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Tumour necrosis factor-α plus interleukin-10 low producer phenotype predicts acute kidney injury and death in intensive care unit patients

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Summary

Genetic polymorphism studies of cytokines may provide an insight into the understanding of acute kidney injury (AKI) and death in intensive care unit (ICU) patients. The aim of this study was to investigate whether the genetic polymorphisms of −308 G < A tumour necrosis factor (TNF)-α, −174 G > C interleukin (IL)-6 and −1082 G > A IL-10 may predispose ICU patients to the development of AKI and/or death. In a prospective nested case–control study, 303 ICU patients and 244 healthy individuals were evaluated. The study group included ICU patients who developed AKI ($n = 139$) and 164 ICU patients without AKI. The GG genotype of TNF-α (low producer phenotype) was significantly lower in the with AKI than without AKI groups and healthy individuals (55 *versus* 62 *versus* 73%, respectively; $P = 0{\cdot}01$). When genotypes were stratified into four categories of TNF-α/IL-10 combinations, it was observed that low TNF-α plus low IL-10 producer phenotypes were more prevalent in patients with AKI, renal replacement therapy and death ($P < 0{\cdot}05$). In logistic regression analysis, low TNF-α producer plus low IL-10 producer phenotypes remained as independent risk factors for AKI and/or death [odds ratio (OR) = 2·37, 95% confidence interval (CI): 1·16–4·84; $P = 0{\cdot}02$] and for renal replacement therapy (RRT) and/or death (OR = 3·82, 95% CI: 1·19–12·23; $P = 0{\cdot}02$). In this study, the combination of low TNF-α plus low IL-10 producer phenotypes was an independent risk factor to AKI and/or death and RRT and/or death in critically ill patients. Our results should be validated in a larger prospective study with long-term follow-up to emphasize the combination of these genotypes as potential risk factors to AKI in critically ill patients.

Keywords: cytokines/interleukins, polymorphism, renal immunology/disease

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Introduction

Acute kidney injury (AKI) is one of the main complications in the intensive care unit (ICU), especially in patients with sepsis [1]. Despite therapeutic and technological advances, AKI remains responsible for the higher mortality rates observed in ICU patients [2,3]. The involvement of systemic inflammatory response syndrome (SIRS), one of the main risk factors in the development of AKI, plays a central role in the high mortality rate observed in these patients [4–6]. This host defence response to different factors, such as bacterial, endotoxin, ischaemia–reperfusion and complement activation, leads to the release of biologically active mediators in the host [7,8], including tumour necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6 and IL-10, which are potent inflammatory mediators and are associated with poor outcome [4,9]. In addition, these inflammatory mediators have been reported to contribute to renal vasculature injury and the consequent development of AKI [10]. Thus, the balance between pro- and anti-inflammatory cytokines has an important influence on the clinical outcome of patients with acute inflammatory conditions. Although human studies report cytokine serum levels as important mediators of kidney injury, the role of these cytokine genes remains unclear.

In recent years, the study of polymorphisms of genes involved in host immune responses, including cytokines and other modulators of the inflammatory response, has

been the subject of interest, as these genetic markers may be potentially responsible for susceptibility or severity of acute disease [11–13].

Also, the study of genetic cytokine polymorphisms may provide an important perspective in the evaluation of ICU patient outcome. Thus, the aim of this study was to identify the prevalence of polymorphisms of TNF-α, IL-6 and IL-10 in ICU patients and investigate whether these polymorphisms could be associated with the development of AKI and/or death.

Materials and methods

Subjects

This was a prospective, nested case–control study which comprised 303 patients from Hospital Israelita Albert Einstein/Sao Paulo, Brazil who had been in the ICU for less than 48 h; 244 healthy individuals from the Hypertension and Cardiovascular Metabolism Center – UNIFESP/Sao Paulo, Brazil, were included into the control group. Overall, individuals were aged more than 18 years. Of the 303 ICU patients, 139 patients developed AKI (AKI group) and 164 patients did not (no-AKI group). AKI was confirmed by the Acute Kidney Injury Network (AKIN) [14] and Risk, Injury, Failure, Lesion Renal and End-stage-Renal Disease (RIFLE) criteria [15], defined as a threefold increase of serum creatinine compared with baseline serum levels of creatinine or the creatinine clearance < 60 ml/min/mm^3, calculated by the Levey equation (Modification of Diet in Renal Disease: MDRD) according to K/DOQI [16].

