

**A69** **TIMP-1 PRODUCTION BY MONOCYTES MAY CONTRIBUTE TO SSC SKIN FIBROSIS**

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**Objective** Systemic sclerosis is a connective tissue disease characterised by vasculopathy, tissue infiltration of mononuclear cells and fibrosis of skin and inner organs. Mechanisms of fibrogenesis are unknown so far. Under normal circumstances matrix metalloproteinases and their inhibitors (TIMPs) regulate accumulation of extra cellular matrix components in skin. This regulation seems to be disturbed in SSc. Our aims are to investigate the effect of SSc patient serum on healthy circulating monocytes and to identify serum factors that contribute to the TIMP/MMP imbalance. Osteopontin has been identified to be more abundant in patients with SSc and lung fibrosis. As osteopontin has been described to induce TIMP-1 in peripheral blood mononuclear cells (PBMCs) it is of potential interest in TIMP regulation. The results might give us some insight into the role of monocytes in SSc skin fibrosis. As novel gene which may be of interest in SSc we have identified TWIST-1 as potential candidate. TWIST-1 has been described as transcription factor and negative regulator of TIMP-1.

**Methods** We collected PBMCs and serum from SSc patients (limited and diffuse). CD14 monocytes were isolated by

positive selection and mRNA levels of TIMP-1 were determined with q-RT-PCR. Monocytes from healthy donors were cultured with serum from SSc patients and TIMP-1 and TWIST-1 were subsequently investigated by q-RT-PCR and ELISA, respectively, or q-RT-PCR only (TWIST-1). Furthermore we investigated osteopontin (OPN) as a candidate cytokine for inducing TIMP-1 expression.

**Results** mRNA levels of TIMP-1 in SSc CD14 monocytes were significantly upregulated compared to healthy controls. Serum from SSc patients, but not from RA patients or healthy controls, induced TIMP-1 production of healthy monocytes. Osteopontin was more abundant in SSc serum than in healthy controls and we have identified OPN as potential activator for TIMP-1 production. Furthermore we found TWIST-1 expression levels in healthy monocytes treated with SSc serum less expressed than in controls. Interestingly OPN seems to repress TWIST-1 expression which could explain the increasing TIMP-1 expression.

**Conclusion** Osteopontin seems to be an important candidate and must be investigated further. As TWIST-1 is a negative regulator of TIMP-1 it is possible that the more abundant OPN levels in SSc serum repress TWIST-1 and thereby promote TIMP-1 expression. This would point OPN out as potential drug target to treat SSc patients.