

# **Oxidative Stress inside the pathogenesis of Ascending Aorta Aneurysms: a clearer Vision for Identifying Promising Biomarkers and Therapeutic targets**

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**Abstract:** Ascending aorta aneurysm (AsAA) is a complex disease, currently defined an inflammatory disease. In the sporadic form, AsAA has, indeed, a complex physiopathology with a strong inflammatory basis, significantly modulated by genetic variants in innate/inflammatory genes, acting as independent risk factors and as largely evidenced in our recent studies performed during the last 10 years. Based on these premises, here, we want to revise the impact of reactive oxygen species (ROS) and oxidative stress on AsAA pathophysiology and consequently on the onset and progression of sporadic AsAA. This might consent to add other important pieces in the intricate puzzle of the pathophysiology of this disease with the translational aim to identify biomarkers and targets to apply in the complex management of AsAA, by facilitating the AsAA diagnosis currently based only on imaging evaluations, and the treatment exclusively founded on surgery approaches.

**Keywords:** Ascending aorta aneurysm (AsAA); reactive oxygen species (ROS); oxidative stress; onset and progression of sporadic AsAA; management of AsAA; biomarkers and targets

## 1. Introduction

Advances, in the care of patients affected by the major number of cardiovascular diseases (CVD), have been achieved [1]. They have facilitated their management, and particularly their diagnosis with the appropriate use of biomarkers, and favoured the application of effective treatments, both pharmacological and surgical, improving the life's quality of patients [1]. Conversely, the aneurysms, and, particularly the sporadic ascending aorta aneurysms (AsAA), are currently characterized by a difficult management, as well as a limited prediction based essentially on classical risk factors, because of the missing of accurate molecular predictors [2,3]. Given the increasing incidence of AsAA in our population, and especially in elderly [4], the search for appropriate biomarkers with diverse applications from risk prediction and screening to diagnosis and prognosis, and the creation of specific algorithms useful in preclinical and clinical settings are imperative [4]. A part of AsAA biomarkers might be represented by inflammatory molecules, given their crucial role in both AsAA development and progression, and as largely evidenced by the results of our studies and summarized in a recent review [5-7]. But triggers of inflammatory responses, as well as optimal mediators (e.g., in the respiratory burst of professional phagocytes) [7], also are the high levels of reactive oxygen species (ROS), which evocate oxidative stress (OS) [8]. Recent evidence reports that ROS-induced OS is strongly linked to remodeling and degeneration of aorta wall, and especially of the aortic media [8]. They result by the evocation of important pathological conditions, including the apoptosis of smooth muscle cells, the fragmentation of elastic fibers, the degradation of collagen fibers, and the infiltration of inflammatory cells. Furthermore, excessive ROS levels have been demonstrated to induce the release of matrix metalloproteinases (MMP) and the evocation apoptosis of aortic smooth muscle cells (SMCs) [8-12].

Based on these premises, we want to revise the impact of ROS and oxidative stress on AsAA pathogenesis, and, consequently, on the onset and progression of sporadic AsAA. This might consent to add other important pieces in the intricate puzzle of the pathophysiology of this disease with the translational aim to identify biomarkers and targets to apply in the complex management of

AsAA, by facilitating the AsAA diagnosis currently based only on imaging evaluations, as well as the treatment exclusively founded on surgery approaches.

## **2. OS and AsAA: mechanisms and pathways involved as potential biomarkers**

### *2.1 Physiological actions of ROS in aorta wall and evocation of OS with the immediate effects on cellular components*

ROS have a relevant physiological role in aorta cells, whose levels result from a fine balance between ROS producers and ROS scavenge enzymatic systems [13-16] (see Table 1). Accordingly, ROS regulate the cellular homeostasis, cell differentiation and growth, and intracellular signaling molecules, such as phosphatases and kinases [17-24](see Fig. 1A). When such balance is absent, like when ROS production is abnormal, and/or when ROS scavenge (enzymatic) systems are impaired, OS occurs followed by an irreversible cell damage or death caused by the boosted lipid peroxidation of the biofilms of organoid and cell membranes, augmented intracellular calcium levels, denaturation of proteins, decremented activity of several enzymes, breakage of DNA and the consequent chromosome aberration, and the activation of inflammatory responses accompanied by the release of related mediators [17-24] (see Fig. 1A). Accordingly, elevated levels both of isoprostane, malondialdehyde and oxidized low-density lipoproteins (ox-LDL) related to lipid peroxidation, and nitrotyrosine, chlorotyrosine, carbonylation and S-glutathionylation have been assessed in patients affected by aorta disorders, such as aorta dilation and dissection [25-31]. Likewise, elevated levels of oxidative stress have been also detected in aortic tissues from animal models, and cases affected by Marfan and bicuspid aortic valve syndromes, and AsAA [25-31].

