

## Red blood cell distribution width changes in septic patients

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### SUMMARY

*The role of leucocytes in inflammatory response is well known, nevertheless the rheologic changes of red blood cells (RBC) and their physiopathological role during inflammation are not completely understood. Previous studies have founded important alterations in RBC shape and functional disturbances during sepsis and inflammation. This has brought to discussion the hypothesis that RBC alterations during shock and sepsis may contribute to multiple organ dysfunction syndrome (MODS). The RBC distribution width (RBC-DW) is an indirect measurement of the variation of RBC size. Our objective was to study the variations of RBC distribution width in patients with sepsis admitted to a medical/surgical ICU (MS-ICU).*

**Material and methods:** Two groups of patients admitted to the MS-ICU were included. One of patients with sepsis, severe sepsis and septic shock, the second one patients admitted without sepsis. There was also a group of healthy blood donors as control group. The variables studied included: RBC-DW, APACHE II and SOFA scores. The RBC-DW was measured with a computerized system (Sysmex xt2000-i) in a sample of whole blood (7.5 mL) anticoagulated with acid ethylenediaminetetracetic (EDTA) during the first 24 hours of admission. The inclusion criteria were: Patients between 18 and 60 years old and patients with sepsis criteria.

**Results:** There were 184 patients included with the following distribution: Septic group 58, without sepsis group 63 and control group 64 patients. The mean age in the septic group was  $48 \pm 11$  years, without sepsis group  $46 \pm 8$  years and control group  $43 \pm 6$  years. The mean of RBC-DW in the sepsis group was  $18.23 \pm 2.01$  vs  $14.03 \pm 1.36$  ( $p > 0.05$ ;  $t$  1.47, IC 95%) in the without sepsis group and  $12 \pm 0.27$  ( $p < 0.05$ ;  $t$  3.580, IC 95%) in the control group. The mean severity scores in the sepsis group were APACHE II  $17.52 \pm 8.51$  and SOFA

### RESUMEN

El papel de los leucocitos en la respuesta inflamatoria está bien establecido, sin embargo no conocemos con precisión el papel de los eritrocitos durante el estado de inflamación.

Estudios previos han encontrado una importante alteración en la forma y función de las células rojas de la sangre durante la sepsis y la inflamación. Esto ha traído a discusión la hipótesis de que las alteraciones de los eritrocitos durante el choque y la sepsis pueden contribuir al síndrome de disfunción orgánica múltiple. El ancho de los eritrocitos es una medición indirecta a la variación en el tamaño de los eritrocitos. Nuestro objetivo era estudiar la variación del ancho de los eritrocitos en pacientes con sepsis ingresados a una unidad de cuidados intensivos.

**Material y métodos:** Dos grupos de pacientes ingresados en la UCI fueron incluidos. Uno de pacientes con sepsis, sepsis severa y choque séptico, el segundo conformado por pacientes sin sepsis, también se tomó en cuenta un grupo con donadores de sangre sanos y éste fue de control. Las variables estudiadas incluían, la distribución en el ancho de las células rojas de la sangre, APACHE II y SOFA. La distribución en el ancho de las células rojas de la sangre fue medida con un programa de computadora (Sysmex xt-2000) en una muestra de sangre (7.5 mL) anticoagulada con (EDTA) durante las primeras 24 horas de ingreso. El criterio para los pacientes era, estar entre los 18 y 60 años de edad y presentar sepsis.

**Resultados:** Se incluyeron 184 pacientes con la siguiente distribución: grupo séptico 58, grupo sin sepsis 63 y grupo de control 63 pacientes. La media de edad en el grupo séptico era de  $48 \pm 11$  años, en el grupo sin sepsis  $46 \pm 8$  años y en el grupo de control  $43 \pm 6$  años. La media de la distribución en el ancho de las células rojas de la sangre en el grupo séptico fue  $19.23 \pm 2.01$

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$9.47 \pm 5.43$ ; in the without sepsis group APACHE II  $6.10 \pm 7.01$  and SOFA  $2.44 \pm 3.76$ .

**Conclusions:** The RBC-DW is higher in septic patients compared with patients without sepsis and healthy subjects. A relation between RBC-DW and higher morphologic alterations in RBC in patients with sepsis is probable. This measure is also statistically higher in patients with the highest SOFA values, but not APACHE II. We need to study a bigger population to acutely describe an association between this variables and RBC-DW. The relationship between RBC-DW and severity assessments scores has to be defined with future studies.

