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The cutoff value of presepsin for diagnosing sepsis increases with kidney dysfunction, a cross-sectional observational study

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Abstract

As presepsin levels increase with kidney dysfunction (KD), our aim was to establish cutoff points for presepsin adapted to the level of KD in order to avoid bacterial infection overdiagnosis, antibiotic overprescription, and risk of bacterial resistance. This is a unicenter retrospective study, which included all patients admitted on an emergency basis to 2 departments of a teaching hospital during a 2-year interval to whom presepsin level was determined at the emergency department prior to admission. Serum creatinine (sCrt) was employed to estimate the severity of KD using 3 thresholds (1.5, 2, and 4 mg/dL) resulting in 4 degrees of severity: KD_1, KD_2, KD_3, KD_4. There is an ascending exponential relationship between presepsin and sCrt: presepsin = $600.03e^{0.212sCrt}$. Presepsin levels are significantly different between the patients with KD_1, KD_2, KD_3, and KD_4. In the receiver operating characteristic curves exploring the usefulness of presepsin in sepsis diagnosis, the area under the curve was satisfactory for KD_1 (0.78), KD_2 (0.78), and KD_3 (0.82), but unacceptably low for KD_4 (0.59), while the optimal cutoff points were (depending on the computational method) 700/ 982, 588/ 1125, 1065, and 2260 pg/mL for KD_1, KD_2, KD_3, and KD_4 respectively. The threshold for abnormal presepsin should be about 600, 1000, and 1300 pg/mL in patients with KD_1, KD_2, and KD_3, respectively. In patients with KD_4, presepsin has a poor discriminating power for sepsis diagnosis. If, notwithstanding, it is used for this purpose, the cutoff point should be at least at 2200.

Abbreviations: AKI = acute kidney injury, CD = cluster of differentiation, CKD = chronic kidney disease, GFR = glomerular filtration rate, KD = kidney dysfunction, LPS = lipopolysaccharide, mCD14 = membrane-bound CD14, ROC = receiver operating characteristic, sCD14 = soluble CD14, sCrt = serum creatinine.

Keywords: bacteria, drug resistance, infections, leukocytes, medical overuse

1. Introduction

Presepsin (sCD14-subtypes) is a biomarker for sepsis that functions as a receptor of lipopolysaccharide (LPS)-LPS binding protein complexes.[1] LPS (aka endotoxin) is a component of the gram-negative bacteria membrane, that binds to cluster of differentiation 14 (CD14) and initiates a systemic inflammatory response.[1,2] CD 14 is a glycoprotein receptor and has 2 forms: membrane-bound CD14 (mCD14) and soluble CD14 (sCD14).[1,3]The former (mCD14) is attached to the membrane of monocytes, macrophage, and neutrophils by a glycosylphosphatidylinositol tail, the absence of which makes sCD14 free to circulate in the blood, where it can be assessed as presepsin.[1] LPS binding to mCD14 leads to the activation of Toll-like receptor 4 and, further, of various tyrosine and mitogen-activated protein kinases, cascading downstream into a cytokine production surge.[1] The sCD14 in plasma may have 2 origins: cleavage of the glycosylphosphatidylinositol-anchor (sCD14α) or secretion by the monocytes, macrophages, or granulocytes (sCD14β).[3] This soluble form too can link to LPS, thereby engendering a complex that may lead to cell activation by means of the same Toll like receptor 4,[3,4] resulting in an immune response in both CD14 negative cells (endothelial and epithelial cells) and CD14 positive cells.[1] The 13 kDa fragments that various proteases chop off from the N-terminus of sCD14 are highly correlated with bacterial infection, hence the name presepsin.[1,5] It was noticed that presepsin level rises in the setting of kidney dysfunction (KD) (both acute and chronic) without signifying sepsis.[6,7] A study conducted on 71 patients with chronic kidney disease

DD and MIG contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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(CKD), 13 of which were on dialysis, demonstrated higher plasma concentration of presepsin in dialysis patients to levels similar to those induced by severe sepsis in patients with normal kidney function. It was noticed that presepsin level increased as glomerular filtration rate (GFR) decreased.[8] Presepsin metabolization by proximal tubular cells explains the increase in plasma presepsin associated with declining kidney function.[9] Another study performed on 47 healthy subjects and 85 patients with CKD attempted to establish reference ranges of presepsin depending on CKD stages but the number of patients was insufficient for reaching the set goal.[7] A similar study with 170 patients confirmed that presepsin level increases exponentially with kidney function decline but it had the same limitation, the number of patients.[5] Presepsin is a sepsis biomarker, with a higher specificity and sensitivity for bacterial infection than other inflammatory markers (C reactive protein, fibrinogen, erythrocyte sedimentation rate) or procalcitonin.[10,11] Presepsin allows differentiation between sepsis and noninfectious systemic inflammatory response syndrome, but this can be difficult when kidney failure is associated.[12] Presepsin level can be high in patients with impaired renal function, without signifying sepsis. Therefore, new reference ranges should be established for patients with KD in order to avoid sepsis overdiagnosis and excessive antibiotic prescription, that may potentially result in a higher rate of antibiotic-resistant bacterial strains.[13]

2. Methods

2.1. Study design and ethical issues

The research was conducted as a unicenter retrospective study, in respect of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of University Emergency Hospital, Bucharest. Written informed consent was obtained from all participants in the study.

2.2. Study population

The study included all patients admitted on an emergency basis to 1st Internal Medicine and Nephrology departments of University Emergency Hospital, Bucharest between January 1, 2018 and December 31, 2020 to whom presepsin level was determined at the Emergency Department prior to admission, irrespective of age or diagnosis. Patients with coronavirus disease-2019 were excluded. A total of 519 patients were enrolled, but 9 patients were eliminated due to extreme levels of leukocyte count, either too high (>70.000/μL, all of which had a myeloproliferative disorder) or too low (<1.000/μL, all of which had chemotherapy-induced leukopenia). We were left with 510 patients, 280 females and 230 males, with ages between 24 and 98 years, average ± standard deviation of 71.83 ± 13.55 years, a median of 73 years, and an interquartile interval of 64 to 82 years.

2.3. Laboratory studies

Presepsin concentration was determined (in pg/mL) by chemiluminescent enzyme immunoassay using an automatic analyzer PATHFAST (Manufactured by: LSI Medience Corporation, Japan; Authorized Representative: Mitsubishi Chemical Europe GmbH, Germany). The reference levels were: <200 = L (low), 300 to 500 = H (high), 500 to 1000 = 2H, and >1000 = 3H. It should be noticed that presepsin values >20.000 were recorded as ">20.000," with no definite value being provided – this was the case for 7 patients. For computing purposes, a value of 20.001 was attributed to these patients. Standard laboratory methods were employed for complete blood count and biochemical parameters, including creatinine.