**Table 1. The most important enzymes and molecules involved in the ROS production**

ENZYMES	Biological Effect
NADPH OXIDASES (NOX1-NOX2)	Generate ROS. Major sources of ROS in the artery wall. The NOX2 phagocyte plays a role in catalysing the respiratory burst. NOX2 facilitating oxygen reduction, resulting the formation of superoxide.
MPO (ENZYME MYELOPEROXIDASE)	MPO is a peroxidase. Excessive levels of these toxic molecules cause tissue damage. MPO-producing macrophages can infiltrate TAA and are likely involved in the progression of TAA disease. MPO can exacerbate mechanisms: damage to the ECM and DNA, activations of inflammatory signaling and an increase in endothelial dysfunctions.
ERK1/2	Increased MMP expression. Increased oxidative stress due to inflammation. Can disrupt aortic wall homeostasis by altering the characteristics of VSMCs. Promote human aortic VSMC migration
3-NITROTYROSINE	Markers of MPO-mediated oxidative damage. Promote human aortic VSMC migration. Contribute to VSMC dysfunction
3-CHLOROTHYROSINE	Markers of MPO-mediated oxidative damage. Promote human aortic VSMC migration
MAPK SIGNALING PATHWAY	Expression of relevant target genes, including upregulation of MMPs.
MPO-DERIVED OXIDANTS	DNA modification, causing DNA damage. Affect VSMC DNA.
MPO-DERIVED HOCL	Damage to specific DNA bases. Inhibit DNA repair enzymes.
NITRIC OXIDE (NO)	Important regulator of vascular tone. Reduced NO production: Endothelial dysfunction
NOX4	Reduced elastin fragmentation, less endothelial dysfunction, and an increase in contractile markers

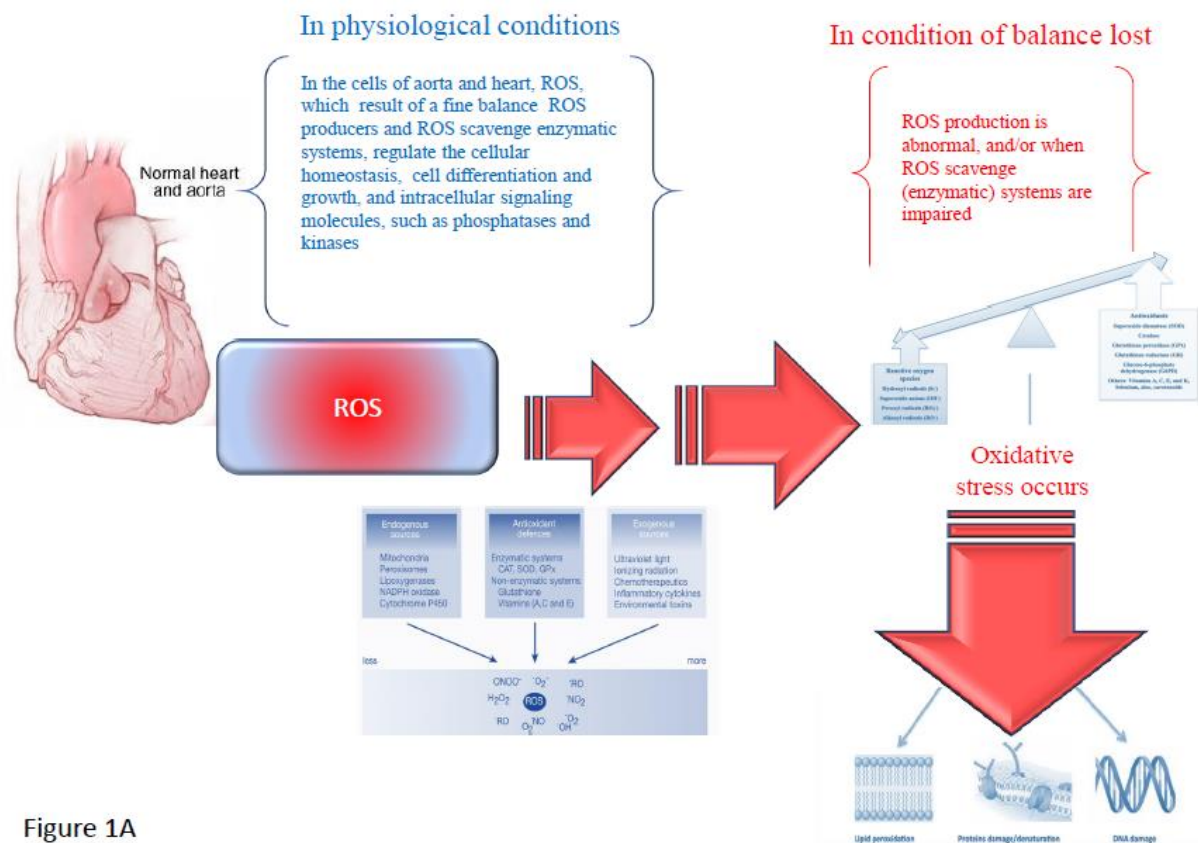


Figure 1A

Fig 1A. Ros in aorta in physiological conditions and in case of the lost of the balance between ROS producers and ROS scavenge enzymatic systems

