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Clinical oxidation parameters of aging

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Abstract

Aging is a complex progressive physiological alteration of the organism which ultimately leads to death. During the whole life a human being is confronted with oxidative stress. To measure how this oxidative stress is developing during the aging process and how it changes the cellular metabolism several substances have been pronounced as biomarkers including lipid peroxidation (LPO) products, protein oxidation products, antioxidative acting enzymes, minerals, vitamins, glutathione, flavonoids, bilirubin and uric acid (UA).

But none of them could develop to the leading one which is accepted by the whole scientific community to determine the life expectancy of the individual person or biological age or age-related health status. Further there are many conflicting data about the changes of each single biomarker during the aging process.

There are so many different influences acting on the concentration or activity of single substances or single enzymes that it is not possible to measure only one clinical marker and determine how healthy an individual is or to predict the life expectancy of the corresponding person. Therefore, always a set or pattern of clinical biomarkers should be used to determine the oxidation status of the person. This set should include at least one marker for the LPO, the protein oxidation and the total antioxidative status and ideally also one for DNA damages.

Keywords: *Biomarker, aging, oxidative stress, lipid peroxidation*

Abbreviations: *8OHdG, 8-hydroxy-2′-deoxyguanosine; AAS, aminoadipic semialdehyde; BCAA, branched-chain amino acid; BPDE-I, benzo[a]pyrene diolepoxide-I (I-compound); CAT, catalase; DNPH, 2,4-dinitrophenylhydrazine; E_{hc}, half-cell potential; ELISA, enzyme-linked-immunosorbent-assay; ESCODD, European Standards Committee on Oxidative DNA Damage; GC, gas chromatography; GC–MS, gas chromatography coupled with mass spectroscopy; GGS, γ-glutamic semialdehyde; GPx, glutathione peroxidase; GSH, reduced form of glutathione; GSH-S-T, glutathione S-transferase; GSSG, glutathione disulfide; GSSG-R, glutathione reductase; HNE, 4-hydroxy-2,3-trans-nonenal; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography coupled with mass spectroscopy; LPO, lipid peroxidation; MDA, malondialdehyde; MS, mass spectroscopy; PCO, protein bound carbonyls; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TLC, thin layer chromatography; UA, uric acid; UV, ultraviolet spectroscopy*

Introduction

Aging is a very complex progressive physiological alteration of the organism which ultimately leads to the death of the organism. The aging process is accompanied by a continuous accumulation of DNA mutations and missfolded proteins, changing capacities of the antioxidative systems where a lot of enzyme activities are affected as well. This all together makes the aged organism more vulnerable to diseases especially to degenerative diseases [1].

A lot of different factors such as nutrition, lifestyle, genetic background and smoking affect the aging process and therefore, there exist a whole variety of

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hypotheses which try to elucidate the mechanisms of aging [2–6]. These entire theses are based on the measurement of different biomarkers which change with the age of the investigated persons but none of the theories has been generally accepted [7]. As the aging process as well as several severe neurodegenerative diseases are known to be connected with oxidative stress we want in this review to summarize the effect of the aging process on the clinically most important biomarkers.

During the whole life the organism of a human being is confronted with oxidative stress on the one side from intrinsic origins as the mitochondrial power generation is leaking reactive oxygen species (ROS) and on the other side from extrinsic origins like the increasing air combustion in the cities by the traffic and industrialization [8,9]. To evaluate how this oxidative stress is developing during the aging process and how it changes the cellular metabolism several substances have been used. In the literature a wide multitude of potential biomarkers are pronounced including lipid peroxidation (LPO) products, protein oxidation products, antioxidative acting enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx) and glutathione reductase, minerals like Se, Mn, Cu and Zn, vitamins like vitamin A, C and E, glutathione, flavonoids, bilirubin and uric acid (UA) [10]. But none of them could develop to the leading one which is accepted by the whole scientific community to determine the life expectance of the individual person, its biological age or its age-dependent health status [11–15].

