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Endothelial function in patients with metabolic syndrome and erectile dysfunction: a question of Angiopoietin imbalance?

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SUMMARY

Erectile dysfunction (ED) is a highly prevalent disease whose aetiology is mostly vasculogenic. It is nowadays considered a marker of future cardiovascular events reflecting the underlying endothelial dysfunction, the common link with the metabolic syndrome (MetS). The recent association between MetS, endothelial dysfunction and peripheral artery disease, but not with coronary artery disease (CAD), suggests that the pathophysiologies of CAD and peripheral artery disease may be distinct. Moreover, several recent studies support an emerging role for an imbalance of angiogenic growth factor levels like Angiopoietin 1 and 2 in cardiovascular disease, considering its intimate association with chronic low-grade inflammation. We aim to find a correlation between Angiopoietins levels and systemic and local endothelial function in MetS and ED patients. Forty-five MetS patients with ED were enrolled. ED severity was assessed by International Index of Erectile Function questionnaire (IIEF5) and penile duplex Doppler ultrasound (PDDU). Endothelial function was assessed by Peripheral arterial tonometry (PAT), and serum asymmetric dimethylarginine (ADMA), Ang1 and Ang2 levels. Obesity and hypertension were the most frequent MetS parameters (91.1 and 88.9% respectively). Severe ED was present in 35.6% of patients. Penile haemodynamic was impaired in 77.5%. Endothelial dysfunction (PAT criteria) was present in 40.9% of patients. Ang2 levels were significantly higher in men with abdominal obesity. We observed an inverse correlation between Ang1 and peak systolic velocity, and in patients with penile arterial dysfunction, Ang1 levels were significantly higher and Ang2/Ang1 ratio significantly lower, than patients with normal arterial function. Neither ADMA nor PAT parameters were correlated with ED severity evaluated by IIEF5 or PDDU exam. In conclusion, there is an imbalance of angiopoietins in MetS and ED patients. The absence of correlation with PAT or ADMA levels suggests that angiopoietins may be early markers of endothelial dysfunction in this population of higher cardiovascular risk.

INTRODUCTION

Erectile dysfunction (ED), the inability to achieve and/or maintain an erection sufficient to permit satisfactory intercourse, is a highly prevalent disease whose aetiology is mostly vasculogenic Brunner *et al.* (2005), McKinlay (2000). It is associated with subclinical coronary artery disease (CAD) and is nowadays considered a marker of future cardiovascular events Böhm *et al.* (2010), Dong *et al.* (2011), Montorsi *et al.* (2003). The higher prevalence of ED in patients with cardiovascular risk factors (CVRF) reflects the underlying endothelial dysfunction, defined as the reduction in nitric oxide (NO) bioavailability.

Metabolic syndrome (MetS) which represents a cluster of several CVRF, as abdominal obesity, dyslipidaemia, hypertension (HTA) and glucose intolerance, it is also associated with an increased risk of diabetes and cardiovascular diseases, with a positive correlation with ED Esposito *et al.* (2005), Grundy (2007), Tomada *et al.* (2011). Nevertheless, the CODAM study demonstrated an unequivocal association between MetS, endothelial dysfunction and peripheral artery disease, but not with CAD Jacobs *et al.* (2011). This observation suggests that the pathophysiologies of CAD and peripheral artery disease may therefore be distinct.

Several recent studies support an emerging role for an imbalance of angiogenic growth factor levels in disease processes including tumour growth, diabetes and cardiovascular disease Augustin *et al.* (2009), Iribarren *et al.* (2011), Lim *et al.* (2005).

Angiopoietin–Tie system, of which Angiopoietin 1 (Ang1) and Angiopoietin 2 (Ang2) have been the most studied, is an endothelial cell-specific ligand–receptor axis that regulates vasculogenesis and angiogenesis. It is also profoundly involved in the pathogenesis of inflammation, Ang1 and Ang2 presenting a counteracting relationship: Ang1 seems to have an anti-inflammatory role, whereas Ang2 works opposite by exerting a proinflammatory effect Imhof & Aurrand-Lions (2006). In healthy individuals, serum Ang1 levels are normally high, whereas serum Ang2 levels are low. In view of the contrasting roles of Ang1 and Ang2, a deregulation of the balance between Ang1 and Ang2 may be associated with disease states that cause inflammation and vascular permeability.

