

Editorial

Vascular Damage in Systemic Sclerosis

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INTRODUCTION

Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by microangiopathy, fibrosis of the skin and internal organs, and disturbances in the humoral and cellular immune responses. The pathogenesis of SSc is complex and is based on microvascular damage, activation of the immune system, and progressive fibrosis.

Vascular dysfunction can be found very early in SSc and is thought to be the primary event. This idea is supported by the fact that Raynaud’s phenomenon (RP) is one of the most characteristic SSc symptoms, which is observed in more than 95% of the patients. RP is characterized by blanching, turning blue and red, aching of the acral parts of the body during exposure to cold or provoked by emotional stress. Moreover, RP may precede other features of the disease by years. RP affects hands, feet, and less frequently, the tip of the nose, the earlobes, and the tongue. RP in SSc is severe, and often presents with digital ulcers. Small areas of ischemic necrosis or ulcerations of the fingertips are a frequent finding, often leaving pitting scars. In a part of the SSc patients, digital necrosis of the terminal portions may also develop [1].

THE ROLE OF ENDOTHELIUM IN MICROCIRCULATION

The severe RP in SSc is due to the profound endothelial damage of the microcirculation. The microcirculation includes arterioles, capillaries and venules. The capillaries are covered by endothelial cells whose amount in the human body is estimated to be over 720 grams and 600 grams from this quantity are the capillary endothelial cells [2]. The overall surface of endothelium in humans is over 3000-6000 m² [3]. The large endothelial surface of the capillaries facilitates exchange of nutrients and hormones. In relation to their role as a *gatekeeper between blood and tissues*, endothelial cells possess several other specific functions

The endothelium *controls tone of blood vessels* via production of vasodilators, vasoconstrictors, cytokines and growth factors. The main physiological vasodilator is nitric oxide (NO) produced by endothelial cells. A diffusible factor, which mediates relaxation, was discovered in 1980 by Robert Furchgott and John Zawaski, and has been termed endothelial derived relaxing factor. In 1987, Moncada and colleagues have found that this factor is NO. Dr. Furchgott with two other colleagues received a Nobel Prize in 1998 for their contribution in the field of NO research. Another potent vasodilator, which acts predominantly when NO synthase activity is inhibited or impaired, is prostacycline – PGI₂, which is a product of the enzyme cyclooxygenase-2. PGI₂ is a vasodilator and an inhibitor of platelet activation and aggregation. Various other vasodilators derived from endothelium have been identified, e. g. hydrogen peroxide (H₂O₂), carbon monoxide (CO), hydrogen sulfide (H₂S), sulfur dioxide (SO₂), as well as cytochrome P450 products such as epoxyeicosatrienoic acids, C-type natriuretic peptide. H₂O₂ may act also as a vasoconstrictor. Other endothelium-derived contracting factors are endothelin (ET), angiotensin II and thromboxane A₂ [3]. Three isoforms of ET are known - ET-1, ET-2, ET-3. The main isoform in humans is ET-1. The cells do not store ET. Its synthesis is stimulated by different chemical and physical factors, e.g. hypoxia, low temperatures, angiotensin II, growth factors, and is inhibited by NO, prostacyclin and increased blood flow. It acts as a local hormone, which binds to smooth muscle cells short after its release from the endothelial cells. The receptors for ET-1 are two types: ET-A and ET-B, whose production is regulated in a similar fashion like that of ET. ET-A receptors dominate on the surface of the smooth muscle cells and cause vasoconstriction via increased intracellular concentration of calcium [4]. In healthy state, balance between endothelium-derived vasodilators and vasoconstrictors exists [3].

The healthy endothelium *prevents thrombus formation* via several mechanisms. The endothelium is an important site of PGI₂ production, which is a potent inhibitor of platelet aggregation. The natural glycoprotein thrombomodulin, which is located in the membrane of endothelial cells, inhibits the procoagulant function of thrombin. *Von Willebrand factor (vWF)* is a mediator that is mainly secreted by endothelial cells in the circulation, and a part of it is stored in a specific organelles of these cells – the bodies of Weibel – Palade. It binds the platelets with the subendothelial surface and thus mediates platelet adhesion and aggregation [5]

Endothelial cell-derived tissue-plasminogen-activator (t-PA) is released either from stored pools or is synthesized de novo, and is a key mediator of intravascular fibrinolysis converting plasminogen to plasmin. The physiological inhibitor of t-PA (t-PAI) is synthesized in endothelial cells as well as in liver and in adipose tissue [3].