2.4. Data analysis

It is known that the equations commonly employed for estimating GFR (such as Chronic Kidney Disease Epidemiology Collaboration and Modification of Diet in Renal Disease equations) are not reliable for assessing GFR in critically ill patients developing acute kidney injury (AKI).[14] Therefore, only serum creatinine (sCrt) (in mg/dL) at admission was used for evaluating kidney function. The thresholds 1.5, 2, and 4 mg/dL were chosen for sCrt, resulting in 4 degrees of severity labeled as KD_1 (sCrt ≤ 1.5 mg/dL), KD_2 (sCrt > 1.5 and ≤ 2 mg/dL), KD_3 (sCrt > 2 and ≤ 4 mg/dL), and KD_4 (sCrt > 4 mg/dL). In each patient, the sCrt taken into account was the one measured closest to the determination of presepsin level. In most cases the blood samples for creatinine and presepsin assessment were taken no more than a few minutes apart. In a minority of cases, the blood sample for creatinine measurement was taken >1 hour (at most 3 hours) before the blood sample for presepsin measurement.

The statistical analysis included: the calculation of the Pearson correlation coefficient when exploring the correlation between 2 numerical values, such as presepsin (or log [presepsin]), sCrt, age, and neutrophil and leukocyte count; the calculation of the various quartiles (minimum, first quartile, median, third quartile, and maximum) for numerical values (mainly presepsin); the performance of Mann–Whitney test with continuity correction in order to compare continuous parameters for various categories of patients, such as presepsin (or log [presepsin]) for the various stages of CKD or for the various clinical course/outcome categories. It should be noted that Mann–Whitney test cannot be employed for sample sizes of 7 or less, as it will yield a *P* value >.05 irrespective of the magnitude of the difference between the compared groups.[15] When multiple comparisons were performed, the significance level (commonly set at 0.05) was lowered according to Bonferroni correction: the corrected significance level was 0.05 divided by the number of comparisons.[16] All the statistical calculations and all but one of the graphical representations were performed using the R language Microsoft Excel 2019 MSO (Version 2211 Build 16.0.15831.20098) and environment for statistical computing and graphics , version 4.0.3 (copyrighted by The R Foundation for Statistical Computing). Microsoft Excel 2019 MSO (Version 2211 Build 16.0.15831.20098) was employed to graphically represent the relation between presepsin and sCrt (Fig. 2), the corresponding equation being provided by "Display Equation on chart" option. In order to achieve a more compact representation, 2 extreme values of sCrt (21.0 and 23.3 mg/dL) were excluded as outliers, as the next highest value was 16.7 mg/dL. Given the wide range of presepsin values, encompassing several orders of magnitude (from 60 to over 20.000 pg/mL), log (presepsin) was used in some of the statistical calculations and in some of the graphical representations. It should be noted that this did not alter the results provided by Mann–Whitney test (given the non-parametric nature of this test). The correlation tests were performed for

Table 1

Results of Mann–Whitney test regarding the factors that influenced antibiotic prescription and the influence of gender on presepsin level.

Parameters compared	Statistic	*P* value
Presepsin in patients prescribed antibiotic ~ presepsin in patients not prescribed antibiotic	8400	2E–06
Leukocytes in patients prescribed antibiotic ~ leukocytes in patients not prescribed antibiotic	7249.5	4E–09
Neutrophils in patients prescribed antibiotic ~ neutrophils in patients not prescribed antibiotic	6463.5	3E–11
Presepsin in female patients ~ presepsin in male patients	31340	.6

~ = compared to.

both presepsin and log (presepsin). We have chosen the decimal logarithm (rather than the natural one), because its results are more easily understandable (powers of 10 are currently used to express various biological parameters, while powers of Euler number hardly at all). The terms "positive/direct correlation" and "positive/direct relationship" are equivalent and will be used interchangeably. The same holds for terms "negative/ inverse correlation" and "negative/ inverse relationship." In this article, the terms threshold and cutoff point will also be used interchangeably. Receiver operating characteristic (ROC) curves were used to explore whether the usefulness of presepsin in diagnosing sepsis depends on the severity of KD at presentation. Sepsis was defined according to 2016 SCCM/ESICM task force.[17] Several methods for calculating the optimal cutoff point were used, all of them relying on the maximization (sum of sensitivity and specificity, product of sensitivity and specificity, Youden index, chi-square statistic) or minimization (the Euclidean distance between the ROC curve and the [0.1] point) of a certain function.[18]

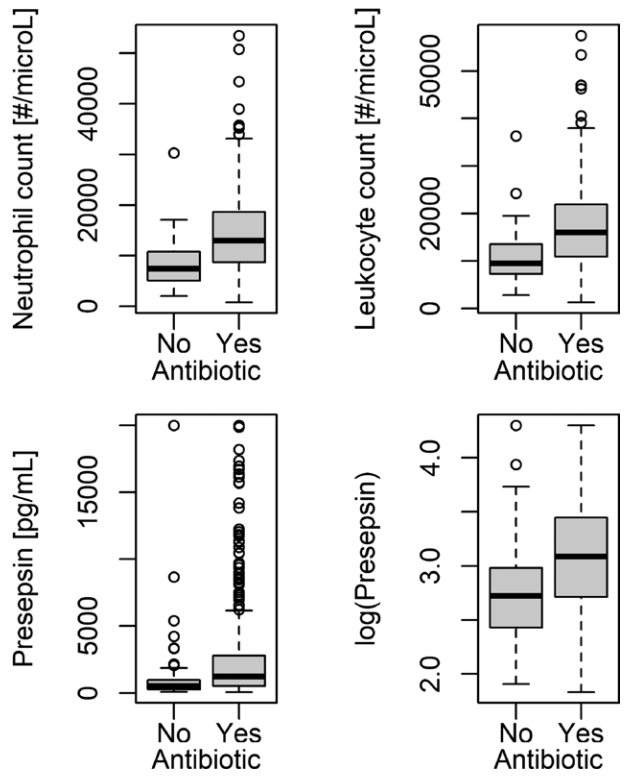

Figure 1. Factors correlated with antibiotic prescription.

Table 2

Correlations among the relevant variables.

