Free Radicals, Reactive Oxygen Species, and Their Biomarkers

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Abstract

Free radicals (FRs) and/or reactive oxygen species (ROS) are bioactive substances generated inevitably during the metabolic process of organisms. To combat excessive free radical and/or reactive oxygen production, living organisms have evolved many sophisticated peroxide-antioxidant defense systems. These systems are located in a dynamic equilibrium state under normal physiological conditions, while the body antioxidant system could be unbalanced and lead to oxidative stress in pathological states. Oxidative stress is closely related to the occurrence and development of various diseases, including cancer. Therefore, FRs and/or ROS involved in pathological reactions can be used as markers of oxidative stress. Although most oxidation-antioxidant markers are not difficult to be measured by modern medical detection technology separately, the detection of each oxidation-antioxidant substance is not only time- and energy-consuming but also inaccurate. One of the reasons for inaccuracy is the incomplete

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understanding and detection of oxidation-antioxidant substances in the organism. The other is the superposition effect produced by various oxidation-antioxidant substances which have a synergistic effect in the same system. In view of this, only combined total oxidant status (TOS) with total antioxidant status (TAS) and oxidant stress index (OSI) can accurately assess the oxidant stress status of subjects.

Keywords

Reactive oxygen species · Free radical · Oxidative stress · Biomarker · Tumor · Cancer

Introduction

Oxygen is an essential element to maintain the life activities of the organism. An adult is required to consume about 500 L of oxygen a day to maintain ATP energy metabolism for life energy generation. However, the oxygen absorbed by the organism is not merely used for energy metabolism, and a small part (approximately 2%) will be not completely reduced to generate ROS and FRs (Umeno et al. 2017). These active substances can react with various biological components such as proteins in blood and thiol groups in biological molecules or nucleic acids, subsequently playing physiological functions or leading to pathological effects. In normal physiological state, excessive ROS and FRs produced by the organism can be cleverly eliminated by antioxidant defense system of itself, so these substances generally do not accumulate in large quantities and cause damages to the organism. Unfortunately, ROS and FRs may be excessively produced or inadequately consumed under various pathological conditions, which will exceed the defense capacity of the organism, resulting in oxidative damage of cells and subsequently to oxidative stress (Lushchak and Gospodaryov 2012). Cellular oxidative damage is a well-established general mechanism for cell, tissue, and organ injury. A series of studies have shown that cellular oxidative damage is caused primarily by ROS and FRs.

Cancer often goes through two stages: activation (also known as trigger) and promotion, in which both ROS and FRs play major roles (Ebrahimi et al. 2020). ROS and FRs are associated with tumor growth and metastasis by inducing gene stability changes, promoting malignant transformed cells' proliferation, inhibiting malignant transformed cells' apoptosis, accelerating tumors' invasion and metastasis, and increasing tumors' treatment tolerance. Therefore, many ROS and FRs may be potential biomarkers of tumor diagnosis, treatment and prognosis, which can be called oxidative stress markers (Marrocco et al. 2017).

Biomarker refers to the biochemical indicators that can reflect the structural or functional changes or possible changes of system, organ, tissue, cell, and subcellular. It can be used as biomarkers for health examination, disease diagnosis, disease staging, treatment monitoring, prognosis evaluation, safe and effective drug

development, as well as the effective evaluation of drugs, food, beverage, and health products (Niki 2014). Oxidative stress markers are biological indicators reflecting the level of oxidation or antioxidation of the organism, which can be affirmed in blood or body fluid samples. The quantitative detection methods of oxidative stress markers can be roughly divided into five categories (Marrocco et al. 2017): (1) direct determination; (2) determination of compounds modified by reactive oxygen species; (3) determination of the amount of enzymes and antioxidants eliminated by reactive oxygen species; (4) determination of oxidative stress markers containing transcription factors; (5) determination of the intermediates or metabolites involved in the reaction. However, these methods only reflect the changes of one or several markers but do not necessarily represent the total oxidation-antioxidant levels of individuals or samples. TOS refers to the sum of all the oxidation substances in the organism, which is the general index reflecting the level of peroxidation of the organism. TAS is the total of enzymatic and nonenzymatic antioxidants in the organism, and is an overall index reflecting the antioxidant capacity of the organism. Only TOS, TAS, and the ratio of them (OSI) can correctly reflect the oxidative stress status of subjects (Wang et al. 2011; Feng et al. 2016)

Free Radicals and Reactive Oxygen Species

Radicals and Free Radicals

The term "radical" is commonly used in chemistry to denote different groups of atoms, such as SO_4^{2-}, HCO_3^-, and -CHO. "Free radical" means that it can exist independently and contains one or more unpaired electrons in an atom or group of atoms, such as HO•(hydroxyl radical), RO• ("R-" for alkyl group), L• ("L" for lipid), and LO•(lipid alkoxyl radicals) (Valko et al. 2006).

Due to the biochemical reactions in the body, there are many FRs in the body. Therefore, as a by-product of normal metabolism, FRs are continuously produced in living organisms, which are essential for cells. When a covalent bond of compound breaks, the paired electrons are divided equally between the two atoms. This process is called homolytic fission. The covalent bonds are split, such as the break between –C–C–, –C–H, –C–O–, etc. This is a process of free radical generation.

If A and B are formed by two atoms covalently bonded (* represents electrons), the homolytic fission can be expressed as follows.

$$A^*_*B \rightarrow A^* + B^*$$

A^* is A radical, indicated by A•, B* is B radical, indicated by B•. When a covalent bond in a water molecule is homolytic fission, hydrogen radicals (H•) and hydroxyl radicals (•OH) are generated. Heterolytic fission is corresponding to the homolytic fission. When the covalent bond is heterolytic fission, an atom receives a pair of electrons, and heterolytic fission can be expressed as follows.

$$A_*^*B \rightarrow A_*^{*-} + B^+$$

A gets an electron and is negatively charged, B loses an electron and is positively charged. For example, the heterolytic fission of water can generate H^+ and OH^-, which are called hydrogen ions and hydroxide ions, respectively. None of them have unpaired electrons, so neither is a free radical.

