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# Sex-related biomarkers in cardiovascular and neurodegenerative disorders

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**Abstract**
Despite considerable advances in the treatment of human inflammatory diseases, such as cardiovascular and neurological disorders, they remain the leading cause of death in developed countries. From a clinical perspective, an active area of investigation focuses on the identification of diagnostic and prognostic biomarkers, because preventing events in those at risk of chronic inflammatory disease is likely to have a substantial impact on the overall public-health burden. The sex difference has not been considered previously as important in the evaluation of biomarkers of human diseases, notwithstanding it is now ascertained that the severity of these disorders is correlated with sex hormones which modulate the inflammatory response. The aim of the present brief review is to report and comment the state of art regarding the sex-related biomarkers in cardiovascular and neurodegenerative disorders, focusing on those compounds showing potential prognostic- and diagnostic values, and/or acting as indicators of the therapeutic treatment efficacy.

**Key words**
- sex differences
- biomarkers
- cardiovascular disease
- neurodegenerative disease
- oxidative stress

## BIOMARKER: DEFINITION AND CHARACTERISTICS

To predict cardiovascular and neurodegenerative risk, numerous biomarkers have been developed. Some of them are simple traditional biomarkers based on lipid profile and risk factors. Biomarkers are used in medicine to facilitate diagnosis, assess risk, direct therapy and determine efficacy of treatment.

In the World Health Organization definition a biomarker is any substance, structure, or process that can be measured in the body. A clinically useful biomarker must be able to meet one of the following criteria: i) show specificity and sensitivity for a certain disease (diagnostic); ii) have prognostic value; and iii) correlate with disease activity. A biomarker may be measured on a biosample (as blood, urine, or tissue test), it may be a recording obtained from a person (blood pressure, ECG, or Holter), or it may be an imaging test (echocardiogram or CT scan).

## SEX-RELATED BIOMARKERS IN CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the number one cause of death in industrialized countries. To estimate CVD risk, the Framingham Risk Score lists eight biomarkers considered classical risk factors [1]. Moreover, an increasing number of novel biomarkers, particularly markers of both inflammation and atherosclerotic burden, have been added to the classical risk factors. The classical risk factors include, some such as hypertension, diabetes, abnormal cholesterol, smoking, physical inactivity, and obesity that are considered modifiable; others, such as age, family history, and sex that are considered non modifiable. Novel biomarkers include molecules circulating in the blood or urine that can be used for both the diagnosis and the prevention of CVD. These molecules can be classified on the basis of their function (*e.g.*, marker of exposition, markers of effects, etc.) or in their biochemical or biologic properties (*e.g.*, proteins metabolites, hormones, cytokines, etc.).

Several epidemiological studies, in particular the Framingham [1], have investigated into the evolution of CVD hypothesizing the presence of a sex difference in the pathogenetic and progression determinants detectable in women and men.

Women and men differ in their manifestations of CVD in a multitude of ways. For instance, women develop CVD when they are about 10 years older than men and typically after the menopause [2]. Marked sex differences have been identified in the clinical manifestations of atherosclerosis and in the pattern of symptoms in the two sexes. In men, cholesterol has a main role than in women, in whom arterial hypertension, diabetes, and their combination has a greater importance in determining cardiovascular risk.

In women, the control of blood pressure and glucose

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metabolism should be a priority. Angina, the most common manifestation of coronary heart disease (CHD), is frequently uncomplicated in women, whereas in men it tends to evolve to an acute coronary syndrome. The clinical presentation of acute ischemic syndromes is also different in men and women and, because of the frequent atypical symptoms, women tend to underestimate the importance of them. However, considering that women are often excluded from research studies, sex differences in CVD remain a frontier for discovery. In this review only novel biomarkers sex-associated will be reported (*Table 1*).

