



Red blood cell distribution width as prognostic factor in sepsis: A new use for a classical parameter

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ABSTRACT

Purpose: To evaluate Red blood cell distribution width (RDW) as a sepsis prognostic biomarker.

Methods: 203 septic patients admitted to the ICU. Analysis of RDW dynamics, hospital mortality discrimination ability and the added value when incorporated to the SOFA, LODS, SAPS-II and APACHE-II scores using the AUC-ROC.

Results: Non-survivors presented higher RDW values during the first week after ICU admission ($p = 0.048$). Only SOFA and RDW were independently associated with mortality when adjusted by Charlson, immunosuppression, nosocomial infection, NEWS2, SAPS-II, septic shock and haemoglobin ($p < 0.05$). After adjustment, AUC-ROC was 0.827, 0.822, 0.824, 0.834 and 0.812 for each model including admission, 24, 48 and 72-h and 7-days RDW, respectively. When added to the scores, 24-h RDW and admission RDW improved their discrimination ability (SOFA AUC-ROC = 0.772 vs 0.812 SOFA + admission RDW, $p = 0.041$; LODS AUC-ROC = 0.687 vs 0.710, $p = 0.002$; SAPS-II AUC-ROC = 0.734 vs 0.785, $p = 0.021$; APACHE-II AUC-ROC = 0.672 vs 0.755, $p = 0.003$). Admission RDW with SOFA presented the better discrimination ability for mortality.

Conclusion: RDW is an independent prognostic marker of death in septic patients admitted in the ICU that improves SOFA, LODS, APACHE-II and SAPS-II discrimination ability. This parameter could be incorporated to the prognostic scores as a marker of systemic dysfunction and dysregulated inflammatory response.

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Abbreviations: APACHE-II, Acute Physiology and Chronic Health Evaluation-II; ARDS, Acute Respiratory Distress Syndrome; AUC-ROC, Area Under the Receiver Operating Characteristic Curve; DAG, Directed Acyclic Graphs; CRP, C reactive protein; FiO₂, Fraction of Inspired Oxygen; HIV, Human immunodeficiency virus; ICU, Intensive Care Unit; LODS, Logistic Organ Dysfunction System; MAP, Mean arterial pressure; NEWS2, National Early Warning Score 2; PaO₂, Arterial oxygen tension; PCT, Procalcitonin; RDW, Red blood cell distribution width; SAPS-II, Simplified Acute Physiology Score-II; SIRS, Systemic Inflammatory Response Syndrome; SOFA, Sequential Organ Failure Assessment; SOT, Solid organ transplantation; SCT, Stem cell transplantation; qSOFA, quick-SOFA.

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1. Background

Sepsis remains an important cause of death worldwide [1]. As a matter of fact, it could be considered one of the main causes of hospital death, with mortality rates reaching 18.7% in hospitalized patients and up to 55.7% in the Intensive Care Units (ICUs) [2–4]. Despite the acknowledgment represented by the Third Consensus Definition for Sepsis and Septic shock (Sepsis-3) and the Surviving Sepsis Campaign, there are still important gaps in the diagnosis and identification of sepsis [5,6]. In this setting, several markers and molecules have been assessed to anticipate sepsis recognition, to confirm the diagnosis and to guide the management of these patients, but unfortunately, to date there are no parameters consolidated for this role [7].

Red blood cell distribution width (RDW) is a parameter routinely reported as part of a complete blood count. It measures the size

Table 1
Patient's characteristics.

Characteristic	Global (n = 203)	Survivors (n = 139)	Non-survivors (n = 64)	p-value
Male gender N (%)	129 (63.6%)	91 (65.5%)	38 (64.9%)	0.402
Age Years (SD)	63.1 (14.3)	61.8 (14.5)	65.9 (11.6)	0.147
Nosocomial N (%)	81 (39.9%)	46 (33.1%)	35 (54.7%)	0.004
Surgical intervention N (%)	111 (54.7%)	73 (52.5%)	38 (59.3%)	0.362
Septic shock N (%)	161 (79.31%)	103 (74.1%)	58 (90.6%)	0.007
ARDS N (%)	29 (14.29%)	18 (12.9%)	11 (17.2%)	0.423
Comorbidities				
Heart disease N (%)	65 (32.0%)	36 (25.9%)	29 (45.3%)	0.001
Peripheral artery disease N (%)	28 (13.8%)	21 (15.1%)	7 (10.9%)	0.413
Connective tissue disease N (%)	25 (12.3%)	12 (8.6%)	13 (20.3%)	0.019
Cerebrovascular disease N (%)	25 (12.3%)	13 (9.4%)	12 (18.8%)	0.058
Pulmonary disease N (%)	66 (32.5%)	39 (28.1%)	27 (42.2%)	0.028
Chronic liver disease N (%)	30 (14.8%)	16 (11.5%)	14 (21.9%)	0.120
Chronic kidney disease N (%)	43 (21.2%)	26 (18.7%)	17 (26.6%)	0.270
Neoplasm N (%)	86 (42.4%)	55 (39.6%)	31 (48.4%)	0.109
Haematological disease N (%)	37 (18.2%)	21 (15.1%)	16 (25%)	0.084
Diabetes N (%)	64 (31.5%)	39 (28.1%)	25 (39.1%)	0.116
Obesity N (%)	25 (12.3%)	18 (12.6%)	7 (10.9%)	0.911
Alcoholism N (%)	26 (12.8%)	17 (12.2%)	9 (14.1%)	0.298
Immunocompromise N (%)	74 (36.5%)	43 (30.9%)	31 (48.4%)	0.018
SOT N (%)	24 (11.8%)	13 (9.4%)	11 (17.2%)	0.108
SCT N (%)	14 (6.9%)	7 (5%)	7 (10.9%)	0.123
Charlson comorbidity index Mean (SD)	6.17 (3.98)	5.78 (4.31)	7.03 (3.01)	0.005
Sources of infection				
Respiratory	79 (38.9%)	54 (38.8%)	25 (39.1%)	0.977
Abdominal	73 (36%)	49 (35.3%)	24 (37.5%)	0.756
Urinary	25 (12.3%)	20 (14.4%)	5 (7.8%)	0.185
Skin and soft tissue	14 (6.9%)	10 (7.2%)	4 (6.3%)	0.805
Catheter-related bacteremia	8 (3.9%)	3 (2.2%)	5 (7.8%)	0.054
Endocarditis	8 (3.9%)	3 (2.2%)	5 (7.8%)	0.054

SD: Standard deviation, ICU: Intensive care unit, HIV: Human immunodeficiency virus, SOT: Solid organ transplantation, HSCT: Hematopoietic stem cell transplantation. Statistically significant results are remarked in bold.

