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Capillaroscopic pattern in systemic sclerosis — an association with dynamics of processes of angio- and vasculogenesis

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ABSTRACT

Microcirculation is the main environment of the pathologic processes in systemic sclerosis (SSc). Nailfold capillaroscopic abnormalities in SSc are highly specific. It is well known that capillaroscopic examination reveals different changes in the different stages of SSc. Dilated, single giant capillaries and haemorrhages, preserved arrangement are the characteristic features of the "early" capillaroscopic phase; frequent giant capillaries and haemorrhages are the findings in the "active" phase, while in the "late" stages extensive avascular areas are usually found. Although tissue hypoxia normally is a strong inducer of neovascularization, there is no evidence of significant vascular recovery in SSc patients. Formation of new blood vessels is possible through two different mechanisms – angiogenesis (formation of new vessels from differentiated endothelial cells of prior vessels) – and vasculogenesis (from endothelial progenitor cells—EPCs). Disturbed function of EPCs has been found in all the stages of the disease. Only EPCs from SSc patients in the early phase of the disease have shown preserved ability for differentiation and maturation to endothelial cells in vitro. The recovery of the injured microvessels is also disturbed due to a predominance of angiogenic inhibitors. The capillaroscopic changes in the different stages of SSc mirror the dynamics in processes of angio- and vasculogenesis. The formation of new capillaries after therapeutic influence of vascular recovery by transplantation of autologous bone-marrow derived stem cells supports this conclusion.

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Introduction

Systemic sclerosis (SSc) is a chronic, multisystem connective tissue disorder, which affects the microcirculation, the skin and various internal organs. It is characterized by a triad of widespread microangiopathy, fibrosis, and autoimmune disturbances with activation of the humoral as well as cellular immune responses. There is an increasing evidence, which indicates that vascular damage is a primary event in the pathogenesis of SSc. The progressive vascular injury includes persistent endothelial damage, intimal thickening, vessel narrowing and obliteration. These changes lead to a reduced capillary blood flow with subsequent tissue ischemia and severe

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clinical manifestations such as digital ulceration, pulmonary arterial hypertension and scleroderma renal crisis. Although tissue hypoxia is a strong inducer of neovascularization, there is no evidence of significant vascular recovery in SSc patients. Different pathogenetic pathways lead to chronic tissue ischemia and result in irreversible structural changes of vascular architecture at different sites, capillary loss and inadequate, inefficient, reparative vascular recovery. The imbalance between pro-angiogenic and angiostatic factors may also contribute to the impaired angiogenic response in SSc. Although different proangiogenic stimuli exist in SSc, they do not elicit the appropriate angiogenic response (Manetti et al., 2010). Either angiogenic or vasculogenic mechanisms may potentially become in the future the target of therapeutic strategies to promote capillary regeneration in SSc (Miniati et al., 2009). Capillaroscopic pattern in SSc is specific and is observed in more than 90% of patients with overt SSc (Cutolo et al., 2000, 2005; Maricq et al., 1980). It allows specific capillaroscopic abnormalities to be used for the early diagnosis of SSc. The specific pattern in SSc is characterized by presence of dilated and giant capillaries, haemorrhages, avascular areas and neoangiogenesis. It was described for the first time by Maricq et al. (1980) and was termed — "scleroderma type" capillaroscopic pattern (Maricq et al., 1980). It is well-known that capillaroscopic findings in the early and late stages of the disease are distinctly diverse. Capillaroscopic changes in SSc are usually classified into three subtypes: – an "early" pattern – with appearance of few dilated and/or giant capillaries and few haemorrhages, relatively preserved distribution without loss of capillaries; an "active" pattern – with higher number of giant capillaries and haemorrhages, moderate loss of capillaries, slight derangement, diffuse pericapillary oedema and – a "late" pattern characterized by extensive avascular areas, severe derangement, ramified and bushy capillaries (Cutolo et al., 2000). The confluence of the avascular areas in the late stages of the disease leads to the characteristic desert-like appearance. Meandering capillaries, presence of more than one capillary loop in a single dermal papilla, ramified and bushy capillaries are the characteristic features of neoangiogenic capillaries, which are a proof of inadequate and abortive angiogenesis and can be found in the advanced stages of the disease.