Correlated parameters	Correlation coefficient	95% confidence interval	Statistic	*P* value
Presepsin ~ creatinine	**0.481**	**0.411–0.545**	**12.351**	**8E–31**
Log (presepsin) ~ creatinine	**0.536**	**0.471–0.595**	**14.304**	**3E–39**
Presepsin ~ age	**–0.181**	**–0.263––0.095**	**–4.14**	**4E–05**
Log (presepsin) ~ age	–0.061	–0.148–0.025	–1.388	.17
Creatinine ~ age	–0.059	–0.145–0.028	–1.334	.18
Presepsin ~ leukocytes	0.021	–0.066–0.108	0.474	.64
Presepsin ~ neutrophils	0.038	–0.049–0.124	0.85	.4
Leukocytes ~ creatinine	0.057	–0.03–0.143	1.276	.2
Neutrophils ~ creatinine	0.054	–0.033–0.14	1.223	.22

The statistically significant correlations are highlighted by bold typing.
~ = correlated with.

3. Results

Our data suggests that the prescription of an antibiotic is influenced by presepsin level at admission, as it is, expectedly, by the neutrophil and leukocyte count (Table 1 and Fig. 1). As the number of neutrophils (and leukocytes) is not correlated with the level of presepsin (Table 2), these 2 determinants of the decision to prescribe an antibiotic should be considered as independent from each other. There was no correlation between neutrophil or leukocyte count and sCrt, but there was a highly statistically significant direct correlation between presepsin (and log [presepsin]) and sCrt (Table 2). Mann–Whitney test indicates statistically significant differences for log (presepsin) between each successive degrees of severity of KD (Table 3). The corresponding quartiles of presepsin are displayed in Table 4. There is an exponential relationship between presepsin and sCrt, modeled by the equation presepsin = $600.03e^{0.212sCrt}$ (Fig. 2). Other factors that may influence the relationship between presepsin level and kidney function were taken into account: gender, age, and the clinical course/outcome of the patient. Gender did not influence the level of presepsin (Table 1). There is a statistically significant correlation between age and presepsin (Table 2), but surprisingly it is a negative one, albeit weak. Interestingly, there was no correlation between age and sCrt, probably because in the majority of patients KD, if present, was mainly or exclusively acute.

The clinical course/outcome of the patients was quite diverse. About 45% (i.e., 229 out of 510) died during their hospital stay; their survival time (in days) followed an exponential distribution

Table 3

Results of Mann–Whitney test used to compare log (presepsin) between each successive degrees of severity of kidney dysfunction in a 4-degree system.

KD severity degrees compared in terms of log (presepsin)	Statistic	*P* value
Log (presepsin) in patients with KD_1 ~ log (presepsin) in patients with KD_2	3875.5	0.0002
Log (presepsin) in patients with KD_2 ~ log (presepsin) in patients with KD_3	1831	3E–05
Log (presepsin) in patients with KD_3 ~ log (presepsin) in patients with KD_4	4228	1E–08

KD_1: sCrt ≤ 1.5 mg/dL, KD_2: sCrt > 1.5 and ≤ 2 mg/dL, KD_3: sCrt > 2 and ≤ 4 mg/dL, KD_4: sCrt > 4 mg/dL.
~ = compared to, KD = kidney dysfunction, sCrt = serum creatinine.

Table 4

The median, interquartile interval (first to third quartile), and range (minimum to maximum) of presepsin (in pg/mL) for the 4 degrees of severity of kidney dysfunction (as evaluated by means of serum creatinine).

sCrt [mg/dL] (KD severity degree)	Minimum	25% quartile	Median	75% quartile	Maximum
≤1.5 (KD_1)	68	274	494	914	8878
>1.5 and ≤ 2 (KD_2)	146	504	974	1372.75	3438
>2 and ≤ 4 (KD_3)	85	951	1603	2757	20001
>4 (KD_4)	248	1724.5	3361	8214.5	20001

KD = kidney dysfunction, sCrt = serum creatinine.