**Key words:** Sepsis, septic shock, multiple organ dysfunction syndrome, red blood cell distribution width.

## INTRODUCTION

Severe sepsis and septic shock are the main causes of mortality in the intensive care units (UCI) worldwide. The role of leucocytes in the inflammatory response is well known, nevertheless the rheologic changes of red blood cells (RBC) and their physiopathological role during inflammation are not completely understood. Anemia is a common finding in septic patients; the contributors include: alterations in erythropoiesis, iron metabolism and bone marrow suppression.<sup>1</sup> The nutritional status and feeding during sepsis are of concern as causes of anemia. The studies of Von Ahsen<sup>1</sup> founded normal values of vitamin B12 among patients in the ICU, but low levels of iron and folic acid; none of these vitamins were correlated with alterations in the RBC shape. In other studies by Rodriguez and col.<sup>2</sup> Iron deficiency was observed in 9%, vitamin B12 2% and folic acid only 2% suggesting that these disturbances may play a minor role in the development of anemia seen in the ICU patients.

Studies by electronic microscopy have founded important alterations in RBC shape during the refractory phase of shock.<sup>3,4</sup> They also showed morphologic and functional changes during sepsis regarding RBC population. This has brought to discussion the hypothesis that RBC alterations during shock and sepsis may contribute to multiple organ dysfunction syndrome (MODS). It has been reported previously that the flexibility of RBC may be dysfunctional due

vs  $14.03 \pm 1.36$  ( $p > 0.05$ ;  $t$  1.47, IC 95%) en el grupo sin sepsis. Y  $12.72 \pm 0.27$  ( $p < 0.05$ ;  $t$  3.580, IC 95%) en el grupo de control. Las medias de los puntajes en el grupo de sepsis fueron APACHE II  $17.52 \pm 8.51$  y SOFA  $9.47 \pm 5.43$ ; en el grupo sin sepsis APACHE II  $6.10 \pm 7.01$  y SOFA  $2.44 \pm 3.76$ .

**Conclusión:** La distribución del ancho en los eritrocitos es más alta en pacientes con sepsis comparada con pacientes sin sepsis y sujetos sanos. Una relación entre la distribución del ancho en las células rojas de la sangre y mayores alteraciones en las células rojas de la sangre en pacientes con sepsis es probable. Esta medida es también estadísticamente más alta en pacientes con valores de SOFA más elevados pero no APACHE II. Necesitamos estudiar una mayor población para describir con precisión la asociación entre estas variables y la distribución del ancho en las células rojas de la sangre. La relación entre la distribución del ancho en las células rojas de la sangre y los puntajes tienen que ser definidas en futuros estudios.

**Palabras clave:** Sepsis, choque séptico, síndrome de disfunción orgánica múltiple, la distribución del ancho en los eritrocitos.

to the endotoxins of bacteria in septic shock. The RBC exposed to endotoxin decreased their deformability and showed increased hydroministic acid content, which is a component of bacterial endotoxin, suggesting a relationship.<sup>5-7</sup>

The RBC distribution width (RBC-DW) is an indirect measurement of the RBC size with a normal value of 11.5 to 14.5; higher values suggest variation in the RBC size or a heterogeneous population of RBC.<sup>8-10</sup> Our objective was to study the variation of the RBC-DW in patients with sepsis admitted to a medical/surgical ICU (MS-ICU). This measurement was also correlated with severity scores of SOFA (Sepsis-related Organ Failure Assessment) and APACHE II (Acute Physiologic And Chronic Health Evaluation) to determine if a correlation between severity scores exist. We hypothesized that RBC-DW is higher in patient with sepsis compared with patients without sepsis and healthy subjects.

## MATERIALS AND METHODS

Two groups of patients admitted to the MS-ICU were included. One of patients with sepsis, severe sepsis and septic shock, the other patients admitted without sepsis. There was also a group of healthy blood donors as a control group. This study was performed from June 2005 to July 2006 in a MS-ICU or a referral Medical Center in Mexico City. The variables studied included: RBC-DW, APACHE II and SOFA scores.

