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Letter to the editor

Evidence for oxidative stress in plasma of patients with Marfan syndrome ☆

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Marfan syndrome (MFS) is an inherited connective tissue disorder with multisystemic clinical manifestations subdivided in major and minor criteria. The presence of two major criteria in two organs/systems and one minor criterion in another allows the diagnosis of MFS [1]. Recently, our group described the accumulation of oxidation products in fibroblasts from subjects affected by MFS [2]. Although an excess of reactive oxygen species (ROS) has been invoked as a pathogenetic mechanism in aortic aneurysm and in other manifestations occurring in MFS [3–5] no study, till now, has specifically investigated the occurrence of oxidative stress in this pathological condition. Thus, we planned to measure protein carbonyl content (protein CO), accounting for ROS attack on proteins, and total antioxidant capacity (TAC), an index of total ROS scavengers, in the plasma of patients affected by MFS.

We investigated 32 patients (F = 13, M = 19; median age = 34.8 y, range 13–76 y), diagnosed according to Ghent criteria, who were referred to the Centre for Marfan Syndrome of the Azienda Ospedaliero-Universitaria, Careggi, Florence. Selection criteria were age >13 years, absence of previous aortic intervention, hypertension, diabetes mellitus, dyslipidemia, renal insufficiency and other major disease states. Thirty healthy unrelated volunteers (F = 13, M = 17; median age 36 y, range 15–71 were recruited. None of these subjects was a smoker or was receiving vitamin supplementation or other drugs. The selection criteria of controls were the absence of a family history of MFS, aortic aneurysm, sudden death and those previously indicated for Marfan patients. Clinical score was built up by assigning 2 points to a major criterion and 1 point to a minor criterion. The authors state that the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the institution's human research committee.

Blood withdrawal was performed after overnight fasting. Plasma was separated within 1 h and stored at −80 °C until determinations. TAC was assessed by a chemiluminescence assay [6] using a L-ascorbic acid standard curve. The levels of protein CO were assessed using 2-4 dinitrophenylhydrazine [7], those of Hcy (free and protein bound) with a fluorescence polarization immunoassay (IMX Abbott Laboratories, Oslo, Norway). Protein concentration was measured by the Bradford assay [8] .

All experiments were performed in triplicate. Owing to the symmetry of the distribution, data were summarized as means ± SD, or (for age and Hcy concentrations) as medians (ranges).The statistical significance of the differences was assessed by the ANOVA-Bonferroni test, for age and Hcy by the Kruskal–Wallis test. The relationships between the clinical score and the plasma levels of protein CO and TAC were analyzed using the Pearson test; for Hcy the same relationships were analyzed by the Spearman test. A $P<0.05$ was accepted as statistically significant.

In our Marfan patients cardiovascular system was involved in 88% of cases as a major criterion (28/32) and in 9% as a minor criterion (3/32). Skeletal and ocular apparatuses were involved as major criteria in 41% (13/32) and 35% (11/32) of cases, respectively. Positive family history was present in 75% (24/32) of cases; when considering the minor criteria, striae distensae (skin) were often present (72%, 23/32), while pneumothorax was associated to 12% (4/32) of patients, namely dural ectasia. As only part of the patients agreed to an MRI scan for detecting dural ectasia, the involvement of CNS was not utilized for scoring. The distribution of patients on the basis of their clinical score, is shown in Fig. 1. No significant difference was observed among the median age of each subgroup of patients.

The results about oxidative stress markers are reported in Fig. 2. Protein CO was significantly higher in Marfan patients with respect to controls (0.68 ± 0.20 *vs* 0.14 ± 0.043 nmol/mg protein), while TAC was significantly lower (718.9 ± 281.5 *vs* 1505 ± 400 ascorbate equivalent units/mg protein). Protein CO values of patients presenting major involvement of cardiovascular and skeletal apparatuses (0.82 ± 0.20 nmol/mg protein) or major involvement of cardiovascular and ocular apparatuses (0.78 ± 0.21 nmol/mg protein) were significantly higher than those observed in the remaining patients (0.61 ± 0.16 and 0.63 ± 0.18 nmol/mg protein; $P=0.02$ and $P=0.03$, respectively). In any case, the increase in clinical score matched a parallel increase in the levels of plasma protein CO, and a significant correlation ($r=0.85$; $P<0.0001$, Fig. 3) was found between these parameters. On the other hand, neither significant differences nor correlation were found for TAC values in the same subgroups of subjects ($r=0.33$; $P>0.05$).