Reactive Oxygen Species and Oxygen Free Radicals

Reactive oxygen species (ROS) refers to the general term for oxygen-containing substances in the body that are composed of oxygen and active in nature, including metabolic products of oxygen and oxygenated products of reactions: (i) single electron reductants of oxygen such as superoxide anion radical ($O_2^{\bullet-}$) and $O^{\bullet-}$, as well as their protonic hydrogen peroxide radical ($HO_2^{\bullet}$) and hydroxyl radical (OH•); (ii) hydrogen two-electron reductant hydrogen peroxide (H_2O_2); (iii) alkane peroxidation ROOH and its homogenous products oxygen organic free radical (RO•), organic peroxide radical (ROO•);(iv) oxygen in excited state, singlet oxygen, and carbonyl compounds. Table 1 shows the ROS with damage significance. ROS is

Table 1 Reactive oxygen species with the significance of oxidative stress

Species	Terminology	Characteristics
$O_2^{\cdot-}$	Superoxide anion	The single electron reduction state; formed by many oxygen reactions (such as flavin protein, redox cycle)
$HO_2^{\cdot}$	Hydrogen peroxy	Formed by the protonation of $O_2^{\cdot-}$; enhanced fat solubility
H_2O_2	Hydrogen peroxide	Two-electron reduced state; formed by disproportionation of $O_2^{\cdot-}(HO_2^{\cdot})$, or directly formed by O_2
HO·	Hydroxyl radical	Three-electron reduction state; formed by Fenon reaction and metal-catalyzed Haber–Weiss reaction; is highly active.
RO·	R-oxygen radical, alkoxy radical	Oxygen organic free radicals (such as lipids)
ROO·	R-Peroxy radical, alkyl peroxy radical	Formed from organic hydroperoxide (ROOH), such as lipid, by hydrogen extraction (or homolysis)
ROOH	R-Hydroperoxide	Organic hydroperoxides (such as fatty acids and thymine hydroperoxides)
O_2^* or $^1O_2$	Singlet oxygen	First excitation; higher than ground state oxygen (O); red (bimolecular) or infrared (monomolecular) light emission
$^3R'R''CO$ ($R'R''CO^*$)	Triplet carbonyl	Excited carbonyl compounds, blue-green light emission (i.e., via dioxane intermediates)

Note: Strictly speaking, $^1O_2$ and H_2O_2 are ROS, not oxygen radicals

characterized by containing oxygen, and its chemical properties are more active than ground state oxygen (Jakubczyk et al. 2020).

Some ROS are FRs (Kundu et al. 2019). If the unpaired electrons of these FRs are located in oxygen, it is called oxygen free radicals (OFR). Other ROS are non-free radical oxygenates. The characteristic of non-free radical ROS is that it can be produced in the free radical reaction. In addition, it can also trigger the free radical reaction directly or indirectly.

In terms of chemical activity, OFR is synonymous with ROS, but there are exceptions. For example, ground state oxygen is a double radical, but its chemical activity is not strong, not a ROS. Excited molecular oxygen and singlet oxygen are not free radicals, but their activity is higher than that of double-radical ground oxygen and oxygen-containing organic compounds in the excited state, such as excited carbonyl compounds and dioxane, and ozone (Conrad and Pratt 2019). All of them are to biologically significant ROS. More than 95% of the total FRs in the human body are OFR, which are usually active groups that trigger the generation of other FRs.

Metal Free Radicals

Some metal elements play a broad biological role in the metabolism of substances in the human body. They can constitute the active centers of many biological enzymes and participate in various electron transfer reactions. Thus, they are indispensable substances for maintaining human life activities. The transition metal elements (d-block) of the periodic table contain unpaired electrons except zinc, so they are all FRs. Copper does not fully meet the definition of transition element because its 3d–orbit is full. However, it is easy to lose two electrons to form Cu^{2+} ions, one electron comes from 4S orbit and the other electron comes from 3d orbit. This forms unpaired electrons. The transition elements are all metals. From the perspective of FRs, their most important feature is change of valence. Thus, they involve the change of the oxidation state of an electron.

Iron

Iron has two common valences, and their electron arrangement is: Fe^{3+} is an oxidant, Fe^{2+} is a weak reducing agent, and Fe^{2+} can be oxidized by a single electron. O_2 captures an electron to form $O_2\overline{\bullet}$. When the Fe^{2+} (ferrous sulfate) solution is exposed to the air, it can be slowly oxidized to Fe^{3+}, and the O_2 dissolved in the solution is reduced to $O_2\overline{\bullet}$.

$$Fe^{2+} + O_2 \rightleftharpoons Fe^{2+} — O_2 \rightleftharpoons Fe^{3+} — O_2^- \rightleftharpoons Fe^{3+} + O_2^{\cdot -}$$

Both Fe^{2+} and Fe^{3+} can interact with H_2O_2 to generate $OH^{\bullet}$ and $O_2^{\cdot -}$, respectively; they can be further oxidized by the generated $OH^{\bullet}$ and $O_2^{\cdot -}$, reducing to generate the corresponding OH^- and O_2.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2^{\cdot -} + 2H^+$$

$$OH^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^-$$

$$O_2 + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

$$\text{net reaction} : 2H_2O_2 \overset{\text{Iron ions}}{\rightarrow} 2H_2O + O_2$$

Copper

Copper has two valence states, Cu^+ (cuprous) and Cu^+ (cupric)

$$Cu^{2+} + O_2\overline{\bullet} \rightarrow Cu^+ + O_2$$

$$Cu^+ + O_2\overline{\bullet} \rightarrow Cu^{2+} + O_2^{2-}$$

$$O_2^{2-} + 2H^+ \rightarrow H_2O_2$$

$$\text{net reaction} : O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow \text{copper ion} \rightarrow 2H_2O + O_2$$

The copper salt changes the two molecules $O_2^{\cdot -}$ into H_2O_2 and O_2, due to the change in price, and the copper salt acts as a catalyst. Copper salts can also react with H_2O_2 to form OH.

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{\bullet}$$

Manganese

The most stable valence state of manganese in solution is Mn^{2+}. Manganese can also be oxidized to Mn^{3+}, Mn^{4+}, and Mn^{7+}. Mn^{2+} can also participate in free radical reactions:

$$Mn^{2+} + O_2^{\cdot -} + 2H^+ \rightarrow Mn^{3+} + H_2O_2$$

Zinc

There is only one valence state of zinc, Zn^{2+}, and cannot participate in free radical reactions. However, zinc can inhibit certain free radical reactions in the body because it can replace other metal ions, such as the iron ion in the binding site with a catalytic reaction.

Transition metals can effectively catalyze many redox reactions by changing the valence. They often catalyze such reactions at the active site of the enzyme. This free radical reaction catalyzed by transition metals can overcome the spin limitation when oxygen reacts directly with non-free radical compounds.