Patients who developed AKI were matched for age and gender to the no-AKI and control group. The exclusion criteria were: patients not considered for resuscitation, kidney transplant patients, patients undergoing dialysis and patients who had participated previously in this study.

This study was approved by the Research Ethics Committee of the Universidade Federal de São Paulo – UNIFESP, ref. number: CEP 1520/05 and by the Ethics Committee of the Hospital Israelita Albert Einstein, ref. number 06/381.

Materials

Thirty ml of blood in ethylenediamine tetraacetic acid (EDTA) anti-coagulant was collected from each individual for renal function analysis (urea and creatinine; Labtest®, Lagoa Santa, MG, Brazil and the Jaffe modified method, respectively), lipid profile [cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, automated method equipment, Cell-Dyn Ruby; Abbott Diagnostics, Lake Forest, IL, USA], markers of SIRS [C-reactive protein (CRP) and albumin, Immunlite 1000 immunoassay system, Erlangen, Germany and the colorimetric method in automated equipment; Olympus AU400, Dallas, TX, USA, respectively] and DNA extraction was performed in order to study the polymorphisms of TNF-α, IL-6 and IL-10 (genotyping tray; One Lambda, Inc., Canoga Park, CA, USA).

Polymorphism of TNF-α, IL-6 and IL-10

Genomic DNA was prepared from peripheral blood using the standard technique and was extracted using the dodecyltrimethylammonium bromide/cetyltrimethylammonium bromide (DTAB/CTAB) method [17]. The polymorphisms of genes of the A allele of the −308 polymorphism gene for TNF-α, −1082 G > A gene IL-10 and −174 G > C gene IL-6 were determined by polymerase chain reaction–sequence-specific primer (PCR-SSP) (One Lambda Kit; One Lamda, Inc.). Genotype detection was performed on the purified DNA using a commercially available cytokine genotyping tray (One Lambda, Inc.). Genotypes for polymorphism were separated by electrophoresis in 2% agarose gel and the bands were visualized under ultraviolet light and photographed using the gel electrophoresis imaging system (Kodak Digital Science – Electrophoresis Documentation and Analysis System 120). Subsequently, the results were entered into the cytokine worksheet provided along with the manual kit. Quality checks were carried out by independent rating of the results by two investigators in order to ensure the accuracy of the genotypes.

Three different possible genotypes at the −308 G > A position of the TNF-α gene promoter can be defined and associated with high (AA and GA) and low (GG) phenotype profiles. To −1082 G > A gene IL-10 gene promoter the possible genotypes were defined as high (GG), intermediate (GA) and low (AA) phenotypes. Three different possible genotypes at the −174 G > C gene IL-6 gene promoter were also defined as high (GG and GC) and low (CC) phenotypes. These production phenotypes for IL-6, TNF-α and IL-10 were based on previously published *in-vitro* transfection studies using constructs of the relevant alleles, studies on whole blood, peripheral blood mononuclear cell cultures stimulated with endotoxin and *in-vivo* studies measuring plasma levels of the relevant cytokines [18].

Statistical analysis

Data were analysed using the statistical software SPSS version 20·0 (SPSS, Inc., Chicago, IL, USA). The results were expressed as mean and standard deviation and the distribution of genotypes and phenotypes were given as percentages. Clinical characteristics and cytokine gene polymorphisms were compared using the χ^2 test and Fisher's exact test when needed. To compare the differences between the groups, *t*-tests were used for independent samples. To examine whether the genotype frequencies were in Hardy–Weinberg equilibrium, the goodness-of-fit χ^2 test was used. Allele frequencies were compared using a 2×2

Table 1. Biochemical and epidemiological data according the development to acute kidney injury (AKI) ($n = 303$) and healthy individuals (control) ($n = 244$).