Recently, we demonstrated Ang1 and Ang2 upregulation in healthy human-aged penile tissue Tomada *et al.* (2010). Furthermore, Ang1 has been shown to induce successful therapeutic angiogenesis in animal model. Intracavernous delivery of the recombinant COMP-Ang1 protein successfully restored the endogenous NO-cGMP pathway through the regeneration of healthy and non-leaky cavernous endothelium and inhibition of reactive oxygen species-mediated cavernous endothelial cell apoptosis Jin *et al.* (2011). Moreover, in animal myocardial infarction and reperfusion models, shifting the Ang2/Ang1 ratio to favour Ang1 by administration of adenovirus expressing Ang1 could prevent myocardial and endothelial cell apoptosis, and reduce infarct size. However, shifting the Ang2/Ang1 ratio to favour Ang2 results in a significant increase in myocardial infarct size Tuo *et al.* (2008). It is therefore plausible that angiopoietins could exert both protective and deleterious effects in the development of cardiovascular disease.

In this context, considering the intimate association of chronic low-grade inflammation with atherosclerosis, Angiopoietin levels and Ang2/Ang1 ratio may be early markers of endothelial dysfunction, and its assessment may represent a new and accurate test to early detection of cardiovascular risk. Although abnormal serum angiopoietin levels have been reported in various cohorts with concomitant vascular disease, patients with MetS and ED have not been investigated to date. In addition, there is no known previous study on the association between angiopoietins and different markers of endothelial function systemic and local, resulting in a comprehensive evaluation of the endothelium in this particular group of patients. Our aim in this study was to find a correlation between Angiopoietin levels and systemic and local endothelial function in patients with high risk of cardiovascular disease, both MetS and ED.

METHODS

Patients

We enrolled 45 consecutive white patients with MetS [National Cholesterol Education Program – Third Adult Treatment Panel; Expert Panel on Detection, Evaluation, and Treatment of High Blood cholesterol in Adults (NCEP-ATPIII) criteria] referred to our department for ED between August 2010 and January 2012 NCEP Expert Panel (2002). This prospective study protocol was approved by the Local Ethical Committee of our Hospital and written informed consent form was obtained from all participants. Exclusion criteria included recent cardiovascular event (<6 months), pelvic surgery or trauma, liver and/or renal failure, chronic and acute inflammatory diseases, tumours and urogenital abnormalities. A standardized health questionnaire covering medical history, including sexual history, CVRF and current medications was obtained. Patients were also asked to complete the abridged five-item version of the International Index of Erectile Function (IIEF-5) Rosen *et al.* (1999). The possible scores for the IIEF-5 range from 5 to 25, and ED was classified into five categories based on the scores: severe (5–7), moderate (8–11), mild to moderate (12–16), mild (17–21) and no ED (22–25). All patients underwent a standardized physical examination protocol. Anthropometric evaluation, including weight, height and waist circumference (WC), was acquired by the same technician, with the subjects in light clothing and barefoot. Body mass index (BMI) was calculated as weight in kilograms divided by square of the height in metres (kg/m^2). WC measurement was obtained using an anthropometric tape (to the nearest 0.1 cm) at the end of normal expiration at the level of the midpoint between the lower end of the 12th rib and upper end of iliac crest.

Systolic and diastolic blood pressures were measured in the right arm using an automatic manometer (DINAMAP Procare 300: GE, Buckinghamshire, UK) in the sitting position after a 10-min rest period. HTA was defined as systolic blood pressure 130 mmHg and/or diastolic blood pressure 85 mmHg. All patients were studied between 8 and 10 AM after a 12-h overnight fast. Venous blood was sampled for measurements of plasma glucose, total and high-density lipoprotein cholesterol (HDL), triglycerides (Trig), by routine laboratory methods. Total testosterone (TT) levels were determined by electrochemiluminescence in an immunoassay analyser (Cobas; Roche Diagnosis GmbH, Mannheim, Germany) with a normal range 9.7–27.8 nmoL/L. Free testosterone (FT) levels were calculated according to Vermeulen formula as previously described Vermeulen *et al.* (1999).

Hypogonadism was defined as TT below 8 nmoL/L or FT under the lower limit of range when the serum TT level was between 8 and 12 nmoL/L, according to the International Society of Andrology, International Society for the Study of Aging Male, European Association of Urology, European Academy of Andrology and American Society of Andrology recommendations Wang *et al.* (2009).