Oxidative Stress Biomarkers in Cancer

Oxidative stress (OxS) refers to the pathological process of excessive production of ROS in the body and/or reduction of the body's antioxidant capacity. The balances of prooxidative systems and antioxidative systems are disordered (Sánchez-Rodríguez and Mendoza-Núñez 2019). This leads to tissue cell damage, which can be a latent pathological process (Gupta et al. 2014). OxS is involved in the pathogenesis of several diseases (Rajasekaran 2020), like cancer, cardiovascular disease, amyotrophic lateral sclerosis, diabetes, atherosclerosis, hypertension, autoimmune disorders, arthritis, neurodegenerative disorders, and pulmonary, kidney, and hepatic diseases (Fig. 1).

Prooxidative systems that generate ROS are mainly mitochondria, cytochrome p450, neutrophils, and macrophages (Kreuz and Fischle 2016). Under normal circumstances, ROS from various types of cells are mainly produced by mitochondria within the cell through the mitochondrial respiratory chain and monoamine oxidase. Phagocytic prooxidative systems refer to NAD(P)H oxidase, myeloperoxidase (MPO), in addition to the more stable oxygen species hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$). Under pathological conditions or during aging process, when the increase of ROS exceeds the primary antioxidant defense capacity of the cell, it causes oxidative damage to lipids, proteins, and DNA (Lee and Paull 2020). The removal mechanism of ROS includes level 1 antioxidant defense system and level 2 antioxidant defense system. The former removes ROS, the latter repairs damaged biomolecules (Villamena 2013). OxS comes from an

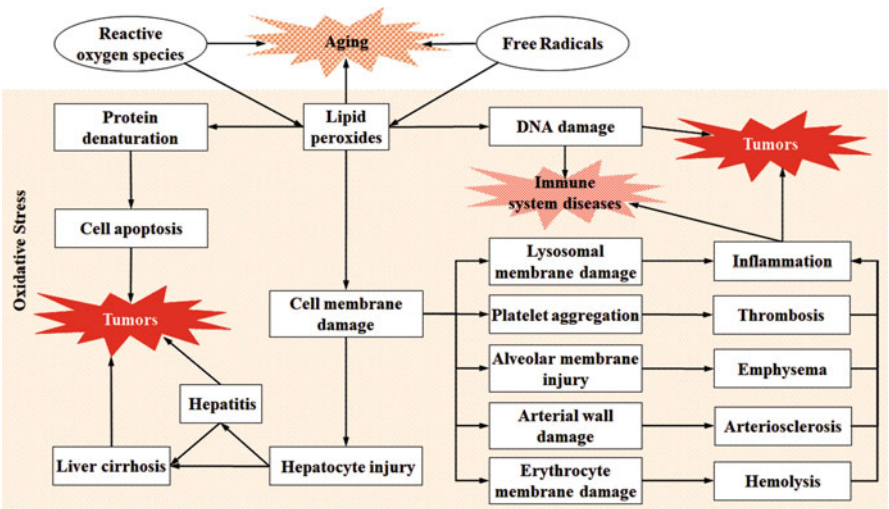

Fig. 1 Reactive oxygen species, free radicals, oxidative stress, and diseases occurrence Excess reactive oxygen species and free radicals in vivo can cause lipid peroxidation of intracellular lipids, resulting in cell membrane damage, vascular endothelial injury, and then cause aging, inflammation, and various diseases, including tumor and/or cancer

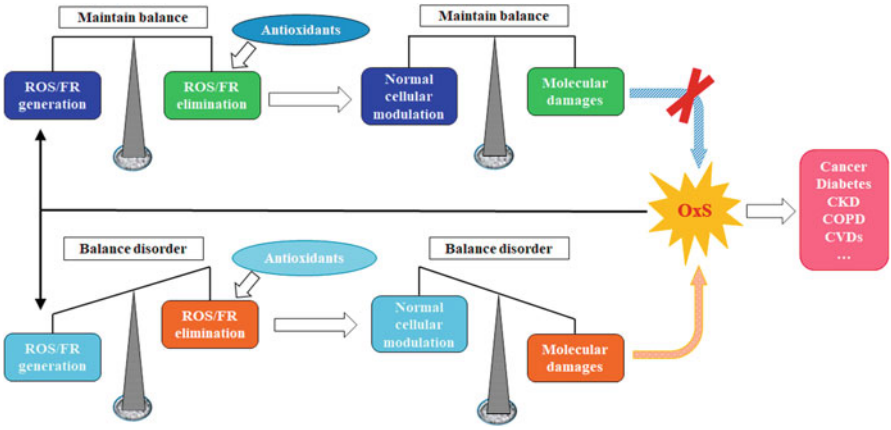

Fig. 2 The balance between oxidative stress and prooxidative-antioxidative systems There is an oxidation-antioxidant system composed of composed of oxidants such as reactive oxygen species(ROS)/free radicals(FR) and antioxidants such as enzymes in the human body. Under normal physiological conditions, the system maintains a dynamic balance. Oxidative stress (OxS) does not occur in the body. In pathological state, the imbalance of the system can result in the damage of various biological macromolecules in tissues and cells. If OxS becomes excessive or a permanent condition, it may lead to a disease, such as cancer, diabetes, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and cardiovascular diseases (CVDs)

imbalance between the generation of FRs by cellular aerobic metabolism and the capacity for removal of these species (Frijhoff et al. 2015). The generation and removal mechanism of ROS in the body is shown in Fig. 2.

OxS plays an important role in some physiological conditions and in disease processes including carcinogenesis (Tsukahara 2007). Tumors are multisystemic or called systemic diseases. Therefore, all FRs and/or ROS involved in the development of OxS may be potential tumor markers, including all kinds of ROS, total oxidant/antioxidant status, and some end products of lipid peroxidation (Hwang and Kim 2007).

