Original Article

Red blood cell distribution width as a marker of hyperinflammation and mortality in COVID-19

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Background: Red blood cell distribution width (RDW) could reflect interleukin-6 (IL-6) systemic activity since anisocytosis represents the inhibition of erythropoiesis, leaded by the hyperinflammatory background. Our objective was to analyze RDW performance to predict outcome in coronavirus disease 2019 (COVID-19) acute respiratory distress syndrome (ARDS).

Methods: Retrospective observational study including 173 patients with COVID-19-associated ARDS. Data was analyzed at hospital admission, inclusion in the TOCICOV Study (day 0), days 1, 3, 7 and 15 post-inclusion.

Results: Overall, 57% patients received tocilizumab. Overall mortality was 20.8%. RDW was higher in non-survivors compared to survivors at admission (13.53% *vs.* 14.35, P=0.0016), day 0 (13.60% *vs.* 14.42, P=0.026), day 3 (13.43% *vs.* 14.36, P<0.001) and day 7 (13.41% *vs.* 14.31, P=0.046), presenting better discrimination ability for mortality than other prognostic markers [area under the curve-receiver operating characteristic (AUC-ROC) =0.668 for admission RDW, 0.680 for day 0 RDW, 0.695 for day 3 RDW and 0.666 for day 7 RDW]. RDW values did not vary significantly according to tocilizumab treatment. When adjusted by hemoglobin and tocilizumab treatment, only RDW at admission, day 0, day 3 and C reactive protein (CRP) at day 0 and day 1 were associated with mortality (P<0.05). Only in non-tocilizumab treated patients, IL-6 levels at day 0 were correlated with day 3 RDW (r=0.733, P=0.004) and with day 3 CRP (r=0.727, P=0.022). Both parameters showed significant statistical correlation (r=0.255 for day 1 RDW and CRP in the overall cohort and r=0.358 for day 3 RDW and CRP in patients not treated with tocilizumab, P<0.015).

Conclusions: RDW predicts COVID-19-associated ARDS mortality and reflects the hyperinflammatory background and the effects of cytokines such as IL-6, irrespective of tocilizumab treatment.

Keywords: Coronavirus disease 2019 (COVID-19); red blood distribution width; interleukin-6 (IL-6); tocilizumab

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Introduction

The coronavirus disease 2019 (COVID-19) has resulted in a pandemic that will determine worldwide society probably for generations, and far from being controlled, there are still great gaps in knowledge about its physiopathology and epidemiology (1). Since the initial outbreak in December 2019, risk factors for developing acute respiratory distress syndrome (ARDS), central axis of its mortality and morbidity, have been identified. Besides age and comorbidities, parameters such as neutrophilia, lymphocytopenia and thrombopenia, data suggestive of organ injury [bilirubin, Aspartate Aminotransferase, urea, creatinine or lactate dehydrogenase (LDH)] and coagulation function abnormalities [D-dimer (DD) or prothrombin activity] have been associated with the risk of developing ARDS, intensive care unit (ICU) admission and death (2-4). Furthermore, and in relation to the "cytokine storm" that seems to determine the disease pathophysiology and ARDS, interleukin-6 (IL-6), IL-10, ferritin and C reactive protein (CRP) have been also identified as prognostic factors (5-8). In fact, the key role that IL-6 seems to play in the immunological imbalance and hyperactivation has led to consider its blockage as a specific target in treatment, with satisfying results (9-12).

Among all the processes in which IL-6 participates, including T-cell differentiation, B-cell maturation, synthesis and secretion of immunoglobulins, its main role in the pathophysiology of anemia of chronic disease is of particular interest (13,14). In addition to diminishing the erythropoietin response and reducing erythrocyte survival, IL-6 inhibits erythropoiesis and hemoglobin synthesis in the bone marrow. Moreover, it induces hepatic cells hepcidin release through binding to the STAT3 receptor. Hepcidin is a protein that controls iron metabolism since it regulates the expression of divalent metal transporter (DMT-1) and enhances the breakdown of ferroportin, which leads to blockade of the duodenal iron transfer. As a result, hepcidin blocks intestinal iron absorption and iron recycling by macrophages. In the same way, IL-6 induces the transcription of ferritin, that also leads to increased iron retention and overload within reticulo-endothelial cells (15,16). Accordingly, the elevated ferritin, despite iron overload and increased iron storage, reflects iron dysregulation and the inhibition of erythropoiesis.

Red blood cell distribution width (RDW) is a parameter routinely reported as part of a complete blood count, and measures the size variability of circulating erythrocytes. RDW is calculated as standard deviation of red blood cell volume divided by mean corpuscular volume, expressed as a percentage. Traditionally, it has been considered for the differential diagnosis of iron deficiency anemia and anemia of chronic disease (17). Recently, multiple studies demonstrating that RDW values correlate with inflammation in clinical scenarios as diverse as cardiovascular disease (18), rheumatoid arthritis (19) and systemic lupus erythematosus (20) have been published. Furthermore, RDW has been studied as a mortality risk factor in sepsis and ARDS (21-24). In the COVID-19 setting, other reports have analyzed this high availability parameter as a prognostic marker of severity and death in hospitalized patients with COVID-19 (25,26).

Therefore, and based in the aforementioned pathophysiological considerations, since RDW reflects anisocytosis probably determined by the hyperinflammatory background and the effects of cytokines such as IL-6, we performed the present study with the objective to analyze its role and performance to predict outcome in COVID-19 ARDS. Moreover, we evaluated the relationship between the parameters that have shown a prognostic value and the chronology of their variation during the first days of the cytokine storm, both in patients treated and not treated with tocilizumab. We present the following article in accordance with the STROBE reporting checklist (available at https://apm.amegroups.com/article/view/10.21037/apm-22-119/rc).

Methods

Study design and participants

The study population consisted of the subset of the patients from the TOCICOV Study (described elsewhere) (9) that were included at Hospital Puerta de Hierro-Majadahonda. This was a retrospective observational study designed to compare mortality and ICU admission between patients treated with corticoids and tocilizumab versus controls, during the first wave of the pandemic. The study was approved by the Research Ethics Committee of Hospital

Puerta de Hierro Majadahonda (No. FIB-TOC-2020-01) and a waiver for informed consent was granted. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

Inclusion criteria for the TOCICOV study were as follow: adult patients (≥18 years) with COVID-19, confirmed by polymerase chain reaction (PCR) on nasopharyngeal swab, who were consecutively admitted to our hospital between March 3th and April 20th, hospitalized outside the ICU and presented documented interstitial pneumonia with severe respiratory failure (respiratory severity scale BRESCIA-COVID =2) (27) or rapid deterioration of respiratory exchanges without immediate possibility of invasive ventilation (respiratory severity scale COVID =3) and at least one of the following parameters: IL-6 >40 pg/mL, increasing LDH or LDH > twice upper normal limit, increasing CRP, DD >1,500 ng/mL, lymphocytes <1,200/μL or ferritin >500 ng/mL. Patients who died within 24 hours after admission to the hospital or after developing inclusion criteria were excluded.