Penile duplex Doppler ultrasound exam

All patients underwent PDDU examination using a standard protocol, as recommended in the 'Standard Practice in Sexual Medicine', produced by the International Society for Sexual Medicine Standards Committee, as previously described Tomada *et al.* (2011). Patients were not under acute or chronic use of phosphodiesterase type 5 inhibitors for at least 30 days until PDDU assessment. Briefly, penile Doppler measurements of spectral waveforms were performed including peak systolic velocity (PSV), end diastolic velocity (EDV) and resistive index (RI). Measurements were made at 5, 10 and 20 min, and the mean of the latest values were recorded. Values of PSV higher than 35 cm/sec, EDV lower than 5 cm/sec and RI >1 were considered normal response. Diagnostic criteria for an abnormal response included arterial insufficiency for PSV <35 cm/sec or PSV asymmetry >10 cm/sec, and cavernous venous-occlusive disease for PSV ≥35 cm/sec and EDV ≥5 cm/sec. The degree of erectile response was estimated according to a graded scale: 0 (no response), 1 (minimal tumescence and no rigidity), 2

(moderate tumescence and no rigidity), 3 (full tumescence and moderate rigidity) and 4 (full rigidity).

Peripheral arterial tonometry evaluation

The PAT evaluation was performed with patient in a supine position in a dimly lit, noise and temperature-controlled room Patvardhan *et al.* (2010), Hamburg *et al.* (2008). All vasoactive medications were discontinued 24 h prior to testing. Patients were instructed to have a light meal on the morning of the exam and to refrain from exercise, smoking and caffeine consumption on that day. Peripheral wave amplitude was assessed before and during reactive hyperaemia (RH) by PAT (Endo-PAT 2000; Itamar Medical Ltd., Caesarea, Israel) as previously described Aversa *et al.* (2011). Following a 10-min acclimatization period, 5-min RH-PAT baseline signals were recorded using plethysmographic finger cuffs, placed on the index finger of both hands. The blood pressure cuff was then inflated rapidly to 200 mmHg and remained at that level for 5 min before being quickly released, and then PAT tracing was recorded for another 5 min. RT-PAT index was calculated as the ratio of the average amplitude of the PAT signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the PAT signal of a 3.5-min time period before cuff inflation. The index was calculated through a computer algorithm automatically normalizing for baseline signal and indexed to the contralateral arm. Endothelial dysfunction was defined using a 1.67 RH-PAT cut-off Ohno *et al.* (2010). Arterial stiffness was also measured through the augmentation index (AI) calculated as the ratio between the amplitude difference of the two systolic peaks and the amplitude of the first peak. The larger the value of AI, the stiffer the arterial system, negative values being possible when first peak is higher than second.

Angiopoietins and asymmetric dimethylarginine quantification

Ang1 and Ang2 were quantified by ELISA (DANG10 and DANG20 Immunoassay; R&D Systems, Abingdon, UK respectively) in 50 μL of human serum according to the manufacturer's instructions. Quantifications were performed at 450 and 550 nm using a plate reader (Multiskan Ascent; Thermo Electron Corporation, Waltham, MA, USA). ADMA was also quantified by ELISA (EA201/96 Immunoassay; DLD Diagnostika GMBH, Hamburg, Germany) in 20 μL of human serum according to the manufacturer's instructions. Quantifications were performed at 450 and 600 nm using a plate reader (Multiskan Ascent; Thermo Electron Corporation). These ELISA assays were performed by an investigator blinded to the sources of the samples.

Statistical analysis

All descriptive data are expressed as mean value ± standard deviation for the continuous variables and as percentage for the categorical variables. Pearson correlation was performed to evaluate the association between continuous variables including between the different markers of endothelial function and markers of endothelial function and clinical variables, MetS criteria, severity of ED and PDDU haemodynamic findings. Some specific correlations are presented with scatter plot graphs with a line of best fit. Statistical analysis was performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at p-level <0.05.

RESULTS

The characteristics of the study population are described in Table 1. Mean age, BMI and WC were 55.4 ± 7.8 years, 31.6 ± 5 kg/m^2 and 112 ± 11.4 cm respectively. Insulin resistance, HTA, hypercholesterolaemia, hypertriglyceridaemia and HDL below 1.03 mmol/L were identified in 71.1, 88.9, 75.6, 75.6 and 64.4% of patients respectively. Three, four and five components of MetS were present in 33.3, 42.2 and 24.4 of the patients respectively.

Hypogonadism was present in 44.4% of the patients, being severe (TT below 8 nmoL/L) in 13.3%. A history of tobacco use was present in 71.1% of the patients. Relevant current medical treatment included angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, diuretic agents, statins, acetylsalicylic acid and antidepressives in 35.6, 33.3, 28.9, 44.4, 55.6, 48.9 and 6.7% of the patients respectively. Obesity defined by BMI ≥30.0 kg/m^2 was present in 97.8%.