To be a reliable and clinically used biomarker for the monitoring of oxidative stress the potential parameter has to fulfill some requirements. First of all the measurement has to be highly reproducible. The value should be stable over the time from the sample drawing until the measurement and ideally even longer so that it would be possible at later time points to control or add further measurements. Freezing and thawing the sample again should not change the readout of the measurement.

As the treatment of ill patients is often dependent on the status of the related biomarker the measurement should be fast. Short times for a single measurement are also necessary for a high throughput, which makes it possible to screen several patients at the same time. This could then be done by an automated system. By comparing the lifespan of different species Ingram et al. [16] developed a strategy for identifying biomarkers of aging in long lived species.

As we are searching for a parameter which reflects the impact of oxidative stress on the aging process this value should not be influenced by other factors. For all so far used biomarkers it is known that they are influenced by either nutrition, lifestyle (smoking, sports and physical exercise), the individual genetic background or diseases and the medications against them. Therefore, it is almost impossible to compare the values of biomarkers between patients directly and this is the reason why large numbers of individuals are necessary to get significant differences in studies monitoring the change of biomarkers with age.

The influence of other factors is also the reason why flavonoids and polyphenols which are known to reduce the oxidative stress in the aging process and several age-related diseases cannot be used as clinical biomarkers for aging [17–20]. The concentrations of flavonoids and polyphenols in human tissues are much more dependent on the nutrition of the individual and therefore age-related changes are of minor impact and cannot be significantly differentiated.

Clinical biomarkers of aging

As there are a lot of factors which influence the different biomarkers of the aging process we will focus in this review only on the most important clinically used markers. These parameters are related to changes in the structure of proteins, DNA or lipids, or to the total antioxidative capacity of the individual. Table I and Figure 1 give an overview over the most commonly used biomarkers, the advantages and disadvantages and their change during aging. As this review is about the clinical use of biomarkers for oxidative stress during the aging process we will focus on studies of healthy human beings.

The unborn child is protected within the mother from oxidative noxes of the environment. Directly after birth the newborn is confronted for the first time with the oxygen of the surrounding air and the antioxidative mechanisms are not fully matured [21]. Therefore, it is not astonishing that some groups reported high values of oxidative biomarkers in newborns especially in preterm infants [22,23]. As this is only a temporary effect we will not discuss this phenomenon further.

Another problem is that the values for the oxidative stress in very old persons are often underestimated because of the fact that individuals with high oxidative stress and low antioxidative capacity either died before they get old or expire severe diseases so that they can not contribute to studies where healthy people are investigated [24,25]. Therefore, it is not astonishing that even very old individuals especially centenarians expire extremely low amounts of oxidative stress and a high antioxidative capacity [25–27].

As the oxidative markers are not always measured in the same organs and different methods are used by the different labs it is not surprising that the results are often contradictory. But even if the same samples are measured the results can differ by several orders of magnitude as the example of the ESCODD-study shows [28–33]. During that study aliquots of the samples with the same concentrations of 8-hydroxy-2-deoxyguanosine (8OHdG) either pure or within DNA

Table I. Overview of the clinical oxidation biomarkers of aging.

