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Elevated serum levels of macrophage migration inhibitory factor and stem cell growth factor β in patients with idiopathic and systemic sclerosis associated pulmonary arterial hypertension

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SUMMARY

Pulmonary arterial hypertension (PAH) can be idiopathic or secondary to autoimmune diseases, and it represents one of the most threatening complications of systemic sclerosis (SSc). Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with proinflammatory functions that appears to be involved in the pathogenesis of hypoxia-induced PH. In SSc patients, high serum levels of MIF have been associated with the development of ulcers and PAH. Stem cell growth factor β (SCGF β) is a human growth factor that, together with MIF, is involved in the pathogenesis of chronic spinal cord injury. The aim of our study was to measure serum levels of MIF in patients with idiopathic and SSc-associated PAH.

We enrolled 13 patients with idiopathic PAH and 15 with SSc-associated PAH. We also selected 14 SSc patients without PAH and 12 normal healthy controls, matched for sex and age. PAH was confirmed by right hearth catheterism (mPAP>25 mmHg). MIF and SCGF β levels were measured by ELISA.

We found significantly higher circulating levels of MIF and of SCGF β in patients with idiopathic PAH (P=0.03 and P=0.004) and with PAH secondary to SSc (P=0.018 and P=0.023) compared to SSc patients without PAH. Higher levels of MIF were found in those patients with an higher New York Heart Association (NYHA) class (P=0.03).

We can hypothesize that MIF and SCGF β are able to play a role in PAH, both idiopathic or secondary, and in the future they may be evaluated as useful biomarkers and prognostic factors for this serious vascular disease.

Key words: *Pulmonary arterial hypertension, Systemic sclerosis, Macrophage migration inhibitory factor, Stem cell growth factor β, Biomarkers.*

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is an important vascular disease, potentially lethal. It's defined as the elevation of median pulmonary arterial pressure (mPAP) above 25 mmHg with a pulmonary capillary wedge pressure <15 mmHg at the right hearth catheterization (1). According to the recent classification of Dana Point 2008, there are many forms of PAH. In particular we distinguish idiopathic PAH (iPAH) and PAH associated with connective tissue diseases (2) whereas this vascular disease is a serious complication of systemic sclerosis (SSc) (3). The pathogenesis of PAH is still unknown, but there are many evidences suggesting a role for inflammation in the vascular remodelling of the precapillary pulmonary arteries. In particular cytokines and chemokines are involved in the endothelial dysfunction, in the hypertrophy and hyperplasia of pulmonary artery smooth muscle cells (PASMCs) and in the neointima formation (4). Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with chemokine-like functions. It has an isomerase enzymatic activity that contributes to chronic inflammation (5). It also acts as endocrine mol-

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ecule and as chaperon-like protein. MIF is expressed in a wide variety of cells in particular in immune system cells, epithelial and endothelial cells, SMC and endocrine system cells (6). MIF has many functions: it works against the immunosuppressive action of glucocorticoids, activates CXCR2 and CXCR4 dependent chemotactic responses, increases the production of pro-inflammatory cytokines such as tumour necrosis factor-α and inhibits the activation-induced apoptosis (7). It is also involved in the pathogenesis of atherosclerosis (8) and of many autoimmune diseases (6) and it plays a key, but controversial, role in wound repair (9). In SSc, it seems to contribute to sclerodermic vasculopathy. In fact MIF serum and tissue levels were significantly increased in SSc patients in comparison to healthy controls. In particular patients with PAH and recurrent digital ulcers showed higher MIF levels than patients without these manifestations (10, 11).

Besides, a particular genetic polymorphism, MIF-173 promoter polymorphism, is associated with the diffuse form of SSc (12, 13). Recent evidences show a role of MIF in the pathogenesis of hypoxia-induced PH with higher levels in patients with idiopathic and with interstitial lung disease associated PH respect to healthy controls (14). MIF may induce the proliferation of PASMCs and may enhance the vasoconstriction of pulmonary arteries in murine models and in human *in vitro* models (15, 16). Because of its role of mediator of pathogenic mechanisms in many systemic and organ-specific autoimmune diseases, neutralizing the action of endogenous MIF with monoclonal antibodies or small chemical inhibitors of its tautomerase activity, such as ISO-1, may be effective in the control of several models of autoimmune diseases (6, 7).