The mean IIEF-5 score was 10.4 ± 5.2. ED severity as assessed by this questionnaire was as follows: no ED-6.7%, mild ED-4.4%, mild to moderate ED-24.4%, moderate ED-28.9%, and severe ED-35.6%.

The PDDU was normal in 22.5%, arterial insufficiency was present in 55% of the cases and veno-occlusive dysfunction in 22.5%.

Table 1 Characteristics of the study population. Data are expressed as mean ± standard deviation when normally distributed and as percentages when categorical

Age (years)	55.4 ± 7.8
Weight (kg)	91.4 ± 17.3
Waist circumference (cm)	112.0 ± 11.4
BMI (Kg/m^2)	31.6 ± 5.0
TT (nmol/L)	13.22 ± 4.62
SHBG (nmol/L)	34.8 ± 19.1
cFT (pmol/L)	260 ± 94
Bioavailable testosterone (nmol/L)	6.42 ± 2.26
SBP (mmHg)	140.6 ± 17.5
DBP (mmHg)	82.7 ± 9.1
Glucose (mmol/L)	6.93 ± 3.12
Cholesterol (mmol/L)	4.70 ± 0.85
Triglyceride (mmol/L)	1.99 ± 0.87
HDL cholesterol (mmol/L)	1.07 ± 0.21
IIEF-5	10.4 ± 5.2
No ED (%)	6.7
Mild ED (%)	4.4
Mild to moderate ED (%)	24.4
Moderate ED (%)	28.9
Severe ED (%)	35.6
Metabolic syndrome components (NCEP-ATP III) (%)	
HTA (>130/85 mmHg) or treatment	88.9
Hypertriglyceridaemia (>1.7 mmol/L) or treatment	75.6
Insulin resistance (fasting plasma glucose ≥6.1 mmol/L)	71.1
Low HDL (<1.03 mmol/L) or treatment	64.4
Waist circumference >102 cm	91.1
Smokers (%)	71.1
Current medical treatment (%)	
ACE inhibitors	35.6
AR blockers	33.3
Beta blockers	28.9
Diuretic agents	44.4
Statins	55.6
Acetylsalicylic acid	48.9
Antidepressives	6.7
Hypogonadism (%)	44.4

BMI: body mass index, TT: total testosterone, SHBG: Sex Hormone–Binding Globulin, cFT: calculated free testosterone, ED: erectile dysfunction, IIEF-5: abridged 5-item version of the International Index of Erectile Function, HTA: hypertension, HDL: high-density lipoprotein, ACE: angiotensin-converting enzyme, AR: angiotensin receptor.

Endothelial dysfunction defined by PAT was present in 40.9% of the patients. Mean RH-PAT and AI were 1.8 ± 0.4 and 8.6 ± 21.1 respectively.

Mean levels of ADMA, Ang1 and Ang2 were 0.56 ± 0.24 μmol/L, 11.10 ± 1.95 ng/mL and 3.0 ± 0.56 ng/mL respectively.

Clinical correlations

ED severity, evaluated by IIEF-5 score, increased with age ($r = -0.386$; $p = 0.009$). Bioavailable testosterone was associated with BMI ($r = -0.348$; $p = 0.032$), unlike TT ($r = -0.222$; $p = 0.153$) or calculated FT ($r = -0.288$; $p = 0.079$).

Correlations between markers of endothelial function and clinical variables

Among all parameters of EndoPAT only AI correlated inversely with BMI ($r = -0.400$; $p = 0.007$) and positively with systolic arterial blood pressure values ($r = 0.486$; $p = 0.001$). There were no correlations between ADMA and clinical variables (data not shown). Ang1 presented an inverse correlation with ageing ($r = -0.368$; $p = 0.020$). Ang2 presented a positive correlation with BMI ($r = 0.422$; $p = 0.007$) and an inverse correlation with TT, bioavailable testosterone and calculated FT ($r = -0.428$, $p = 0.006$; $r = -0.398$, $p = 0.018$; and $r = -0.415$, $p = 0.013$; respectively).

Correlations between markers of endothelial function and MetS criteria

We failed to observe a correlation between EndoPAT parameters, ADMA or Angiopoietins with increasing criteria for MetS. There was also no correlation of any of these markers with any single parameter of MetS, with the exception of Ang2 levels being significantly higher in patients with WC >102 cm ($p = 0.047$) and a trend to be elevated in patients with HDL levels <40 mg/dL ($p = 0.057$) (Table 2).

