# AUTOIMMUNE AND INFLAMMATORY DISEASES

Jonathan A. Leff
Assistant Professor of Medicine
Webb-Waring Institute for Biomedical Reearch
University of Colorado School of Medicine
4200 East Ninth Avenue - Box C322
Denver, Colorado 80262

## INTRODUCTION

Toxic oxygen radicals (collectively called reactive oxygen species, ROS) are intricately related to a vast number of disease processes including a variety of autoimmune and inflammatory disorders[1]. It is difficult to directly measure ROS because of their extremely short half-life. However, the reactions in which they participate often leave measurable markers ("footprints") that reflect oxidant activity. Primarily four strategies have evolved to detect oxidant activity for diagnostic purposes: 1) measuring products of oxidation, for example lipid peroxides or oxidized glutathione (GSSG); 2) measuring the release of products from damaged cells, for example the release of hepatic enzymes during acute hepatitis; 3) measuring antioxidant enzyme activities which may reflect the systemic compensation to oxidant stress; 4) ROS can be directly measured although the technology is complex and imperfect, the methodology somewhat complicated, and the equipment cost often prohibitive. In this review, I will discuss sources of selected oxidant markers, methodologies involved in their measurement, and their relevance to the diagnosis and management of several autoimmune and inflammatory diseases.

## SELECTED MARKERS OF OXIDANT INDUCED DISEASE

### Extracellular Catalase

**Methodology of Measurement.** Catalase is an enzyme which catalyzes the destruction of hydrogen peroxide ($H_2O_2$) by one of two reactions. In the first, $H_2O_2$ is converted to oxygen and $H_2O$ (catalatic reaction). In the second $H_2O_2$ oxidizes a variety of substrates including methanol, ethanol, formic acid, thiols, nitrites, quinones and phenols (peroxidatic reaction). Which of these two pathways predominates is currently unclear. There are many assays for the measurement of catalase activity, usually based on the rate of liberation of oxygen, the disappearance rate of $H_2O_2$, or the heat liberated by the reaction[2]. The rate of liberation of $O_2$ can be measured polarographically with an oxygen electrode, manometrically, volumetrically, or with an

*Free Radicals in Diagnostic Medicine*, Edited by
D. Armstrong, Plenum Press, New York, 1994

approximate paper disk method. Alternatively, the disappearance rate of $H_2O_2$ can be determined spectrophotometrically, polarographically, electrochemically, or by titration. Since $H_2O_2$ absorbs at 240 nm, its destruction can be followed spectrophotometrically[2]. The disadvantage of this approach is its theoretical non-specificity since any consumption of $H_2O_2$ by other peroxidases, or by non-specific means will appear as catalase activity. In addition, this assay is not particularly sensitive. The polarographic assay offers the advantage of being specific for catalase (no other peroxidase liberates oxygen) and being more sensitive than the spectrophotometric assay[3].

**Source of extracellular catalase.** The presence of catalase activity in serum or plasma may be easily overlooked due to the massive quantities of catalase present in erythrocytes and the frequent contamination of serum and plasma with lysed erythrocytes during sample preparation. However, elevated serum and plasma catalase activity has been associated with several disease processes (Table 1). The source of extracellular catalase is frequently unclear. In hemolytic diseases and acute pancreatitis the source appears to be lysed erythrocytes[4,5]. However, in other inflammatory diseases the source remains unknown[6]. In one case, a lymphocyte T-cell line has been shown to release catalase which protects the cell from programmed cell death[7].

## Extracellular Manganese Superoxide Dismutase (MnSOD)

**Methodology of measurement.** Mammalian systems possess three forms of superoxide dismutases. All catalyze the same reaction; the dismutation of superoxide anion to hydrogen peroxide. The first, described in 1969 by McCord and Fridovich, is a copper and zinc containing enzyme present in the cytosol (Cu,Zn-SOD)[8]. The second is also a copper containing enzyme present in extracellular fluid, called extracellular SOD (ECSOD)[9]. Lastly, a manganese containing SOD (MnSOD) located mainly in the matrix of the mitochondria is present[10]. While all catalyze the same reaction they are products of separate genes and have differing sensitivities to inhibitors which can help distinguish their activity *in vitro*. For example, Cu,Zn-SOD is sensitive to hydrogen peroxide and cyanide while MnSOD is not. While assays based on enzymatic activities are widespread, they suffer from several disadvantages. First, all three enzymes have similar activity making it difficult to distinguish their separate activities. Second, many tissues contain compounds with SOD-like activity[11]. Finally, assays based on activity require careful tissue preparation and storage as enzyme activity declines with time and improper storage. Assays based on immunologic recognition with antibodies have the advantage of demonstrating no cross-reactivity since the SOD enzymes are structurally and antigenically unique. These assays are reproducible, quick and convenient for clinical and laboratory work. Since these assays are specific and detect immunogenicity and not activity, for the particular enzyme, they are unaffected by tissue inhibitors or activators. Several types of immunochemical assays are used including immunodiffusion, radioimmunoassay or a sandwich type enzyme-linked immunosorbent assay (ELISA)[11]. The accuracy of these assays is, of course, dependent on the selectivity of the antibodies. Both polyclonal and monoclonal antibodies may be used but given the cross species similarities these antibodies are difficult to raise. These antibodies have indeed been produced and hopefully will be commercially available. One ELISA utilizing a monoclonal antibody has a sensitivity of 2 ng/ml with a working range of 2-200 ng/ml[12]. The coefficient of variation is 3% and within run reproducibility 5%.