In addition, endothelial cells are involved in the healing process after traumatic damage or inflammation being a vector of *angiogenesis* i.e. the creation of new blood vessels that is essential for proper formation of granulation tissue and tissue repair [2]. Although the endothelium includes a single cell type – endothelial cells, it is considered to function as an endocrine organ, which controls the processes of thrombosis, inflammation, metabolism, vascular remodeling and angiogenesis [2, 3].

The endothelial dysfunction, which is thought to be the first step in SSc, may be explained by the following processes (Fig. 1):

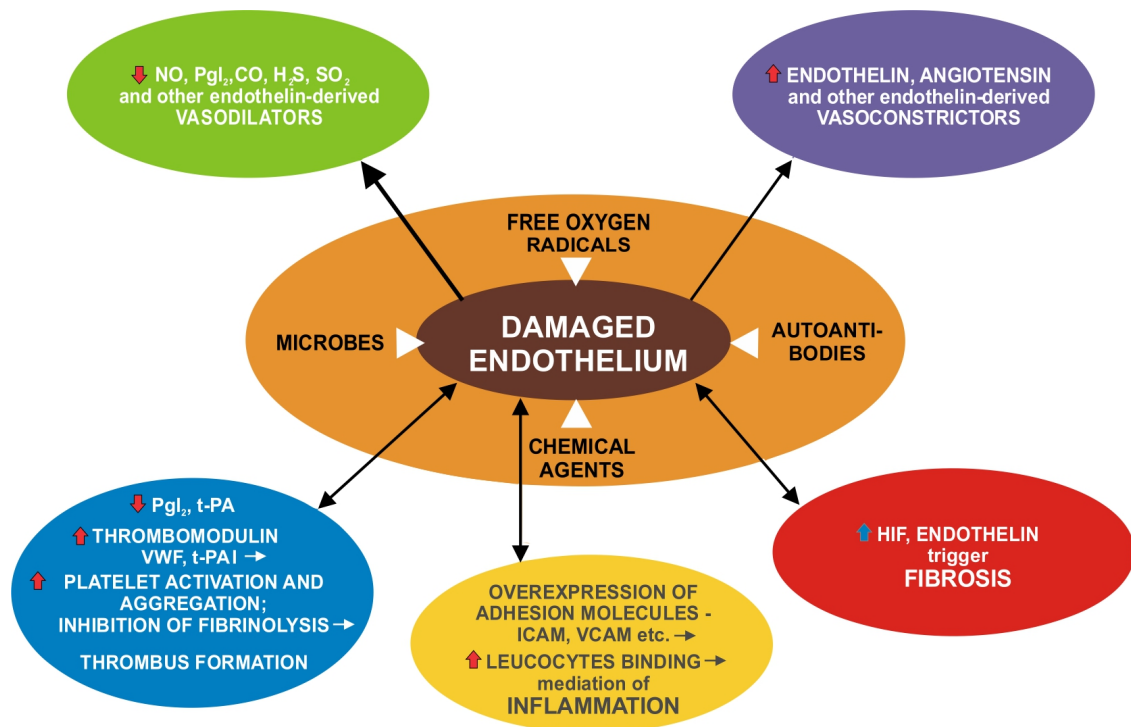


Fig. (1). Consequences of endothelial dysfunction in systemic sclerosis.

1st Step – Endothelial Cell Activation and Apoptosis

In secondary RP in SSc as a result of recurrent ischemia and reperfusion, there is production of *free oxygen radicals*, which mediates tissue injury and endothelial cell activation [6]. In addition, specific *anti-endothelial antibodies* are detected in 40% of SSc patients with diffuse skin involvement and in 13% of those with limited form of the disease. A correlation between their level and the severity of RP, as well as with the presence of trophic changes of the fingers and pulmonary arterial hypertension has been found [7].

Several infectious agents (e.g. herpes viruses, retroviruses, and human cytomegalovirus) that trigger molecular mimicry have also been supposed to be causative agents in SSc. This hypothesis has been supported by the presence of IgG anti-human cytomegalovirus antibodies and sequence homologies between retroviral proteins and the target of anti-Scl-70 antibody – a topoisomerase I antigen [8].

