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Evaluation of Oxidative Stress in Serum of Critically Ill Patients by a Commercial Assay and Gas Chromatography–Mass Spectrometry, *Giuliana Cighetti,[1*] Rita Paroni,[2] Silvia Marzorati,[3] Erica Borotto,[3] Riccardo Giudici,[3] Gabriele Magnanini,[2] and Gaetano Iapichino*[3] ([1] Department of Medical Chemistry, Biochemistry and Biotechnology; [2] Department of Medicine, Surgery and Dental Science; and [3] Institute of Anaesthesia and Intensive Care Medicine, University of Milan, Milan, Italy; * address correspondence to this author at: Department of Medical Chemistry, Biochemistry and Biotechnology, University of Milan, Via Saldini 50, 20133 Milan, Italy; fax 39-250316040, e-mail giuliana.cighetti@unimi.it)

The formation of reactive oxygen species (ROS), as a result of an imbalance of the oxidant/antioxidant system, and their reactivity toward various molecular targets lead to oxidative damage contributing to different human pathologies *(1, 2)*. Nonneutralized ROS trigger the lipid peroxidation process of cell membranes, thus generating hydroperoxides (intermediate compounds) and malondialdehyde (MDA), the most abundant carbonyl-terminal molecule in the circulation. The direct in vivo detection of ROS is difficult because of their very short lifetimes; therefore, changes in hydroperoxide and/or MDA concentrations are often used as an indicator of oxidative stress in clinical laboratory settings *(3)*. MDA exists in 2 forms in tissues and blood: free and bound to −SH and/or $-NH_2$ groups of proteins, nucleic acids, and lipoproteins *(4)*. Free MDA (F-MDA), the chemically active form, serves as an indicator of recent damage *(4, 5)*, and the bound fraction excreted by urine is indicative of an older injury *(6)*.

The analytical assay generally used to quantify MDA, based on detection of the product from the reaction between MDA and thiobarbituric acid (TBARS), measures only total MDA (T-MDA; free + bound) *(4, 7)*. This method, although criticized for its low specificity, is often used in clinical laboratories despite its complexity. Thus, the commercial availability of a specific, simple, rapid, and low-cost assay to measure oxidative stress by assay-

ing hydroperoxide derivatives would be useful in hospital laboratories.

The aim of the present study was to compare a commercial assay (D-ROMs Test™; Diacron) for assessing oxidative stress in sera of healthy persons and critically ill patients admitted to an intensive care unit (ICU) with an isotope-dilution gas chromatography–mass spectrometry (ID-GC-MS) method *(8)*, the most specific and sensitive method in this field for measuring F- and T-MDA concentrations *(9, 10)*.

The assay, which detects serum hydroperoxides as their derivatives (D-ROMs), is based on the reaction of serum with transition metal ions (ferrous sulfate) to form alkoxy and peroxy radicals. The in vitro–formed radicals react with a chromophore compound, generating a radical cation detectable spectrophotometrically at 505 nm; the resulting D-ROMs concentrations are reported in Carratelli units (U.CARR) *(11, 12)*.

To study the overt oxidative status, we selected the most critically ill patients among those consecutively admitted to a 6-bed adult general ICU from September 2002 to June 2003. Patients over 18 years of age judged by attending physicians to need acute artificial ventilation for more than 3 days (to exclude patients not in a sufficiently critical state and to select those with a consistent ROS generation process) were eligible. Of those, we consecutively enrolled patients admitted for acute respiratory failure (pneumonia, n = 7; pulmonary edema, n = 9) or cardiorespiratory failure attributable to cardiogenic shock (n = 4), cardiac arrest (n = 9), septic shock (n = 15), or of hypovolemic origin (diabetes; n = 1). The San Paolo Hospital (Milan, Italy) ethics committee approved the protocol, and patients or their next of kin gave informed consent. Exclusion criteria were referrals from other ICUs, severe liver failure, oligo- or anuria, and an underestimated ventilation period. Patient characteristics, diagnoses, Simplified Acute Physiology Score II (SAPS II) *(13)*, and Sequential Organ Failure Assessment (SOFA) score *(14)* were recorded at ICU admission. Length of stay (LOS) and vital statistics determined in the ICU/hospital were also recorded. Septic shock was defined according to the American College of Chest Physicians and Society of Critical Care Medicine criteria *(15)* and pneumonia according to CDC criteria *(16)*. Central venous blood samples were collected at ICU admission, and serum was stored at −80 °C until biochemical measurements. Healthy volunteers were enrolled as controls [n = 25 (12 males and 13 females); mean (SD) age, 62.1 (5.6) years].

Results were analyzed by a 2-tailed unpaired Student *t*-test. Multiple regression analysis was done to relate oxidative stress markers with patient characteristics, SAPS II, and SOFA score at ICU admission and ICU LOS and mortality, and the relationships between D-ROMs and F- or T-MDA and between F- and T-MDA were analyzed by Spearman rank-order correlation. Significance was set at $P < 0.05$. Statistical analyses were done with the Stata 7.0 statistical package (Stata). Values are reported as the mean (SD) or median (25th–75th percentile), depending on whether the distribution was gaussian or skewed, respectively, or as the number (proportion).