Biomarker	Abbreviation	Aging	Method	Advantage	Disadvantage	Literature
Lipid-based						
Polyunsaturated fatty acids	PUFA	↓	GC/HPLC	Easy handling *in vitro* and *in vivo*	Only little changes, sampling problems	[34]
Conjugated dienes		↑	UV/VIS	Easy to perform	Unspecific for purines, pyrimidines, heme proteins	[34]
Lipid hydroperoxides		↑	Iodometric, MS, electrochemical	Very specific and sensitive	Unstable products, expensive equipment	[34]
Hydrocarbon gases		↑	GC		Sample collection	[76,118,119]
F2-isoprostanes	PGF2	↑	GC–MS LC–MS(–MS)	Highly precise and accurate	Expensive, numerous isomers	[35–40]
Oxystyrols			GC, GC–MS		Time consuming	[120]
Thiobarbituric acid reactive substances	TBARS	↑	Fluorometrically,	Easy to measure, fast	Non specific for LPO, overestimation possible	[5,24,25,42–47]
Malondialdehyde	MDA	↑↓	HPLC		Metabolized rapidly	
4-Hydroxy-2-trans-nonenal	HNE	↑ ↓	UV	Very rapid	Only with fresh samples	[24,48–53]
			TLC, HPLC	High reproducible	Low recovery, time consuming	
Protein-based						
Protein bound carbonyls	PCO	↑↓	ELISA	High throughput	Time consuming	[24,43–45,49,60–68]
			Western blot	Very sensitive		
γ-Glutamic-semialdehyde &	GGS	↑	HPLC	Very sensitive	Expensive equipment	[54]
Aminoadipic-semialdehyde	AAS	↑	GC–MS	Very sensitive	Expensive equipment	[70]
Branched-chain amino acids	BCAA				No reference values	[56]
Lipofuscin		↑	Autofluorescence	Easy to perform		[58,67,71–76,78]
DNA-biomarkers						
8-Hydroxy-2′-deoxyguanosine	8OHdG	↑↓	Comet assay, HPLC, GC–MS ELISA	Very sensitive	Low sensitivity, time- and cost-consuming, expensive	[15,17,29–33,38,41,80–86]
DNA-adducts (I-compounds)		↑↓	32P-post-labelling		Radioactive lab necessary, large adduct spectrum influenced by many factors	[85,87–91]
Telomere length		↓	Electrophoresis		No reference values available	[92–96]
Antioxidative status						
Reduced glutathione	GSH	↓	Spectroscopic	Sensitive		[21,24,98,101–103]
Oxidized glutathione	GSSG	↑	Fluorometrically	Sensitive		[21,24,98,101–103]
Glutathione ratio	GSSG/(GSH + 2GSSG)	↑				[24]
Half-cell potential	E_{hc}	↑			Low illustrative value; recalculation recommended	[24,99]

Table I – *continued*

Biomarker	Abbreviation	Aging	Method	Advantage	Disadvantage	Literature
Uric acid	UA	↑ ↓	Spectroscopic		Influenced by nutrition	[24,25,107–112]
Vitamin A, C and E		↑ ↓	HPLC		Influenced by nutrition in high amounts, toxic	[43,47,113,114]
Antioxidative acting enzymes						
Superoxide dismutase	SOD	↑ ↓		Simple, sensitive	Inconsistent literature data	[11,45,46,48,68,102,115–117]
Glutathione peroxidase	GPx	↑ ↓		Enzyme assays;	Inconsistent literature data	[11,21,45,46,49,102,115,116]
Glutathione reductase	GSSG-R	↓		High throughput		[21,115]
Glutathione S-transferase	GSH-S-T	↓		Possibly due to		[115]
Catalase	CAT	↑ =		Microchips		[11,45,68,102,116]

Table I shows the most important biomarkers used in the past to evaluate oxidative stress. Their changes during the aging process and their advantages and disadvantages are also listed.

were measured in different laboratories with different methods.

Lipid peroxidation as biomarker

LPO may lead to severe disturbances in cellular structure and metabolism as lipids have important functions as membrane constituents, steroid hormones, retinoic acids and prostaglandins. Unstable carbon radicals from fatty acids can rearrange to short alkanes and conjugated dienes which are exhaled or react with oxygen further to peroxyl radicals and finally by hydrogen abstraction to result in lipid hydroperoxides. These first oxidation products are limited in their function as biomarkers in the clinical routine as they are either volatile or highly reactive. Some of them can easily react to secondary oxidation products like malondialdeyhde (MDA), 4-hydroxy-2,3-trans-nonenal (HNE), isoprostanes or oxysterols [34]. These secondary oxidation products influence the gene expression and protein synthesis and they can lead to further damage by crosslinking proteins.