**Source of extracellular MnSOD.** MnSOD is a component of the mitochondrial matrix. Since erythrocytes contain no MnSOD serum measurements are unaffected by hemolysis. The source of serum MnSOD is presumed to be mitochondrial in all cases (Table 2). In acute myocardial infarction the specific source has been shown to be

release from cardiac tissue[11]. In other disease states, presumably affected organs are responsible for the increase in serum values. The mechanism of release of MnSOD from mitochondria remains unknown. Serum MnSOD has a half-life of 6 hours[11].

### Hydrogen Peroxide ($H_2O_2$)

**Methodology of measurement.** Although $H_2O_2$ is not a free radical, it is a potent oxidant and plays a central role in the metabolism of oxygen free radicals generating several toxic free radicals in the process. It is metabolized by glutathione peroxidase, catalase and participates in other reactions as well. It is the most stable of the primary reactive oxygen species and, as such, can sometimes be detected in human blood, urine, and since it is relatively volatile, even expired breath[13-20]. The detection of $H_2O_2$ in blood, however, is the subject of some controversy[15]. Many techniques have been adapted to measure small quantities of $H_2O_2$, often in the picomole range. These include high-performance liquid chromatography, isoluminol chemiluminescence, fluorescence spectroscopy among others[21].

**Source of $H_2O_2$.** Hydrogen peroxide can be formed by the dismutation of superoxide anion. Neutrophils and other inflammatory cells as well as xanthine oxidase are significant sources. The precise source of $H_2O_2$ in specific disease processes has not been elucidated.

## SELECTED DISEASES

### Sepsis and the adult respiratory distress syndrome (ARDS)

**Serum catalase activity in sepsis and ARDS.** Both sepsis and ARDS represent syndromes with an altered oxidant-antioxidant balance. Many investigators have revealed evidence of an increased oxidant burden in ARDS[22]. Less attention has been paid to the status of the enzymatic antioxidant defense systems. We studied septic patients with and without established ARDS[6]. We found that serum catalase activity was elevated in those septic patients with ARDS compared to those without (Figure 1). Serum catalase activity was also elevated in both septic groups compared to healthy control subjects (normal value 7.3 U/ml, s.d. 3.5). In contrast, serum activity of another antioxidant enzyme, glutathione peroxidase, was similar in septic patients with or without ARDS and control subjects.

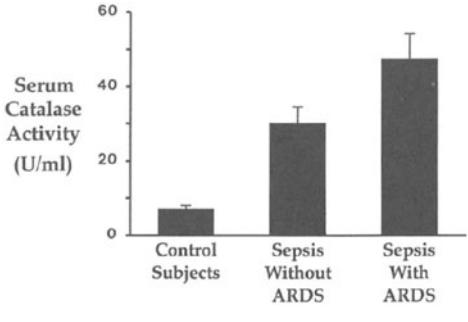


Figure 1. Serum catalase activity was increased ($p \leq 0.05$) in septic patients with ARDS ($n=9$) compared with septic patients without ARDS ($n=20$). Serum from healthy control subjects had less ($p \leq 0.05$) serum catalase activity compared to both septic groups. Values are mean ± SEM. Modified from reference 6.

We also studied several less acute pulmonary diseases and found normal values for serum catalase in pulmonary fibrosis, alpha-1-antitrypsin deficiency, sarcoidosis, and cystic fibrosis. To investigate erythrocyte (RBC) hemolysis as a potential source of serum catalase in these septic patients we evaluated serum haptoglobin levels and a RBC fragility index. Serum haptoglobin levels were similar in septic patients with or without ARDS and healthy control subjects. The RBC fragility index (the tonicity of saline required to lyse 50% of RBC) was also similar in all three groups. While not ruling out subtle hemolysis as a source of the elevated serum catalase activity, we could find no evidence to support this premise. Several alternative explanations are possible including tissue injury with release of catalase, increased cellular excretion of catalase, or upregulation of catalase activity in the face of an oxidant stress.

Since septic patients with ARDS have elevated serum catalase activity, we explored the possibility that this elevation might occur before the clinical diagnosis of ARDS, and thus provide a blood test that might predict the development of ARDS in septic patients. We studied patients admitted to an intensive care unit with the diagnosis of sepsis[23]. Roughly twenty percent of these patients subsequently developed ARDS. Serum was withdrawn at study entry and collected every six to twenty four hours thereafter. Since some of the septic patients at study entry were destined to develop ARDS but had not yet done so, we were able to ask the question whether serum antioxidant enzyme levels could predict who was destined to develop ARDS before they had done so clinically. We found that serum catalase activity measured with a sensitive and specific polarographic assay was elevated in septic patients compared to serum catalase activity in healthy control subjects. We also found that serum catalase activity was higher in septic patients who subsequently developed ARDS compared to septic patients who did not (Figure 2). Elevated serum catalase activity (>30 units/ml) in septic patients had a 83% sensitivity and 65% specificity for the subsequent development of ARDS. The positive predictive value was 42% while the negative predictive value was 93%. Despite significant overlap in the groups, blood markers predicting various processes will be an important area of future research. In this way a highly selected group of patients more likely to develop ARDS or other syndromes can be identified and subjected to experimental therapies. Treating all at-risk patients for ARDS would be too impractical since the vast majority (<10%) do