Data collection

Clinical, pharmacological, laboratory and radiologic data was extracted from medical records using a standardized data collection form. Patients follow-up, corticosteroid use, blood examinations and serum biochemical tests were done according to clinical practice and each physicians criteria. Data was collected from the first day at hospital admission, at the inclusion in the TOCICOV study according to the criteria specified above (day 0) and days 1, 3, 7 and 15 post-inclusion. All data was included by a primary reviewer and subsequently checked by three senior physicians. Routine blood examinations included a complete blood count, coagulation tests including DD, serum biochemical tests including lactate dehydrogenase, CRP, serum ferritin and IL-6. Chest radiographs or computed tomography scans were also done for all inpatients.

Biomarkers analysis and dynamics

We analyzed the association of RDW and IL-6 with in-hospital mortality during the first 7 days of the study period, the main study outcome. Besides, we considered other blood count parameters, coagulation tests and biomarkers including LDH, CRP and ferritin. In order to measure the parameters accuracy, we determined the area under the curve (AUC)-"receiver operating characteristic"-(ROC). Secondly, we analyzed the influence of tocilizumab treatment on the values of the mentioned parameters, and performed a multivariable analysis considering tocilizumab treatment and hemoglobin values to confirm the independent prognostic ability of the inflammatory parameters. Thirdly, we studied the correlations among the different biomarkers to better understand their role and dynamics as well as the pathophysiology of the cytokine storm. Parameters were considered at admission, at inclusion (day 0) and during the first week of inclusion (days 1, 3 and 7).

Statistical analysis

Data were expressed as mean and standard deviation or number (percentage) as appropriate. The Kolmogorov test was used to evaluate data distribution and as data did not follow a parametric distribution, statistical analysis was performed using Spearman rank's test to analyze correlations and Mann-Whitney U-test to assess differences between groups. Levene's test was used for the homogeneity of variance test. The χ^2 test (with the two-sided Fisher's exact test) was used to compare categorical variables. The discrimination ability was evaluated following an approach based on the AUC-ROC. Finally, we performed a multivariable analysis considering tocilizumab treatment and hemoglobin for the parameters associated with mortality in the univariate analysis since IL-6 values can rise after IL-6 blockade and because anemia status could influence RDW values (17). For all analyses, significance was defined as a P value below 0.05. Statistical analysis was performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics

Overall, 173 patients with COVID-19 and respiratory insufficiency or increased inflammatory parameters were included. Their baseline characteristics and comorbidities, in addition to the treatment received according to outcome (survivors versus non-survivors) are shown in *Table 1*. Mean age was 66.6 years and 67.1% patients were male. Mean time from symptom onset to patient inclusion was 10 days, and mean time from hospital admission to inclusion was 2.8 days. At the time of inclusion, 66.7% were receiving oxygen therapy through nasal cannula or Venturi mask and 31.4%

Table 1 Patients' characteristics, comorbidities, treatment received and outcomes

Characteristics	Global (n=173)	Survivors (n=137)	Non-survivors (n=36)	P value
Age, mean ± SD	66.6±13.6	63.6±12.5	78±11.5	<0.001
Gender (men), n (%)	116 (67.1)	91 (66.4)	25 (69.4)	0.843
Charlson score, mean ± SD	3.49±2.5	2.9±2.1	5.4±2.8	<0.001
Underlying medical conditions, n (%)				
Arterial hypertension	94 (54.3)	56 (40.9)	23 (63.9)	0.015
Diabetes	49 (28.3)	31 (22.6)	18 (50.0)	0.003
Cardiovascular disease	44 (25.4)	25 (18.2)	19 (52.8)	<0.001
Chronic lung disease	31 (17.9)	20 (14.6)	11 (30.6)	0.048
Neurological disease	21 (12.1)	10 (7.3)	11 (30.6)	0.001
Liver disease	13 (7.5)	5 (3.6)	8 (22.2)	0.001
Chronic kidney disease	15 (8.7)	6 (4.4)	9 (25.0)	<0.001
Onco-hematologic	8 (4.6)	5 (3.6)	3 (8.3)	0.365
HIV	3 (1.7)	3 (2.2)	0	1
Transplant (SOT/SCT)	3 (1.7)	1 (0.7)	2 (5.6)	0.110
Clinical characteristics, mean ± SD				
SBP	117±21	115±19	138±37	0.038
DBP	68±11	68±11	66±13	0.654
Temperature	37±1	37±1	37±1	0.111
Respiratory rate	26±7	25±7	28±1	0.112
$SapO_2$	93±4	93±3	92±6	0.097
FiO_2	53±28	53±28	55±27	0.769
$SapO_2/FiO_2$	202±84	206±85	188±78	0.266
Treatment received, n (%)				
Lopinavir/ritonavir	143 (82.7)	120 (83.9)	23 (63.9)	0.002
Hydroxychloroquine	170 (98.3)	135 (79.4)	35 (93.2)	0.506
Interferon	102 (59.0)	92 (67.2)	10 (72.2)	0.000
Azithromycin	75 (43.4)	54 (39.4)	21 (58.3)	0.058
Ceftriaxone	84 (48.6)	68 (49.6)	16 (44.4)	0.708
Levofloxacin	24 (13.9)	23 (16.8)	1 (2.8)	0.030
Steroids	156 (90.2)	124 (90.5)	32 (88.9)	0.757
Tocilizumab	108 (62.4)	93 (67.9)	15 (41.7)	0.006
Outcomes, n (%)				
Infectious complications (confirmed)	15 (8.7)	12 (8.8)	3 (8.3)	1

SD, standard deviation; HIV, human immunodeficiency virus; SOT, solid organ transplantation; SCT, stem cell transplantation; SBP, systolic blood pressure; DBP, diastolic blood pressure; $SapO_2$, saturation by pulse oximetry; FiO_2, fraction of inspired oxygen; $SapO_2/FiO_2$, saturation by pulse oximetry/fraction of inspired oxygen ratio.