variability of circulating erythrocytes (anisocytosis) and has traditionally been used in the differential diagnosis of iron deficiency anaemia and anaemia of chronic disease [8]. Anisocytosis reflects the dysregulation of the iron metabolism and inhibition of erythropoiesis resulting in chronic disease anaemia, mediated by diverse cytokines, mainly IL-6 [9,10]. In fact, Allen et al. described the association between RDW and a biochemical profile suggestive of impaired iron mobilization, typical of anaemia of chronic disease, in a cohort of patients with heart failure [11]. Moreover, these changes in the red blood cell physiology are particularly common in critical illness, in patients admitted to the ICU and in hyperinflammatory states [12]. In consequence, and since it is a fast and available parameter, several studies have considered RDW as an inflammatory marker or a predictor of mortality in diverse clinical settings including chronic inflammatory diseases, cardiovascular disease, infections and acute respiratory distress syndrome (ARDS) [13]. Therefore, and based on the aforementioned pathophysiologic considerations, the aim of the present study was to understand RDW dynamics and to better analyze its prognostic role in sepsis, when compared to other biomarkers.

2. Material and methods

2.1. Study population

The study population consisted of a retrospective cohort of consecutive patients admitted to the ICU due to sepsis at a 620-bed tertiary University Hospital from January 1st to 31st December 2019. Patients were included if sepsis was the reason for admission and/or sepsis criteria were met during ICU admission. Sepsis was considered if the patient presented a confirmed or suspected infection with organ dysfunction, represented by an increase of SOFA score of 2 or more points,

according to Sepsis-3 criteria [5]. The study was approved by the local Research Ethics Committee (PI_222–20). According to Spanish law and the Ethics Committee, a waiver for informed consent was granted.

2.2. Data collection and scores calculation

The following data were extracted from medical records using a standardised data collection form: epidemiological and baseline conditions and comorbidities, physiological, laboratory and microbiological parameters, supportive treatment received, ICU and hospital stay. Furthermore, RDW, C-reactive protein (CRP) and procalcitonin (PCT) values were analysed at ICU admission, at 24, 48, 72 h and 7 days later; and considering the maximum values identified during the ICU and hospital admission if the patient was discharged from the ICU.

The prognostic scores (quick-SOFA, National Early Warning Score 2 [NEWS2], Logistic Organ Dysfunction System [LODS], Sequential Organ Failure Assessment [SOFA], Acute Physiology and Chronic Health Evaluation-II [APACHE-II] and Simplified Acute Physiology Score-II [SAPS-II]) were calculated considering the worse or more relevant values within the first 24 h of ICU admission [5,14–20]. If the patient suffered from chronic conditions with previously altered values, scores were calculated considering the difference between baseline and admission values (ie. creatinine or bilirubin in kidney or liver disease, respectively). Furthermore, if any parameter or value was missing, no contribution was made to the score and thus it was considered in the normal range. According to their definition, patients met the qSOFA criteria if 2 of the 3 variables were present (Glasgow Coma Score of 13 or less, systolic blood pressure of 100 mmHg or less and respiratory rate of 22 per minute or greater) [5,15] and Systemic inflammatory response syndrome (SIRS) was considered if at least 2 of the following 4 criteria were met (temperature > 38 °C or < 36 °C, heart rate > 90

Table 2

ICU admission clinical parameters, analytical values, and prognostic scores.

Parameter	Overall (n = 203)	Survivors (n = 139)	Non-survivors (n = 64)	p-value
Glasgow coma score Mean (SD)	14 (2.5)	14 (2.2)	13 (2.9)	<0.001
Temperature (°C) Mean (SD)	37.1 (1.6)	37.4 (1.5)	36.5 (1.7)	0.002
MAP (mmHg) Mean (SD)	70 (20.7)	70 (21.8)	69 (18.2)	0.829
Heart rate (bpm) Mean (SD)	107 (23)	107 (21)	107 (27)	0.862
Respiratory rate (breaths per minute) Mean (SD)	28 (8)	27 (8)	30 (7)	0.026
SpO ₂ (%) Mean (SD)	93 (6)	93 (6)	93 (7)	0.280
Vasopressors N (%)	164 (80.8)	105 (75.5)	59 (92.2)	0.007
Mechanical ventilation N (%)	91 (44.8)	46 (33.1)	45 (70.3)	<0.001
PaO ₂ /FiO ₂ ratio (Mean,SD)	232 (157)	230 (155)	237 (163)	0.793
Diuresis (ml/24 h) N (%)				<0.001
<200	33 (16.3)	15 (10.8)	18 (12.5)	
200–500	35 (17.3)	16 (11.5)	19 (29.7)	
>500	134 (66.3)	108 (77.7)	26 (40.63)	
Renal replacement therapy N (%)	57 (28.1)	24 (17.3)	33 (51.6)	<0.001
Blood transfusion N (%)	50 (24.6)	27 (42.2)	23 (16.5)	<0.001
Arterial pH (Mean, SD)	7.30 (0.11)	7.319 (0.11)	7.260 (0.11)	<0.001
Bicarbonate (mmol/L) (Mean, SD)	19.9 (4.5)	20.2 (4.2)	19.1 (5.1)	0.145
Lactate (mmol/L) (Mean, SD)	3.8 (3.1)	3.5 (2.3)	4.7 (4.4)	0.505
pO ₂ (mmHg) (Mean, SD)	94 (37.5)	91 (38.1)	100 (35.6)	0.047
pCO ₂ (mmHg) (Mean, SD)	40 (12.2)	39 (11.6)	43 (13.1)	0.034
Urea (mg/dL) (Mean, SD)	93 (61)	85 (60)	111 (60)	<0.001
Creatinine (mg/dl) (Mean, SD)	2.15 (1.66)	2.06 (1.72)	2.35 (1.51)	0.037
Glucose (mg/dl) (Mean, SD)	173 (90)	175 (81)	169 (107)	0.278
Sodium (mmol/L) (Mean, SD)	138 (6)	137 (5)	139 (8)	0.249
Potassium (mmol/L) (Mean, SD)	4.39 (0.95)	4.31 (0.90)	4.56 (1.04)	0.150
Bilirubin(mg/dL) (Mean, SD)	2.20 (3.79)	1.92 (3.21)	2.81 (4.78)	0.114
Leukocytes (x10E3/mm3)(Mean, SD)	16.645 (14.595)	16.914 (11.525)	16.061 (19.788)	0.041
Neutrophils (x10E3/mm3) (Mean, SD)	13.954 (12.564)	14.228 (10.112)	13.359 (16.784)	0.052
Lymphocytes (x10E3/mm3) (Mean, SD)	961 (2109)	884 (896)	1129 (3530)	0.013
Platelets (x10E3/mm3) (Mean, SD)	185.305 (138.294)	196.878 (127.674)	160.172 (157.095)	<0.001
Haemoglobin (g/dL) (Mean, SD)	11.60 (2.65)	12.00 (2.53)	10.73 (2.72)	<0.001
Haematocrit (%) (Mean, SD)	35.15 (8.05)	36.33 (7.42)	32.57 (8.81)	<0.001
Prothrombin activity (%) (Mean, SD)	53.96 (21.12)	56.23 (20.77)	49.01 (21.17)	0.014
RDW (%) (Mean, SD)	15.66 (2.88)	15.06 (2.82)	16.95 (2.58)	<0.001
CRP (mg/L) (Mean, SD)	166 (90)	167 (86)	162 (98)	0.820
PCT (ng/mL) (Mean, SD)	18.9 (32.3)	17.8 (27.2)	21.8 (42)	0.513
Score				
qSOFA N (%)	66 (32.5)	42 (30.2%)	24 (37.5)	0.304
NEWS2 Mean (SD)	9.72 (3.36)	9.21 (2.91)	10.83 (3.98)	0.008
SIRS N (%)	158 (77.8)	110 (79.1)	48 (30.75)	0.510
SOFA Mean (SD)	9.06 (3.58)	7.99 (3.24)	11.39 (3.19)	<0.001
LODS Mean (SD)	7.92 (2.181)	7.37 (1.69)	9.09 (2.64)	<0.001
APACHE-II Mean (SD)	19.94 (5.94)	18.65 (5.14)	22.72 (6.62)	<0.001
SAPS-II Mean (SD)	51.42 (13.93)	47.68 (12.13)	59.53 (14.22)	<0.001