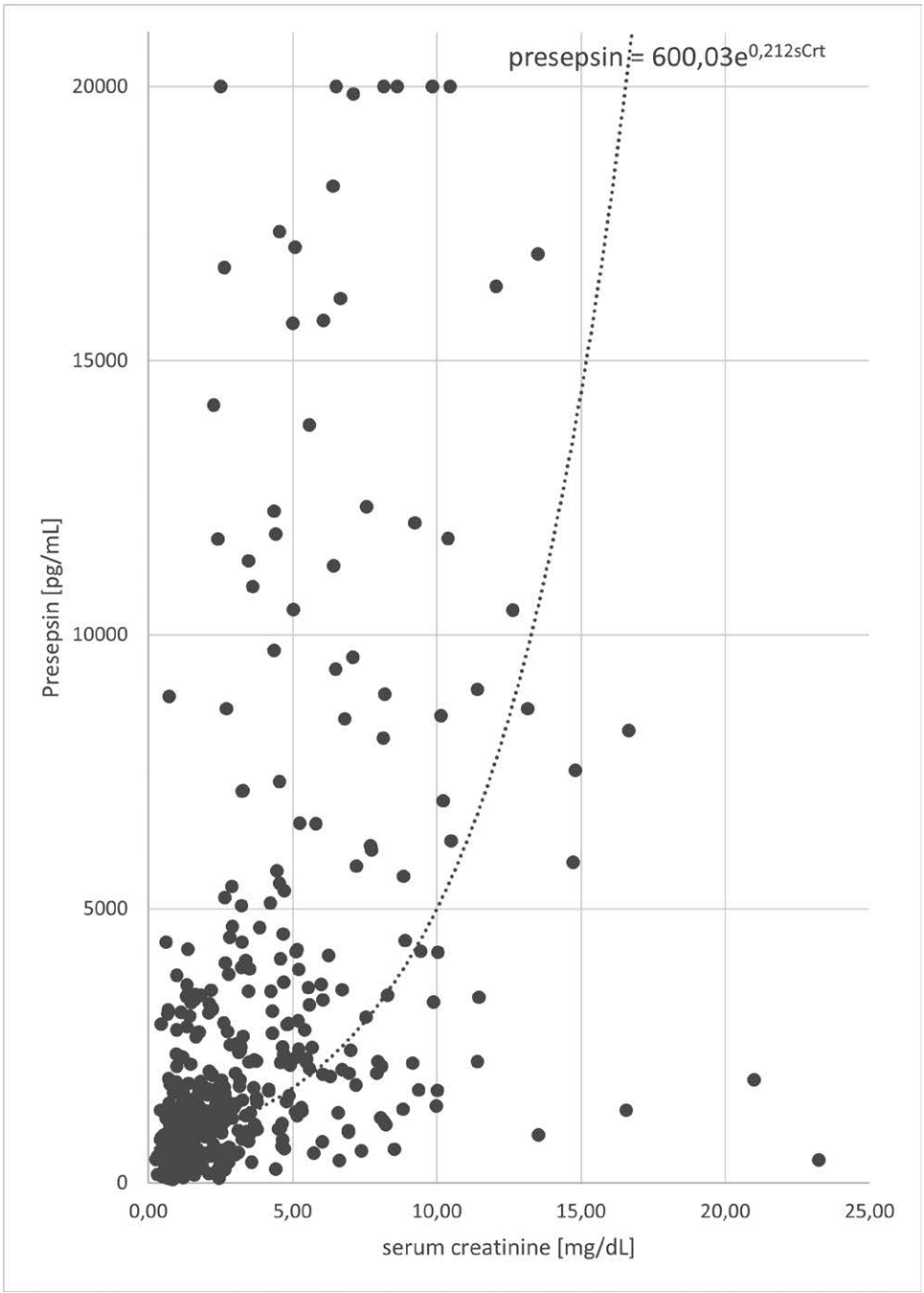

Figure 2. The exponential relationship between presepsin and serum creatinine at presentation.

with a median of 2 days, an interquartile range of 1 to 7 days, and a maximum of 40 days. Consequently, we divided our sample of patients into 2 groups: survivors and non-survivors. We classified the patients in each of these 2 groups according to the sCrt variation during their hospital stay: increase, decrease, no variation, and dialysis. In agreement with the definition of risk category in the risk, injury, failure, loss of kidney function, and end-stage kidney disease (RIFLE) system,[19] we defined "increase" as at least 50% increase from the baseline, and "decrease" as at least 33% decrease from the baseline. The dialysis category included patients requiring dialysis, either newly initiated or on a chronic basis (patients already on dialysis). The non-survivors group included a further category: those in which there was no follow-up sCrt measurement as death occurred too soon (Table 5). Presepsin levels were compared among these categories (Fig. 3) by means of Mann–Whitney test (only the comparisons with a P value <.05 are listed in Table 6). However, only 1 comparison survives the Bonferroni correction. Indeed, after the elimination of the comparisons involving groups of 7 or less (as Mann–Whitney test cannot be employed with such small samples), we were left with 34 comparisons. Therefore, by applying the Bonferroni correction, the significance level for P values was lowered to $0.05/34 \approx 0.0015$. Only the comparison between non-survivors and survivors with no variation in sCrt among the KD_1 patients has a P value <0.0015. Hence, we may conclude that for each category of KD, the clinical course/outcome (at least in terms of survival and creatinine variation) was not significantly correlated with the level of presepsin (but for 1 exception).

ROC curves depicting the usefulness of presepsin in sepsis diagnosis are shown in Figure 4, one for each category of KD severity (as reflected by sCrt at presentation). The

performances of the various methods employed for calculating the optimal cutoff points (Table 7) were very diverse, those of maximum achievable value of the chi-square statistic and of point closest to (0,1) corner in the ROC plane being uniformly dismal – consequently the corresponding results were placed in a separate table (Table 8) and the only results taken into consideration were those provided by sum of sensitivity and specificity, product of sensitivity and specificity, and Youden index. As the product of sensitivity and specificity yields an optimal cutoff point for KD_3 (1065) lower than for KD_2, an attempt was made to find a higher cutoff point with acceptable accuracy, sensitivity, and specificity (Table 7). There is a significant difference between presepsin levels in patients with and without sepsis in the 3 lower categories of severity of KD, but not in the patients with sCrt > 4 mg/dL (KD_4) (Table 9 and Fig. 5).

Table 5

Clinical course/outcome in terms of survival and serum creatinine (sCrt) variation in each of the 4 categories of severity of kidney dysfunction (KD).

sCrt [mg/dL]	Non-survivors					Survivors			
	Too short	Incr.	Decr.	No var.	Dial.	Incr.	Decr.	No var.	Dial.
≤1.5	7	9	4	62	1	3	18	109	0
>1.5 and ≤2	5	7	2	8	0	0	21	11	0
>2 and ≤4	20	6	12	22	1	1	34	14	3
>4	8	0	23	24	8	0	36	6	25

The cells of the table contain the number of patients, for example, 7 patients with a sCrt ≤ 1.5 mg/dL at presentation (KD_1) survived too short a time to have a follow-up sCrt determined (too short = the survival was too short for a follow-up sCrt to be measured).
Decr. = significant decrease in sCrt (i.e., at least 33% decrease from the baseline), Dial. = dialysis (requiring dialysis, either acute or chronic), Incr. = significant increase in sCrt (i.e., at least 50% increase from the baseline), No var. = no significant variation in sCrt.

4. Discussion

When admitted to the emergency room, a patient with KD has frequent dyspnea and rales. Consequently, a chest x-ray is performed, which usually reveals areas of consolidation – the clinician is faced with a difficult decision: are these areas only the expression of uremic lung (combination of vascular congestion with increased permeability of the alveolo-capillary membrane due to metabolic alterations and inflammation[20]) or is there also a bacterial infection looming underneath.