	AKI			
	Yes ($n = 139$)	No ($n = 164$)	Control ($n = 244$)	*P*
Age (years)	67 ± 17	65 ± 18	60 ± 10	0·67
Gender (male)	91 (66%)	97 (60%)	154 (61%)	0·26
APACHE II score	20 ± 7	17 ± 6	–	0·001
MDRD	70 ± 51	100 ± 49	81 ± 13	0·001
Creatinine (mg/dl)	1·07 ± 1·20	0·40 ± 0·70	1·00 ± 0·2	0·0001
Albumin (mg/dl)	3·06 ± 0·25	3·24 ± 0·54	4·5 ± 0·3	0·22
CRP (mg/dl)	7·89 ± 8·61	7·20 ± 8·47	0·56 ± 0·69	0·48
Vasoactive drug	24 (17%)	6 (4%)	–	0·0001
Mechanical ventilation	29 (21%)	11 (7%)	–	0·0001
Sepsis	44 (32%)	35 (21%)	–	0·04
Coronary artery disease/heart failure	26 (19%)	31 (19%)	24 (10%)	0·96
Stroke	7 (5%)	13 (8%)	–	0·31
Hypertension	54 (39%)	66 (40%)	73 (29%)	0·80
Diabetes mellitus	25 (18%)	31 (19%)	32 (13%)	0·83
Chronic kidney disease	1 (0·01%)	3 (0·02%)	–	0·60
Mortality rate (%)	12 (9%)	7 (4%)	–	0·12

Comparison between AKI and no-AKI: Student's *t*- and χ^2 tests. MDRD: modification of diet in renal disease; APACHE: acute physiology and chronic health evaluation; CRP: C-reactive protein.

contingency table using Fisher's exact test. Binary logistic regression analysis was used to identify independent variables associated with AKI and death. All results were considered significant at $P < 0·05$.

Results

Biochemical and epidemiological data

The AKI patients had a lower initial renal function (calculated by MDRD equation) compared to the no-AKI and control groups (70 ± 51 *versus* 100 ± 49 and 81 ± 13; $P < 0·001$, respectively) and higher serum levels of creatinine (Table 1). According to RIFLE criteria, AKI patients were classified as follows: 41% as injury, 17% as failure, 15% as loss and 23% as end-stage. Among the overall cohort (AKI and no-AKI patients, $n = 303$), patients with AKI ($n = 139$) had a higher acute physiology and chronic health evaluation (APACHE) score II (20 ± 7 *versus* 17 ± 6; $P = 0·001$), a higher incidence of vasoactive drug use (17 *versus* 4%, $P = 0·0001$), were more frequently on mechanical ventilation (21 *versus* 7%, $P = 0·0001$) and had a higher incidence of sepsis (32 *versus* 21%, $P = 0·04$). Age, gender, coronary artery disease, heart failure, stroke, hypertension and diabetes mellitus were not significantly different between the studied groups (Table 1). In the univariate analysis, only APACHE II score [odds ratio (OR) = 1·07, 95% confidence interval (CI): 1·04–1·10; $P = 0·0001$] and mechanical ventilation (OR = 0·53, 95% CI: 0·31–0·90; $P = 0·02$) were risk factors for AKI.

Biochemical and epidemiological data according to mortality

With respect to mortality, an association was observed between a higher APACHE II score (27 ± 7 *versus* 18 ± 6; $P = 0·0001$), higher serum creatinine (1·66 ± 1·49 *versus* 0·64 ± 0·95; $P = 0·0001$), higher CRP (13·78 ± 12·58 *versus* 7·10 ± 8·04; $P = 0·001$), use of vasoactive drug (42% *versus* 8%, $P = 0·0001$), mechanical ventilation (53% *versus* 11%, $P = 0·0001$), sepsis (68% *versus* 23%; $P = 0·0001$) and higher death rates (Table 2). In logistic regression analysis all variables remained as markers of risk for this outcome with the exception of CRP and mechanical ventilation.