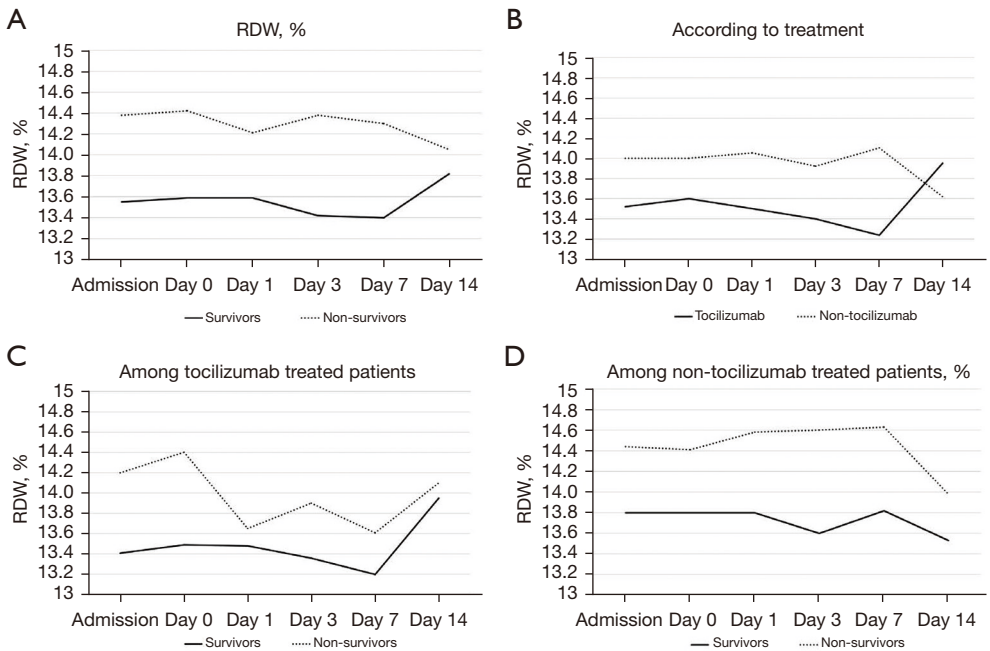

Figure 1 RDW dynamics. The figure shows RDW values among survivors and non-survivors (A), RDW values according to treatment (B), RDW among survivors and non survivors in the patients treated with tocilizumab (C) and not treated with tocilizumab (D). RDW, red blood distribution width.

through oxygen mask with reservoir bag; mean oxygen saturation by pulse oximetry ($SapO_2$)/fraction of inspired oxygen (FiO_2) ratio (SaO_2/FiO_2) was 202. The 90.2% of patients received steroids and 57.4% tocilizumab. Among patients treated with tocilizumab, 87.2% received it at the day of inclusion (day 0). Infectious complications were identified and confirmed in 15 patients. Global mortality was 20.8%.

Comparison of biomarkers in survivors versus non-survivors

Blood count and biochemical parameters, in addition to coagulation tests at admission, at the moment of inclusion (day 0) and during admission (days 1, 3 and 7) were analyzed and compared according to death. RDW and IL-6 dynamics are shown in *Figures 1,2*, respectively, while the other parameters are illustrated in the Figures S1,S2.

At day 0, the patients who ultimately died presented a higher RDW (13.60% *vs.* 14.42%, P=0.026). No significant differences were found when other parameters were analyzed at day 0 (*Table 2*). During overall admission, RDW at hospital admission (13.53% *vs.* 14.35%, P=0.0016), day 3 (13.43% *vs.* 14.36%, P<0.001) and day 7 (13.41% *vs.* 14.31%, P=0.046) was higher in non-survivors (*Figure 1A*). Otherwise, non-survivors presented higher admission CRP (105 *vs.* 136 mg/L, P=0.0021), day 1 CRP (128 *vs.* 176 mg/L, P=0.003), day 7 CRP (23 *vs.* 55 mg/L, P=0.01), day 1 IL-6 (243 *vs.* 454 pg/mL, P=0.046) and day 3 ferritin (1,444 *vs.* 1,934 ng/mL, P=0.029). Neither lymphocyte counts, neutrophil counts, NLR, platelet counts, hemoglobin, PA, fibrinogen, DD or LDH showed significant differences among survivors and non-survivors (Figures S1,S2).

In order to compare the performance of the different biomarkers associated with mortality, an ROC-AUC analysis was performed (*Figures 3,4*, *Table 3*). Admission, days 0, 3 and 7 RDW values showed AUC beyond 0.650, being day 3 RDW the best predictor with AUC 0.695. Both day 1 CRP and day 1 IL-6 also showed AUC under 0.650.

Variation of biomarkers according to tocilizumab treatment.

The mentioned parameters were analyzed according to tocilizumab treatment to understand the influence of IL-6 blockade on them (Figures S3,S4). Patients who received tocilizumab presented a higher day 0 CRP (127 *vs.* 155 mg/L, P=0.038) but a lower day 3 CRP (92 *vs.* 43 mg/L, P<0.001)

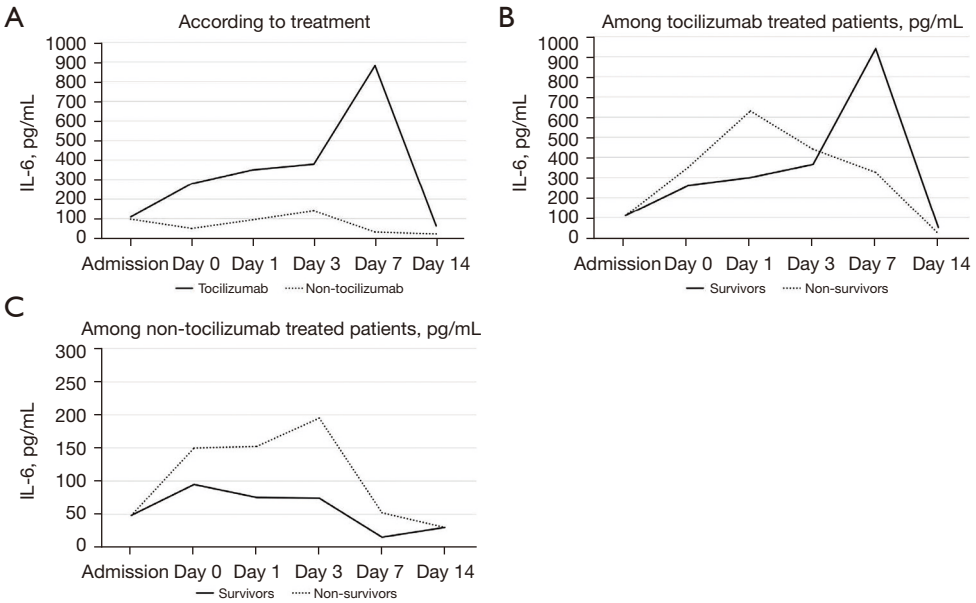

Figure 2 IL-6 dynamics. The figure shows IL-6 levels according to treatment group (A) and among survivor and non survivors considering tocilizumab treatment (B) and non-tocilizumab treatment (C). IL-6, interleukin-6.