Among novel biomarkers considered inflammatory markers, C-reactive protein (CRP), soluble CD40 ligand (sCD40L), IL-18, monocyte chemotactic protein-1 (MCP-1), and fibrinogen, have been typically linked to endothelial cell (EC) activation. In human plasma, CRP levels are one of the most powerful predictors of atherosclerosis and vascular death, and have been associated with risk of acute myocardial infarction, angina, and stroke. Higher CRP levels in women than in men have been reported [3, 4]. sCD40L, released in the peripheral blood from ECs, macrophages, activated T lymphocytes, and platelets, is involved in the pathogenesis of atherosclerosis via its inflammatory and prothrombotic properties. An association between high levels of sCD40L and CRP with microalbuminuria has been found in hypertensive premenopausal women and not in men [5].

IL-18, a member of the IL-1 cytokine family, is highly expressed in atherosclerotic plaques. After post-myocardial infarction its concentration has been found higher in men [6]. MCP-1 is a chemokine responsible for the recruitment of monocytes to sites of inflammation that appears to play a critical role in the promotion of plaque instability [7]. Different levels of MCP-1 have been found between men and women. In particular, it has been shown that the subjects with high estrogen status have significantly lower plasma MCP-1 levels than subjects with low estrogen status [8].

Fibrinogen is a glycoprotein mainly synthesized in hepatic cells and in megakaryocytes. It can bind to GpIIB/IIIa surface proteins creating bridges between platelets. Moreover, being involved in the coagulation cascade, fibrinogen stimulates smooth muscle cell migration, promotes platelet aggregation, and increases blood viscosity [9]. Fibrinogen is associated with atherosclerosis and thrombosis and is considered an independent risk factor for CHD, myocardial infarction (MI), and stroke. It has been reported that fibrinogen levels are generally higher in women than in men of the same age, and that in women plasma fibrinogen rises with menopause [10].

**Table 1**
Sex-associated biomarkers in cardiovascular diseases

| Men | Women |
|---|---|
| IL-18 | CRP |
| MCP-1 | sCD40 |
| sP-selectin | Fibrinogen |
| ox-LDL | s-ICAM |
| | ADMA |
| | BNP |
| | Troponin |

It is known that endothelial dysfunction is a risk factor for ischemic events such as stroke, myocardial infarction, unstable angina pectoris, ventricle fibrillation, and death from cardiovascular reasons. It has been hypothesized that some soluble molecules play an important role in the pathogenesis of EC injury and progressive formation of atherosclerotic lesions. Among these molecules, soluble intercellular adhesion molecule-1 (sICAM-1), soluble P-selectin (sP-selectin), asymmetric dimethylarginine (ADMA), and oxidized low-density lipoprotein (ox-LDL) are sex-associated biomarkers.

sICAM-1 and sP-selectin have been demonstrated to promote the adherence of monocytes and lymphocytes to ECs contributing to atheroma formation [11].

sICAM-1 has been associated with increased risk of CVD-associated death in women but not in men [12].

sP-selectin mediates the rolling of blood cells on the surface of the endothelium and initiates the attachment of leukocytes circulating in the blood to platelets, ECs, and other leukocytes at sites of tissue injury and inflammation. Its levels have been found higher in the hypertensive patients, and significantly higher in the hypertensive patients with diabetes [13]. In relation to sex disparity, higher levels of sP-selectin have been detected in plasma from men with metabolic syndrome [14].

ADMA is an endogenous competitive nitric oxide synthase (NOS) inhibitor derived from the hydrolysis of methylated proteins and is constantly produced in the course of normal protein turnover in many tissues, including vascular ECs. An increase of ADMA plasma levels induces endothelium dysfunction, which becomes clinically evident by impaired endothelium-dependent vasodilation, platelets hyperaggregability, and enhanced monocyte adhesion [15, 16]. Interestingly, sex- and age-dependent differences have been found in ADMA plasmatic concentration (*i.e.* increased in plasma of women at the onset of menopause with respect to age-matched men) [17].

Ox-LDL may be formed by oxidative processes during migration of the LDL particles in the vessel wall. Its plasma levels are correlated with the presence of thrombotic lesion morphology in patients with unstable angina. Ox-LDL binds to a lectin-like specific receptor, a membrane glycoprotein expressed in ECs, macrophages, VSM cells, and platelets, and induces atherosclerosis by stimulating monocyte infiltration and smooth muscle cell migration and proliferation. Its plasma levels are correlated with the presence of thrombotic lesion morphology in patients with unstable angina. Increasing levels of ox-LDL seem to be preferentially associated with loss of systolic function in men (with less impairment of diastolic function). By contrast, in women, systolic function remains better preserved but with decreasing diastolic function.