Genotype and phenotype frequency of the polymorphisms of TNF-α, IL-10 and IL-6 in patients with and without AKI

Genotype frequencies for TNF-α, IL-6 and IL-10 were similar to previous reports in the Caucasian population and the observed Hardy–Weinberg equilibrium. The genotype frequencies were not associated with gender and renal function initial (measured by MDRD) (data not shown). Our study showed that the GG genotype (low producer phenotype) of TNF-α, the GA genotype (intermediate producer phenotype) of IL-10 and the GG genotype (high producer phenotype) of IL-6 were more frequent in the total studied population (Table 3). There was no association between disease category at ICU admission and the cytokine genetic profile in the studied population (Table 4). Also, we did not observe any association between genotype profile and

Table 2. Biochemical and epidemiological data according to mortality ($n = 303$).

	Mortality		
	Yes ($n = 19$)	No ($n = 284$)	*P*
Age (years)	74 ± 14	65 ± 18	0·04
Gender (male)	10 (52·6%)	178 (62·7%)	0·38
APACHE II score	27 ± 7	18 ± 6	0·0001
Creatinine (mg/dl)	1·66 ± 1·49	0·64 ± 0·95	0·0001
Albumin (mg/dl)	3·00 ± 0·02	3·18 ± 0·46	0·43
CRP (mg/dl)	13·78 ± 12·58	7·10 ± 8·04	0·001
Vasoactive drug	8 (42%)	22 (8%)	0·0001
Mechanical ventilation	10 (53%)	30 (11%)	0·0001
Sepsis	13 (68%)	66 (23%)	0·0001
Coronary artery disease/heart failure	4 (21%)	53 (19%)	0·80
Stroke	1 (5%)	19 (7%)	0·80
Hypertension	4 (21%)	116 (41%)	0·08
Diabetes mellitus	5 (26%)	51 (18%)	0·36

Student's *t*- and χ^2 tests. APACHE: acute physiology and chronic health evaluation; CRP: C-reactive protein.

specific underlying diseases or clinical conditions that occurred after ICU admission that might contribute to the course of AKI (Tables 5 and 6).

The separate analysis of IL-6 and IL-10 genotypes did not show an association with AKI occurrence (Table 3). When phenotype combinations for TNF-α, IL-10 and IL-6 were stratified into four categories for all polymorphisms and respective phenotypes, the low TNF-α plus low IL-10 producer phenotype was the unique combination associated with AKI ($P = 0·03$), renal replacement therapy (RRT) ($P = 0·04$) and AKI and/or death ($P = 0·01$) (Table 7). Combinations of IL-6 and IL-10 and IL-6 and TNF-α were not statistically significant different among the groups studied (data not shown). In logistic regression analysis, adjusted for variables such as age, gender, ethnicity, APACHE II score, sepsis, albumin and CRP, the low TNF-α plus low IL-10 producer phenotype remained an independent risk factor for AKI and/or death (OR = 2·37, 95% CI: 1·16–4·84; $P = 0·02$) and for RRT and/or death (OR = 3·82, 95% CI: 1·19–12·23; $P = 0·02$) (Table 8).

Discussion

In the present study, we did not observe a specific association of TNF-α, IL-6 and IL-10 polymorphisms in ICU patients who developed AKI and/or death. However, patients who had a combination of low TNF-α plus low IL-10 producer phenotype showed an independent association with AKI and/or death and RRT and/or death.

In accordance with our study, Pappachan *et al.* observed an association with high mortality rate in ICU patients who were lower TNF-α producer phenotype carriers [19]. Our group had already demonstrated previously a significant

Table 3. Frequency of phenotypes and genotypes (%) of tumour necrosis factor (TNF)-α, interleukin (IL)-10 and IL-6 polymorphisms in intensive care unit (ICU) patients according acute kidney injury (AKI) and in the control group.