Environmental factors that have been proposed to be SSc causative agents include organic solvents, vinyl chloride, silica, metal dust, certain pesticides, hair dyes, and toxins [4].

In addition, the endothelium may be *activated by hypoxia, metabolic stress and inflammatory cytokines*, the last acting as *triggers of the innate and acquired immune response*. Via expression adhesion molecules on their surface, endothelial cells control the recruitment of leucocytes in the areas where these cells are needed.

Anti-endothelial cell antibodies have shown to induce adhesion molecule expression on the surface of the endothelial cells and to stimulate leucocyte attachment. They can also lead to endothelial cell damage and apoptosis [9]. Increased levels of free oxygen radicals and cytokines produced by activated lymphocytes contribute to this process [10, 7]. The endothelial injury in SSc results in decreased production of vasodilators such as NO and prostacyclin (which also inhibits platelet aggregation), and increased levels of vasoconstrictors such as ET. The exposition of the subendothelium to the bloodstream may induce platelet adhesion and intravascular thrombus formation. Pathomorphologic alterations of the blood vessels in SSc include perivascular inflammatory infiltrates, intimal proliferation, narrowing of the blood vessel lumen, a damaged capillary network with decreased capillary density number and capillary derangement, which cause a reduced blood flow [4, 11, 12, 13]. In conclusion, endothelial cells in SSc are supposed to be activated by the following possible triggers – free oxygen radicals, microbes, chemical agents, autoantibodies.

2nd Step – Inflammatory Infiltration

Opening of the endothelial contacts – tight junction and infiltration of lymphocytes in the vessel wall is the potential next step as a result of endothelial cell activation. Afterwards the passage of leucocytes through the vessel wall (diapedesis) leads to formation of perivascular inflammatory infiltrates from macrophages, T- and B-lymphocytes with CD4+ T lymphocytes being the predominant cell type. The process of interaction between endothelial cells and leucocytes is mediated by overexpression of adhesion molecules, which are structures from the surface of endothelial cells that bind leucocytes and mediate the inflammatory response. Adhesion molecules of endothelial cells are several type, e.g. E-selectin, P-selectin, ICAM (intercellular adhesion molecules), VCAM (vascular adhesion molecules), etc. [3]

In patients with SSc and secondary RP, an overexpression of adhesion molecules can be found. Increased levels of their soluble forms – sICAM, sVCAM, and sE-selectin are found in SSc and patients with other connective tissue diseases with secondary RP in comparison with primary RP individuals both in basal conditions and after exposure to low temperatures [14].

Consequences of Endothelial Damage – A Dysbalance Between Vasodilators and Vasoconstrictors

Decreased levels of NO, which is a key mediator of vasodilation in SSc, is a characteristic feature in pathogenesis of SSc. Increased level of the mediator *asymmetric dimethylarginine*, also synthesized by the endothelial cells, which is an endogenous inhibitor of the NO synthesis, has been found in patients with secondary RP in connective tissue diseases as compared with primary RP. As a result of the endothelial injury in SSc with secondary RP, a production of prostacycline is reduced, which leads to vasospasm and activation of platelets [15].

The ET level is found to be significantly increased in patients with SSc as compared to primary RP [15]. Apart of its property to cause vasospasm, ET also induces inflammation, leads to hypertrophy of vessel wall, thus reducing the vessel lumen, and stimulating fibrosis [3]. ET is supposed to be the key connecting mediator in SSc between the processes of inflammation and fibrosis.

In the context of endothelial damage in SSc, dysbalance between the levels of vasodilators and vasoconstrictors is observed, with a distinct prevalence of vasoconstrictors.

Consequences of Endothelial Damage – Thrombus Formation – 3rd Step

In patients with secondary RP in SSc, a significantly higher level of *thrombomodulin* could be found. It is a glycoprotein in the membrane of the endothelial cells. In cases of endothelial injury, its fragments are released in the circulation, so its increased level is a marker of endothelial dysfunction. The concentration of *vWF* is found to be increased in SSc with secondary RP and its level correlates with the severity and frequency of the vasospastic attacks. In SSc with secondary RP, increased activation and aggregation of platelets can be found. It is thought to be as a result of endothelial dysfunction and deficiency of platelet aggregation inhibitors, e. g. prostacyclin. An increased level of different substances derived from platelets could be found such as – thromboxane A₂, serotonin, platelet factor 4, thrombospondine, etc. [7, 11]. In addition, inhibition of *fibrinolysis* has been observed in secondary RP in SSc. Disturbed release of the *t-PA* and increased level of *t-PAI* has been detected in SSc patients [16]. The chronic inhibition of NO synthesis induces the expression of t-PAI and angiotensin converting enzyme in vascular tissues, and angiotensin II induces t-PAI in vascular endothelial cells [3].