Table 2 Analytical parameters at day 0

Laboratory values	Overall (n=173), mean ± SD	Survivors (n=137), mean ± SD	Non survivors (n=36), mean ± SD	P value
Lymphocytes/μL	869±473	868±488	869±421	0.990
Neutrophils/μL	6,381±3,588	6,477±3,520	6,028±3,864	0.520
N/L ratio	9.43±7.95	9.55±7.80	8.98±8.59	0.715
Platelets (10^3/μL)	228±85	235±85	204±82	0.06
Hb (g/dL)	13.58±1.96	13.55±1.99	13.72±1.84	0.308
RDW (%)	13.76±1.84	13.60±1.81	14.42±1.87	0.026
Prothrombin activity (%)	87.01±16.50	86.74±18.20	86.53±13.40	0.954
Fibrinogen (mg/dL)	676±143	682±148	653±113	0.399
DD (ng/mL)	1,395±1,700	1,330±1,658	1,646±1,858	0.336
CRP (mg/L)	144±82	142±86	154±67	0.462
AST (U/L)	71±112	69±106	79±134	0.659
LDH (UI/L)	404±174	396±178	437±155	0.213
IL-6 (pg/mL)	199±228	186±221	293±274	0.286
Ferritin (ng/mL)	1,478±1,638	1,394±834	1,933±3,793	0.344

SD, standard deviation; N/L, neutrophils to lymphocytes ratio; Hb, hemoglobin; RDW, red blood cell distribution width; DD, D-dimer; CRP, C reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; IL-6, interleukin-6.

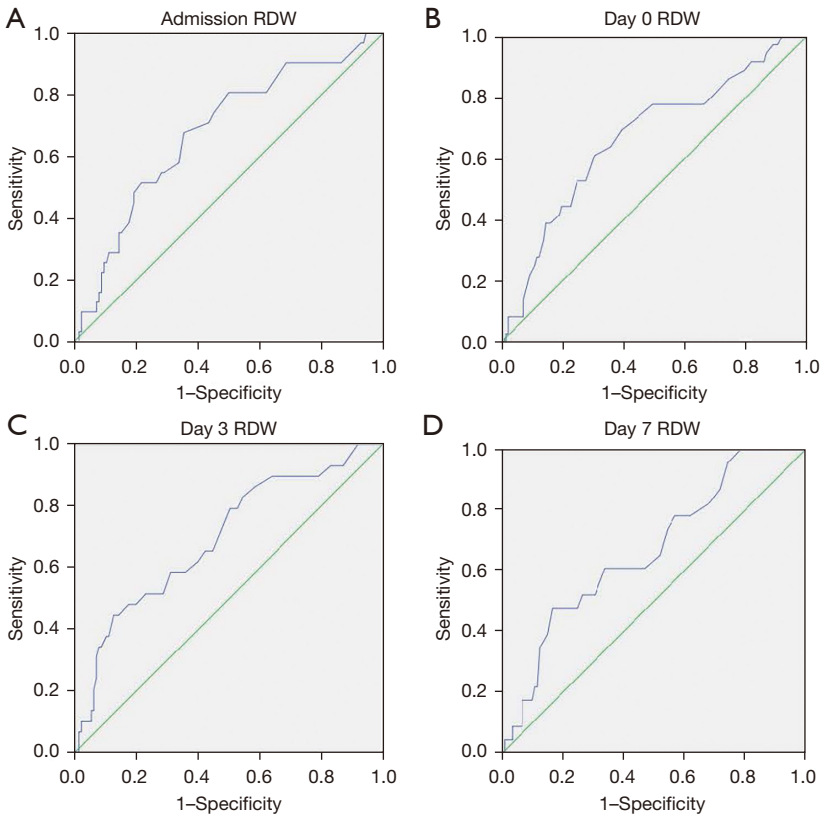

Figure 3 Receiver-operating characteristic curve of admission RDW (A), day 0 RDW (B), day 3 RDW (C) and day 7 RDW (D). RDW, red blood distribution width.

and day 7 CRP (55 *vs.* 12.5 mg/L, P<0.001). Furthermore, IL-6 levels were higher in the patients who received tocilizumab at day 0 (98 *vs.* 270 pg/mL, P=0.008), day 1 (95 *vs.* 346 pg/mL, P=0.004) and day 3 (141 *vs.* 379 pg/mL, P=0.016) (*Figure 2A*). There were no differences in RDW, ferritin, lymphocyte counts, neutrophil counts, NLR, platelet counts, hemoglobin, PA, fibrinogen, DD or LDH according to tocilizumab treatment (Figures S3,S4).

In addition, we performed different multivariable analyses according to tocilizumab treatment and hemoglobin levels to assess the independent prognostic role of the parameters associated with mortality in the univariate analysis. Admission RDW (OR =1.23, 95% CI: 1.01–1.49, P=0.041), day 0 RDW (OR =1.22, 95% CI: 1–1.49, P=0.05), day 3 RDW (OR =1.25, 95% CI: 1.01–1.56, P=0.047), day 0 CRP (OR =1.01, 95% CI: 1–1.01, P=0.043) and day 1 CRP (OR =1.01, 95% CI: 1.01–1.02, P=0.001) were independently prognostic of mortality, while day 7 CRP, day 1 IL-6 and day 3 ferritin were not.

Correlation between the inflammatory parameters

The correlation between the inflammatory parameters was analyzed to understand the physiopathology of the cytokine storm (Table S1). IL-6 at admission showed significant correlation with day 0 CRP (r=0.332, P=0.007) and day 1 CRP (r=0.303, P=0.015) while IL-6 at day 0 was correlated with day 0 CRP (r=0.297, P=0.003) and day 0 ferritin (r=0.539, P=0.008). In parallel, day 1 CRP was correlated with admission RDW (r=0.186, P=0.026), day 0 RDW (r=0.275, P=0.002), day 1 RDW (r=0.255, P=0.04) and day 3 RDW (r=0.277, P=0.001). Similarly, day 3 CRP was correlated with day 0 RDW (r=0.188, P=0.03), day 1 RDW (r=0.245, P=0.001) and day 3 RDW (r=0.266, P=0.001). Day 7 CRP showed correlation with day 7 RDW (r=0.218, P=0.017).