SD: Standard deviation, MAP: Mean arterial pressure, bpm: beats per minute, ICU: Intensive Care Unit, SD: Standard deviation, RDW: Red blood cell distribution width, CRP: C reactive protein, PCT: Procalcitonin, qSOFA: quick-SOFA, NEWS2: National Early Warning Score 2, SIRS: Systemic Inflammatory Response Syndrome, SOFA: Sequential Organ Failure Assessment, LODS: Logistic Organ Dysfunction System, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, SAPS-II: Simplified Acute Physiology Score-II. Statistically significant results are remarked in bold

beats per minute, respiratory rate > 20 breaths per minute or PaCO₂ < 32 mmHg and white blood count >12,000/mm³, <4000/mm³, or >10% band) [14].

On the other hand, septic shock was defined by the vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/L in the absence of hypovolemia, according to Sepsis-3 criteria [5] while Acute

Respiratory Distress Syndrome was (ARDS) was defined according to the Berlin definition [21].

2.3. Statistical analysis

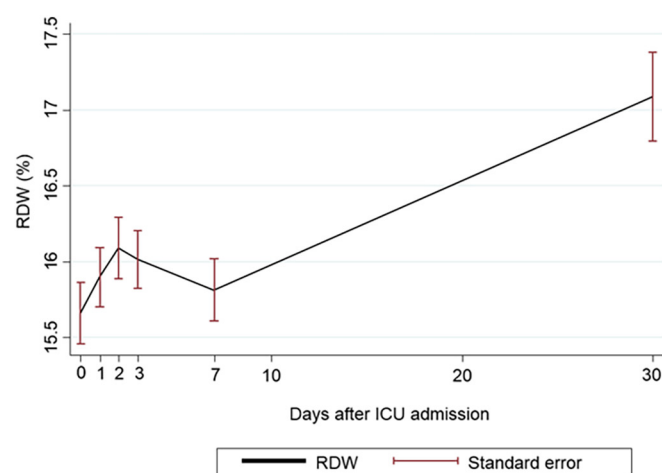
In-hospital mortality was the main study outcome, and the comparison between the groups and the statistical analyses were performed

Table 3

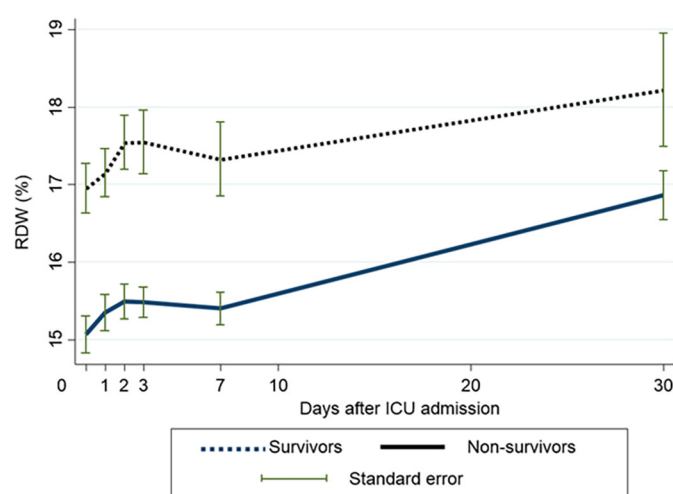
Red blood distribution width values.

	Overall (n = 203)	Survivors (n = 139)	Non-survivors (n = 64)	p-value	AUC-ROC
RDW at admission (Mean %) (SD)	15.66 (2.88)	15.06 (2.82)	16.95 (2.58)	<0.001	0.737
RDW 24 h (Mean %) (SD)	15.90 (2.76)	15.35 (2.73)	17.15 (2.41)	<0.001	0.737
RDW 48 h (Mean %) (SD)	16.09 (2.80)	15.49 (2.66)	17.54 (2.61)	<0.001	0.750
RDW 72 h (Mean %) (SD)	16.02 (2.50)	15.48 (2.18)	17.55 (2.74)	<0.001	0.747
RDW 7 days (Mean %) (SD)	15.81 (2.66)	15.40 (2.45)	17.33 (2.86)	<0.001	0.740

RDW: Red blood cell distribution width, SD: Standard deviation, AUC-ROC: area under the curve receiving operating characteristic.



A. Red blood cell distribution width (RDW) dynamics 30 days after ICU admission. Data are expressed as mean and standard error.



B. Red blood cell distribution width (RDW) dynamics 30 days after ICU admission among survivors and non-survivors. Data are expressed as mean and standard error.