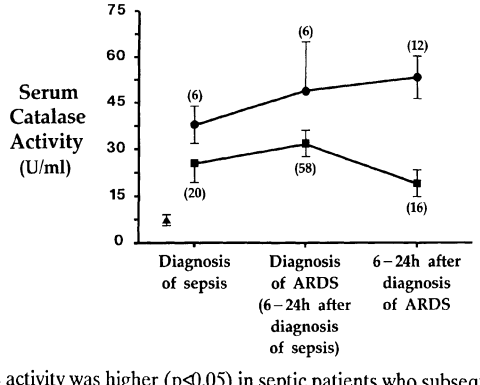


Figure 2. Serum catalase activity was higher ($p \leq 0.05$) in septic patients who subsequently developed ARDS (closed circles) compared to septic patients who did not develop ARDS (closed squares). Both septic groups had higher ($p \leq 0.05$) serum catalase activity compared to healthy control subjects (closed triangles). Serum catalase activity continued to rise in septic patients who developed ARDS and decreased over time in septic patients without ARDS. Values are mean ± SEM with numbers of patients in parentheses. Modified from reference 23.

not proceed to ARDS. The serum catalase activity in septic patients with ARDS continued to rise during the course of their illness while declining in the septic patients who did not develop ARDS (Figure 2). Serum catalase activity did not predict survival in this series of patients. Serum catalase activity also appears to be elevated in patients with ARDS associated with other risk factors (unpublished observations).

**Serum manganese superoxide dismutase (MnSOD) in sepsis and ARDS.** Serum levels of MnSOD measured by ELISA have recently emerged as another marker of sepsis and ARDS. In the study mentioned above MnSOD levels were also measured in the same septic patients some of whom subsequently developed ARDS[23]. Similar to serum catalase activity, we found that the serum MnSOD level measured by ELISA was elevated in septic patients compared to the serum MnSOD level in healthy control subjects. We also found that the serum MnSOD level was higher in septic patients who subsequently developed ARDS compared to septic patients who did not (Figure 3). If a cutoff of 450 ng/ml was used, an elevated serum MnSOD level in a septic patient without ARDS had a 67% sensitivity, 88% specificity, 67% positive predictive value and a 88% negative predictive value for the subsequent development of ARDS. In septic patients who did not develop ARDS, the serum MnSOD level rose over time and then decreased into the normal range. In septic patients after the development of ARDS, the serum MnSOD level remained elevated during the course of the study. Serum MnSOD levels also did not predict patient survival in this study.

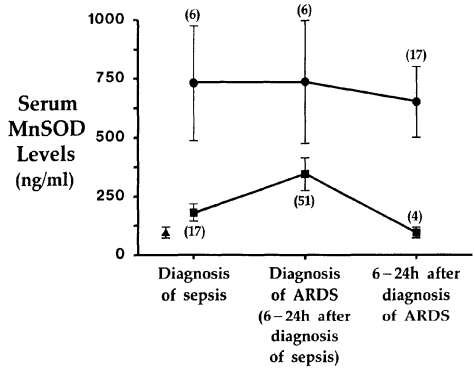


Figure 3. The serum manganese superoxide dismutase (MnSOD) level measured by ELISA was higher ($p \leq 0.05$) in septic patients who subsequently developed ARDS (closed circles) compared to septic patients who did not develop ARDS (closed squares). Both septic groups had higher ($p \leq 0.05$) serum MnSOD levels compared to healthy control subjects (closed triangles). Serum MnSOD levels remained elevated in septic patients who developed ARDS and decreased over time in septic patients without ARDS. Values are mean ± SEM with numbers of patients in parentheses. Modified from reference 23.

**Exhaled breath $H_2O_2$ in ARDS.** There have been three reports of exhaled breath $H_2O_2$ during the course of ARDS[17,19,24]. In the first Baldwin and associates studied 43 hospitalized patients, 16 with a diagnosis of ARDS and 27 without. All patients were supported on mechanical ventilation. The 16 patients with ARDS had elevated levels of breath $H_2O_2$ on the day of diagnosis of ARDS compared to the levels seen in patients without ARDS (Figure 4).

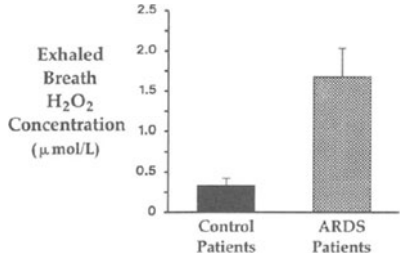


Figure 4. Exhaled breath $H_2O_2$ concentrations were increased ($p \leq 0.05$) in 16 patients on the day of diagnosis of ARDS compared to $H_2O_2$ concentrations in 27 patients without ARDS. Values are mean ± SEM. Replotted from reference 17.

The correlation of breath $H_2O_2$ concentrations with plasma lysozyme levels (a marker of neutrophil turnover) suggested activated neutrophils as the source of $H_2O_2$ in these patients. In another study, Sznajder and colleagues confirmed these findings[19]. Fifty-five ventilated patients with ARDS had a mean breath $H_2O_2$ concentration of 2.34 ± 1.15 (sd) μmol/L compared to 0.99 ± 0.72 (sd) μmol/L in patients without ARDS. These are difficult studies since the $H_2O_2$ concentrations are near the limits of detection and since bacteria and saliva both contain catalase thus making interpretation difficult.

**Other markers.** Various other markers of oxidative damage in ARDS have been proposed including oxidized glutathione (GSSG) in alveolar fluid, depressed plasma vitamin E levels, increased plasma lipid peroxidation products, as well as other oxidized proteins in lavage fluid (e.g. alpha-1-protease inhibitor)[25-29].

