Pharmacology and perspectives in erectile dysfunction in man

Emma Mitidieri, Giuseppe Cirino, Roberta d'Emmanuele di Villa Bianca, Raffaella Sorrentino

PII: S0163-7258(20)30021-8

DOI: https://doi.org/10.1016/j.pharmthera.2020.107493

Reference: JPT 107493

To appear in: *Pharmacology and Therapeutics*

Please cite this article as: E. Mitidieri, G. Cirino, R. d'Emmanuele di Villa Bianca, et al., Pharmacology and perspectives in erectile dysfunction in man, *Pharmacology and Therapeutics*(2020), https://doi.org/10.1016/j.pharmthera.2020.107493

P&T #23442

Pharmacology and perspectives in erectile dysfunction in man

Emma Mitidieri, Giuseppe Cirino, Roberta d'Emmanuele di Villa Bianca*, Raffaella Sorrentino

Department of Pharmacy, School of Medicine and Surgery, University of Naples, Federico II, Via D. Montesano, 49, 80131, Naples, Italy.

*Corresponding Author: Department of Pharmacy, School of Medicine and Surgery, University of Naples, Federico II, Via D. Montesano, 49, 80131, Naples Italy, +39-081878457, +39-081678107, email demmanue@unina.it

Abstract

Penile erection is a perfect example of microcirculation modulated by psychological factors and hormonal status. It is the result of a complex neurovascular process that involves the integrative synchronized action of vascular endothelium; smooth muscle; and psychological, neuronal, and hormonal systems. Therefore, the fine coordination of these events is essential to maintain penile flaccidity or allow erection; an alteration of these events leads to erectile dysfunction (ED). ED is defined as the consistent or recurrent inability of a man to attain and/or maintain a penile erection sufficient for sexual activity. A great boost to this research field was given by commercialization of phosphodiesterase-5 (PDE5) inhibitors.

Indeed, following the discovery of sildenafil, research on the mechanisms underlying penile erection has had an enormous boost, and many preclinical and clinical papers have been published in the last 10 years. This review is structured to provide an overview of the mediators and peripheral mechanism(s) involved in penile function in men, the drugs used in therapy, and the future prospective in the management of ED. Indeed, 30% of patients affected by ED are classified as "nonresponders," and there is still an unmet need for therapeutic alternatives. A flowchart suggesting the guidelines for ED evaluation and the ED pharmacological treatment is also provided.

Keywords: Human corpus cavernosum; erectile function; erectile dysfunction; peripheral mechanisms; risk factors, cardiovascular disease.

Table of Contents

Abbreviations

3-mercaptopyruvate sulfurtransferase (3-MST);
Adenylyl cyclase (AC);
Angiotensin II (AngII);
ATP-dependent potassium channels (K_{ATP});
American Urological Association (AUA)
Bone marrow-mononuclear cells (BM-MNCs)
Botulinum neurotoxin (BoNT);
Cardiovascular disease (CVD);
Coronary artery calcium (CAC);
Cyclic adenosine monophosphate (cAMP);
Cyclic guanosine monophosphate (cGMP);
Cystathionine β-synthase (CBS);
Cystathionine γ-lyase (CSE);
Dopamine (D);
Electric field stimulation (EFS);
Erectile dysfunction (ED);
Follicle-stimulating hormone (FSH)
Gonadotropin-releasing hormone (GnRH);
Heme oxygenase (HO);
Human corpus cavernosum (HCC);
Hydrogen sulfide (H_2S);
Hypothalamic–pituitary–gonadal (HPG);
Index for Erectile Function (IIEF);
Luteinizing hormone (LH);
Metabolic syndrome (MS);
Myosin light chain (MLC);
Nitric oxide (NO);
Nonadrenergic noncholinergic (NANC);
Noradrenaline (NA);
NO synthase (NOS)
Oro-dispersible tablet (ODT);
Phosphodiesterase 5 (PDE5);
Prostacyclin (PGI2);
Prostaglandin D2 (PGD2),
Prostaglandin E2 (PGE2)
Prostaglandin F2α (PGF2α);
Protein kinase G (PKG);
Pyridoxal-5-phosphate- (PLP);
Radical prostectomy (RP);
Renin–angiotensin system (RAS);
Serotonin (5-HT);
Sexual Health Inventory for Men (SHIM)
Soluble guanylate cyclase (sGC);
Thromboxane A2 (TXA2);
Vasoactive intestinal peptide (VIP).

1. INTRODUCTION – PHYSIOLOGY OF PENILE FUNCTION

1.1 Penile erection and detumescence

Penile erection is a perfect example of microcirculation modulated by psychological factors and hormonal status. It is the result of a complex neurovascular process that involves the integrative synchronized action of vascular endothelium; smooth muscle; and psychological, neuronal, and hormonal systems (Gratzke et al., 2010). Therefore, the fine coordination of these events is essential to maintain penile flaccidity or allow erection. During sexual stimulation, nerve impulses release neurotransmitters from cavernous nerve terminals and relaxing factors from the endothelial cells, resulting in the relaxation of vascular smooth muscle and an increase of blood flow to the penis (Lue, 2000; Martinez-Salamanca et al. 2010). Simultaneously, the relaxation of the trabecular smooth muscle facilitates the rapid filling and the expansion of the sinusoidal system. The final result is the compression of the subtunical venular plexus between the tunica albuginea and the peripheral sinusoids that blocks the venous outflow. This process is known as the erection veno-occlusive mechanism (Udelson, 2007). In extreme synthesis, the blood is trapped within the corpora cavernosa, and this, in turn, causes an increase in the intra-cavernous pressure that, together with the contraction of ischiocavernous muscle, leads to the erect state of the penis (Dean & Lue, 2005). In parallel, the ischiocavernous and bulbocavernous muscles vigorously compress the spongiosum and penile veins, contributing to a further increase in the intracavernosal pressure and penile rigidity. During this phase, the inflow and the outflow of blood temporarily cease. During the detumescence, the contraction of the trabecular smooth muscle allows free flow to the emissary veins leading to the flaccidity state. Penis detumescence is characterized by three different phases: i) a transient increase in intracavernous pressure, associated with the beginning of smooth muscle contraction against the closed venous system, ii) a slow pressure decrease, due to a slow reopening of the venous channel, and iii) a fast pressure decrease with fully restored venous outflow capacity (Bosch et al., 1991).

1.2 Central and peripheral control of penile erection

Although penile erection is a vascular event that relies on the veno-occlusive mechanism described above, the central and peripheral nervous systems play a major role. Indeed, penile erection is primarily initiated by the supraspinal centers in response to auditory, visual, olfactory, tactile, and imaginative stimuli that can be elicited by peripheral neural pathways arising in either the sacral parasympathetic or the thoracolumbar sympathetic nuclei of the spinal cord (Dail et al., 1989). In particular, the sacral parasympathetic divisions (S2-S4) regulate penile tumescence, and inputs of the thoracolumbar sympathetic divisions (T11-L2) mediate detumescence.

The innervation of the penis is both autonomic (sympathetic and parasympathetic) and somatic (sensory and motor). From the neurons in the spinal cord and peripheral ganglia, the sympathetic and parasympathetic nerves merge to form the cavernous nerves, which enter the corpora cavernosa and corpus spongiosum and affect the neurovascular events during erection and detumescence. The somatic nerves are primarily responsible for sensation and the contraction of the bulbocavernosus and ischiocavernosus muscles. The nerve population, commonly classified as adrenergic, cholinergic, and nonadrenergic noncholinergic (NANC), modulates the functional state of the penis by releasing mediators that act on the vascular endothelium and/or the smooth muscle component of corpora cavernosa (Ignarro et al., 1990). During the state of flaccidity, a tonic sympathetic activity predominates. This involves the release of noradrenaline (NA) and other agonists leading to smooth muscle cell contraction within the corpora cavernosa.

Smooth muscle contraction and relaxation are principally regulated by cytosolic free calcium (Ca^{2+}). Indeed, through the activation of membrane receptors, NA and other mediators increase the intracellular messengers inositol triphosphate and diacylglycerol, leading to Ca^{+2} release from the intracellular stores. The binding of Ca^{2+} to calmodulin favors the interaction with myosin light chain (MLC) kinase and then the phosphorylation of MLC(s), followed by the activation of myosin ATPase, which, in turn, hydrolyzes ATP, thereby providing energy for muscle contraction. Once the cytosolic Ca^{2+} concentration returns to the basal levels, the calcium-sensitizing pathways

prevail. One such mechanism of contraction is the activation of excitatory receptors coupled to G-proteins, which increase Ca^{2+} sensitivity without any change in cytosolic Ca^{2+} levels. This pathway involves the activation of Rho-kinase, which, by inhibiting the regulatory subunit of smooth muscle myosin phosphatase and preventing dephosphorylation of myofilaments, maintains the contractile tone (Somlyo & Somlyo, 2000).

When penile erection occurs, there is relaxation of the smooth muscle component that, at the molecular level, involves a decrease in free Ca^{2+} levels in the sarcoplasma through different steps. The reduction in the Ca^{2+}/calmodulin complex leads to a decrease in MLC kinase activity, which results in the reduction of phosphorylated MLC levels, breakdown of actin/myosin cross bridges, and cavernosal smooth muscle relaxation (Walsh, 1991). Indeed, upon sexual stimulation, the cavernous nerves release nitric oxide (NO) that starts the erectile response. They also release acetylcholine that stimulates the endothelium to generate a more sustained release of NO. Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), the second messengers involved in smooth muscle relaxation, activate cAMP- and cGMP-dependent protein kinases, which, in turn, phosphorylate certain proteins and ion channels. This cascade of events provokes the opening of the potassium channels and hyperpolarization, an increase in intracellular Ca^{2+} by the endoplasmic reticulum, and the blockade of Ca^{2+} influx through the inhibition of voltage-dependent Ca^{2+} channels. The consequence is a drop in cytosolic free Ca^{2+} and smooth muscle relaxation.

2. MEDIATORS IN ERECTILE FUNCTION

Several mediators produced by the endothelium or the smooth muscle component play an important role in maintaining the erection or leading to detumescence. A fine balance between vasodilating and vasoconstrictor mediators within the penis is crucial to ensure penile erection (Fig. 1). An unbalance between the contractile and the relaxant tone can lead to erectile dysfunction (ED). The

following paragraphs briefly summarize the information known on the role of these mediators in erectile function in men.

2.1 Contracting mediators

2.1.1 Noradrenaline

Preclinical experiments suggest a modulator role for NA in the control of penile erection either in the brain or in the spinal cord. On this basis, a new therapeutic approach to ED has been proposed using yohimbine. This substance is an α2 antagonist that was used in the past in an anecdotal manner for ED therapy. Yohimbine showed poor therapeutic efficacy and had questionable effects in men with organic ED, leading, in 1996, to a statement by the American Urological Association suggesting that "*yohimbine does not appear to be effective for erectile dysfunction and, thus, it should not be recommended as treatment for the standard patient*" (Montague et al., 1996). This has led to create doubts on the role at the central level of the noradrenergic system in erectile function. However, the lack of efficacy could be ascribed to the peripheral contractile effect on the penile musculature (Smith et al., 1987). More recently, it has been suggested that yohimbine may be useful in subsets of men with mild disease or few risk factors (Guay et al., 2002); however, it is clear that it will never be a first-line drug for ED.

The sympathetic preganglionic neurons are located in the intermediolateral gray matter of the spinal cord. Postganglionic neurons are located in the sympathetic chain ganglia, the inferior mesenteric, hypogastric and pelvic ganglia, and the ganglia near the target organ. Sympathetic fibers can be found in the pelvic, cavernous, and pudendal nerves. Stimulation of the sympathetic pathways to the penis may, however, also produce erection (Andersson et al., 2000).

At the peripheral level, the activation of sympathetic adrenergic nerves causes release of NA, acting on α1-adrenoceptors either on the trabecular smooth muscle of the corpus cavernosum or in penile vessels. This effect is mediated by Ca^{+2} entry involving both L-type and 2-aminoethoxydiphenyl borate-sensitive receptor-operated channels. Further, Ca^{+2} sensitization mechanisms mediated by protein kinase C, tyrosine kinases, and Rho kinase are also involved. Penile erection in men is

accompanied by a significant reduction in NA in cavernosal blood while adrenaline is increased (Becker et al., 2000a). Among α1-adrenoceptor subtypes, human erectile tissues express mRNAs of all the three subtypes (α1A, α1B, and α1D) with high affinity for prazosin, although the α1A-adrenoceptor is thepredominant (Traish, et al., 1995). A small clinical trial on selective α1A-adrenoceptor antagonist failed to show any improvement in ED (Choppin, et al., 2001). More recently, a fourth receptor, known as α1L or α1A/L, considered a functional phenotype of the α1A-adrenoceptor, was been identified, and its role in human erectile tissues of patients undergoing urethroplasty was suggested (Davis, et al., 2018). In particular, this study demonstrates that the α1L-adrenoceptor subtype, at both the functional and the protein level, is present in human erectile tissues, and it plays a major role in mediating erectile smooth muscle contraction. Interestingly, the α1L-adrenoceptor is also present in the human prostate, and it is the target of current α1A/L adrenoceptor antagonists used to treat lower urinary tract symptoms. Although retrograde ejaculation has long been assumed to be a common side effect of specific α1-blocker therapy, recent investigations have shown that what was previously thought to be a peripheral effect causing retrograde ejaculation is actually a centrally mediated process (Wolters & Hellstrom, 2006).

However, each α1-blocker has different effects on sexuality, and this issue should be considered when this therapy is prescribed. Therefore, it is necessary that future clinical studies will be designed to define a therapeutic algorithm to drive the right choice among the different α1-blocker subtypes in case of bladder/prostate diseases and/or hypertension in sexually active men.

2.1.2 Serotonin

Serotonin (5-HT) receptor role in ED has been addressed in several studies. 5-HT has several receptors, and selective inhibitors are available (Pytliak et al., 2011). It has been shown that the activation of the 5-HT receptors, i.e., 5-HT1A, 5-HT2A, and 5-HT4, inhibits penile erection (Uckert et al., 2003; Lau et al., 2006). In vitro, the 5-HT-induced contraction of isolated human corpus cavernosum (HCC) strips is abolished by NAN-190, a 5-HT1A-receptor antagonist; ketanserin, a 5-

HT2A receptor antagonist; and SB 203186, a 5-HT4 receptor antagonist (Uckert et al., 2003; Lau et al., 2006). In vitro, 5-HT did not affect the relaxation of NA-stimulated HCC induced by electric field stimulation (EFS) (Uckert et al., 2003). Conversely, the EFS-induced cavernosal contraction was reduced by ketanserin, indicating that preterminal neuronal storage of 5-HT released following EFS acts on 5-HT2A receptors (Lau et al., 2006). Thus, 5-HT was released by neuronal storage functions as a contractile neurotransmitter in addition to NA. This evidence is in contrast with Uckert and coworkers (2003) who, although suggest a role for 5-HT in maintaining the penis flaccidity and in facilitating detumescence, conclude that it is unlikely that 5-HT contributes to neuronal-derived function of the HCC. Further studies are required to address the role of 5-HT in the neuronal control of human penile erection and to define whether it is possible to exploit the possible use of 5-HT selected subtype inhibitors in ED therapy in nonresponder patients to phosphodiesterase 5 (PDE5) inhibitors.

2.1.3 Endothelin

Endothelin(s) (ET) are potent vasoconstrictor peptides that cause strong, slowly developing, but sustained, contraction of trabecular smooth muscles cells of the corpora cavernosa. There are three isoforms of the peptide (identified as ET-1, -2, and -3) and at least four endothelin receptors, namely, ETA, ETB1, ETB2, and ETC. In HCC, endothelium expresses ET-1 mRNA, and ET-like immunoreactivity was observed in sinusoid (Saenz de Tejada I, et al., 1991). Both ETA and ETB receptors were found in human tissues (vascular and trabecular). ET-1 induces potent vasoconstriction in HCC together with ET-2 and ET-3, although both are less potent than ET-1. Multiple mechanisms of action have been proposed, including transmembrane calcium flux, mobilization of inositol triphosphate-sensitive intracellular Ca^{2+} stores, and Ca^{2+} sensitization through the Rho-Rho kinase pathway (Holmquist et al., 1990; Holmquist et al., 1992).

Several clinical studies addressed the possible role of ET-1 in the pathophysiology of erection, but no data clearly supporting this function are available. The ET levels were evaluated in the plasma of diabetic patients with or without ED, and no differences in plasma concentration of ET-1 between

the two groups examined were found (Francavilla et al., 1997). The possible role of circulating ET has also been investigated using cavernosal blood. In this clinical study, no changes in ET-1 or ET-2 were observed either in systemic or in cavernosal blood during penile tumescence, rigidity, and detumescence in healthy volunteers. In patients with ED, plasma ET-1/-2 levels during penile flaccidity and detumescence were found to be higher in the systemic circulation than in the cavernosal blood. No differences in the plasma levels of ET-1/-2 were found between patients with organogenic *versus* psychogenic ED. During detumescence, the ET-1/-2 levels were lower in the cavernosal blood collected from patients than those in healthy volunteers. Therefore, there is a different profile of ET-1/-2 in the cavernosal blood between healthy subjects and patients with ED. However, there is no clear definition of the role played by ET-1 in the control of penile flaccidity and detumescence (physiological role) or in its involvement in the pathophysiology of ED (Becker et al., 2001). The lack of a role of ET-1 in ED is supported by the finding obtained from a pilot study using BMS-193884, an ET-A receptor-selective antagonist. In this study, performed in 53 men diagnosed with mild-to-moderate ED, BMS-193884 failed to show any significant improvement in erectile function either during office visits or home use when compared to the placebo (Kim et al., 2002).

It should be stressed that there is a big disparity between preclinical (in vitro and in vivo animal studies) and clinical studies and results. This issue implies that there are significant differences among species to what concerns the role of ET in erectile function. Overall, the lack of clear data concerning the exact role of ET in penile erection has hampered the progress in this area. It is possible that the ET system may only be relevant to ED under certain conditions where global endothelial dysfunction exists. In this context, it has been suggested that ET antagonists could be useful in these patient groups (Ritchie & Sullivan, 2011). Most likely, ET antagonist may result useful in combined therapy for patients with severe ED that are weak or no-responders to PDE5 inhibitor therapy.

2.1.4 Renin-angiotensin system

The role of the renin–angiotensin system (RAS) in ED observed in preclinical studies is clearly confirmed either in human in vitro studies or in clinical studies. The RAS plays an active role within the erectile tissues (Fraga-Silva et al., 2013). It is expressed in cavernosal tissues and may act in a paracrine manner, and its involvement is sustained by the finding that angiotensin II (AngII) plasma level in cavernous blood is significantly higher in the flaccid or the detumescence phase than in the systemic circulation (Becker, at al., 2001). AngII role has also been shown under pathological conditions, suggesting that its plasma levels could be a useful tool in the diagnosis of organogenic ED. In this study, patients with ED of either organogenic or psychogenic etiology were exposed to visual and tactile erotic stimuli to elicit penile tumescence. The control group consisted of healthy subjects where stimulation caused rigidity (firm erection). In healthy volunteers, the AngII levels in the cavernous plasma increased by 28%. A weaker increase was also found in the peripheral plasma. During penile flaccidity, the AngII levels in the systemic and cavernous blood were higher in the group of organogenic patients than in the control group. Thus, AngII might be involved in triggering penile detumescence in men (Becker et al., 2001). As AngII is a product of angiotensin-converting enzyme (ACE), the above-mentioned results stimulated the investigation of the possible involvement of ACE in the pathogenesis of ED. A significant increase in systemic venous levels of ACE activity was found in psychogenic or organogenic ED subgroups as compared to those in controls. In addition, the cavernosal level of ACE activity was significantly higher in diabetic patients with ED as compared to psychogenic patients with ED. Of particular interest is the finding that systemic blood ACE activity negatively correlates with the NO level in diabetic patients with ED. A significant increase in the systemic levels of ACE was also associated with reduction in the systemic levels of growth hormone, NO, and cGMP in both diabetic patients with ED and nondiabetic patients with ED. These disturbances indicate that endothelial dysfunction and the imbalance among vasoactive mediators might contribute to ED (Hamed et al., 2003).

The importance of RAS in erectile function is indirectly further confirmed by data published by Fogary (2001) where a comparison between two antihypertensive treatments, namely, valsartan and

cavedilol, on sexual activity in hypertensive men never treated before for hypertension was addressed. The age of the enrolled patients was 40-49 years, all married and without any previous sexual dysfunction. After a 4-week placebo period of treatment, the patients were divided into two groups: a) 120 patients were randomized to receive carvedilol 50 mg once daily or valsartan 80 mg once daily for 16 weeks according to a double-blind, cross-over design; after another 4-week placebo period, the patients were crossed over to the alternative regimen for a further 16 weeks; b) 40 patients were treated with placebo according to a single-blind design for 16 weeks. At the screening visit and every 4 weeks thereafter, blood pressure was evaluated and the patients were interviewed through a questionnaire about their sexual activity. Blood pressure was lowered significantly by both treatments, with 48% of patients showing normalization with valsartan and 45% with carvedilol. During the first month of therapy sexual activity (assessed as the number of sexual intercourse episodes per month) declined with both drugs as compared to that at baseline. The decrease was statistically significant in the carvedilol group but not in the valsartan group. As the study progressed, the sexual activity further worsened in the carvedilol group, while the valsartan group fully recovered and, even more relevant, improved. These results were confirmed by the cross-over protocol. In particular, ED was a complaint of 15 patients receiving carvedilol (13.5%), one patient receiving valsartan (0.9%), and one patient receiving placebo. These findings suggest that carvedilol, a β1, β2, and α1 antagonist, induces chronic worsening of sexual activity, whereas valsartan does not significantly worsen sexual activity but may improve it (Fogari et al., 2001). The advantage of using AngII antagonist, as an antihypertensive drug, in relation to sexual function/dysfunction was also confirmed by another independent study (Llisterri et al., 2001). In this study, there was improvement in the erectile function (i.e., both satisfaction and frequency of sexual activity) in hypertensive patients receiving losartan. This has highlighted the possibility to perform therapeutic management of hypertension by adding positive impact on quality of life. More recently, in 2012, it has been shown that in diabetic patients, the combination of losartan and tadalafil is more effective than the single-use of losartan or tadalafil. This was the first clinical trial

designed to assess the effect of losartan therapy on diabetic patients with ED. Losartan seems to be effective and well tolerated in diabetic patients with ED, especially for mild to moderate conditions. Thus, the combination therapy of losartan and tadalafil appeared to be more effective than monotherapy (Chen et al., 2012). Of particular interest is also the effect reported in patients with ED caused by nerve-sparing radical retropubic prostatectomy. In these patients, treatment with irbesartan significantly increased sexual activity and ameliorated the loss of stretched penile length, which occurs postoperatively (Segal et al., 2012).

To date, knowledge of the RAS cascade has expanded because of the discovery of additional components such as Ang (1-7), ACE2, and, even more interesting, the Mas receptor, a G protein-coupled receptor of Ang (1-7). Therefore, the RAS has two branches: one deleterious branch triggered by the Ang II/AT1 receptor and the other protective branch triggered by the Ang (1-7)/Mas receptor (Ferreira et al., 2010). In addition, Ang-(1-7) activity seems to be linked to eNOS phosphorylation/activation through Akt-dependent pathways and thus to NO (Sampaio et al., 2007). Currently, studies on the role of the Ang-(1-7)/Mas receptor in erectile function and its therapeutic potential for treating ED have been addressed in preclinical studies only, and human data are not available either in vivo or in vitro.

2.1.5 Rho-Kinase pathway

The RhoA/Rho-kinase pathway is present in many tissues throughout the body, and it is involved in regulating various functions. The activation of this pathway causes Ca^{2+} mobilization, thereby promoting contraction. The activation process involves the dissociation of RhoA that translocates from the cytosol to the membrane, enabling the downstream activation of various effectors such as Rho kinase. Phosphorylation of the regulatory subunit of MLC phosphatase by Rho kinase causes inhibition of phosphatase activity, which enhances the contractile response (de Godoy & Rattan, 2011). An in vitro study showed the presence of the expression of Rho-kinase in primary cultures of human cavernosal smooth muscle cells (Rees et al., 2002). Interestingly, the Rho-kinase inhibitor, namely, SAR407899, relaxed HCC strips in vitro, and this effect was not modified by L-NAME, an

NO-synthase (NOS) inhibitor. Interestingly, without L-NAME treatment, sildenafil was significantly less potent and effective than SAR407899. The potency, particularly the efficacy of sildenafil, was even lower in the presence of L-NAME. Thus, when the endothelium is damaged, for example, in chronic pathologies such as hypertension/diabetes and when patients do not successfully respond to PDE5 inhibitors, inhibition of this pathway may represent a valuable alternative therapy (Guagnini et al., 2012). Presently, two Rho-kinase inhibitors have been approved for clinical use in Japan (fasudil and ripasudil) and one in China (fasudil). In 1995, fasudil was approved for the treatment of cerebral vasospasm, and more recently, ripasudil, in 2014, was approved for the treatment of glaucoma. Currently, there are no studies available on the use of these inhibitors in ED, but it would be of interest to have clinical studies addressing their possible use in ED associated with diabetes and/or hypertension where an upregulation of this pathway has been demonstrated (for more details, see section 9.3 on Rho/RhoA Kinase inhibitor).