Despite the afore-mentioned data, no correlation was found between IL-6 and RDW in the overall cohort. However, in the patients not treated with tocilizumab, IL-6 levels at day 0 were strongly correlated with day 3

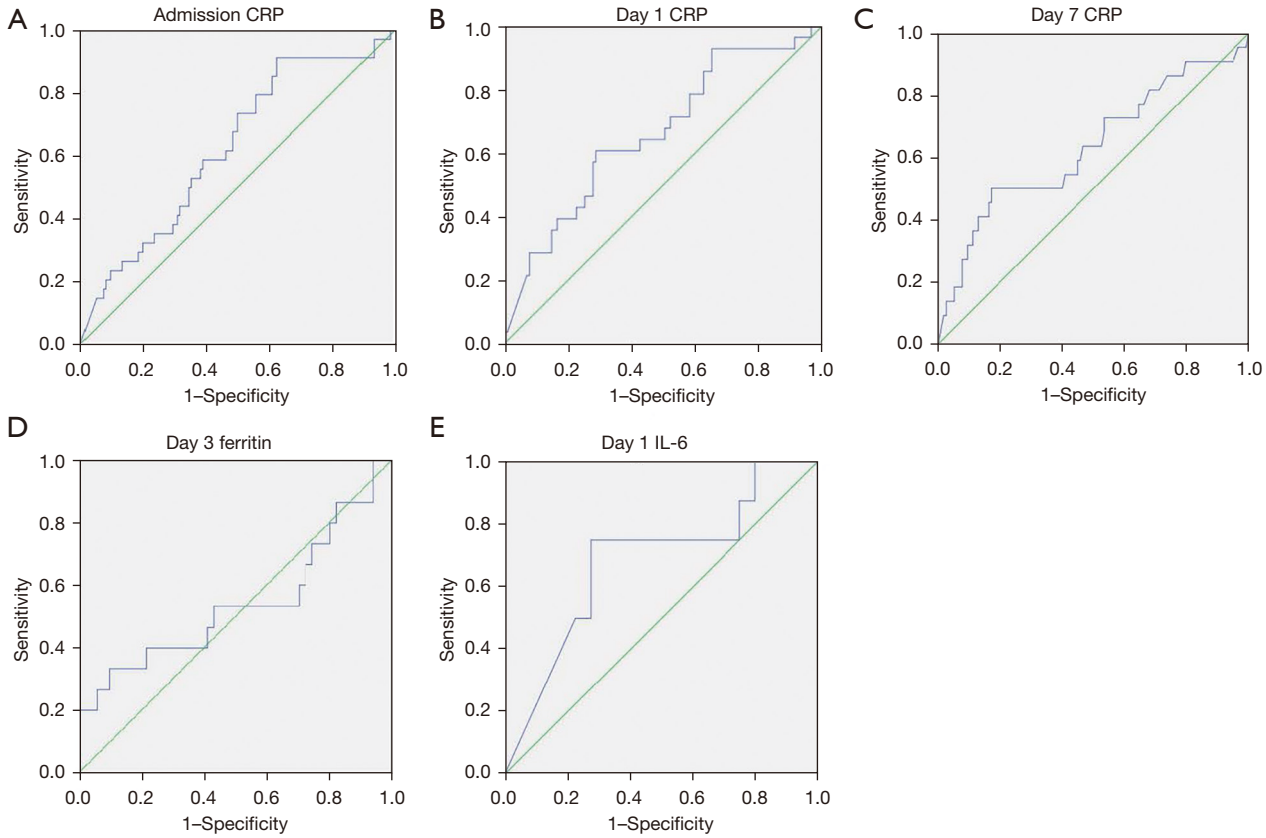

Figure 4 Receiver-operating characteristic curve of admission CRP (A), day 1 CRP (B), day 7 CRP (C), day 3 ferritin (D) and day 1 IL-6 (E). CRP, C reactive protein; IL-6, interleukin-6.

Table 3 Inflammatory parameters discrimination ability for mortality

Parameter	AUC-ROC	95% confidence interval
Admission RDW	0.668	0.567–0.769
Day 0 RDW	0.680	0.574–0.785
Day 3 RDW	0.695	0.587–0.803
Day 7 RDW	0.666	0.548–0.783
Admission CRP	0.625	0.523–0.727
Day 1 CRP	0.662	0.549–0.775
Day 7 CRP	0.633	0.494–0.773
Day 1 IL-6	0.681	0.471–0.892
Day 3 ferritin	0.540	0.348–0.731

AUC-ROC, area under the curve receiving operating characteristic; RDW, red blood cell distribution width; CRP, C reactive protein; IL-6, interleukin-6.

RDW (r=0.733, P=0.004) and with day 3 CRP (r=0.727, P=0.022) (*Figure 5A,5B*). In these patients, day 3 RDW and day 3 CRP also showed a significant correlation (r=0.358, P=0.005) (*Figure 5C*).

Discussion

Our results indicate that RDW may predict COVID-19-associated ARDS mortality with better discrimination ability than other parameters, reflecting the hyperinflammatory background and the effects of cytokines such as IL-6, irrespective of tocilizumab treatment.

RDW is a parameter routinely reported as part of a complete blood count, measuring the size variability of circulating erythrocytes (anisocytosis) (17). During the recent years, several studies have confirmed that RDW adequately assesses inflammation in diverse clinical

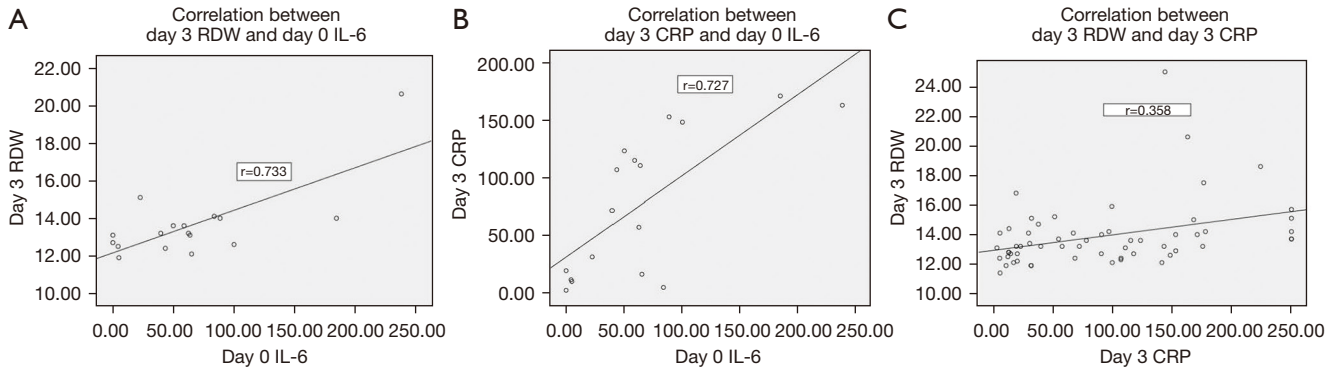

Figure 5 Correlation between IL-6, day 3 RDW and day 3 CRP among non-tocilizumab treated patients. The figure shows the correlations among day 0 IL-6 and day 3 RDW (A) and with day 3 CRP (B). (C) shows correlation between day 3 RDW and CRP in the patients non treated with tocilizumab. RDW, red blood distribution width; IL-6, interleukin-6; CRP, C reactive protein.