F2-isoprostanes. A family of prostaglandin F2-isomers also called F2-isoprostanes is the result of a radical peroxidation of arachidonic acid. They can be measured in urinary excretion by the use of GC–MS,

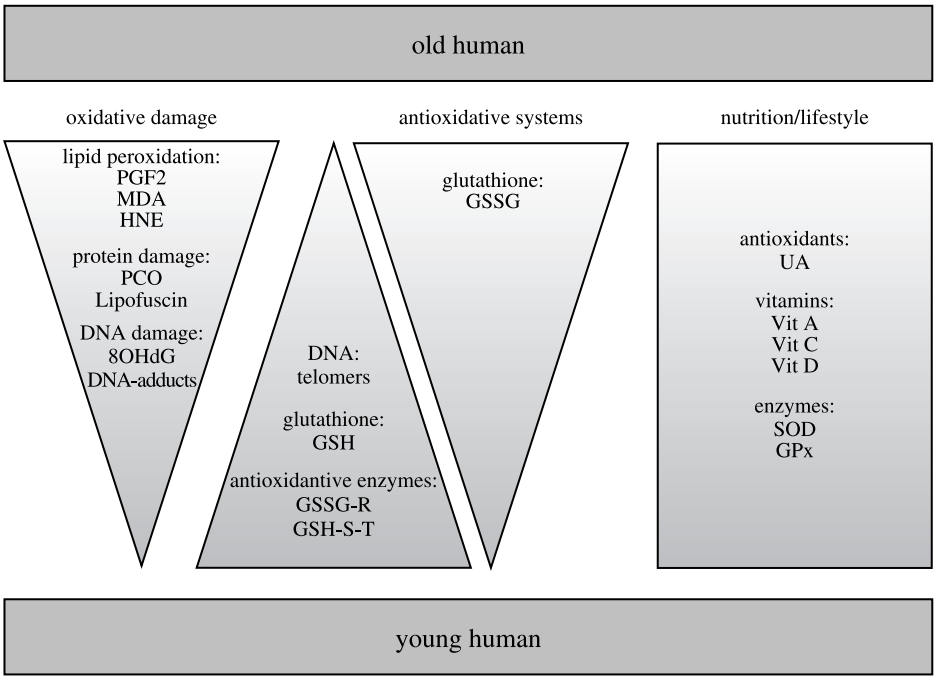

Figure 1. The changes of the most important biomarkers of oxidative stress during the aging process are shown.

LC–MS or LC–MS–MS [35–37]. As the method is highly precise and accurate it can be used as well in epidemiological studies as in clinical routine. The disadvantages of the method are the expensive equipment and there exist several isomers which have to be separated before quantification. During the aging process the concentration of F2-isoprostanes is increased in the urinary excretion [15,38–40]. Also during diseases like ischemic heart disease, diabetes and hepatic cirrhosis, cardiac failure the levels of isoprostanes are elevated. In contrast, Sacheck et al. (2003) reported similar increases in serum F2-isoprostane concentration of young and older men after eccentric exercise [41].

Therefore, F2-isoprostanes can be used routiniously as a sensitive and accurate biomarker of LPO for measuring the age-related changes of oxidative damage to membranes.

Thiobarbituric acid reactive substances including malondialdehyde. One of the most often used biomarker to investigate the oxidative damage on lipids is the fluorometrical measurement of thiobarbituric acid reactive substances (TBARS) which include MDA as their major compound. The measurement of this biomarker according to Wong et al. [42], is very easy to perform, fast and cheap. Nevertheless, the specificity of TBARS assay is low. Plasma is the most often used biological fluid for the determination of MDA. Plasma samples were therefore cooked with thiobarbituric acid, separated by high performance liquid chromatography (HPLC) and detected fluorimetrically. Several studies revealed a significant age-related increase in the plasma concentration of MDA [5,11,24,25,43–49].