2.2 Relaxing mediators

2.2.1 Gasotransmitters

Penile vascular tone is controlled by a series of events coordinated at the level of the central and the peripheral nervous system, and after sexual stimulation, activation of pro-erectile mechanisms occurs. Consequently, adequate inflow of blood to the erectile tissues is achieved after arterial vasodilation and sinusoidal smooth muscle relaxation. In this complex mechanism, gasotransmitters have been recognized to cover a key role, in particular NO and, more recently, hydrogen sulfide (H_2S).

2.2.1.1 Nitric Oxide

NO has a key role in the relaxation of HCC smooth muscle and vasculature, it is a well-consolidated concept (Burnett et al., 1993; Toda et al., 2005). NO is synthesized in the nerve terminals and in the endothelium by the action of the tissue-specific enzyme NOS, which catalyzes the production of NO and citrulline from oxygen and L-arginine. NO passively diffuses into cavernous smooth muscle cells, where it binds and activates the soluble guanylate cyclase (sGC), which catalyzes the

breakdown of guanosine triphosphate into cGMP. In parallel, NANC, as reported above, plays an important role in erectile function, as they are involved in the relaxation of corpus cavernosum. Studies involving pharmacological modulation have indicated that NANC neurotransmission in the HCC muscle is nitrergic, i.e., involves NO as the major mediator (Kimura et al., 1993; Adaikan & Ng., 2000).

The existence and location of the constitutive isoform endothelial NOS (eNOS) and neuronal NOS (nNOS) in the HCC have been widely investigated. eNOS is expressed within smooth muscle cells of the fibromuscular stroma and vessels of HCC (Bloch et al., 1998). The presence of the constitutive eNOS in cultures of human cavernosal smooth muscle cells has also been reported by Anfrossi and coworkers (2002). Smooth muscle cells are not only targets of NO derived from endothelial cells or from nerve endings but also a source of NO conceivably involved in the mechanism of erection. In addition, insulin can activate the constitutive eNOS, in turn, increasing cGMP and cAMP and influencing the penile vascular homeostasis and therefore penile erection (Anfrossi et al., 2002).

In large veins, there is weaker expression of eNOS and nitrergic innervation, while the small intracavernosal helicine arteries expressed large quantities of eNOS and intense nitrergic innervation (Bloch et al., 1998).

The nNOS is present in pelvic plexus, cavernous nerve, and adventitia of the arteries in men. A double immunolabeling for nNOS and vesicular acetylcholine transporter has demonstrated that acetylcholine and NO coexist in the same parasympathetic cholinergic neurons (Burnett et al., 1993). The human cavernosal smooth muscle cells expressed both eNOS and the inducible form of NOS (iNOS) (Rajasekaran et al., 1998). Exposure of human cavernosal smooth muscle cells to glucose, mimicking a diabetic condition, showed increased expression of eNOS and iNOS. Their expression significantly increased in the presence of insulin and C-peptide, suggesting a synergistic effect on the NO regulation; thus, the body, through this mechanism, tries to counterbalance the

endothelial damage occurring in ED. Therefore, the replacement of peptide C in patients with insulin-dependent diabetes may have therapeutic effects (Li et al., 2004).

eNOS has a complex regulation that can modify its activity. Caveolin-1 is an endogenous modulator of eNOS (Fulton et al., 2001). Caveolin 1 and caveolin 3 are present in HCC. In particular, caveolin-1 is expressed in the smooth muscle and endothelial cells of corpus cavernosum while caveolin 3, which binds to nNOS, is located close to the NAPDH-positive fibers (Tsutsui et al., 1999). The existence and distribution of two key enzymes of the NO/cGMP pathway, namely, the cGMP-dependent kinase I (cGK I) and the sGC in HCC tissue, were examined in healthy volunteers and patients with ED (Koltz et al., 2000). sGC and cGK I are highly expressed in smooth muscle cells of vessels and in the fibromuscular stroma. The endothelium of the cavernosal sinus, the cavernosal arteries, and the cavernosal nerve fibers also contains sGC. cGK I is present on smooth muscle cells as compared to the endothelium. However, there are no changes in the immunoreactivity and cellular distribution between healthy volunteers and patients with ED. The expression of different isoforms of sGC, namely, sGC α1/β1 and α2/β1, has been found in HCC. In addition, the existence of the β2 subunit has been found, but the role/pharmacological properties of the enzyme containing β2 remains to be elucidated (Beherends et al., 2000). In this regard, it has been reported that the opening of Ca^{2+}-activated potassium channels is most likely mediated by the activation of sGC, leading to an increase in cGMP levels and consequent activation of protein kinase G (PKG) rather than a direct NO effect on human smooth muscle cells (Lee & Kang, 2001).

The NO levels in the peripheral and cavernosal blood did not appreciably change during and immediately following an erection in a study performed on 15 healthy adult male volunteers (Moriel et al., 1993). This result did not exclude abnormalities in the synthesis and release of NO in ED, but rather, alternative testing methods may be required.

NO plays a crucial role in erectile function, and nitrosothiols such as S-nitrocysteine, S-nitroso-N-acetylcysteine, and S-nitrogluthatione and NO-donors such as nitroglycerine and isosorbide dinitrate cause relaxation in isolated HCC strips (Heaton, 1989; Mirone et al., 2000; Filippi et al.,

2003), which suggests possible application of NO for the management of ED. Indeed, when NO donors are applied topically or intracavernously in men, they can induce erection (Owen et al., 1989, Stief et al., 1992; Truss et al., 1994). Despite these studies, this category of drugs has never been studied for the treatment of ED. In addition, there are no studies supporting oral therapy of NO donors probably due to their low efficacy and/or adverse effects.

The effect of L-arginine, the NO precursor, has been studied in HCC (Gur et al., 2007). L-arginine relaxed in a concentration- and a time-dependent manner in isolated HCC strips through several mechanisms including the stimulation of NO/sGC/cGMP/PKG, the opening of Ca^{2+}-activated small conductance channels, and inhibition of the Rho-kinase system. On this basis, it has been speculated that L-arginine supplementation could have a role in ED therapy by increasing the endogenous NO production, but there are no clinical studies supporting this hypothesis. Among other substances that can increase NO availability, it is worth reporting that sphingosine-1-phosphate (S1P) can modulate eNOS activity in HCC tissues by modulating its phosphorylation operated by Akt. Indeed, both HCC and the penile artery express the S1P receptors, and exogenous S1P can increase HCC relaxation induced by a subliminal concentration of acetylcholine (d'Emmanuele di Villa Bianca et al., 2006). Further studies are needed to better define the S1P/NO axis in erectile function.

2.2.1.2 Carbon monoxide/Heme Oxygenase

Heme Oxygenase (HO) has the physiological role to degrade heme. Three isoforms of HO have been identified, namely, HO-1, HO-2, and HO-3. HO-3 has no activity and is not expressed in humans, HO-2 is a constitutive form, and HO-1 is inducible. HO is present in the endothelium and smooth muscle cells, and particular interest has focused on the antioxidant and anti-inflammatory properties of the inducible HO-1 isoform in the vascular endothelium (Calay & Mason, 2014). HO-1 and HO-2 degrade heme and generate carbon monoxide (CO) and biliverdin, simultaneously releasing iron, which is stored within the iron-binding protein ferritin. These products exert signaling and cytoprotective activities that mitigate apoptosis and inflammation, regulate vasomotor tone, and exert antioxidant and immunomodulatory functions. The protective role of HO-1 and its

products in the heart and cardiovascular system is unequivocal (Otterbein et al., 2016), and the importance of this stress inducible enzyme in cellular homeostasis in humans was supported by the identification of the first case of HO-1 human deficiency (Yachie et al., 1999). More recently, another case of human HO-1 deficiency has been reported (Radhakrishnan et al., 2011). These patients had several systemic pathologies, and symptoms included abnormal coagulation–fibrinolysis associated with elevated levels of von Willebrand factor, reflecting the presence of endothelial injury and dysfunction.

Of the three products of heme catabolism, CO remains the main focus of vascular-related scientific research, and studies on the major actions of CO focus on its cardiovascular and neurological effects.

Owing to these specific actions, it is easy to hypothesize a role for CO in sexual function and/or ED. To date, CO involvement has been extensively investigated in preclinical studies, while few data are available in humans (Shamloul, 2009). In particular, the expression of HO-1 and HO-2 was detected in the endothelium lining human penile arteries and in sinusoidal walls by immunohistochemistry. Interestingly, as occurs for NO, CO relaxes HCC and spongiosum strips. The relaxation induced by CO was not accompanied by increases in the intracellular level of cyclic GMP, suggesting that endothelial-derived NO and the HO/CO system may have a complementary role in penile erection (Hedlund et al., 2000). This evidence suggests a possible role of the HO/CO pathway in aging, considered the most important risk factor of ED, but studies on this aspect are lacking both at the preclinical and clinical levels.

As translocation of CO through the human body is difficult, small molecules known as CO-releasing molecules (CORMs) that deliver controlled amounts of CO to biological systems have been characterized; these molecules are of great interest from a medical point of view. Among them, SANGUINATE is a CO-releasing/oxygen transfer agent being developed for the treatment of anemic and ischemic hypoxia. This trial established the safety of SANGUINATE and permitted its

advance to phase II trials (Misra et al., 2017). This and other ongoing clinical studies may effort the use of CO- releasing molecule as therapeutic approach for ED.

2.2.1.3 Hydrogen sulfide

H_2S is produced by two pyridoxal-5-phosphate (PLP)-dependent enzymes, namely, cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), starting from L-cysteine or homocysteine as substrates. In addition, H_2S is synthesized by 3-mercaptopyruvate sulfurtransferase (3-MST) with cysteine aminotransferase (CAT) in a PLP-independent manner (Kimura, 2011). A role for H_2S signaling in the urogenital tract has been defined (d'Emmanuele di Villa Bianca et al., 2017). The first study showing the existence of the H_2S pathway in the HCC has been published in 2009 (d'Emmanuele di Villa Bianca et al., 2009). CSE is localized either within the muscle component or at peripheral nerves, while CBS was found exclusively at the muscle level in the human tissue. L-cysteine, the substrate, or an H_2S donor, relaxes precontracted HCC strips in an endothelium-independent manner, suggesting a "back role" for the H_2S pathway in the case of endothelium damage. Electric stimulation of HCC strips under resting conditions caused an increase in tension that was enhanced by inhibiting CSE and/or CBS, implying also that the H_2S pathway contributes to the penile homeostasis. This contribution most likely involves the RhoA/Rho-kinase pathway and the ATP-dependent potassium channel (K_{ATP}). The effect on K_{ATP} is of particular interest, as the opening of these channels leads to relaxation of the corporal smooth muscle, which is essential during erection (Király et al., 2013; Insuk et al., 2003; Lee et al., 1999).

β_3 adrenoceptor stimulation is known to relax HCC strips in a cGMP-dependent and endothelium/NO-independent manner (Cirino et al., 2003). Recently, it has been shown that H_2S is involved in β_3-induced relaxation in isolated HCC strips and in penile artery in men (Mitidieri et al., 2017). Indeed, BRL37344, a selective β_3 agonist, causes increase in H_2S production that is reverted by inhibition using either CSE or a β_3 receptor antagonist. Considering that β_3 receptors are mainly localized on the muscle component, the β_3/H_2S/cGMP pathway may act as a valid alternative to NO. Another finding that relates to the link between the H_2S pathway and the cyclic nucleotides is

the discovery that incubation of the human bladder with two stable analogues of cGMP and cAMP causes an increase in H_2S production (Fusco et al., 2012; d'Emmanuele di Villa Bianca et al., 2015). In conclusion, these findings indicate the H_2S pathway as a feasible therapeutic target to develop new therapies for ED.

2.2.2 Prostanoids

Prostanoids are a heterogeneous group of substances with different physiological effects on the body. Penile erectile tissue is able to locally synthesize and metabolize most of these substances (Jeremy et al., 1986; Khan et al., 1999; Minhas et al., 2000; Moreland et al., 2001). Production of thromboxane A2 (TXA2), prostaglandin F2α (PGF2α), prostacyclin (PGI2), prostaglandin D2 (PGD2), and prostaglandin E2 (PGE2) has been reported in HCC. It is well established that they can modulate both contractile and relaxant effects in human trabecular and arterial smooth muscle (Hedlund & Andresson, 1985; Angulo et al 2002). The effects of prostanoids are mediated by specific receptors, namely, TP (TXA2), FP (PGF2α), IP (PGI2), DP (PGD2), and EP (PGEs). The EP receptor family has been further divided into four different subtypes (Breyer et al, 2001). EP1 receptors cause elevation in intracellular Ca^{2+}; EP2 and EP4 receptors increase cyclic-AMP through the activation of adenylyl cyclase (AC); EP3 receptors activate AC and drive Ca^{2+} mobilization (Woodward et al., 2011; Coleman et al., 1994). TXA2 and PGF2α, through the activation of the TP or FP receptor, and PGE, through EP1 and EP3, mediate the contraction of erectile tissue (Angulo et al., 2002). PGD2, PGE, and PGI2 can induce smooth muscle relaxation (Pierce et al., 1995). The major relaxing prostanoid in corpus cavernosum smooth muscle is PGE (PGE1 and PGE2), which induces relaxation by binding to G-protein-coupled receptors (EP2-EP4) and by increasing cAMP synthesis (Lue & Dahiya 1997). Forskolin, a potent activator of AC, increases cAMP production induced by PGE, suggesting a synergistic effect (Traish et al., 1997). Indeed, the intracavernous injection of forskolin in patients with ED who had failed to respond to standard PGE1 injection therapy improved erection in 61% of them (Mulhall et al., 1997), probably for the enhancement of the relaxant corporal effects of PGE1. Another possibility to enhance the effect of PGE1 is the

combination with αAR antagonists such as doxazosin (Kaplan et al., 1998). Intracavernous injection of PGE1 (alprostadil) has been largely used before the advent of the oral inhibition of PDE5 despite the penile pain being a common adverse event (Wespes et al 2000). Alprostadil, the first and only drug approved for the intracavernous treatment of ED (Eardley et al., 2010), is used as monotherapy at a dose of 5-40 µg (Hatzimouratidis et al., 2017) or in combination: i) papaverine and alprostadil (Zaher 1998); ii) ketanserine and alprostadil (Mirone et al., 1996) iii) phentolamine and alprostadil (Mehinardt et al1996). Currently, the most effective intracavernous therapy is a three-drug mixture therapy "trimix" containing papaverine (8-16 mg) plus phentolamine (0.2-0.4 mg) plus alprostadil (10-20 µg). This triple combination has the highest efficacy rates, reaching 92%. Furthermore, the trimix has similar side effects as alprostadil monotherapy but a lower incidence of penile pain due to the presence of a lower dose of alprostadil (Hatzimouratidis et al., 2017; He et al., 2011). In addition, in patients who do not respond to the trimix alone, the combination with sildenafil is suggested (Park et al., 2008). To bypass the adverse event of intracavernous injection and ameliorate the compliance of patients, a medical urethral system for erection (intraurethral PGE1) has been developed (Hellstrom et al., 1996; Padma-Nathan et al., 1997). Nehra et al., in 2002, performed a clinical study using the intraurethral PGE1 in combination with sildenafil (inhibitor of PDE5) in patients where the use of the single agent failed. This combination proved to be well tolerated and effective (Nehra et al., 2002). More recently, alprostadil has been marketed in the form of a cream, a noninvasive treatment that combines PGE1 with a skin enhancer that improves the local absorption (Moncada & Cuzin, 2015). This formulation has a favorable pharmacodynamic profile and is poorly absorbed in systemic circulation, thereby allowing a reduced risk of adverse systemic effects. This local formulation represents a useful therapeutic tool in several clinical settings where a systemic administration is not indicated by the risk-benefit ratio. More recently, the pharmacological and physiological activities of novel EP and DP receptor agonists have been characterized in humans (Brugger et al., 2008). EP agonists showed no consistent correlation between their pharmacological profile, and their effect in HCC; AS702224, a potent DP1 selective

agonist, showed high activity in causing the relaxation of human cavernosal tissue, stimulating pro-erectile responses. The clinical development of DP1 receptor agonists as intracavernosal agents may provide a more desirable alternative to intracavernosal PGE1 (Brugger et al., 2008).

2.2.3 Dopamine

The physiological actions of dopamine (D) are mediated by five distinct but closely related G protein-coupled receptors that are divided into two major groups: the D1 and D2 (Vallone et al., 2000; Andersen et al., 1990). In detail, D receptors in mammalians have been classified as D1-like (D1 and D5) and D2-like (D2, D3, and D4) according to their binding affinity and their ability to modulate AC activity (Beaulieu & Gainetdinov, 2011). Beyond the D role in the central regulation of penile erection, an involvement at the peripheral level has been reported in HCC (d'Emmanuele di Villa Bianca et al., 2005). Both D1- and D2-like receptors are expressed in HCC, and they are mainly localized on the smooth muscle cell component. Interestingly, apomorphine (a nonselective dopaminergic receptor agonist) relaxed in a concentration-dependent and endothelium-independent manner precontracted HCC strips. In addition, the authors have demonstrated, by using selective D1 and D2 agonists, that D1 receptors are mainly involved in penile erection, and this effect is partially mediated by NO. The exact role of D receptors, especially D1 receptors, in the corpus cavernosum has not been clearly defined if further studies will clarify its role, D1 receptor may represent a feasible therapeutic target for ED.

2.2.4 Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is a 28-amino acid-peptide neurotransmitter with potent vasodilatory properties involved in penile erection in humans (Ottensen et al., 1984; Ehmke et al., 1995). In a study conducted on nine men, i.e., two normal volunteers and seven patients with ED, the concentration of VIP in cavernous blood resulted in an increase of up to 20-fold during tumescence or erection, while no changes were observed within the peripheral circulation, suggesting local release of the polypeptide (Virag et al., 1982; Ottensen et al., 1984). Conversely, a study in healthy volunteers showed contrasting results (Becker et al., 2002). The authors did not

find any increase in VIP plasma levels in the systemic and cavernous blood when the flaccid penis reached rigidity. During penile detumescence, cavernous VIP levels increased, whereas VIP remained unaltered in the systemic circulation. Interestingly, following ejaculation, VIP levels in the cavernous blood increased, whereas, in the systemic blood, no significant changes were recorded. Overall, these studies suggest a role for VIP in erectile function.

In vitro functional studies on isolated HCC strips VIP caused a concentration-dependent relaxation through an increase in cAMP without affecting cGMP levels (Hedlund et al., 1995), and this effect was blocked by VIP antiserum (Adaikan et al., 1986). Immunohistochemical studies have demonstrated the presence of VIP in autonomic nerves in the smooth muscle of HCC and in penile vessels (Polak et al., 1981). The presence of VIP signaling has also been found by immunoreactivity in the human circumflex vein, and its functional role has been reported. VIP relaxed the circumflex vein precontracted with NA (Kirkeby et al., 1992). In addition, human penile erectile tissue has been found to contain high concentrations of VIP (Polak et al., 1981; Shirai et al., 1990, Hedlund et al., 1995). In particular, the VIP content in cavernous tissues was determined by radioimmunoassay in 18 patients with ED and 5 healthy controls. A lower penile VIP content was found in patients with organic ED than in healthy individuals, suggesting a negative correlation between ED severity and VIP content (Shirai et al., 1990). In line with this finding, the number of VIP-immunoreactive nerves and the concentration of VIP immunoreactivity in the penises of ED men were considerably reduced compared with those in healthy men (Gu et al 1984). These lines of evidence indicate that a deficiency in VIP might be cause ED. Numerous NO synthase (NOS) and VIP-containing axons have been found in the human penis. More than 50% of the perivascular nerve fibers and more than 90% of the trabecular nerve fibers within the corpus cavernosum stained positive for both NOS and VIP. NOS/VIP-immunoreactivity was reduced in diabetes or absent in case of lesion of the cavernous nerve in penile tissue, supporting the concept that NO and VIP act as neural comediators of penile erection in humans (Ehmke et al., 1995). Finally, the effect of castration on VIP immunostaining in HCC was studied in patients with chemical or surgical castration. The authors

conclude that VIP is not an androgen-dependent neuromediator and could be responsible for sexually induced erection in castrated patients (Cormio et al., 2005).

The findings that the intracavernous injection of VIP caused various degrees of tumescence within few minutes and lasted 15-20 minutes associated with a significant increase in penile circumference suggested VIP as a possible pharmacological approach (Ottensen et al., 1984; Adaikan et al., 1986; Kiely et al., 1989). However, Wagner and Gerstenberg, in 1987, have shown that intracavernous injection of VIP did not induce erection in men *per se*. Improvement in erectile response was obtained only when VIP was injected intracavernously in combination with papaverine or phentolamine (Kiely et al., 1989). On this basis, two clinical studies were conducted, showing that the combination of VIP with phentolamine mesylate was safe and effective in men with ED (McMahon, 1996; Dinsmore et al., 1999). It is worth reporting that Invicorp®, which is a combination of 25 μg VIP and 1 or 2 mg phentolamine mesylate for intracavernous use, has been registered for ED in Denmark.

2.2.4 Urotensin-II

Urotensin-II (U-II) is a cyclic peptide originally isolated from the teleost neurosecretory system and subsequently identified in other species including humans (Russel 2008). It is an important factor of cellular homeostasis. Indeed, U-II plays an important role in the pathogenesis of several acute and chronic diseases and in the development of cardiovascular disorders, metabolic syndrome (MS), inflammation, liver cirrhosis, renal failure, diabetic nephropathy, and reproductive dysfunction (Svistunov et al., 2018). At the cardiovascular level, it causes both vasoconstriction and vasodilation depending on the concentration and route of administration, the vascular district, and the species. Its vasoactive effect is mediated by binding to a G-protein-coupled receptor (UT receptor) (Russell, 2008). U-II and the UT receptor were found in HCC. Interestingly, U-II relaxed precontracted HCC in an endothelium-dependent manner, and the inhibition of eNOS reduced the relaxing effect, suggesting the involvement of NO (d'Emmanuele di Villa Bianca et al., 2010,

d'Emmanuele di Villa Bianca et al., 2015). These data may suggest U-II as a novel target in erectile function.

3. ERECTILE DYSFUNCTION

ED is a common disorder that affects quality of life. ED can negatively affect a man's mental health, his relationship, and his general well-being. The presence of ED, therefore, provides an opportunity to potentially address multiple issues that affect a man's general health (Burnett et al., 2018); https://www.auanet.org/guidelines/male-sexual-dysfunction-erectile-dysfunction-(2018)#x8049).

ED is prevalent in more than half of males aged above 60 years and it is defined as the persistent (at least 6 months) inability to achieve and maintain penile erection sufficient to allow a satisfactory sexual performance (The National Institutes of Health (NIH) Consensus Development Panel on Impotence. 1993; Hatzimouratidis et al. 2010). ED has affected more than 150 million men worldwide, and this number will reach approximately 322 million by 2025 (Ayta et al., 1999).ED affects an estimated 150 million men globally and up to 30 million men in the United States (Burnett et al., 2018).

As already discussed, erection is thought to be a process that is regulated by hormones and neurovascular mechanisms at the cerebral and peripheral levels. The causes of ED may be primary or secondary (Fig. 2). Impairment in the sexual hormone level, particularly the lack (or a significant reduction) of them during the early developmental stage of male children, is the major cause of primary ED. The majority of secondary ED is vasculogenic, and ample evidence indicates that ED is a risk marker for the presence of treatable underlying medical conditions that, left untreated, reduce quality and length of life (e.g., undiagnosed diabetes and cardiovascular disease [CVD]) (Saigal et al., 2006). In fact, ED can be an early manifestation of coronary artery disease and/or peripheral vascular disease (see section "4. ERECTILE DYSFUNCTION AND CARDIOVASCULAR DISEASE").

3.1 Erectile dysfunction and risk factors

3.1.1 Smoking

Smoking is positively associated with an increased incidence of ED, in particular vasculogenic ED (Sullivan et al., 2001; Polsky et al., 2005). Both the direct use of tobacco and secondhand exposure are considered a consolidated risk factor for ED (Kupelian et al., 2007). The most well-understood signal transduction mechanism underlying ED, with regard to smoking, involves NOS isoforms (Butler et al., 2001; Demady et al., 2003). Indeed, smoking has been shown to impair endothelial NOS-mediated vascular dilation in young men (Huang et al., 2015; Celermajer et al., 1993). In addition, smoking also causes intrinsic damage to vessels by altering the elastin of the extracellular matrix and by inducing calcification of medial elastic fibers, thereby producing arterial stiffness (Guo et al., 2006). Smokers are 1.5 times more likely to suffer from ED than nonsmokers (Dorey, 2001), and the negative impact of smoking on erectile function is dose dependent and cumulative (Chew et al., 2009; Wu et al., 2012). Indeed, former smokers and ever smokers have significantly higher odds of ED than never smokers. In particular, Chew and coworkers evaluated data from 2868 men and observed that the odds of ED, adjusted for age, square of age, and CVD, were significantly higher among current smokers (odds ratio [OR] = 1.40; 95% confidence interval [CI] 1.02, 1.92) and ever smokers (OR = 1.57; 95% CI 1.02, 2.42) than among never smokers. In addition, the adjusted odds of severe ED were significantly higher among former smokers and the odds of ED increased with the number of cigarettes smoked among current smokers (Chew et al., 2009).