In the present review, we have aimed to analyze the recent knowledge about the impaired vascular repair (e. g. angiogenesis and vasculogenesis) in the different stages of SSc.

Results and discussion

Nailfold capillaroscopy in SSc

Nailfold capillaroscopy is a non-invasive, inexpensive and easy-to-repeat technique, which is of substantial value for the evaluation of microcirculation in vivo (Bollinger and Fagrell, 1990; Cortes and Cutolo, 2007; Shore, 2000). It is the only method for the evaluation of nutritional capillaries of the nailfolds. In the nailfold area, the capillary loops become more parallel to the skin surface, and in the last row they can be observed in their full length. The capillaries consist of an arterial and a venous part, and an apical loop. Normally nailfold capillaries are hair-pin shaped, with parallel, regular arrangement; a single capillary loop is localized in a single dermal papilla.

The main indication for performing capillaroscopy in rheumatology is the presence of Raynaud's phenomenon. The capillaroscopic pattern of the same digit remains constant for an extended period of time in healthy individuals, while the appearance of abnormal findings inherits a positive predictive value for the development of a connective tissue disease (Bollinger and Fagrell, 1990; Herrick and Clark, 1998). Among rheumatic disease the most specific capillaroscopic pattern is the "scleroderma" pattern in SSc. Microcirculation is the main environment of the pathologic processes in SSc. Raynaud's phenomenon is one of the most common features in SSc. It is observed in about 90–95% of SSc patients and usually precedes the other features of the disease by years. Nailfold capillaroscopy is the method of choice for the evaluation of microcirculation in SSc. It is useful for the early diagnosis of SSc and also allows a follow-up of the microvascular modifications (Bergman et al., 2003; Cortes and Cutolo, 2007; Cutolo et al., 2000, 2005). Of note, the authenticity of capillaroscopic changes is confirmed histologically (Silver et al., 2005). "Scleroderma type" capillaroscopic pattern is a characteristic feature in the vast majority of SSc patients. Bergman et al. found this type of specific capillaroscopic changes in 70,4% of the examined patients (19/27) (Bergman et al., 2003). Other authors observed the "scleroderma type" capillaroscopic pattern in 87.5% of patients with diffuse SSc and in 61.6% of cases with a limited form of the disease among examined 102 SSc patients (Nagy and Czirjac, 2004). In an own study, among 19 SSc patients, we found an abnormal capillaroscopic pattern in 18 cases (94.74%). An "early" pattern was found in 77.7% of the cases in the early stage of the disease with duration of SSc less than 2 years. In the subgroup of patients in the late stage (mean duration of SSc 18.8 ± 10 years), we observed a "late" pattern with the characteristic avascular areas in 90% of the cases (Lambova et al., 2009). The appearance of dilated, giant capillaries and haemorrhages is of crucial importance for the early diagnosis of SSc. The inclusion of abnormal capillaroscopic changes as a diagnostic criterion together with the specific for SSc authoantibodies improve the sensitivity of American College of Rheumatology criteria for SSc (Hudson et al., 2007; Le Roy, 2001; Lonzetti et al., 2001).