Correlations between markers of endothelial function and severity of ED evaluated by IIEF5

We could not demonstrate a correlation between Ang1, Ang2, Ang2/Ang1 ratio, ADMA and EndoPAT parameters RH-PAT and AI with the severity of ED as evaluated by IIEF5 questionnaire ($r = -0.019$, $p = 0.907$; $r = -0.277$, $p = 0.084$; $r = -0.156$, $p = 0.336$; $r = -0.221$, $p = 0.171$; $r = -0.038$, $p = 0.805$; $r = -0.156$, $p = 0.313$; respectively).

Correlations between markers of endothelial function and PDDU haemodynamic findings

We observed an inverse correlation between Ang1 and PDDU haemodynamic parameters PSV ($r = -0.451$, $p = 0.005$) (Fig. 1) and EDV ($r = -0.524$, $p = 0.003$), whereas Ang2/Ang1 ratio presented a direct correlation ($r = 0.350$, $p = 0.034$; $r = 0.465$, $p = 0.010$ respectively). There were no correlations between Ang2 ($r = 0.002$, $p = 0.990$; $r = 0.097$, $p = 0.610$ respectively), ADMA ($r = 0.116$, $p = 0.494$; $r = 0.294$, $p = 0.114$ respectively), RH-PAT ($r = 0.155$, $p = 0.347$; $r = 0.34$, $p = 0.855$; respectively) and AI ($r = 0.138$, $p = 0.401$; $r = -0.029$, $p = 0.877$; respectively) with those same parameters.

When we considered patients with arterial dysfunction, Ang1 levels were significantly higher ($p = 0.015$) and Ang2/Ang1 ratio significantly lower ($p = 0.048$), than patients with normal arterial function in PDDU examination.

Correlations between the different markers of endothelial function

The ADMA presented an inverse correlation with Ang1 ($r = -0.452$, $p = 0.003$) and a positive correlation with Ang2/Ang1 ratio ($r = 0.429$, $p = 0.006$). There were no correlations

Figure 1 Correlation between Ang1 serum levels and peak systolic velocity ($r = -0.45$, $p = 0.005$).

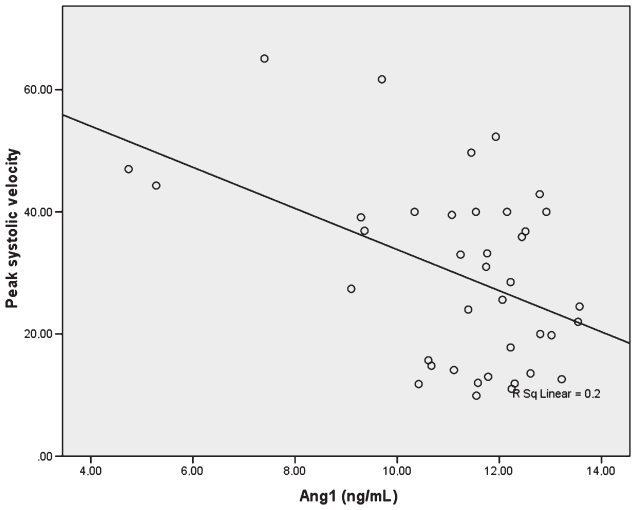


Table 2 Results of analysis of the relationship between EndoPAT parameters, ADMA and Angiopoietins with MetS parameters. There was no correlation of any of these markers with any single parameter of MetS, with the exception of Ang2 levels being significantly higher in patients with WC >102 cm ($p = 0.047$)

Metabolic syndrome components (NCEP-ATP III)	Waist circumference >102 cm	HTA (>130/85 mmHg) or treatment	Insulin resistance (fasting plasma glucose ≥6.1 mmol/L)	Hypertriglyceridaemia (>1.7 mmol/L) or treatment	Low HDL (<1.03 mmol/L) or treatment
RH-PAT	0.790	0.862	0.371	0.118	0.646
AI	0.554	0.691	0.587	0.878	0.844
ADMA	0.795	0.228	0.644	0.488	0.665
Ang1	0.788	0.261	0.692	0.112	0.274
Ang2	**0.047**	0.954	0.587	0.467	0.057
Ang2/Ang1	0.176	0.472	0.855	0.229	0.624

HTA: hypertension, HDL: high-density lipoprotein, RH-PAT: reactive hyperaemia by peripheral arterial tonometry, AI: augmentation index, ADMA: Asymmetric dimethylarginine, Ang1: Angiopoietin 1, Ang2: Angiopoietin 2.
Bold indicates statistically significant value.

between EndoPAT parameters and angiopoietins or ADMA serum levels (data not shown).