Phenotype	Genotype	With AKI ($n = 139$)	Without AKI ($n = 164$)	Control ($n = 244$)	$P^\dagger$
TNF-α (−308 G > A)					<0·01
Low producer	GG	76 (55%)	102 (62%)	178 (73%)	
High producer	GA	60 (43%)	52 (32%)	54 (23%)	
	AA	3 (2%)	10 (6%)	8 (4%)	
		With AKI ($n = 136$)	Without AKI ($n = 162$)	Control ($n = 240$)	*P*
IL-10 (−1082 G > A)					0·10
Low producer	AA	43 (32%)	63 (38%)	109 (45%)	
Intermediate producer	GA	80 (59%)	88 (54%)	113 (47%)	
High producer	GG	13 (9%)	11 (8%)	18 (8%)	
		With AKI ($n = 125$)	Without AKI ($n = 138$)	Control ($n = 239$)	*P*
IL-6 (−174 G > C)					<0·001
Low producer	CC	32 (26%)	33 (24%)	18 (8%)	
High producer	GC	33 (26%)	40 (29%)	72 (30%)	
	GG	60 (48%)	65 (47%)	149 (62%)	
TNF-α + IL-10 combination phenotype					0·03
TNF-α low + IL-10 low producer		40 (30%)	20 (15%)		
TNF-α low + IL-10 intermediate + high producer		6 (5%)	9 (7%)		
TNF-α high + IL-10 low producer		23 (18%)	23 (18%)		
TNF-α high + IL-10 intermediate + high producer		5 (4%)	4 (3%)		

$^\dagger\chi^2$/Fisher's exact test.

Table 4. Frequency of genotypes (%) of tumour necrosis factor (TNF)-α,interleukin (IL)-10 and IL-6 polymorphisms in patients with and without acute kidney injury (AKI) according disease category at intensive care unit (ICU) admission.

		With AKI* ($n = 139$)				Without AKI* ($n = 164$)			
Disease category		Clinical	Surgical	Sepsis	Other	Clinical	Surgical	Sepsis	Other
TNF-α (−308 G>A)*									
Low producer	GG	23 (16%)	3 (2%)	47 (34%)	3 (2%)	31 (19%)	12 (7%)	50 (30%)	9 (5%)
High producer	GA	18 (13%)	5 (4%)	35 (25%)	2 (2%)	19 (11%)	4 (2%)	23 (14%)	6 (3%)
	AA	1 (1%)	0	1 (1%)	1 (1%)	2 (1%)	2 (1%)	6 (3%)	–

		With AKI ($n = 136$)				Without AKI ($n = 162$)			
Disease category		Clinical	Surgical	Sepsis	Other	Clinical	Surgical	Sepsis	Other
IL-10 (−1082 G>A)*									
Low producer	AA	18 (13%)	0	25 (13%)	1 (1%)	19 (12%)	5 (3%)	35 (21%)	5 (3%)
Intermediate producer	GA	20 (15%)	7 (5%)	50 (36%)	4 (4%)	27 (16%)	12 (7%)	40 (25%)	9 (5%)
High producer	GG	5 (4%)	0	7 (5%)	1 (1%)	6 (3%)	1 (1%)	3 (2%)	1 (2%)

		With AKI ($n = 126$)				Without AKI ($n = 138$)			
Disease category		Clinical	Surgical	Sepsis	Other	Clinical	Surgical	Sepsis	Other
IL-6 (−174 G>C)*									
Low producer CC		12 (9%)	1 (10%)	18 (1%)	1 (1%)	13 (9%)	5 (4%)	13 (9%)	2 (2%)
High producer	GC	8 (6%)	2 (18%)	21 (16%)	3 (2%)	13 (9%)	6 (4%)	16 (11%)	5 (4%)
	GG	17 (13%)	4 (17%)	37 (29%)	2 (1%)	19 (14%)	4 (3%)	35 (25%)	7 (5%)