Single Oxidation or Antioxidant

Aerobic organisms have evolved an antioxidant defense system that can remove FRs and ROS (Gadoth and Göbel 2010). The system can be divided into three levels: (i) primary antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione S-transferase (GSH), and paraoxonase 1 (PON1) enzyme, whose function is to prevent the production of new FR and/or ROS; (ii) secondary antioxidants, such as vitamin (Vit) A, Vit C, Vit E, uric acid, glutathione, α-lipoic acid, carotene, trace elements copper, zinc, manganese, and selenium, whose function is to remove FRs and/or ROS before FRs and/or ROS trigger lipid peroxidation chain reaction; (iii) tertiary antioxidants, such as DNA repair enzymes and methionine oxysulfide reductase, whose function is to repair nucleic acid chains damaged by FRs and/or ROS oxidation and maintain the normal

physiological function of cells (Hensley and Floyd 2003). However, in some pathological conditions, the body cannot defend the increase of oxidations or decrease of antioxidants. In addition, the balance between oxidation and antioxidation is transformed to the oxidative state, which will inevitably lead to OxS reaction.

All biochemical antioxidants involved in the body's antioxidant defense system are biomarkers of OxS. In order to evaluate the antioxidant status in vivo, it is necessary to detect the antioxidants in the body (Rahman and Biswas 2004). These antioxidants such as SOD, GSH-Px, CAT, GSH, PON1, Vit A, Vit C, Vit E, and uric acids in the oxidation defense system can be detected separately (Yin 2008). In the past, many researchers often used these indicators to reflect the body's antioxidant status (Cuffe et al. 2017). However, due to the presence of a large number of different antioxidants in plasma, serum, urine, or other biological samples, it is difficult to implement a single determination of various antioxidants.

In the body's oxidation-antioxidation system, the opposite of antioxidants is oxidations, which are mainly FRs and oxidants (Cherubini et al. 2005). The FRs that can be generated in organisms through enzymatic and/or non-enzymatic reactions include: (i) ROS, such as $O_2^{\cdot -}$, $OH^{\bullet}$; (ii) RNS, such as $NO^{\bullet}$, NO_2, $ONOO^{-}$. Commonly used oxidants are $^1O_2$ and H_2O_2. The FRs and oxidants in the study are shown in Table 1. Strictly, $^1O_2$ and H_2O_2 are not OFR, but active oxygen (Gelpi et al. 2016).

The following oxidizing substances are all OxS biomarkers (Table 1), but oxidizing substances are less easy to detect than anti-oxidizing substances, and are more difficult to detect individually.

Tables 2 and 3 list the biomarkers of oxidative stress commonly used in clinical or scientific research or reported in the literature (except for the nitro-oxidative stress class). Among them, many markers have been commonly recognized as OxS biomarkers of diseases (including tumors). Many studies on cancer patients have shown that these single OxS biomarkers are various in different tumor types and/or stages (Wang et al. 2011; Xiang et al. 2019). The results reported by the researchers are inconsistent, and there are even completely different results. One reason is that the research methods are different, such as the selection of sampling methods, the condition of the subjects, and the differences between the bodies. Another important reason is that the "oxidation-antioxidation system" in the body is extremely complicated (Ghezzi et al. 2017). In addition, the components are only partially recognized, and more are not recognized. Therefore, the subject's oxidative stress state can only be correctly judged when all OxS biomarkers are tested. The reason is that the oxidation and anti-oxidation substances can fluctuate, and ultimately maintain a dynamic balance.

Total Oxidant Status, Total Antioxidant Status, and Oxidant Stress Index

There are two types of antioxidant systems in the human tissues and cells (Lauridsen 2019). The first type is the enzyme antioxidant system, including SOD, CAT, and glutathione peroxidase (GSH-Px). The second type is the non-enzyme antioxidant

Table 2 OxS biomarkers commonly used in clinical practice or research (antioxidants)

Abbreviation	Full name	Abbreviation	Full name
AOPP	Advanced Oxidation Protein Products	Mel	Melatonin
ALA	α-Lipoic acid	Myase	Myeloperoxidase
apoA-I	Apolipoprotein A-I	OMP	Oxidatively modified protein
ADMA	Asymmetric dimethyl-L-arginine	DHN	1,4-Dihydroxynonene
CbP	Carbonylproteine	Anti-oxLDL	Ox-LDL antibody
ACR	Carotene	PHPA	Para-hydroxyphenylacetic acid
CAT	Catalase	PON1	Paraoxonase 1
CoQ10	Coenzyme Q10	PMN-Elae	Polymorphonuclear leukocyte elastase
Cu	Copper	GSH	Reduced glutathione
CRP	C-reactive protein	Se	Selenium
COX	Cyclooxygenase	SOD	Superoxide dismutase
8-OHdG	8-Hydroxy-20-deoxyguanosine	SDMA	Symmetrisches dimethylarginin
F_2-IsoP	F_2-Isoprostane	Try	Tryptophan
FB	Free biotin	DNP	2,4-Dinitrophenylhydrazine
GSSG	Glutathione disulfide	Ubi	Ubiquinone
GSHPx	Glutathione peroxidase	UA	Uric acid
GSH	Glutathione S-transferase	VitA	Vitamin A
Hp	Haptoglobin	VitB	Vitamin B6
IDO	Indolamin-2,3-dioxygenase	VitB12	Vitamin B12
Kyn	Kynurenin	VitAC	Vitamin C
Lyso	Lysozyme	Vit/E	Vitamin E
Mn	Manganese	Zn	Zinc

system, including Vit C, Vit E, glutathione, melatonin, alpha-lipoic acid, carotenoids, trace elements copper, zinc, selenium, etc. Most antioxidants in the antioxidant system can be independently detected with existing biochemical and/or molecular biology technologies. However, detection of each antioxidant separately is time-consuming, laborious, expensive, complicated, and inaccurate. The reason for this inaccuracy is that the antioxidants have a synergistic effect in the same system and will produce a superimposed effect (Akki et al. 2019). In addition, both the oxidizing substance and the antioxidant substances have properties that we do not yet know. Therefore, the determination of one and/or several oxidative or antioxidant substances or their metabolites cannot correctly evaluate the status of oxidation or antioxidants in the body (Wang et al. 2011). In addition, oxidative/antioxidant substances can be classified into known and unknown. Using existing medical laboratory testing methods, the known oxidation/antioxidants can be detected, but it is time-consuming and laborious. The unknown ones still cannot be detected. Otherwise, the effects of different oxidation/antioxidants can be superimposed (Feng

Table 3 OxS biomarkers commonly used in clinical practice or research (oxidants)