## Human Immunodeficiency Virus (HIV) Infection

HIV infection is an insidiously progressive disorder whose pathogenesis in part involves oxygen radicals[30]. The immunological defense of these patients is further hindered by deficiencies in several antioxidants including glutathione, vitamin E, selenium, and manganese-containing superoxide dismutase[30-33]. Buhl and colleagues studied 14 individuals with asymptomatic HIV infection measuring glutathione (GSH)

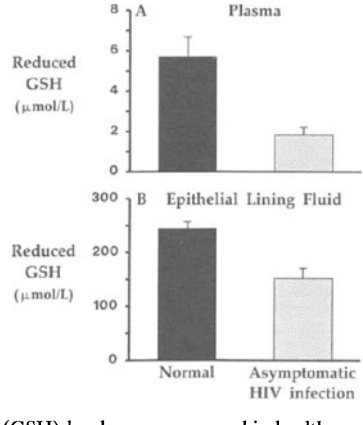


Figure 5. Reduced glutathione (GSH) levels were measured in healthy control subjects and patients with asymptomatic human immunodeficiency virus (HIV) infection. Reduced GSH levels were significantly ($p < 0.05$) reduced in both plasma and epithelial lining fluid of individuals with asymptomatic HIV infection compared to healthy control subjects. Values are mean ± SEM. Replotted from reference 31.

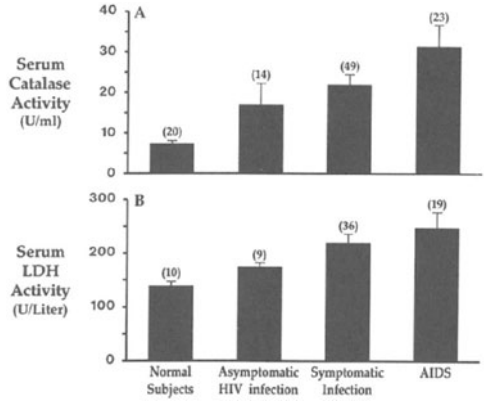


Figure 6. Serum catalase activity was increased ($p \leq 0.05$) in patients with AIDS compared to other HIV-infected individuals and healthy control subjects. Individuals with AIDS and symptomatic infection had greater ($p \leq 0.05$) serum catalase activity than healthy control subjects (panel A). Serum LDH activity was increased ($p \leq 0.05$) in all HIV-seropositive individuals compared to healthy control subjects (panel B). Values are mean ± SEM with numbers of patients in parentheses. Modified from reference 34.

levels in plasma and epithelial lining fluid (obtained by bronchoscopy)[31]. They found that individuals with asymptomatic HIV infection had significantly depressed levels of reduced GSH in both plasma and epithelial lining fluid (Figure 5).

This appears to be an early manifestation of HIV infection and may influence the progression of the process. Given this perturbed oxidant-antioxidant balance we examined the serum catalase activity in HIV infected patients[34]. Serum was collected during routine clinic visits in patients free of acute disease. Patients were classified as having asymptomatic infection, symptomatic infection, or AIDS by standard criteria. We found that serum catalase activity increased progressively as HIV infection progressed (Figure 6). We found a similar pattern with serum lactate dehydrogenase activity (LDH, Figure 6). In contrast, serum glutathione peroxidase activity was not elevated in HIV infected individuals and did not increase with progression of infection.

Serum haptoglobin levels and RBC fragility indices were similar in HIV infected individuals and healthy control suggesting that RBC hemolysis was not the source of serum catalase in these patients. The fact that serum catalase activity correlated ($r=0.67$, $p \leq 0.05$) with serum LDH activity suggested that progressive tissue injury with release of catalase may be a source of serum catalase activity in these patients[34].

**Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. Although RA is often a multisystem disease, it is primarily associated with intermittent acutely inflamed joints. An actively inflamed rheumatoid joint is massively infiltrated with neutrophils and neutrophil-derived products such as lysozyme and stable prostaglandins. These activated neutrophils would be expected to release toxic oxygen metabolites and in fact there is a great deal of evidence that oxygen radicals play an important part in the inflammation of the rheumatoid joint. Interleukin-1 (IL-1) present in inflamed joints may prime neutrophils[35] for oxidant release and activate other local cells such as synoviocytes and chondrocytes to release proteolytic enzymes. Besides neutrophils, activated macrophages may produce oxygen radicals in the inflamed rheumatoid joint[36]. It has also been proposed that an ischemia-reperfusion

injury may occur in RA joints providing substrate and conditions favorable for xanthine oxidase-derived free radical generation[37]. Many markers of this oxidant-induced damage have been measured both in serum and joint (synovial) fluid and may be important in understanding the pathogenesis of RA as well as following disease activity (Table 3).

Lunec and associates studied 58 patients with RA and compared their sera and synovial fluid to healthy control subjects and patients with degenerative (non-inflammatory) joint disease (mainly osteoarthritis)[38]. Using techniques to measure conjugated dienes, visible and ultraviolet fluorescence, they found that greater than 90% of synovial fluids from all patients had evidence of free radical oxidation (peroxidation) products. In particular, however, patients with RA had greater free radical oxidation products than patients with non-inflammatory joint disease and healthy controls. Free radical oxidation products were also elevated in serum of RA patients compared to healthy controls and tended to decrease when patients were treated with a non-steroidal anti-inflammatory agent (feprazone) or gold (Figure 7).