## 2.2 OS and aorta media degeneration and remodeling: associated pathways

Elevated levels of ROS [8, 25-31] have been also demonstrated to induce both the release of matrix metalloproteinases (MMP), and the evocation of apoptosis of aortic SMCs. Such results in the media degeneration and aorta wall remodeling, which represent the typical pathological conditions significantly associated with the augment of dimension of aorta wall, that is with the aneurysm onset, and dissection [32]. Angiotensin (Ang) II has been demonstrated to be involved in the release of MMPs in OS condition (see Fig. 1B). On the other hand, Ang II perfusion represents the optimal strategy for creating mouse models of AsAA, because Ang II evokes the production of ROS in aorta cells and inflammatory cells, promoting mechanisms determining the formation of aortic dissection or aneurysm [17], i.e., the release of MMPs [32]. Accordingly, it has been demonstrated in mouse models that the reduction or better the deficiency of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1 (NOX1) significantly reduce the incidence of aortic dissection

induced by Ang II perfusion via the significant decrease of MMPs [32]. In addition, it has been also reported that the treatment with ursodeoxycholic acid prevents Ang II-induced NOX1 expression and evokes the inhibition of apoptosis of SMCs [32,33] (see Fig. 1B). Similar results have been obtained by using human brain vascular SMCs stimulated with recombinant secreted protein acidic and rich in cysteine (SPARC) *in vitro* [34]. The results found have revealed that SPARC proteins induce an increase expression of NOX proteins, and precisely of NOX4 via TGF- $\beta$ 1-dependent signaling pathway. This causes oxidative stress, pro-inflammatory matrix behaviour and apoptosis in human brain vascular SMCs. Thus, these proteins may be involved in the onset of intracranial aneurysms, by evoking OS and release of MMPs via activation of TGF- $\beta$ 1-dependent signaling pathway [34]. Precisely, the TGF- $\beta$ 1/ROS/NF- $\kappa$ B pathway has been demonstrated to mediate these effects, but also vascular SMC senescence and aneurysm formation in AsAA, BAV and Marfan Syndrome patients [5,6, 34-38]. Consistent with this, growing evidence reports that OS can activate NF- $\kappa$ B pathway, which is a well-recognized inflammatory driver of pathogenesis of AsAA. This promotes to release of cytokines, which contribute to further recruitment of circulating monocytes to the middle aorta and differentiate into active macrophages able to secrete MMPs and other ECM-degrading proteins and to hasten the onset and progression of AsAA [5,6, 34-38] (see Fig. 1B).

*In vitro* and *ex vivo* studies on human aorta tissues have also shown that ROS accumulation is significantly associated with the increased expression of the connective tissue growth factor (CTGF), whose levels appear to correlate with media degeneration [39]. Accordingly, CTGF has been demonstrated to regulate the synthetic phenotype of vascular SMC. This datum has been validated by using a murine model of TAA (C57BL/6J) based on Ang II infusion. The findings obtained have revealed that medial thickening and luminal expansion of the proximal aorta is associated with the vascular SMC synthetic phenotype as found in human aorta tissue samples [39].

Moreover, it has been also demonstrated that NOX, xanthine oxidase (XO), myeloperoxidase, (MPO), lipoxygenase (LOX), cyclooxygenase (COX), uncoupled endothelial nitric oxide synthase (eNOS), other amine oxidases, and non-enzymic sources including electron leakage from the

mitochondrial electron transport chain, result altered in expression and function in patients with AsAA(see Table 2) [40].