The effect of smoking cessation on erectile function has been largely examined. In a prospective study on 2837 smokers (aged 30-60 years), for whom a strong association between the intensity of cigarette smoking and the degree of ED was observed, it has been demonstrated that 1 year after smoking cessation, patients who successfully stopped smoking had a 25% improvement in erectile function (Pourmand et al., 2004). Similarly, a change in penile tumescence has been examined in a cohort of young men (age ≤40 years). In fact, the erectile tumescence response was significantly enhanced and had faster onset to reach maximum subjective sexual arousal in successful quitters

than in those who relapsed (Harte & Meston, 2012). Furthermore, the cessation of cigarette smoking immediately improved penile hemodynamic. Indeed, within 24 to 36 hours of the cessation of cigarette smoking, color Doppler parameters demonstrated a significant decrease in end-diastolic velocity and a trend toward an increase in peak systolic velocity (Sighinolfi et al., 2007).

3.1.2 Alcohol

The role of alcohol in the progression of ED is not completely understood. It is commonly asserted that alcohol consumption can modulate men sexuality depending on the amount. Indeed, moderate alcohol intake may foster the initiation of sexual activity through a vasodilatory effect and inhibition of anxiety, but persistent and chronic use of alcohol can cause central sedation and decrease libido and thus ED (Sadock, 2005; Graham & Bancroft, 2009). In particular, in men who have chronic alcohol use, the common dysfunctions reported were lack of sexual desire, premature ejaculation, and ED (Arackal & Benegal, 2007; Dackille et al., 2008). It has been demonstrated that in young adults, binge drink can heighten the risk for cardiovascular events such as stroke, myocardial infarction, increased mortality, and atherosclerosis (Sundell et al., 2008; Mukamal et al., 2005; Marques-Vidal et al., 2001; Kauhanen et al., 1999). The effect of alcohol on cardiovascular risk may be due to changes in vascular biology such as endothelial dysfunction, which represents the primary pathogenesis of ED. However, it has also been reported that regular of low/moderate alcohol intake may decrease the risk of adverse cardiovascular events. Indeed, a cross-sectional study based on large population showed that minimal to moderate alcohol use significantly reduces the risk of ED in young and middle-age males (Weber et al., 2013). In line with these data, a recent meta-analysis reported that moderate alcohol consumption lowered the risk of ED, but regular and heavy alcoholic beverage intake was associated with higher ED prevalence (Wang et al., 2018).

3.1.3 Metabolic syndrome and obesity

MS comprises a set of conditions including obesity, hypertension, hypertriglyceridemia, low levels of high-density lipoprotein–cholesterol, and hyperglycemia. MS has been defined as a cluster of at least three of the five following metabolic abnormalities: abdominal circumference >102 cm,

hypertriglyceridemia >150 mg dl^{-1}, high-density lipoprotein–cholesterol <40 mg dl^{-1}, blood pressure >130/85 mmHg, or glycemia >110 mg dl^{-1} (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; Alberti et al., 2009). One of the major risk factors for MS is obesity, a pathological condition that increases the incidence of diabetes, dyslipidemia, and hypertension. A large body of evidence indicates that abnormal abdominal adiposity is associated with insulin resistance, leading to diabetes; insulin resistance and the associated hyperglycemia and adipokines can lead to abnormal lipid profile, hypertension, and vascular inflammation, all of which promote the development of atherosclerosis (Meigs et al 2003; Esposito & Giugliano, 2011). Atherosclerotic disease underlying MS and obesity is associated with an increasing likelihood to develop ED. Atherosclerosis associated with obesity and MS affects the vascular tissue of the penis as well as generates structural damage within the penis tissue (Talavera-Garcia et al., 2016; Kloner & Speakman, 2002; Tsujimura et al., 2017). The association between MS and ED was elegantly described in some studies (Gunduz et al., 2004; Esposito et al., 2005). In particular, in a cohort of 100 men affected by MS, the prevalence of ED was 26.7% and there was an increase in the presence of growing numbers of metabolic abnormalities, suggesting a link between ED and the cumulative burden of cardiovascular risk (Esposito et al., 2005). Several reports have defined that the risk to develop ED is 70-95% higher in obese men than in normal-weight subjects. A study on 1130 Finnish men with no ED at baseline demonstrated that obese men showed a relative risk of 1.7 to develop ED after 5 years compared to men with normal weight (Shiri et al., 2004). Among 570 men followed up for 25 years, the incidence of ED was higher in obese men than in those who had normal weight (RR¼ 1.7, 95% CI: 1.1–2.5) with a 90% higher risk in overweight men. It has also been reported that the development of ED is significantly higher in men with a high body mass index (BMI >30 kg/m^2) than in men with a low BMI (<25 kg/m^2) (Larsen et al., 2007). Therefore, body weight has been proposed as an independent risk factor for ED (Fung et al., 2004). Moreover, endothelial dysfunction, associated with MS, leads to a decrease in vascular NO levels, thereby resulting in impaired vasodilation, leading to ED. Further, the well-

documented relationship between obesity and androgen deficiency suggests that reduction in testosterone levels, followed by NO biosynthesis impairment, is exacerbated by MS and obesity (Kalyani & Dobs 2007; Diaz-Arjonilla et al., 2009; Kaplan et al., 2006). On the other hand, hyperglycemia associated with obesity and MS, which increases the production of reactive oxygen species, not only promotes atherosclerotic damage to the vascular walls but also causes glycation of penile cavernosal tissue, leading to collagen turnover impairment and later on ED (Beckman et al., 2001; Jiaan et al., 1995).

3.1.4 Diabetes

Diabetes is positively correlated to an increased ED risk. Indeed, a threefold increased risk of ED has been documented in diabetic men (Giuliano et al., 2004; Giugliano et al., 2010; Thorve et al., 2011). Epidemiological studies that describe the prevalence of ED in diabetes do not generally distinguish between type 1 and type 2 diabetes (Bacon et al., 2002; Kalter-Leibovici et al., 2005), whereas other studies have reported an increased ED risk in men with type 1 diabetes as compared to type 2 diabetes (Fedele et al., 2001). However, ED in diabetic patients develops 10–15 years earlier than ED in the average nondiabetic patients (Feldman et al., 1994). ED secondary to diabetes is more severe (Penson et al., 2003) and more resistant to medical management with oral drugs (Goldstein et al., 1998; Corona et al., 2013), leading to a significant decrease in quality of life (De Berardis et al., 2002). An association exists between glycemic control and ED because, as reported in a systematic review of five cross-sectional studies, a poor glycemic control elevates ED risk in type 2 diabetes (Binmoammar et al., 2016). A clinical study on 571 men with type 1 diabetes clearly showed that a period of intensive glycemic control significantly reduces ED prevalence in these patients (Wessels et al., 2011).

The pathogenesis of ED in diabetes is multifactorial and involves not only organic but also psychological factors. Indeed, diabetes negatively affects self-esteem of men, leading to depression and anxiety, and the management of the psychological factors is clearly beneficial (Phè & Rouprêt, 2012). The other complications associated with diabetes, i.e., vasculopathy, neuropathy, visceral

adiposity, hormonal imbalance, insulin resistance, and hypogonadism, are also recognized as organic risk factors for ED (Ponholzer et al., 2005; Bortolotti et al., 2001; Giuliano et al., 2004; Nicolosi et al., 2003; Rosen et al., 2009). Diabetic vasculopathy, due to chronic insult of hyperglycemia, results in atherosclerotic damage, leading to vascular ED (Esposito et al., 2005; Giugliano et al., 2010; Guay, 2007). Endothelial dysfunction also decreases NO, thereby impairing the relaxation of the vascular smooth muscle of the corpora cavernosa, i.e., erection, the key element (Thorve et al., 2011; Malavige & Levy, 2009). In addition, vascular diseases could determine autonomic and peripheral neuropathy contributing to diabetes-induced ED through the impairment of both sensory impulses from the penis to the erotexogenic erectile center and of parasympathetic activity necessary for relaxation of the smooth muscle of the corpus cavernosum (Nehra & Moreland, 2001; Sáenz de Tejada et al., 2003). Finally, visceral adiposity and insulin resistance, peculiar of type 2 diabetes, lead to ED due to a pro-inflammatory state, which results in decreased availability and activity of NO (Esposito & Giugliano, 2011).

3.1.5 Depression

The association of depression with ED has been firmly established, but it may be difficult, in some cases, to distinguish between the cause and the effect, i.e., whether ED causes depression, or depression causes ED, in an individual patient. The incidence of ED in depression appears to be higher than in healthy men (Shiri et al., 2007). Moreover, therapy for depression has been associated with a high prevalence of ED, and this negative consequence may affect compliance with therapy and ultimate depression treatment success (Gregorian et al., 2002).

A recent systematic review and meta-analysis suggest that patients reporting ED should be routinely screened for depression, and patients presenting with symptoms of depression should be regularly assessed for ED. In this regard, policymakers, clinicians, and patients should attend to the association between depression and ED (Liu et al., 2018).

4. ERECTILE DYSFUNCTION AND CARDIOVASCULAR DISEASE

CVD and ED share the same risk factors (Fig. 3). ED is now recognized for most men to be of vascular etiology, with endothelial dysfunction as the common denominator (Solomon et al., 2003). A study performed on 2126 men aged >20 years old showed that the prevalence of ED was ~50% in individuals with diabetes (51.3%, 95% CI, 41.9-60.7) or a history of CVD (50.0%, 95% CI, 41.7-58.3). The prevalence of ED was 13.1% (95% CI, 10.7-15.4) among current smokers and 21.8% (95% CI, 16.6-27.1) among obese individuals (BMI >30 kg/m^2). Slightly less than half of all individuals with treated hypertension (44.1%, 95% CI, 37.2-51.1) or a self-reported history of benign prostate enlargement (42.6%, 95% CI, 33.3-51.9) were affected by ED. The authors compared the crude and age-adjusted estimates, suggesting that age is an important confounder, but the prevalence of ED among individuals with cardiovascular risk factors remained high even after adjustment for age (Selvin et al., 2007). Endothelial dysfunction enhances the intimal proliferation, resulting in vascular disease and hence causing specific organ impairment or systemic pathology depending on its grade and extension. ED often precedes CVD and is often present in men with known CVD, leading to the concept that a man with ED, and no CVD symptoms, is a cardiac or vascular patient until proven otherwise, and a man with known CVD should be routinely asked about his erectile function. The first relevant study that drew attention to the four main arterial risk factors (hypertension, diabetes, smoking, and hyperlipidemia) in men with ED was published in 1985 (Virag et al., 1985a, b). In these studies, (men aged 46-48 years), the authors observed that the frequency of organic ED increased from 49% in the absence of any of the four risk factors to 100% when 3 or 4 of them were present (Virag et al, 1985b).

To date, many studies have been published on this issue contributing to clarify that a link between ED and CVD does exist, and they have led to put forward, for example, the *artery-size hypothesis*. This hypothesis assumes that i) atherosclerosis is a systemic disease that should, in theory, affect all major vascular beds to a similar degree; ii) the rate of occurrence of atherosclerotic symptoms in these vascular beds is dependent on differences in the size of the supplying arteries; iii) larger vessels can better tolerate the same degree of plaque deposition as smaller vessels. The femoral

arteries are 5–7 mm and coronary arteries are 3–4 mm, while the penile artery is 1–2 mm in diameter. Thus, endothelial dysfunction and plaque burden in the small penile arteries can cause the appearance of the ED symptoms well before they affect blood flow in larger arteries (Montorsi et al., 2005). In addition, smaller arteries that supply the penile tissues and sinusoids need to dilate up to 80% to provide blood flow necessary for sufficient venous compression to sustain penile erection than other arteries that dilate up to 15% (Patel & Lees, 1999). On this basis and with evidence showing that the degree of ED correlates with the severity of CVD, it has been postulated that ED is a sentinel symptom in patients with occult CVD. This makes penile arteries a sort of "sentinel" for vascular dilation activity in men. The first evidence of a strong association between ED and subsequent development of clinical cardiovascular events was formulated in a study by Thompson published in 2005. This study analyzed men aged 55 years or older who were randomized to the placebo group (n=9457) in the Prostate Cancer Prevention Trial at 221 US centers, and they were evaluated every 3 months for CVD and ED between 1994 and 2003. Of these 9457 eligible men, randomized to the placebo group, 8063 (85%) had no CVD at study entry; 3816 (47%) were excluded from the analyses assessing the association of incident ED with cardiovascular events, as they reported some level of ED at study entry. Among the 4247 men with no ED at study entry, 2420 (57%) reported incident ED after 5 years, and this increased to 65% at 7 years. Acknowledging this association during a 5-year period and the high prevalence of vasculogenic/atherogenic etiologies in men of this age, the presenting symptom of ED should prompt assessment of cardiovascular risk factors and vigorous interventions, as appropriate. Therefore, it is now widely accepted that while a full cardiovascular evaluation is not necessary in response to findings of ED in asymptomatic patients, such findings should prompt diligent observation of at-risk men for cardiovascular risk factors (Thompson et al., 2005). Indeed, epidemiological and clinical evidence has established that ED precedes the occurrence of CVD symptoms by 2-5 years (Hodges et al., 2007).

Of note, the relation between ED and subclinical CVD is even more relevant for the clinical practice; in general, subclinical vascular disease can be assessed by multiple modalities: cardiac computed tomography, carotid ultrasound, brachial artery flow-mediated dilation, cardiac MRI, and ankle-brachial indices. A significant association has been demonstrated between ED and subclinical disease markers such as flow-mediated dilatation and carotid intima-media thickness, which, together with coronary artery calcium (CAC) scoring, can predict incident ED. In the Multi-Ethnic Study of Atherosclerosis (MESA), advanced CAC and carotid plaque have been proposed as markers of early detection of subclinical atherosclerosis to provide opportunities for predicting the onset of vascular ED and thus even earlier predict the CVD. Men with higher levels of subclinical atherosclerosis and vascular stiffness/dysfunction at baseline had a higher prevalence of ED 9 years later, and only CAC>100 and carotid plaque >2 (odds ratios: 1.43 and 1.33, respectively) were independent significant risk factors for ED beyond a comprehensive set of cardiovascular risk factors (Feldman et al., 2016). Moreover, a significant negative correlation between IIEF and CAC score was observed, implying a positive correlation between ED severity and CAC levels (Yaman et al., 2008).

In summary, while the relationship of ED and subclinical CVD is less certain, the evidence that ED is an independent risk factor for CVD is a wealth of evidence (Uddin et al., 2018; Orimoloye et al., 2019). In a meta-analysis of 12 prospective studies, it is reported that the incidence of ED is 42.0–57.0% in men with CAD and 33.8% in those who have diabetes with silent ischemia compared with 4.7% in men without silent ischemia. Compared to men without ED, men with ED experienced a significantly increased risk of 48% for CVD, 46% for CAD, 35% for stroke, and 19% for all-cause mortality.

Interestingly, ED evaluation could have more relevant impact in the intermediate cardiovascular risk group and among younger men (Dong et al., 2011), as, to date, these subgroups are less monitored for elective preventive treatment. An asymptomatic lipid-rich plaque in the coronary arteries carries the risk of rupture that leads to acute coronary syndrome or death; thus, for these

subgroups, ED may be predictive of serious events in the absence of cardiac symptoms (Ibrahim et al., 2018).

Despite general agreement, ED is not mentioned in US risk prediction guidelines (Goff et al., 2014). In line with US guidelines, neither the European SCORE risk chart nor the ACC/AHA Pooled Cohort Equations include ED as a variable to include in the calculation of global CVD risk. Of the common CVD risk calculators, ED status is only included in the recently updated United Kingdom QRISK-3 10-year CVD risk prediction algorithm, where its presence was shown to be associated with a 25% increased risk of CVD (Hippisley-Cox et al., 2017). While the inclusion of ED in global multivariable risk prediction models may be a subject of controversy, ED assessment may have potential utility when adopted as a risk-enhancing factor whose presence may signal the need for a more detailed assessment or aggressive therapy, independent of these risk scores.

Certain expert and guideline committees such as the Princeton Consensus III, and more recently, the 2018 American Urological Association (AUA) guidelines on ED, have adopted this approach, highlighting the presence of ED as an important risk marker for underlying CVD that may warrant further evaluation and treatment (Burnett et al., 2018; Nehra et al., 2012). The urologist's involvement in these consensuses, recommendations, and guidelines is highly encouraged, with an aim to improve patient quality care and overall health. The decision to treat with preventive therapy (i.e., statins) is now well accepted for individuals with a very high (>20%) 10-year Atherosclerotic Cardiovascular Disease (ASCVD) score. On the other hand, a significant number of men fall into an "intermediate" (5-20%) score category, where preventive therapy is not always adopted; in this case, stratification is needed. To this aim, the CAC evaluation has demonstrated significant utility for both reclassification and therapeutic decision (Greenland et al., 2018). Moreover, individuals who are in the low-risk group (<5%) but have concerning "risk-enhancing factors" such as ED may benefit from additional screening.

In summary, the Princeton Consensus III (Expert Panel) Conference recommends assessing cardiovascular risk in all patients with ED and CVD. This refers to estimating the risk of mortality

and morbidity associated with sexual activity. The current recommendations classify patients into low-, intermediate-, and high-risk on the basis of their New York Heart Association class. The consensus also recommends that all patients with ED and CVD should undergo lifestyle changes such as exercise, smoking cessation, healthy diet, and weight reduction. These measures are likely to reduce cardiovascular risk and improve erectile function. Moreover, patients with ED at high risk of cardiovascular events should refrain from sexual activity until they have a stable cardiovascular condition. Their management should be under close supervision from a cardiologist. The guideline suggests starting the evaluation with a noninvasive assessment, i.e., stress testing, ankle-brachial indices, or carotid intima-media thickness evaluation, in men above 30 years of age. However, the specific test ordered is ultimately guided by the evaluating primary care physician, men's health expert, or cardiologist. For patients with clinical CVD, the expert consensus recommends assessment of cardiovascular risk associated with sexual activity; elective stress testing for low-risk patients, standardized stress testing for intermediate-risk patients, and cardiology referral for high-risk patients. Interestingly, the CAC score can also be used in low-risk patients (<5% risk) who have a strong family history of heart disease (Nehra et al., 2012; Gowani et al., 2017).

We also suggest to the reader the review by Orimoloye OA, et al., where the link between ED and CVD has been deeply analyzed, referred to relevant clinical studies (Orimoloye et al., 2019).

5. TESTOSTERONE AND ERECTILE DYSFUNCTION

In healthy adult men, testosterone production is regulated by the hypothalamic–pituitary–gonadal (HPG) axis. Higher cortical centers in the brain signal the hypothalamus to secrete gonadotropin-releasing hormone (GnRH) in a pulsatile manner. GnRH stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary, which, in turn, modulates testosterone production from the Leydig cells and spermatogenesis by the Sertoli cells. As testosterone levels increase, negative feedback suppression is exerted on the androgen receptors in the hypothalamic neurons and pituitary gland, thereby inhibiting the release of GnRH, FSH, and

LH (Basaria, 2014). Testosterone, among androgens, is mainly involved in the development and growth of the penis as well as in the regulation of physiological mechanisms during erection in men. In fact, it plays a crucial role in penile response to sexual stimuli and in the maintenance of libido. In hypogonadal patients, testosterone supplementation stimulates both sleep-related erections and erectile function (Kwan et al., 1983; Montorsi & Oettel, 2005). Men with erectile disorder who have decreased serum levels of testosterone respond well to testosterone treatment, whereas the efficacy is associated exclusively with high levels of LH and low values of the T/LH (testosterone/LH) ratio (Rakic et al., 1997). Notably, in healthy men, testosterone administration enhances the rigidity of nocturnal penile tumescence (Carani et al., 1990). In addition, a recent review concluded that "normalizing" testosterone levels, in men with low testosterone, ameliorates libido and erectile function when used as monotherapy in men with mild ED. Testosterone therapy may be ineffective in men with moderate and severe ED, as the etiology for these more severe pathologies often includes diabetes, radical pelvic surgery, or severe neurologic damage. In these cases, the hormonal factor is often not the primary cause of dysfunction and other treatments are likely to be more effective (Rizk et al., 2017).

A relationship between plasma hormones and flow-mediated vasodilation (FMD) of the brachial artery by using ultrasonography has also been reported. In detail, total and free testosterone and dehydroepiandrosterone-sulfate (DHEA-S) showed significant correlation with FMD%. A low plasma testosterone level was associated with endothelial dysfunction in men, independent of other risk factors, suggesting a protective effect of testosterone on the endothelium (Akishita et al, 2007). Additionally, a well-conducted clinical study showed that precise combined dynamic penile vascular and hormonal evaluation of patients with ED can unmask subtle endocrine differences between organic and psychogenic patients. Indeed, patients with organic ED had lower free testosterone levels than patients with psychogenic ED that correlated well with erectile function and the degree of trabecular smooth muscle relaxation, measured by the resistive index at dynamic

duplex ultrasound. Thus, a strong correlation exists between free testosterone and compliance of cavernous arteries in patients with organic ED (Aversa et al., 2000).

Testosterone, in addition to its central action, can affect erectile function at the peripheral level. Systemic testosterone levels increase in healthy subjects during the tumescence and rigidity (Becker et al., 2000b; Stoleru et al., 1993). Similarly, an increase in cavernous testosterone levels was found in healthy volunteers during penile erection (flaccidity to tumescence), and this increase was less pronounced in patients with ED (Becker et al., 2001). All these lines of evidence are in line with the concept of peripheral action of testosterone. This is not surprising as it is known that HCC expresses androgen receptors that are localized in stromal and in endothelial cells together with estrogen-alpha receptors (Schultheiss et al., 2003). In particular, HCC samples obtained from biopsies of adult potent patients undergoing penile deviation surgery or male-to-female transsexual surgery, receiving either estrogens or androgens or their combination, show neither significant difference in androgen receptor distribution nor any correlation with age. A nongenomic effect of testosterone has been reported in HCC and human cavernosal artery strips. Testosterone caused relaxation in both HCC and human cavernosal arteries precontracted with NA. To exclude the local conversion of testosterone into 17β estradiol by aromatase, dihydrotestosterone was used. It caused a relaxation that mimicked testosterone in magnitude and time. Notably, no change in cAMP or cGMP content was found in human tissues stimulated with testosterone (Waldkirch et al., 2008). A study published in 2009 clearly demonstrated that testosterone relaxed HCC strips in a concentration-dependent manner, and this effect was inhibited by blocking K_{ATP}. Therefore, acute vasorelaxant, i.e., the nongenomic effect of testosterone, is mediated in part by increasing potassium efflux through K_{ATP} channels (Yilidiz al., 2009).

6. ERECTILE DYSFUNCTION DIAGNOSIS

AUA and EAU guidelines propose a schematic approach for diagnostic evaluation and treatment of ED (Hatzimouratidis et al., 2019; Burnett et al., 2018; Hatzimouratidis et al., 2017, https://uroweb.org/wp-content/uploads/14-Male-Sexual-Dysfunction_LR.pdf).

The EAU scheme guidelines for ED diagnostic evaluation are reported as follows:

Basic workup

1) Sexual history
2) Physical examination
3) Laboratory testing
4) Cardiovascular system and sexual activity: the patient at risk
    a. Low-risk category
    b. Intermediate- or indeterminate-risk category
    c. High-risk category

Specialized diagnostic tests

1) Nocturnal penile tumescence and rigidity test
2) Intracavernous injection test
3) Duplex ultrasound of the penis
4) Arteriography and dynamic infusion cavernosometry or cavernosography
5) Psychiatric assessment
6) Penile abnormalities

Patient education – consultation and referrals

AUA scheme guidelines are reported as follows:

1) Men presenting with symptoms of ED should undergo a thorough medical, sexual, and psychosocial history; physical examination; and selective laboratory testing.
2) For men with ED, validated questionnaires are recommended to assess the severity of ED, to measure treatment effectiveness, and to guide future management.

3) Men should be counseled that ED is a risk marker for the underlying CVD and other health conditions that may warrant evaluation and treatment.
4) In men with ED, morning serum total testosterone levels should be measured.
5) For some men with ED, specialized testing and evaluation may be necessary to guide treatment.