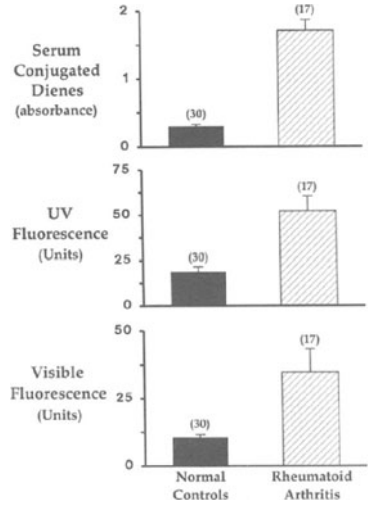


Figure 7. Serum was withdrawn from healthy control subjects and patients with rheumatoid arthritis. Conjugated dienes, UV fluorescence and visible fluorescence (as measures of free radical oxidation products) were all increased ($p \leq 0.05$) in RA patients compared to normal control subjects. Values are mean ± SEM with numbers of patients in parentheses. Replotted from reference 38.

There also was a correlation between serum free radical oxidation products and clinical activity of the disease. This provides a rationale, if corroborated, for using serum markers to follow disease activity and response to therapy. Measurement of lipid peroxidation products is, however, fraught with difficulties. The techniques of measurement are tedious, artifacts are frequent, the products measured are unstable and subject to degradation, and the exact products to measure are frequently unknown in specific conditions.

Rowley and co-workers extended these observations by measuring thiobarbituric acid-reactive (TBAR) material in serum and synovial fluid from RA patients[39]. TBAR material is thought to largely measure malondialdehyde (MDA), a product of lipid peroxidation. Although widely used as an index of lipid peroxidation, the measure is subject to artifact and is non-specific. Nonetheless, in many systems it can be a useful marker. They found detectable TBAR material in the serum and synovial fluid of RA

patients. Of interest, they found correlations of TBAR material in joint fluid with clinical activity of disease.

Ambanelli and colleagues studied serum markers in 25 RA patients and compared the results to values from 15 subjects matched for sex and age[40]. They found elevations in "serum antioxidant activity" and decreases in serum SH groups (thiols) in RA patients compared to controls. Moreover, serum SH levels correlated inversely with disease activity (r=-0.57). In addition, RA patients who responded to therapy had increased SH levels compared to pre-therapy values (Figure 8).

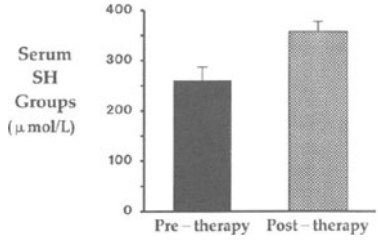


Figure 8. Serum SH groups (thiols) were measured before and after therapy (with tiopronin) in patients with rheumatoid arthritis. Non-responders did not increase serum SH levels (not shown), however, SH levels in responders increased ($p \leq 0.05$) after therapy compared to values before therapy. Values are mean ± SEM. Replotted from reference 40.

Imadaya and associates investigated antioxidant enzyme levels in erythrocytes from RA patients and found reduced levels of superoxide dismutase, catalase and glutathione peroxidase compared to patients with degenerative arthritis and healthy controls[41]. This conflicts with previous work[42] but has been confirmed by others [43].

Situnayake and co-workers evaluated the ability of serum from RA patients to resist attack in vitro by controlled peroxidation induced by peroxyl radicals (the "TRAP" assay)[44]. They found that serum from RA patients had reduced ability to resist attack by peroxyl radicals (TRAP) compared to normal controls. The major determinant of TRAP in RA patients was found to be uric acid while in control serum it was vitamin E. They also found reduced plasma ascorbic acid, serum vitamin E and serum sulfhydryl levels in RA patients compared to healthy controls. In these studies, no relation between these free radical markers and disease activity were noted, however.

Lunec and colleagues have studied serum and synovial fluid from RA patients[45]. They found that when human immunoglobulin G (IgG) was exposed *in vitro* to free radicals, auto-fluorescent monomeric and polymeric IgG was formed. These IgG aggregates can stimulate neutrophils to release toxic oxygen metabolites *in vitro*. Using high-performance liquid chromatography (HPLC) they were able to isolate these complexes from sera and synovial fluid from RA patients. The results suggest neutrophils invading the rheumatoid joint may release oxygen radicals causing aggregation of IgG which in turn activates other neutrophils to release more oxygen radicals creating a perpetuating cycle of inflammation and damage.

Grootveld and associates have suggested both synovial fluid formate levels and a hyaluronate-derived low molecular mass oligosaccharide species as novel markers of reactive oxygen radical activity in the inflamed rheumatoid joint during exercise detecting these species using proton-nuclear magnetic resonance imaging[46]. In separate studies Grootveld and Halliwell have shown that allantoin, the product of free radical attack on uric acid, is elevated in serum and synovial fluid of RA patients[47]. The practical value of these studies remains to be shown. In summary, numerous alterations

in free radical markers are present in rheumatoid disease. Many of these markers correlate with clinical disease activity and some decrease after effective treatment. It is not yet clear whether these markers add anything to the routine clinical assessment of these patients. Further study will hopefully answer this question.

**Primary Biliary Cirrhosis (PBC)**

PBC is a chronic disorder characterized by clinical and laboratory evidence of impaired bile excretion and progressive liver destruction centered around intrahepatic bile ducts. PBC is associated with a number of specific and nonspecific immunologic abnormalities including anti-mitochondrial antibodies, elevated serum IgM levels, antibodies directed against bile canaliculi, and impaired lymphocyte transformation. This and other evidence suggests that PBC represents an autoimmune disorder. There is mounting evidence that oxygen free radicals may be important in its pathogenesis. Hepatic copper levels are greatly elevated (albeit late) in PBC and copper can reduce $O_2$ to superoxide anion as one potential mechanism. Ono and colleagues measured serum MnSOD levels in patients with PBC and found them to be greatly elevated (407 ± 35 ng/ml) compared to control patients (<150 ng/ml)[48]. This was a very early finding in the disease. This is interesting since antibodies directed against mitochondria are present in this disease and MnSOD is a mitochondrial product. Cytokines such as interleukin-1 and tumor necrosis factor induce MnSOD gene expression and also induce an intracellular oxidant stress in endothelial cells and neutrophils possibly relating these observations[49,50].