As the measurement of MDA is so easy and fast to perform and the results are very reproducible it is one of the most valuable factors for the evaluation of the status of LPO.

4-Hydroxy-2,3-trans-nonenal. The total amount of HNE in organisms is usually very low. Therefore, a very sensitive method is necessary for the quantification. HNE can be measured either direct photometrically at 223 nm or after derivatisation by HPLC or GC–MS. The direct determination is very rapid but it is only possible with "fresh" samples. Stored samples require the derivatisation with 2,4-dinitrophenylhydrazine (DNPH), separation by thin layer chromatography (TLC), organic extraction, evaporation/concentration and quantification after HPLC separation [50–52]. This complex isolation method is accompanied by a low but reproducible recovery. Even though the HNE measurement is personal-, material- and time-consuming it is used because HNE is specific for LPO. As HNE is a very reactive substance it reacts very fast with different biomolecules and the accumulation in cells is inhibited by HNE metabolic enzymes which exist in all cell types [34].

While Diamond et al. [53] found a slight decrease of HNE with age in thyroarytenoid muscle our investigations showed significant positive correlation between the HNE concentration in plasma and the age of the individual [24]. The HNE values correlate positively with the MDA values and negatively with the antioxidative status of the glutathione system for the individual test persons.

Protein-based biomarker

Oxidation of proteins can lead to a whole variety of amino acid modifications like formation of protein bound carbonyl-, dityrosine-, methioninesulfoxide-formation. A detailed overview over several oxidative amino acid modifications, specific methods to detect them and their impact on cell metabolism is given in the reviews from Requena et al. [54] and Stadtman and Levine [55].

A quite new substance which is now investigated as biomarker is the assessment of branched-chain amino acid (BCAA). BCAA play an important role in protein and neurotransmitter synthesis. The big disadvantage of this marker is that it is dependent on many clinical states and optimal levels have not been published so far [56].

Beside the analysis of changes in single amino acid level like the determination of 3-nitrotyrosine, which is a (rather unspecific) marker for peroxinitrite mediated damage [57] there exist two more general biomarkers of protein oxidation. These are the formation of protein bound carbonyls (PCO) by the oxidation of the aliphatic side chains of alanine, valine, leucine and the acid side chain of aspartate which can be removed from the protein backbone via β-scission and second the accumulation of protein aggregates also called lipofuscin [58].

Protein bound carbonyls. It is estimated that almost every third protein in a cell of older animals is dysfunctional as enzyme or structural protein due to oxidative damage [59]. Therefore, the measurement of the protein oxidation is a clinically important factor for the prediction of the aging process and age-related diseases. The protein bound carbonyls are derivatized with DNPH and the corresponding hydrazone is analyzed by an ELISA-based method [60,61] or immunohistochemically as described by Mehlhase et al. [62] and by Chevion [63]. An age related increase in the PCO concentration was already reported for tissues like heart, muscle or brain [64–68] as well as in plasma of healthy people [24,44,45,49,69].

Traverso et al. [43] measured lower levels of protein bound carbonyls in the over 90-year-old people compared to younger. This controversial effect may be explained by the fact that often only very healthy

people with generally low oxidation status survive so long and take part in scientific studies.

It can be concluded that the measurement of protein bound carbonyls comprise a cheap, fast and reliable method to determine oxidative stress on protein level as the oxidative damage correlates well with aging and ever though with the severity of some diseases [63,69].

γ-Glutamic semialdehyde and aminoadipic semialdehyde. The two major compounds which comprise the group of PCO are γ-glutamic semialdehyde (GGS) and aminoadipic semialdehyde (AAS). They are the main oxidation products of proteins after metal catalyzed oxidation and can reach 55–100% of the total carbonyl content [54]. Daneshvar et al. (1997) developed a HPLC-based method to detect these two compounds [70]. Along with Requena et al. [55] they measured an increase of the concentration of oxidatively modified proteins with age which is complementary to the increase of PCO.