Brain natriuretic peptide (BNP), a member of the natriuretic peptide family, is a biomarker reflecting left

ventricular dysfunction. In healthy populations, women tend to have higher levels of BNP than do men, perhaps as a result of estrogen-mediated stimulation and androgen-mediated suppression. This sex-specific difference seems to be less pronounced in the settings of heart failure and other disease states in which natriuretic peptides are upregulated.

Troponin is a component of the contractile apparatus in the myocardium. Its circulating levels are essential for diagnostic assessment and risk prediction in patients with symptoms of unstable coronary artery disease.

In both sexes, higher levels of troponin predict an increased risk of death or MI, although troponin seems to be a stronger marker of recurrent MI in women than in men.

## SEX-RELATED BIOMARKERS IN NEURODEGENERATIVE DISEASES

The mammalian brain is sexually dimorphic, exhibiting significant structural and functional differences between the sexes [18] as well as different vulnerabilities to Central Nervous System (CNS) disorders including some neurodegenerative diseases (ND), such as Alzheimer's disease (AD) and Parkinson's disease (PD) [18]. Accurate diagnosis of ND will be crucial to treatment development and ultimately, to the ability to offer earlier effective therapeutic interventions. Definitive diagnosis in non-genetic ND can currently be made with histopathological confirmation, and usually only at post-mortem examination [19]. *In vivo* clinical diagnosis of ND is difficult especially in the earliest stages: specialist centers typically only achieve an accurate premortem diagnosis in 70-90% of cases [19]. Tremendous efforts have been made in recent years to identify the neuropathological, biochemical, and genetic biomarkers of the diseases so that the diagnosis could be established in the earlier stages [20]. At the time, some blood, plasma, serum or cerebrospinal fluid (CSF) biomarkers for detection and tracking of the preclinical and clinical stages of AD have been proposed. These methods include CSF detecting levels of amyloid-β (Aβ42), total tau (t-tau) and phospho-tau (p-tau) [21], for AD, while probably alpha-synuclein is the most promising assay for diagnosis and evolution of PD [22]. Furthermore, neuroimaging techniques may provide useful adjunctive information for the early diagnosis of neurodegenerative disorders: magnetic resonance imaging (MRI), functional MRI and positron emission tomography (PET) scan imaging, as well as neuropsychological tests for cognitive performance. However, there is a pressing need for efficient, cost-effective biomarkers that can help to diagnose patients earlier and more accurately to improve the therapeutic prospects.

Sex differences in vulnerability to neurological disorders might be due to sexual dimorphisms established during development as well as to adult brain levels of the steroid hormones 17β-estradiol (E2), progesterone, testosterone and their metabolites. Research has been extensive in trying to understand the interactions of sex chromosomes and hormones and the underlying mechanisms of these devastating disorders [23].

The 2015 World Alzheimer Report updates data on the prevalence, incidence, cost and trends of dementia worldwide. With reference to Italy (the country with the largest number of studies), approximately 1.2 million of Italians are affected by Dementias [25].

AD is a chronic degenerative disease characterized by extracellular amyloid plaques resulting from the accumulation of Aβ42 and intracellular neurofibrillary tangles due to the aggregation of tau proteins [25]. Diagnosis of AD is based on subjective neuropsychological tests supplemented by late-stage biomarkers in CSF [26]. The early diagnosis may be important before the irreversible brain damage or mental decline has occurred. AD is more prevalent in women than in men. Although the incidence of AD in men and women increases robustly with age [27, 28], these age-related changes are not known, but many studies revealed that there has been surprisingly little research into the effect of sex on dementia. For instance, women getting AD also demonstrate greater cognitive declines compared with men. This even includes verbal skills that are stronger in healthy females compared with males [29-31]. These conditions suggest that there may be important sex differences among the possible biomarkers for AD, more specific in women or in men and that their discovery is important not only for the diagnosis, but also when studying the pathways involved in AD pathology. An Italian study comparing the results of 18FDG-PET imaging in men and women with equally severe AD showed that women had significantly higher glucose metabolism in the areas primarily involved in the pathological process of AD (the right inferior frontal, superior temporal and insular cortices, and the hippocampus), suggesting a sex difference in the disease process [32, 33].