Classification	Abbreviation	Full name
Oxidant	ALE	Advanced lipoxidation end product
	8-iso-$PGF_2\alpha$	8-Iso-prostaglandin $F_2\alpha$
	Fe^{2+}	Ferrous ion
	4-HNE	4-Hydroxy-2-nonenal
	HNA	4-Hydroxynonenoic acid
	HydrP	Hydroperoxide
	DDG	7,8-Dihydro-8-oxo-20-deoxyguanosine
	LO	Lipid alkoxyl radical
	LOOH	Lipid hydroperoxide
	LOO	Lipid peroxyl radical
	MDA	Malondialdehyd
	Nrf2	Nuclear factor-like 2
	OMP	Oxidatively modified proteins
	oxLDL	Oxidierte LDL
	di-Tyr	O,o′-Dityrosine
	ProC	Protein carbonyl
	SNST	S-Nitrosothiols
	NHPA	3-Nitro-4-hydroxyphenylacetic acid
	Cl-Tyr	3-Chlorotyrosine
Integral	OSI	Oxidant stress index
	TAS	Total antioxidant status
	TOS	Total oxidant status

et al. 2016). The detection of only a few oxidative/antioxidant substances does not represent a change in overall levels, because the changes of other oxidative/antioxidant substances are not clear.

Therefore, the concepts of TAS and TOS are derived (Zhang et al. 2019). TAS represents the overall level of enzymes and non-enzyme antioxidants in the organism (Toczewska et al. 2020). It is also called total antioxidant capacity (TAC), total antioxidant activity (TAA), total antioxidant power (TAOP), total antioxidant response (TAR) or total reactive antioxidant potential (TRAP), etc. TAS is synonymous with the body's total antioxidant level. It not only represents the sum of enzymes and non-enzyme antioxidants in the body but also reflects the relationship of mutual connection and synergism between the antioxidants. There is a close relationship between the strength of the body's antioxidant defense system and its health and disease status. When it decreases, it will inevitably cause inflammation, tumors, and immune system diseases. Therefore, the TAS level reflects the comprehensive information of the body's antioxidant capacity in different states.

Compared with TAS, TOS represents the overall level of all oxidants in the oxidation-antioxidant system that maintains the body's antioxidant defense capabilities (Toczewska et al. 2020). It is also named total peroxide (TP), serum oxidation activity (SOA), reactive oxygen metabolites (ROM), oxygen radical absorbance

capacity (ORAC), or some other synonyms. TOS is synonymous with the total oxidation level of the body (Morvaridzadeh et al. 2020). Like the TAS, it not only represents the sum of oxidations in the body but also reflects the relationship of mutual connection and synergism between oxidations. Oxidation is an essential component of the human body's antioxidant defense system. There is a close relationship between the strength of the body's antioxidant defense system and health or disease states. When the oxidation is elevated, it will inevitably cause inflammation, tumor and immune system diseases. Therefore, the TOS level reflects comprehensive information about oxidizing ability in different states (Taravati and Tohidi 2018).

TAS and TOS are necessary detection indicators to fully reflect the antioxidant effect of the human body. At present, both TAS and TOS can realize automatic detection, with high precision and good reproducibility. This can be used for the detection of any biological sample and is easy to popularize (Jansen and Ruskovska 2015).

Taking Hitachi automatic biochemical analyzer as an example, the instrument setting parameters for automatic detection of TAS and TOS are shown in Tables 3 and 4, respectively. Other brands and models of automatic biochemical analyzers can refer to this parameter and instrument performance, and settings can be easily modified (Peluso and Raguzzini 2016).

Measurement of Total Antioxidant Status

The following is a brief introduction of TAS full-automatic detection method.

Detection Principle

The TAS assay relies on the ability of antioxidants in the sample to inhibit the oxidation of the peroxidase methemoglobin from ABTS (2,2′-azino-di-3-ethylbenzthiazoline sulfonate) to $ABTS^{+}$. The amount of $ABTS^{+}$ produced can be monitored at 600 nm using an automatic biochemical analyzer. Under the reaction conditions, the antioxidants in the sample suppress the absorbance at 600 nm to an extent proportional to the concentration.

Instrument Settings

It is easy to adjust the test parameters according to the principle of the experiment, reagent composition, and instrument performance on different biochemical analyzers or spectrophotometers. The overall antioxidant levels in the samples are calculated using a certain concentration of antioxidant. TAS values are expressed as mmol Trolox equivalent/L (mmol Trolox equiv/L).

Measurement of Total Oxidant Status

The following is a brief introduction to TOS full-automatic detection method.

Detection Principle

The various oxidation substance in samples can oxidize ferrous ion (Fe^{2+}) into high iron ion (Fe^{3+}) in an acidic medium, and then react with xylenol orange to produce

Table 4 Serum TOS/TOS/OSI level before treatment of some in situ tumor [median(P25, P75)]

Tumor(n/male)	age	TOS	TAS	OSI
Liver cancer (107/73)	47.7 ± 16.6	19.4(15.2, 26.6)	1.34(1.08, 1.66)	1.38(1.01, 2.43)
Gastric Carcinoma (119/76)	54.5 ± 13.1	20.9(16.1, 28.4)	1.28(0.86, 1.70)	1.51(1.14, 3.32)
Colorectal cancer(120/80)	52.4 ± 15.7	18.8(14.3, 26.2)	1.12(0.82, 1.49)	1.80(1.00, 3.39)
Breast cancer (128/0)	52.4 ± 17.1	21.1(15.3, 27.5)	1.38(1.02, 1.75)	1.64(0.89, 2.67)
Lung cancer (150/131)	49.3 ± 15.8	20.4(16.0, 27.8)	1.14(0.82, 1.45)	1.90(1.25, 2.77)
Esophageal cancer(117/78)	53.4 ± 14.6	22.2(16.4, 28.0)	1.29(0.93, 1.66)	1.46(1.06, 2.89)
Brain cancer (115/74)	47.2 ± 15.9	18.5(14.6, 23.9)	1.17(0.82, 1.43)	1.72(1.06, 2.65)
Kidney cancer (156/89)	53.4 ± 12.8	18.8(15.3, 24.4)	1.23(0.91, 1.53)	1.59(1.06, 2.42)
Lung cancer (44/21)	61.2(50.0, 70.0)	3.32(0.89, 36.3)	1.52(1.12, 2.02)	2.07(0.66, 26.7)
Breast cancer (91/0)	47.4 ± 8.3	23.12(17.18, 26.55)	1.45(1.01, 1.77)	1.61(1.05, 2.53)
Lung cancer (94/54)	57.34 ± 10.14	22.32(10.90, 33.90)	1.50(0.63, 2.00)	1.55(0.67, 3.43)
Healthy control (178/95)	52.4 ± 13.5	14.6(10.3, 18.0)	1.59(1.19, 1.92)	0.85(0.65, 1.49)

color. The color intensity is directly proportional to the concentration of TOS. The TOS concentration in the sample can be obtained by comparing with the hydrogen peroxide calibrator (unit: μmol/L) with a certain concentration handled under the same conditions. The results were expressed in μmol H_2O_2 equivalent/L (μmol H_2O_2 equiv/L).