Fig. 1. Red blood cell distribution width (RDW) dynamics. The figure shows RDW dynamics 30 days after ICU admission among the cohort (A) and among survivors and non-survivors (B). Data are expressed as mean and standard error.

accordingly. Quantitative variables were expressed as mean and standard deviation or as median and p25–p75 as appropriate; qualitative variables were expressed as frequency and percentage. Survivors were compared to non-survivors. Numerical variables were compared using the *t*-test or Mann-Whitney's U where appropriate, and categorical variables were compared using the chi-square test. In addition, significant variables were entered in the different multivariable logistic regression analyses to identify factors associated with hospital mortality. The discrimination ability was evaluated following an approach based on the area under the curve (AUC) - "receiver operating characteristic"-ROC. A value of AUC-ROC of 0.5 indicates random predictions, and a value of 1 indicates perfect discrimination. A model with an AUC-ROC roughly above 0.7 is considered to be useful to predict the responses of individual subjects [22]. Stata's *roccomp* command was used to compare more than two ROC areas.

Regarding analysis of RDW dynamics, firstly, we studied the association of RDW with hospital mortality along the different time points (admission, 24, 48, 72 h and 7 days), using a generalized estimating

Table 4
Factors statistically associated with a higher hospital mortality after adjustment.

	OR*	95% Confidence interval
Analysis considering admission RDW		
SOFA	1.28	1.10–1.49
RDW	1.18	1.03–1.34
Analysis considering 24-h RDW		
SOFA	1.28	1.10–1.49
RDW	1.17	1.01–1.34
Analysis considering 48-h RDW		
SOFA	1.30	1.11–1.51
RDW	1.21	1.04–1.39
Analysis considering 72-h RDW		
SOFA	1.31	1.09–1.56
RDW	1.31	1.10–1.56
Analysis considering 7-days RDW		
SOFA	1.30	1.08–1.57

SOFA: Sequential Organ Failure Assessment, RDW: Red blood cell distribution width. OR = Odds ratio.

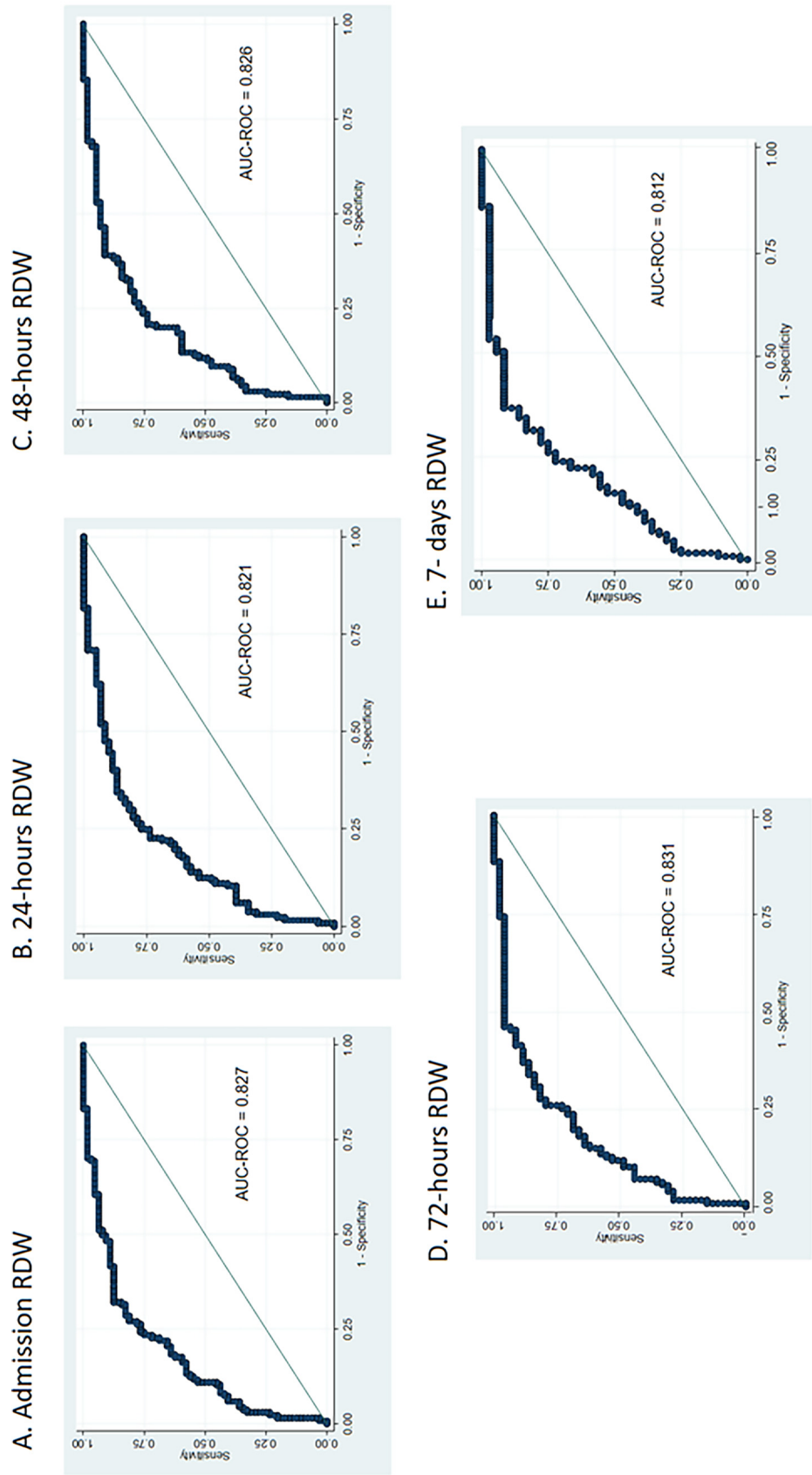
* All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Simplified Acute Physiology Score-II, septic shock and haemoglobin.

equation (GEE) analysis. As the dependent variable was mortality, we used the binomial family and the logit as the link function. RDW at the different time points as well as the time points variable were introduced as independent variables. Secondly, we performed different mortality multivariable logistic analyses considering a pool with the next variables: Charlson Comorbidity Index, immunocompromise, nosocomial infection, NEWS2, SOFA, SAPS-II, septic shock and haemoglobin; and testing one of the measures for RDW variable each time point (at admission, 24, 48, 72 h and 7 days after in addition to the maximum values identified). These variables were chosen to evaluate age and comorbid conditions (Charlson Comorbidity Index, immunocompromise and nosocomial infection), clinical situation at admission (NEWS2), organ damage (SOFA) and disease severity (septic shock and SAPS-II). NEWS2, SOFA and SAPS-II were used over qSOFA, LODS and APACHE-II according to a discrimination ability analysis described elsewhere [23]. Besides, haemoglobin and blood transfusions before and during admission were considered, since they could modify RDW values and significance [8,24]. However, when the causal pathways between haemoglobin, transfusion and mortality were evaluated by DAG (Directed Acyclic Graphs), we confirmed that transfusion is a consequence of the haemoglobin levels or the anaemia status [25]. Therefore, its inclusion in the model would not be appropriate and was consequently discarded. Accordingly, we evaluated the discrimination ability for each one of the models by AUC-ROC. Finally, we explored the discrimination ability of SOFA, LODS, APACHE-II and SAPS-II scores by themselves alone, or adding the RDW at admission or at 24 h after ICU admission. For all the analyses, a significance level of 0.05 was set. Stata v16 software was used (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.)