scenarios (17-24). In this context, it has been hypothesized that anisocytosis reflects the dysregulation of the iron metabolism and the inhibition of erythropoiesis that result in the anemia of the chronic disease, which in turn is attributable to the effect of diverse cytokines, mainly IL-6 (13-16). Interestingly, other authors have found that inflammatory anemia-associated parameters changed significantly in parallel to RDW in patients with sepsis or heart failure, due to the effect of cytokines on erythropoiesis (16,21-23,28,29). Since IL-6 is one of the key molecules of the COVID-19 disease pathophysiology and because tocilizumab has demonstrated to improve survival in this setting, the aim of our study was to analyze the RDW prognostic role and to understand the effects of IL-6 blockade on this parameter.

To date, several publications support that the RDW is higher in more severe COVID-19 patients and those with worse outcomes (25,26,30-35). In fact, Hornick et al confirmed that RDW was associated with mortality after adjusting for age, sex, race, cardiovascular disease or hemoglobin (36). In addition, this interesting report identified that RDW was associated with certain pro-inflammatory cytokines such as TNF-α or IL-6. Similarly, Martínez-Urbistondo *et al.* noticed higher RDW values, among other inflammatory markers, in the patients with higher IL-6 levels (37). However, there are still important unanswered questions about the mechanisms or the usefulness of this parameter. We believe our study helps to clarify the previous findings but additionally presents some strengths and novelties that, to our knowledge, have not been previously mentioned by others. On the one hand, we evaluated not only RDW but other additional biomarkers such as ferritin, CRP, IL-6 and LDH, among others, both during the course of the disease and after tocilizumab treatment. Accordingly, we studied their role as biomarkers, their dynamics and relations, providing a more comprehensive picture of the inflammatory environment during the cytokine storm. In addition, we considered the effect of tocilizumab treatment and its potential role as a confounding factor of the prognostic ability of the mentioned parameters. This point is particularly important in the case of IL-6, which is directly altered by the use of tocilizumab. Finally, we would like to highlight that we evaluated all these biomarkers in a homogeneous population with severe disease, with less selection bias when compared to other cohorts including a wide array of patients with different degrees of clinical severity, from outpatients to patients admitted to ICU. Besides, the analysis in this homogenous cohort, while limiting the possibility to identify or compare to other prognostic markers, would yield more strong and robust results once the differences and statistical significances would be found.

In our cohort, we found that the prognostic value of RDW was dynamic, useful both from hospital admission to seven days after the respiratory worsening, irrespective of hemoglobin values and tocilizumab treatment. In addition, RDW showed better discrimination ability for mortality than other inflammatory parameters such as lymphocyte count, DD, LDH or ferritin. Besides, some authors have described that RDW constantly increases from symptom onset in COVID-19 disease while Kim *et al.* and Ku *et al.* identified a higher mortality risk in the patients whose RDW increased within 72 hours after the admission because of sepsis or after the onset of gram-negative bacteremia,

respectively (22,38-40). Similarly, in our cohort, day 3 RDW after respiratory worsening was the best mortality predictor, beyond CRP, ferritin or IL-6. As a matter of fact, day 3 RDW was strongly correlated with IL-6 values at inclusion but only in the patients not treated with tocilizumab, suggesting that the IL-6 blockade prevented the IL-6 inflammatory effects and dysregulation of the red blood cells synthesis and therefore there were no changes on day 3 RDW values after tocilizumab treatment. In the same way, CRP, a clearly identified marker and prognostic in COVID-19 disease, was the only other parameter independently associated with mortality. At the same time, day 3 CRP was also strongly determined by IL-6 at inclusion and was correlated with RDW during the study period. Therefore, we believe that RDW, and mainly day 3 RDW after the respiratory worsening, reflects the hyperinflammatory state and the cytokine storm that leads to COVID-19 severe disease, in parallel to CRP, being a surrogate marker of the pleiotropic effects of IL-6. In addition, these findings suggest that RDW could be useful to monitor the inflammatory environment and the effect of the active IL-6, even when IL-6 levels are increased as a result of the detection of blocked IL-6 after tocilizumab administration, and no longer reflect inflammation.

However, there are recent reports that have called into question the lead role of IL-6 in COVID-19.

In the interesting report from Leisman et al, the elevations of IL-6 were remarkably lower than those reported in patients with ARDS unrelated to COVID-19, sepsis, and the chimeric antigen receptor (CAR) T cell-induced cytokine release syndrome (CRS) (41). However, several biomarkers, including DD, CRP, ferritin or LDH were elevated to a similar or greater extent in patients with COVID-19 than in patients with the compared disorders. These findings are in the same direction of the report from Zizzo *et al.*, who alternatively proposes IL-33 as the key player in driving all stages of COVID-19 (42). From this perspective, IL-6 as well as other cytokines such as IL-1b, IL-7 or IL-2, would be mediators in the damage produced by IL-33. We consider that our results do not contradict these hypotheses but rather reinforce the concept that COVID-19 pathophysiology differs from the classical hyperinflammatory states as sepsis, CRS, macrophage activation syndrome (MAS) or hemophagocytic lymphohistiocytosis (43). Consequently, and despite the previous reports, IL-6 in our cohort was not an independent prognostic factor of mortality. On the one hand, this result confirms that IL-6 levels after tocilizumab treatment do not reflect inflammation or activity once the receptor is blocked. On the other hand, reinforces the hypothesis that IL-6 is probably not the only and initial factor but could be a key mediator that, along with other cytokines, determines the cytokine storm. As a result, these findings provide more consistency to the utility and applicability of RDW as a surrogate marker of the cytokine storm and not only of the IL-6 effects. All the same, further studies are needed to characterize and determine COVID-19 complex pathophysiology that not necessarily has to be parallel to the hyperinflammatory syndromes aforesaid.