**Table 1.** Disorders Associated With Elevated Serum or Plasma Catalase Activity.

| Disorder | Reference |
|---|---|
| Fatty liver | 51 |
| Acute alcoholic hepatitis | 51 |
| Toxic hepatitis | 51 |
| Acute pancreatitis | 4,52 |
| Acute cholecystitis | 52 |
| Congestive heart failure | 51 |
| Sepsis | 6,23 |
| Adult respiratory distress syndrome | 6,23 |
| Human immunodeficiency virus (HIV) infection | 34 |
| Skin burn | 53 |
| Hemolytic anemia | 55 |

## CONCLUSION

The involvement of oxidant species in human autoimmune and inflammatory diseases is now undeniable. With the advent of new techniques to measure either these reactive species themselves or their reaction products we are now in a position to assess the utility of these oxidant markers in the diagnosis and management of these challenging diseases.

**Table 2.** Disorders Associated With Elevated Serum MnSOD Levels.

| Disorder | % Elevated* | Reference |
|---|---|---|
| Myocardial infarction | 63 | 12 |
| Liver disease | | |
| Primary biliary cirrhosis | 97 | 11 |
| Hepatoma | 60 | 11,48 |
| Hepatitis | ? | 11,48 |
| Cirrhosis | 33 | 11,48 |
| Gastric cancer | 27 | 11 |
| Lung cancer | 33 | 11 |
| Lymphoma | 17 | 11 |
| Sepsis | 35 | 23 |
| Adult respiratory distress syndrome | 89 | 23 |
| Acute myeloid leukemia | 60 | 11,55 |
| Acute lymphocytic leukemia | 27 | 11,55 |
| Ovarian cancer | 62 | 11,55 |
| Cervical cancer | 19 | 11 |
| Endometrial cancer | 22 | 11 |
| Benign ovarian tumors | 13 | 11 |

Normal values: Females 99.8 ± 24.8 (s.d.) ng/ml ; Males 88.8 ± 20.8 (s.d.) ng/ml
* Abnormal value defined as >2 s.d. above mean.

**Table 3.** Free Radical Markers in Rheumatoid Arthritis.

| Marker | Reference |
|---|---|
| Lipid peroxidation products in blood and synovial fluid | 38,39 |
| Reduced serum thiols | 40,44 |
| Increased serum ceruloplasmin levels | 40 |
| Decreased erythrocyte antioxidants | 41-43 |
| Decreased plasma and synovial fluid ascorbic acid levels | 44 |
| Decreased serum "antioxidant" activity | 44 |
| Decreased serum vitamin E levels | 44 |
| Increased neutrophil oxidant activity | 56 |
| Increased synovial fluid hyaluronate-derived degradation products | 46 |
| Increased synovial fluid formate levels | 46 |
| Increased serum and synovial fluid allantoin levels | 47 |
| Aggregated serum and synovial fluid IgG | 45 |

## REFERENCES


1. C.E. Cross, Oxygen radicals and human disease, Ann. Intern. Med. 107:526-545 (1987).
2. H. Aebi, Catalase in Vitro, in: "Methods in Enzymology: Oxygen radicals in biological systems. Volume 105", L. Packer., ed., Academic Press, Inc., Orlando, pp. 121-126 (1984).
3. J.A. Leff, M.A. Oppegard, L.S. Terada, E.C. McCarty, and J.E. Repine, Human serum catalase decreases endothelial cell injury from hydrogen peroxide, J. Appl. Physiol. 71(5):1903-1906 (1991).
4. L. Goth, Origin of serum catalase activity in acute pancreatitis, Clin. Chim. Acta 186:39-44 (1989).
5. L. Goth, H. Nemeth, and I. Meszaros, Serum catalase activity for detection of hemolytic diseases [letter], Clin. Chem. 29:741-743 (1983).
6. J.A. Leff, P.E. Parsons, C.E. Day, E.E. Moore, F.A. Moore, M.A. Oppegard, and J.E. Repine, Increased serum catalase activity in septic patients with the adult respiratory distress syndrome, Am. Rev. Respir. Dis. 146:985-989 (1992).
7. P.A. Sandstrom and T.M. Buttke, Autocrine production of extracellular catalase prevents apoptosis of the human CEM T-cell line in serum-free medium, Proc. Natl. Acad. Sci. U. S. A. 90:4708-4712 (1993).
8. J.M. McCord and I. Fridovich, Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem. 244:6049-6055 (1969).
9. S.L. Marklund, Human copper-containing superoxide dismutase of high molecular weight, Proc. Natl. Acad. Sci. USA 79:7634-7638 (1982).
10. B.B. Keele,Jr., J.M. McCord, and I. Fridovich, Superoxide dismutase from escherichia coli B: A new manganese-containing enzyme, J. Biol. Chem. 245:6176-6181 (1970).