Sex differences in the brain, such as in brain anatomy, age-related declines in brain volume, and brain glucose metabolism, have been documented and may be important in understanding AD etiology. Some genes involved in Familial Alzheimer's Disease (FAD) as presenilin 1 (PSEN1) and presenilin 2 (PSEN2) are widely expressed during brain development. Several mutations in these proteins have been associated with autosomal-dominant inherited forms of AD [34-40]. Their expression is regulated by various cellular and extracellular factors, which change with age and sex. Both age and sex are key risk factors for AD, but the issue of whether the expression of presenilins (PSENs) was influenced by the sex during brain development had been poorly investigated. Our study showed that both the transcript levels of PSENs, and the subset of neurons expressing these proteins in various brain areas of the developing post-natal brain have a different distribution in male and female rats, suggesting that their function(s) may contribute to sexual dimorphism in the brain, both at morphological and functional levels [41].

However, the full impact of sex as a basic biologic variable on this pathology remains elusive [42]. AD pathogenesis in women may be influenced by metabolic changes induced by gonadal hormones, such as estrogen, which is known to have a protective effect on the brain. Loss of estrogen during menopause could, in part, lead to the deficits seen in brain metabolism in

Mild Cognitive Impairment (MCI) and AD. Transgenic animal studies have provided evidence that both male and female gonadal hormones regulate AD pathogenesis. [43-45]. Current diagnosis methods are helpful in detecting ND when CNS damage has already occurred. The use of effective biomarkers has great significance for the prediction, diagnosis, monitoring, treatment, and prognosis of many diseases. Should be important to detect AD at very early stages to improve the therapeutic prospects.

In the last few years, some sex specific biomarkers for the identification of AD have been described (*Table 2*). Koran and colleagues found a significant interaction between sex and Aβ42 and total tau on longitudinal hippocampal atrophy and longitudinal decline in memory and executive function. They described that women with Aβ42 and total tau levels indicative of worse pathological changes, showed more rapid hippocampal atrophy and cognitive decline (46). Moreover, Ribeiro and colleagues showed that transthyretin (TTR) decreased in the CSF of AD patients suggesting that TTR could be a potential CSF biomarker in AD. In details, in MCI and AD groups, women showed significantly lower plasma TTR levels when compared to MCI and AD men, respectively, and to women control group. In the AD women group, TTR levels correlated with disease stage, reflecting disease severity. Moreover, the plasma estradiol levels in women and men showed a reduction in both groups. Thus, this study prompts TTR as an early plasma biomarker in AD indicating that disease modulation by TTR is sex dependent [47].

Another protein of interest, the progranulin (PGRN), constitutes a potentially invaluable biomarker for ND including frontotemporal lobar degeneration (FTLD) and AD. In fact, our study trying to assess the plasma PGRN levels in 107 AD patients, 36 FTLD patients, and 107 controls, showed that in female AD patients there was a positive correlation between PGRN levels and age. Although no significant differences were found between patients and controls, we showed higher levels of PGRN in females compared to males; in AD patients, a positive correlation between PGRN levels and age was observed in females, suggesting a sex-related involvement of PGRN in the pathogenesis of AD [48].