Stem cell growth factor (SCGF) is a human growth factor for hematopoietic progenitor cells, which belongs to the C-type lectin superfamily (17). There are two isoforms (α and β) and SCGF β exhibits a burst-promoting activity and granulocyte/macrophage colony stimulating activity on erythroid and granulocyte/macrophage progenitor cells (18). It seems to have a prognostic role in Chagas' disease and idiopathic dilated cardiomyopathy (19). Its serum levels seem to increase following stem cell transplantation (20), and together with MIF, it is involved in the pathogenesis of chronic spinal cord injury (21). No data are actually available concerning its role in SSc and in PAH.

MATERIALS AND METHODS

Objectives

We dosed MIF and SCGF β serum levels in patients affected by PAH, both idiopathic and secondary to SSc, to determine their potential role in the development of this important vascular disease and any potential difference between the two forms of PAH.

Methods

We enrolled 13 consecutive patients affected by iPAH who had been referred to the Pulmonary Hypertension Center of our Hospital and 15 consecutive patients referred to our Rheumatology Unit affected by the SSc-associated form of PAH. As controls we enrolled 14 patients affected by SSc without PAH and 12 normal healthy controls (NHC) sex and age matched. SSc patients were classified according the American College of Rheumatology (ACR) classification criteria of 1980 (22) and the classification was confirmed with the new ACR/European League Against Rheumatism (EULAR) Criteria of 2013 (23). The diagnosis of PAH was confirmed by right hearth catheterization and it was defined as a precapillary pulmonary hypertension (mPAP >25 mmHg, pulmonary wedge pressure <15 mmHg) (1). All patients underwent to clinical and laboratory evaluation, including the assessment of the New York Heart Association (NYHA) functional class. SSc patients organ involvement was defined as previously described (24): lung = bibasilar pulmonary fibrosis on chest radiography; isolated PH = clinical evidence of PH and increased systolic pulmonary ar-

terial pressure (>35 mmHg), indirectly assessed by echocardiography, in the absence of severe pulmonary interstitial fibrosis; oesophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arrhythmias requiring treatment. High-resolution computed tomography was performed in each case showing negative x-ray and the diagnosis of pulmonary fibrosis was done by a radiologist, blinded reading, basing on the presence either of bilateral basilar reticulonodular changes on x-ray or of *ground glass* appearance of the lung parenchyma on HRTC. The cutaneous evaluation included: presence/absence of Raynaud's phenomenon, modified Rodnan skin score (25), presence/absence of digital ulcers, defined as a loss of epithelialization and tissues involving the epidermis, the dermis and the subcutaneous tissue, teleangiectasia, calcinosis, defined as deposits of calcium in soft tissues eye visible or confirmed by x-ray (26). All patients underwent a nailfold videocapillaroscopy as previously described (27). Antinuclear antibodies including anti-centromere antibodies were detected by indirect immunofluorescence using HEp-2 cell line as substrate (Bio-Rad Lab., Hercules, CA, USA). Antibodies against topoisomerase I (anti-Scl70) were measured using enzyme-linked immunosorbent assays (ELISA) (Diamedix, Miami, FL, USA). Serum sample was obtained from all patients and sera were stored at -20°C. Levels of MIF and SCGF β were detected by enzyme-linked immunoassay (Bio-Rad Lab.). Assays were performed following the manufacturer's instructions.

Statistical analysis

The Mann-Whitney U test was used for comparison of groups. For correlation analysis, Spearman's rank correlation test was employed. P values≤0.05 were considered significant.

RESULTS

All SSc patients were female, 15 with PAH (mean age 67 yrs, range 50-75; mean disease duration 230 months; range 4-696) and 14 without PAH (mean age 62.5 yrs, range 47-79; mean disease duration 154.6 months, range 84-502). Among those patients with PAH, 10 had the limited form of SSc and 5 had the diffuse form, the mean mRSS was 13 (range 4-30), 2 had digital ulcers, 5 were anti-centromere positive and 1 anti-Scl70 positive, while in the group without PAH, 12 had the limited form and 2 had the diffuse form, the mean mRSS was 11 (range 4-24), 4 had digital ulcers, 10 were anti-centromere positive and 4 anti-Scl70 positive (Tab. I). The 13 patients with iPAH were 9 female and 4 male (mean age =55.7 yrs; mean disease duration = 5.48 yrs) and the 12 NHC were sex and age matched.

We found significantly higher circulating levels of MIF and of SCGF β in patients with idiopathic PAH (MIF median 270 pg/mL *vs* 175 pg/mL, P=0.03; SCGF β median 18,845 pg/mL *vs* 12,054 pg/

Table I - Main clinical-demographic and laboratory features of systemic sclerosis (SSc) patients, with and without pulmonary arterial hypertension (PAH).