11. N. Taniguchi, Clinical significances of superoxide dismutases: changes in aging, diabetes, ischemia, and cancer, Adv. Clin. Chem. 29:1-59 (1992).
12. T. Kawaguchi, K. Suzuki, Y. Matsuda, T. Nishiura, T. Uda, M. Ono, C. Sekiya, M. Ishikawa, S. Iino, Y. Endo, and N. Taniguchi, Serum Mn-superoxide dismutase: Normal values and increased levels in patients with acute myocardial infarction and several malignant diseases determined by enzyme-linked immunosorbent assay using a monoclonal antibody, J. Immunol. Meth. 127:249-254 (1990).
13. B. Frei, Y. Yamamoto, D. Niclas, and B.N. Ames, Evaluation of an isoluminol chemiluminescence assay for the detection of hydroperoxides in human blood plasma, Anal. Biochem. 175:120-130 (1988).
14. Y. Yamamoto, B. Frei, and B.N. Ames, Assay of lipid hydroperoxides using high-performance liquid chromatography with isoluminol chemiluminescence, Methods Enzymol. 186:371-380 (1990).
15. A. Nahum, L.D.H. Wood, and J.I. Sznajder, Measurement of hydrogen peroxide in plasma and blood, Free Radic. Biol. Med. 6:479-484 (1989).
16. S.D. Varma and P.S. Devamanoharan, Excretion of hydrogen peroxide in human urine, Free Rad. Res. Comms. 8:73-78 (1990).
17. S.R. Baldwin, R.H. Simon, C.H. Grum, L.H. Ketai, L.A. Boxer, and L.J. Devall, Oxidant activity in expired breath of patients with adult respiratory distress syndrome, Lancet 1:11-14 (1986).
18. M.D. Williams and B. Chance, Spontaneous chemiluminescence of human breath: spectrum, lifetime, temporal distribution and correlation with peroxide, J. Biol. Chem. 258:3628-3631 (1983).
19. J.I. Sznajder, A. Fraiman, J.B. Hall, W. Sanders, G. Schmidt, G. Crawford, A. Nahum, P. Factor, and L.D.H. Wood, Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure, Chest 96:606-612 (1989).
20. J.A. Leff, C.P. Wilke, B.M. Hybertson, P.F. Shanley, C.J. Beehler, and J.E. Repine, Post-insult treatment with N-acetylcysteine decreases interleukin-1-induced lung neutrophil sequestration and oxidative lung leak in rats, Am. J. Physiol. 265:L501-L506 (1993).
21. J.J. Hageman, A. Bast, and N.P.E. Vermeulen, Monitoring of oxidative free radical damage in vivo: Analytical aspects, Chem. Biol. Interactions 82:243-293 (1992).
22. J.E. Repine, Scientific perspectives on adult respiratory distress syndrome, Lancet 339:466-469 (1992).
23. J.A. Leff, P.E. Parsons, C.E. Day, N. Taniguchi, M. Jochum, H. Fritz, F.A. Moore, E.E. Moore, J.M. McCord, and J.E. Repine, Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis, Lancet 341:777-780 (1993).
24. W.C. Wilson, J.F. Swetland, J.L. Benumof, P. Laborde, and R. Taylor, General anesthesia and exhaled breath hydrogen peroxide, Anesthesiology 76:703-710 (1992).
25. G.R. Bernard, B.B. Swindell, M.J. Meredith, F.E. Carroll, and S.B. Higgins, Glutathione (GSH) repletion by N-acetylcysteine in patients with the Adult Respiratory Distress Syndrome, Am. Rev. Resp. Dis. 139:A221 (1989) (Abstract).
26. E.R. Pacht, A.P. Timerman, M.G. Lykens, and A.J. Merola, Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome, Chest 100:1397-1403 (1991).
27. E. Bunnell and E.R. Pacht, Oxidized glutathione is increased in the alveolar