4th Step – Intimal Hyperplasia

Endothelial dysfunction and induced inflammation lead to intimal hyperplasia of the small arterioles in SSc. All of these processes cause tissue hypoxia and are thought to be a trigger of *fibrosis (5th step)*, which itself disturbs perfusion and accelerates endothelial dysfunction.

An Association Between Vascular Damage, Fibrosis and Disturbed Vascular Recovery in SSc

The reduced capillary flow leads primarily to a decrease in oxygen and nutritional supply. Tissue hypoxia itself increases the production of hypoxia inducible factor (HIF). Increased level of HIF and ET (the latter being released from endothelial cells) trigger the fibrotic process. In addition, HIF increases the level of vascular endothelial growth factor (VEGF) [13, 17, 18]. VEGF is a potent proangiogenic factor, which takes part in different phases of the compensatory but inadequate new blood vessel formation that occurs through two different processes - angio- and vasculogenesis. Angiogenesis is a multistage process of new blood vessel formation from differentiated endothelial cells of prior vessels, while vasculogenesis is vascular formation from endothelial progenitor cells (EPCs) derived from the bone marrow independent from pre-existing vessels [13, 19]. Significantly higher levels of VEGF in SSc patients have been detected both in the early and in the late stages of the disease. Of note, its levels have been found to be higher in the late stages as compared with those in early SSc [13, 19,20]. VEGF increases the vascular permeability, stimulates the migration and proliferation of endothelial cells and induces tube formation [13, 19]. It also causes proliferation and organization of EPCs through interaction with their surface receptor - VEGFR-2. A prolonged overexpression of VEGF is believed to produce unfavourable effects such as vascular fusion and formation of a chaotic vessel network, appearance of giant capillaries, which is analogous to the observed phenomena in SSc [13, 21, 22]. Microangiopathic abnormalities in SSc include initially capillary dilation, appearance of giant capillaries and haemorrhages. Subsequently in the late stages extensive capillary loss and abortive reparative neoangiogenesis develop. An excessive accumulation of extracellular matrix components produced by the highly activated fibroblasts, that derive from specific progenitor bone marrow cells is another characteristic finding in SSc. Normally, the tissues hypoxia is a stimulus for new blood vessel formation. Despite the stimulatory effects of the hypoxia in SSc the vascular recovery is inadequate [13, 23]. In addition to the above mentioned phenomena, which disturb vascular recovery in SSc, imbalance between pro-angiogenic and angiostatic factors have been found in SSc with a predominance of angiogenic inhibitors. The latter are known to be products of cleavage of extracellular matrix components and of circulating proteins - angiostatin (a product of plasminogen cleavage), endostatin (of collagen type XV), tumstatin (of collagen type V), canstatin (of collagen type A2- V) [13, 16, 24]. Thus, fibrosis in SSc is not only stimulated by the endothelial injury through the subsequent tissue hypoxia and increased level of ET, but it itself deteriorates the vascular state via suppression of the vascular recovery.

CONCLUSION

Microangiopathy is the hallmark of SSc. Its clinical manifestations are telangiectasia, RP, digital pitting scars and ulcerations, extensive tissue necrosis in a part of the cases, pulmonary arterial hypertension, scleroderma renal crisis, etc. The microcirculation is of key importance for tissue homeostasis due to its specific localization and functions. Thus, the endothelial injury of the microcirculation leads to organ damage and chronic disease. In rheumatology, the SSc is a prototype of a disease, in which the endothelium is a primary target and a trigger for de-

velopment of the other pathological processes. Endothelial damage mediates and stimulates the processes of inflammation and fibrosis, which itself disturb the endothelial function that finally leads to devascularization and organ dysfunction.