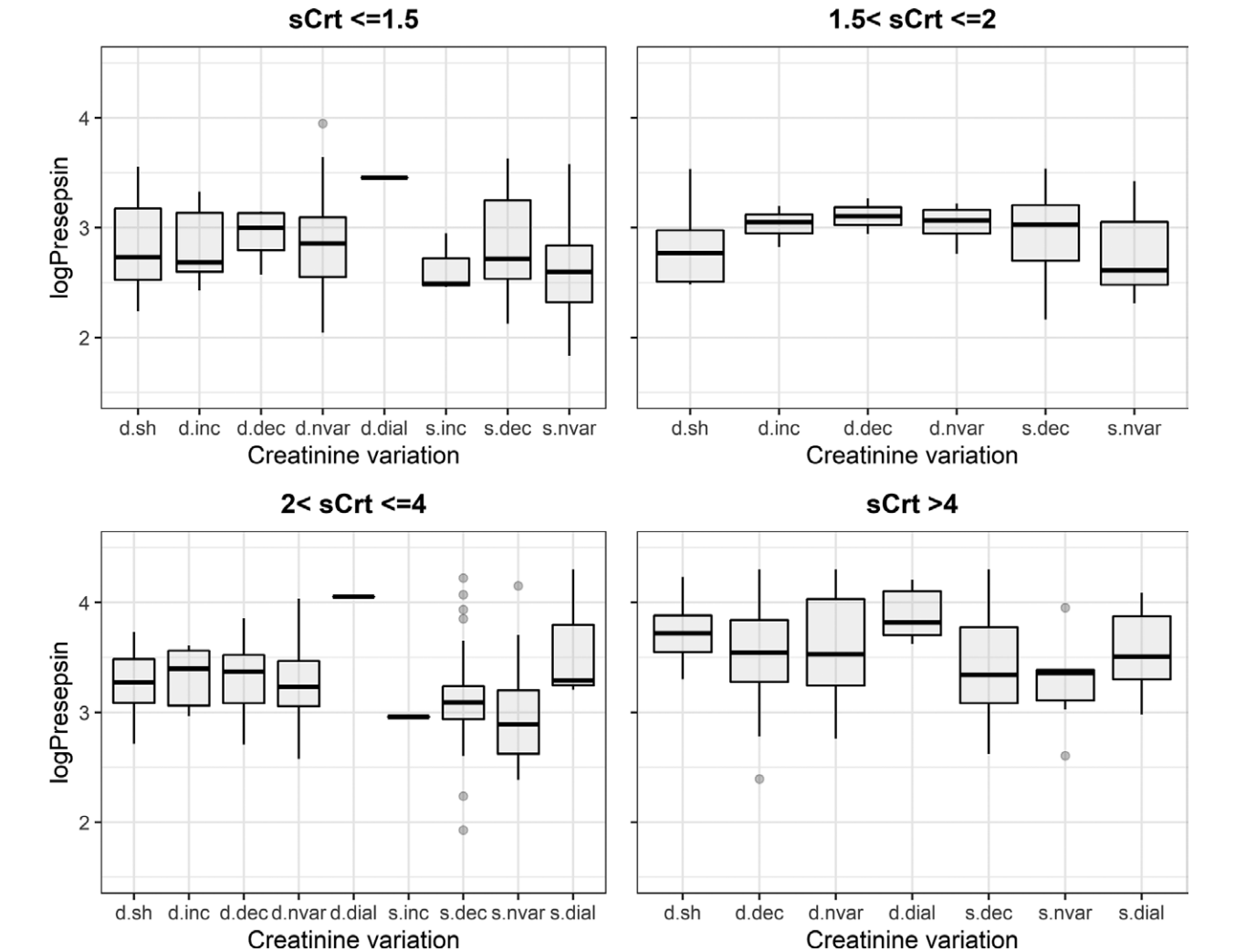

Figure 3. Comparison of the presepsin levels among the various clinical course/outcome categories. d. = dead/non-survivors, dec = significant decrease in sCrt (i.e., at least 33% decrease from the baseline), dial = dialysis (requiring dialysis, either acute or chronic), inc = significant increase in serum creatinine (i.e., at least 50% increase from the baseline), nvar = no significant variation in sCrt, s. = survivors, sCrt = serum creatinine, sh = the survival was too short for a follow-up serum creatinine to be measured.

Table 6

Comparison of the presepsin levels (by means of Mann–Whitney test) among the various clinical course/outcome categories.

sCrt [mg/dL]	Categories compared in terms of presepsin levels	*P* value
≤1.5 (KD_1)	Non-survivors with no variation in sCrt ~ survivors with no variation in sCrt	**.0001**
	Survivors with decrease in sCrt ~ survivors with no variation in sCrt	.042
>2 and ≤ 4 (KD_3)	Non-survivors too short ~ survivors with no variation in sCrt	.036
	Non-survivors with decrease in sCrt ~ survivors with no variation in sCrt	.027
	Non-survivors with no variation in sCrt ~ survivors with no variation in sCrt	.03
>4 (KD_4)	Non-survivors with decrease in sCrt ~ non-survivors on dialysis	.03
	Non-survivors on dialysis ~ survivors with decrease in sCrt	.027
	Non-survivors on dialysis ~ survivors on dialysis	.013

Only the differences with a *P* value <.05 are listed. For clarity sake, Mann–Whitney statistics are not listed. By lowering the significance threshold for *P* values to .0015 (for reasons explained in the text), only the first comparison remained statistically significant – it was highlighted by bold typing. ~ = compared to, KD = kidney dysfunction, sCrt = serum creatinine.

Sometimes, the febrile reaction to infection is blunted in an uremic patient.[21] Many clinicians consider that KD dampens the neutrophil rise in response to infection, although we could not find a corroborating study (in uremic patients neutrophils are dysfunctional and more apoptosis-prone, but not necessarily depressed in number[22]). Therefore, the clinician often resorts to a more reliable marker of bacterial infection such as presepsin. Unfortunately, the lower the kidney function, the higher the normal level of presepsin,[8] hence the reference values for presepsin employed for patients with normal kidney function are not applicable in those with KD. The aim of the present article was not only to prove that the normal range for presepsin should be scaled up in patients with KD, but also to propose different cutoff points for presepsin depending on the degree of severity of KD. Although RIFLE classification was used by other researchers,[23] we consider it as impractical in many clinical situations as it relies on the changes in sCrt and on urine output, which are generally not readily available for the patient admitted to the emergency department, in whom a decision regarding the initiation of antibiotic therapy must be reached and applied in <3 hours, preferably within an hour of presentation.[24] Therefore, it is not possible to wait for the 6, 12, or 24 hours needed for defining (based on the urine