The Alzheimer's Disease Neuroimaging Initiative (ADNI) program hypothesized that plasma leptin, a 16 kDa peptide hormone synthesized and secreted specifically from white adipose cells, in individuals with MCI or AD could be a good biomarker. In fact, its levels were found lower than those of subjects with normal cognition (NC). Approximately 70% of both men and women with MCI have plasma leptin levels lower than the median values of NC. Additionally, half of these subjects carry at least one apolipoprotein-E4 (APOE-ε4) allele. A subgroup of participants also had CSF leptin measured. Plasma leptin typically reflected the levels of the peptide in CSF in all groups (Control/MCI/AD) in both sex. The data suggest that plasma leptin deficiency provides an indication of potential CNS leptin deficiency, further supporting the exploration of plasma leptin as a diagnostic marker for MCI or AD. The important question is whether leptin deficiency plays a role in the causation of AD and/or its progression. If this is the case, individuals with early AD or MCI with low plasma leptin may benefit from leptin replacement therapy. Thus, these data indicate that trials of leptin in low leptin MCI/early-stage AD patients should be conducted to test the hypothesis. However, when analyzed by sex, plasma leptin values in men were approximately half those of women regardless of group (NC, MCI or AD). MCI men had significantly lower plasma leptin than NC men [49].

Also in PD, in which a higher incidence and prevalence in men is observed, there are some sex differences. In fact, PD occurs more often in men than in women, such as described in an interesting meta-analysis performed on seven studies and reporting an increased relative risk of 1.5 [50]. Sexual dimorphisms in non-diseased basal ganglia and *substantia nigra* may partly explain this sex-specific risk. Estrogen, neuroprotective to the dopaminergic system may account for some of these differences. In addition, chromosome differences may contribute to the sex differences noted in PD, with interplay between chromosomal factors and gonadal hormone factors [51]. As well known, the histopathological hallmark of PD is the presence of fibrillar aggregates called Lewy bodies in the *substantia nigra*. The observation that α-synuclein is the main protein component of these aggregates and the discovery that mutations in the α-synuclein (SNCA) gene can cause PD suggest a central role for α-synuclein in the disease process. Hence, α-synuclein in human body fluids was hypothesized as being a good disease-linked candidate biomarker for PD [52]. The progressive accumulation and spread of α-synuclein pathology in patients with PD led to the hypothesis that CSF α-synuclein could particularly serve as a marker of progression. In fact, CSF α-synuclein levels have been shown to correlate inversely with PD severity. In our observational cross-sectional study performed on 69 patients with AD and 110 controls, we observed a significant sex difference in plasma α -synuclein concentrations [53]. In men, α-synuclein concentrations differed significantly according to disease progression. An explanation of these results could be related to the fact that the intracellular α-synuclein aggregation differs in men and women owing to a protective hormonal effect in women; in fact, estrogens

**Table 2**
Sex-associated biomarkers in neurodegenerative diseases

| Disease | Men | Women |
|---|---|---|
| Alzheimer | Leptin | Amyloid-β42 |
| | | Total tau |
| | | Transthyretin |
| | | Progranulin |
| Parkinson | Alpha-synuclein | LRRK2 |
| | Urate | |
| | DJ-1 | |
| Amyotrophic lateral sclerosis | | MTHFR |
| | | Testosteron |

dose-dependently inhibit α-synuclein aggregation and in particular, estriol and estradiol destabilize preformed fibrillar α-synuclein *in vitro*. Moreover, some *in vitro* evidences suggest that sex hormones may alter α-synuclein expression in the brain. Studying the sex-associated genetic expression in dopaminergic neurons, Cantuti-Castelvetri and colleagues observed a female-associated gene up-regulation in signal transduction and neuronal maturation [54]. They also observed a men-associated up-regulation of genes directly involved in PD pathogenesis, including α-synuclein: men therefore seem to produce more central nervous system α-synuclein than women. Caranci and colleagues also described an association between plasma α-synuclein concentrations and cognitive impairment, hallucinations, psychosis, apathy, sleep disturbances in men but not in women [52]. Indeed, some studies have highlighted gender-related cognitive dysfunction, such as prevalent deficits in verbal fluency and facial expression recognition in males, and visual-spatial deficits in women [55]; furthermore, the association with sleep disturbances only in male patients could reflect the male prevalence in these disorders so that several studies have shown an correlation between REM sleep behavior disorders and male PD patients.