The selected critically ill patients [n = 45 (31 males and 14 females); mean (SD) age, 62.0 (16.7) years; median SAPS II, 38 (31.5–55.0); median SOFA score, 6 (4–9)] were admitted from the emergency ward (n = 29; 64.4%), operating theater (n = 8; 17.8%), or hospital wards (n = 8; 17.8%). Admission to the ICU was medical for 36 (80.0%) and surgical for 9 (20.0%) patients; their ICU LOS was 10 (4–19) days, and the ICU and hospital mortality rates were 37.8% and 46.7%, respectively. The severity and organ failure scores, the ICU LOS, and the high mortality of the case mix fit with our selection aim.

In control individuals, serum F- and T-MDA values (Table 1) were within the previously reported reference intervals *(5, 8, 10)*, and the D-ROMs indicated a lack of any significant oxidative condition, as suggested by the manufacturer's cutoff (≤300 U.CARR). In critically ill patients, the significant increases in all tested indices with respect to controls ($P = 0.0001$) confirmed the expected oxidative stress, in agreement with other reports *(17–21)*. However, the increase in serum D-ROMs (1.4-fold vs controls) was consistently less than the increases in F-and T-MDA (4- and 10-fold increases, respectively, vs controls). Moreover, F- and T-MDA concentrations, higher than those we had observed in patients affected by acute coronary artery disease *(5)*, confirmed the presence of severe oxidative status in our patients. We found no correlation between oxidative stress marker values on admission and severity or organ failure scores, in contrast to results reported previously *(18, 19, 21)*. This discrepancy might be explained by our patient selection criteria.

When we pooled the controls and patients, the D-ROMs concentrations were significantly correlated only to F-MDA values ($r = 0.472$; $P = 0.0003$; Fig. 1). The dashed lines in Fig. 1, which show the control cutoff values for D-ROMs and F-MDA, identify 4 areas of distribution indicating concordance or discordance between the 2 tests. For both tests, no oxidative stress was observed in 28 individuals (24 controls and 4 ill patients), and oxidative stress was confirmed in 23 patients; thus, both tests exhibited concordance in 73.9% of the total samples. The group for whom there was appreciable discordance (26.1%) between the markers of oxidative stress included

Table 1. Serum concentrations of F-MDA, T-MDA, and D-ROMs in controls and patients.[a]

	F-MDA, μmol/L	T-MDA, μmol/L	D-ROMs, U.CARR
Controls (n = 25)	0.40 (0.36–0.45)	1.67 (1.41–1.83)	245 (215–279)
Patients (n = 45)	1.24 (0.66–2.15)	17.97 (12.80–23.22)	332 (263–436)

[a] Results are reported as median (25th–75th percentile). Controls vs patients: $P = 0.0001$ for all indices.

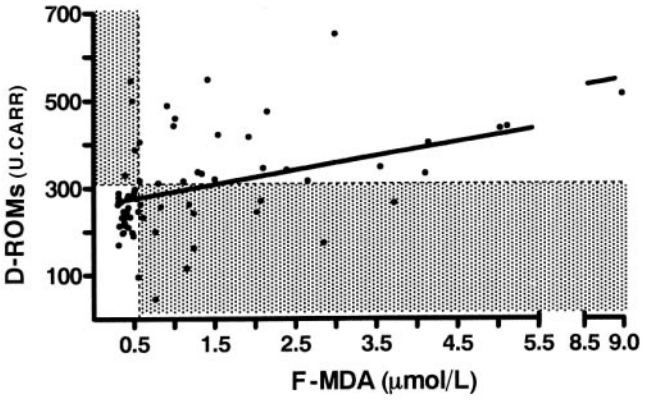

Fig. 1. Linear correlation of serum concentrations of F-MDA and D-ROMs in controls and critically ill patients at ICU admission.

Total of 70 patients and controls. Equation for the line: $y = 32.1x + 261.0$ ($r = 0.429$; $S_{y|x} = 102.5$). The *dashed lines* limiting the areas of point distribution are defined by the cutoff values (mean ± 2 SD) calculated on the control population (F-MDA, 0.56 μmol/L; D-ROMs, 313.7 U.CARR).

4 patients in whom D-ROMs, but not MDA, indicated oxidative stress; 13 patients in whom F-MDA (concentrations 4- to 5-fold higher than the F-MDA cutoff), but not D-ROMs, indicated oxidative stress; and 1 borderline control. This discrepancy, mainly attributable to an underestimation of D-ROMs, might be explained by the fact that F-MDA formed during in vivo oxidative stress is evaluated directly by ID-GC-MS. In contrast, the hydroperoxides formed in vivo are indirectly evaluated after their in vitro transformation in alkoxy and peroxyl radicals by the D-ROMs test, and this last reaction might be affected by endogenous antioxidants or drugs.

D-ROMs were not related to T-MDA values, which were consistently high in every patient. The high T-MDA value might suggest active ROS production some time before the onset of acute organ insufficiency because the patients had pathologic processes eliciting oxidative stress before the organ failure that caused the ICU admission. Differences in the duration of the pathophysiologic process and severity of condition at the time of blood collection might also explain the loss of relationship between F- and T-MDA.

In conclusion, the expected presence of oxidative stress in the selected ICU patients was confirmed by each of the tested indices, although the information on the strength of oxidative status provided by each test was not of the same extent. Our results showed that the D-ROMs test is poorly correlated to F-MDA measurements. Although the commercial D-ROMs assay has been useful in some studies *(11, 12)*, in critically ill patients, compared with F-MDA measured by ID-GC-MS, its performance was not satisfactory.

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