3. Results

3.1. Patient's characteristics

203 patients were admitted to the ICU due to sepsis during the study period. Baseline characteristics and the site of infection are shown in Table 1. 63.6% were males with a mean age of 63.1 years (SD = 14.3%). 78.3% were outpatients, 19.2% were transferred from another institution and 2% from nursing homes. Nosocomial infections were identified in 39.9% of patients. Fifteen patients (7.4%) presented more than one site of infection. The most frequent source of the infection was



The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) values for admission Red blood cell distribution width (RDW) (A), 24-hours RDW (B), 48-hours RDW, 72-hours RDW (D) and 7-days RDW (E). All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Sequential Organ Failure Assessment, Simplified Acute Physiology Score-II and haemoglobin.

Fig. 2. Discrimination ability for the hospital mortality adjusted models. The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for the models including admission Red blood cell distribution width (RDW) (A), 24-h RDW (B), 48-h RDW, 72-h RDW (D) and 7-days RDW (E). All the analyses were adjusted by the Charlson Comorbidity Index, immunosuppression, nosocomial infection, National Early Warning Score 2, Sequential Organ Failure Assessment, Simplified Acute Physiology Score-II and haemoglobin.

Table 5
Discrimination ability for the hospital mortality prognostic model comprised by RDW when added to the prognostic scores.

	AUC-ROC	95% Confidence interval	p-value for comparison between AUC-ROCs
SOFA	0.772	0.700–0.844	
+ Admission RDW	0.812	0.748–0.876	0.041
+ 24-h RDW	0.808	0.744–0.873	0.042
LODS	0.687	0.605–0.768	
+ Admission RDW	0.771	0.702–0.839	0.002
+ 24-h RDW	0.770	0.701–0.839	0.002
SAPS-II	0.734	0.660–0.809	
+ Admission RDW	0.785	0.721–0.849	0.021
+ 24-h RDW	0.781	0.716–0.846	0.030
APACHE-II	0.672	0.588–0.756	
+ Admission RDW	0.755	0.682–0.827	0.003
+ 24-h RDW	0.752	0.678–0.826	0.004

SOFA: Sequential Organ Failure Assessment, RDW: Red blood cell distribution width, LODS: Logistic Organ Dysfunction System, SAPS-II: Simplified Acute Physiology Score-II, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, AUC-ROC: Area Under the Receiver Operating Characteristic Curve.

respiratory (38.9%), followed by abdominal (36%), urinary (12.3%), skin or soft tissue infection (6.9%), catheter-related bacteraemia (3.9%) and endocarditis (3.9%). 79.3% of patients met the septic shock criteria and 14.3% the criteria for ARDS. Mean ICU stay was 16 days and mean hospitalization length of-stay was 38 days. Hospital mortality was 31.5%.

Table 2 shows clinical parameters and analytical values at ICU admission. The different diagnostic and prognostic scales were calculated accordingly. 332.5% of patients met qSOFA criteria (30.2% among survivors versus 37.5% among non-survivors, $p = 0.304$) while 77.8% met SIRS criteria (79.1% among survivors vs 75% among non-survivors, $p = 0.510$). NEWS2, SOFA, LODS, APACHE-II and SAPS-II were significantly higher in non-survivors ($p < 0.01$). The prognostic scores mortality discrimination ability was evaluated by an AUC-ROC comparison for the subsequent analysis. NEWS2 presented significantly higher AUC-ROC than qSOFA (0.615 vs 0.536, $p = 0.039$) and SOFA than LODS (0.776 vs 0.693, $p = 0.01$). SAPS presented higher discrimination ability than APACHE-II but without statistical significance (AUC-ROC = 0.738 vs 0.673, $p = 0.08$).

3.2. RDW dynamics and hospital mortality

Table 3 shows RDW values at the different time points and its dynamics during the first 30 days of ICU admission. The highest RDW values were seen on 16.1 days after ICU admission (mean RDW 17.70%), while during the first week the highest average RDW values were at 48-h (mean RDW 16.09%) and 72-h (mean RDW 16.02%) (Fig. 1. A). RDW was higher in non-survivors at all the time points ($p < 0.001$), (Fig. 1.B). RDW mortality discrimination ability, evaluated through the AUC-ROC analysis, showed that AUC-ROC was above 0.700 for all the measures. The longitudinal analysis confirmed that, during the first week after ICU admission, RDW was associated with a higher in-hospital mortality risk (**OR = 1.05, 95% CI 1.01; 1.10, $p = 0.048$**). Neither CRP (OR = 0.99, 95% CI 0.99–1.00, $p = 0.957$) nor PCT (OR = 1.01, 95% CI 0.99–1.02, $p = 0.130$) were associated with hospital mortality along time.

In order to identify at which time point RDW was a better predictor of hospital mortality, different multivariable logistic regression analyses were tested. A pool of independent variables, such as the Charlson comorbidity index, immunocompromise, nosocomial infection, NEWS2 score, SOFA score, SAPS-II score, septic shock and haemoglobin were entered into the models, in addition to RDW at admission, 24, 48 and 72 h and 7 days after ICU admission and maximum RDW, one by one. In the different multivariable logistic regression analysis for each time point

RDW value, only SOFA and RDW were statistically associated with a higher hospital mortality risk (Table 4). AUC-ROCs for the adjusted models were as follows: 0.827 for the model considering admission RDW, 0.822 for the model considering 24-h RDW, 0.824 for the model considering 48-h RDW, 0.834 for the model considering 72-h RDW and 0.812 for the model considering 7-days RDW (Fig. 2).

3.3. RDW discrimination ability when added to the prognostic scores

Finally, we explored the discrimination ability of RDW when added to the widely used prognostic scores SOFA, LODS, APACHE-II and SAPS-II (Table 5, Fig. 3). When RDW values at admission and 24 h later were added to the scores, their discrimination ability uniformly improved ($p < 0.05$). The prognostic model comprised by SOFA score and RDW at admission presented the higher discrimination ability for mortality (AUC-ROC = 0.812).