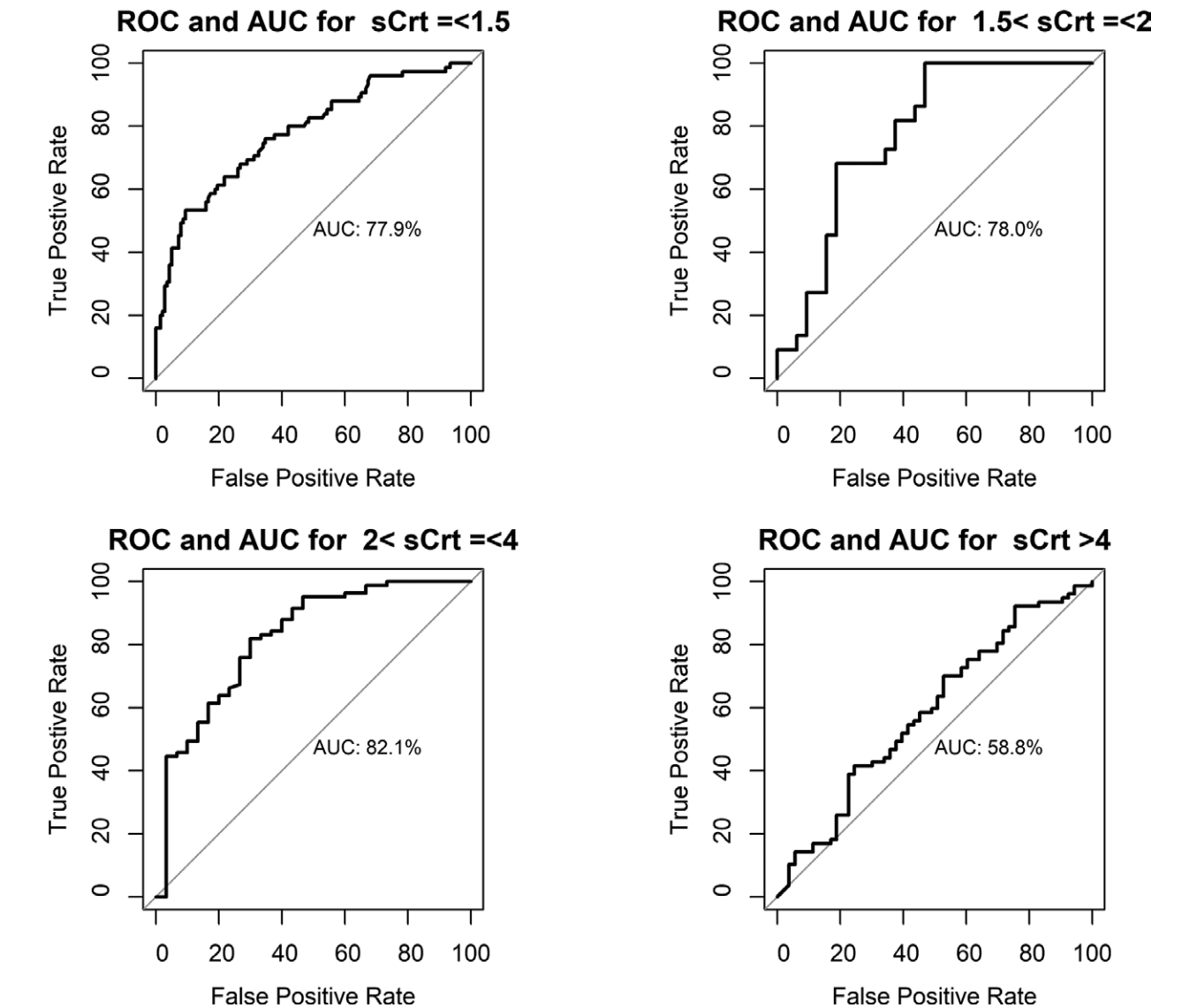

Figure 4. Receiver operating characteristic (ROC) curves depicting the usefulness of presepsin in sepsis diagnosis, one for each category of kidney dysfunction severity (as reflected by serum creatinine at presentation). AUC = area under the curve, sCrt = serum creatinine.

Table 7

Optimal cutpoints for presepsin (in pg/mL) on the receiver operating characteristic (ROC) curves corresponding to the 4 degrees of severity of kidney dysfunction (KD).

sCrt [mg/dL]	Method	Cutpoint	Accuracy	Sensitivity	Specificity	AUC
≤1.5 (KD_1)	Sum_sens_spec	**982**	0.77	0.53	0.91	0.78
	Prod_sens_spec	**700**	0.73	0.64	0.78	0.78
	Youden index	**982**	0.77	0.53	0.91	0.78
>1.5 and ≤ 2 (KD_2)	Sum_sens_spec	**588**	0.72	1	0.53	0.78
	Prod_sens_spec	**1125**	0.76	0.68	0.81	0.78
	Youden index	**588**	0.72	1	0.53	0.78
>2 and ≤ 4 (KD_3)	Sum_sens_spec	**1065**	0.79	0.82	0.7	0.82
	Prod_sens_spec	**1065**	0.79	0.82	0.7	0.82
	Prod_sens_spec	1200	0.75	0.76	0.73	0.82
	Prod_sens_spec	1300	0.72	0.71	0.73	0.82
	Prod_sens_spec	1400	0.69	0.67	0.73	0.82
	Prod_sens_spec	1500	0.68	0.64	0.8	0.82
	Prod_sens_spec	1600	0.66	0.61	0.8	0.82
	Youden index	**1065**	0.79	0.82	0.7	0.82
>4 (KD_4)	Sum_sens_spec	**2260**	0.61	0.7	0.47	0.59
	Prod_sens_spec	**2260**	0.61	0.7	0.47	0.59
	Youden index	**2260**	0.61	0.7	0.47	0.59

Evaluated by means of serum creatinine (sCrt) as calculated by the various methods: sum of sensitivity and specificity (sum_sens_spec), product of sensitivity and specificity (prod_sens_spec), and Youden index. Optimal cutpoints are highlighted by bold typing. For reasons explained in the text, for KD_3 patients several other cutpoints (beside the optimal one) are listed for prod_sens_spec.
AUC = area under the curve.

Table 8

"Optimal" cutpoints for presepsin (in pg/mL) on the receiver operating characteristic (ROC) curves corresponding to the 4 degrees of severity of kidney dysfunction (KD).

sCrt [mg/dL]	Method	Cutpoint	Accuracy	Sensitivity	Specificity	AUC
≤1.5 (KD_1)	Closest_to_roc01	infinite	0.65	0	1	0.78
	P_chisquared	81	0.36	1	0.01	0.78
>1.5 and ≤ 2 (KD_2)	Closest_to_roc01	infinite	0.59	0	1	0.78
	P_chisquared	2754	0.59	0.09	0.94	0.78
>2 and ≤ 4 (KD_3)	Closest_to_roc01	20001	0.26	0	0.97	0.82
	P_chisquared	11743	0.28	0.04	0.97	0.82
>4 (KD_4)	Closest_to_roc01	404	0.58	0.99	0	0.59
	P_chisquared	11259	0.44	0.17	0.83	0.59

Evaluated by means of serum creatinine (sCrt)] as calculated by means of chi-square statistic (p_chisquared) and the point nearest to the (0,1) corner in the receiver operating characteristic (ROC) plane (closest_to_roc01).
AUC = area under the curve.