Plasma Hcy in Marfan patients was moderately, but significantly ($P=0.03$), higher (11.6 μmol/L; range 6.2–68.8) than in controls (10.6 μmol/L; range 6.9–15.2). Hcy levels, however, were correlated neither with protein CO ($r=0.14$; $P=0.43$) nor with TAC values ($r=-0.04$; $P=0.82$).

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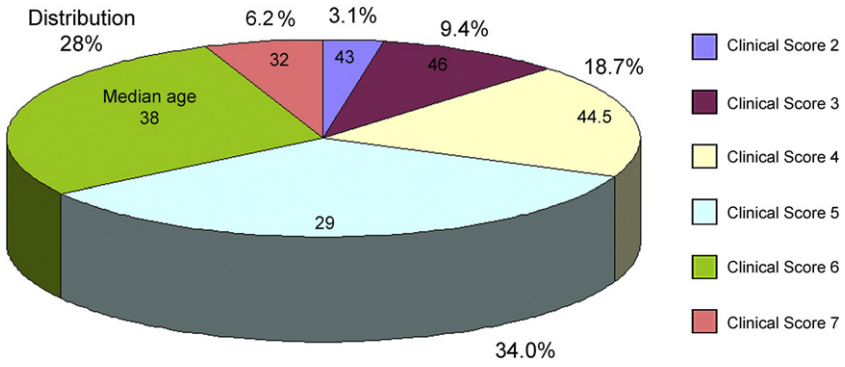

Fig. 1. Pie chart representing the Marfan patients enrolled in this study distinguished on the basis of the assigned Clinical Score: numbers inside and outside the sector arcs indicate respectively, the median age and the numerousness (as%) of each group.

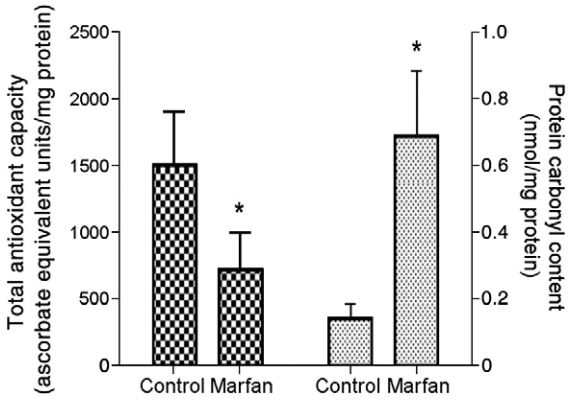

Fig. 2. Oxidative stress markers in plasma of Marfan patients ($n=32$) and control subjects ($n=30$). TAC is expressed as ascorbate equivalent units/mg protein, protein CO as nmol/mg protein. For each sample three independent determinations were performed in triplicate. Values are reported as means ± SD. *Significant difference ($P<0.05$) *vs* control subjects by the ANOVA-Bonferroni test.

Our results represent the first report about plasmatic signs of oxidative stress in MFS. Another interesting finding emerging from our study is the well-defined and statistically significant positive correlation between plasma protein CO levels and clinical involvement, thus suggesting that in MFS the intensity of oxidative stress may be a marker of the clinical severity and of the number of organs/systems affected. TAC levels did not exhibit similar relationships with the clinical manifestations but, in this regard, it has to be considered that the antioxidant status may be altered by several factors, such as lifestyle and daily diet [9]. The fact that we lack comprehensive nutritional information in our patients is a limitation of this study.

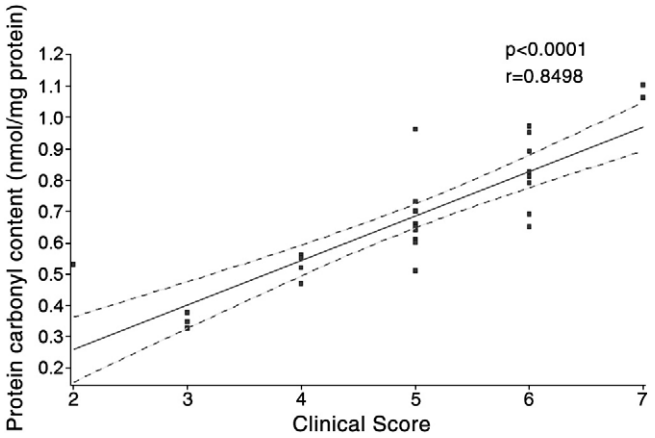

Fig. 3. Relationship between the assigned clinical score and the plasma protein CO in the same patients. Analysis by the Pearson test indicated a good and significant positive correlation between these occurrences. The dashed line indicates the 95% confidence interval.