Lipofuscin. Oxidatively modified proteins are normally repaired or degraded by proteasome and lysosomes and thereby replaced by *de novo* synthesized proteins. The repair of oxidatively modified proteins is restricted to the reversion of few modifications on cystein and methionine residues. The major process is the degradation by the proteasome. If the oxidative damage is faster than the proteolysis rate the oxidized proteins accumulate within the cells. Depending on their location and composition these aggregates are called inclusion bodies, plaques, lipofuscin, ceroid, aggresomes, Lewy body or age pigment. Waste material which is stored in lysosomes is called lipofuscin. As lipofuscin comprises autofluorescence properties the quantification can easily be done by fluorescence measurement [71].

As the proteasomal and lysosomal systems are unable to degrade these aggregates, the accumulation is a progressive and continuously ongoing process for the whole life of the cell. In addition to this the high molecular weight aggregates are inhibitors of the proteasomal proteases and therefore they enhance their own formation as the removal of damaged proteins is slowing down. Furthermore, defective mitochondria by which the oxidative stress is even amplified are accumulated because of lacking proteasome in mitochondria [58,67,72–77]. Because of their ability to bind transition metals like iron and copper and as lipofuscin is a fluorochrome, it seems to sensitize lysosomes and cells to blue light a process which might be important for the pathogenesis of age-related macular degeneration [71,78].

A steady increase of accumulation of lipofuscin for postmitotic cells is a known inevitable hallmark of aging and also some neurodegenerative diseases are linked to elevated levels of proteinous material aggregation. As the measurement of the autofluorescence is fast and easy lipofuscin formation is a good clinical biomarker for age-related impairments in protein turnover by the proteasomal and lysosomal system.

DNA based biomarkers

Beside lipids and proteins also DNA is prone to oxidative stress and if the repair capacity is lower than the damage mutations which accumulate during the normal aging process are the outcome [64]. If the mutated genes are then transcribed and translated more and more malfunctioning proteins are the result.

8-Hydroxy-2′deoxyguanosine. The main ROS based DNA damage is 8OHdG an oxidative lesion of guanosine, which leads in the end to a point mutation due to misspairing [78]. Up to 200 oxidative modifications of guanine bases per cell and day occur [80]. 8OHdG is often used as biomarker for oxidative DNA damage because it is easy to measure by different methods like GC–MS, HPLC, LC–MS, immunohistochemical assay and spectroscopy. But as already mentioned above no consensus exists about normal values in healthy people [28–32,81]. 8OHdG lesions can be efficiently repaired. Imbalances between rates of oxidative modification of guanosine and of repair mechanisms may be the reason for conflicting results on 8OHdG accumulation. Whereas some workgroups found an age related increase in damaged bases [15,33,79,82–85] others detected no change or even a decrease in the 8OHdG level of elderly persons [41,80,86]. Other factors which are influencing the amount of detectable DNA damage are diet and the metabolic rate of the individuals [17,80]. Furthermore, those investigations were carried out in different organs and it is possible that the 8OHdG damage is accumulating in one or a few organs, while in the others the repair is more active and no DNA modification can be observed. As the energy metabolism is most intensive in the mitochondria the mt-DNA is exposed to more ROS in comparison with the nuclear DNA. Nevertheless, the role of mitochondrial dysfunction in the aging process is not yet fully understood [79].

Further 8OHdG is only one of approximately 20 described oxidative DNA modifications and for some of them no efficient repair system exists so that these damages accumulate with aging [80,82]. To our knowledge no studies exist concerning these damages in relation to age and therefore they cannot be used as clinical biomarker for the oxidative stress in aging.

DNA adducts. Reactive organic molecules can react irreversibly with nucleic acids and the results are covalently bound DNA adducts. The common method

for the detection of these adducts is the 32P-post-labelling method.