*No statistical differences between groups; $P > 0{\cdot}05$.

reduction in spontaneous release of IL-10 and TNF-α by peripheral blood mononuclear cells (PBMC) isolated from critically ill patients compared to healthy controls. In this study, we suggest that a proinflammatory environment results in the preactivation of immune cells from critically ill patients, resulting in decreased spontaneous IL-10 and TNF-α production [20]. In fact, studies have shown down-regulation of nuclear factor-kappa B (NF-κB) in PBMC from critically ill patients, due to an imbalance between its active (p65p50) and inactive (p50p50) forms and to a weak cytoplasmic expression of its inhibitor (IκBα), that may be related with the lower TNF-α producer profile observed in the present study [21]. Therefore, it is possible that genetic polymorphisms may influence this imbalance, more so in critically ill patients. Accordingly, in the present study we observed that low TNF-α plus low IL-10 producer phenotype combinations were associated with poor outcome, reinforcing the hypothesis that these phenotypes may reflect an immuno-incompetent response in critically ill patients.

Table 5. Frequency of genotypes of the polymorphisms of tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10 in acute kidney injury (AKI) patients according to underlying disease.

	HAS*		DM*		MDRD*	
Polymorphism	No	Yes	No	Yes	< 60	≥ 60
TNF-α						
AA	1 (1%)	2 (4%)	3 (3%)	0 (0%)	3 (4%)	0 (0%)
GA	37 (44%)	23 (43%)	50 (44%)	10 (40%)	27 (37%)	33 (51%)
GG	47 (55%)	29 (54%)	61 (54%)	15 (60%)	44 (60%)	32 (50%)
IL-6						
CC	16 (19%)	16 (30%)	23 (20%)	9 (36%)	7 (10%)	16 (25%)
GC	25 (30%)	8 (15%)	31 (27%)	2 (8%)	16 (22%)	17 (26%)
GG	36 (42%)	24 (44%)	49 (43%)	11 (44%)	16 (22%)	25 (39%)
IL-10						
AA	26 (31%)	17 (32%)	36 (32%)	7 (28%)	24 (32%)	19 (30%)
GA	53 (50%)	27 (50%)	13 (59%)	13 (52%)	40 (55%)	40 (52%)
GG	4 (5%)	9 (17%)	10 (9%)	3 (12%)	8 (11%)	5 (8%)

*No statistical differences between groups; $P > 0{\cdot}05$.

Table 6. Frequency of genotypes of tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10 polymorphisms in the acute kidney injury (AKI) group according mechanical ventilation, vasoactive drug and sepsis after intensive care unit (ICU) admission.

	Mechanical ventilation*		Vasoactive drug*		Sepsis*	
Polymorphism	No	Yes	No	Yes	No	Yes
TNF-α						
AA	3 (3%)	0 (0%)	3 (3%)	0 (0%)	3 (3%)	0 (0%)
GA	49 (44%)	11 (38%)	47 (41%)	12 (50%)	43 (45%)	17 (39%)
GG	58 (53%)	18 (62%)	64 (56%)	12 (50%)	49 (52%)	27 (61%)
IL-6						
CC	24 (22%)	8 (28%)	25 (22%)	7 (30%)	22 (23%)	10 (23%)
GC	29 (26%)	4 (14%)	28 (25%)	4 (17%)	18 (19%)	15 (34%)
GG	45 (41%)	15 (52%)	48 (42%)	12 (50%)	43 (45%)	17 (39%)
IL-10						
AA	32 (29%)	11 (33%)	34 (30%)	9 (38%)	29 (31%)	14 (32%)
GA	65 (59%)	15 (52%)	66 (58%)	13 (44%)	56 (59%)	24 (55%)
GG	10 (9%)	3 (10%)	11 (10%)	2 (8%)	7 (8%)	6 (14%)

*No statistical differences between groups or genotype/phenotype; $P > 0{\cdot}05$.