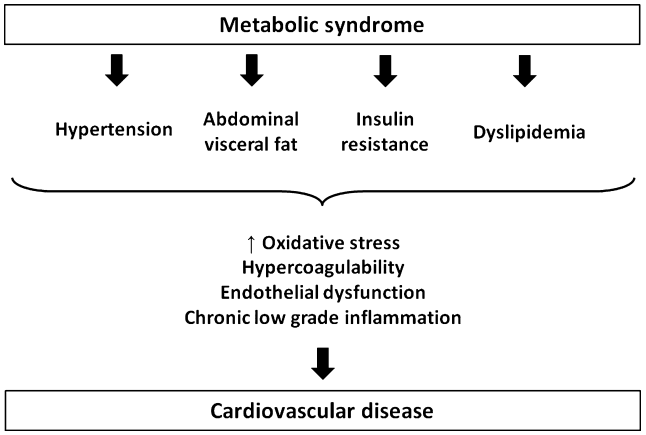
DISCUSSION

The MetS is a cluster of metabolic risk factors, responsible for a greater risk of developing cardiovascular disease and diabetes. ED often coexists with CVRF and has been recently recognized as a predictor of future cardiovascular events Böhm *et al.* (2010). Moreover, the prevalence of ED is increased in men with MetS compared with the general population Lee *et al.* (2012). Several evidences have revealed the relationship existing between the earliest stages of atherosclerosis, such as the impairment of endothelial function, and MetS components (Fig. 2). The perivascular smooth muscle relaxation dependence of the NO produced by endothelial NO synthase (eNOS) is regulated by insulin. Insulin-resistant conditions such as diabetes, MetS and obesity significantly contribute to endothelial dysfunction by disrupting the NO cascade, resulting first in a loss of arterial capacity to dilate and finally in increased atherosclerosis, with subsequent vascular compromise Ryan & Gajraj (2012), Vaudo *et al.* (2007). This endothelial dysfunction is believed to be the common key mechanism in the pathogenesis of ED and MetS. Understanding this pathophysiological pathway has particular clinical relevance in terms of recognizing early micro- and macrovascular dysfunction manifesting as ED.

We recently showed that MetS in ED patients is associated with deterioration in the haemodynamic parameters of the cavernosal arteries, particularly with a diminished PSV, as the number of MetS criteria increased Tomada *et al.* (2011). Moreover, increased fibrinogen and resting heart rate as well as impaired arterial elasticity were more evident among subjects with both conditions, highlighting the higher cardiovascular risk for MetS patients presenting with ED Pohjantähti-Maaroos *et al.* (2011). Thus, there is an opportunity for the development of new clinical methods/markers for subclinical atherosclerosis, fundamental for public health purposes.

Endothelium evaluation may comprise a multitude of methods. Local response of cavernous arteries to a pharmacologic vasodilator stimuli as evaluated by PDDU is an important tool for the diagnosis and classification of patients with vasculogenic ED, especially of arteriogenic origin, which reliability in this context has been repeatedly demonstrated Kendirci *et al.* (2007), La Vignera *et al.* (2011), Tomada *et al.* (2011). More recently, physiological measurements as EndoPAT or systemic methods with serum markers as ADMA emerged as promising new alternatives.

Figure 2 Mechanisms responsible for the increased risk of cardiovascular disease in patients with metabolic syndrome.



The EndoPAT device uses a finger probe to assess digital volume changes accompanying pulse waves after inducing reactive hyperaemia with a blood pressure cuff on the upper arm. RH-PAT has been extensively correlated with early and clinically relevant CAD. In addition, it is similar to flow-mediated dilation in representing a true physiological reflection of peripheral endothelial function Tamler & Bar-Chama (2008). Recently, its application in men with ED was tested, but an increased arterial stiffness rather than an impaired reactive hyperaemia demonstrated by RH-PAT was found in patients with vascular ED independently of CVRF presence Aversa *et al.* (2011).

In our study, a RH-PAT less than 1.67 was observed only in 41% of patients, which means that the majority did not present with endothelial dysfunction by this method. We could not demonstrate a correlation between EndoPAT parameters neither with increasing severity of ED evaluated by IIEF5 questionnaire or PDDU, nor with increasing MetS criteria. Taken together, this may represent a state of vascular impairment that precedes the classical detection of endothelial dysfunction by RH-PAT. More information regarding the variability in the PAT hyperaemic response with disease status, and a higher threshold for identifying vascular ED, may be necessary.