Thus, the evaluation of men with ED requires a full medical, personal, and sexual history and focused clinical examination. It is important to obtain careful history to determine the extent of symptoms and their association with chronic diseases, medication use, and psychosocial issues. The first approach to evaluate the onset of symptoms, severity degree of impact on daily life, and situational factors that exacerbate symptoms is represented by validated questionnaires used to help both diagnose and track treatment effectiveness for patients with ED. Indeed, validated psychometric questionnaires such as Index for Erectile Function (IIEF) or its short version and the Sexual Health Inventory for Men (SHIM) help to assess not only the different sexual function domains but also the potential impact of specific treatment (Hatzimouratidis et al., 2017). IIEF, which consists of 15 items and 5 domains, is a psychometrically valid and reliable instrument that was developed through consultations with an international panel of experts for defining the grade and area of sexual dysfunction. In addition, the IIEF has high sensitivity for detection of treatment effects and has been adopted to monitor changes related to treatment. On the other hand, the SHIM, also called IIEF-5, is an abridged and slightly modified five-item version of the IIEF, designed for easy use by clinicians to diagnose the presence and severity of ED in clinical settings. The IIEF it is intended to complement the physical examination and patient history as a means of detecting ED.

Although most patients with ED can be managed within the primary care setting, some circumstances require specific diagnostic testing, as reported in Table 1, according to EAU guidelines (2019). The specific diagnostic tests are summarized in Table 2. In particular, evaluation of nocturnal penile tumescence (NPTR) and rigidity testing using RigiScan (Hatzimouratidis et al., 2010) are recommended. NPTR was reported to occur in all men of different age groups during

periods of rapid eye movement (REM) sleep (Karacan et al., 1975), assuming that, during sleep, psychological factors cannot interfere with nocturnal erection, whereas organic factors can interfere variously. The RigiScan device, considered one of the most reliable tools to diagnose ED and to differentiate psychogenic from organic cases, allows to measure penile tumescence and rigidity continuously through two loops, one to be placed around the base of the penis and the other toward the tip, that tighten every 15 or 30 seconds. In addition to thorough full medical, personal, and sexual history, diagnosis of ED requires appropriate physical examination. First, given the association of ED with obesity and hypertension, it is advantageous to assess pulse, blood pressure, and weight (Montorsi et al, 2010). The International Consultation on Sexual Medicine of the International Society for Sexual Medicine proposes that laboratory tests for men with ED include fasting glucose level, fasting lipid profile, and, in select cases, hormonal tests (Montorsi et al., 2010; Qaseem et al., 2009). Hormonal tests include early morning total testosterone evaluation. If indicated, the bio-available or calculated free testosterone may be needed to corroborate total testosterone measurements (Hatzimouratidis et al., 2017). In addition, to distinguish between psychogenic and organic ED, several guidelines suggest the intracavernous injection test. A vasoactive drug, usually prostaglandin E1, i.e., alprostadil, is administered as an intracavernous injection to assess penile rigidity after 10 minutes (Hatzichristou et al., 1999; Hackett et al., 2008). Its use as a diagnostic test for ED is limited because a positive result can also be found in patients with both normal and mild vascular disease (Hatzichristou et al., 1999). Indeed, after the intracavernous injection, a duplex Doppler study of the penis should be requested, if clinically warranted (Hatzimouratidis et al., 2017). The use of duplex Doppler provides information about penile hemodynamic discriminating between arterial insufficiency and veno-occlusive dysfunction from other causes of ED (Sikka et al., 2013).

More recently, it has been demonstrated that platelet cGMP level may be used as a biomarker for ED diagnosis and PDE5 inhibitor efficacy (Mirone et al., 2009). Patients with ED (IIEF<26) were unrolled and randomized to 6 weeks of vardenafil, 5 mg/d or placebo. Platelet cGMP was measured

in both placebo and vardenafil groups before starting the protocol and after the 6 weeks of treatment. Similarly, patients completed the IIEF and underwent visual sexual stimulation coupled with RigiScan. Platelet cGMP content was higher in patients taking vardenafil versus placebo. Vardenafil was not superior to placebo in improving IIEF but ameliorated visual sexual stimulation-RigiScan. Interestingly, platelet cGMP content displayed a weak association with IIEF but a significant correlation with visual sexual stimulation-RigiScan. Although the measurement of platelet cGMP is not a routine laboratory test, it may represent, in the future, an important parametric measure that helps to interpret and/or correct the data obtained by IIEF and other psychometric questionnaire in clinical practice for ED diagnosis and PDE5 inhibitor efficacy (d'Emmanuele di Villa Bianca et al., 2011). Further studies, in a larger cohort, are needed to support this hypothesis.

7. PHARMACOLOGICAL THERAPY

Basing on the fact that ED and CVD share common risk factors as reported also in the recent EAU 2019 guidelines (Hatzimouratidis et al. 2019), it is recommended to approach the following lifestyle adjustments:

- weight loss in case of overweight and adoption of a diet to prevent or counteract the negative effect of risk factors on ED development,
- sodium dietary restriction,
- regular aerobic physical activity,
- moderate alcohol consumption (two or less alcoholic beverages/day),
- smoking cessation.

The changes in lifestyle could, in some cases, improve the erectile function, while, in the case of established ED related to other risk factors or organ damage, a pharmacological therapy must be associated. Several studies have shown some evidence that lifestyle modification and pharmacotherapy for CVD risk factors may be of help in improving sexual function in men with

ED. Indeed, regular physical exercise improves erectile function through different mechanisms involving glucose and lipid metabolism, regulation of arterial pressure, production of NO, and hormonal modulation. Exercise shows a synergistic effect with the drugs commonly used in the treatment of ED. Of note, the evaluation of individual cardiovascular risk is mandatory before prescribing physical exercise, as many patients with ED may have underlying CVD. When exercise is not contraindicated, the most appropriate protocol must be chosen, considering the individual characteristics of the patient. Both aerobic and anaerobic/resistance protocols have proven effective. Meta-analysis studies show that aerobic exercise with moderate-to-vigorous intensity is most effective in improving erection (Duca et al., 2019). However, it should be emphasized that more controlled perspective studies are necessary to determine the effects of exercise or other lifestyle changes in the prevention or treatment of ED (Gupta et al., 2011).

7.1 Oral Therapy

7.1.1 Phosphodiesterase inhibitors

PDEs are enzymes responsible for the hydrolysis of cyclic nucleotides, namely, cAMP and cGMP. In particular, PDE5 inhibitors significantly enhance the effect of the endogenous NO, released following sexual stimulation. When NO is released, inhibition of PDE5 leads to accumulation of cGMP levels, causing smooth muscle relaxation through the activation of intracellular PK and modulation of intracellular Ca^{2+} as well as resulting in influx of blood, thereby facilitating penile erection (Corbin, 2004; Francis, 2010). These drugs do not induce a direct and erogenous stimulus; hence, the erection occurs only if the stimulation is associated with natural stimulation (Corbin, 2004). This peculiar characteristic gives a connotation of "spontaneity" that also represents an advantage from the psychological point of view. To date, PDE5 inhibitors represent a mainstay in the therapy of ED, effective and well tolerated in more than 70% of cases (Hatzimouratidis et al., 2017; Burnett, 2018). To date, there are currently seven available PDE5 inhibitors (sildenafil, tadalafil, vardenafil, avanafil, lodenafil, mirodenafil, and udenafil) with different dosages and

formulation. The review focuses on sildenafil, vardenafil, tadalafil, and avanafil approved by the US FDA.

PDE5 inhibitors are metabolized by the liver; thus, their metabolism can be altered by CYP3A4 inhibitors and inducers; they possess vasorelaxant properties and exert systemic hemodynamic effects, which need to be considered when other cardiovascular drugs are co-administered. Special caution is needed with alpha-blockers, while they are contraindicated in the case of nitrate therapy, such as nitroglycerin or isosorbide dinitrate. Indeed, the combination with one of these two drugs would create sustained vasodilation, which could be extremely dangerous (Doumas et al., 2015). Although a causal relationship has not been established, the administration of PDE5 inhibitors in patients with comorbidities such as diabetes, hypertension, and dyslipidemia appears to be associated with non-arteritic anterior ischemic optic neuropathy as a side effect. In addition, headache, dyspepsia, flushing of the face, dizziness, nasal congestion, vomiting, diarrhea, vision disorders, eye pain/inflammation, back pain, and myalgia are reported as side effects. These common adverse events are caused by the inhibition of the other isoforms of PDE in tissues outside of the penis (Burnett et al., 2018). The most significant difference among these drugs is the serum half-life. Table 3 summarizes the pharmacokinetic profiles of PDE5 inhibitors.

Sildenafil

Sildenafil is the first PDE5 inhibitor used for the treatment of ED; administered orally, at doses of 25, 50, and 100 mg, sildenafil is rapidly absorbed with a bioavailability of 40%, reaching the maximum plasma concentrations after 60 minutes (Hatzimourratidis, 2006). It is metabolized by the liver, mainly by the cytochrome isoenzymes CYP3A4 (primary route) and CYP2C9 (secondary route), to an active metabolite with similar properties, which contributes approximately 20% to the overall activity. Sildenafil should not be used in patients with severe cardiovascular disorders such as angina pectoris or severe heart failure. It is also contraindicated in cases of hypotension (blood pressure <90/50 mmHg), severe hepatic impairment, retinitis pigmentosa, and recent history of stroke or myocardial infarction. Furthermore, sildenafil can also interact with certain foods and

beverages, particularly with grapefruit-based juice or supplements. Therefore, it is always preferable to avoid the consumption of this fruit, which tends to interfere with the correct absorption of the drug (Mehrota et al., 2007).

Vardenafil

Vardenafil is the second PDE5 inhibitor commercialized in 2003. Vardenafil is a structural modification of the parent molecule sildenafil. Nevertheless, it has been demonstrated that PDE5 inhibitors might have some beneficial effects in men with lifelong premature ejaculation (Gökçe et al., 2011); in particular, the safety and efficacy of vardenafil or sertraline in premature ejaculation have been investigated in a randomized trial (Mathers et al., 2009). Vardenafil and sertraline showed a statistically relevant improvement of premature ejaculation. This result has been confirmed by other groups using sildenafil (Abdel-Hamid et al., 2001).

Tadalafil

Tadalafil came on the market in February 2003. The onset of the therapeutic effect of tadalafil occurs within 30 minutes following administration, with peak efficacy after approximately two hours. Efficacy is maintained for up to 36 hours and not affected by food (Brock et al., 2002). Tadalafil is administered on demand at doses of 10 and 20 mg or, alternatively, in a daily dose of 5 mg. The recommended starting dose is 10 mg and must be adapted depending on the patient's response and side effects. Daily tadalafil 5 mg therapy has also been approved for the treatment of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia Therefore, it is useful in concomitant patients with ED and LUTS (Hatzimouratidis et al., 2017). It has a highly selective action on PDE5, and no effect is observed on the cardiovascular system, liver function, and other organs. Tadalafil does not modify the sperm quality and does not cause changes in the levels of testosterone, FSH, and LH in the blood. Tadalafil is metabolized by the cytochrome P450 enzyme group, particularly by the isoenzyme CYP3A4 (Brock et al., 2002).

Avanafil

Avanafil is the fourth drug of the PDE5 inhibitors class approved for the treatment of ED marketed in 2013 at dosages of 50, 100, or 200 mg (Goldestein et al., 2012). After oral administration, it is rapidly absorbed from the gastrointestinal tract, and the maximum plasma concentration is reached from approximately 30 to 45 minutes after administration. In the body, approximately 99% of the drug is bound to plasma proteins. The molecule is extensively metabolized by the cytochrome P450 isoenzyme CYP3A4 and, to a lesser extent, by the CYP2C isoform.

Oro-dispersible tablets

Oro-dispersible tablets (ODTs) are innovative drug delivery systems formulated to dissolve rapidly when placed in the mouth, without the need for water (Kathpalia & Gupte, 2013). Several drugs have been successfully formulated as ODTs including the PDE5 inhibitors vardenafil and sildenafil. A rapidly disintegrating ODT formulation of vardenafil 10 mg has been developed and is marketed in Europe, the United States, and other countries. The efficacy and safety of the vardenafil 10 mg ODT formulation, administered on demand, were established in the POTENT I and POTENT II randomized, placebo-controlled trials (Gittelman et al., 2010; Sperling, et al., 2011). In both studies, vardenafil ODT therapy showed significantly higher results than placebo for all primary and secondary measures of erectile function. Similarly, an ODT formulation of sildenafil citrate offers an alternative pharmaceutical form to the marketed film-coated tablet (Damle et al., 2014). The ODT formulation of sildenafil improves treatment adherence, thereby enhancing the sexual health and sense of psychological well-being of patients and their partners (Scaglione et al., 2017). Accordingly, Cocci and coworkers (2017) have reported that almost all patients enrolled in the study stated that they recommend this new formulation, as it has greater rapidity of absorption indeed, the median action time is 20 minutes, and the only side effect recorded is related to the bad taste. In addition, basing on the results of the food-effect study, sildenafil ODT should be taken on an empty stomach (Damle et al., 2014).

7.2 Intracavernous therapy

With the advent of PDE5 inhibitors as oral therapy, intracavernous injection of vasoactive agents has been relegated to second-line therapy (Porst, 2000). Prostaglandin E-1 (PGE-1), phentolamine, and papaverine remain the most used injectable vasoactive drugs and are efficacious for vascular and nonvascular (psychogenic, hormonal, and neurogenic) forms of ED. According to the AUA guidelines, an in-office intracavernous injection test dose should be administered to all men to optimize the dose and to ensure the patient does not develop priapism or adverse effects. In patients with a history of recurrent priapism, Peyronie's disease, and bleeding disorders, the decision to use this medication should be carefully evaluated (Belew et al., 2015).

7.2.1 Prostaglandin E-1

In 1996, PGE-1 became the first and only FDA-approved penile injectable for ED treatment. It acts by increasing cAMP in the penis and reducing free Ca^{2+} concentration and then smooth muscle relaxation. Once injected, PGE-1 is rapidly metabolized during the first passage through the lung. Plasma half-life of PGE-1 is approximately 1 min (Porst, 1996). Side effects include burning sensation at the time of injection, priapism (1%), and penile fibrosis (1-3%) (Hauck et al., 1999; Porst et al., 1998). It has been suggested that long-term use of intracavernous injection of vasoactive agents could promote spontaneous erection. It has been demonstrated that long-term PGE-1 intracavernous injection may provide subjective improvement in erectile function in some men (Maniam et al., 2001). With the advent of PDE5 inhibitors on the market, the use of PGE-1 is restricted to patients who have failed first-line oral pharmacotherapy. However, in addition to its therapeutic role, intracavernous PGE1 is commonly used as a diagnostic option to assess the vascular flow in men with ED. Lack of response in this test suggests the presence of a venous-occlusive mechanism leakage (Elhanbly et al., 2002).

7.2.2 Papaverine

Papaverine is an opium alkaloid originally isolated from the poppy *Papaver somniferum*. It is primarily used with spasmolytic indications in therapy. In 1982, a French surgeon, Ronald Virág, discovered in Paris that papaverine could be administered as an intracavernosal injection.

Papaverine injection was used for a long time as a second-line treatment, but currently, it is not indicated because of more discomfort or side effects to the patient (Glina et al, 2010). It relaxes cavernous smooth muscle by nonspecific inhibition of PDEs, increasing cAMP and cGMP levels. Papaverine has essentially hepatic metabolism with a plasma half-life of 1-2 h. Finally, as it occurs for PGE-1, papaverine is used as diagnostic tool to exclude vasculogenic causes (Mutnuru et al., 2017)

7.2.3 Phentolamine

Phentolamine was discovered in 1978 (Domer, 1978). It is a nonselective alpha-adrenergic antagonist that inhibits smooth muscle cell contraction. It has weak efficacy as monotherapy and can be used in combination therapy only.

7.2.4 Vasoactive intestinal peptide

VIP increases the activity of adenosine cyclase with consequent cavernous smooth muscle relaxation and penile erection. Side effects include flushing and headaches. Generally, it is used in combination with phentolamine when the monotherapy is ineffective (Dinsmore & Wyllie, 2008). The combination, named Invicorp® approved in Denmark, shows a reduction in the incidence of priapism, pain, and fibrosis.

7.3 Intraurethral therapy

7.3 Prostaglandin E-1

PGE-1 is also available as an intraurethral suppository. Patients who wish to avoid oral or injectable medications may choose this route of administration. While intraurethral administration of PGE-1 ameliorates erectile function over placebo, it is less effective than the intracavernous injection (Dinsmore & Wyllie, 2008). The most common adverse effect is penile or urethral pain (Costa & Potempa, 2012). This medication should be used with caution in patients with increased risk for priapism or in patients with urethral disease. A condom should be used during sexual activity with a pregnant female partner to minimize the potential risk of fetal exposure to prostaglandin.

7.4 Topical therapy

7.4 Prostaglandin E-1

A multicenter, open-label, long-term study in 1,161 patients with ED evaluated the safety and efficacy of topical alprostadil that was administered in the penis meatus before intercourse (Rooney et al., 2009). It was considered effective and safe by most patients and their partners, with most adverse events limited to the application site. The delivery of alprostadil offers an improved alternative.

7.5 Low-intensity Extracorporeal Shockwave Therapy

Low-intensity extracorporeal shockwave therapy (LI-ESWT) is included as first-line therapy for ED. In fact, it has been proven to be useful for various medical conditions such as neovascularization in myocardial ischemia, nonhealing wounds, ED, and chronic pelvic pain syndrome. In this scenario, several studies suggest that LI-ESWT can improve the IIEF-5 and the Erection Hardness Score of patients with mild ED (Lu et al., 2017).

8. PREFERENCES AND SWITCH STUDIES: ARE ALL THE PDE5 INHIBITORS THE SAME?

The discovery and introduction of therapy of the PDE5 inhibitors have revolutionized the treatment and the management of ED. PDE5 inhibitors are considered an efficient and well-tolerated treatment option (Eardley et al., 2010; Hatzimouratidis et al., 2016; Ventimiglia et al., 2016). The satisfaction of patients receiving ED therapy is a multifaceted and personal matter, and there are no clearly recognized and reliable criteria for selecting among PDE5 inhibitors. Indeed, there are no significant differences for what concerns safety and efficacy. These inhibitors differ mainly in half-life, duration of action, and dietary effects on absorption (Forgue et al, 2006; Nichols et al., 2002; Stark et al., 2001). In addition, what makes even more complicated the panorama is that the adherence of patients to the PDE5 therapy cannot be exclusively based on the erectile response and the side effects. Indeed, how the treatment meets the needs and expectations of patients and how it affects the relationship between the partners must be considered. In other words, patients using

different PDE5 inhibitors have a similar perception of improvement after treatment (Jannini et al., 2009). To define possible difference among these inhibitors, several preference studies comparing PDE5 inhibitors have been performed. Most of these studies have compared sildenafil and tadalafil, and only few have addressed vardenafil, even less udenafil and avanafil. Albeit, the same being observational, sponsored by industry, or biased, they suggest a general preference for tadalafil (Smith et al., 2013; Govier et al., 2003; von Keitz et al., 2004; Eardley et al., 2007; Tolrà et al., 2006). Similarly, Mirone and coworkers (2009) have reported that there is lack of direct comparative studies, and preferences studies are inconclusive and biased. Therefore, it is complex to select the most favorable PDE5 inhibitor (Mirone et al., 2009). Moreover, up to 50% of patients stop treatment for the cost, inadequate efficacy, or adverse events. Indeed, the International Consultation on Sexual Medicine Reports concluded that, despite the favorable clinical data, high dropout rates exist, and it is extremely difficult to define the "best" PDE5 inhibitor (Hatzimouratidis et al., 2016). Nevertheless, a more recent well-conducted meta-analysis examines the efficacy of sildenafil and tadalafil and provides important evidence for the selection of PDE5 inhibitor for the clinical treatment of ED (Gong et al., 2017). Tadalafil and sildenafil exhibit comparable efficacy, safety, and satisfaction for the treatment of ED, and tadalafil significantly improved the psychological outcomes. The adherence and persistence rates for tadalafil and sildenafil were equal. Both men and women preferred tadalafil to sildenafil for the treatment of ED. Thus, tadalafil may be a better choice for ED treatment. On the other hand, a meta-analysis published in 2015 included randomized controlled studies comparing at least one PDE5 inhibitor with placebo or with other PDE inhibitors, whereas various dosages of avanafil, lodenafil, sildenafil, tadalafil, udenafil, and vardenafil were used, and it concluded that for men prioritizing high efficacy, sildenafil 50 mg appears to be the treatment of choice. Men wishing to optimize tolerability should take tadalafil 10 mg or switch to udenafil 100 mg in the case of insufficient efficacy (Chen et al., 2015). In conclusion, there is no evidence-based guidance for either clinicians or patients regarding PDE5 inhibitor choice. Corona and coworkers have tried to define a simple algorithm as a useful tool to be

used in daily clinical practice to facilitate the PDE5 inhibitor choice. The algorithm identifies factors influencing the PDE5 inhibitor choice during patients' assessment, for example, age, food, alcohol, partner needs/requirements, habit frequency *versus* nonsignificant factors, i.e., anamnestic factors (Corona et al., 2011). The situation is further complicated by the fact that often patients switch from one PDE5 inhibitor to another, and this does not allow to perform real-world study. In addition, there is a high discontinuation rate. An inadequate treatment response could be responsible for this, but additional factors have been associated with a higher probability of discontinuing treatment, including age above 60 years, presence of comorbidities (Souverein et al., 2002), and severe ED (Sato et al., 2007). Likewise, emotional and relationship factors as well as financial reasons have also been mentioned as the cause for discontinuation. In this context, a study conducted on patients with ED, self-reported as nonresponders to either tadalafil or vardenafil, demonstrates that often an increase in "nonresponders" to a selected PDE5 therapy is related to insufficient information on i) the timing of intercourse, ii) food instructions, and iii) fewer doses/attempts performed by the patients (Hatzimouratidis et al., 2006). The authors conclude that by using a continuous administration scheme, a consistent number of "nonresponders" to PDE5 inhibitor therapy may be salvaged.

Thus, for the reasons described above in detail, it is not suitable to use these studies to define, or better unmask, possible differences among the PDE5 inhibitors that could help to define different therapeutic approaches, i.e., the more appropriate PDE5 inhibitor for each ED patient category.

9. FUTURE THERAPEUTIC APPROACHES

9.1 Stem cell therapy

Preclinical studies have investigated the use of intracavernous stem cells for the treatment of ED following radical prostectomy (RP) with encouraging results. Recently, stages I and II of a phase I/II clinical trial of intracavernous injection of autologous bone marrow mononuclear cells (BM-MNCs) in humans have been reported (NCT01089387) (Yiou, 2017). In stage I, four doses of

intracavernous injection of BM-MNCs were tested in 12 patients, and the safety and the efficacy were evaluated at scheduled time points (1, 3, 6, and 12 months). Overall, BM-MNC injection improved most of the sexual function scores at 6 months. In stage II, six additional patients received the optimal dose identified in stage I (10*9 BM-MNCs), and the results of 12 patients included in stage I were reported. The study suggests that intracavernous injection of BM-MNCs is safe and improves erectile function. Interestingly, significant improvement of erectile function has been found using injection of autologous adipose-derived cells freshly isolated by liposuction in patients with RP (Haahr et al., 2016). Therefore, both approaches appear to be promising interventional therapies of ED following RP.

9.2 sGC stimulators and activators: BAY 60-4552

A direct stimulation of sGC can be considered as an alternative approach, especially for patients who do not respond to PDE5 inhibitors. BAY 60-4552, a heme-dependent stimulator, was considered as a promising molecule from preclinical studies. A phase II study examined the efficacy and safety of the combination of BAY 60-4552 (1 mg) and vardenafil (10 mg) versus vardenafil (20 mg) in patients who did not respond to standard 20 mg vardenafil. The combination was effective compared to placebo but was not superior to vardenafil alone. A prospective, randomized, double-blind, double-dummy, placebo, active controlled, multicenter study assessed the efficacy and safety of the combination BAY 604552/vardenafil compared to vardenafil (20 mg) for the treatment of ED, not sufficiently responsive to standard therapy with PDE5 inhibitors (Mónica & Antunes, 2018).

9.3 Rho/RhoA Kinase inhibitor

SAR 407899 has been characterized as a novel and potent selective Rho-kinase inhibitor with promising antihypertensive activity (Löhn et al., 2009). Later, it has been demonstrated that SAR407899 both in isolated HCC strips and in an in vivo animal model showed greater potency and longer duration of action than sildenafil (Guagnini et al., 2012). SAR407899 has been tested in a clinical study in patients with ED (NCT00914277) but discontinued due to the lack of efficacy.

More recently, the relaxant effect of Y-27632 in HCC strips has been demonstrated, which was enhanced in the presence of vardenafil (Uvin et al., 2017). Therefore, the combination of inhibition of Rho-kinase and PDE5 inhibitor may represent a promising oral therapeutic approach for the treatment of ED.

9.4 Maxi-potassium channel activators

It is a well-consolidated concept that potassium channels contribute to the penile erection (Archer, 2002). To date, andolast (Rottapharm BioTech, Monza, Italy) is the only maxi-potassium channel-activating drug still in clinical development. It is in phase III trial for the treatment of allergic rhinitis, asthma, and chronic obstructive pulmonary disease (Maherba et al., 2015). A phase I trial has investigated the effect of a single-dose corpus cavernosum injection of thw transfer of the naked DNA sequence of the alpha-subunit of the maxi-potassium channel in eleven patients with moderate-to-severe ED. Unfortunately, the efficacy cannot be drawn from these results, as, in the phase I trial, no control group was included. However, the promising primary safety outcomes of the study and preliminary indications of effectiveness provide evidence that maxi-potassium gene transfer is a viable approach to the treatment of ED and that further studies investigating the efficacy of maxi-potassium in patients with ED should be performed (Melman et al., 2006).