Table 7. Association between tumour necrosis factor (TNF)-α plus interleukin (IL)-10 low producer phenotype with acute kidney injury (AKI) occurrence, renal replacement therapy (RRT) and death.

	TNF-α plus IL-10 producer combination		
	TNF-α + IL-10 low producer	Other combination	P*
AKI occurrence	36 (27·7%)	20 (15·4%)	0·03
RRT	11 (8·5%)	3 (2·3%)	0·04
Death	4 (3·1%)	2 (1·5%)	0·51
AKI and/or death	38 (29·2%)	20 (15·4%)	0·01
RRT and/or death	15 (11·5%)	4 (3·0%)	0·01

*Univariate analysis. Other combination: TNF-α low plus IL-10 high; TNF-α high plus IL-10 high and TNF-α high plus IL-10 low producer.

TNF-α plays a central role in the physiological response; its secretion is necessary for efficient innate and adaptive immune responses [22]. It has been reported that low production of TNF-α reduces the expression of adhesion molecules on the vascular endothelium and less leucocyte migration to local injury [23,24]. Hence, lower migration of neutrophils and macrophages to the site of tissue injury contributes to lower, or lack of, initial immune response. In fact, when homozygous TNF-α gene knock-out mice (no TNF-α production) were infected with *Corynebacterium parvum*, there was little or no initial response but the mice went on to develop a severe and fatal inflammatory reaction [25]. Sepsis is the main contributory cause of the development of AKI. Therefore, it is possible that this disequilib-

Table 8. Relationship between tumour necrosis factor (TNF)-α plus low interleukin (IL)-10 producer and risk of acute kidney injury (AKI) and/or death and renal replacement therapy (RRT) and/or death.

Outcomes	OR	95% CI	P
AKI and/or death			
Unadjusted	2·37	1·16–4·84	0·02
Adjusted for age (years)	2·34	1·14–4·80	0·02
Adjusted for age and gender (male)	2·40	1·16–4·95	0·02
Adjusted for age, gender and ethnicity (Caucasian)	2·83	1·32–6·07	0·007
Adjusted for age, gender, ethnicity and APACHE score II	2·87	1·29–6·38	0·009
Adjusted for age, gender, ethnicity, APACHE II score and sepsis	3·05	1·36–6·85	0·007
Adjusted for age, gender, ethnicity, APACHE II score, sepsis and albumin	3·05	1·35–6·87	0·007
Adjusted for age, gender, ethnicity, APACHE II score, sepsis, albumin and CRP	3·05	1·35–6·91	0·007
RRT and/or death			
Unadjusted	3·82	1·19–12·23	0·02
Adjusted for age (years)	3·93	1·22–12·68	0·02
Adjusted for age and gender (male)	3·97	1·23–12·80	0·02
Adjusted for age, gender and ethnicity (Caucasian)	4·61	1·25–16·95	0·02
Adjusted for age, gender, ethnicity and APACHE II score	4·76	1·22–18·52	0·02
Adjusted for age, gender, ethnicity, APACHE II score and sepsis	7·15	1·66–30·67	0·008
Adjusted for age, gender, ethnicity, APACHE II score, sepsis and albumin	6·65	1·51–29·30	0·01
Adjusted for age, gender, ethnicity, APACHE II score, sepsis, albumin and CRP	6·74	1·52–29·76	0·01

APACHE: acute physiology and chronic health evaluation; CRP: C-reactive protein; OR: odds ratio; CI: confidence interval.

rium of the production of TNF-α in patients who are TNF-α low producer genotype carriers show a reduced response to infectious challenge agents in critically ill patients. Although, in the present study, we observed 42 patients with sepsis who developed AKI, this sample size and their respective polymorphisms are not enough to prove this hypothesis. Instead, the high TNF-α local production reflects leucocyte activation, greater inflammation and tissue injury. Jaber *et al*. demonstrated an association between high TNF-α producer phenotypes and death in AKI patients who required dialysis [26], but this study did not demonstrate if this phenotype was a predictor of AKI occurrence or direct renal injury. Balakrishnan *et al*. also showed that a high TNF-α producer phenotype was associated with morbidity and low serum albumin levels in peritoneal dialysis patients [27]. The results mentioned above suggest that high TNF-α producer phenotypes are risk markers associated with poor outcome in dialysis patients. However, this association has not yet been reported prospectively in critically ill patients, taking AKI occurrence into consideration.