As mentioned above, the NO pathway is of critical importance for the physiological regulation of penile erectile function and its production can be affected by endogenous NOS inhibitors like ADMA. In various pathological states, such as hypercholesterolaemia, hyperglycaemia, hyperhomocysteinaemia, HTA and CAD, plasma levels of ADMA may be increased and lead to inhibition of NO synthesis and endothelial dysfunction Elesber *et al.* (2006), Wierzbicki *et al.* (2006). Thus, ADMA could be used as a biochemical indicator of endothelial dysfunction, as it seems to be responsible for the pathophysiological link between this condition and ED Wierzbicki *et al.* (2006). Nevertheless, there are reports of absence of relationship between ADMA and the development of the age-related impairment of endothelium-dependent vasodilations Gates *et al.* (2007). Recently, Ioakeimidis *et al.* (2011) showed that in patients with vasculogenic ED, ADMA levels were significantly higher in subjects with arterial insufficiency than in men with venous leakage alone, while there was also an independent inverse association between ADMA level and PSV. However, in our sample, there was no correlation of ADMA levels with arteriogenic ED, and, similar to Wierzbicki *et al.* (2006), neither with markers of ED severity. Inhibition of ADMA synthesis or intensification of metabolism of this compound might indirectly lower ADMA. Several medications like angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, acetylsalicylic acid and also some hypolipaemic, hypoglycaemic and beta-adrenoreceptor blocking drugs decrease ADMA levels Trocha *et al.* (2010). Considering the higher percentage of consumption of these substances in our patients, its influence cannot be excluded.

The vascular endothelium is considered a systemically disseminated organ that is maintained in a quiescent state in healthy individuals. The loss of quiescence is associated with severe pathology. Although cardiovascular events such as myocardial infarction and stroke are often sudden conditions, long-term subclinical structural and functional changes in the

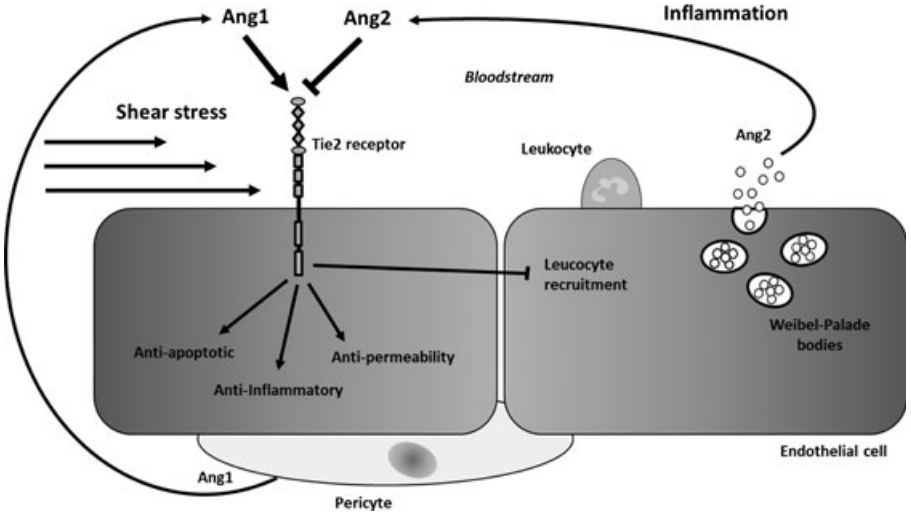
vascular wall precede overt atherosclerotic disease. This vascular remodelling is at least in part mediated by vascular growth factors that may be a link between CVRF and atherosclerosis Kaess *et al.* (2012). Angiopoietins, a distinct family of angiogenic proteins, have recently been shown to play fundamental physiological roles in the maintenance of vascular integrity Augustin *et al.* (2009). Ang1 and Ang2 play divergent roles in mediating cell survival, vascular quiescence and inflammation (Fig. 3). Indeed, Ang1 has antiapoptotic, antipermeability and anti-inflammatory properties, whereas Ang2 seems to have opposite effects. In healthy individuals, serum Ang1 levels are normally high, contrasting with low serum levels of Ang2. Accordingly, a deregulation of the balance between Ang1 and Ang2 may be associated with disease states that cause inflammation and vascular permeability, functioning like true biomarkers of cardiovascular disease Augustin *et al.* (2009), Imhof & Aurrand-Lions (2006), Iribarren *et al.* (2011). Despite the promising value of new endothelial dysfunction biomarkers such as circulating endothelial progenitor cells, as well as, endothelial microparticles secreted after endothelial cell activation, its detection in blood is still laborious and technically difficult, particularly considering that the origin of the EPCs has not yet been fully clarified La Vignera *et al.* (2011). Ang–Tie receptor–ligand system emerges then as a critical regulator of vascular function. In this setting, circulating levels of Ang1 and Ang2 may provide valuable information regarding the status of endothelial dysfunction, which is supported by our findings of angiopoietin levels significant correlation with ADMA. Notwithstanding the abnormal serum angiopoietin concentrations found in the spectrum of vascular diseases, its status in a particular sample of patients with both MetS and ED have not been investigated to date.