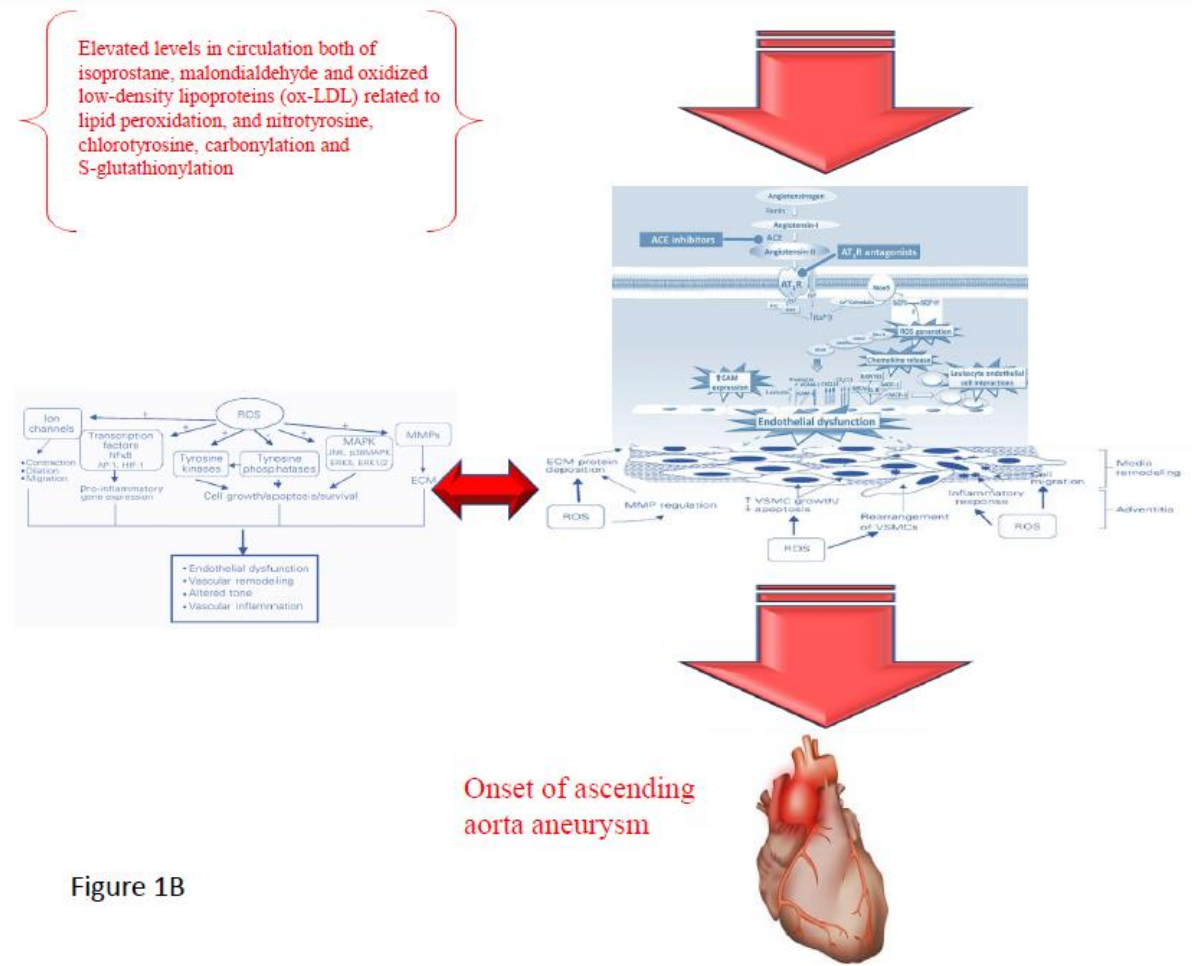


Figure 1B

Fig.1B The Redox-dependent signaling pathways activated by Ang II in endothelial and vascular smooth muscle cells. Intracellular reactive oxygen species (ROS) modify the activity of tyrosine kinases, such as Src, Ras, JAK2, Pyk2, PI3K, and EGFR, as well as mitogen-activated protein kinases (MAPK), particularly p38MAPK, JNK and ERK5. ROS may inhibit protein tyrosine phosphatase activity, further contributing to protein tyrosine kinase activation. ROS also influence gene and protein expression by activating transcription factors, such as NF-kB, activator protein-1 (AP-1) and hypoxia-inducible factor-1 (HIF-1). ROS stimulate ion channels, such as plasma membrane  $Ca^{2+}$  and  $K^{+}$  channels, leading to changes in cation concentration. Activation of these redox-sensitive pathways results in numerous cellular responses which, if uncontrolled, could contribute to hypertensive vascular damage. -, inhibitory effect; +, stimulatory effect; ECM, extracellular matrix; MMPs, matrix metalloproteinases.

Table 2. Biomarkers of oxidative stress involved in thoracic ascending aneurysm

Biomarkers	Biological Effect
Glutathione (GSH) system	Prevent the initiation of oxidative stress. Endothelial and smooth muscle cell dysfunction
Metallothionein (MT)	Prevent the initiation of oxidative stress increased aortic MMP-9 expression
Superoxide dismutases (SOD)	Transform RNS/RNS into less reactive species



Glutathione peroxidases (GPx)	Transform RNS/RNS into less reactive species
Peroxiredoxins (PRX)	Transform RNS/RNS into less reactive species
Nox4	Oxidative damage to multiple cytoskeletal and contractile proteins and elastic fragmentation
Xanthine oxidase (XO)	Alteration of contraction and relaxation, ECM degradation and aortic wall remodelling
SmgGDS (Small GTP-Binding Protein GDP Dissociation Stimulator)	Maintains the contractile phenotype of VSMCs. The deficiency induces severe aortic dilatation and severe elastic fragmentation, higher levels of ROS, MMPs and inflammatory cell migration
Myeloperoxidase (MPO)	Increased MMP-2 and MMP-9 expression, increased ECM fragmentation and apoptosis

### 2.3 Molecules and pathways related to OS attenuation

Sirtuin3 (Sirt3), a histone deacetylase having the function of regulating many cellular processes, has been demonstrated to reduce ROS levels, inflammation, and apoptosis of aorta SMCs. Sirt3 deficiency has been investigated and reported to be significantly related to an enhanced oxidative stress. Mice with thoracic aorta dissection have been shown to have a significantly decreased expression of Sirt3 respect to normal mice. Mice with Sirt3 knockout display a significant increased incidence of thoracic aorta dissection and aorta dilatation. In addition, the augmented Sirt3 overexpression has been significantly associated with a significant reduction in Ang II-induced ROS production, NF- $\kappa$ B activation, and apoptosis in human aortic SMCs. Consequently, Sirt3 overexpression diminishes aneurysm formation and reduces aortic expansion. Thus, Sirt3 deficiency is significantly associated with the increased susceptibility to of thoracic aorta dilation and dissection, because the Sirt-3-induced anti-ROS effects result attenuated versus a significant augment of apoptosis of aorta SMCs and inflammation [41].

In contrast of CTGF activity and the enzymes abovementioned, and similarly to the actions mediated of Sirt3[41], diverse intracellular enzymes, including superoxide dismutases, catalase, glutathione peroxidases, and peroxiredoxins (Prdxs), have the role of maintaining the ROS levels in a precise balance for reducing the eventual increase during the pathogenesis of diseases, such as AsAA [amply quoted in 42]. But evidence has recently reported that an impaired functioning of these enzymes, as well as a mitochondrial dysfunction, characterize patients affected by AsAA, as elegantly summarized by Portelli and co-workers [40]. Particular attention has been also given to



Prdxs, antioxidant enzymes involved in the regulation of OS and H<sub>2</sub>O<sub>2</sub>-mediated intracellular signaling, by other researcher groups. Accordingly, studies have confirmed an impaired activity and expression of Prdxs, a particularly of Prdx2 in patients affected by AsAA [42].