Nevertheless, we have to consider some limitations. Our study was a single-center, observational, retrospective study, where the population size was relatively small. Therefore, the present data need confirmation in other populations. Second, the homogeneity of the population, since all patients presented severe disease, can limit the generalizability of the study to mild or non-severe COVID-19. Another pitfall of the study is the fact that over 90% of the patients in our cohort received steroids. Therefore, our data represent mainly what occurs under steroid treatment, but, given the present evidence and recommendations (44,45), that will probably be the most frequent scenario in patients with severe COVID-19 ARDS and is not necessarily a confounding factor.

In summary, RDW may predict mortality in severe COVID-19 pneumonia and reflects the hyperinflammatory background and the effect of cytokines such as IL-6. This readily available parameter could help clinicians to identify patients at risk, those who are potential candidates to IL-6 blockade and to monitor treatment response to tocilizumab, discerning ongoing inflammation among patients who present evenly elevated IL-6.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm.amegroups.com/article/view/10.21037/apm-22-119/coif). The authors have no conflicts of interest to declare.

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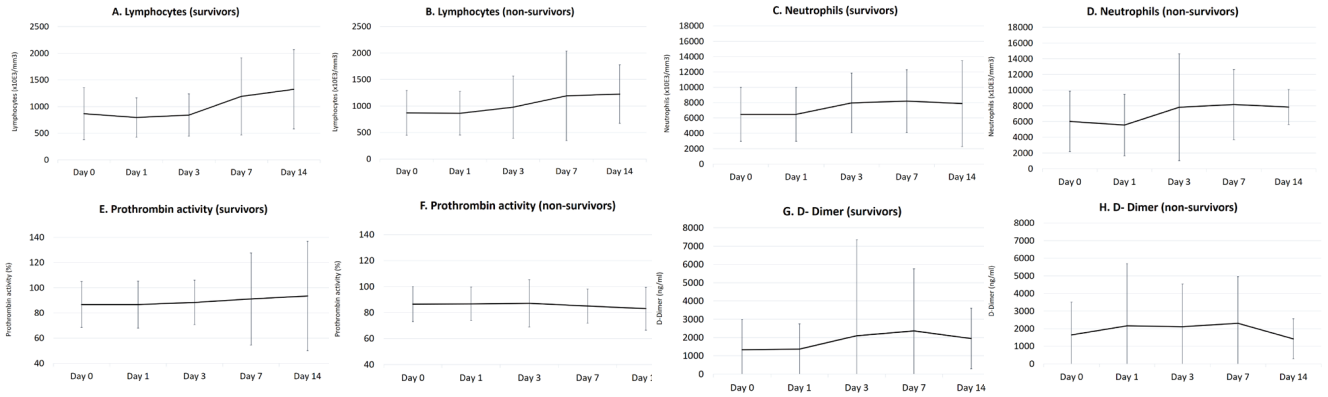

Figure S1 Blood and coagulation tests values among survivors and non-survivors. Lymphocytes (A,B), Neutrophils (C,D), Prothombin activity (E,F) and D-Dimer (G,H) are expressed as mean and standard deviation (error bars).

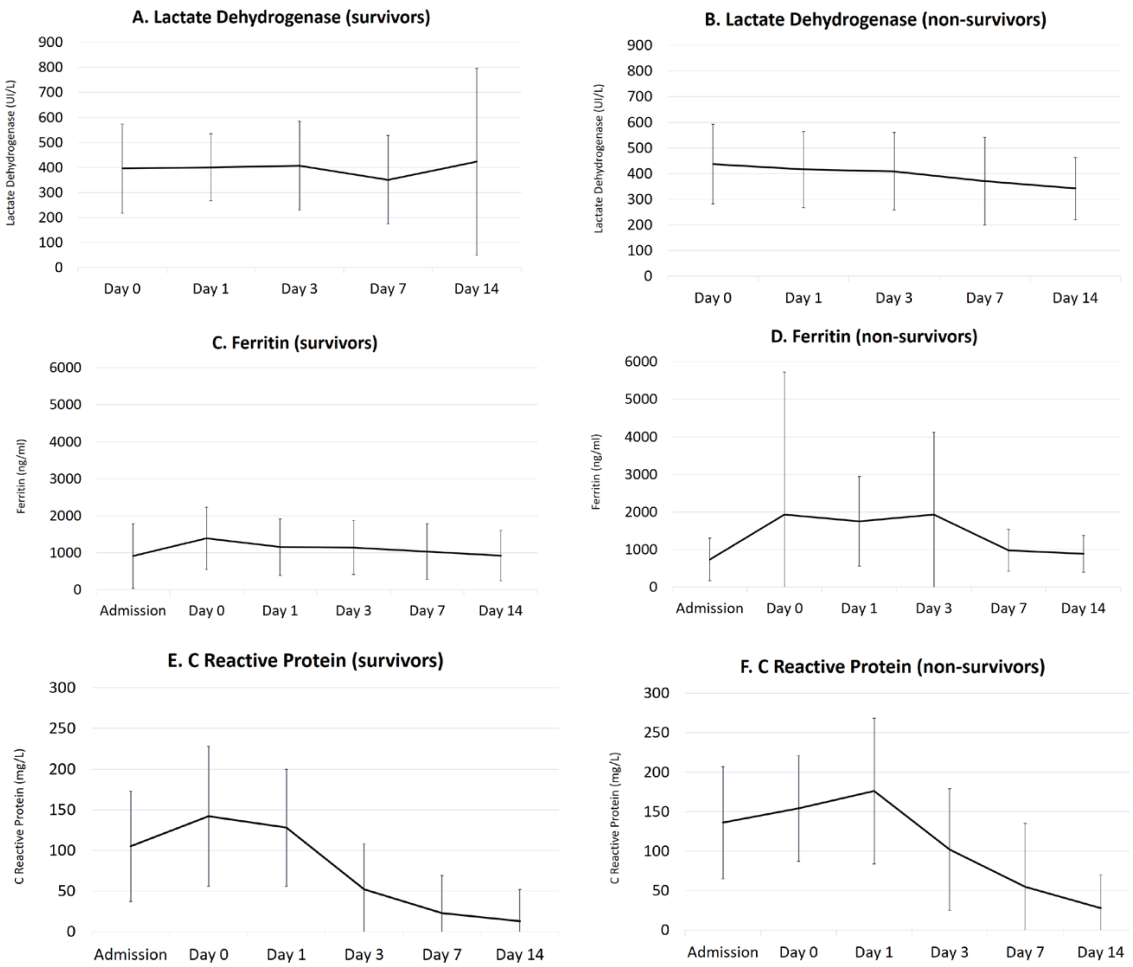

Figure S2 Inflammatory markers among survivors and non-survivors. Lactate Dehydrogenase (A,B), Ferritin (C,D) and C reactive protein (E,F) are expressed as mean and standard deviation (error bars).