Adipose tissue is a highly metabolic active and well-vascularized organ, dependent of the presence of angiogenic factors for its growth and maintenance. In this study, a positive correlation between BMI and Ang2 levels was found, corroborating the higher circulating levels of Ang2 observed in overweight and obese compared with lean individuals Silha *et al.* (2005). Owing to BMI limitations on predicting total adiposity mass and its distribution, and considering the relation of abdominal obesity with insulin resistance risk, WC gained wide acceptance as a component of MetS. A direct and significant relation between Ang2 levels and WC was also observed, highlighting the link with visceral adipose tissue. This make sense, attending that expansion of this tissue may not be as well vascularized. In this context, the following hypoxia, a powerful inducer of Ang2 exocytosis from Weibel-Palade Bodies, may justify the higher serum levels of Ang2 Gealekman *et al.* (2011).

Adipose tissue constantly experiences expansion and regression throughout adult life. This plasticity requires continuous remodelling of the vasculature that controls energy expenditure, metabolic exchange, transport of adipokines, adipocyte hypertrophy and hyperplasia, or even hormones like testosterone and estradiol Xue *et al.* (2008). Obesity was present in 98% of our patients and was significantly associated with reduced levels of bioavailable testosterone. This may explain the inverse correlation, reported here for the first time, between Ang2 levels and testosterone measurements in patients with MetS.

We previously demonstrated that HTA is the most important component of MetS impacting penile peak systolic velocities Tomada *et al.* (2011). HTA in the microcirculation is associated with vessel rarefaction. However, it is not clear whether this is caused by destruction of the microvessels because of the increased blood pressure, or caused by impaired angiogenesis, as abnormalities in indices of angiogenesis, such increased plasma Ang1 are present in hypertensive patients Nadar *et al.* (2005). Although a correlation of Ang1 with HTA could not be found in our sample, we observed an inverse correlation between Ang1 levels and PSV, and that in patients with arteriogenic ED there were a significant increase in Ang1 levels. Angiopoietins are regulated by a number of factors, including hypoxia and inflammation. It is likely that in MetS, these angiogenic factors are increased as a compensatory mechanism. In particular may be a role for Ang1 as it is considered an endothelial survival factor and vasculoprotective agent. High levels of Ang1 were protective against graft coronary arteriopathy in a preclinical model and, in addition, Ang1 also increases mobilization of

Figure 3 Angiopoietin signalling in quiescent endothelial cells covered by pericytes that constitutively express and secrete Ang1. Ang1 induces Tie2 activation at endothelial cell–cell junctions, thus promoting EC survival and vessel stabilization. Ang2, which is released from Weibel-Palade bodies upon inflammatory stimuli, binds to Tie-2 and behaves as a competitive antagonist by blocking further binding of Ang1.



endothelial progenitor cells Aicher *et al.* (2005), Nykänen *et al.* (2003). Consistent with this reparative potential of Ang1, our results demonstrated that increased Ang1 may be responsible for a compensatory attempt for vessel stabilization before cardiovascular events take place.

CONCLUSIONS

Our observations that Ang1 and Ang2 are deregulated in patients with ED and MetS support the hypothesis that they may be involved in the pathogenesis of this syndrome. And more importantly, our results reinforce the role of ED as a more reliable predictor of cardiovascular disease considering that in patients with MetS, ED represents a marker of subclinical atherosclerosis demonstrated by Angiopoietins correlation with local endothelial dysfunction and not with systemic markers. However, the cross-sectional design of this study prevents any definitive causal inferences. In addition, we realize that it is a small sample and thus additional research is warranted to determine whether the observed associations are clinically meaningful.

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