#### *2.4 Suggestions and recommendations for further investigations*

Several pathways mediate, thereby, the close relationship between OS and AsAA. They might be used as potential biomarkers of AsAA management. However, all these observations suggest that further scientific efforts are needed for the identification of biomarkers related to OS for AsAA and targets for the development of antioxidant therapies. To this goal, further advances may be realized by studying molecules associated with OS pathways through a new technological appraisal based on innovative approaches and systems. Integration of multi-omics analyses on genomics, epigenomics, transcriptomics, and proteomic profiles, with the recent metabolomics, microbiomics, and nutrigenomics investigations might be encouraged [43-45]. This might attenuate the limitations evidenced on the current use of myriad available methods for detecting ROS and ROS-damage, as recently suggested in the recommendations from American Heart Association [46].

### **3. From the experimental aspects to translational medicine: antioxidant treatments and targets**

Several pharmacological treatments and natural compounds with antioxidant proprieties exist. We describe below some of these, by evidencing the beneficial effects and limitations.

#### *3.1 Natural compounds and Mediterranean diet*

Natural compounds principally obtained from plants, but also from animals and microorganisms, have been well recognized, for thousands of years, as treatments of prevention and therapy for many human diseases [47, 48]. Of note are the phytochemicals, chemical compounds produced by plants for resisting to pathogens and having beneficial proprieties on human health. Accordingly, they have biological effects on diverse mechanisms, such as epigenetic modifications, modulation of signal transduction and metabolic pathways, and regulation of antioxidant enzymes activity [47, 48]. In support of their advantageous effects, several data have been reported in litera-

ture, and particularly for their anticancer activities [49] and the benefits for delaying or escaping the onset and progression of CVD [50] or other chronic pathologies [51]. The phytochemicals with antioxidant-rich proprieties comprise the polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, and fibers. They are generally utilized as functional food, soft drinks, and many other food items, and show a good nutrient value [52,53]. In animal AsAA models, their action has been revealed as effective, while human clinical trial studies have failed to demonstrate this evidence [53-56]. Precisely, Davies and Holt, by adopting two modelling techniques, that is Gillespie's Stochastic Simulation Algorithm (using Kinetiscope) and a discrete Markov chain, have evidenced the reasons, such as the rate of reaction between the free radical and the non-enzymatic antioxidant, which is considered a necessary threshold for evaluating the effect of an antioxidant than the free radical defence systems naturally present, able to dwarf their action [56]. In addition, it has been also evidenced that many times the active natural compound can result inactive because of isolation method [56]. Accordingly, the development of recent analytical and computational techniques has offered/ is offering new possibilities for treating and processing complex natural compounds for obtaining new and innovative drugs [57]. The use of quantum computing, computational software, and databases able in simulating molecular connections and calculating characteristics and factors required for the development of drugs, even if of natural, but also for evaluating their pharmacokinetic and pharmacodynamics, can facilitate this difficult aim, as recently evidenced by Thomford and coworkers [57].

Despite these limitations, new flavonoids have been tested, among these the diosmetin. The diosmetin is a citrus flavonoid with antioxidant and anti-inflammatory effects, and experimental studies have shown its capacity to ameliorate vascular dysfunction and remodeling by influencing the Nrf2/HO-1 and p-JNK/p-NF- $\kappa$ B expression in hypertensive rats [58]. Other natural compounds of recent examination also are, for example, the essential oils, which represent alternative drugs to natural and synthetic antioxidant agents [59,60]. Among these last, the essential oil of *Inula Montana*, containing the E,E-Farnesyl acetate results to be a new inhibitor with potent activity towards

the superoxide Dismutase (SODs) and ctDNA inhibition [61]. Of recent interest also is the hydroxytyrosol (HT), the major phenolic compound in olive oil. HT has beneficial properties, such as a remarkable antioxidant and anti-inflammatory power [62]. Accordingly, it has been recently studied the role of HT in the formation of advanced glycation end-products (AGEs), associated with the onset of diabetes, neurodegenerative and cardiovascular diseases. The data obtained have demonstrated the HT capacity of selectively inhibiting the protein glycation reaction in human insulin, and of counteracting the AGE-induced cytotoxicity by acting on sirtuins levels and oxidative stress, as well as on inflammatory response [62]

Consistent of these recent discoveries on HT, it has been also reported that Mediterranean diet may positively impact the cardiovascular system thanks to its antioxidant effects [63]. Precisely, two studies have tested the risk of abdominal aortic and cerebral aneurysms in relation to Mediterranean diet adherence [64, 65]. They have been conducted on very large sample size and have demonstrated that a Mediterranean diet based on high consumption of fruits, vegetables, wholegrains, legumes, nuts, fermented dairy products, fish, use of olive and/or rapeseed oil, moderate consumption of alcohol, and low consumption of processed and unprocessed red meat, may represent a positive factor for the prevention of two forms of aneurysm [64, 65]. No studies there are about AsAA and Mediterranean diet until now. However, the positive results, here described, encourage to execute this type of studies. On the other hand, preclinical studies, population studies and clinical trials recommend the MD adherence, with precise consumption of foods having high content of polyphenols, such as bio-phenols, including red wine, extra virgin olive oil (EVOO), green tea, spices, berries, and aromatic herbs [66, 67]. Although present the bio-phenols in low quantities in these foods, their quotidian consumption during the life of an individual may result in a significant reduction in the incidence of cardiovascular diseases, such as AsAA [66, 67].