Another molecule that could influence the clinical progression of PD is urate, a natural oxidant and iron chelator. Several studies have found lower plasma urate levels in women than in men. The impact of sex on the association between urate and PD is controversial. In fact, plasma urate in men is described to have an inverse correlation with the risk of PD in two prospective cohorts, but no sex-specific results were presented in another study by De Lau *et al* [56]; then, more studies are needed in the future to define the real influence of sex on the association between urate and PD [57].

Among biomarkers having sex-differences in PD, DJ-1 was found increased in urine exosome of male with PD. DJ-1 is an antioxidant protein that is autoxidized when exposed to oxidative stress, protects cellular contents, and regulates the gene expression of defense. Because oxidative stress is suspected as one of major causes of PD, DJ-1 and oxidized DJ-1 were extensively studied for their potentials as PD biomarkers using various biofluids such as CSF, blood, and saliva. Moreover, levels lower in female for leucine-rich repeat kinase 2 (LRRK2), another protein associated to PD were also found [58]. Mutations in the (LRRK2) gene are the most common genetic determinant of Parkinson disease (PD) identified to date. Orr-Urtreger and colleagues described a mutation of LRRK2 gene (G2019S) over-represented in women of Ashkenazi Jews with Parkinson disease, suggesting a gender effect on genetic frequencies of this mutation [59].

Amyotrophic lateral sclerosis (ALS), so called Lou Gehrig's disease, is a rapidly progressive, invariably fatal neurological disease that involves the death of neurons responsible for controlling voluntary muscles. The disease belongs to a group of disorders known as motor neuron diseases, which are characterized by the gradual degeneration and death of motor neurons.

In ALS most studies have found that sex has no effect on the disease outcome, but a higher frequency of bulbar onset disease was found among older women [60]. The search of biomarkers useful for monitoring disease progression and assessing treatment effectiveness is particularly imperative in ALS. Changes in circulating steroids throughout the course of ALS might influence the neuroprotective response against neurodegenerative damage. Gargiulo-Monachelli and colleagues showed that steroids in ALS bear a different profile in relation to gender: postmenopausal female patients showed significantly higher total (TT) and free testosterone (FT) serum levels compared to controls, as well as a reduction of progesterone/FT and dehydroepiandrosterone sulfate (DHEAS)/cortisol ratios [61]. Familial and sporadic ALS (SALS) have also influenced by genetics and some polymorphisms associated to the pathology have been found to be gender-specific. A study on methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms showed an association between female SALS and MTHFR T677T [62].

## SEX-RELATED BIOMARKERS OF OXIDATIVE STRESS IN CVD AND ND

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) participate in the normal aging process as well as in age-related diseases, such as atherosclerosis and ND. ROS/RNS can cause direct damage to the vascular wall, triggering several redox-sensitive signaling pathways, which ultimately cause pro-atherogenic changes. In clinical practice, the analytical measurement of ROS/RNS is very difficult due to their short half-life and to the applicability of the determination methods. The localization and effects of oxidative stress, as well as information regarding the nature of the ROS/RNS, may be gleaned from the analysis of discrete biomarkers of oxidative stress damage isolated from tissues and biological fluids.

Clinical studies have demonstrated a relationship between oxidative stress and inflammatory biomarkers. Inflammation plays a major role in life, allowing the organism to restore homeostatic balance in the case of infection and traumatic or ischemic tissue damage. Human inflammatory disorders, such as CVD and ND, are subjected to redox control being strongly modulated by the levels of ROS/RNS [63-66]. In inflamed tissues, ROS/RNS formation is increased by the activation of the related ROS/RNS-producing enzymes, and trigger diseases: i) by reaching concentrations that exceed cellular antioxidant defense mechanisms, ii) being produced in inappropriate cellular compartments, or iii) through a shift in the type of ROS/RNS being formed. Under these conditions, better known as oxidative stress, all the major cellular components (proteins, lipids, carbohydrates, nucleic acids, antioxidants) undergo oxidation and typical reversible/irreversible post-transcriptional modification reactions occur (*i.e.*, oxidation, nitrosation, nitration). Consequently, cell undergoes to death not only for the increase of irreversible oxidative damage, but also for the antioxidant inability to reduce the reversible modifications, which accumulate. In view of these events, clinical relevance of oxidative stress biomarkers has been critically analyzed for their ability to be used in both healthy and pathological conditions,

with prognostic- and diagnostic values, as well as indicators of the therapeutic treatment efficacy [64-66].