Hypoxia consequences

Vascular injury is the hallmark of SSc. Microangiopathia in SSc ranges from considerable initial endothelial derangement, haemorrhages, dilation, to finally extensive capillary loss and abortive reparative neoangiogenesis. Specific histological findings in SSc are perivascular inflammatory infiltrates, a damaged capillary network with reduced number and irregular distribution. In addition, there is an excessive accumulation of extracellular matrix components produced by fibroblasts, which are highly activated in SSc and derive from specific pull progenitor cells from the bone marrow (Manetti et al., 2010). The reduced capillary flow leads primarily to a decrease in oxygen and nutritional supply. Tissue hypoxia increases the production of hypoxia inducible factor (HIF) and triggers fibrosis. HIF itself, increases the level of vascular endothelial growth factor (VEGF) (Mulligan-Kehoe and Simons, 2008; Sweiki et al., 1992). Normally, the tissues hypoxia is a stimulus for new blood vessels formation. In SSc the vascular recovery is disturbed despite the stimulatory effects of the hypoxia (Manetti et al., 2010).

Vascular repair — angiogenesis and vasculogenesis

Angiogenesis

Formation of new blood vessels is possible through two different mechanisms — angio- and vasculogenesis. *Angiogenesis* is a complex, multistage process, which is characterized by formation of new vessels from the differentiated endothelial cells of prior vessels. It is controlled by angiogenic stimulators and inhibitors, whose level is balanced in healthy tissues and in normal conditions there is no enhanced angiogenesis. Angiogenesis plays a pivotal role during embryonal development and later, in adult life, it is observed in several physiological processes such as endometrial regeneration, corpus luteum formation and wound healing. Angiogenesis is the hallmark of several pathological conditions such as tumours, chronic inflammation and macular degeneration. Endothelial cells death is a typical feature of diseases such as atherosclerosis, allograft vasculopathy, heart failure, diabetic retinopathy and SSc (Manetti et al., 2010; Mulligan-Kehoe and Simons, 2008). Sprouting angiogenesis encompasses an increase in vasopermeability, leading to the extravasation of

plasma proteins. Matrix metalloproteinases, which are secreted by the endothelium, break down the vascular basement membrane and allow the invasion of the surrounding stroma by endothelial cells. The influence of the pro-angiogenic stimuli result in proliferation and the organization of the endothelial cells in three dimensional tubular structures. Lumen formation and pericytes vessel wall stabilization are the final processes of sprouting angiogenesis and lead to the formation of a functional network of new capillary vessels (Manetti et al., 2010), (Fig. 1).

Of note, an imbalance between pro-angiogenic and angiostatic factors was described in SSc with a predominance of *angiogenic inhibitors*. The latter are 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) (Mulligan-Kehoe et al., 2007; Mulligan-Kehoe and Simons, 2008). It was demonstrated that the exposure of normal human microvascular dermal endothelial cells to plasma derived from SSc patients resulted in a decreased cell migration and tube formation. Plasma level of angiostatin in SSc patients was reported to be 2.9-fold higher as compared with healthy controls (Mulligan-Kehoe et al., 2007). The expression of kallikrein 9, 11 and 12, all of which are pro-angiogenic factors, was found to be decreased in SSc patients as compared with healthy controls, while the antiangiogenic factor–kallikrein 3 was found to be increased (Giusti et al., 2005).

Vasculogenesis

Vasculogenesis is a formation of new vessels from endothelial progenitor cells (EPCs) derived from the bone marrow, independent from pre-existing vessels (Distler et al., 2002). It is known that this process occurs exclusively during vascular development in embryogenesis. Previously, it has been hypothesized that vascular regeneration and repair in adults is limited to the proliferation of existing differentiated cells within vascular tissue, but the recent knowledge was substantially expanded and it was proven, that postnatal vasculogenesis in human adults can be found. EPCs contribute to vascular healing in response to vascular injury or ischemia in adults by homing to the site of injury (Caplice and Doyle, 2005; Gill et al., 2001; Gomer, 2008; Guiducci et al., 2008; Hur et al., 2004; Nevskaya et al., 2009; Zammaretti and Zisch, 2005). The release of EPCs from the bone marrow into the bloodstream is stimulated by different molecules — cytokines, angiogenic growth factors such as granulocyte colony stimulating factor (GM-CSF) and VEGF (Distler et al., 2002). In healthy adults, EPCs are a very rare circulating cell population (one cell out of 10^6 circulating leukocytes) (Distler et al., 2002; Kuwana et al., 2004; Peichev et al., 2000).