Of note also are the recent data on quercetin, a natural flavonoid, commonly existing in nature, and precisely in tea, coffee, apples, and onions [68]. Precisely, very promising are the studies which have shown how the combination of quercetin with small antioxidant agents, such as resvera-

trol, luteolin, arctigenin, trehalose, curcumin, etc., can improve the therapeutic effect at lower doses by preventing the possible toxicity and its consequent effects during the treatment [68]. Likewise, it has been recently observed that the effect of other antioxidant agents can be increased by fusing or combining two or more compounds, having several properties [69]. An example can be given by a recent study, which has tested the effect of non-natural compounds, but of non-steroidal anti-inflammatory drugs fused with the antioxidant fractions 3,5-di-tert-butyl-4-hydroxybenzoic acid (BHB), its reduced alcohol 3,5-di-tert-butyl- 4-hydroxybenzyl alcohol (BHBA), or 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), a hydrophilic analogue of  $\alpha$ -tocopherol [69]. For validating the anti-inflammatory and antioxidant effects of these compounds, Machine learning algorithms were used. Fortunately, interesting results were obtained, that have evidenced significantly more increased antioxidant and anti-inflammatory activities of the new fused molecules than the parent molecules [69].

Thus, the combination and design of multifunctional compounds might potentially be more advantageous and represent a more efficient therapeutic approach for the disease discussed in this paper.

### *3.1 Innovative Technologies as targeted antioxidant treatments: nanomedicine and its benefits and limitations*

Modern technologies have been recently developed for treating OS and preventing the onset of cardiovascular diseases, such as AsAA. Among these, the use of nanomaterials and nanotechnologies [70, 71] consents to apply the nanomedicine, a specific targeted treatment able to improve the delivery of targeted drugs (i.e., natural antioxidant compounds or pharmacological drugs) using nanoparticles (NPs) (i.e., liposomes and niosomes to polymers, lipid and organic polymer hybrids and precursors, carbon nanotubes, quantum dots, metal, metal oxides), their bioavailability, as well as to reduce associated toxicity or side effects, and costs. Such innovative approach has been used, for example, for increasing bioavailability, stability, and the consequent beneficial effects of curcumin versus inflammatory related diseases [72]. Likewise, pH and ROS dual-responsive NPs engi-

neered by integrating pH- and ROS-responsive cyclodextrin materials with resveratrol have been employed as an efficient and secure nanoplatform for therapeutic delivery to sites of vascular inflammation, in view of the presence of acidosis and OS at inflammatory vascular sites [73, 74]

The promising results, which are obtaining, lead to believe that this approach can be used for ameliorating the ROS levels and reduce the susceptibility to aorta diseases, such as AsAA.

However, in the application of nanomedicine some considerations have be evaluated because it shows benefits, but also diverse limitations [70, 71]. Precisely, the nanomedicine based on use of the nanoparticles encapsulated with therapeutic compounds offers the advantage of overcoming the biological body barriers and improving the way for delivering compounds to specific tissues and organs, such as aorta with high levels of ROS, in our specific case. Furthermore, nanomedicine technology has another advantage to enhance the efficacy of therapeutic compounds, by reducing their toxicity or other side effects. Nanoparticles constitute one of the major technologies of nanomedicine, because combinations of physical, chemical, and biological technologies can be used for improving the *in vivo* performance of this therapeutic approach of last generation [70, 71]. Another important aspect of nanomedicine is the assessment of the biodistribution of the nanoparticles following their *in vivo* administration in animals and humans [70, 71]. It constitutes a very challenge despite the large range of techniques available for detecting nanoparticle biodistribution, including histology, electron microscopy, liquid scintillation counting (LSC), indirectly measuring drug concentrations, *in vivo* optical imaging, computed tomography (CT), magnetic resonance imaging (MRI), and nuclear medicine imaging. Thus, further investigations need for making this approach very effective [75].

### 3.2 New drugs: metformin, melatonin, and the necessity of validating the positive evidence

For stopping or retarding the development of AsAA and relieving its progression into aortic dissection, potential pharmacological treatments are investigating. At present,  $\beta$ -adrenergic blocking agents, losartan, irbesartan, angiotensin-converting-enzyme inhibitors, statins, antiplatelet agents, doxycycline represent the elective treatments used for AsAA [2,3]. However, as abovementioned

other drugs, such as those with antioxidant action, are object of study. Among these, metformin, a usual drug used in diabetes therapy, is emerging as a potent antioxidant agent [76]. Studies on model organisms have, indeed, demonstrated that metformin can ameliorate OS status and health status of cardiovascular system, aorta included, via diverse mechanisms: (a) reducing insulin and IGF-1 signaling; (b) inhibiting mTOR; (c) reducing the levels of ROS; (d) lowering inflammation, (e) reducing DNA damage, and (f) activating AMPK/acetyl-CoA carboxylase (ACC) pathway [76]. The last effect on the AMPK/acetyl-CoA carboxylase (ACC) pathway has attracted particular attention [76]. Consistent with this, Li and coworkers have recently demonstrated that metformin inhibits the onset of intracranial aneurysm and its progression by controlling the vascular smooth muscle cell phenotype switching via the AMPK/ACC pathway [77]. In addition to the recent work from Li group, other studies suggest that metformin can suppress progression of early aneurysms, such as abdominal aortic aneurysms [78]. This aspect has been recently pointed from Yu group, by performing a systematic review and metanalysis on eight studies and 29587 participants [78]. Relevant also appears, indeed, that in every population examined of eight studies an inhibitory effect on aneurysm growth in affected cases prescribed metformin for type 2 diabetes management has been significantly evidenced with a variation only in the magnitude [78]. Another interesting aspect reported from Yu group regards the little results existing in literature on efficiency of metformin in controlling the aneurysm onset in nondiabetic patients, even if various clinical trials are examining novel endpoints in other cardiovascular disorders, cancer and other pathologies related to OS status [79, 80]. Thus, such aspect needs to be cleared, although clinical trials on metformin for this objective are also planned or ongoing in European and American populations [80].