Table 9

Comparison between the presepsin levels in patients with and without sepsis for the 4 categories of severity of kidney dysfunction.

sCrt [mg/dL]	No sepsis	Sepsis	Statistic	*P* value
≤1.5	372 (211,5–662)	1039 (505,5–1794,5)	2290	2E–11
>1.5 and ≤ 2	560,5 (327,75–1073,5)	1252 (946,25–1639,25)	155	.0004
>2 and ≤ 4	727,5 (424,5–1368,5)	1805 (1215,5–3504,5)	445,5	2E–07
>4	2890 (1343–5598)	3623 (2082–8654)	1679,5	.09

Columns "No sepsis" and "Sepsis" contain the median followed by the interquartile interval (in parentheses) of presepsin (and not of log [presepsin], which is the parameter used in Fig. 5).
sCrt = serum creatinine.

output criteria) the risk, injury, or failure stages, respectively in the RIFLE classification system. Moreover, in many/most cases information is lacking about a baseline sCrt essential for defining the increase in sCrt necessary for the classification in one of the first 3 stages of the RIFLE system or of Acute Kidney Injury Network classification system.

Significant differences have been found for the range of presepsin values between the patients with various degrees of KD, as evaluated by sCrt. Remarkably, the median value for KD_1 group (sCrt < 1.5 mg/dL) was, 494 pg/mL—Table 4, close to the threshold commonly admitted for abnormal values of presepsin in the general population (600 pg/mL[25,26]). Statistical analysis demonstrated that presepsin levels were significantly different between the patients pertaining to each successive degrees of severity of KD (Table 3), with a clear ascending trend of the median and both the first and especially the third quartile (Table 4), which does not seem to be the consequence of the higher propensity to infection of patients with KD,[27] as there was no correlation between neutrophil or leukocyte count and sCrt. The reasons for choosing the median (and not, say, the first quartile) as the threshold for abnormal values were: the median for presepsin in KD_1 patients, about 500 pg/mL (Table 4), was close to the accepted threshold of 600 for general population[25,26]; the predominant tendency is to overprescribe antibiotics, often despite the lack of any clinical and laboratory signs of infection, in many patients with KD, the only argument being "high" presepsin – therefore a more exigent threshold would much better serve the practical purpose of avoiding unnecessary and potentially harmful antibiotic treatment. It should be stressed that in clinical settings presepsin should not be the only parameter employed for deciding the initiation of antibiotic therapy – other clinical and laboratory signs of infection should also be considered, such as fever, local signs of infection, neutrophil count etc. Therefore, at this stage, we concluded that the threshold for abnormal presepsin should be about 950 pg/mL for KD_2 patients, about 1600 pg/mL for KD_3 patients, and about 3400 pg/mL for KD_4 patients (Table 4). Based on the 3 quartiles, we even thought that we could define 3 thresholds for each degree of severity of KD – see Table 10. A "slightly increased" level of presepsin would serve only as a weak argument for prescribing

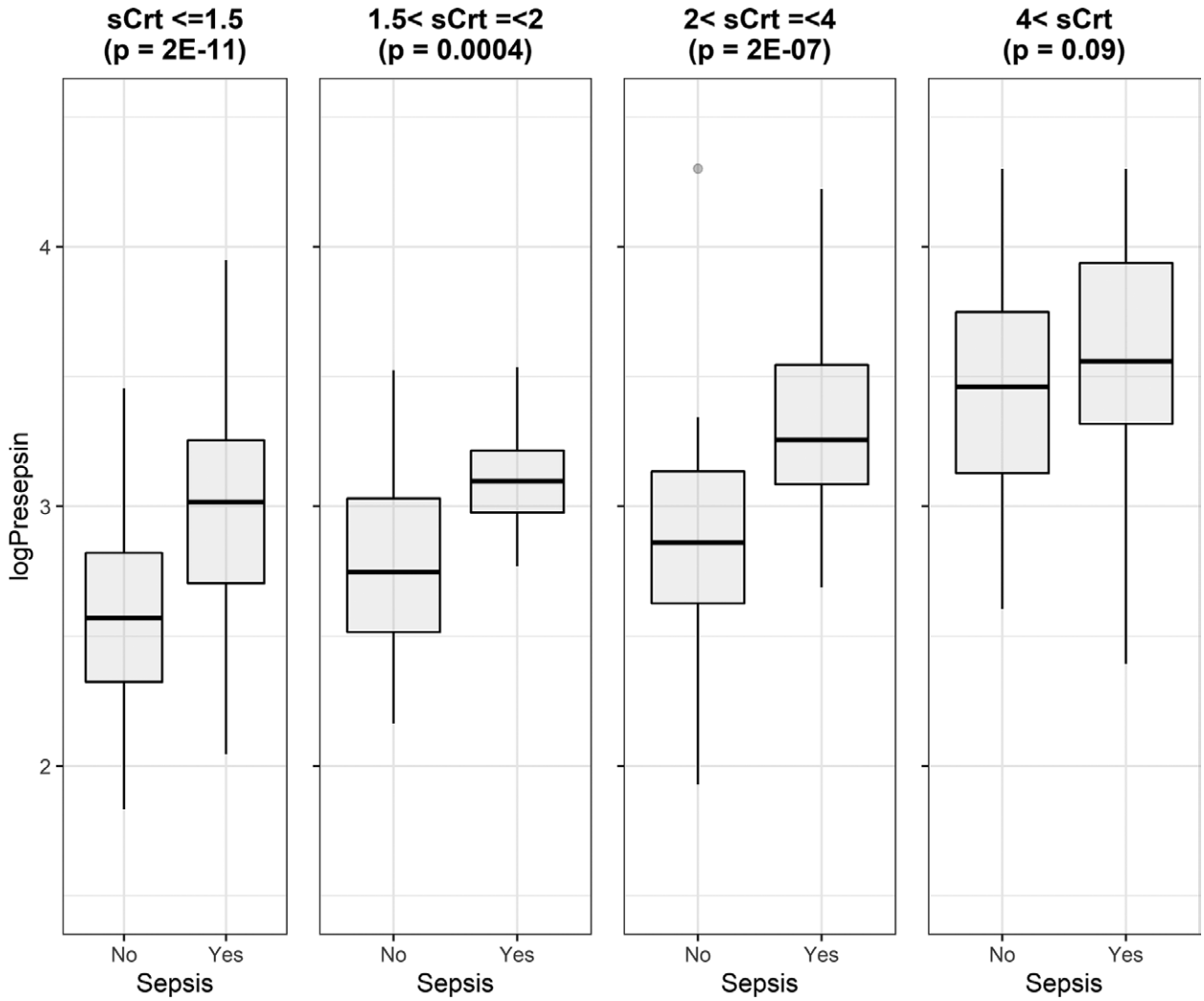

Figure 5. Log (presepsin) in patients with and without sepsis divided according to the severity of kidney dysfunction (as reflected by serum creatinine at presentation). sCrt = serum creatinine.