VEGF and EPCs

VEGF is a well-known proangiogenic factor, which takes a part in different phases of angio- and vasculogenesis. It increases the vascular permeability, stimulates the migration and proliferation of endothelial cells and induces tube formation. Even slight changes in the level of VEGF have pronounced effects on angiogenesis (Distler et al., 2002). In addition, VEGF causes proliferation and organization of EPCs through interaction with their surface receptor — VEGFR-2 (Fig. 1). Different research groups have found significantly higher levels of VEGF in SSc patients both in the early and in the late stages of the disease. In the late stages of the disease it was found higher level as compared with the level in patients with recent onset (Del Papa et al., 2004; Distler et al., 2002). A correlation between level of VEGF and fingertip ulcers was found with a higher level in SSc patients without fingertip ulcers. This suggests that VEGF may have a protective effect regarding the development of digital ulcers in SSc patients (Del Papa et al., 2006; Distler et al., 2002). In addition, it was found that if the up-regulation of VEGF is too short, the newly formed vessels are not stable. A prolonged overexpression of VEGF has also unfavourable effects, because the vessels fuse and form a chaotic vessel network, with appearance also of giant capillaries, which is a picture that resembles those in SSc (Distler et al., 2006; Dor et al., 2002). In a recent study of the EUSTAR (EULAR Scleroderma Trials and Research) network, which included 416 SSc patients, an association between three VEGF gene polymorphisms and the disease phenotype was not found (Allanore et al., 2007). On condition that the level of VEGF in SSc is decreased, a disturbed formation of new vessels is a classic finding. This suggests a defect of the effector cells. The bone marrow in healthy adults is a rich reservoir for tissue specific stem and progenitor cells. A small population of them are EPCs. EPCs can also be found in peripheral blood, but in healthy individuals their level is low. It increases under distinct circumstances such as tissue hypoxia, vascular injury and formation of tumours. EPCs migrate and locate to the sites of vascular injury, differentiate into mature endothelial cells, interact with the preserved local endothelial cells and take a part in the vascular recovery e. g. neovascularization. EPCs are positive for the following surface markers — CD34 +, CD133 + and VEGFR-2 and they can be differentiated from the other progenitor cells by flowcytometry (Del Papa et al., 2004). The evaluation of the specificity of these markers demonstrated, that both EPCs and mature endothelial cells can express similar endothelial-specific markers such as VEGFR-2. Identification of the differences between the EPCs and the circulating mature endothelial cells is further complicated by the fact that other markers such as CD34 are expressed both on hematopoietic progenitor cells, and on the endothelial cells. However, it can be found that a distinct population of circulating human CD34 cells that coexpress CD133 and VEGFR-2 and have the capacity to migrate and differentiate into adherent, mature endothelial cells. CD133 is expressed on a subset of EPCs, but not on the mature differentiated endothelial cells (Peichev et al., 2000). In the current EUSTAR recommendations, 2008 it was approbated that the endothelial phenotype of EPCs should be identified with expression of CD133, VEGFR2 and CD34 in combination (Distler et al., 2009). It was suggested that the presence of mature circulating EPCs in peripheral blood of SSc patients particularly in the early stages might be a result of shedding from affected blood vessel walls (Del Papa et al., 2004). A number of studies, addressing the level of EPCs in peripheral blood of SSc patients, were performed. Kuwana et al. (2004) found a significantly lower level of EPCs in the peripheral circulation in comparison with healthy controls and patients with rheumatoid arthritis. A disturbed maturation of EPCs into mature endothelial cells after cultivation with VEGF was observed. It was speculated that the use of cytokines, that can mobilize EPCs into the circulation, may be beneficial to SSc patients (Kuwana et al., 2004). On the other hand, Avouac et al. (2008a,b) found higher level of circulating EPCs in 50 SSc patients as compared with healthy controls. This increased level may result from their increased mobilization from the bone marrow (Avouac et al., 2008a). Del Papa et al. (2006) also found significantly higher level of EPCs than in healthy individuals. As they further compared the level of EPCs in different stages of SSc, significantly lower levels were found in patients in the early phase of the disease as compared with those in the late phase. Of note, the analysis of bone-marrow material in SSc patients demonstrated disturbed function of EPCs in all of the stages of the disease and only EPCs from SSc patients in the early phase of the disease showed a preserved ability for differentiation and maturation into endothelial cells in vitro (Del Papa et al., 2006). The interaction between VEGF and the surface receptor of the EPCs is of crucial importance. The expression of this receptor is decreased in SSc patients, and this phenomenon is considered to be involved in the pathophysiology of the disease (Avouac et al., 2008b; Cipriani et al., 2007). Due to these data, EPCs as well as their mobilization from the bone marrow are potential targets for future therapies in SSc. For example, mobilization of EPCs after administration of G-CSF (Huang et al., 2005), statins