In the complex, the literature existing data consent to suggest that the metformin currently represents the most likely and efficient candidate as antioxidant and protective agent of all the forms of aneurysm, even if insignificant or better no data do exist on AsAA. Such question should be definitively addressed by performing an adequate number of clinical trials.

Another emerging antioxidant molecule is the melatonin (N-acetyl-5-methoxytryptamine), an indoleamine molecule highly and generally identified in the major number of plant and animal organisms, including human [81, 82]. Melatonin is synthesized from the essential amino acid L-tryptophan thanks to the action of four enzymes, and in vertebrates, including human, it is known as a secretory product of the pineal gland, even if it is a physiological cell component of other tissues, such as retina, skin, immune system, gastrointestinal tract, and reproductive tract [82]. In these last, melatonin is present at diverse levels with a higher density within the membranes and the mitochondria, where it has several functions, including the interaction with lipids, stabilization of all cellular membranes, reduction of lipid peroxidation, and increase of ATP production. In addition, it evokes several beneficial effects in context of antioxidant activities. Precisely, it has antioxidant and free radical scavenging capacity against ROS and reactive nitrogen species (RNS), that consent to melatonin to protect proteins and mt-DNA from OS [82]. In addition, it increases the activity of endogenous antioxidant enzymes and has anti-inflammatory properties related principally to SIRT1 activation [83]. Accordingly, melatonin has displayed, in apolipoprotein E-deficient mice, the ability to reduce the endothelial damage, the loss of SIRT1 and endothelial nitric oxide synthase, and the p53 and endothelin-1 expression. In addition, it has been also noted that melatonin confers a cardioprotective effect against myocardial ischemia-reperfusion injury, by reducing oxidative stress damage via activation of SIRT1 signaling in a receptor-dependent manner [83]. Likewise, it has been recently demonstrated in thoracic aortic aneurysm and dissection (TAAD) mouse models, that melatonin shows therapeutic effects against TAAD by reducing OS and VSMC loss via activation of SIRT1 signaling in a receptor-dependent manner, thus evidencing a novel therapeutic strategy for TAAD. Of course, both experimental basic investigations and clinical trials are necessary for its future clinical applications in AsAA [84].

#### **4. Prebiotics, Probiotics, and Synbiotics as other OS and aneurysm therapeutic opportunity**

The gut microbiota is described to have a substantial influence on the health of an individual, and its alterations, recognized with the term of dysbiosis, play a key role in the in pathogenesis



of several CVD, such as aneurysms, contributing to systemic endotoxemia and inflammation, atherosclerosis, and hypertension [85-88]. Accordingly, a recent study conducted in C57BL ApoE<sup>-/-</sup> mice with abdominal aortic aneurysms, evoked with angiotensin II (Ang II) (1000 ng/min per kg), demonstrated that the composition of gut microbiomes between control and AAA mice was diverse and correlated with abdominal aortic aneurysm diameter, as well as Linear discriminant analysis effect size of the genera Akkermansia, Odoribacter, Helicobacter and Ruminococcus correlated with the progression of abdominal aortic aneurysm [89]. Thus, the gut microbial dysbiosis could contribute to pathogenesis and progression of abdominal aortic aneurysm and might represent a potential target for further research [89]. Recent evidence also suggests that the origin of CVD, such as aneurysm can derive from early life. Accordingly, an increased number of studies reports that the health status of gut microbiota in early life is significantly associated with the onset of CVD in later life. In turn, growing evidence remarks the close connection between mitochondria, the gut microbiome and ROS [85-87]. The imbalance of gut microbiome leads a mitochondrial dysfunction and elevated levels of ROS. These last, reciprocally impacts human health, homeostasis of gut cells, and the gastrointestinal microbial community's biodiversity [85-87]. All this evidence has led to delve gut microbiota-based treatment modalities, including probiotics, prebiotics, and synbiotics,[90] which can restore symbiosis. Probiotics are represented by beneficial living microorganisms that can improve the intestinal microbiota profile. Prebiotics are constituted by non-digestible substrates that can increase the growth of beneficial gut microorganisms, such as resident microorganisms and probiotic strains. The combinations of probiotics and prebiotics leads to synbiotics that can impact in a synergic manner the gastrointestinal tract [91-95]. Experimental and clinical studies are reporting promising results [90, 96]. Precisely, it has been evidenced that probiotics reduce cholesterol levels, increase bile salt synthesis and bile acid deconjugation. Comparable effects have also been detected for prebiotics and synbiotics [90, 96]. However, probiotics also seem to have anti-oxidative, anti-platelet and anti-inflammatory properties. In addition, probiotics show beneficial effects in all the studied models, *in vitro* and in animal models, and in humans, where the probiotics supplementation

diminishes the OS and CVD risk [90, 96]. However, the probiotics, prebiotics and synbiotics produced in commerce have proprieties which remain unidentified, and consequently additional experimental investigation is required, so that these substances can be used for preventing and treating OS and CVD. Well-designed clinical trials are particularly mandatory to assess the impact of probiotics on trimethylamine-N-oxide (TMAO), which is supposed to be a CVD marker, and to elucidate the long-term effects, and action, of probiotic, prebiotic and synbiotic supplementation in combination with other drug therapy (for example, aspirin). Others are evaluating if probiotics can upregulate genes that are altered in CVD, such as intracranial aneurysms, and promising data have been obtained [97].