9.5 NO donors

A recent study evaluated the efficacy and tolerability of MED2005, a 0.2% glyceryl trinitrate topical gel, formulated into an enhanced absorption topical delivery system administered on demand, in the treatment of ED. The primary outcome measure was the IIEF score. The start of erection was noticed within 5 and 10 minutes in 44.2% and 69.5%, respectively, of all intercourse attempts with MED2005. In total, 23.1% of patients showed a clinically relevant increase in IIEF-EF scores (>3 points) after treatment with MED2005 compared to 14.5% of patients compared to placebo. Patients and partners showed significant preferences for MED2005 over placebo. This study suggests that topical glyceryl trinitrate could be a useful treatment option in mild ED (Ralph et al., 2018). Further studies including higher doses of MED2005 are needed to better clarify the

clinical significance particularly in case of moderate and severe ED. In relation to MED2005, there is an ongoing study of phase III clinical trial (see "10. ONGOING CLINICAL TRIALS" section). Clinical studies have been also performed using L-arginine, the NO precursor. The efficacy and the safety of 8 g L-arginine aspartate combined with 200 mg of adenosine monophosphate with placebo alone for intermittent treatment of mild-to-moderate ED were compared (Neuzillet et al., 2013). This pilot phase II study showed that the on-demand oral administration of the combination may be effective in patients with mild-to-moderate ED, and this treatment was very well tolerated. These preliminary results need to be confirmed in a larger size phase III study.

9.6 Melanocortin agonist

The efficacy and safety of synthetic melanotropic peptides MTII and PT-141 have been investigated in the treatment of ED. Subcutaneous injection of the MTII peptide (0.025 mg/kg) elicited significant erection in 8 /10 men with psychogenic ED assessed by real-time RigiScan compared to placebo. These findings were supported by a double-blind placebo-controlled study where the administration of MTII to 20 men with psychogenic and organic ED resulted in penile erection in 17/20 men in the absence of video sexual stimulation. Therefore, the latter result suggests that MTII agonist acts with a mechanism that does not require sexual stimulation to achieve an erection as it occurs for a PDE5 inhibitor. The most frequent side effects noted were yawning, nausea, and decreased appetite (Wessells et al., 2000). The clinical study leads to the development of PT-141, a molecule that possesses efficacy comparable to MTII but with an improved tolerability profile and a more rapid onset of action. PT-141, a cyclic heptapeptide melanocortin analog, was evaluated following intranasal administration in healthy male subjects and patients with ED. In the phase IIA study, a clinically significant erectile response was observed in patients with ED following single-dose PT-141 administration compared to placebo. The pharmacokinetics and pharmacodynamics data collected in these studies indicate that PT-141 is a promising candidate for further evaluation as an ED therapy (Diamond et al., 2004; Rosen et al., 2004). The contribution of the melanocortin system to the sexual function is well established, and the role of the melanocortin-4 receptor

subtype has received particular interest. In a pilot clinical study in patients with ED, a potent and selective melanocortin-4 receptor agonist displayed a similar level of efficacy to sildenafil (Lansdell et al., 2010). Albeit preclinical and clinical studies showing the melanocortin system as a promising target for pharmacological treatment for ED, there have been no further studies to support this to date.

9.7 Botulinum toxin

Botulinum neurotoxin (BoNT) is one of the most potent toxins known to humans. It is produced by *Clostridium botulinum*, an anaerobic spore-forming, gram-positive bacterium. Poisoning with BoNT can cause botulism, resulting in generalized paralysis including respiratory arrest and death. There are seven distinct biochemical and serological forms of BoNT (A, B, C1, D, E, F, and G). BoNT-A, BoNT-B, and BoNT-E can cause botulism in humans, whereas the remaining BoNT forms can cause disease only in animals. BoNT-A is the most commonly used form in medicine. However, during the past four decades, BoNT-A has been used to relax muscles to treat several striated and smooth muscle disorders in addition to its wide use in esthetic medicine. More recently, it has been investigated whether the muscle relaxing capacity of BoNT-A could be used within the corpora cavernosa to enhance penile erections, introducing a possible new line of treatment for ED. Two human studies are available from Egypt. The first is a phase I pilot randomized controlled trial of 24 patients that was completed and presented at meetings of the International Society for Sexual Medicine and affiliated societies (Ghanem et al., 2016). The second is a phase II randomized controlled trial of 160 patients that is in progress (Ghanem et al., 2017). The efficacy of BoNT-A was evaluated by penile color Doppler and Erection Hardness Score associated with the SHIM questionnaire and Sexual Encounter Profile questions 2 and 3 at baseline and 4 weeks after treatment. The treatment improved the vascular parameters and SHIM scores, and the NCT03102762 clinical trial is still ongoing (see "10. ONGOING CLINICAL TRIALS" section). Therefore, BoNT-A could represent a promising therapy in vasculogenic ED.

10. ONGOING CLINICAL TRIALS

10.1 A Study to Assess the Safety and Tolerability Profile of TR399 in Healthy Volunteers and Patients with Erectile Dysfunction

TR399 is a topical preparation of 5% vardenafil $HCl{\cdot}3H_2O$. Nonclinical studies have shown that the topical use of TR399 can enhance erection and sexual behavior in animal models without causing irritancy and photo toxicity. An ongoing phase I/IIA study is assessing the safety and the tolerability profile of TR399 in healthy volunteers and patients with ED. This study will be conducted in a single-arm and open-label fashion, and it is designed to assess the safety and the pharmacokinetics of TR399 in both healthy volunteers and patients with ED (**NCT03102398;** https://clinicaltrials.gov/ct2/home).

10.2 Botulinum Toxin for Erectile Dysfunction

The purpose of this trial is to evaluate the safety and the efficacy of intracavernosal botulinum toxin injection as an alternative line of treatment in patients with ED not responding to oral PDE5 inhibitors through cavernosal smooth muscle relaxation. This study is a phase II trial designed to confirm the phase I results in a larger group of men (NCT03102762; https://clinicaltrials.gov/ct2/home).

10.3 Clinical Trial of Topically applied Glyceryl Trinitrate (GTN) for the Treatment of Erectile Dysfunction

This phase III trial will be a dose-ranging, multicenter, randomized, double-blind, placebo-controlled, home use, parallel-group clinical trial of topically applied GTN MED2005. This study will recruit approximately 1,000 patients at European centers with mild, moderate, or severe ED and compare the efficacy of 0.2%, 0.4%, and 0.6% w/w GTN doses of MED2005 against that of placebo using IIEF-EF clinical endpoints. The trial will be conducted throughout Eastern Europe with a three-month study period for each patient (NCT02495467; https://clinicaltrials.gov/ct2/home).

10.4 Very Small Embryonic-like Stem Cells for Erectile Dysfunction

The aim of this clinical trial is to assess the safety and the efficacy of autologous very small embryonic-like stem cells to organic ED, such as those associated with MS or the treatment of prostate cancer**.** This study will document for the first time the safety and the efficacy of underlying penile cellular damage. The efficacy will be evaluated by using validated scores and color duplex Doppler ultrasound (NCT03973021; https://clinicaltrials.gov/ct2/home).

11. CONCLUSION

The prevalence of ED increases with advanced age and with the presence of a systemic disease; in fact, the vasculogenic ED is a complex multifactorial event linked to many morbidity factors, of which the most important are risk factors common with that indicated for CVD. Thus, clinical evaluations may not always be enough for the assessment of ED, and it is widely accepted that laboratory testing such as fasting blood glucose, HbA1c, and lipid profile or the use of penile Doppler ultrasonography should be considered for the evaluation of penile vascular structures in patients with ED. Moreover, ED in the absence of CVD is now considered a "sentinel symptom" in patients with occult undiagnosed CVD where the endothelial dysfunction has a key role.

The introduction of sildenafil on the market in 1998 as an oral remedy for ED has opened an entirely new field of research that has been flourishing in the past 20 years. This increase in interest contributed to a better understanding of the pathophysiology of erection function. As has been described and discussed in the above sections, we now have much information and we are in the process of identifying new therapeutic targets that should allow us to cure those cohorts of patients who are nonresponsive to PDE5 therapy. Another important issue is that there is still an unmet need for objective parameters, instead of validated questionnaires, to better classify the grade of ED. The development of these objective markers will also be instrumental to increase our knowledge of the efficacy of PDE5 inhibitors and even more relevant the relative potency of the drug under

examination. In this regard, the measurement of cGMP levels in platelets could be considered one such marker, as it can be determined in the peripheral blood in case of PDE5 inhibitor treatments.

The review focuses on data collected from human preclinical and clinical research only. The reader will realize that there are still many unanswered questions that need to be addressed and more efforts need to be made to improve ED therapy. The emerging strategies from basic science toward the anticipation of features, treatment of ED, and new treatments using pharmacological and innovative therapies could represent a real change in ED therapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Abdel-Hamid, I.A., Naggar, E.A., Gilany, A.H. (2001). Assessment of as needed use of pharmacotherapy and the pause-squeeze technique in premature ejaculation. *Int J Impot Res,*13, 41–45.

Adaikan, P. G., & Ng, S. C. (2000). Physiological significance of nitrergic transmission in human penile erection. *Asian Journal of Androogy*, 2, 51-56

Adaikan, P. G., Kottegoda SR, & Ratnam S. S. (1986). Is vasoactive intestinal polypeptide the principal transmitter involved in human penile erection? *The Journal of Urology*, 135,638-640.

Akishita, M., Hashimoto, M., Ohike, Y., Ogawa, S., Iijima, K., Eto, M., et al. (2007). Low testosterone level is an independent determinant of endothelial dysfunction in men. *Hypertension Research*, 30, 1029-1034

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., et al. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, 120, 1640–1645.

Andersen, P. H., Gingrich, J. A., Bates, M. D., Dearry, A., Falardeau, P., Senogles, S. E., et al. (1990). Dopamine receptor subtypes: beyond the D1/D2 classification. *Trends in Pharmacological Sciences*, 11, 231-236.

Andersson, K. E., Hedlund P., & Alm P. (2000). Sympathetic pathways and adrenergic innervation of the penis. *International Journal of Impotence Research*, 12, S5-S12.

Anfossi, G., Massucco, P., Mattiello, L., Balbo, A., Russo, I., Doronzo, G., et al. (2002). Insulin influences the nitric oxide cyclic nucleotide pathway in cultured human smooth muscle cells from corpus cavernosum by rapidly activating a constitutive nitric oxide synthase. *European Journal of Endocrinology*, 147, 689-700.

Angulo J., Cuevas, P., La Fuente, J. M., Pomerol, J. M., Ruiz-Castañé, E., Puigvert, A., et al. (2002). Regulation of human penile smooth muscle tone by prostanoid receptors. *British Journal of Pharmacology*, 136, 23-30.

Arackal, B. S., & Benegal, V. (2007). Prevalence of sexual dysfunction in male subjects with alcohol dependence. *Indian Journal of Psychiatry*, 49,109–112.

Archer, S. L. (2002). Potassium channels and erectile dysfunction. *Vascular Pharmacology*, 38, 61-71.

Aversa, A., Isidori, A. M., De Martino, M. U., Caprio, M., Fabbrini, E., Rocchietti-March, M., et al. (2000). Androgens and penile erection: evidence for a direct relationship between free testosterone and cavernous vasodilation in men with erectile dysfunction. *Clinical Endocrinology (Oxf)*, 53, 517-522.

Ayta, I. A., McKinlay, J. B., & Krane, R. J. (1999). The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. *British Journal Urology International*, 84, 50-56.

Bacon, C. G., Hu, F. B., Giovannucci, E., Glasser, D. B., Mittleman, M. A., & Rimm, E. B. (2002). Association of type and duration of diabetes with erectile dysfunction in a large cohort of men. *Diabetes Care*, 25, 1458-1463.

Basaria, S. (2014). Male hypogonadism. *Lancet*, 383, 1250-1263.

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*, 63, 182-217.

Becker, A. J., Uckert, S., Stief, C. G., Scheller, F., Knapp, W. H., Hartmann, U., et al. (2001). Plasma levels of angiotensin II during different penile conditions in the cavernous and systemic blood of healthy men and patients with erectile dysfunction. *Urology*, 58, 805-810.

Becker, A. J., Uckert, S., Stief, C. G., Scheller, F., Knapp, W. H., Machtens, S. A., et al. (2002). Systemic and cavernous plasma levels of vasoactive intestinal polypeptide during sexual arousal in healthy males. *World Journal of Urology*, 20, 59-63.

Becker, A. J., Uckert, S., Stief, C. G., Truss, M. C., Hartmann, U., & Jonas, U. (2001). Systemic and cavernosal plasma levels of endothelin (1-21) during different penile conditions in healthy males and patients with erectile dysfunction. *World Journal of Urology,* 19, 371-376.

Becker, A. J., Uckert, S., Stief, C. G., Truss, M. C., Machtens, S., Scheller, F., et al. (2000a) Plasma levels of cavernous and systemic norepinephrine and epinephrine in men during different phases of penile erection. *The Journal of Urology*, 164, 573–577.

Becker, A. J., Uckert, S., Stief, C. G., Truss, M. C., Machtens, S., Scheller, F., et al. (2000b). Cavernous and systemic testosterone levels in different phases of human penile erection. *Urology*, 56,125-129.

Beckman, J. A., Goldfine, A. B., Gordon, M. B., & Creager, M. A. (2001). Ascorbate restores endothelium‐dependent vasodilation impaired by acute hyperglycemia in humans. *Circulation*, 103,1618‐1623.

Behrends, S., Steenpass, A., Porst, H., & Scholz, H. (2000). Expression of nitric oxide-sensitive guanylyl cyclase subunits in human corpus cavernosum. *Biochemical Pharmacology*, 59,713-717.

Belew, D., Klaassen, Z., & Lewis, R.W. (2015). Intracavernosal Injection for the Diagnosis, Evaluation, and Treatment of Erectile Dysfunction: A Review. *Sexual Medicine Reviews*, 3,11–23.

Binmoammar, T. A., Hassoulah, S., Alsaad, S., Rawaf, S., & Majeed, A. (2016). The impact of poor glycaemic control on the prevalence of erectile dysfunction in men with type 2 diabetes mellitus: a systematic review. *Journal of the Royal Society of Medicine*, 7,2054270415622602.

Bloch, W., Klotz, T., Sedlaczek, P., Zumbé, J., Engelmann, U., & Addicks, K. (1998). Evidence for the involvement of endothelial nitric oxide synthase from smooth muscle cells in the erectile function of the human corpus cavernosum. *Urological Research*, 26, 129-135.

Bortolotti, A., Fedele, D., Chatenoud, L., Colli, E., Coscelli, C., Landoni, M., et al. (2001). Cigarette smoking: a risk factor for erectile dysfunction in diabetics. *European Urology*, 40, 392–396

Bosch, R. J., Benard, F., Aboseif, S. R., Stief, C, G., Lue, T. F., & Tanagho, E. A. (1991). Penile detumescence: characterization of three phases. *The Journal of Urology*, 146, 867-871

Breyer, R. M., Bagdassarian, C. K., Myers, S. A., & Breyer M. D. (2001). Prostanoid receptors: subtypes and signaling. *Annual Review of Pharmacology and Toxicology*; 41, 661–690.

Brock, G. B., McMahon, C. G., Chen, K. K., Costigan, T., Shen, W., Watkins, V, et al. (2002). Efficacy and safety of tadalafil for the treatment of erectile dysfunction: results of integrated analyses. *The Journal of Urology,* 168, 1332-1336.

Brugger, N., Kim, N. N., Araldi, G. L., Traish, A. M., & Palmer, S. S. (2008). Pharmacological and functional characterization of novel EP and DP receptor agonists: DP1 receptor mediates penile erection in multiple species. *The Journal of Sexual Medicine,* 5, 344-356.

Burnett, A. L., Nehra, A., Breau, R. H., Culkin, D. J., Faraday, M. M., Hakim, L. S., et al. (2018). Erectile Dysfunction: AUA Guideline. *The Journal of Urology,* 200: 633-641.

Burnett, A. L., Tillman, S. L., Chang, T. S., Epstein, J. I., Lowenstein, C. J., Bredt, D. S., et al. (1993) Immunohistochemical localization of nitric oxide synthase in the autonomic innervation of the human penis. *The Journal of Urology*, 150, 73-76.

Butler, R., Morris, A. D., & Struthers, A. D. (2001) Cigarette smoking in men and vascular responsiveness. *British Journal of Clinical Pharmacology*, 52, 145–149.

Calay, D., & Mason, J. C. (2014). The multifunctional role and therapeutic potential of HO-1 in the vascular endothelium. *Antioxidants & Redox Signaling*, 20, 1789-1809.

Carani, C., Scuteri, A., Marrama, P., & Bancroft, J. (1990). The effects of testosterone administration and visual erotic stimuli on nocturnal penile tumescence in normal men. *Hormones and Behavior,* 24, 435-441.

Celermajer, D. S., Sorensen, K. E., Georgakopoulos, D., Bull, C., Thomas, O., Robinson, J., et al. (1993). Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*, 88, 2149-2155.

Chen, L., Staubli, S. E., Schneider, M. P., Kessels, A. G., Ivic, S., Bachmann, L. M., et al. (2015). Phosphodiesterase 5 inhibitors for the treatment of erectile dysfunction: a trade-off network meta-analysis. *European Urology, 68,* 674-680.

Chen, Y., Cui, S., Lin, H., Xu, Z., Zhu, W., Shi, L., et a., (2012). Losartan improves erectile dysfunction in diabetic patients: a clinical trial. *International Journal of Impotence Research,* 24, 217-220.

Chew, K. K., Bremner, A., Stuckey, B., Earle, C., & Jamrozik, K. (2009). Is the relationship between cigarette smoking and male erectile dysfunction independent of cardiovascular disease? Findings from a population-based cross-sectional study. *The Journal of Sexual Medicine*, 6, 222-231.

Choppin, A., Blue, D. R., Hegde, S. S., Gennevois, D., McKinnon, S. A., Mokatrin, A., et al. (2001). Evaluation of oral ro70-0004/003, an alpha(1A)-adrenoceptor antagonist, in the treatment of male erectile dysfunction. *International Journal of Impotence Research,* 13, 157-161.

Cirino, G., Sorrentino, R., d'Emmanuele di Villa Bianca, R., Popolo, A., Palmieri, A., Imbimbo, C., et al. (2003). Involvement of beta 3-adrenergic receptor activation via cyclic GMP- but not NO-dependent mechanisms in human corpus cavernosum function. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 5531-5536.

Cocci, A., Capece, M., Cito, G., Russo, G.I., Falcone, M., Timpano, M., et al. (2017). Effectiveness and Safety of Oro-Dispersible Sildenafil in a New Film Formulation for the Treatment of Erectile Dysfunction: Comparison Between Sildenafil 100-mg Film-Coated Tablet and 75-mg Oro-Dispersible Film. *J Sex Med*, 14, 1606-1611.

Coleman, R.A., Smith, W.L., & Narumiya, S. (1994). International union of pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacological Reviews*, 46, 205–29.

Corbin, J.D. (2004). Mechanisms of action of PDE5 inhibition in erectile dysfunction. *International Journal of Impotence Research*, 16 (Suppl. 1), S4-S7.

Cormio, L., Gesualdo, L., Maiorano, E., Bettocchi, C., Palumbo, F., Traficante, A., et al. (2005). Vasoactive intestinal polypeptide (VIP) is not an androgen-dependent neuromediator of penile erection. *International Journal of Impotence Research*, 17, 23-26.

Corona, G., Giorda, C. B., Cucinotta, D., Guida, P., Nada, E. (2013). Gruppo di studio SUBITO-DE. The SUBITO-DE study: sexual dysfunction in newly diagnosed type 2 diabetes male patients. *Journal of Endocrinological Investigation*, 36, 864–868.

Corona, G., Mondaini, N., Ungar, A., Razzoli, E., Rossi, A., & Fusco, F. (2011). Phosphodiesterase type 5 (PDE5) inhibitors in erectile dysfunction: the proper drug for the proper patient. *The Journal of Sexual Medicine*, 8, 3418-3342.

Costa, P., & Potempa, A. J: (2012). Intraurethral alprostadil for erectile dysfunction: a review of the literature. *Drugs*, 72, 2243–2254.

d'Emmanuele di Villa Bianca, R., Mitidieri, E., Donnarumma, E., Fusco, F., Longo, N., De Rosa, G. (2015). A new therapeutic approach to erectile dysfunction: urotensin-II receptor high affinity agonist ligands. *Asian J Androl*, 1, 81-85.

d'Emmanuele di Villa Bianca, R., Mitidieri, E., Mirone, V., Fusco, F., Imbimbo, C., Cirino, G., et al., (2011). An ex vivo standardized assay to measure human platelet cGMP. *Journal of Pharmacological and Toxicological Methods*, 64, 164-167.

d'Emmanuele di Villa Bianca, R., Sorrentino, R., Sorrentino, R., Imbimbo, C., Palmieri, A., Fusco F, Maggi M, et al. (2006). Sphingosine 1-phosphate induces endothelial nitric-oxide synthase activation through phosphorylation in human corpus cavernosum. *Journal of Pharmacology and Experimental Therapeutics*, 316, 703-708.

Dachille, G., Lamuraglia, M., Leone, M., Pagliarulo, A., Palasciano, G., Salerno, M.T., et al. (2008). Erectile dysfunction and alcohol intake. *Urologia*, 75, 170–176.

Dail, W. G., Trujillo, D., de la Rosa, D., & Walton, G. (1989). Autonomic innervation of reproductive organs: analysis of the neurons whose axons project in the main penile nerve in the pelvic plexus of the rat. *The Anatomical Record*, 224, 94-101.

Damle, B., Duczynski, G., Jeffers, B. W, Crownover, P, Coupe, A., & LaBadie, R. R. (2014). Pharmacokinetics of a novel orodispersible tablet of sildenafil in healthy subjects. *Clinical Therapeutics*, 36, 236-244.

Davis, B. J., Chapple, C. R., Sellers, D. J., Naylor, A. L., Sillar, D., Campbell, A., et al. (2018). α(1L)-adrenoceptors mediate contraction of human erectile tissue. *Journal of Pharmacological Sciences*, 137, 366-371.

De Berardis, G., Franciosi, M., Belfiglio, M., Di Nardo, B., Greenfield, S., Kaplan, S.H., et al. (2002). Quality of Care and Outcomes in Type 2 Diabetes (QuED) Study Group. Erectile dysfunction and quality of life in type 2 diabetic patients: a serious problem too often overlooked. *Diabetes Care*, 25, 284–291.

de Godoy, M. A, & Rattan, S. (2011). Role of rho kinase in the functional and dysfunctional tonic smooth muscles. *Trends in Pharmacological Sciences*, 32, 384-393.

Dean, R. C., & Lue, T. F. (2005). Physiology of penile erection and pathophysiology of erectile dysfunction. *Urologic Clinics of North America*, 32, 379-395.

Demady, D. R., Lowe, E. R., Everett, A. C., Billecke, S. S., Kamada, Y., Dunbar, A. Y., et al. (2003). Metabolismbased inactivation of neuronal nitric-oxide synthase by components of cigarette and cigarette smoke. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, 31, 932–937.

d'Emmanuele di Villa Bianca, R., Cirino, G., Mitidieri, E., Coletta, C., Grassia G, Roviezzo F et al. (2010). Urotensin II: a novel target in human corpus cavernosum. *J Sex Med*, 7:1778-1786.

d'Emmanuele di Villa Bianca, R., Fusco, F., Mirone, V., Cirino, G., & Sorrentino, R. (2017). The Role of the Hydrogen Sulfide Pathway in Male and Female Urogenital System in Health and Disease. *Antioxidants & Redox Signaling*, 27, 654-668.

d'Emmanuele di Villa Bianca, R., Mitidieri, E., Esposito, D., Donnarumma, E., Russo, A., Fusco, F., et al. (2015). Human Cystathionine-β-Synthase Phosphorylation on Serine227 Modulates Hydrogen Sulfide Production in Human Urothelium. *PLoS One*, 10, e0136859.

d'Emmanuele di Villa Bianca, R., Sorrentino, R., Maffia, P., Mirone, V., Imbimbo, C., Fusco, F., et al. (2009). Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. *Proceedings of the National Academy of Sciences of the United States of America,* 106, 4513-4518.

d'Emmanuele di Villa Bianca, R., Sorrentino, R., Roviezzo, F., Imbimbo, C., Palmieri, A., De Dominicis, G., et al. (2005). Peripheral relaxant activity of apomorphine and of a D1 selective receptor agonist on human corpus cavernosum strips. *International Journal of Impotence Research*, 17, 127-133.

Diamond, L.E., Earle, D.C., Rosen, R.C., Willett, M.S. & Molinoff, P.B. (2004). Double-blind, placebo-controlled evaluation of the safety, pharmacokinetic properties and pharmacodynamic effects of intranasal PT-141, a melanocortin receptor agonist, in healthy males and patients with mild-to-moderate erectile dysfunction. *International Journal of Impotence Research,* 16, 51-59.