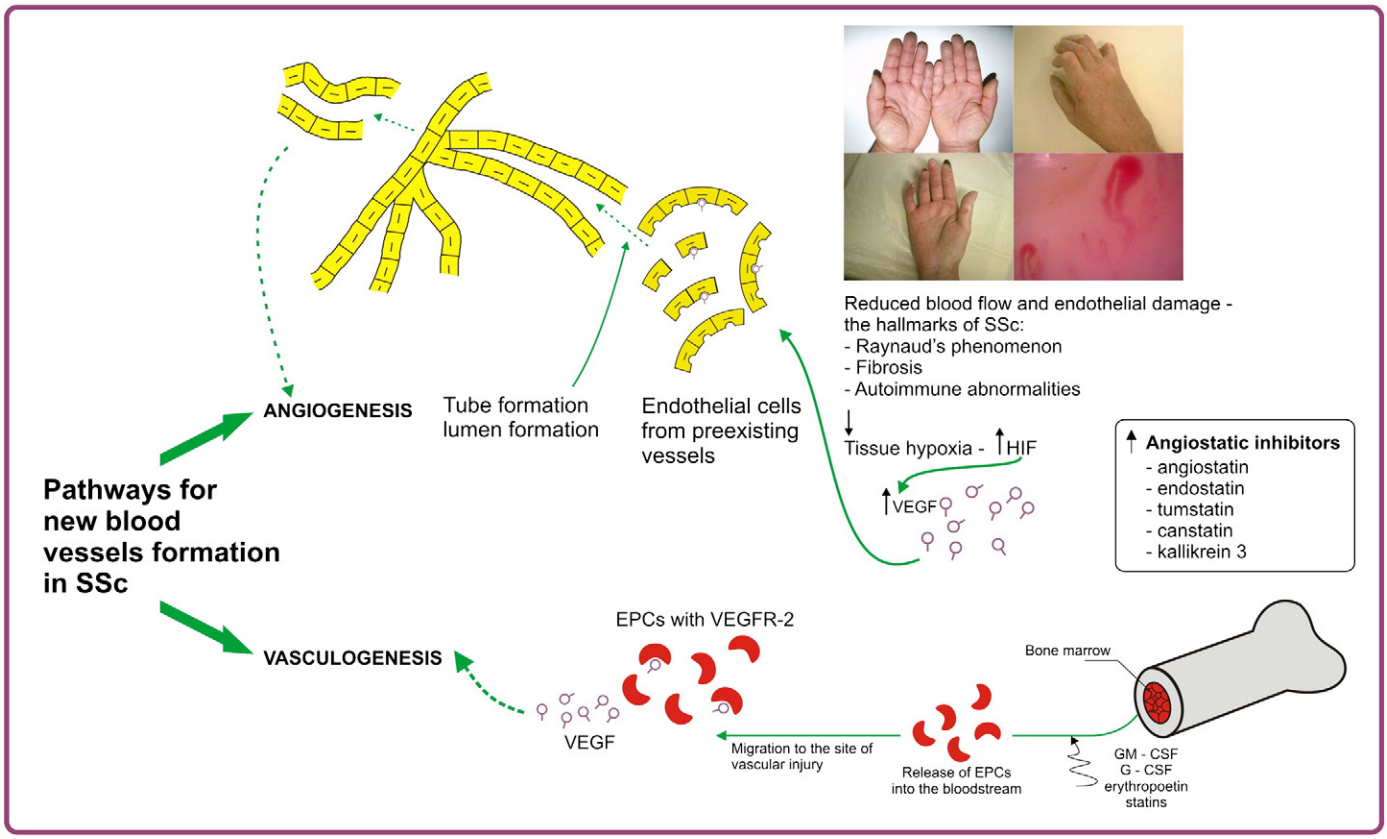

Fig. 1. Pathways of new blood vessels formation and the role of different cells and mediators in SSc.

(Kuwana et al., 2006; Llevadot et al., 2001) and erythropoietin (Ferri et al., 2007; Heeschen et al., 2003) can be found. Autologuos transplantation of G-CSF led to clinical improvement in diabetic critical limb ischemia (Huang et al., 2005). Increased level of EPCs was found after treatment of SSc patients with atorvastatin — 10 mg daily for a 12 week-period without reaching the level in healthy individuals. Furthermore, the authors observed an improvement in the manifestation of Raynaud's phenomenon (Kuwana et al., 2006). These studies led to the hypothesis, that the abnormal response and function of mononuclear cells from the bone marrow are responsible for the disturbed angiogenesis and the inadequate tissue repair in SSc (Distler et al., 2006; Mulligan-Kehoe and Simons, 2008). In addition, two different types of EPCs have been described: the — so-called "early-" and "late-outgrowth" EPCs, according to their time-dependent appearance when isolated with cell culture-based methods. These two types of EPCs have different cell surface markers (CD14 + "early-outgrowth" EPCs, CD14—"late-outgrowth" EPCs), morphologies, gene expression profiles and survival behaviours. However, both types of EPCs appear to contribute to neovasculogenesis in vivo. In particular, while "early-outgrowth" EPCs are mainly involved in the secretion of pro-angiogenic cytokines and vascular growth factors, "late-outgrowth" EPCs may proliferate and differentiate at sites of vascular injury after integration with the vessel wall, thus supplying a sufficient number of mature endothelial cells for vascular repair (Hur et al., 2004; Manetti et al., 2010). Upon considering the data about dynamics in capillaroscopic changes and in processes of angio- and vasculogenesis in the different stages of SSc, the following conclusions can be made:

i) Early phase of SSc
In the early phases of SSc there is inflammatory response, increased levels of VEGF, a partially preserved response of EPCs, which result in stimulated angio- and vasculogenesis. The capillaroscopic pattern in the early phase of SSc reflects these phenomena. In capillaroscopic examination, microhaemorrhages, dilated and giant capillaries are the classic findings. These findings are considered to be a result from a tissue hypoxia and the effects of proangiogenic mediators on the endothelium (Mulligan-Kehoe and Simons, 2008) (Fig. 2).

ii) Late phase of SSc
The stage of a stimulated angio- and vasculogenesis is followed by the phase of strongly depressed new blood vessels formation in the late stages. In the advanced stages of SSc, a reduced number and disturbed function of EPCs can be found, as well as the