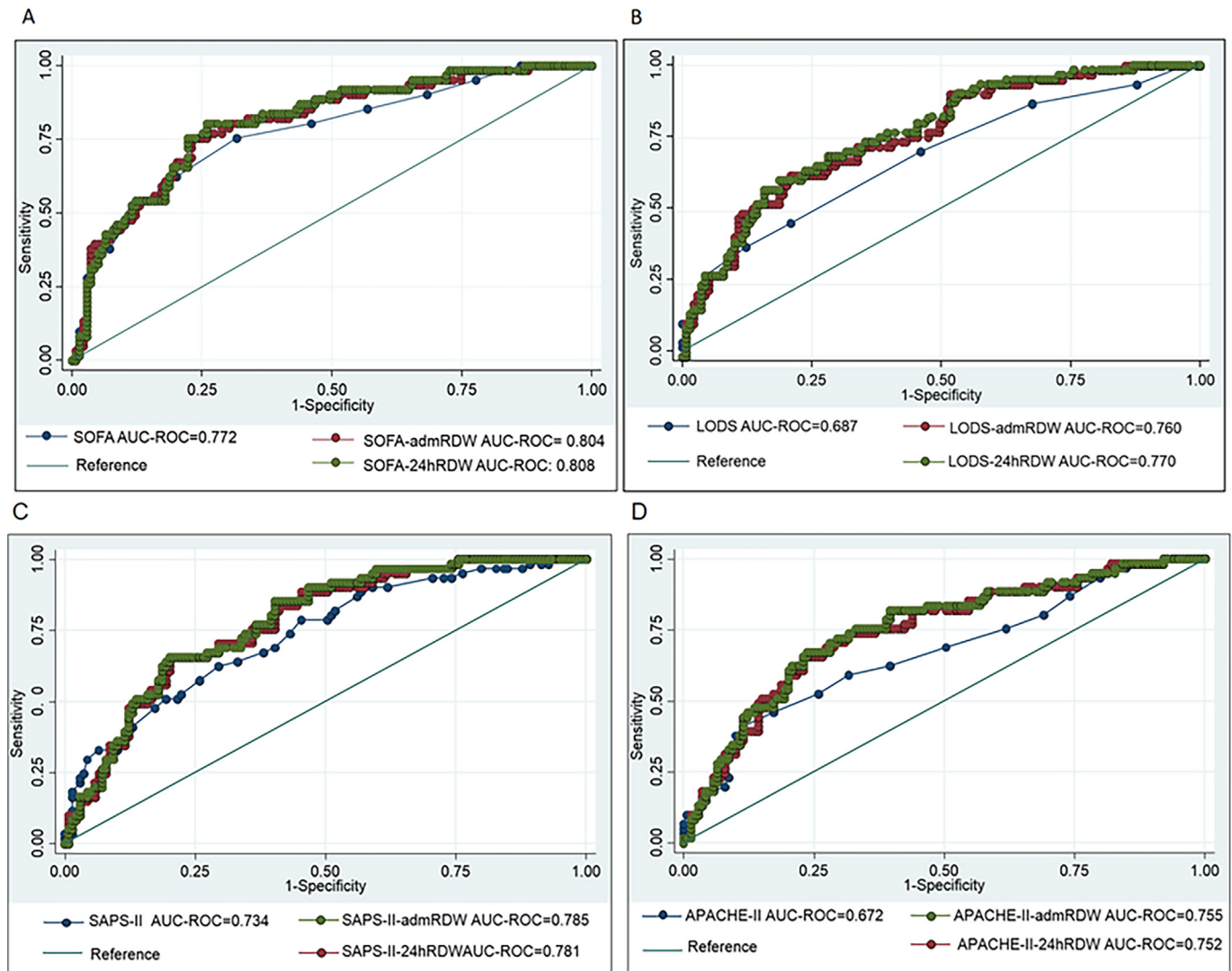
4. Discussion

The present study shows that RDW is a prognostic biomarker of hospital mortality in septic patients admitted to the ICU, since RDW was independently associated with mortality throughout time. On top of that, RDW improved the discrimination ability of SOFA, LODS, APACHE-II and SAPS-II, placing it as a potential parameter to be included in these prognostic scores.

Previous reports have already found that RDW independently predicts mortality in septic patients, in the emergency department and ICUs [15,26–44]. As a matter of fact, some authors have confirmed that RDW has better discrimination ability than CRP, PCT or even lactate, wide applied parameters in sepsis [29,38,39]. However, other authors have questioned the prognostic role of RDW [45]. From this perspective, Fontana *et al* even showed that RDW was not associated with microcirculatory alterations or outcomes in septic patients [46]. Our findings, based on a well-defined homogeneous cohort, and after adjustment by baseline conditions, haemoglobin and other clinical and analytical parameters, identified RDW as a potential marker of inflammation and prognosis in this setting. In addition, we confirmed that RDW is a strong and independent predictor of mortality during the first week of ICU admission while CRP and PCT were not. Consequently, despite the large use of CRP and PCT among other biomarkers, to date there is still conflicting evidence to support any of these parameters in sepsis [6,7,47].

Of note, RDW is a dynamic parameter whose variations have shown to predict mortality, running in parallel to other parameters such as malondialdehyde (MDA), tumour necrosis alpha factor or SOFA score [32,35,41,42]. In our cohort, we show that in addition that RDW values during the first week present a robust and independent association with mortality, RDW values 48 and 72 h after admission were the highest observed during the first week after admission and the strongest predictors of mortality. Yi Lang *et al*. described that RDW increased on day 3 after ICU admission in non-survivors patients with sepsis along with other inflammatory anaemia-associated parameters such as ferritin, IL-6 or hepcidin [27]. Similarly, Kim *et al*. identified a higher mortality risk in septic patients whose RDW increased within 72 h of the emergency department admission, while Ku *et al*. observed that RDW 72 h after the onset of the gram-negative bacteraemia was higher in non-survivors [35,48]. These data highlight that RDW reflects the aforementioned systemic inflammation and the pleiotropic effects of the cytokines during the altered immune response after the sepsis onset. Consequently, the dynamic character of RDW turns it into an appropriate and useful biomarker.

Some authors have studied and compared RDW with prognostic scores such as SOFA, SIRS or APACHE-II, showing that RDW correlates with APACHE-II and SOFA [27,30,32,41,42] and that it could even be a better prognostic tool than APACHE-II or SIRS [29,36,38,43]. Besides, Sadaka *et al*. demonstrated that, when adding RDW to APACHE-II, the AUC-ROC for hospital mortality in septic shock significantly increased



The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for SOFA score (A), LODS (B), SAPS-II score (C) and APACHE-II (D) and the added value of admission Red blood cell distribution width (RDW) and 24-hours RDW.

Fig. 3. Discrimination ability for the hospital mortality prognostic model comprised by RDW when added to SOFA, LODS, SAPS-II and APACHE-II. The figure shows the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) for SOFA score (A) and LODS (B) and the added value of admission Red blood cell distribution width (RDW) and 24-h RDW.