fluid of patients with the adult respiratory distress syndrome, Am. Rev. Resp. Dis. 148:1174-1178 (1993).
28. C. Richard, F. Lemonnier, M. Thibault, M. Couturier, and P. Auzepy, Vitamin E deficiency and lipoperoxidation during adult respiratory distress syndrome, Crit. Care. Med. 18:4-9 (1990).
29. C.G. Cochrane, R.G. Spragg, and S.D. Revak, Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid, J. Clin. Invest. 71:754-758 (1983).
30. B. Halliwell and C.E. Cross, Reactive oxygen species, antioxidants, and acquired immunodeficiency syndrome. Sense or speculation? Arch. Intern. Med. 151:29-31 (1991).
31. R. Buhl, K.J. Holroyd, A. Mastrangeli, A.M. Cantin, H.A. Jaffe, F.B. Wells, C. Santini, and R.G. Crystal, Systemic glutathione deficiency in symptom-free HIV-seropositive individuals, Lancet 2:1294-1298 (1989).
32. J.J. Javier, M.K. Fodyce-Baum, R.S. Beach, M. Gavancho, C. Cabrejos, and E. Mantero-Atienza, Antioxidant micronutrients and immune function in HIV-1 infection, FASEB Proc. 4:A940 (1990).
33. B.M. Dworkin, W.S. Rosenthal, G.P. Wormser, and L. Weiss, Selenium deficiency in the acquired immune deficiency syndrome, J. Parenter. Ent. Nutr. 10:405-407 (1986).
34. J.A. Leff, M.A. Oppegard, T.J. Curiel, K.S. Brown, R.T. Schooley, and J.E. Repine, Progressive increases in serum catalase activity in advancing human immunodeficiency virus infection, Free Radical Biol. Med. 13:143-149 (1992).
35. Y. Ozaki, T. Ohashi, and S. Kume, Potentiation of neutrophil function by recombinant DNA-produced interleukin-1a, J. Leukocyte Biol. 42(6):621-627 (1987).
36. B. Halliwell, J.R. Hoult, and D.R. Blake, Oxidants, inflammation, and anti-inflammatory drugs, FASEB J. 2:2867-2873 (1988).
37. J. Unsworth, J. Outhwaite, D.R. Blake, C.J. Morris, J. Freeman, and J. Lunec, Dynamic studies of the relationship between intraarticular pressure, synovial fluid oxygen tension and lipid peroxidation in the inflamed knee: an example of reperfusion injury, Annu. Clin. Biochem. 25:8S-11S (1988).
38. J. Lunec, S.P. Halloran, A.G. White, and T.L. Dormandy, Free-radical oxidation (peroxidation) products in serum and synovial fluid in rheumatoid arthritis, J. Rheumatol. 8:233-245 (1981).
39. D. Rowley, J.M.C. Gutteridge, D. Blake, M. Farr, and B. Halliwell, Lipid peroxidation in rheumatoid arthritis: thiobarbituric acid-reactive material and catalytic iron salts in synovial fluid from rheumatoid patients, Clin. Sci. 66:691-695 (1984).
40. U. Ambanelli, A. Spisni, and G.F. Ferraccioli, Serum antioxidant activity and related variables in rheumatoid arthritis. Behaviour during sulphydrylant treatment, Scand. J. Rheumatol. 11:203-207 (1982).
41. A. Imadaya, K. Terasawa, H. Tosa, M. Okamoto, and K. Toriizuka, Erythrocyte antioxidant enzymes are reduced in patients with rheumatoid arthritis, J. Rheumatol. 15:1628-1631 (1988).
42. P. Scudder, J. Stocks, and T.L. Dormandy, The relationship between erthrocyte superoxide dismutase activity and erythrocyte copper levels in normal subjects and in patients with rheumatoid arthritis, Clin. Chem. Acta 69:397-403 (1976).
43. J.C. Banford, D.H. Brown, R.A. Hazelton, C.J. McNeil, R.D. Sturrock, and W.E. Smith, Serum copper and erythrocyte superoxide dismutase in rheumatoid disease, Ann. Rheum Dis. 41:458-462 (1982).
44. R.D. Situnayake, D.I. Thurnham, S. Kootathep, S. Chirico, J. Lunec, M. Davis, and B. McConkey, Chain breaking antioxidant status in rheumatoid arthritis:

clinical and laboratory correlates, Ann. Rheum Dis. 50:81-86 (1991).
45. J. Lunec, D.R. Blake, S.J. McCleary, S. Brailsford, and P.A. Bacon, Self-perpetuating mechanisms of immunoglobulin G aggregation in rheumatoid arthritis, J. Clin. Invest. 76:2084-2090 (1985).
46. M. Grootveld, E.B. Henderson, A. Farrell, D.R. Blake, H.G. Parkes, and P. Haycock, Oxidative damage to hyaluronate and glucose in synovial fluid during exercise of the inflamed rheumatoid joint. Detection of abnormal low-molecular-mass metabolites by proton-n.m.r. spectroscopy, Biochem. J. 273:459-467 (1991).
47. M. Grootveld and B. Halliwell, Measurement of allantoin and uric acid in human body fluids- A potential index of free radical reactions in vivo? Biochem. J. 243:803-808 (1987).
48. M. Ono, C. Sekiya, M. Ohhira, M. Namiki, Y. Endo, K. Suzuki, Y. Matsuda, and N. Taniguchi, Elevated level of serum Mn-superoxide dismutase in patients with primary biliary cirrhosis: possible involvement of free radicals in the pathogenesis in primary biliary cirrhosis, J. Lab. Clin. Med. 118:476-483 (1991).
49. T. Matsubara and M. Ziff, Increased superoxide anion release from human endothelial cells in response to cytokines, J. Immunol. 137:3295-3298 (1986).
50. S.J. Klebanoff, M.A. Vadas, J.M. Harlan, L.H. Sparks, J.R. Gamble, J.M. Agosti, and A.M. Waltersdorph, Stimulation of neutrophils by tumor necrosis factor, J. Immunol. 136:4220-4225 (1986).
51. L. Goth, I. Meszaros, and H. Nemeth, Serum catalase enzyme activity in liver diseases, Acta. Biol. Hung. 38:287-290 (1987).
52. I. Meszaros, L. Goth, and G. Vattay, The value of serum catalase activity determinations in acute pancreatitis, Digestive Diseases 18:1035-1041 (1973).
53. J.A. Leff, L.K. Burton, E.M. Berger, B.O. Anderson, C.P. Wilke, and J.E. Repine, Increased serum catalase activity in rats subjected to thermal skin injury, Inflammation 17:199-204 (1993).
54. T. Nishiura, K. Suzuki, T. Kawaguchi, H. Nakao, N. Kawamura, M. Taniguchi, Y. Kanayama, T. Yonezawa, S. Iizuka, and N. Taniguchi, Elevated serum manganese superoxide dismutase in acute leukemias, Cancer Lett. 62:211-215 (1992).
55. M. Ishikawa, Y. Yaginuma, H. Hayashi, T. Shimizu, Y. Endo, and N. Taniguchi, Reactivity of a monoclonal antibody to manganese superoxide dismutase with human ovarian carcinoma, Cancer Res. 50:2538-2542 (1990).
56. P. Suryaprabha, U.N. Das, G. Ramesh, K.V. Kumar, and G.S. Kumar, Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus, Prostaglandins Leukot. Essent. Fatty. Acids 43:251-255 (1991).