Diaz-Arjonilla, M., Schwarcz, M., Swerdloff, R.S., & Wang, C. (2009). Obesity, low testosterone levels and erectile dysfunction. *International Journal of Impotence Research*, 21,89-98.

Dinsmore, W. W., Gingell, C., Hackett, G., Kell, P., Savage, D., Oakes, R., et al. (1999). Treating men with predominantly nonpsychogenic erectile dysfunction with intracavernosal vasoactive intestinal polypeptide and phentolamine mesylate in a novel auto-injector system: a multicentre double-blind placebo-controlled study. *British Journal Urology International*, 83,274-279.

Dinsmore, W.W., & Wyllie, M.G. (2008). Vasoactive intestinal polypeptide/phentolamine for intracavernosal injection in erectile dysfunction. *British Journal Urology International*, 102,933-937.

Domer, F. R., Wessler, G., Brown, R. L., & Charles, H. C. (1978). Involvement of the sympathetic nervous system in the urinary bladder internal sphincter and in penile erection in the anesthetized cat. *Investigative Urolology,* 15,404-407.

Dong, J. Y., Zhang, Y. H., & Qin, L. Q. (2011). Erectile dysfunction and risk of cardiovascular Disease. *Journal of the American College of Cardiology*, 58,1378–1385.

Dorey, G. (2001). Is smoking a cause of erectile dysfunction? A literature review. *British Journal of Nursing*, 10,455-465.

Doumas, M., Lazaridis, A, Katsiki, N., & Athyros, V. (2015). PDE-5 inhibitors: clinical points. *Current Drug Targets*, 16,420-426.

Duca, Y., Calogero, A.E., Cannarella, R., Giacone, F., Mongioi, L.M., Condorelli, R.A., et al. (2019). Erectile dysfunction, physical activity and physical exercise: Recommendations for clinical practice. *Andrologia*, 51, e13264.

Eardley, I., Donatucci, C., Corbin, J., El-Meliegy, A., Hatzimouratidis, K., McVary, K., et al. (2010). Pharmacotherapy for erectile dysfunction. *The Journal of Sexual Medicine*, 7, 524-450.

Eardley, I., Montorsi, F., Jackson, G., Mirone, V., Chan, M.F., Loughney, K., et al. (2007). Factors associated with preference for sildenafil citrate and tadalafil for treating erectile dysfunction in men naïve to phosphodiesterase 5 inhibitor therapy: post hoc analysis of data from a multicentre, randomized, open-label, crossover study. *British Journal Urology International*, 100:122-129.

Ehmke, H., Jünemann, K.P., Mayer, B., & Kummer, W. (1995). Nitric oxide synthase and vasoactive intestinal polypeptide colocalization in neurons innervating the human penile circulation. *International Journal of Impotence Research*, 7,147-156.

Elhanbly, S., Schoor, R., Elmogy, M, Ross, L., Hegazy, A., & Niederberger, C. (2002). What nonresponse to intracavernous injection really indicates: a determination by quantitative analysis. *The Journal of Urology*, 167,192-196.

Esposito, K., & Giugliano, D. (2011). Obesity, the metabolic syndrome, and sexual dysfunction in men. *Clinical Pharmacology & Therapeutics*, 90,169–173.

Esposito, K., Giugliano, F., Martedì, E., Feola, G., Marfella, R., D'Armiento, M, et al. (2005). High proportions of erectile dysfunction in men with the metabolic syndrome. *Diabetes Care*, 28,1201–1203.

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP)

Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *The Journal of the American Medical Association*, 285,2486–2497.

Fedele, D., Coscelli, C., Cucinotta, D., Forti, G., Santeusanio, F., Viaggi, S., et al. (2001). Incidence of erectile dysfunction in Italian men with diabetes. *The Journal of Urology*, 1661368–1371.

Feldman, D. I., Cainzos-Achirica, M., Billups, K. L., Defilippis, A. P., Chitaley, K., Green-land, P., et al. (2016). Subclinical vascular disease and subsequent erectile dysfunction: the Multiethnic Study of Atherosclerosis (MESA). *Clinical Cardiology*, 39,291-298.

Feldman, H. A., Goldstein, I., Hatzichristou, D. G., Krane, R. J., & McKinlay, J. B. (1994). Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *The Journal of Urology*, 151,54–61.

Ferreira, A. J., Santos, R. A., Bradford, C. N., Mecca, A. P., Sumners, C., Katovich, M. J., et al. (2010). Therapeutic implications of the vasoprotective axis of the renin-angiotensin system in cardiovascular diseases. *Hypertension*, 55,207–213.

Filippi, S., Crescioli, C., Vannelli, G.B., Fazzini, A., Natali, A., Riffaud, J.P, et al. (2003). Effects of NCX 4050, a new NO donor, in rabbit and human corpus cavernosum. *International Journal of Andrology*, 26,101-108.

Fogari, R., Zoppi, A., Poletti, L., Marasi, G., Mugellini, A., & Corradi, L. (2001). Sexual activity in hypertensive men treated with valsartan or carvedilol: a crossover study. *American Journal of Hypertension*, 14,27-31.

Forgue, S. T., Patterson, B. E., Bedding, A. W., Payne, C. D., Phillips, D. L., Wrishko, R. E., et al. (2006). Tadalafil pharmacokinetics in healthy subjects. *British Journal of Clinical Pharmacology*, 61,280-288.

Fraga-Silva, R. A., Montecucco, F., Mach, F., Santos, R. A., & Stergiopulos N. (2013). Pathophysiological role of the renin-angiotensin system on erectile dysfunction. *European Journal of Clinical Investigation*, 43,978-985.

Francavilla, S., Properzi, G., Bellini, C., Marino, G, Ferri, C., & Santucci, A. (1997). Endothelin-1 in diabetic and nondiabetic men with erectile dysfunction. *The Journal of Urology*, 158,1770–1774.

Francis, S. H., Busch J. L., & Corbin J. D. (2010). cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. *Pharmacological Reviews*, 62,525–563.

Fulton, D., Gratton, J. P., & Sessa, W. C. (2001). Post-translational control of endothelial nitric oxide synthase: why isn't calcium/calmodulin enough? *Journal of Pharmacology and Experimental Therapeutics*, 299,818-824.

Fung, M. M., Bettencourt, R., & Barrett-Connor, E. (2004). Heart disease risk factors predict erectile dysfunction 25 years later: the Rancho Bernardo Study. *Journal of the American College of Cardiology*, 43,1405-1411.

Fusco, F., d'Emmanuele di Villa Bianca, R., Mitidieri, E., Cirino, G., Sorrentino, R., & Mirone, V. (2012). Sildenafil effect on the human bladder involves the L-cysteine/hydrogen sulfide pathway: a novel mechanism of action of phosphodiesterase type 5 inhibitors. *European Urology*, 62,1174-1180.

Ghanem, H., Abd, E.l. Raheem, A. & Soliman I. (2017). Botox for erectile dysfunction. https://clinicaltrials.gov/ct2/show/study/NCT03102762.

Ghanem, H., Soliman, I. & Abdu.Hamid, M. (2016). Intracavernosal injec-tion of botulinum toxin type A in the treatment of vascular erectile dysfunction. https://clinicaltrials.gov/ct2/show/NCT02584686.

Gittelman, M., McMahon, C. G., Rodríguez-Rivera, J. A., Beneke, M., Ulbrich, E., & Ewald, S. (2010). The POTENT II randomised trial: efficacy and safety of an orodispersible vardenafil formulation for the treatment of erectile dysfunction. *International Journal of Clinical Practice*, 64,594-603.

Giugliano, F., Maiorino, M., Bellastella, G., Gicchino, M., Giugliano, D., & Esposito, K. (2010). Determinants of erectile dysfunction in type 2 diabetes. *International Journal of Impotence Research*, 22,204-209.

Giuliano, F. A., Leriche, A., Jaudinot, E. O., & de Gendre, A. S. (2004). Prevalence of erectile dysfunction among 7689 patients with diabetes or hypertension, or both. *Urology*, 64,1196-1201.

Glina, S., Virag, R., Luis Rhoden, E., & Sharlip, I. D. (2010). Intracavernous Injection of Papaverine for Erectile Failure R. Virag. *The Journal of Sexual Medicine*, 7,1331–1335.

Goff, D. C., Lloyd-Jones, D. M, Bennett, G., Coady, S., D'Agostino, R. B., Gibbons, R, et al., (2014). ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Journal of the American College Cardiologi*. 63, 2935-2959.

Gökçe, A., Halis, F., Demirtas, A., & Ekmekcioglu, C. (2011). The effects of three phosphodiesterase type 5 inhibitors on ejaculation latency time in lifelong premature ejaculators: a double-blind laboratory setting study. *British Journal Urology International*, 107,1274-1277.

Goldstein, I., Lue, T. F., Padma-Nathan, H., Rosen, R. C., Steers, W. D., & Wicker, P. A. (1998). Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. *The New England Journal of Medicine*, 338,1397-1404.

Goldstein, I., McCullough, A. R., Jones, L. A, Hellstrom, W. J., Bowden, C. H., Didonato, K., et al. (2012). A randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of avanafil in subjects with erectile dysfunction. *The Journal of Sexual Medicine*, 9,1122-1133.

Gong, B., Ma, M., Xie, W., Yang, X., Huang, Y., Sun, T., et al. (2017). Direct comparison of tadalafil with sildenafil for the treatment of erectile dysfunction: a systematic review and meta-analysis. *International Urology and Nephrology*, 49,1731-1740.

Govier, F., Potempa, A. J., Kaufman, J., Denne, J., Kovalenko, P., & Ahuja, S. (2003). A multicenter, randomized, double-blind, crossover study of patient preference for tadalafil 20 mg or sildenafil citrate 50 mg during initiation of treatment for erectile dysfunction. *Clinical Therapeutics*, 25,2709-2723.

Gowani, Z., Uddin, S. M. I., Mirbolouk, M, Ayyaz, D., Billups, K. L., Miner, M., et al. (2017). Vascular Erectile Dysfunction and Subclinical Cardiovascular Disease. *Current Sexual Health Reports*, 9,305-312.

Graham, C. A., & Bancroft, J. (2009). The sexual dysfunction. In: Gelder MG, Andreasen CN, Lopez-Ibor JJ, Geddes JR, New Oxford Textbook of Psychiatry. (2nd ed). (pp. 821–832). Oxford, UK: Oxford University.

Gratzke, C., Angulo, J., Chitaley, K., Dai, Y.T., Kim, N.N., Paick, J.S., et al., (2010). Anatomy, physiology, and pathophysiology of erectile dysfunction. *Journal of Sexual Medicine*, 7, 445-475.

Greenland, P., Blaha, M. J., Budoff, M. J., Erbel, R., & Watson, K. E. (2018). Coronary calcium score and cardiovascular risk. *Journal of the American College of Cardiology*, 72,434-447.

Gregorian, R.S., Golden, K.A., Bahce, A., Goodman C. Kwong, W. J., Khan, Z.M. (2002). Antidepressant-induced sexual dysfunction. *Ann Pharmacother*,36, 1577–1589.

Gu, J., Polak, J. M., Lazarides, M., Morgan, R. Pryor, J. P., Marangos, P. J., et al. (1984). Decrease of vasoactive intestinal polypeptide (VIP) in the penises from impotent men. *Lancet*, 2, 315-318.

Guagnini, F., Ferazzini, M., Grasso, M., Blanco, S., & Croci, T. (2012). Erectile properties of the Rho-kinase inhibitor SAR407899 in diabetic animals and human isolated corpora cavernosa. *Journal of Translational Medicine*, 10, 59-70.

Guay, A. T. (2007). ED2. erectile dysfunction = endothelial dysfunction. *Endocrinology & Metabolism Clinics of North America*, 36,453-463.

Guay, A. T., Spark, R. F., Jacobson, J., Murray, F. T., & Geisser, M. E. (2002). Yohimbine treatment of organic erectile dysfunction in a dose-escalation trial. *International Journal of Impotence Research*, 14,25-31.

Gündüz, M. I., Gümüs, B. H., & Sekuri, C. (2004). Relationship between metabolic syndrome and erectile dysfunction. *Asian Journal of Andrology*, 6,355-358.

Guo, X., Oldham, M. J., Kleinman, M. T., Phalen, R. F., & Kassab, G. S. (2006). Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries. *American Journal of Physiology-Heart and Circulatory Physiology*, 291, H2354–2361.

Gupta, B.P., Murad, M.H., Clifton, M.M., Prokop, L., Nehra, A., Kopecky, S.L. (2011). The effect of lifestyle modification and cardiovascular risk factor reduction on erectile dysfunction: a systematic review and meta-analysis. *Arch Intern Med*, 171, 1797-803.

Gur, S., Kadowitz, P. J., Trost, L., & Hellstrom, W. J. (2007). Optimizing nitric oxide production by time dependent L-arginine administration in isolated human corpus cavernosum. *The Journal of Urology*, 178,1543-1548.

Haahr, M.K., Jensen, C.H., Toyserkani, N.M., Andersen, D.C., Damkier, P., Sørensen, J.A. et al., (2016). Safety and Potential Effect of a Single Intracavernous Injection of Autologous Adipose-Derived Regenerative Cells in Patients with Erectile Dysfunction Following Radical Prostatectomy: An Open-Label Phase I Clinical Trial. *EBioMedicine*, 5, 204-210.

Hackett, G., Kell, P., Ralph, D., Dean, J., Price, D., Speakman, M., et al. (2008). British Society for Sexual Medicine guidelines on the management of erectile dysfunction. *The Journal of Sexual Medicine*, 5,1841-1865.

Hamed, E. A., Meki, A. R., Ja.far, A. A., & Hamed, S. A. (2003). Role of some vasoactive mediators in patients with erectile dysfunction: their relationship with angiotensin-converting enzyme and growth hormone. *International Journal of Impotence Research*, 15,418-425.

Harte, C. B., & Meston, C. M. (2012). Association between smoking cessation and sexual health in men. *British Journal Urology International*, 109:888-896.

Hatzichristou, D. G., Hatzimouratidis, K., Apostolidis, A., Ioannidis, E., Yannakoyorgos, K., & Kalinderis, A. (1999). Hemodynamic characterization of a functional erection. Arterial and corporeal veno-occlusive function in patients with a positive intracavernosal injection test. *European Urology*, 36,60-67.

Hatzimouratidis, K. (2006). Sildenafil in the treatment of erectile dysfunction: an overview of the clinical evidence. *Clinical Interventions in Aging*, 1,403-414.

Hatzimouratidis, K., (Chair), Giuliano, F., Moncada, I., Muneer, A., Salonia, A., (Vice-chair), Verze P. (2019). Guideline Associates: A. Parnham, E.C. Serefoglu. EAU Guidelines. Edn. presented at the EAU Annual Congress Barcelona, ISBN 978-94-92671-04-2.

Hatzimouratidis, K., Amar, E., Eardley, I., Giuliano, F., Hatzichristou, D., Montorsi, F., et al. (2010). Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. *European Urology*, 57,804-814.

Hatzimouratidis, K., Giuliano, F., Moncada, I., Muneer, A., Salonia, A., & Verze, P. (2017). Guideline Associates: Parnham A., Serefoglu E.C. EAU guidelines on Erectile Dysfunction, Premature Ejaculation, Penile Curvature and Priapism. European Association of Urology, https://uroweb.org/wp-content/uploads/14-Male-Sexual-Dysfunction_LR.pdf.

Hatzimouratidis, K., Moysidis, K., Bekos, A., Tsimtsiou, Z., Ioannidis, E., Hatzichristou D. (2006). Treatment strategy for "non-responders" to tadalafil and vardenafil: a real-life study. *European Uroogy*, 50, 126-133.

Hatzimouratidis, K., Salonia, A., Adaikan, G., Buvat, J., Carrier, S., El-Meliegy, A., et al. (2016). Pharmacotherapy for Erectile Dysfunction: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015). *The Journal of Sexual Medicine*, 13,465-488.

Hauck, E. W., Altinkilic, B. M., Schroeder-Printzen, I., Rudnick, J., & Weidner, W. (1999). Prostaglandin E1 long-term self-injection programme for treatment of erectile dysfunction--a follow-up of at least 5 years. *Andrologia*, 31,99-103

He, L., Wen, J., Jiang, X., Chen, H., & Tang, Y. (2011). Long-term efficacy and safety of self-intracavernous injection of prostaglandin E1 for treatment of erectile dysfunction in China. *Andrologia*, 43,208-212.

Heaton, J. P. (1989). Synthetic nitrovasodilators are effective, in vitro, in relaxing penile tissue from impotent men: the findings and their implications. *Canadian Journal of Physiology and Pharmacology*, 67,78-81.

Hedlund, H. & Andresson, K. E. (1985). Contraction and relaxation induced by some prostanoids in isolated human penile erectile tissue and cavernous artery. The *Journal of Urology*, 134,1245-1250.

Hedlund, P., Alm, P., Ekström, P., Fahrenkrug, J., Hannibal, J., Hedlund, H., et al. (1995). Pituitary adenylate cyclase-activating polypeptide, helospectin, and vasoactive intestinal polypeptide in human corpus cavernosum. *British Journal of Pharmacology*, 116,2258-2266.

Hedlund, P., Ny, L., Alm, P., & Andersson, K. E. (2000). Cholinergic nerves in human corpus cavernosum and spongiosum contain nitric oxide synthase and heme oxygenase. *The Journal of Urology*, 164,868-875.

Hellstrom, W. J., Bennett, A. H., Gesundheit, N., Kaiser, F. E., Lue, T. F, Padma-Nathan, H., et al. (1996). A double-blind, placebo-controlled evaluation of the erectile response to transurethral alprostadil. *Urology*, 48: 851–856.

Hippisley-Cox, J., Coupland, C., & Brindle, P. (2017). Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *The British Medical Journal*, 357:j2099.

Hodges, L. D, Kirby, M., Solanki, J., O'Donnell, J., & Brodie, D. A. (2007). The temporal relation-ship between erectile dysfunction and cardiovascular disease. *International Journal of Clinical Practice*, 61,2019–2025.

Holmquist, F., Andersson, K. E., & Hedlund, H. (1990). Actions of endothelin on isolated corpus cavernosum from rabbit and man. *Acta Physiologica Scandinavica*, 139,113–122.

Holmquist, F., Kirkeby, H. J., Larsson, B., Forman, A., & Andersson, K. E. (1992). Functional effects, binding sites and immunolocalization of endothelin-1 in isolated penile tissues from man and rabbit. *Journal of Pharmacology and Experimental Therapeutics*, 261,795–802.

Huang, Y. C., Chin, C. C., Chen, C. S., Shindel, A. W., Ho D. R., Lin C. S., et al. (2015). Chronic cigarette smoking impairs erectile function through increased oxidative stress and apoptosis, decreased nNOS, endothelial and smooth muscle contents in a rat model. *PLoS One*, 10, e0140728.

Ibrahim, A., Ali, M., Kiernan, T. J., & Stack, A. G. (2018). Erectile Dysfunction and Ischaemic Heart Disease. *European Cardiology Review*, 13,98-103.

Ignarro, L. J., Bush, P. A., Buga, G. M., Wood, K. S., Fukuto, J. M., & Rajfer, J. (1990). Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochemical and Biophysical Research Communications*, 170,843-850.

Insuk, S. O., Chae, M. R., Choi, J. W., Yang, D. K., Sim, J. H., & Lee S. W. (2003). Molecular basis and characteristics of KATP channel in human corporal smooth muscle cells. *International Journal of Impotence Research*, 15,258-266.

Jannini, E. A., Isidori, A. M., Gravina, G. L., Aversa, A., Balercia, G., Bocchio, M., et al. (2009). The ENDOTRIAL study: a spontaneous, open-label, randomized, multicenter, crossover study on the efficacy of sildenafil, tadalafil, and vardenafil in the treatment of erectile dysfunction. *The Journal of Sexual Medicine*, 6,2547-2560.

Jeremy, J. Y., Morgan, R. J., Mikhailidis, D. P., & Dandona, P. (1986). Prostacyclin synthesis by the corpora cavernosa of the human penis: evidence for muscarinic control and pathological implications. *Prostaglandins Leukotrienes and Medicine*, 23,211-216.

Jiaan, D. B., Seftel, A. D., Fogarty, J., Hampel N., Cruz, W., Pomerantz, J., et al. (1995). Age-related increase in an advanced glycation end product in penile tissue. *World Journal of Urology*, 13,369-375.

Kalter-Leibovici, O., Wainstein, J., Ziv, A., Harman-Bohem, I., Murad, H., Raz, I., et al. (2005). Clinical, socioeconomic, and lifestyle parameters associated with erectile dysfunction among diabetic men. *Diabetes Care*, 28,1739-1744.

Kalyani, R. R., & Dobs, A. S. (2007). Androgen deficiency, diabetes, and the metabolic syndrome in men. *Current Opinion in Endocrinology, Diabetes and Obesity*, 14,226-234.

Kaplan, S. A., Meehan, A. G., & Shah, A. (2006). The age related decrease in testosterone is significantly exacerbated in obese men with the metabolic syndrome. What are the implications for the relatively high incidence of erectile dysfunction observed in these men? *The Journal of Urology*, 176,1524-1528;

Kaplan, S. A., Reis, R. B, Kohn, I. J., Shabsigh, R., & Te, A. E. (1998). Combination therapy using oral alpha-blockers and intracavernosal injection in men with erectile dysfunction. *Urology*, 52,739-743.

Karacan, I., Williams, R. L., Thornby, J. I., & Salis, P. J. (1975). Sleep-related penile tumescence as a function of age. *American Journal of Psychiatry*, 132, 932-937.

Kathpalia, H., & Gupte, A. (2013). An introduction to fast dissolving oral thin film drug delivery systems: a review. *Current Drug Delivery*. 10,667-684.

Kauhanen, J., Kaplan, G. A., Goldberg, D. E., Salonen, R., & Salonen, J. T. (1999). Pattern of alcohol drinking and progression of atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 19,3001–3006.

Khan, M. A., Thompson, C. S., Sullivan, M. E., Jeremy, J. Y., Mikhailidis, D. P., & Morgan, R. J. (1999). The role of prostaglandins in the aetiology and treatment of erectile dysfunction. *Prostaglandins Leukot Essent Fatty Acids*, 60,169–174.

Kiely, E. A., Bloom, S. R., & Williams, G. (1989). Penile response to intracavernosal vasoactive intestinal polypeptide alone and in combination with other vasoactive agents. *British Journal of Urology*, 64,191-194.

Kim, N. N., Dhir, V., Azadzoi, K. M., Traish, A. M., Flaherty, E., & Goldstein, I. (2002). Pilot study of the endothelin-A receptor selective antagonist BMS-193884 for the treatment of erectile dysfunction. *Journal of Andrology*, 23,76-83.

Kimura, H. (2011). Hydrogen sulfide: its production, release and functions. *Amino Acids*, 41,113-121.

Kimura, K., Takahashi, M., Naroda, T., Iriguchi, H., Miyamoto, T., Kawanishi, Y., et al. (1993). The relaxation of human corpus cavernosum caused by nitric oxide. *Nihon Hinyokika Gakkai Zasshi*, 84,1660-1664. Japanese.

Király, I., Pataricza, J., Bajory, Z., Simonsen, U., Varro, A., Papp, J.G., et al. (2013). Involvement of large-conductance Ca(2+) -activated K(+) channels in both nitric oxide and endothelium-derived hyperpolarization-type relaxation in human penile small arteries. *Basic Clinical Pharmacology and Toxicology*, 113,19-24.

Kirkeby, H.J., Fahrenkrug, J., Holmquist, F., & Ottesen, B. (1992). Vasoactive intestinal polypeptide (VIP) and peptide histidine methionine (PHM) in human penile corpus cavernosum tissue and circumflex veins: localization and in vitro effects. *European Journal of Clinical Investigation*, 22, 24-30.

Kloner, R.A., & Speakman, M. (2002). Erectile dysfunction and atherosclerosis. *Current Atherosclerosis Reports*, 4, 397-401.

Klotz, T., Bloch, W., Zimmermann, J., Ruth, P., Engelmann, U., & Addicks K. (2000). Soluble guanylate cyclase and cGMP dependent protein kinase I expression in the human corpus cavernosum. *International Journal of Impotence Research*, 12,157-164.

Kupelian, V., Link, C. L., & McKinlay, J. B. (2007). Association between smoking, passive smoking, and erectile dysfunction: results from the Boston Area Community Health (BACH) Survey. *European Urology*, 52, 416–422.

Kwan, M., Greenleaf, W. J., Mann, J., Crapo, L., & Davidson, J. M. (1983). The nature of androgen action on male sexuality: a combined laboratory-self-report study on hypogonadal men. *The Journal of Clinical Endocrinology and Metabolism*, 57, 557-562.

Lansdell, M.I., Hepworth, D., Calabrese, A., Brown, A.D., Blagg, J., Burring, D.J., et al., (2010). Discovery of a selective small-molecule melanocortin-4 receptor agonist with efficacy in a pilot study of sexual dysfunction in humans. *Journal of Medicinal Chemistry*, 53, 3183-3197.