REFERENCES

- [1] Seibold JR, Steen VD. Systemic sclerosis. In: Klippel JH, Dieppe PA. *Rheumatology*. London: Mosby; 1994: 6.8- 6.11.
- [2] Van Hinsbergh VW. Endothelium-role in regulation of coagulation and inflammation. *Semin Immunopathol* 2012; 34: 93-106.
- [3] Triggie CR, Samuel SM, Ravishankar S, *et al*. The endothelium: influencing vascular smooth muscle in many ways. *Can J Physiol Pharmacol* 2012; 90: 713-38.
- [4] Mayes MD. Endothelin and endothelin receptor antagonists in systemic rheumatic diseases. *Arthritis Rheum* 2003; 48: 1190-9.
- [5] Kontinen YT, Mackiewicz Z, Ruuttila P, *et al*. Vascular damage and lack of angiogenesis in systemic sclerosis skin. *Clin. Rheumatol* 2003; 22: 196-202.
- [6] Herrick AL, Cerrinic MM. The emerging problem of oxidative stress and the role of antioxidants in systemic sclerosis. *Clin Exp Rheumatol* 2001; 19:4-8.
- [7] Kahaleh B, Meyer O, Scorza R. Assessment of vascular involvement. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S9-S14.
- [8] Nambodiri AM, Rocca KM, Pandey JP. IgG antibodies to human cytomegalovirus late protein UL94 in patients with systemic sclerosis. *Autoimmunity* 2004; 37: 241-4.
- [9] Sgonc R, Gruschwitz M, Dietrich H, *et al*. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest* 1996; 785-92.
- [10] Herrick AL, Matucci-Cerinic M. The emerging problem of oxidative stress and the role of antioxidants in systemic sclerosis. *Clin Exp Rheumatol* 2001; 19: 4-8.
- [11] Block JA, Sequeira W. Raynaud's phenomenon. *Semin, Lancet* 2001; 357: 2042-2048.
- [12] Ho M and Belch JFF. Raynaud's Phenomenon: State of the Art 1998. *Scand J Rheumatol* 1998; 27: 319-22.
- [13] Lambova S, Müller-Ladner U. Capillaroscopic pattern in systemic sclerosis – an association with processes of angiogenesis and vasculogenesis. *Microvasc Res* 2010; 80(3): 534-9. 2010; 80(3): 534-9.
- [14] Brevetti G, De Caterina M, Martone VD, *et al*. Measurement of soluble adhesion molecules in primary Raynaud's phenomenon and in Raynaud's phenomenon secondary to connective tissue diseases. *Int J Clin Lab Res* 2000; 30: 75-81.
- [15] Rajagopalan S, Pfenninger D, Kehrer C, *et al*. Increased asymmetric dimethylarginine and endothelin 1 levels in secondary Raynaud's phenomenon. *Arthritis Rheum* 2003; 48: 1992-2000.
- [16] Ames PR, Lupoli S, Alves J, *et al*. The coagulation/fibrinolytic balance in systemic sclerosis: evidence for a haematological stress syndrome. *Br J Rheumatol* 1997; 36: 1045-50.
- [17] Mulligan-Kehoe MJ, Drinane MC, Mollmark J, *et al*. Antiangiogenic plasma activity in patients with systemic sclerosis. *Arthritis Rheum* 2007; 56: 3448-58.
- [18] Sweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992; 359: 843-5.
- [19] Distler O, Del Rosso A, Giacomelli R, *et al*. Angiogenic and angiostatic factors in systemic sclerosis: increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. *Arthritis Res* 2002; 4: R11.
- [20] Del Papa N, Colombo G, Fracchiolla N, *et al*. Circulating endothelial cells as a marker of ongoing vascular disease in systemic sclerosis. *Arthritis Rheum* 2004; 50: 1296-1304.
- [21] Distler JHW, Gay S, Distler O. Angiogenesis and vasculogenesis in systemic sclerosis. *Rheumatol* 2006; 45(Suppl 3): 26-7.
- [22] Dor Y, Djonov V, Abramovitch R, *et al*. Conditional switching of VEGF provides new insights into adult neovascularization and proangiogenic therapy. *EMBO J* 2002; 21: 1939-47.
- [23] Manetti M, Guiducci S, Ibba-Manneschi L, *et al*. Mechanisms in the loss of capillaries in systemic sclerosis: angiogenesis versus vasculogenesis. *J Cell Mol Med* 2010; 14: 1241-54.
- [24] Mulligan-Kehoe MJ, Simons M. Vascular disease in scleroderma: angiogenesis and vascular repair. *Rheum Dis Clin North Am* 2008; 34: 73-9.

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