The adduct spectrum is wide and ranking from simple methylation of guanine to bulky adducts like benzo[a]-pyrene diolepoxide-I-DNA adducts (BPDE-I-DNA) also called I-compounds. Several researchers described an age related increase of I-compounds with age [85,87–89]. In contrast thereof the group of Hemminki [90,91] found no difference in the concentration of 7-methyl-, 7-(2-hydroxyethyl)-guanine and other adducts in peripheral lymphocytes of persons at different ages.

Besides aging, several other factors influence the amount and profile of DNA adducts. The profile of I-compound DNA adducts is species-, sex-, tissue- and diet-dependent [89].

The wide spectrum of DNA adducts and the special radioactive laboratory which is necessary for the 32P-post-labelling limit the clinical use of this biomarker of aging.

Telomere shortening. The length of telomeres, DNA sequences at the ends of linear chromosomes which contain repeated sequences, can be a valuable biomarker of oxidative stress during the aging process. In dividing cells telomeres are shortened by each cell division as DNA polymerases are not able to fully duplicate the ends of the chromosomes. In addition to that the telomeres can be shortened by oxidative damage and/or mutations. In human beings the telomeres shorten during aging and therefore they are also called molecular clocks. Even though several studies linked the cumulative oxidative stress to the length of the telomeres, reference values as well as the impact of other factors which influence length of telomeres are still missing [92–96]. Therefore, the role of telomere shortening in aging is not clearly presented. Several studies have linked the telomere attrition with the aging process [97].

Antioxidative capacity as biomarker

During the aging process the mitochondrial leakage of ROS is increasing and this leads to an increasing impairment of biological functions. To keep the normal metabolism up evolution has developed several antioxidative acting systems which can buffer the increasing oxidative stress. Therefore, also the concentrations of these substances can be used as readout for the aging process.

Glutathione system. The most important defence system against oxidative stress is the glutathione system. As for the glutathione synthesis cysteine is necessary the thiol pool of human plasma consists mainly out of cysteine. Under oxidative conditions reduced glutathione (GSH) is reversible oxidized to glutathione disulfide (GSSG).

Glutathione in its reduced form and glutathione disulfide can be assayed in a variety of assays including highly specific and easy-to-do assays [98]. Since the total redox capacity is more important than the total amount of reduced GSH often the percentage of oxidized glutathione which is given as GSSG/(GSH + 2 GSSG) is measured. From the concentrations of GSH and GSSG the half-cell reduction potential (E_{hc}) can be calculated according to the method of Schafer and Buettner [99].

During the aging process the plasma thiol concentration is declining while the amount of oxidized cysteine (cystine) is increasing [100]. This leads to an age-related decline in intracellular glutathione concentration and therefore, a decrease of the antioxidative capacity observed by several groups [21,24,101–103]. The decline in total plasma thiol (cysteine) concentration further accounts for an age-related decrease in the total plasma albumin concentration and an increase in the oxidized forms of albumin [104].

In some studies also gender specific differences are reported. The different oestrogen levels between man and women during the aging process should account for a higher antioxidative capacity in elderly women [105,106]. Even it is known that oestrogenic compounds contribute to the upregulation of antioxidative system-associated genes [106] the influences of oestrogens onto the glutathione system are not clear. In contrast, some other investigators found no significant gender specific differences [21,46].

Uric acid. UA as the major hydrophilic radical scavenger is present in high concentration both in intracellular and extracellular compartments. Since UA levels contribute to the antioxidant capacity of plasma this might be an important biomarker. It can be estimated by an enzymatic test using spectrophotometric detection at the absorption maximum of 293 nm [107].

Some authors reported an augmentation of UA levels with age in human plasma [108–110] while others found no significant difference [24] or even lower UA plasma level [25,111,112]. On the other hand, it is well known that UA concentrations are largely influenced by nutrition and therefore, oxidative stress might have a minor influence. This results in non-significant age related changes of UA plasma levels and contradictory data. Changes of the UA concentration in the plasma can therefore give a hint to cellular stress but UA is not applicable as clinical biomarker for the determination of specific oxidative damage during the aging process.