Convincing evidence for the association of oxidative/nitrosative/nitrative stress and acute/chronic diseases lies on validated oxidative stress biomarkers. In general, several markers of oxidative stress still represent a possible biomarker opportunity for clinical use [64-66]. However, visualization of biomarkers measured in human CVD and ND shows that the majority oxidative stress-related target modification is a nuanced phenomenon, and their specificity with regards to a single disease is lost [64, 65]. This phenomenon is conceivable bearing in mind that increased ROS/RNS formation and redox unbalance are the major causes of inflammatory pathological conditions, so that the same oxidative stress-related biomarkers could result altered in different inflammatory diseases.

The evaluation of oxidative stress biomarkers in human healthy and diseased population is particularly important when considering that the severity of inflammatory status appear to be correlated with the levels of female sex hormones [67, 68]. In a healthy population, however, it is not clear whether tissue oxidant status and redox balance are significantly different between males and females. Indeed, conflicting results have been reported regarding the physiological levels of oxidant concentration (superoxide anion- and nitric oxide-derived species), oxidation markers (isoprostanes, malondialdehyde, lipid peroxidation), and antioxidant levels (glutathione, superoxide dismutase, catalase, glutathione peroxidase, etc.) measured *ex vivo* in blood of males and females [69-71].

Nevertheless, it is now evident that male and female hormones differently affect ROS/RNS-driven intracellular activities, thus contributing to explain the gap of knowledge between diagnosis, prognosis, and outcome of diseases characterized by significant alterations of redox state [72]. Indeed, estrogens modulated several ROS/RNS-producing enzymes (*i.e.*, NOS, xanthine oxidase, NADPH oxidases, cytochromes, the pro-oxidant myeloperoxidase) [72], as well as important antioxidant redox-related enzymes [73, 74], among which glutathione peroxidase, whose prognostic value has been recently investigated [73] (*Figure 1*). This hypothesis was