	SSc patients without PAH (n=14)	Patients with SSc-associated PAH (n=15)	P
Sex (F)	14	15	n.s.
Mean age (range)	62.5 yrs (47-79)	67 yrs (50-75)	n.s.
Mean disease duration (range)	154.6 months (84-502)	230 months (4-696)	n.s.
Form (L/D)	12/2	10/5	n.s.
Mean mRSS (range)	11 (4-24)	13 (4-30)	n.s.
Digital ulcers (n. pt.)	4 (28.5%)	2 (13%)	n.s.
Anti-centromere+ve	10 (71%)	5 (33%)	n.s.
Anti-topoisomerase1+ve	4 (29%)	1 (7%)	n.s.

mL, P=0.004) and SSc-associated PAH (MIF median 333 pg/mL *vs* 175 pg/mL, P=0.018; SCGF β median 17,804 pg/mL *vs* 12,054 pg/mL, P=0.023) compared to SSc patients without PAH (Tab. II and III; Fig. 1 and 2). We found higher mean levels of MIF and SCGF β in SSc patients, in particular in those with PAH, and in patients with iPAH compared to normal control but the differences were not statistically significant. We didn't find any significant difference between the two forms of PAH and regarding other vascular manifestations such as digital ulcers and nailfold capillaroscopy features. Besides we found a significant increase of MIF levels in patients with an higher NYHA functional classes (class NYHA 3-4 *vs* class NYHA 1-2) (median 380.69 pg/mL *vs* 244.58 pg/mL, P=0.03) (Fig. 3). We didn't find any significant correlation between MIF and SCGF β and other clinical or laboratory parameters.

Table II - Macrophage migration inhibitory factor (MIF) and stem cell growth factor (SCGF) b serum levels (pg/mL) in patients with idiopathic pulmonary arterial hypertension (iPAH) and in systemic sclerosis (SSc) patients without PAH (SSc controls) (median±standard deviation).

	iPAH	SSc controls	P
MIF	270±636.2	175±68.68	0.03
SCGF β	18,845±5819.7	12,054±3448.5	0.004

Table III - Macrophage migration inhibitory factor (MIF) and stem cell growth factor (SCGF) β serum levels (pg/mL) in patients with systemic sclerosis (SSc)-associated pulmonary arterial hypertension (PAH) and in SSc patients without PAH (SSc controls) (median±standard deviation).

	SSc-associated PAH	SSc controls	P
MIF	333±520	175±68.68	0.018
SCGF β	17,804±8033.2	12,054±3448.5	0.023

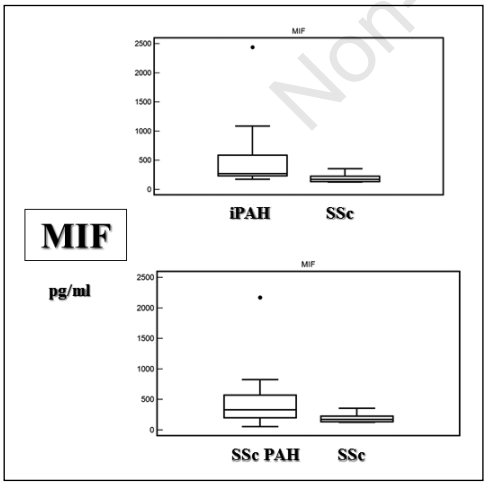

Figure 1 - Macrophage migration inhibitory factor (MIF) serum levels (pg/mL) in patients with idiopathic pulmonary arterial hypertension (iPAH) and systemic sclerosis (SSc)-associated PAH (P=0.03 and P=0.018).

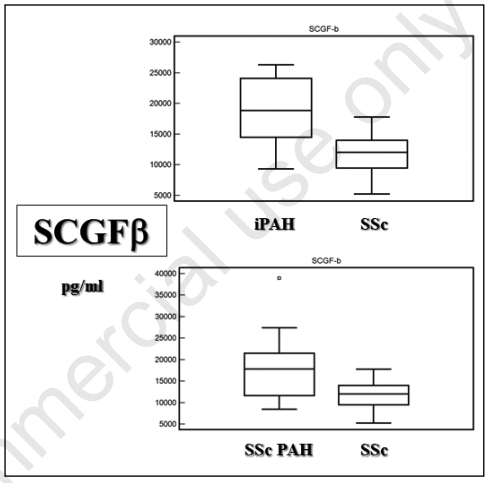

Figure 2 - Stem cell growth factor (SCGF) β serum levels (pg/mL) in patients with idiopathic pulmonary arterial hypertension (iPAH) and systemic sclerosis (SSc)-associated PAH (P=0.04 and P=0.023).

