOFA score over time (Delta SOFA), with assessment of these values in first 24 hours of initiation of renal replacement therapy related with higher risk of early mortality during their ICU admission.[68] The construction of Groups 1 to 4 showed correspondence with biological outcomes described in table 1, with crescent disease severity, according to group. This helps to corroborate the fact that renal dysfunction is associated with higher morbimortality. Our findings of first day SOFA and Delta SOFA scores as independent risk factors for ICU outcome have previously been described in literature.[69] Table 2, otherwise, showed a statistically similar prevalence of genotypes, haplotypes and alleles for the two focused polymorphisms among the Groups 1 to 4. If the genetic inheritance of these polymorphisms have some demonstrable effect in determine SOFA scores or Renal SOFA scores, we should expect a differential prevalence of them in specific groups, what was not demonstrated. Studies involving genetic polymorphisms and clinical conditions in ICU are not so frequent. The majority is about outcomes of acute respiratory distress syndrome (ARDS), with ACE I/D polymorphism as a significant prognostic factor for the outcome of ARDS, some indicating II as a protective genotype,[70] other showing DD genotype as responsible by susceptibility and worst prognosis of ARDS.[11] To our knowledge, no study has previously investigated I/D and -262A > T ACE polymorphisms and mortality risk in individuals with acute renal failure in ICU. Complementarily, in our study, we developed

a new well-succeeded strategy to the -262A > T ACE SNP determination, in which only one step of PCR amplification is necessary. Other authors have been presenting the -262A > T ACE SNP genotyping including two obligatory successive PCR amplifications.[26,28,40]

Conclusion

Our study is the first to attempt to make an association between I/D and -262A > T ACE polymorphisms and acute renal dysfunction among critically ill patients. We showed no significant association between genotypes or allele frequencies and the trend of the renal dysfunction was found. The I/D and -262A > T ACE polymorphisms apparently have no significant impact on the trend of renal function during the first week of ICU stay, neither any influence on mortality in critically ill patients.

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