Instrument Settings

It is easy to adjust the test parameters according to the principle of the experiment, reagent composition, and instrument performance on different biochemical analyzers or spectrophotometer. Similarly, the overall oxidant levels in the samples are calculated using a certain concentration of hydrogen peroxide. TOS values are expressed as μmol H_2O_2 equivalent/L (μmol H_2O_2 equiv./L).

Calculation of Oxidant Stress Index

The OSI is an index that reflects the state of redox balance in the human body. It can be calculated by the following formula (Wang et al. 2011; Feng et al. 2016):

$$\mathrm{OSI} = \mathrm{TOS}/\mathrm{TAS}.$$

When the TOS unit is μmoL H_2O_2equiv/L and the TAS unit is μmoL Troloxequiv/L, the above formula can be converted into:

$$\text{OSI (arbitrary unit)} = [(\text{TOS}, \mu\text{moL } H_2O_2\text{equiv/L})/(\text{TAS}, \mu\text{moL Troloxequiv/L}] \times 100$$

Compared with the level of healthy individuals, the individual test results of one or several oxidizing and/or antioxidant substances may increase or decrease in a physiological state or a pathological state. The most confusing thing is that TAS or TOS may also change (rise or fall) to varying degrees. However, when TAS and TOS simultaneously increase or decrease proportionally, the body will not produce OxS at this time if the OSI ratio does not change significantly (i.e., there is a small fluctuation within the allowable range). Compared with healthy individuals, if there is only one or several oxidants and/or antioxidants, or TAS or TOS levels change significantly, the observer may judge that OxS has occurred. However, if the OSI remains relatively stable, OxS will not occur. Therefore, OSI is the key indicator to judge whether the oxidation-antioxidation balance of the body is disordered, which leads to the occurrence of OxS (Sánchez-Rodríguez and Mendoza-Núñez 2019).

More and more researchers have realized that the correct evaluating method of the OxS status of the patient's body is to comprehensively detect the overall level of oxidative and antioxidant status in the subject. Many tumors have been studied, and the overall levels of serum oxidation and antioxidant status in cancer patients are shown in Table 4.

End Products of Lipid Hydroperoxide

The main target of reactive oxygen species is polyunsaturated fatty acids on the cell membrane, which can trigger lipid peroxidation and cause damage to cell structure and function. In addition, the decomposition of lipid hydroperoxide produces many end products, such as compounds containing aldehyde groups (malondialdehyde, MDA), keto groups, and hydroxyl groups (4-hydroxynonene, 4-HNE), organic hydrocarbons alkane, alkene, and new OFR. These can accelerate biological oxidation in cells (Venditti and Di Meo 2020).

Lipid peroxidation is a free radical chain reaction. There are two types of lipid peroxide formation (Su et al. 2019):

1. Enzymatic reactions: Some lipoxygenases can promote the reaction of oxygen with polyunsaturated fatty acids to form lipid peroxides. For example, 5-lipoxygenase and 12-lipoxygenase can promote the carbon atoms in the fifth and twelfth sites of arachidonic acid to be oxygenated to form 5-hydrogen peroxy-arachidonic acid and 12-hydrogen peroxy-arachidonic acid.
2. Non-enzymatic reaction: Polyunsaturated fatty acids have multiple double bonds, and more active hydrogen atoms are located on the methylene group between the two double bonds. For example, the dissociation energy of a CH bond in a

methylene group that is not affected by double bonds is 393.56 kJ/mol. The dissociation energy of the CH bond located in the methylene group and affected by two double bonds is 355.85 kJ/mol mole. Therefore, when polyunsaturated fatty acids are exposed to light, radiation, FRs, etc., they can easily remove hydrogen atoms from the methylene group located between the two double bonds to form lipid radicals. Then, double bonds and unpaired electron sites are transferred to form relatively stable conjugated double bonds; they react with oxygen to form products, such as lipid peroxy radicals and lipid peroxides.

Under the condition of light, radiation, or FRs, lipid molecules (LH) remove 1 hydrogen atom to form lipid FRs (L). Lipid FRs react with oxygen to form lipid peroxyl radicals (LOO·). Then, LOO· radicals attack other lipid molecules and seize their hydrogen atoms to generate lipid radicals (L) and lipid hydrogen peroxide (LOOH). Repeating the reaction in this way results in continuous consumption of lipids and mass production of lipid peroxides (Bayır et al. 2020).

RO, RO_2, and ROOH are lipid peroxidation products. However, the content of these lipid peroxidation products in the human body is extremely low under normal physiological conditions. Although there is a chance of lipid peroxidation, their products will be converted to harmless substances. Lipid peroxide can be decomposed into aldehydes, ketones, alcohols, ethers, carboxylic acids, and alkanes, of which malondialdehyde is the most representative lipid peroxidation product. Therefore, many researchers tested malondialdehyde to determine whether a lipid peroxidation reaction has occurred in a system (Zhang et al. 2019). However, in terms of the human body, it is extremely one-sided to determine whether OxS occurs in the human body in this way.

Lipid peroxidation products are commonly used as biomarkers of OxS or oxidative stress/damage (Su et al. 2019). Lipid peroxidation generates a variety of relatively stable end products for decomposition, which can then be measured as an indirect biomarker of OxS in biological samples (Conrad and Pratt 2019).

The antioxidant activity in vivo can be estimated by the changes in lipid, protein, and/or DNA oxidative damage markers in biological samples. However, most of these markers are nonspecific, and their detection may also be interfered by compounds from non-peroxidative origin. There are many methods available for detecting oxidative damage to human lipid, protein, and DNA (Zińczuk et al. 2020). A series of peroxidative products involved in the methods have been applied, including thiobarbituric acid-reactive substances (TBARS), converged dienes, hydrocarbons, lipid peroxides, F_2-iso standards, protein carbonyls, 8-hydrodeoxyguanosine, etc.