Larsen, S. H., Wagner, G., & Heitmann, B. L. Sexual function and obesity. *International Journal of Obesity (Lond)*, 2007 Aug;31(8):1189-98.

Lau, D. H., Thompson, C. S., Bellringer, J. F., Thomas, P. J., Mumtaz, F. H., Morgan, R. J., et al. (2006). Doxazosin and serotonin (5-HT) receptor (1A, 2A, and 4) antagonists inhibit 5-HT-mediated human cavernosal contraction. *Journal of Andrology*, 27,679-685.

Lee, S. W., & Kang, T. M. (2001). Effects of nitric oxide on the Ca2+-activated potassium channels in smooth muscle cells of the human corpus cavernosum. *Urological Research*, 29,359-365.

Lee, S. W., Wang, H. Z., & Christ, G. J. (1999). Characterization of ATP-sensitive potassium channels in human corporal smooth muscle cells. *International Journal of Impotence Research*, 11,179-188.

Li, H., Xu, L., Dunbar, J. C., Dhabuwala, C. B., Sima, A. A. (2004). Effects of C-peptide on expression of eNOS and iNOS in human cavernosal smooth muscle cells. *Urology*, 64,622-627.

Liu, Q., Zhang, Y., Wang, J., Li, S., Cheng, Y., Guo, J. (2018). Erectile dysfunction and depression: A systematic review and meta-analysis. *J Sex Med*,15,1073-1082

Llisterri, J. L., Lozano Vidal, J. V., Aznar Vicente, J., Argaya Roca, M., Pol Bravo, C., Sanchez Zamorano, M. A., et al. (2001). Sexual dysfunction in hypertensive patients treated with losartan. *The American Journal of the Medical Sciences*, 321,336-341.

Löhn. M., Plettenburg, O., Ivashchenko, Y., Kannt, A., Hofmeister, A., Kadereit, D., et al. (2009). Pharmacological characterization of SAR407899, a novel rho-kinase inhibitor. *Hypertension*, 54,676-83.

Lu, Z., Lin, G., Reed-Maldonado, A., Wang, C., Lee, Y.C., Lue, T.F.(2017). Low-intensity Extracorporeal Shock Wave Treatment Improves Erectile Function: A Systematic Review and Meta-analysis. *Eur Urol*. 71:223-233.

Lue, T. F. (2000). Erectile dysfunction. *The New England Journal of Medicine*, 342,1802-1813.

Lue, T. F., & Dahiya, R. (1997). Molecular biology of erectile function and dysfunction. *Molecular Urology*, 1,55-64.

Malavige, L. S., & Levy, J. C. (2009). Erectile dysfunction in diabetes mellitus. *The Journal of Sexual Medicine*, 6,1232-1247.

Malerba, D., Amato, M., Radaeli, A., Giacovelli, G., Rovati, L., Arshad, S.H., et al., (2015). Efficacy of Andolast in Mild to Moderate Asthma: A Randomized, Controlled, Double-Blind Multicenter Study (The Andast Trial). *Curr Pharm Des*, 21, 3835-3843.

Maniam, P., Seftel, A. D., Corty, E. W., Rutchik, S. D., Hampel, N., & Althof, S. E. (2001). Nocturnal penile tumescence activity unchanged after long-term intracavernous injection therapy. *The Journal of Urology*, 165,830-833;

Marques-Vidal, P., Arveiler, D., Evans, A., Amouyel, P., Ferrieres, J., & Ducimetiere, P. (2001). Different alcohol drinking and blood pressure relationships in France and Northern Ireland: the PRIME study. *Hypertension*, 38,1361–1366.

Martínez-Salamanca, J. I., Martínez-Ballesteros, C., Portillo L., Gabancho, S., Moncada, I,. & Carballido, J. (2010). Physiology of erection. *Archivos españoles de urología*, 63,581-888.

Mathers, M.J., Klotz, T., Roth, S., Lümmen, G., Sommer, F. (2009). Safety and efficacy of vardenafil versus sertraline in the treatment of premature ejaculation: a randomised, prospective and crossover study. *Andrologia*, 41,169-175.

McMahon, C. G. (1996). A pilot study of the role of intracavernous injection of vasoactive intestinal peptide (VIP) and phentolamine mesylate in the treatment of erectile dysfunction. *International Journal of Impotence Research*, 8,233-236.

Mehrotra, N., Gupta, M., Kovar, A., Meibohm, B. (2007). The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy. *Int J Impot Res*. 19,253-264.

Meigs, J. B., Wilson, P. W., Nathan, D. M., D'Agostino, R. B. Sr, Williams, K., & Haffner, S. M. (2003). Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring Studies. *Diabetes*, 52,2160-2167.

Meinhardt, W., de la Fuente, R.B., Lycklama à Nijeholt, A.A., Vermeij, P., & Zwartendijk, J. (1996). Prostaglandin E1 with phentolamine for the treatment of erectile dysfunction. *International Journal of Impotence Research*, 8, 5-7.

Melman, A., Bar-Chama, N., McCullough, A., Davies, K., Christ, G. (2006). hMaxi-K gene transfer in males with erectile dysfunction: results of the first human trial. *Hum Gene Ther*, 17, 1165-1176.

Minhas, S., Cartledge, J., & Eardley, I. (2000). The role of prostaglandins in penile erection. *Prostaglandins Leukot Essent Fatty Acids*, 62,137–146.+

Mirone, V., d'Emmanuele di Villa Bianca, R., Mitidieri, E., Imbimbo, C., Fusco F, Verze P, et al. (2009) Platelet cyclic guanosine monophosphate as a biomarker of phosphodiesterase type 5 inhibitor efficacy in the treatment of erectile dysfunction: a randomized placebo-controlled study. *European Urology*, 56,1067-1073.

Mirone, V., Fusco, F., Rossi, A., Sicuteri, R., & Montorsi, F. (2009). Tadalafil and vardenafil vs sildenafil: a review of patient-preference studies. *British Journal Urology International*, 103,1212-1217.

Mirone, V., Imbimbo, C., Fabrizio, F., Longo, N., & Palmieri, A. (1996). Ketanserin plus prostaglandin E1 (PGE-1) as intracavernosal therapy for patients with erectile dysfunction unresponsive to PGE-1 alone. *British Journal of Urology*, 77, 736-739.

Mirone, V., Sorrentino, R., d'Emmanuele di Villa Bianca, R., Imbimbo, C., Palmieri, A., Fusco, F., et al. (2000). A standardized procedure for using human corpus cavernosum strips to evaluate drug activity. *Journal of Pharmacological and Toxicological Methods*, 44,477-482.

Misra, H., Bainbridge, J., Berryman, J., Abuchowski, A., Galvez, K. M., Uribe, L. F., et al. (2017). A Phase Ib open label, randomized, safety study of SANGUINATE™ in patients with sickle cell anemia. *Revista Brasileira de Hematologia e Hemoterapia*. 39,20-27.

Mitidieri, E., Tramontano, T., Gurgone, D., Imbimbo, C., Mirone, V., Fusco, F., et al. (2017). β(3) adrenergic receptor activation relaxes human corpus cavernosum and penile artery through a hydrogen sulfide/cGMP-dependent mechanism. *Pharmacological Research*, 124,100-104.

Moncada, I., & Cuzin B. (2015). Clinical efficacy and safety of Vitaros©/Virirec© (Alprostadil cream) for the treatment of erectile dysfunction. *Urologia*, 82, 84-92.

Mónica, F. Z. & Antunes, E. (2018). Stimulators and activators of soluble guanylate cyclase for urogenital disorders. *Nature Reviews Urology*, 15, 42–54.

Montague, D. K., Barada, J. H., Belker, A. M., Levine, L. A., Nadig, P. W., Roehrborn, C. G., et al. (1996). Clinical guidelines panel on erectile dysfunction: summary report on the treatment of organic erectile dysfunction. The American Urological Association. *The Journal of Urology*, 156,2007-2011.

Montorsi, F., & Oettel, M. (2005). Testosterone and sleep-related erections: an overview*. *The Journal of Sexual Medicine*, 2,771-784.

Montorsi, F., Adaikan, G., Becher, E., Giuliano, F., Khoury, S., Lue, T. F., et al. (2010). Summary of the recommendations on sexual dysfunctions in men. *The Journal of Sexual Medicine*, 7,3572-3588.

Montorsi, P., Ravagnani, P. M., Galli, S., Rotatori, F., Briganti, A., Salonia, A., et al. (2005). The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. *American Journal of Cardiology*, 96, 19–23.

Moreland, R. B., Albadawi, H., Bratton, C, Patton, G., Goldstein, I., Traish, A., et al. (2001). O2-dependent prostanoid synthesis activates functional PGE receptors on corpus cavernosum smooth muscle. *American Journal of Physiology-Heart and Circulatory Physiology*, 281, H552-558.

Moriel, E. Z., Gonzalez-Cadavid, N., Ignarro, L. J., Byrns, R., & Rajfer, J. (1993). Levels of nitric oxide metabolites do not increase during penile erection. *Urology*, 42, 551-553.

Mukamal, K. J., Maclure, M., Muller, J. E., & Mittleman, M. A. (2005). Binge drinking and mortality after acute myocardial infarction. *Circulation*, 112, 3839–3845.

Mulhall, J. P., Daller, M., Traish, A. M., Gupta, S., Park, K., Salimpour, P., et al., (1997). Intracavernosal forskolin: role in management of vasculogenic impotence resistant to standard 3-agent pharmacotherapy. *The Journal of Urology.*, 158, 1752-1759.

Mutnuru, P. C., Ramanjaneyulu, H. K., Susarla, R., Yarlagadda, J., Devraj, R., & Palanisamy P. (2017). Pharmaco Penile Duplex Ultrasonography in the Evaluation of Erectile Dysfunction. *Journal of Clinical and Diagnostic Research*, 11, TC07-TC10.

Nehra, A., & Moreland, R. B. (2001). Neurologic erectile dysfunction. *Urologic Clinics of North America*, 28, 289–308.

Nehra, A., Blute, M. L., Barrett, D. M., & Moreland, R. B. (2002). Rationale for combination therapy of intraurethral prostaglandin E(1) and sildenafil in the salvage of erectile dysfunction patients desiring noninvasive therapy. *International Journal of Impotence Research*, 14 Suppl 1, S38-42.

Nehra, A., Jackson, G., Miner, M., Billups, K. L., Burnett, A. L., Buvat, J., et al. (2012). The Princeton III consensus recommendations for the management of erectile dysfunction and cardiovascular disease. *Mayo Clinic Proceedings*. 87, 766-778.

Neuzillet, Y., Hupertan, V., Cour, F., Botto, H., & Lebret, T. (2013). A randomized, double-blind, crossover, placebo-controlled comparative clinical trial of arginine aspartate plus adenosine monophosphate for the intermittent treatment of male erectile dysfunction. *Andrology*, 1, 223-228.

Nichols, D. J., Muirhead, G. J., & Harness, J. A. (2002). Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *British Journal of Clinical Pharmacology*. 53 Suppl 1, 5S-12S.

Nicolosi, A., Moreira, E. D., Shirai, M., Bin Mohd Tambi, M. I., & Glasser, D. B. (2003). Epidemiology of erectile dysfunction in four countries: cross-national study of the prevalence and correlates of erectile dysfunction. *Urology*, 61, 201–206.

NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence (1993). *Journal of American Medical Association*, 270, 83-90.

Orimoloye, O. A., Feldman, D. I., & Blaha, M. J. (2019). Erectile dysfunction links to cardiovascular disease-defining the clinical value. *Trends in Cardiovascular Medicine,* S1050-1738, 30001-30005.

Otterbein, L. E., Foresti, R., & Motterlini, R. (2016). Heme Oxygenase-1 and Carbon Monoxide in the Heart: The Balancing Act Between Danger Signaling and Pro-Survival. *Circulation Research.* 118, 1940-1959.

Ottesen, B., Wagner, G., Virag, R., & Fahrenkrug, J. (1984). Penile erection: possible role for vasoactive intestinal polypeptide as a neurotransmitter. *British Medical Journal (Clinical research ed.),* 288, 9-11.

Owen, J. A., Saunders, F., Harris, C., Fenemore, J., Reid, K., Surridge, D., et al., (1989). Topical nitroglycerin: a potential treatment for impotence. *The Journal of Urology.* 141, 546-548.

Padma-Nathan, H., Hellstrom, W. J., Kaiser, F. E., Labasky, R. F., Lue, T. F., Nolten, W. E., et al., (1997). Treatment of men with erectile dysfunction with transurethral alprostadil. *The New England Journal of Medicine,* 336, 1–7.

Park, J.K., Park, J.S., Jeon, S.B., Cui, W.S., Kim, S.Z., Kang, K.K., et al. (2008). Why a combined intracavernosal injection with trimix and oral sildenafil is reliable therapy in the ultrasonographic evaluation of erectile dysfunction. *The British Journal of Urology International,*102, 993-997.

Patel, U., & Lees, W. R. (1999). Pharmacological testing: Doppler. In: Carson CC, Kirby RS, Goldstein I, (Eds), Textbook of Erectile Dysfunction. Oxford, U.K.99 (pp.207–220). Isis Medical Media Ltd.

Penson, D. F., Latini, D. M., Lubeck, D. P., Wallace, K. L., Henning, J. M., & Lue, T. F. (2003). Comprehensive Evaluation of Erectile Dysfunction (ExCEED) database. Do impotent men with diabetes have more severe erectile dysfunction and worse quality of life than the general population of impotent patients? Results from the Exploratory Comprehensive Evaluation of Erectile Dysfunction (ExCEED) database. *Diabetes Care,* 26, 1093–1099.

Phé, V. & Rouprêt, M. (2012). Erectile dysfunction and diabetes: a review of the current evidence-based medicine and a synthesis of the main available therapies. *Diabetes & Metabolism*, 38:1-13.

Pierce, K. L., Gil, D. W., Woodward, D. F., & Regan, J. W. (1995). Cloning of human prostanoid receptors. *Trends in Pharmacological Sciences*, 16, 1-22.

Polak, J. M., Gu, J., Mina, S., & Bloom, S. R. (1981). Vipergic nerves in the penis. *Lancet*, 2, 217-219.

Polsky J. Y., Aronson K. J., Heaton J. P., Adams M. A. (2005). Smoking and other lifestyle factors in relation to erectile dysfunction. *British Journal Urology International*, 96, 1355–1359.

Ponholzer, A., Temml, C., Mock, K., Marszalek, M., Obermayr, R., & Madersbacher, S. (2005). Prevalence and risk factors for erectile dysfunction in 2869 men using a validated questionnaire. *European Urology*, 47, 80–86.

Porst H. (1996). The rationale for prostaglandin E1 in erectile failure: a survey of worldwide experience. *The Journal of Urology*, 155, 802-815.

Porst, H. (2000). Current perspectives on intracavernosal pharmacotherapy for erectile dysfunction. *International Journal of Impotence Research*, 12, S91-S100.

Porst, H., Buvat, J., Meuleman, E., Michal, V., & Wagner, G. (1998). Intracavernous Alprostadil Alfadex--an effective and well tolerated treatment for erectile dysfunction. Results of a long-term European study. *International Journal of Impotence Research*, 10, 225-231

Pourmand, G., Alidaee, M. R., Rasuli, S., Maleki, A., & Mehrsai, A. (2004). Do cigarette smokers with erectile dysfunction benefit from stopping?: a prospective study. *British Journal Urology International*, 94, 1310-1330.

Pytliak, M., Vargová, V., Mechírová, V., & Felšöci, M. (2011). Serotonin receptors – from molecular biology to clinical applications. *Physiology Research*, 60, 15-25

Qaseem, A., Snow, V., Denberg, T. D., Casey, D. E. Jr, Forciea, M. A., Owens, D. K., et al., (2009). Clinical Efficacy Assessment Subcommittee of the American College of Physicians

Hormonal testing and pharmacologic treatment of erectile dysfunction: a clinical practice guideline from the American College of Physicians. *Annals of Internal Medicine*, 151, 639-649.

Radhakrishnan, N., Yadav, S. P., Sachdeva, A., Pruthi, P. K., Sawhney, S., Piplani, T., et al. (2011). Human heme oxygenase-1 deficiency presenting with hemolysis, nephritis, and asplenia. *Journal of Pediatric Hematology Oncology*, 33, 74-78.

Rajasekaran, M., Mondal, D., Agrawal, K., Chen, I.L., Hellstrom, W., & Sikka, S. (1998). Ex vivo expression of nitric oxide synthase isoforms (eNOS/iNOS) and calmodulin in human penile cavernosal cells. *The Journal of Urology*, 160,2210-2215.

Rakic, Z., Starcevic V., Starcevic, V. P., & Marinkovic, J. (1997). Testosterone treatment in men with erectile disorder and low levels of total testosterone in serum. *Archives of Sexual Behavior*, 26,495-504.

Ralph, D.J., Eardley, I., Taubel, J., Terrill, P., Holland, T. (2018). Efficacy and Safety of MED2005, a Topical Glyceryl Trinitrate Formulation, in the Treatment of Erectile Dysfunction: A Randomized Crossover Study. *The Journal of Sexual Medicine*, 15, 167-175.

Rees, R. W., Ziessen, T., Ralph, D. J., Kell, P., Moncada, S., & Cellek S. (2002). Human and rabbit cavernosal smooth muscle cells express Rho-kinase. *International Journal of Impotence Research*, 14,1-7.

Ritchie, R., & Sullivan, M. (2011). Endothelins & erectile dysfunction. *Pharmacological Research*, 63,496-501.

Rizk, P. J., Kohn, T. P., Pastuszak, A. W., & Khera, M. (2017). Testosterone therapy improves erectile function and libido in hypogonadal men. *Current Opinion in Urology*, 27,511-515.

Rooney, M., Pfister, W., Mahoney, M., Nelson, M., Yeager, J., Steidle, C. (2009). Long-term, multicenter study of the safety and efficacy of topical alprostadil cream in male patients with erectile dysfunction. *J Sex Med*, 6,520-523

Rosen, R. C., Wing, R. R., Schneider, S., Wadden, T. A., Foster, G. D., West, D. S., et al. (2009). Erectile dysfunction in type 2 diabetic men: relationship to exercise fitness and cardiovascular risk factors in the Look AHEAD trial. *The Journal of Sexual Medicine*, 6,1414–1422.

Rosen, R.C., Diamond, L. E., Earle, D. C., Shadiack, A. M. & Molinoff, P.B. (2004). Evaluation of the safety, pharmacokinetics and pharmacodynamic effects of subcutaneously administered PT-141, a melanocortin receptor agonist, in healthy male subjects and in patients with an inadequate response to Viagra. *International Journal of Impotence Research*, 16, 135-142.

Russell, F.D. (2008). Urotensin II in cardiovascular regulation. *Vasc Health Risk Manag*, 4,775-785.

Sadock, V. A. (2005). Normal human sexuality and sexual dysfunctions. In: Sadock, B.J., Sadock, V.A., (editors). Kaplan & Sadock's Comprehensive Textbook of Psychiatry. (8th ed). (pp-1925). Philadelphia, PA: Lippincott Williams & Wilkins.

Sáenz de Tejada, I., Angulo, J., Cellek, S., González-Cadavid, N., Heaton, J., Pickard, R., et al. (2005). Pathophysiology of erectile dysfunction. *The Journal of Sexual Medicine*, 2,26–39.

Saenz de Tejada, I., Carson, M. P., de las Morenas, A., Goldstein, I., & Traish, A.M. (1991). Endothelin: localization, synthesis, activity, and receptor types in human penile corpus cavernosum. *American Journal of Physiology*, 261, H1078-1085.

Saigal, C. S., Wessells, H., Pace, J., Schonlau, M., & Wilt, T. J. (2006). Urologic Diseases in America Project. Predictors and prevalence of erectile dysfunction in a racially diverse population. *Archives of Internal Medicine*, 166,207-212.

Sampaio, W. O., Souza dos Santos, R. A., Faria-Silva, R., da Mata Machado, L. T., Schiffrin, E. L, & Touyz, R. M. (2007). Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Aktdependent pathways. *Hypertension*, 49, 185–192.

Sato, Y., Tanda, H., Kato, S., Onishi, S., Nitta, T., & Koroku, M. (2007). How long do patients with erectile dysfunction continue to use sildenafil citrate? Dropout rate from treatment course as outcome in real life. *International Journal of Urology,* 14, 339-342.

Scaglione, F., Donde, S., Hassan, T. A., & Jannini, E. A. (2017). Phosphodiesterase Type 5 Inhibitors for the Treatment of Erectile Dysfunction: Pharmacology and Clinical Impact of the Sildenafil Citrate Orodispersible Tablet Formulation. *Clinical Therapeutics*, 39, 370-377.

Schultheiss, D., Badalyan, R., Pilatz, A., Gabouev, A. I., Schlote, N., Wefer, J., et al. (2003). Androgen and estrogen receptors in the human corpus cavernosum penis: immunohistochemical and cell culture results. *World Journal of Urology*, 21, 320-324.

Segal, R. L., Bivalacqua, T. J., & Burnett, A. L. (2012) Irbesartan promotes erection recovery after nerve-sparing radical retropubic prostatectomy: a retrospective long-term analysis. *British Journal Urology International*, 110, 1782-1786.

Selvin, E., Burnett, A. L, &Platz, E. A. (2007). Prevalence and risk factors for erectile dysfunction in the US. *The American Journal of Medicine*, 120, 151-157.

Shamloul, R. (2009). The potential role of the heme oxygenase/carbon monoxide system in male sexual dysfunctions. *The Journal of Sexual Medicine*, 6, 324-333.

Shirai, M., Maki, A., Takanami, M., Ando, K., Nakamura, K., Yanaihara, N., et al. (1990). Content and distribution of vasoactive intestinal polypeptide (VIP) in cavernous tissue of human penis. *Urology*, 35, 360-363.

Shiri, R., Koskima, J., Hakama, M., Ha¨kkinen, J., Huhtala, H., Tammela, T. L. J., et al. (2004). Effect of life-style factors on incidence of erectile dysfunction. *International Journal of Impotence Research*, 16, 389–394.

Shiri, R., Koskimäki, J., Tammela, T.L., Häkkinen, J., Auvinen, A., Hakama, M. (2007). Bidirectional relationship between depression and erectile dysfunction. *J Urol*, 177, 669–673.

Sighinolfi, M. C., Mofferdin, A., De Stefani, S., Micali, S, Cicero, A. F., & Bianchi, G. (2007). Immediate improvement in penile hemodynamics after cessation of smoking: previous results. *Urology*, 69, 163-165.

Sikka, S. C., Hellstrom, W. J., Brock, G., & Morales, A. M. (2013). Standardization of vascular assessment of erectile dysfunction: standard operating procedures for duplex ultrasound. *The Journal of Sexual Medicine*, 10, 120-129.

Smith, E. R., Lee, R.L., Schnur, S. L., & Davidson, J. M. (1987). Alpha 2-adrenoceptor antagonists and male sexual behavior: II. Erectile and ejaculatory reflexes. *Physiology & Behavior*, 41, 15-19.

Smith, W. B. 2nd, McCaslin, I. R., Gokce, A., Mandava, S. H., Trost, L., & Hellstrom, W. J. (2013). PDE5 inhibitors: considerations for preference and long-term adherence. *International Journal of Clinical Practice*, 67, 768-780.

Solomon, H., Man, J. W., & Jackson, G. (2003). Erectile dysfunction and the cardiovascular patient: endothelial dysfunction is the common denominator. *Heart*, 89, 251-253.

Somlyo, A. P., & Somlyo, A. V. (2000). Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *Journal of Physiology*, 522, 177-185.

Souverein, P. C., Egberts, A. C., Meuleman, E. J., Urquhart, J., & Leufkens, H. G. (2002). Incidence and determinants of sildenafil (dis)continuation: the Dutch cohort of sildenafil users. *International Journal of Impotence Research*, 14, 259-265.

Sperling, H., Gittelman, M., Norenberg, C., Ulbrich, E., & Ewald, S. (2011). Efficacy and safety of an orodispersible vardenafil formulation for the treatment of erectile dysfunction in elderly men and those with underlying conditions: an integrated analysis of two pivotal trials. J *The Journal of Sexual Medicine*, 8, 261-271.

Stark, S., Sachse, R., Liedl, T., Hensen, J., Rohde, G., Wensing, G., et al. (2001). Vardenafil increases penile rigidity and tumescence in men with erectile dysfunction after a single oral dose. *European Urology*, 40, 181-190.

Stief, C. G., Holmquist, F., Djamilian, M., Krah, H., Andersson, K. E., & Jonas U. (1992). Preliminary results with the nitric oxide donor linsidomine chlorhydrate in the treatment of human erectile dysfunction. *The Journal of Urology*, 148, 1437-1440.

Stoléru, S. G., Ennaji, A., Cournot, A., & Spira, A. (1993). LH pulsatile secretion and testosterone blood levels are influenced by sexual arousal in human males. *Psychoneuroendocrinology*, 18,205-218.

Sullivan, M. E., Keoghane, S. R., & Miller, M. A. (2001). Vascular risk factors and erectile dysfunction. *British Journal Urology International*, 87, 838-845.