[31]. In the present study, we analysed the role of RDW not only compared to SOFA and LODS, prognosis scores that define and quantify organ dysfunction, but to the APACHE-II and SAPS-II scores, predictors of hospital and ICU mortality. Our results demonstrate that RDW improves the mortality discrimination ability of the SOFA, LODS and SAPS-II scores. We therefore believe that these novel findings are promising and relevant because they confirm that RDW is a robust marker of the dysregulated inflammatory response that conveys systemic organ dysfunction.

RDW has been criticised because of its lack of specificity, since its values rise related to inflammation, regardless of the cause [13,49,50]. However, we understand that RDW is not a marker of infection itself but an easily obtained parameter that reveals the altered inflammatory response secondary to the infection. Therefore, we believe that its main strength, besides the promptness and the availability, is that it measures and determines the systemic dysfunction related to the mentioned inflammatory environment. As a result, we consider that it could be a marker of organ dysfunction, similar to creatinine, bilirubin or platelets, other unspecific markers for sepsis or infection but which define the

organ dysfunction included in the SOFA, LODS, APACHE-II and SAPS-II scores. In this sense, RDW and SOFA, that were the only two independent factors related to mortality, had the better discrimination ability when the scores were compared, possibly because they represent sepsis dysfunction in a more comprehensive way. Since the SOFA score is the tool to assess organ dysfunction or failure, according to the Third Consensus Definition for Sepsis and Septic shock (Sepsis-3), adding RDW could result in a better performance of the score. Further studies are needed to confirm and validate these findings.

However, our study has several limitations. In addition to being a single-centre, observational and retrospective study, the population size was relatively small. In spite of that, statistical significance with clinical relevance was reached in the analysis. Secondly, the study population consisted only of patients admitted to the ICU, instead of a general population of septic patients. However, almost all septic patients have unequivocally ICU admission criteria if the disease is evolving. Thirdly, RDW was considered and analysed as a continuous variable and not as a categorical, grouped in quartiles or considering a cut-off value. Our analysis allows a more exact, precise and rigorous analysis

of a variable such as RDW, but it limits the determination of a pathological threshold or value that defines a patient or situation as pathological [51]. Therefore, our findings must be evaluated in a prospective cohort, ideally in a multicentre registry, and considering RDW cut-off points that define the severity of the organic damage; similar to how organic damage in SOFA is determined by bilirubin, creatinine, or platelet count. Finally, it should be mentioned that we did not evaluate concomitant conditions such as iron-deficiency anaemia, anaemia related to chronic kidney disease, haemolytic anaemia or B12, folic and ferritin levels, which could have been modifiers of the RDW levels and dynamics [8–10]. Similarly, blood transfusions were not considered in the multivariate analysis since, according to DAG, transfusion is a consequence of the haemoglobin levels or the anaemia status and should have introduced bias, as mentioned [25].

5. Conclusion

In summary, our study suggests that RDW values and their variations along time are an independent prognostic marker of death in septic patients admitted in the ICU and that, when added to SOFA, LODS, APACHE-II and SAPS-II scores, it could contribute to improve their discrimination ability. RDW is an easy to determine and widely available parameter that reflects the dysregulated inflammatory response and systemic dysfunction in septic patients.

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Authors' statement

All authors have approved the submitted version and agree to be personally accountable for the author's own contributions.

VMT designed the work; performed data acquisition, analysis and interpretation of data and drafted the work.

AR designed the work; performed analysis and created the software used in the work.

EM, AG, PM, AO, ST, JG, AM, JC, AFC and AR have made substantial contributions to the conception and design of the work and revised the manuscript.

Declaration of Interest

None.