However, while it cannot be unambiguously indicated whether such supplementation generates benefits in the prevention and treatment of CVDs, it is of note that clinical studies performed up to the present time have revealed a beneficial effect for OS and dysbiosis without any side-effects to use.

## **5. Use news regulators of OS for developing targeted treatments**

As abovementioned, OS can modulate and alter the expression of gene profiles related to homeostasis of a tissue and onset of associated diseases. Concerning expression of genes, RNA binding proteins (RBP) have a key role in RNA expression and metabolism. Consequently, the appropriate control of these proteins is critical for cellular health [98]. Use of a clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9-single guide RNA library and stimulation of cells via paraquat has permitted to identify CSDE1 and STRAP proteins, interacting with each other, that convey sensitivity to OS and that Pumilio homologues (PUM1 and PUM2) convey resistance [98]. Targeting eIF4-E1 and -A1 protected cells from high-dose paraquat, whereas eIF4E2 targeted cells did less well. We also found that G3BP1 promoted sensitivity to a low dose of paraquat but protected cells at a higher dose. Thus, this study emphasizes that the use of genetic screens may consent to identify RBPs and novel genes regulating sensitivity to OS. They may be used for developing targeted treatments for OS [98].

Another class of novel OS regulators are Circular RNAs (circRNAs) [99], endogenous non-coding RNAs characterized by a covalently closed-loop structure generated through a special type of alternative splicing termed back-splicing. At this time, growing evidence has disclosed that circRNAs are evolutionarily conserved across species, stable, and resistant to RNase R degradation. They display cell-specific, and tissue-specific/developmental-stage-specific expression. Their biogenesis appears dissimilar from the canonical splicing of linear RNAs and is controlled by specific cis-acting elements and trans-acting factors [99-102]. CircRNAs act as regulators of diverse biological and pathological processes by sponging miRNAs, binding to RBP, that are as abovementioned regulators of splicing and transcription, modifiers of parental gene expression, and regulators of protein translation or being translated into peptides in various diseases [99-102]. CircRNAs have been detected in exosomes and body fluids, including human blood, saliva, and cerebrospinal fluids, evidencing that these exo-circRNAs can represent both disease biomarkers and novel therapeutic targets. Precisely, exo-circRNAs can be used as biomarkers of OS, because they are regulated by OS and induce ROS production as well as cause ROS-induced cellular death, cell apoptosis, and inflammation. These features make circRNAs as important regulators of diverse diseases, such as atherosclerosis, and other CVD, metabolic disease, and cancers [99-102].

Based on concepts stressed, circRNAs might represent another effective therapeutic approach of OS. The control of the expression of circRNAs in cardiovascular cells might consent the application of anti OS agents for significantly reducing endothelial dysfunction and vascular inflammation associated with the onset of CVD, i.e. aneurysms, and metabolic diseases [99-102].

## 6. Conclusions and Perspectives

It appears evident, today, the role of OS in the pathogenesis of different forms of aneurysm, AsAA included, even if additional studies are required for clearing the pathways involved. This might facilitate the identification of biomarkers. Here, we have reported some of these and suggested as potential targets for developing related treatments. Of note are the RBP proteins and circRNAs, which

are emerging not only as OS regulators, but also as biomarkers and potential targets for treatments. In addition, a large focus has been particularly given to emerging antioxidant agents of natural nature, such as diosmetin, as well as to combination of these, e.g., quercetin with resveratrol, luteolin, arctigenin, trehalose, curcumin, etc. These last have been demonstrated to have therapeutic effects at lower doses. However, it has been also evidenced the possible toxicity and its consequent effects during the treatment [68] as well as their failed action as beneficial for AsAA in human clinical trial studies because of some factors well evidenced. Possible solutions have been suggested, as well as the use more innovative technologies, such as NP or emerging drugs, i.e., metformin or melatonin, although further studies need for validating the beneficial evidence. A particular attention has been also given to Mediterranean diet, as well as to gut microbiota-based treatment modalities, including probiotics, prebiotics, and synbiotics [90, 96] which can restore symbiosis, since a close link has been demonstrated between gut dysbiosis and AsAA.

Certainly, all this evidence points and clears some aspects of the complex link between OS and onset and progression of AsAA, as well as encourages the treatments stressed, but it contemporarily appears not complete. Many gaps and limitations characterize it, as above reported. Consequently, further investigations are needed and possibly of multi-omics nature, which might provide an exhaustive portrait with the translational aim to identify biomarkers and innovative therapies to apply in the complex management of AsAA, by facilitating the AsAA diagnosis currently based only on imaging evaluations, and the treatment exclusively founded on surgery approaches. This might consent to add other important pieces in the intricate puzzle of the pathophysiology of this disease.

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