The RBC-DW was measured with a computerized system (Sysmex xt-2000i) in a sample of whole blood (7.5 mL) anticoagulated with acid etylenediaminetetra-cetic (EDTA) during the first 25 hours of admission the inclusion criteria were: patients between 18 and 60 years old and patients with sepsis defined as:

- Clinical of a bacteriological evidence of infection with two or more signs of systemic inflammatory systemic response syndrome:
  - a. Temperature  $> 38^{\circ}\text{C}$  or  $< 36^{\circ}\text{C}$
  - b. Heart rate  $> 90$  bits per minute.
  - c.  $> 20$  breads per minute or  $\text{PaCO}_2 < 32$  mmHg or need of mechanical ventilation.
  - d. White blood cells count  $> 12,000 \text{ mm}^3$  or  $< 4,000 \text{ mm}^3$
  - e. Platelets  $< 100,000 \text{ mm}^3$  or sepsis-induced cuagulopatya
  - f. Signs of hipoperfusion: oliguria ( $< 0.5 \text{ mL/kg/hr}$ ), altered mental status or serum lactate level  $> 2.2 \text{ mmol/Lt}$
- Patients admitted to the MS-ICU with different diagnosis from sepsis, severe sepsis and septic shock. Without exclusion criteria.
- Healthy blood donors registered in the bank of blood at the hospital Central Sur of PEMEX

The exclusion criteria included:

- Fasting  $> 15$  days.
- Blood products transfusion in the previous week os admission to the MS-ICU.
- Bleeding  $> 10\%$ .
- Medical History of hematological disorders.
- Resent chemotherapy.
- Cardiogenic shock.
- Hepatic cirrhosis.
- Pregnancy.
- Use of drugs known to induce changes in the morphology and rheology of RBC (pentoxiphylline, erythropoietin, aspirin, cyclosporine, nitrovasodilatory drugs).

The statically analysis of the differences in the RBC-DW between two groups was performed by t-student test for quantitative continuous data using Social Package for Social Science software (SPSS) version 12.0, variations and tendencies were evaluated in each group and correlated with APACHE II and SOFA scores, this study was reviewed and accepted from the Research Committee with approval of the Ethics Committee.

## RESULTS

There were 184 patients included with the following distribution: septic group 58, without sepsis group 63 and control group 63 patients. The mean age in the septic group was  $48 \pm 11$  years, without sepsis group  $46 \pm 8$  years and control group  $43 \pm 6$  years.

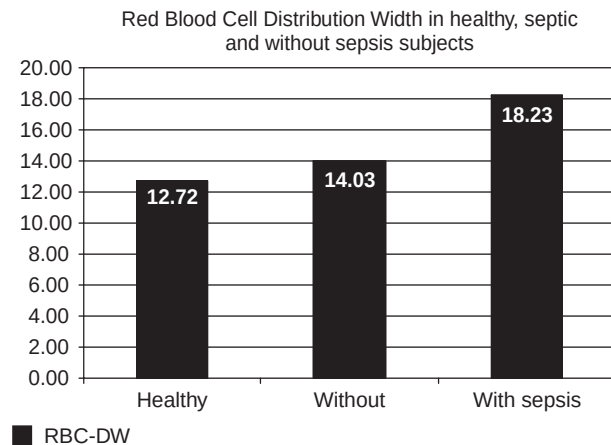
Los diagnósticos de admisión en el grupo séptico fueron: Neumonía 33 pacientes (56.89%), sepsis originada en la sección abdominal, pirocolesisto 3 pacientes (5.17%), infección ósea 2 pacientes (3.45%), colangitis 2 pacientes (3.45%) infección de tejido suave 2 pacientes (3.45%), absceso rectal 1 paciente (1.72%), absceso pulmonar 1 paciente (1.72%), abseso de riñón 1 paciente (1.72%), enfermedad diverticular 1 paciente (1.72%) y pancreatitis infecciosa 1 paciente (1.72%).

El diagnóstico en el grupo sin sepsis fue: embolia cerebrovascular 16 pacientes (25.39%), choque 12 pacientes (19.04%), quemaduras 5 pacientes (7.93%), biopsia cerebral 5 pacientes (7.93%), descompresión medular 4 pacientes (6.34%), biopsia pulmonar con toracotomía 3 pacientes (4.76%), extirpación de adenoma hipoficial 3 pacientes (4.76%), pancreatitis 2 pacientes (3.17%), edema pulmonar 2 pacientes (3.17%), sección medular 1 paciente (1.59%), esofagogastrectomía 1 paciente (1.59%).