References

- Vincent JL, Marshall JC, Namendys-Silva SA, François B, Martin-Loeches I, Lipman J, et al. ICON investigators. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet. Respir Med.* 2014;2:380–6.
- Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievian DR, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the global burden of disease study. *Lancet.* 2020;18(395):200–11.
- Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012. *JAMA.* 2014;311:1308–16.
- Liu V, Escobar GJ, Greene JD, Soule J, Whippy A, Angus DC, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA.* 2014;312:90–2.
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for Sepsis and septic shock (Sepsis-3). *JAMA.* 2016;315:801–10.
- Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis campaign: international guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med.* 2017;43:304–77.
- Pierrakos C, Velissaris D, Bischoff M, Marshall JC, Vincent JL. Biomarkers of sepsis: time for a reappraisal. *Crit Care.* 2020;24:287.
- Aslan D, Gümrük F, Gürgey A, Altay C. Importance of RDW value in differential diagnosis of hypochromic anemias. *Am J Hematol.* 2002;69:31–3.
- Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med.* 2005;352:1011–23.
- Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood.* 2019;133:40–50.
- Allen LA, Felker GM, Mehra MR, Chiong JR, Dunlap SH, Ghali JK, et al. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. *J Card Fail.* 2010;16:230–8.
- Scharte M, Fink MP. Red blood cell physiology in critical illness. *Crit Care Med.* 2003;31:S651–7.
- Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: a simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci.* 2015;52:86–105.
- Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM consensus conference committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest.* 1992;101:1644–55.
- Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for Sepsis: for the third international consensus definitions for Sepsis and septic shock (Sepsis-3). *JAMA.* 2016;315:762–74.
- Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on Sepsis-related problems of the European Society of Intensive Care Medicine. *Intensive Care Med.* 1996;22:707–10.
- Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on “sepsis-related problems” of the European Society of Intensive Care Medicine. *Crit Care Med.* 1998;26:1793–800.
- Le Gall JR, Klar J, Lemeshow S, Saulnier F, Alberti C, Artigas A, et al. The logistic organ dysfunction system. A new way to assess organ dysfunction in the intensive care unit. ICU scoring group. *JAMA.* 1996;276:802–10.
- Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med.* 1985;13:818–29.
- Le Gall JR, Lemeshow S, Saulnier F. A new simplified acute physiology score (SAPS II) based on a European/north American multicenter study. *JAMA.* 1993;270:2957–63.
- Definition Task Force ARDS, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA.* 2012;307:2526–33.
- Hosmer DW, Lemeshow S. Applied logistic regression. 2nd Ed. Chapter 5. New York, NY: John Wiley and Sons; 2000; 160–4.
- Moreno-Torres V, Royuela A, Muñoz E, Ortega A, Gutierrez Á, Mills P, et al. Better prognostic ability of NEWS2, SOFA and SAPS-II in septic patients. *Med Clin (Barc).* 2021;S0025-7753 00675–8.
- Jiang Y, Jiang FQ, Kong F, An MM, Jin BB, Cao D, et al. Inflammatory anemia-associated parameters are related to 28-day mortality in patients with sepsis admitted to the ICU: a preliminary observational study. *Ann Intensive Care.* 2019;9(1):67.
- Lipsky AM, Greenland S. Causal directed acyclic graphs. *JAMA.* 2022;327:1083–4.
- Hu ZD, Lippi G, Montagnana M. Diagnostic and prognostic value of red blood cell distribution width in sepsis: a narrative review. *Clin Biochem.* 2020;77:1–6.
- Zhang L, Yu CH, Guo KP, Huang CZ, Mo LY. Prognostic role of red blood cell distribution width in patients with sepsis: a systematic review and meta-analysis. *BMC Immunol.* 2020;21(1):40.
- Wang AY, Ma HP, Kao WF, Tsai SH, Chang CK. Red blood cell distribution width is associated with mortality in elderly patients with sepsis. *Am J Emerg Med.* 2018;36:949–53.
- Chen CK, Lin SC, Wu CC, Chen LM, Tzeng IS, Chen KF. STARD-compliant article: the utility of red cell distribution width to predict mortality for septic patients visiting the emergency department. *Med (Baltimore).* 2016;95:e3692.
- Mahmood NA, Mathew J, Kang B, DeBari VA, Khan MA. Broadening of the red blood cell distribution width is associated with increased severity of illness in patients with sepsis. *Int J Crit Illn Inj Sci.* 2014;4:278–82.
- Sadaka F, O'Brien J, Prakash S. Red cell distribution width and outcome in patients with septic shock. *J Int Care Med.* 2013;28:307–13.
- Jo YH, Kim K, Lee JH, Kang C, Kim T, Park HM, et al. Red cell distribution width is a prognostic factor in severe sepsis and septic shock. *Am J Emerg Med.* 2013;31:545–8.
- Kim YC, Song JE, Kim EJ, Choi H, Jeong WY, Jung IY, et al. A simple scoring system using the red blood cell distribution width, Delta neutrophil index, and platelet count to predict mortality in patients with severe Sepsis and septic shock. *J Intensive Care Med.* 2019;34:133–9.
- Kim JH, Lee Y, Cho YS, Sohn YJ, Hyun JH, Ahn SM, et al. A modified simple scoring system using the red blood cell distribution width, Delta neutrophil index, and mean platelet volume-to-platelet count to predict 28-day mortality in patients with Sepsis. *J Int Care Med.* 2020;36:873–8. [Jun 9:885066620933245].
- Kim CH, Park JT, Kim EJ, Han JH, Han JS, Choi JY, et al. An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. *Crit Care.* 2013;17:R282.
- Kim S, Lee K, Kim I, Jung S, Kim MJ. Red cell distribution width and early mortality in elderly patients with severe sepsis and septic shock. *Clin Exp Emerg Med.* 2015;2:155–61.
- von Meijenfildt GCI, van der Laan MJ, Zeebregts CJAM, Christopher KB. Red cell distribution width at hospital discharge and out-of hospital outcomes in critically ill non-cardiac vascular surgery patients. *PLoS One.* 2018;13:e0199654.
- Chen KF, Liu SH, Li CH, Wu CC, Chaou CH, Tzeng IS, et al. Development and validation of a parsimonious and pragmatic CHARM score to predict mortality in patients with suspected sepsis. *Am J Emerg Med.* 2017;35:640–6.

- [39] Park SH, Park CJ, Lee BR, Nam KS, Kim MJ, Han MY, et al. Sepsis affects most routine and cell population data (CPD) obtained using the Sysmex XN-2000 blood cell analyzer: neutrophil-related CPD NE-SFL and NE-WY provide useful information for detecting sepsis. *Int J Lab Hematol*. 2015;37:190–8.
- [40] Han YQ, Zhang L, Yan L, Li P, Ouyang PH, Lippi G, et al. Red blood cell distribution width predicts long-term outcomes in sepsis patients admitted to the intensive care unit. *Clin Chim Acta*. 2018;487:112–6.
- [41] Ju XF, Wang F, Wang L, Wu X, Jiang TT, You DL, et al. Dynamic change of red cell distribution width levels in prediction of hospital mortality in Chinese elderly patients with septic shock. *Chin Med J*. 2017 May 20;130:1189–95.
- [42] Lorente L, Martín MM, Abreu-González P, Solé-Violán J, Ferreres J, Labarta L, et al. Red blood cell distribution width during the first week is associated with severity and mortality in septic patients. *PLoS One*. 2014;9:e105436.
- [43] Ghimire R, Shakya YM, Shrestha TM, Neupane RP. The utility of red cell distribution width to predict mortality of septic patients in a tertiary hospital of Nepal. *BMC Emerg Med*. 2020;20:43.
- [44] Zhao C, Wei Y, Chen D, Jin J, Chen H. Prognostic value of an inflammatory biomarker-based clinical algorithm in septic patients in the emergency department: An observational study. *Int Immunopharmacol*. 2020;80:106145.
- [45] Zhang Z, Xu X, Ni H, Deng H. Red cell distribution width is associated with hospital mortality in unselected critically ill patients. *J Thorac Dis*. 2013;5:730–6.
- [46] Fontana V, Spadaro S, Bond O, Cavicchi FZ, Annoni F, Donadello K, et al. No relationship between red blood cell distribution width and microcirculatory alterations in septic patients. *Clin Hemorheol Microcirc*. 2017;66:131–41.
- [47] Lee CC, Chen SY, Tsai CL, Wu SC, Chiang WC, Wang JL, et al. Prognostic value of mortality in emergency department sepsis score, procalcitonin, and C-reactive protein in patients with sepsis at the emergency department. *Shock*. 2008;29:322–7.
- [48] Ku NS, Kim HW, Oh HJ, Kim YC, Kim MH, Song JE, et al. Red blood cell distribution width is an independent predictor of mortality in patients with gram-negative bacteremia. *Shock*. 2012;38:123–7. 100157.
- [49] Moreno-Torres V, Castejón R, Mellor-Pita S, Tutor-Ureta P, Durán-del Campo P. Usefulness of the hemogram as a measure of clinical and serological activity in systemic lupus erythematosus. *J Trans Autoimmun*. 2022;5:100157.
- [50] Moreno-Torres V, Sánchez-Chica E, Castejón R, Caballero Bermejo Antonio-Francisco, Mills P, Diago-Sempere E, et al. Red blood cell distribution width as a marker of hyperinflammation and mortality in COVID-19. *Ann Palliat Med*. 2022; 22:119 In press.
- [51] Altman DG, Royston P. The cost of dichotomising continuous variables. *BMJ*. 2006; 332:1080.