With respect to IL-10, the −1082 G of the IL-10 polymorphism (high producer) has been associated with lower risk of death [23] and the −1082 A (low producer phenotype) has been associated with increased susceptibility to infectious disease [28], as well as an increased incidence of acute rejection of transplanted kidney and pancreas [29].

In a study of patients with AKI requiring dialysis, the −1082 G of IL-10 was associated with high production of IL-10 and lower risk of death [26]. Our study showed a prevalent intermediate producer phenotype for all groups, but there was no difference between the groups studied. Instead, we observed an association between the higher risk of AKI and/or death and RRT and/or death in patients with genotype carriers of low TNF-α plus low IL-10 producer. It has been described that IL-10 requires 10–20 times higher serum concentrations than TNF-α to inhibit the effect of the proinflammatory cytokine. Additionally, it has been reported that TNF-α regulates serum synthesis of IL-10, but it is unclear whether the presence of genotype to TNF-α low producer may be involved in down-regulated IL-10 genotype expression, or if there is any association between the expression of the genotypes of these two cytokines, which is not favoured by their gene location. Furthermore, it is still unclear from our and other data if that association has any bearing on the patient's outcome and whether only this association may be associated with outcome. Lu *et al*., in a systematic review of 35 polymorphisms as genetic determinants of AKI that included −308 of the gene for TNF-α G > A, −1082 G > A gene IL-10 and −174 G > C gene IL-6 polymorphisms, reported that there is no single polymorphism that can be determined conclusively as a risk factor in AKI [24]. At present, there is no report on the direct effect of these polymorphisms on kidney injury among critically ill patients evaluated prospectively.

High serum levels of IL-6, a proinflammatory cytokine, have been associated with a rise in acute phase proteins and inflammation. However, there are conflicting results concerning the influence of the IL-6 genetic polymorphisms with renal disease patients [26,30]. Although we have observed a higher frequency of the IL-6 high producer phenotype for IL-6 polymorphisms in the overall population, we did not observe any association between isolated and/or a combination of this IL-6 genotype with other genetic polymorphisms studied and with AKI and/or death. In accordance with our study, Balakrishnam *et al*. also observed a higher proportion of patients with the IL-6 high producer genotype in haemodialysis patients, but this isolated genotype was not a predictor of death, although it was of diabetes [27]. Thus, there are many biological steps, apart from the influence of the gene polymorphisms on cytokine production, for which the control, production and release of these cytokines and its activity are regulated.

It has been also reported that there are marked discrepancies regarding cytokine production in studies *in vivo* when compared with correspondent genetic polymorphisms [28,29]. These discrepancies may be due, in part, to ethnic differences, with some ethnic groups carrying extremely low frequencies of some genotypes for a determined cytokine [30]. Moreover, the limited sample size impaired the ability to generalize the results in genetic association studies.

To our knowledge, the present study is the first to evaluate genetic polymorphisms of TNF-α, IL-6 and IL-10 in ICU patients as possible risk factors in the development of AKI, RRT or death. Although the combination of low TNF-α plus low IL-10 producer phenotypes were shown to be independent risk factors to AKI and/or death and RRT and/or death, these polymorphisms were not associated separately with these outcomes in ICU patients. This genetic combination seems to reflect an immunoincompetent state in critically ill patients associated with worse prognosis. Our results should be validated in a larger prospective study with long-term follow-up to emphasize the combination of these genotypes as potential risk factors of AKI in critically ill patients.

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Disclosure

All authors state that they have no conflicts of interests.

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