Among them, the method of detecting MDA based on TBARS reaction principle has been widely used because of its simple technology. However, it is interfered by compounds of non-peroxidative origin in human biological samples. It is also affected by Fe content in buffers and reagents. There are significant differences in the values of healthy subjects between different laboratories. High performance liquid chromatography has improved specificity, but it is not easy to be popularized because of the limitations of instrument prices and technical

difficulties. Taking these factors into account, only the spectrophotometry of MDA is introduced here.

Measurement of Malondialdehyde
In this chapter, a modified method for the determination of MDA by thiobarbituric acid spectrophotometry (TBA) is introduced.

Detection Principle
MDA in LPO degradation products can combine with thiobarbituric acid to form a red complex TBARS with the maximum peak at 532 nm. The concentration of MDA in the sample can be calculated by comparing it with the standard of equivalent tests.

Manipulation Steps
Step 1: Deproteination
We first added 200 μl of sample to a clean 5 ml test tube, then added 400 μl of reagent R1. Then, we shake vigorously or use a micro shaker to mix them thoroughly, and centrifuge at 5000 rpm for 10 min.
Step 2: Color reaction
We took 300 μl of supernatant and added 300 μl of Reagent R2. Then, the sample was then boiled in an open water bath for 10 min, removed, and cooled to room temperature.
Step 3: Colorimetric determination
We read the absorbance at the wavelength of 532 nm using a spectrophotometer. The concentration of MDA in the sample can be obtained by calculating the standard tube operated simultaneously.

This method is simple and easy to be popularized. However, it cannot be automatically detected because of the need for centrifugation and boiling, as can be seen from the Manipulation steps.

Conclusions

Oxidative stress refers to the imbalance between oxidation and antioxidant system in the body, which causes a pathological process of oxidative damage of cells and/or tissues. When OxS occurs, the oxidation-antioxidant system tends to be unbalanced in the direction of oxidation, resulting in inflammatory infiltration of neutrophils, this leads to increased secretion of proteases, and the production of a large amount of oxidation intermediates. Therefore, OxS is a negative effect from the FRs in the body. It can not only promote the aging of the body under physiological conditions but also promote the occurrence and development of diseases in pathological conditions. More than 95% of the FRs in the body are OFR, with the characteristics as follows: (i) the human body can not only produce FRs but also scavenge them to keep the dynamic balance. Thus, the body can protect cells, tissues, and organs from oxidative damage; (ii) OFR can not only cause damage to the body but also promote

certain physiological functions of the body; (iii) the production and removal of OFR are in a dynamic balance. If this dynamic balance is broken, it will cause damages to cells, tissues, and/or organs, leading to the occurrence and development of diseases.

Antioxidants are the substances that the body fights against OFR (oxidants). At present, there are many kinds of related biomarkers (i.e., OxS biomarkers) used to reflect the oxidation/oxidation status of the body. But so far, there is no widely accepted, highly specific OxS biomarker as an indicator for clinical disease diagnosis, risk prediction, and prognosis. Many oxidative damages may be cascade reactions. This not only have complicated disease course but also involve special tissue structures. Therefore, the use of a single biomarker of oxidative stress is very limited, because it can only reflect a certain stage or aspect of damage to cells or tissues. TAS, TOS, and OSI can reflect the state of OxS in the system (cell, tissue, organ, or whole body). In addition, the combined detection of them is the best choice to evaluate the OxS system. However, these three indices have no tissue specificity and can only reflect the overall level of the body. With the continuous development of science and technology, more understanding of proteomics, metabonomic, and bioinformatics will promote the development of OxS biomarkers with tissue organ specificity, high accuracy, and sensitivity to provide reliable clinical evidence for disease (including tumors) prevention and treatment.

References

Akki R, Siracusa R, Cordaro M, Remigante A, Morabito R, Errami M, Marino A (2019) Adaptation to oxidative stress at cellular and tissue level. Arch Physiol Biochem 1–11. https://doi.org/10.1080/13813455.2019.1702059

Bayır H, Anthonymuthu TS, Tyurina YY, Patel SJ, Amoscato AA, Lamade AM, Yang Q, Vladimirov GK, Philpott CC, Kagan VE (2020) Achieving life through death: redox biology of lipid peroxidation in ferroptosis. Cell Chem Biol 27(4):387–408. https://doi.org/10.1016/j.chembiol.2020.03.014

Cherubini A, Ruggiero C, Polidori MC, Mecocci P (2005) Potential markers of oxidative stress in stroke. Free Radic Biol Med 39(7):841–852. https://doi.org/10.1016/j.freeradbiomed.2005.06.025

Conrad M, Pratt DA (2019) The chemical basis of ferroptosis. Nat Chem Biol 15(12):1137–1147. https://doi.org/10.1038/s41589-019-0408-1

Cuffe JS, Xu ZC, Perkins AV (2017) Biomarkers of oxidative stress in pregnancy complications. Biomark Med 11(3):295–306. https://doi.org/10.2217/bmm-2016-0250

Ebrahimi SO, Reiisi S, Shareef S (2020) miRNAs, oxidative stress, and cancer: a comprehensive and updated review. J Cell Physiol. https://doi.org/10.1002/jcp.29724

Feng JF, Lu L, Dai CM, Wang D, Yang YH, Yang YW, Liu YS (2016) Analysis of the diagnostic efficiency of serum oxidative stress parameters in patients with breast cancer at various clinical stages. Clin Biochem 49(9):692–698. https://doi.org/10.1016/j.clinbiochem.2016.02.005

Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, Knight AR, Taylor EL, Oettrich J, Ruskovska T, Gasparovic AC, Cuadrado A, Weber D, Poulsen HE, Grune T, Schmidt HH, Ghezzi P (2015) Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal 23(14):1144–1170. https://doi.org/10.1089/ars.2015.6317