Sundell, L., Salomaa, V., Vartiainen, E., Poikolainen, K., & Laatikainen, T. (2008). Increased stroke risk is related to a binge-drinking habit. *Stroke*, 39, 3179–3184.

Svistunov, A.A., Tarasov, V.V., Shakhmardanova, S.A., Sologova, S.S., Bagaturiya, E.T., Chubarev, V.N. et al. (2018). Urotensin II: Molecular Mechanisms of Biological Activity. *Curr Protein Pept Sci*, 19, 924-934.

Talavera-Garcia, E., Delgado-Lista, J., Garcia-Rios, A., Delgado-Casado, N., Gomez-Luna, P., Gomez-Garduño, A., et al. (2016). Influence of Obesity and Metabolic Disease on Carotid Atherosclerosis in Patients with Coronary Artery Disease (CordioPrev Study) *PLoS One*, 11, e0153096. Correction in: *PLoS One*, 11, e0157213.

Thompson, I. M., Tangen, C. M., Goodman, P. J., Probstfield, J. L., Moinpour, C. M., & Coltman, C. A. (2005). Erectile dysfunction and subsequent cardiovascular disease. *The Journal of the American Medical Association*, 294,2996-3002.

Thorve, V. S., Kshirsagar, A. D., Vyawahare, N. S., Joshi, V. S., Ingale, K. G., & Mohite, R. J. (2011). Diabetes-induced erectile dysfunction: epidemiology, pathophysiology and management. *Journal of Diabetes and its Complications*, 25,129-136.

Toda, N., Ayajiki, K., & Okamura, T. (2005). Nitric oxide and penile erectile function. *Pharmacology & Therapeutics*, 106,233-266.

Tolrà, J. R., Campaña, J. M. C., Ciutat, L. F., & Miranda, E. F. (2006). Prospective, randomized, open-label, fixed-dose, crossover study to establish preference of patients with erectile dysfunction after taking the three PDE-5 inhibitors. *The Journal of Sexual Medicine*, 3,901-909.

Traish, A. M., Gupta, S., Toselli, P., de Tejada, I. S., Goldstein, I., & Moreland, R. B. (1995). Identification of alpha 1-adrenergic receptor subtypes in human corpus cavernosum tissue and in cultured trabecular smooth muscle cells. *Receptor*, 5,145e157.

Traish, A. M., Moreland, R. B., Gallant, C., Huang, Y. H., & Goldstein, I. (1997). G-protein-coupled receptor agonists augment adenylyl cyclase activity induced by forskolin in human corpus cavernosum smooth muscle cells. *Journal of Receptors and Signal Transduction*, 7,121-132.

Truss, M. C., Becker, A. J., Djamilian, M. H, Stief, C. G., & Jonas, U. (1994). Role of the nitric oxide donor linsidomine chlorhydrate (SIN-1) in the diagnosis and treatment of erectile dysfunction. *Urology*, 44,553-556.

Tsujimura, A., Hiramatsu, I., Aoki, Y., Shimoyama, H., Mizuno, T., Nozaki, T., et al. (2017). Atherosclerosis is associated with erectile function and lower urinary tract symptoms, especially nocturia, in middle-aged men. *Prostate International*, 5,65-69.

Tsutsui, T., Takeda, M., Hartano, A., Suwa, M., & Takahashi, K. (1999). Different distribution of caveolin-1 and caveolin-3 in the human corpus cavernosum: immunohistochemical study, in comparison with nitric oxide synthase nerve and low affinity nerve growth factor receptor. *The Journal of Urology*, 161,218.

Uckert, S., Fuhlenriede, M. H., Becker, A. J., Stief, C. G, Scheller, F., Knapp, W. H., et al. (2003). Is serotonin significant for the control of penile flaccidity and detumescence in the human male? *Urological Research*, 31,55-60.

Uddin, S. M. I., Mirbolouk, M., Dardari, Z., Feldman, D. I., Cainzos-Achirica, M., DeFilippis, A. P., et al. (2018). Erectile dysfunction as an independent predictor of future cardiovascular events: the Multi-Ethnic Study of Atherosclerosis. *Circulation*, 138,540-542.

Udelson, D. (2007). Biomechanics of male erectile function. *Journal of the Royal Society Interface*, 4,1031–1048.

Uvin, P., Albersen, M., Bollen, I., Falter, M., Weyne, E., Linsen, L., et al., (2017). Additive effects of the Rho kinase inhibitor Y-27632 and vardenafil on relaxation of the corpus cavernosum tissue

of patients with erectile dysfunction and clinical phosphodiesterase type 5 inhibitor failure. *The British Journal of Urology International*, 119, 325-332.

Vallone, D., Picetti, R., & Borrelli, E. (2000). Structure and function of dopamine receptors. *Neuroscience & Biobehavioral Reviews*, 24, 125-132.

Ventimiglia, E., Capogrosso, P., Montorsi, F., & Salonia, A. (2016). The safety of phosphodiesterase type 5 inhibitors for erectile dysfunction. *Expert Opinion on Drug Safety*, 15, 141-152.

Virag, R., Bouilly, P., & Frydman, D. (1985a). About arterial risk factors and impotence. *Lancet*, 1, 1109-1110.

Virag, R., Bouilly, P., & Frydman, D. (1985b). Is impotence an arterial disorder?: a study of arterial risk factors in 440 impotent men. *Lancet*, 325, 181–184

Virag, R., Ottesen, B., Fahrenkrug, J., Levy, C. C. Wagner, G. (1982). VIP release during penile erection in man. *Lancet*, 2,1166.

von Keitz, A., Rajfer, J., Segal, S., Murphy, A., Denne, J., Costigan, T., et al. (2004). A multicenter, randomized, double-blind, crossover study to evaluate patient preference between tadalafil and sildenafil. *European Urology*, 5, 499-509.

Waldkirch, E., Uckert, S., Schultheiss, D., Geismar, U., Bruns, C., Scheller, F., et al. (2008). Non-genomic effects of androgens on isolated human vascular and nonvascular penile erectile tissue. *British Journal Urology International*, 101, 71-75.

Walsh, M. P. (1991). The Ayerst Award Lecture 1990. Calcium-dependent mechanisms of regulation of smooth muscle contraction. *Biochemistry and Cell Biology*, 69, 771-800.

Wang, X. M., Bai, Y. J., Yang, Y. B., Li, J. H., Tang, Y., & Han, P. (2018). Alcohol intake and risk of erectile dysfunction: a dose-response meta-analysis of observational studies. *International Journal of Impotence Research*, 30, 342-351.

Weber, M. F., Smith, D. P., O'Connell, D. L., Patel, M. I., de Souza, P. L., Sitas, F, et al. (2013). Risk factors for erectile dysfunction in a cohort of 108 477 Australian men. *Medical Journal of Australia,* 199, 107-111.

Wespes, E., Sattar, A. A., Noël, J. C., & Schulman, C. C. (2000). Does prostaglandin E1 therapy modify the intracavernous musculature? *The Journal of Urology*, 163, 464-466.

Wessells, H., Levine, N., Hadley, M.E., Dorr, R., Hruby, V. (2000). Melanocortin receptor agonists, penile erection, and sexual motivation: human studies with Melanotan II. *International Journal of Impotence Research,* 12 Suppl 4, S74-79.

Wessells, H., Penson, D. F., Cleary, P., Rutledge, B. N., Lachin, J. M., McVary, K. T., et al. (2011). Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive glycemic therapy on erectile function in men with type 1 diabetes. *The Journal of Urology*, 185, 1828-1834.

Wolters, J. P., & Hellstrom, W. J. (2006). Current concepts in ejaculatory dysfunction. *Reviews in urology*, 8, S18-S25;

Woodward, D. F., Jones, R. L., & Narumiya, S. (2011). International union of basic and clinical pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. *Pharmacological Reviews*, 63, 471-538.

Wu, C., Zhang, H., Gao, Y., Tan, A., Yang, X., Lu, Z., et al. (2012). The association of smoking and erectile dysfunction: results from the Fangchenggang Area Male Health and Examination Survey (FAMHES). *Journal of Andrology*, 33,59-65.

Yachie, A., Niida, Y., Wada, T., Igarashi, N., Kaneda, H., Toma, T., et al. (1999). Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *Journal of Clinical Investigation*, 103,129–135.

Yaman, O., Gulpinar, O., Hasan, T., Ozdol, C., Ertas, F.S., & Ozgenci, E. (2008). Erectile dysfunction may predict coronary artery disease: relationship between coronary artery calcium scoring and erectile dysfunction severity. *International Urology and Nephrology*, 40,117–123.

Yildiz, O., Seyrek, M., Irkilata, H.C., Yildirim, I., Tahmaz, L., & Dayanc. M. (2009). Testosterone might cause relaxation of human corpus cavernosum by potassium channel opening action. *Urology*, 74,229-232.

Yiou R. (2017). Stem-cell therapy for erectile dysfunction. *Bio-Medical Materials and Engineering*, 28, S81-S85.

Zaher, T.F. (1998). Papaverine plus prostaglandin E1 versus prostaglandin E1 alone for intracorporeal injection therapy. *International Urology and Nephrology*, 30, 193-196.

Table 1

Indications for specific diagnostic tests*
Primary ED (not caused by organic disease or psychogenic disorder).
Young patients with a history of pelvic or perineal trauma, who could benefit from potentially curative revascularization surgery or angioplasty.
Patients with penile deformities that might require surgical correction (e.g., Peyronie's disease, congenital penile curvature).
Patients with complex psychiatric or psychosexual disorders.
Patients with complex endocrine disorders.
Specific tests may be indicated at the request of the patient or his partner.
Medico-legal reasons (e.g., implantation of penile prosthesis to document end-stage ED, sexual abuse).

**** Hatzimouratidis et al 2019; EAU guidelines***

Table 2

Specific diagnostic tests*
Nocturnal Penile Tumescence and Rigidity (NTPR) using RigiScan®
Vascular studies:
- Intracavernous vasoactive drug injection
- Penile Dynamic Duplex Ultrasonography
- Penile Dynamic Infusion Cavernosometry and Cavernosography
- Internal pudendal arteriography
Neurological studies (e.g., bulbocavernosus reflex latency, nerve conduction studies)
Endocrinological studies
Specialized psycho-diagnostic evaluation
**** Hatzimouratidis et al 2019; EAU guidelines***

Table 3

Pharmacokinetics of PDE-5 inhibitors				
	Sildenafil	Vardenafil	Tadalafil	Avanafil
Tmax (min)	45-60	45-60	120	30-45
Cmax (min)	30-120	30-120	120	30-45
Vd, mean (l)	105	208	63	
Duration of action (h)	3-5	4-6	17.5	6-17
Metabolism	Hepatic	Hepatic	Hepatic	Hepatic
Principal route	CYP3A4	CYP3A4	CYP3A4	CYP3A4
Secondary route	CYP2C9	CYP3A5 CYP2C		CYP2C9

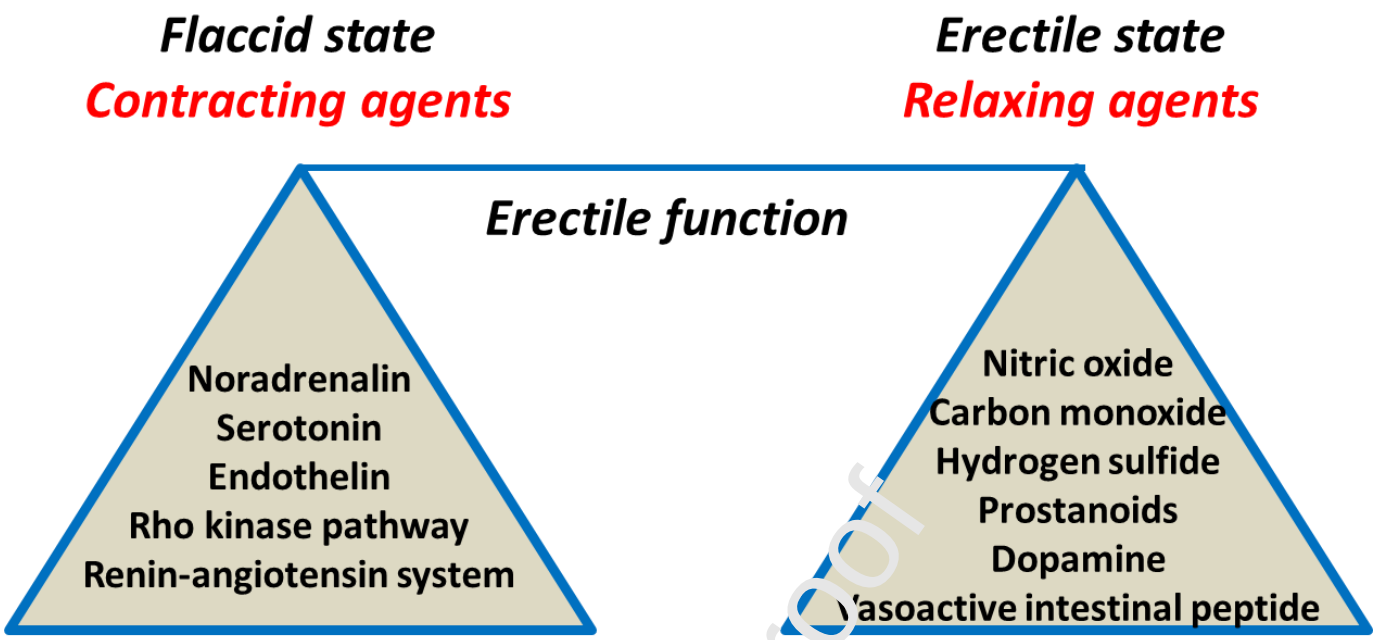

Figure 1. Schematic representation of erectile function. A tight balance between contracturant and vasorelaxant agents concurs to the erectile function in healthy men. The most relevant contracting agents (left side of the balance) and vasorelaxing agents (right side of the balance) involved in the flaccid and erectile states, respectively, are reported.

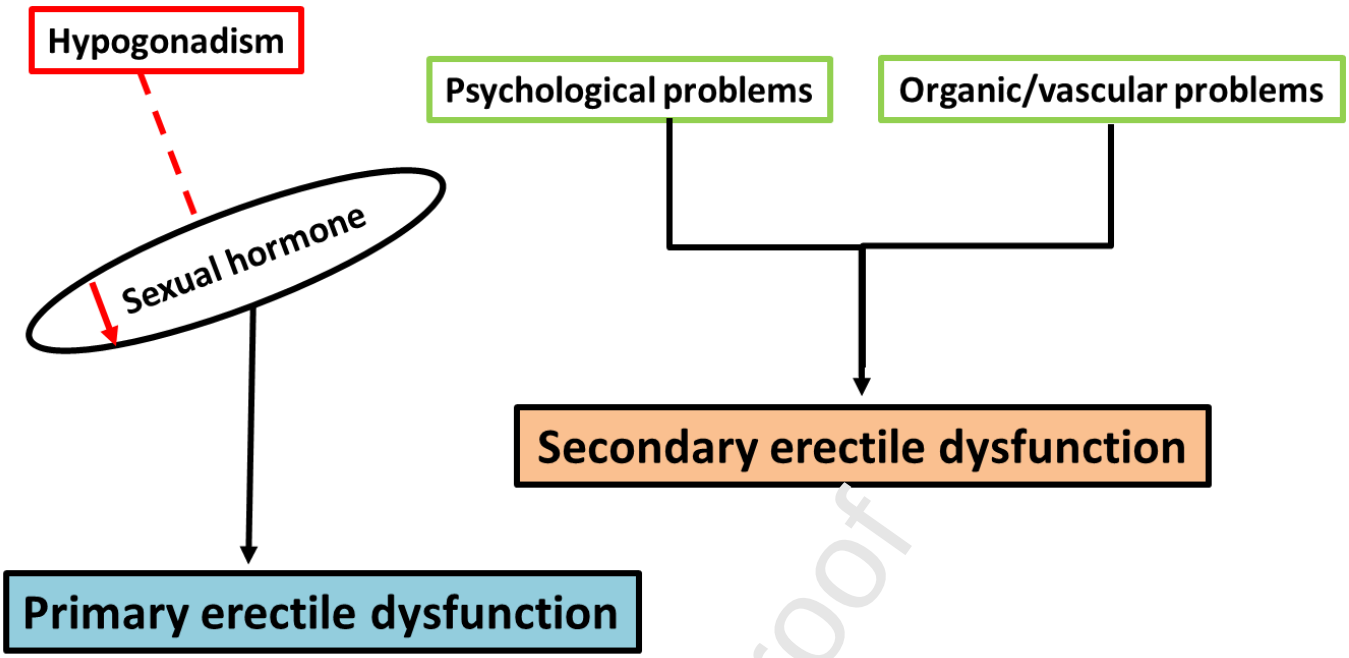

Figure 2. Primary and secondary erectile dysfunction. A scheme representing the most important causes associated with primary or secondary erectile dysfunction. The dotted line indicates inhibition.

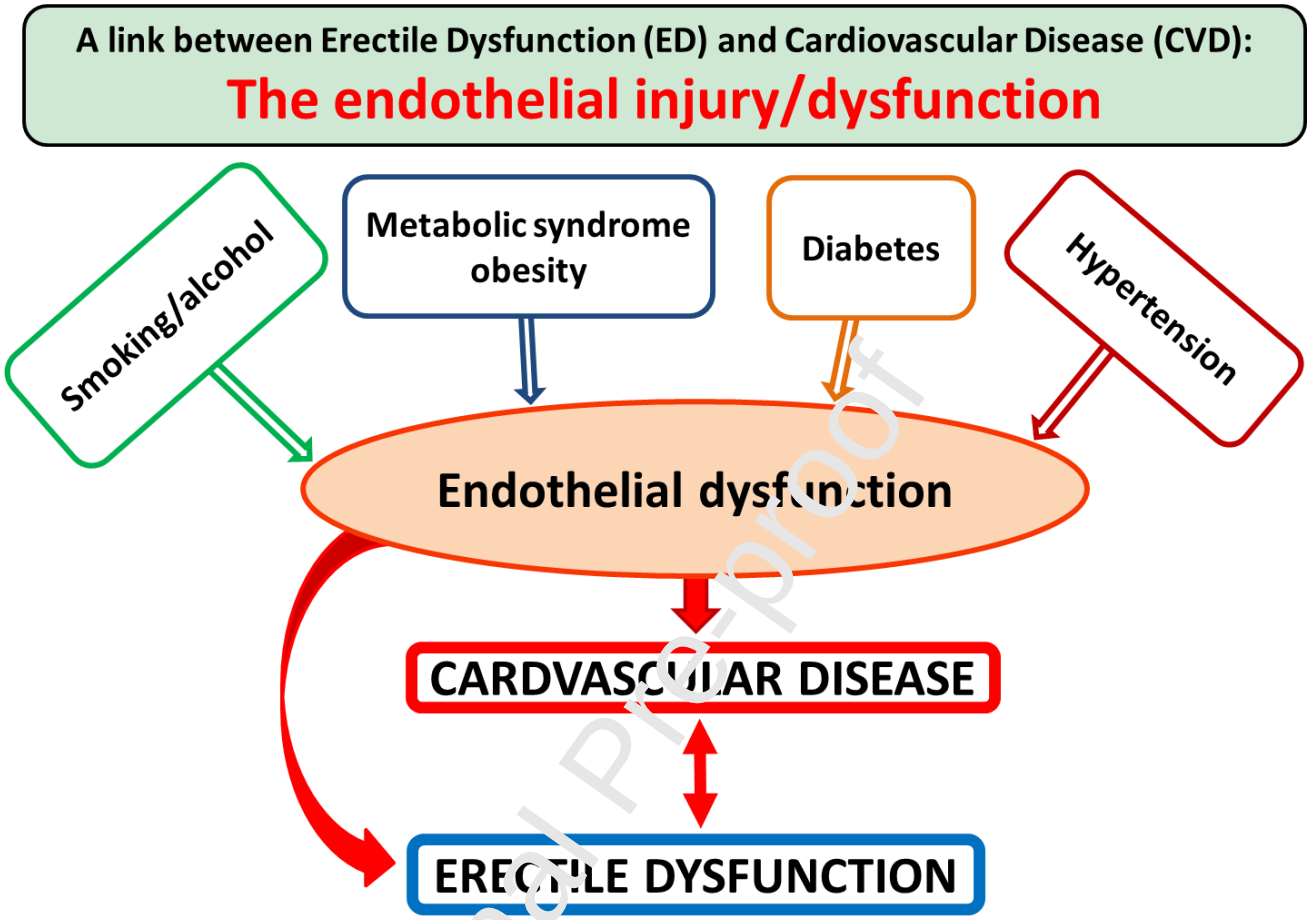

Figure 3. Vascular erectile dysfunction (ED) and cardiovascular disease (CVD). ED and CVD share the same risk factors that cause endothelial dysfunction. In patients with ED who do not suffer from CVD, the ED must be considered as a warning symptom, suggesting a different diagnostic and therapeutic approach. In this case, ED should be considered as a mirror of CVD. This approach will contribute to prevent CVD events and to a better counteract/delay in the worsening of ED.

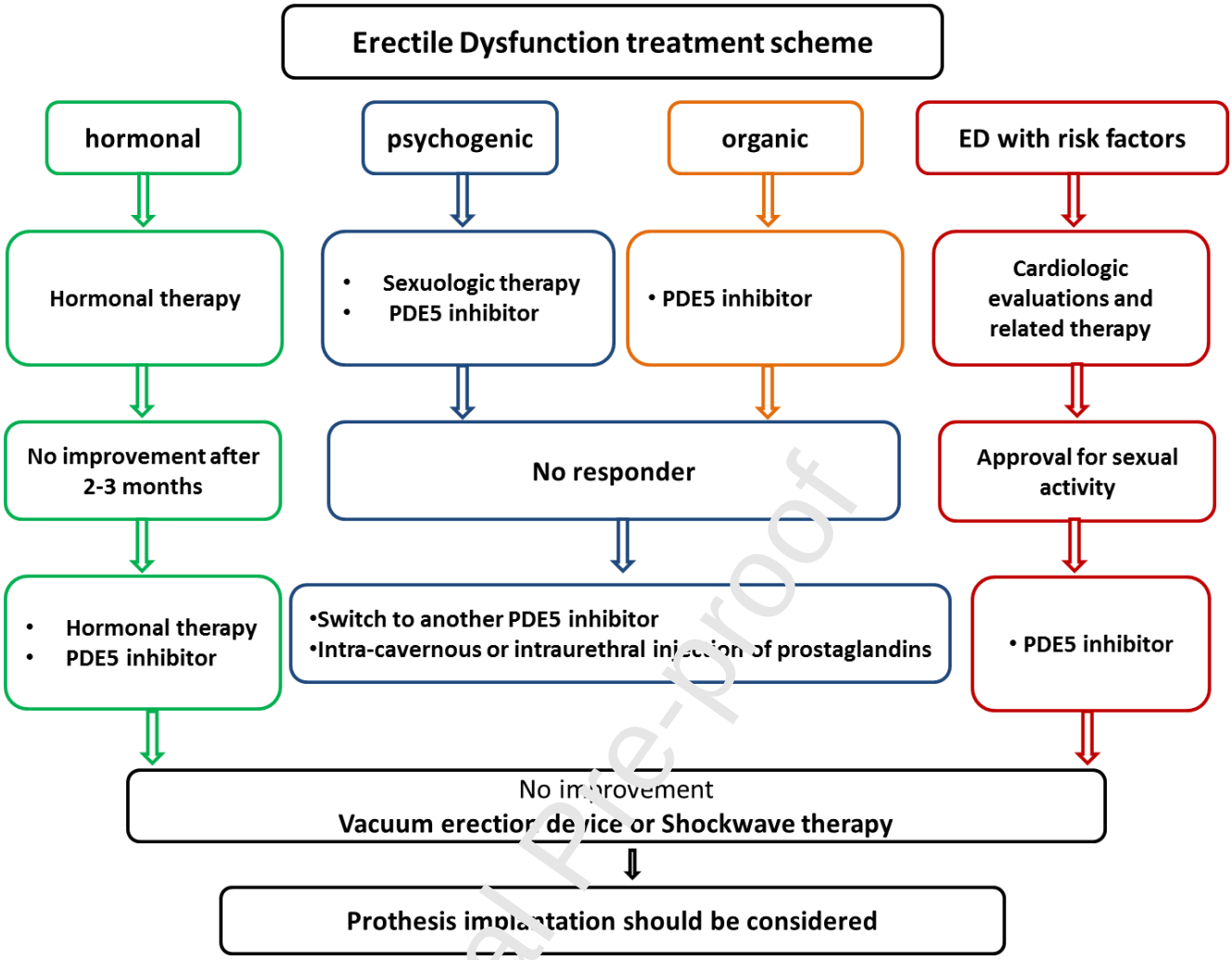

Figure 4. Scheme of erectile dysfunction (ED) treatment. This scheme shows the flowchart suggested by the ED guidelines (EUA and AUA). In case of concomitant presence of ED and risk factors for CVD, the clinicians must also consider treating patients for CVD symptoms/risk factors such as hypertension, hypercholesterolemia, diabetes, and atherosclerosis.