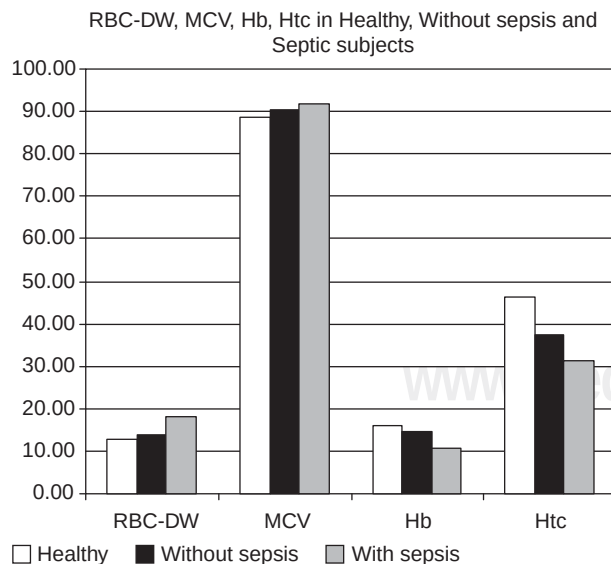
Inestabilidad de la columna 1 paciente (1.59%), artroplastia de la cadera 1 paciente (1.59%), intoxicación con agentes de bloqueo muscular 1 paciente (1.59%), hemicolectomía 1 paciente (1.59%), fractura de fémur 1 paciente (1.59%), disección aórtica 1 paciente (1.59%) COPD 1 paciente (1.59%), ce-toacidosis diabética 1 paciente (1.59%), y aneurisma abdominal de la aorta 1 paciente (1.59%).

The mean of RBC-DW in the sepsis group was  $18.23 \pm 2.01$  versus  $14.03 \pm 1.36$  ( $p > 0.05$ ,  $t$  1.47; IC 95%) in the group without sepsis and  $12.72 \pm 0.27$  ( $p < 0.05$ ,  $t$  3.580, IC 95%) in the control group (figure 1). The mean corpuscular volume (MCV), hemoglobin (Hb) and hematocrit (Htc) in the septic group were  $91.79 \pm 5.35 \text{ fL}$ ,  $10.69 \pm 2.24 \text{ gr/dL}$  and  $31.31 \pm 6.93\%$  respectively; in the group without sepsis  $90.32 \pm 4.58 \text{ fL}$ ,  $14.71 \pm 2.36 \text{ gr/dL}$  and  $37.29 \pm 6.61\%$ ; in the control group  $88.73 \pm 3.27 \text{ fL}$ ,  $15.97 \pm 1.02 \text{ gr/dL}$ ,  $46.09 \pm 2.99\%$  respectively (figure 2). The means severity scores in the septic group were APACHE II  $17.52 \pm 8.51$  and SOFA  $9.47 \pm 5.43$ ; in the group without sepsis APACHE II  $6.10 \pm 7.01$  and SOFA  $2.44 \pm 3.76$  (figure 3).

In the septic group there were 40 patients (68.97%) discharged from the MS-ICU, 18 patients (31.03%) died. In the subgroup of survivors the mean RBC-DW was  $15.90 \pm 1.79$  versus  $16.82 \pm 2.33$  ( $p < 0.05$ ,  $t$  2.219; IC 95%) in the sub-group that died; APACHE II  $16.23 \pm 8.26$  versus  $20.39 \pm 8.58$  ( $p > 0.05$ ,  $t$  1.150; IC 95%) and SOFA  $8.75 \pm 9.34$  versus  $11.06 \pm 5.43$  ( $p > 0.05$ ,  $t$  1.191; IC 95%) (figure 4).



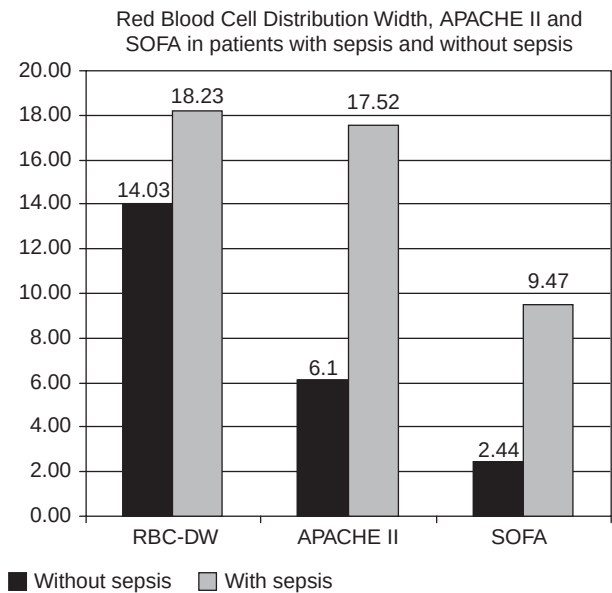
**Figure 1.** Red blood cell distribution width (RBC-DW) in healthy subjects, septic and not septic patients.