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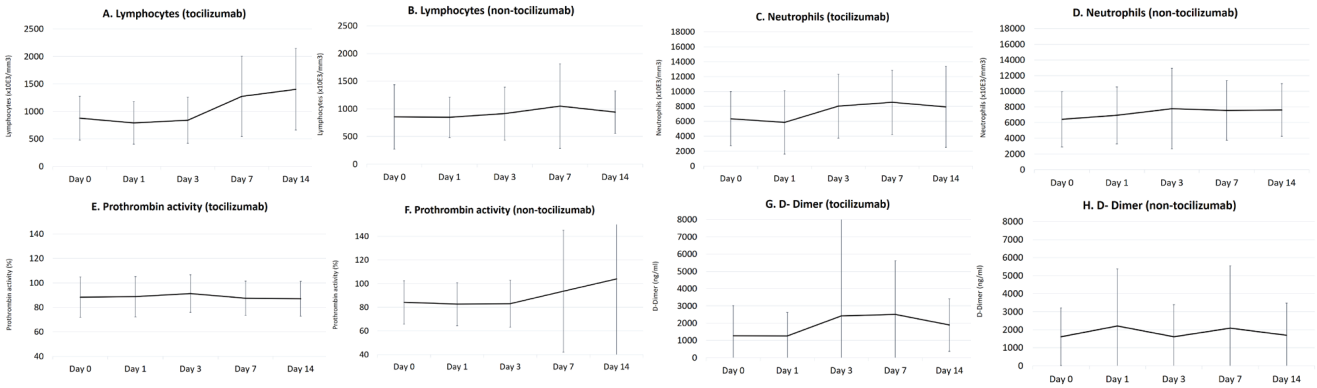

Figure S3 Blood and coagulation tests values according to tocilizumab treatment. Lymphocytes (A,B), Neutrophils (C,D), Prothombin activity (E,F) and D-Dimer (G,H) are expressed as mean and standard deviation (error bars).

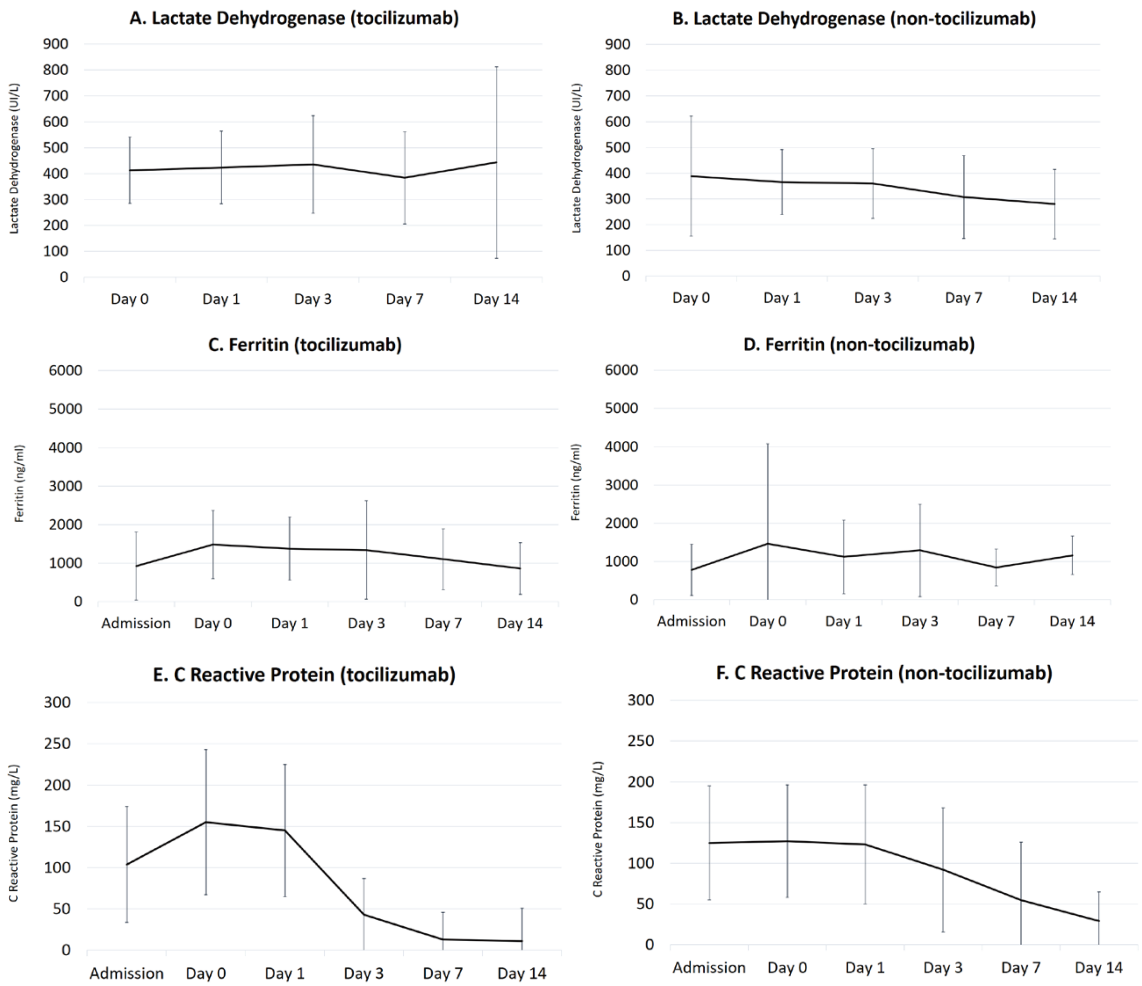

Figure S4 Inflammatory markers according to tocilizumab treatment. Lactate Dehydrogenase (A,B), Ferritin (C,D) and C reactive protein (E,F) are expressed as mean and standard deviation (error bars).

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Table S1 Correlations among the inflammatory parameters

Parameter	Correlation coefficient (r)	P value
Admission IL-6		
Day 0 CRP	0.332	0.007
Day 1 CRP	0.303	0.015
Day 0 IL-6		
Day 0 CRP	0.297	0.003
Day 0 ferritin	0.539	0.008
Day 1 CRP		
Admission RDW	0.186	0.026
Day 0 RDW	0.275	0.002
Day 1 RDW	0.255	0.04
Day 3 RDW	0.277	0.001
Day 3 CRP		
Day 0 RDW	0.188	0.03
Day 1 RDW	0.245	0.001
Day 3 RDW	0.266	0.001
Day 7 CRP		
Day 7 RDW	0.218	0.017

CRP, C reactive protein; RDW, red blood distribution width.

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