Vitamin A, C and E. Another group of substances which protects the cells against oxidative damage are vitamins. Especially vitamin A, C and E have antioxidative properties. Vitamin A also known as retinol is important for the maintenance of the vision. It can be synthesized

by its precursors within the carotenoids. The main field of action of vitamin C (ascorbic acid) is the defence of infections, and the collagen synthesis, too. Vitamin E, also called tocopherol, is necessary for the renewal of cells, to inhibit inflammatory lesions and it has beneficial effects on the immune system.

The concentrations of the vitamins A, C or E are strongly dependent on individual nutrition. This could be the reason why some groups reported higher values of vitamin A in elderly people [113] while others showed no effect [43] of age or even decreasing amounts of vitamin A in the plasma of old people [114]. For vitamin E the same extremes are published [47,113,114].

Further vitamins are not good clinical biomarkers for oxidative stress because they are often supplemented with the diet. But an excessive supplementation with vitamins can even lead to toxic effects as vitamin C for example interacts with the Fenton reaction and therefore it can even enhance the radical production.

Enzymes with antioxidative properties. Several enzymes are influenced by the oxidation status of cells. Some of them are specifically used to maintain the redox balance after oxidative stress. Therefore, changes in the amount or activity of these enzymes can serve as biomarkers for oxidative stress. Sometimes also minerals like selenium, manganese, copper and zinc are seen as antioxidants [10] but the main antioxidative action of these minerals is that they are necessary for the synthesis of antioxidative acting enzymes like SOD. Therefore, we will discuss here only the properties of the enzymes as biomarkers and not the properties of the pure minerals. The quantification of the enzymes can either be done by the measurement of the enzymatic activity, by immunochemical detection of the proteins or by analyzing the expression profiles of the corresponding RNAs. As these assays can be done on microchips several different enzymes can be measured at the same time. Therefore, a lot of clinical samples can be measured in a short time. But nevertheless the data published in the past are very contradictory.

Superoxide dismutases. Detoxification of superoxide is done by SOD. Some investigators found a decrease in SOD activity [11,45,102,115]. Other researchers could not detect an age-related difference [116] or they showed an increase with age [46,117]. These discrepancies can be in part related to the fact that humans express different SODs. Gianni et al. [68] discovered that there is no difference in total activity or CuZn-SOD activity while the activity of Mn-SOD increases with age.

Glutathione peroxidase. GPx oxidizes glutathione and lowers by this the antioxidative potential of the glutathione system. In the past it was reported that aging can lead to an increased GPx activity [11,21,115], a decreased activity [25,45,49,102] or age had no impact on the GPx activity [116].

Glutathione reductase and S-transferase. For glutathione reductase (GSSG-R) and glutathione S-transferase (GSH-S-T) only negative correlations with age were reported [21,115]. This corresponds with a shift of the glutathione system to the oxidized form, though with increasing oxidative stress for the cellular structures.

Catalase. CAT converts hydrogen peroxide and prevents the organism thereby from oxidative damage. Previous investigations found either no significant change [45,102,116] or increasing values of CAT activity with increasing age [11,68].

Conclusions

In the past a lot of molecules have been published as biomarkers for oxidative stress during the aging process but for some of them the data are very conflicting. One reason is that the different research groups take different methods for the quantification, therefore not all published markers are useful as clinical biomarkers. Another reason for the variations in the literature data is that there are so many different influences acting on the single biomarker as well as on the whole body that it is not possible to measure only one clinical marker and determine how healthy an individual is and to predict the life expectancy of the individual person. Therefore, always a set of clinical biomarkers should be used to determine the oxidation status of the person. This set should include at least one marker for the LPO, the protein oxidation and the total antioxidative status and ideally also one for DNA damages. Protein oxidation and proteosomal activity are linked to LPO. Since the proteasome has been shown to be involved in the elimination of oxidative modified proteins and to be inhibited during aging, it would be considered as a good biomarker for the aging process, too [121].

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