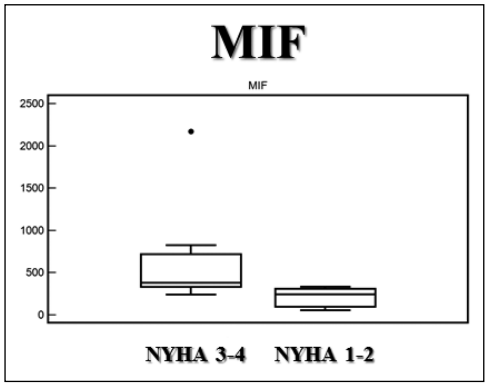

Figure 3 - Macrophage migration inhibitory factor (MIF) serum levels (pg/mL) in patients with the New York Heart Association (NYHA) functional classes 3-4 and NYHA functional classes 1-2 (P=0.03).

CONCLUSIONS

PAH is an important vascular disease, potentially lethal and it can be associated with connective tissue diseases (2). MIF is a pleiotropic cytokine and in SSc patients, serum levels of MIF are increased compared to healthy controls and have been associated with the development of ulcers and PAH (11). Moreover, MIF seems to be involved in the pathogenesis of hypoxia-induced PH, showing higher levels in those patients with idiopathic PH and with interstitial lung disease-associated PH respect to healthy controls (14). SCGF β seems to have a prognostic role in Chagas' disease and idiopathic dilated cardiomyopathy (19) and together with MIF, is involved in the pathogenesis of chronic spinal cord injury (21). In our study, we found significantly higher levels of MIF and SCGF β in patients with iPAH and SSc-associated PAH, without any significant difference between the two forms of PAH. Regarding MIF, unlike other studies (11), we didn't found any significant association with the other clinical manifestation of SSc vasculopathy, such as digital ulcers. In a previous study, we found a significant association between a more severe capillaroscopic score and the presence of PAH (28). Instead, in this study, we didn't find any significant association between the serum levels of MIF and SCGF β and the nailfold capillaroscopic features. Like previous study (10, 11), we found higher mean levels of MIF and SCGF β in SSc patients, in particular in those with PAH, and in patients with iPAH, compared to normal control but the differences were not statistically significant, probably because of the small sample size. Thus, the higher serum levels of MIF seem, in our cohort of patients, to be specific of PAH and these data are consistent with the recent evidences for a role of MIF as a molecular mediator in the pathogenesis of PH in murine and human *in vitro* models (15, 16). No data are actually available regarding the SCGF β involvement in PAH and in SSc pathogenesis. SCGF β has been studied as a prognostic factor in other kinds of cardiovascular diseases (19).

Thus our study presents for the first time an association among this molecule, PAH and SSc. Because of the potential lethality of PAH, there is a need of sensible diagnostic tools to early assess the development of this important vascular disease. The next step of our research will be to verify the real sensibility of these molecules.

We also found that significantly higher serum levels of MIF, but not of SCGF β are present in patients with higher NYHA functional classes (NYHA 3 and 4) probably reflecting a more severe vascular damage and, consequently, a more severe type of PAH. Thus MIF seems to behave as a prognostic marker, to be used for monitoring patients with PAH. Our study confirms the association of MIF, a proinflammatory cytokine, with PAH and these results are consistent with the evidence that suggests a role for inflammation in the vascular remodelling of the precapillary pulmonary arteries, that are among the main features of the pathogenetic mechanism of PAH (4). To establish the possible role of MIF in the pathogenesis of PAH, both idiopathic and secondary, may have important consequences in the current therapeutic strategy for PAH (1). In fact monoclonal antibodies directed against MIF or small chemical inhibitors of its tautomerasic activity, such as ISO-1, are already experienced in many models of autoimmune diseases (6, 7).

In conclusions, with our study we can hypothesize a role for MIF and SCGF β as biomarkers of PAH and, for MIF in particular, as a prognostic marker.

Further studies are needed to identify a clear pathogenetic role for these molecules in the development of PAH, as well as their potential use as therapeutic targets.

Competing interest: none declared

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