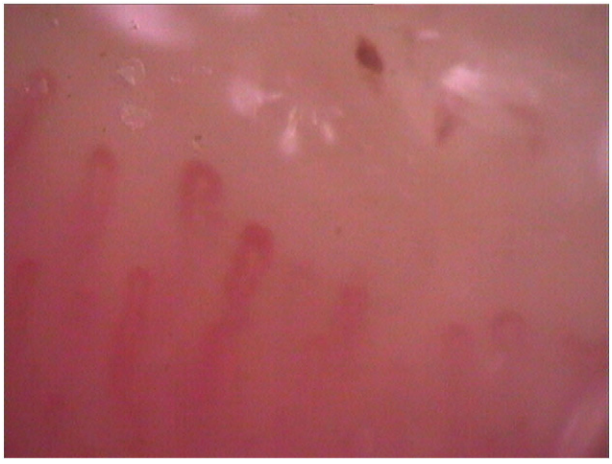

Fig. 2. Scleroderma type capillaroscopic pattern — early phase, that demonstrates significantly dilated capillary loops, single haemorrhage, relatively preserved distribution, magnification 200X , videocapillaroscope Videocap 3.0 (DS Medica, Italy).

predominance of angiogenic inhibitors. The respective capillaroscopic pattern in the late stages of SSc shows reduced capillary density, followed by extensive avascular areas, areas of desertifications, severe architectural derangement, neoangiogenic capillaries (ramified and bushy capillaries, more than one capillary loop in a single dermal papilla), which are a proof of an abortive recovery (Fig. 3).

New treatments in SSc and their influence on microcirculation

Haemopoietic stem cell transplantation (HSCT) have been explored for the treatment of autoimmune diseases including SSc. The clinical trials demonstrated a satisfactory benefit, including long-term improvement in skin thickening and stability of visceral organ involvement. Autologous HSCT is currently applied only in clinical trials for patients with severe SSc refractory to conventional therapies (Mulligan-Kehoe et al., 2007; Xiong and Derk, 2009). Therapeutic influence of angiogenesis after transplantation of autologous bone-marrow derived stem cells was observed (Ishigatsubo et al., 2010; Miniati et al., 2009). Different therapeutic approaches were compared in 16 patients with severe diffuse SSc, as follows: 6 with HSCT (CD34 positive cells) and 10 with monthly pulse cyclophosphamide (1 g for 6 months and then orally with 50 mg/day for further 6 months). Capillaroscopic examination was performed at baseline and at the 3rd month after the beginning of each treatment and then was repeated every 3 months. Before HSCT the capillaroscopic examination revealed “late” type capillaroscopic pattern in all of the patients. At the 3rd month after the HSCT pattern, the capillaroscopic pattern was changed from “late” into “active” with frequent giant capillaries and haemorrhages, absence of avascular areas and angiogenesis phenomena. Microvascular abnormalities were preserved in the active pattern one year after the HSCT, while in patients treated with cyclophosphamid alone no capillaroscopic modifications were observed during 24 months of follow-up and the pattern remained “late”. These observations suggest that the HSCT with a high dose cyclophosphamid may be beneficial for vascular remodelling and recovery (Miniati et al., 2009). Rise of surface temperature, increased blood flow volume, and new capillaries have been found on thermography, capillaroscopy, intra-arterial digital subtraction angiography and laser Doppler flowmetry (Ishigatsubo et al., 2010). There are case reports about successful treatment of digital ulcers in SSc after *local transplantation of autologous stem cells*. Accelerated ulcer healing, pain relief, functional improvement during, reduced activity of Raynaud's phenomenon, improved vessel reactivity, demonstrated by laser Doppler flowmetry was observed during the follow-up (Nevskaya et al., 2009).

Fig. 3. Scleroderma type capillaroscopic pattern – late phase – that demonstrates avascular areas, ramified and bushy (neoangiogenic) capillaries, which are a proof for an abortive recovery, magnification 200X, videocapillaroscope Videocap.

Conclusion

Despite the severe tissue ischemia in SSc, which is a strong stimulus for the formation of new vessels, the vascular recovery in this disease is defective. The capillaroscopic changes in the different stages of SSc mirror the dynamics in processes of angio- and vasculogenesis. A more detailed analysis of the pathways underlying the defective angiogenesis and vasculogenesis in SSc may result in new future therapies.

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