Table 10

Presepsin thresholds (in pg/mL) for the 4 degrees of severity of kidney dysfunction evaluated by means of serum creatinine.

sCrt [mg/dL]	Normal	Slightly increased	Definitely increased	Much increased
≤1.5	≤300	300–500	**500**–1000	>1000
>1.5 and ≤ 2	≤500	500–950	**950**–1400	>1400
>2 and ≤ 4	≤950	950–1600	**1600**–3000	>3000
>4	≤1800	1800–3500	**3500**–8000	>8000

sCrt = serum creatinine.

antibiotics, to be corroborated by other elements, such as high neutrophil count, signs of localized infection, or fever with no other obvious cause. A "definitely increased" level of presepsin might be considered a strong argument for prescribing antibiotics. A "much increased" presepsin level might mean that antibiotic treatment is highly recommended, as long as there were no alternative explanations for the high presepsin level. Of course, a normal presepsin level pleads against prescribing antibiotics. However, the results yielded by the ROC curves appear to overthrow these suppositions. The performance of presepsin in diagnosing sepsis seems to be acceptable in patients in the first 3 categories of KD severity, with area under the curve of 0.78, 0.78, and 0.82 respectively (Table 7), but for the patients with the most severe degree of KD (sCrt > 4 mg/dL) it is poor enough to be almost useless in diagnosing sepsis, which is in agreement with the results of other researchers.[23] Regarding the optimal cutoff points on the ROC curves (Table 7), we only considered the results provided by sum of sensitivity and specificity, product of sensitivity and specificity, Youden index (as explained in the Results section), as it is up to the researcher to select the most clinically relevant method[18] for determining optimal cutoff points. To that end, we also took into account accuracy, sensitivity, specificity.

For the patients with a sCrt ≤ 1.5 mg/dL (KD_1) the best balance between sensitivity and specificity is achieved for a presepsin cutoff point of 700 (Table 7), although the accuracy was slightly better for the 982 cutoff point. Employing the latter cutoff point would greatly improve specificity (91%), but the diagnosis of sepsis would be missed in almost half (47%) of the patients. The 700 threshold is not a far cry from the 500 suggested above. Therefore, 600, which is halfway between, is probably the best choice, in agreement with the values proposed by others researchers.[25,26] For the patients with a sCrt between 1.5 and 2 mg/dL (KD_2), the best accuracy and balance between sensitivity and specificity is provided by the 1125 threshold for presepsin (Table 7). A cutoff point of about 600 seems to offer the almost certainty of not missing any case of sepsis, at the cost of overdiagnosing almost half of

the non-sepsis patients. The cutoff value of about 1100 is not much higher than the 950 proposed in the Table 10 – so an educated guess puts the cutoff point somewhere in between, at about 1000. For the patients with a sCrt between 2 and 4 mg/dL (KD_3), 1065 seems to be the optimal cutoff point for presepsin (Table 7), but this is lower than the optimal cutoff point for KD_2 patients and far lower than the 1600 value suggested above. Consequently, several higher cutoff points between 1200 and 1600 were explored (Table 7). It should be noticed that, while for the cutoff points of 1200 and 1300, accuracy, sensitivity, and specificity dropped, but remained above 0.7, for the cutoff points of 1400, 1500, and 1600, sensitivity and accuracy became unacceptably low, with only a slight improvement in specificity. Consequently, 1300 is our best guess for the appropriate cutoff point in KD_3 patients. Finally, for the patients with a sCrt > 4 mg/dL (KD_4), 2260 appear to be the optimal cutoff point for presepsin (Table 7), which is far less than the 3400 suggested above. On the other hand, presepsin is an unreliable marker of sepsis in patients with severe KD (as shown above). In such patients presepsin would better not be used to diagnose sepsis, but, if used, a threshold of at least 2200 (if not a more conservative one of 2500 or even 3000) should be employed. A potential pitfall might be a possible independent effect of age on presepsin due to a putative increased tendency to infection in elderly patients.[28,29] However, this would result in a direct correlation between presepsin and age, while our data point to an inverse one, if any, while no correlation was found between sCrt and age, most probably due to, as suggested above, the acute nature of KD in most of our patients. Therefore, at least in acute settings, age does not seem to intervene as a confounding factor in the relationship between presepsin level and sCrt. One of the weak points of our study could have been the nonuniformity in terms of clinical course/outcome in our sample of patients. However, we explored this avenue and found no significant difference between the presepsin levels corresponding to the different variants of clinical course/outcome (with only 1 exception, as shown in the Results section).

To our knowledge, this is the first study in which an attempt is made to establish thresholds for significant increases in presepsin level adapted to the various degrees of KD as reflected by the only reliable and readily available marker in acute/emergency settings, which is sCrt. The research performed by Kobayashi et al[5] was conducted on patients with CKD without signs of infection (or other causes of high presepsin) and, albeit valuable in establishing that presepsin levels exponentially increases with the severity of CKD, is of limited applicability in acute settings, when KD is frequently acute or chronic, and information is lacking about the personal history of the patient including recent measurements of sCrt. The study of Nakamura et al[23] was performed on a smaller sample of patients with AKI and yielded results similar to ours, but did not attempt to propose significance thresholds for presepsin adapted to the degree of severity of KD. Moreover, a Japanese equation was employed for estimating GFR, which was, in our opinion, wrong, as the said equation was derived based on data collected mainly from patients with CKD and it was not validated in patients with AKI.[30] Some of the limitations of our study are: relatively low number of enrolled patients, and relative heterogeneity of the sample. Studies conducted on larger samples of patients are clearly needed, with the aim of both defining the presepsin cutoff points corresponding to the different degrees of severity of KD and of establishing whether presepsin is indeed useless in patients with severe KD.

In conclusion, the higher the severity of KD, as reflected by sCrt at presentation, the higher the expected level of presepsin, therefore the higher the cutoff point for significant increase in presepsin. The best cutoff points for presepsin are 600, 1000, and 1300 pg/mL in patients with sCrt ≤ 1.5 mg/dL, between 1.5 and 2 mg/dL, and between 2 and 4 mg/dL, respectively. Regrettably, in patients with a sCrt at presentation >4 mg/dL, presepsin is not a reliable marker for sepsis, and should not be used as an argument for this condition.

Author contributions

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