Gadoth N, Göbel HH (2010) Oxidative stress and free radical damage in neurology (oxidative stress in applied basic research and clinical practice). In: Park SY (ed) Reactive oxygen species in

mitochondrial encephalomyopathy: mechanisms and effects, 1st edn. Humana Press, Totowa, pp 253–292
Gelpi RJ, Boveris A, Poderoso JJ (2016) Biochemistry of oxidative stress: physiopathology and clinical aspects. In: Boveris A (ed) Mitochondria are the main cellular source of O_2^-, H_2O_2 and oxidative stress, 1st edn. Springer, Cham, pp 23–36
Ghezzi P, Jaquet V, Marcucci F, Schmidt H (2017) The oxidative stress theory of disease: levels of evidence and epistemological aspects. Br J Pharmacol 174(12):1784–1796. https://doi.org/10.1111/bph.13544
Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, Gupta PK, Pakuwal U (2014) Oxidative stress and antioxidants in disease and cancer: a review. Asian Pac J Cancer Prev 15(11):4405–4409. https://doi.org/10.7314/apjcp.2014.15.11.4405
Hensley K, Floyd RA (2003) Methods in biological oxidative stress. In: Castegna a protein carbonyl levels – an assessment of protein oxidation, 1st edn. Humana Press, Totowa, pp 161–168
Hwang ES, Kim GH (2007) Biomarkers for oxidative stress status of DNA, lipids, and proteins in vitro and in vivo cancer research. Toxicology 229(1–2):1–10. https://doi.org/10.1016/j.tox.2006.10.013
Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K (2020) Reactive oxygen species – sources, functions, oxidative damage. Pol Merkur Lekarski 48(284):124–127
Jansen E, Ruskovska T (2015) Serum biomarkers of (anti)oxidant status for epidemiological studies. Int J Mol Sci 16(11):27378–27390. https://doi.org/10.3390/ijms161126032
Kreuz S, Fischle W (2016) Oxidative stress signaling to chromatin in health and disease. Epigenomics 8(6):843–862. https://doi.org/10.2217/epi-2016-0002
Kundu S, Sinhababu S, Chandrasekhar V, Roesky HW (2019) Stable cyclic (alkyl)(amino)carbene (cAAC) radicals with main group substituents. Chem Sci 10(18):4727–4741. https://doi.org/10.1039/c9sc01351b
Lauridsen C (2019) From oxidative stress to inflammation: redox balance and immune system. Poult Sci 98(10):4240–4246. https://doi.org/10.3382/ps/pey407
Lee JH, Paull TT (2020) Mitochondria at the crossroads of ATM-mediated stress signaling and regulation of reactive oxygen species. Redox Biol 32:101511. https://doi.org/10.1016/j.redox.2020.101511
Lushchak V, Gospodaryov DV (2012) Oxidative stress and diseases. Intech
Marrocco I, Altieri F, Peluso I (2017) Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxidative Med Cell Longev 2017:6501046. https://doi.org/10.1155/2017/6501046
Morvaridzadeh M, Sepidarkish M, Daneshzad E, Akbari A, Mobini GR, Heshmati J (2020) The effect of pomegranate on oxidative stress parameters: a systematic review and meta-analysis. Complement Ther Med 48:102252. https://doi.org/10.1016/j.ctim.2019.102252
Niki E (2014) Biomarkers of lipid peroxidation in clinical material. Biochim Biophys Acta 1840(2):809–817. https://doi.org/10.1016/j.bbagen.2013.03.020
Peluso I, Raguzzini A (2016) Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. Pathol Res Int 2016:5480267. https://doi.org/10.1155/2016/5480267
Rahman I, Biswas SK (2004) Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues. Redox Rep 9(3):125–143. https://doi.org/10.1179/135100004225005219
Rajasekaran (2020) Oxidative stress mechanisms in the pathogenesis of environmental lung diseases. Oxid Stress Lung Dis 2:103–140. Edited by Chakraborti S, Parinandi NL, Ghosh R, Ganguly NK, Chakraborti T
Sánchez-Rodríguez MA, Mendoza-Núñez VM (2019) Oxidative stress indexes for diagnosis of health or disease in humans. Oxidative Med Cell Longev 2019:4128152. https://doi.org/10.1155/2019/4128152

Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and Ferroptosis. Oxidative Med Cell Longev 2019:5080843. https://doi.org/10.1155/2019/5080843

Taravati A, Tohidi F (2018) Comprehensive analysis of oxidative stress markers and antioxidants status in preeclampsia. Taiwan J Obstet Gynecol 57(6):779–790. https://doi.org/10.1016/j.tjog.2018.10.002

Toczewska J, Maciejczyk M, Konopka T, Zalewska A (2020) Total oxidant and antioxidant capacity of gingival crevicular fluid and saliva in patients with periodontitis: review and clinical study. Antioxidants (Basel) 9(5). https://doi.org/10.3390/antiox9050450

Tsukahara H (2007) Biomarkers for oxidative stress: clinical application in pediatric medicine. Curr Med Chem 14(3):339–351. https://doi.org/10.2174/092986707779941177

Umeno A, Biju V, Yoshida Y (2017) In vivo ROS production and use of oxidative stress-derived biomarkers to detect the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. Free Radic Res 51(4):413–427. https://doi.org/10.1080/10715762.2017.1315114

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Venditti P, Di Meo S (2020) The role of reactive oxygen species in the life cycle of the mitochondrion. Int J Mol Sci 21(6). https://doi.org/10.3390/ijms21062173

Villamena FA (2013) Molecular basis of oxidative stress: chemistry, mechanisms, and disease pathogenesis. In: Franco R (ed) Oxidative stress and redox signaling in carcinogenesis, 1st edn. Wiley, New York, pp 203–236

Wang D, Feng JF, Zeng P, Yang YH, Luo J, Yang YW (2011) Total oxidant/antioxidant status in sera of patients with thyroid cancers. Endocr Relat Cancer 18(6):773–782. https://doi.org/10.1530/erc-11-0230

Xiang M, Feng J, Geng L, Yang Y, Dai C, Li J, Liao Y, Wang D, Du XB (2019) Sera total oxidant/antioxidant status in lung cancer patients. Medicine (Baltimore) 98(37):e17179. https://doi.org/10.1097/md.0000000000017179

Yin H (2008) New techniques to detect oxidative stress markers: mass spectrometry-based methods to detect isoprostanes as the gold standard for oxidative stress in vivo. Biofactors 34(2):109–124. https://doi.org/10.1002/biof.5520340203

Zhang Y, Li Z, Ma Y, Mu Z (2019) Association of total oxidant status, total antioxidant status, and malondialdehyde and catalase levels with psoriasis: a systematic review and meta-analysis. Clin Rheumatol 38(10):2659–2671. https://doi.org/10.1007/s10067-019-04676-1

Zińczuk J, Maciejczyk M, Zaręba K (2020) Pro-oxidant enzymes, redox balance and oxidative damage to proteins, lipids and DNA in colorectal cancer tissue. Is oxidative stress dependent on tumour budding and inflammatory infiltration? Cancers (Basel) 12(6). https://doi.org/10.3390/cancers12061636