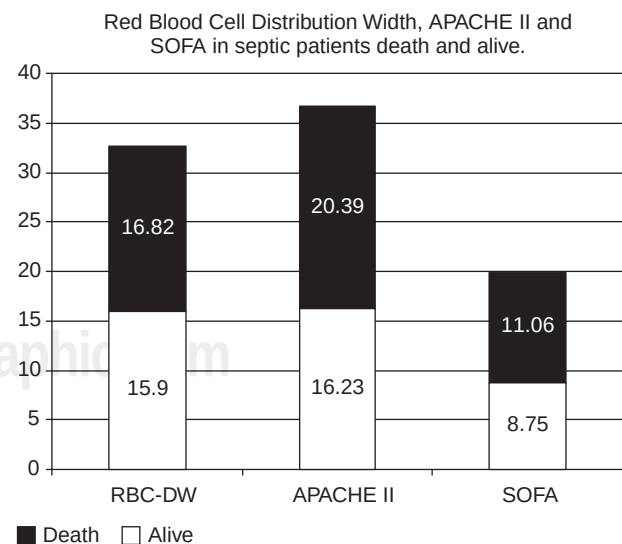
**Figure 2.** Red blood cell distribution width (RBC-DW), mean corpuscular volume (MCV), hemoglobin (Hb) and hematocrit (Htc) in healthy, without sepsis and sepsis subjects.

## DISCUSSION

The RBC-DW expresses the size variation among a red blood cells population when measured by a computerized system, as an indirect value of mean



**Figure 3.** Red blood cell distribution width (RBC-DW) and severity scores APACHE II and SOFA in septic and not septic patients.



**Figure 4.** Red blood cell distribution width (RBC-DW) and severity scores APACHE II and SOFA in septic and not septic patients according to outcome in the Intensive Care Unit.



corpuscular volume (MCV). During sepsis it has been previously established that red blood cells have morphologic and physiologic alterations that may contribute to tissue damage and multiple organ dysfunction syndrome development. These results showed a high RBC-DW in septic patients compared with healthy subjects when admitted to de MS-ICU. When RBC-DW in septic patients was compared with patients admitted to the ICU with different diagnosis to sepsis there was no statistical difference between the two groups; this observation may be due

When data are analyzed as a tendency it is clear that RBC-DW, MCV and the highest APACHE II and SOFA scores are seen in the septic patients group, which may correlate with severity of the disease. In the septic group no one presented normal RBC-DW and the higher tendency correlated with SOFA score, but not APACHE II score. This result could be explained because SOFA score is better for evaluating organ dysfunctions than APACHE II,<sup>11,12</sup> which only considers physiological variables and does not consider the effects of therapeutic interventions such as use of vasopressors or inotropic drugs.

Regarding to mortality, the red blood cell distribution width was statistically higher in those patients that dies in the ICU compared with those discharged alive. No significance was observed when applying the severity score to this sub-group of patients, nevertheless we consider that this may be a consequence of the sample size. It is priority to evaluate a bigger sample to stablish any difference between groups and know if the morphologic and physiological alterations observed in red blood cells correlate with the higher risk of death when it is evaluated by means of APACHE II and SOFA. This observation was not an objective in this study; it is clear that there is not a correlation and we only saw a tendency.

The functional and morphologic alterations in red blood cells during sepsis are consequence of inflammatory response. The RBC-DW is helpful in the determination of morphologic alterations of red blood cells and it can not be used for the evaluation of functional disturbances of these cells. Wheter this disturbances are related to the development of multiple organ dysfunction syndrome is under research now and remains to be determined.

The values of hemoglobin and hematocrit are shown in the results; since there is no correlation of these measurements with the red blood cells size,

no correlation was done in this study. The RBC-DW must be interpreted in this setting as variations in the cellular size in relation to mean corpuscular volume which denotes the mean cell size; this explains the finding of abnormal RBC-DW with normal mean corpuscular volume. In consequence a higher RBC-DW suggests the presence of an heterogeneous populations of red blood cells. Our results also correlates with those previously described with a high incidence of anemia in the patients admitted to the ICU.

## CONCLUSION

The RBC-DW is higher in patients with sepsis compared with patients without sepsis and healthy subjects. A relation between RBC-DW and higher morphologic alterations in red blood cells in septic patients is probable. This measure is also higher in patients with the highest SOFA scores, but not APACHE II. We need to study a bigger population to acutely describe an association between these variables and RBC-DW. The relationship between RBC-DW and severity assessments scores has to be defined in future studies.

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