As for the mechanisms responsible for the onset of oxidative stress, it has been recently demonstrated, in a MFS mouse model [10], a reduced expression of SOD and an increased expression of iNOS, NAD(P)H oxidase, and xanthine oxidase that represent the main sources of ROS in the aortic tissue. Also increased plasma Hcy levels, found in Marfan patients with major cardiovascular involvement, could enhance ROS production via NADPH oxidase [11]; in our patients, however, plasma Hcy was moderately increased, and no significant correlation was found between its levels and those of protein CO or TAC. A meaningful aspect of the present study is that the involvement as major criteria of skeletal and ocular (in addition to cardiovascular) apparatuses was associated with more marked signs of oxidative stress. This agrees with previous studies indicating enhanced ROS production in osteopenia and cataractogenesis [12,13]. Among the factors responsible for the vascular alterations that characterize MFS (hemodynamic forces, transmural inflammation, apoptosis and destructive remodelling), activation of metalloproteinases is thought to play an important role in the degradation of the extracellular matrix of arterial walls [14]. Most importantly, ROS have been recently reported to enhance the activation of TGFbeta 1 [15] whose circulating levels are increased in Marfan patients [16].

In conclusion the signs of oxidative stress detected in the plasma of our patients represent a novel finding that strengthens our previous report about the presence of oxidation products in the fibroblasts of these patients. Moreover, the significant correlation between the intensity of oxidative stress and the severity of the clinical manifestations suggest a systemic oxidative damage, notably that the molecular pathology of this disorder does not only affect the connective tissue.

Further studies aimed to confirm the presence of oxidative stress in MFS, to elucidate the mechanisms responsible for its onset and to assess the pathways of oxidative stress-mediated injury would be of value, even in a prospect of possible therapeutical interventions.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

References

[1] De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996;62:417–26.
[2] Monici M, Basile V, Romano G, et al. Fibroblast autofluorescence in connective tissue disorders: a future tool for clinical and differential diagnosis? J Biomed Opt 2008;13:054025.

[3] Ejiri J, Inoue N, Tsukube T, et al. Oxidative stress in the pathogenesis of thoracic aortic aneurysm: protective role of statin and angiotensin II type 1 receptor blocker. Cardiovasc Res 2003;59:988–96.
[4] Phillippi JA, Klyachko EA, Kenny 4th JP, Eskay MA, Gorman RC, Gleason TG. Basal and oxidative stress-induced expression of metallothionein is decreased in ascending aortic aneurysms of bicuspid aortic valve patients. Circulation 2009;119:2498–506.
[5] Tabakoglu E, Ciftci S, Hatipoglu ON, Altiay G, Caglar T. Levels of superoxide dismutase and malondialdehyde in primary spontaneous pneumothorax. Mediat Inflamm 2004;13:209–10.
[6] Fiorillo C, Becatti M, Pensalfini A, et al. Curcumin protects cardiac cells against ischemia–reperfusion injury: effects on oxidative stress, NF-kappaB, and JNK pathways. Free Radic Biol Med 2008;45:839–46.
[7] Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Meth Enzymol 1994;233:346–57.
[8] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:48–54.
[9] Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. Nutr Rev 2006;64:S27–47.
[10] Yang HH, van Breemen C, Chung AW. Vasomotor dysfunction in the thoracic aorta of Marfan syndrome is associated with accumulation of oxidative stress. Vasc Pharmacol Oct. 29 2009 Epub ahead of print.
[11] Ventura E, Durant R, Jaussent A, et al. Homocysteine and inflammation as main determinants of oxidative stress in the elderly. Free Radic Biol Med 2009;46:737–44.
[12] Moura B, Tubach F, Sulpice M, et al. Multidisciplinary Marfan Syndrome Clinic Group. Bone mineral density in Marfan syndrome. A large case-control study. Joint Bone Spine 2006;73:733–5.
[13] Bhatia RP, Rai R, Rao GR. Role of malondialdehyde and superoxide dismutase in cataractogenesis. Ann Ophtalmol (Skokie) 2006;38:103–6.
[14] Ikonomidis JS, Jones JA, Barbour JR, et al. Expression of matrix metalloproteinases and endogenous inhibitors within ascending aortic aneurysms of patients with Marfan syndrome. Circulation 2006;114:I365–I3670.
[15] Qi S, den Hartog GJ, Bast A. Superoxide radicals increase transforming growth factor-beta1 and collagen release from human lung fibroblasts via cellular influx through chloride channels. Toxicol Appl Pharmacol 2009;237:111–8.
[16] Matt P, Schoenhoff F, Habashi J, et al, The GenTAC Consortium. Circulating transforming growth factor-{beta} in Marfan Syndrome. Circulation 2009;120: 526–32.
[17] Coats AJ. Ethical authorship and publishing. Int J Cardiol 2009;131:149–50.

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