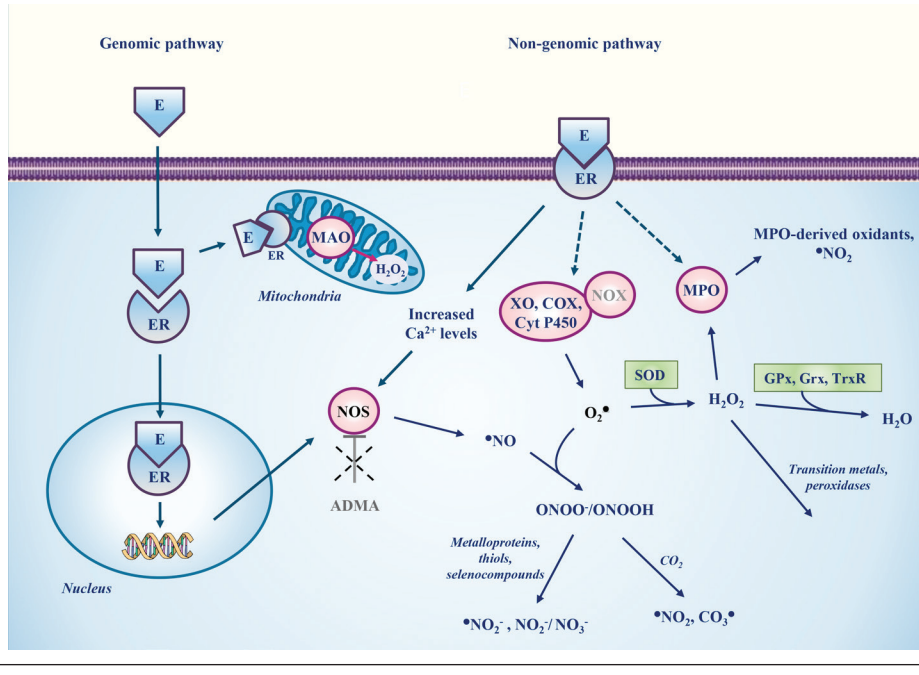


**Figure 1**
Estrogens (E) signaling is thought to affect the activities of ROS/RNS-producing enzymes (pink circles) and redox-related antioxidant enzymes (green rectangles) in human tissues by binding to E receptors (ER). In the genomic pathway, E bind to cytosolic/nuclear ER increasing the transcription of nitric oxide synthase (NOS) leading to increased nitric oxide (·NO) production. In the non-genomic pathway, E bind to mitochondrial- and/or membrane-ER. In the first case, the activation of monoamine oxidase (MAO) could increase $H_2O_2$ production contributing to mitochondrial disfunction. In the second case, the E binding to membrane-associated ER could i) stimulate $Ca^{2+}$ release from the endoplasmic reticulum leading to NOS activation, ii) activate Cycloxygenases (COX), Xanthine Oxidase (XO), Cytochrome P450 (Cyt P450) and Myeloperoxidase (MPO), leading to subsequent superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide ($H_2O_2$) production, strarting chemical cascades generating additional damaging molecules, such as ·OH (generated by the $O_2^{\cdot}/H_2O_2$ reaction with transition metals), peroxynitrite ($ONOO^{\cdot}$) (generated by the $O_2^{\cdot}$ reaction with ·NO), peroxynitrite-derived highly oxidants, *i.e.* the carbonate radical ($^{\cdot}CO_3$) and the nitrating agent nitrogen dioxide ($^{\cdot}NO_2$), and peroxidase ferryl species; iii) inhibit the activity of the $O_2^{\cdot}$-producing enzyme NADPH oxidases (NOX), iv) increase the activity of key antioxidant enzymes, *i.e.* Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), glutaredoxin (Grx), and Thioredoxin Reductase (TrxR), and v) decrease the concentration of asymmetric dimethylarginine (ADMA), the NOS endogenous inhibitor NOS. Dashed lines indicate uncleared mechanisms. Marked in grey: inhibitory effects of E treatments. $NO_2^{\cdot}$ and $NO_3^{\cdot}$: nitrite and nitrate, respectively.

supported by the *in vivo* finding that menopause is associated with significant down regulation of antioxidant gene expression [75]. A possible protective estrogen effect on vasculature *in vivo* has been hypothesized, since in cell cultures estradiol increased nitric oxide release and decreased the concentration of ADMA [76]. Finally, potential understimated oxidative stress biomarkers of importance for human inflammatory diseases could be the two isoforms of monoamine oxidase (MAO). These mitochondrial enzymes generate significant hydrogen peroxide amounts in the catalytic process leading to the oxidative deamination of endogenous/exogenous amines, including neurotransmitters. Their activity has been reported to be increased in CVD and ND and their inhibition allowed beneficial effects in animal experimental models of these diseases [64, 66]. Interestingly, estrogens have been reported to modulate MAO activities (*Figure 1*), which were decreased in amenorrheic and post-menopausal woman after estrogen treatment [77]. It is very difficult to draw general conclusions on the significance of sex-related oxidative stress biomarkers in pathological conditions, because even the sex-unrelated oxidative stress biomarkers currently under study in human diseases still need to be validated in larger sample sizes and/or compared with current clinical standards to establish them as clinical diagnostics. However, we would underline that the growing interest for the redox medicine in human health has recently allowed to consider the disease-triggering ROS/RNS-generating enzymes as suitable clinical therapeutic targets for the disease-relevant oxidative stress [64, 66]. In the light of the reported evidences, a sex-perspective should drive future clinical studies before starting any pharmacological modulation and drug candidate development regarding oxidative stress-related human diseases.

## CONCLUSIONS

In conclusion, research studies that have looked for differences in CDV and ND prevalence, progression, and phenotype support differences between men and women. However, sex has not been considered previously important in diagnostic approaches, but should be considered in both future clinical and laboratory studies. Very little research has been conducted to potentially explain such sex differences in these pathologies. Certainly, no studies of biomarkers have considered sex, and yet it is entirely possible that development of effective treatments may differ by gender.

### *Conflict of interest statement*

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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