

Raynaud's phenomenon: new aspects of pathogenesis and the role of nailfold videocapillaroscopy

Anna Kuryliszyn-Moskal, Jacek Kita, Anna Hryniewicz

Department of Rehabilitation, Medical University of Białystok, Białystok, Poland

Abstract

Raynaud's phenomenon (RP) refers to paroxysmal pallor or cyanosis of the digits of the hands or feet and, infrequently, the tips of the nose or ears (acral parts) owing to cold-induced vasoconstriction of the digital arteries, precapillary arterioles, and cutaneous arteriovenous shunts.

Raynaud's phenomenon reflects an exaggeration of normal central and local vasomotor responses to cold or emotion. Raynaud's phenomenon has been classified as primary or secondary, depending on whether it occurs as an isolated condition or is associated mainly with a connective tissue disease.

Dysregulation of autonomic and sensitive nerve fibers, functional and structural vessel changes, and intravascular alterations can be observed in the pathogenesis of RP.

Nailfold videocapillaroscopy (NVC) is the best non-invasive and repetitive diagnostic technique for detecting morpho-functional changes in the microcirculation. Nailfold videocapillaroscopy is accepted in early diagnosis and monitoring of primary and secondary RP.

Key words: Raynaud's phenomenon, microvascular abnormalities, nailfold videocapillaroscopy.

Raynaud's phenomenon (RP) is characterized by digital ischemic attacks, followed by cyanosis and erythema. It is usually bilateral, symmetric, mostly seen in young women and is considered as a primary (classic, idiopathic) form and a benign clinical problem. Raynaud's phenomenon could be a manifestation of connective tissue disease, vasculitis, cryoglobulinemia, drug-induced, etc. (considered as secondary). The vasospastic attack of RP is classically manifested as triphasic color changes.

Raynaud's phenomenon refers to paroxysmal pallor or cyanosis of the digits of the hands or feet, and, infrequently, the tips of the nose or ears (acral parts), nipples and, very rarely, the tongue owing to cold-induced vasoconstriction of the digital arteries, precapillary arterioles, and cutaneous arteriovenous shunts [1–3]. Characteristically, the fingers change color in the sequence white-blue-red [4]. The white phase (ischemia) is due to excessive vasoconstriction and cessation of regional blood flow. This phase is followed by a cyanotic phase

(blue deoxygenation), as the residual blood in the finger desaturates. The red phase (reperfusion) is due to hyperemia as the attack subsides and blood flow is restored. Structural changes in the arterial walls are absent except late in the course, when intimal thickening can appear. Raynaud's phenomenon reflects an exaggeration of normal central and local vasomotor responses to cold or emotion. The prevalence in the overall population is approximately 3% to 5%; the median age of those affected is 14 years [1].

The course of RP is usually benign, but in long-standing cases atrophy of the skin, can appear subcutaneous tissues, and muscles, ulceration and ischemic gangrene are rare [5].

Raynaud's phenomenon has been classified as primary (pRP) or secondary (sRP), depending on whether it occurs as an isolated condition or is associated with an underlying disease, mainly a connective tissue disease (CTD-RP) [6]. Raynaud's phenomenon may also occur

Address for correspondence:

Jacek Kita, Department of Rehabilitation, Medical University of Białystok, M. Skłodowskiej-Curie 24 A, 15-267 Białystok, Poland, e-mail: jacek.kita@umb.edu.pl

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in other non-CTD (occupational factors, repetitive trauma, malignancies, clotting disease, neurodegenerative cause, nervous system diseases, endocrine diseases, surgical diseases, iatrogenic – drugs, drug addiction).

In contrast to uncomplicated or pRP, sRP refers to arterial insufficiency of the extremities caused by various conditions, including systemic lupus erythematosus, systemic sclerosis (SSc; scleroderma), atherosclerosis, Takayasu's disease or B rger disease [2, 5, 7].

Since RP may be the first manifestation of such conditions, all patients who manifest the condition should undergo evaluation to rule out an underlying cause, which will be apparent at presentation or will develop later in approximately 10% of patients [8]. Features suggestive of secondary RP include age of onset > 30 years, more severe episodes associated with skin lesions, and clinical features of connective tissue disease.

The agents that may cause RP are physical (low temperature, vibration) and psychological (stress).

The pathophysiology of RP is still not completely understood. The key factor in the pathogenesis of RP is thought to be dysregulation in the control mechanisms (i.e., neural, endothelial, and intravascular) of the vascular motility, resulting in an imbalance between vasodilatation and vasoconstriction [4]. Dysregulation of autonomic and sensitive nerve fibers, functional and structural vessel changes, intravascular alterations (platelet activation and impaired fibrinolysis, oxidative stress and reduced red blood cell plasticity, leukocyte hyper-activation) can be observed in the pathogenesis of RP [4].

Neural abnormalities

The autonomic nervous system regulates the vascular tone through a number of neurotransmitters, which trigger either vasodilation (calcitonin gene-related peptide, neurokinin A, substance P, and vasoactive intestinal peptide) or vasoconstriction (adrenergic agonists and nerve growth factor).

Calcitonin gene-related peptide (CGRP), an alternative product of the calcitonin gene, is a very potent vasodilator secreted by nerves that supply blood vessels. CGRP triggers the intracellular synthesis of adenylate cyclase in smooth muscle cells (SMC), which increases cyclic adenosine monophosphate (cAMP), ultimately leading to SMC relaxation. Decreased levels of the vasodilator CGRP in perivascular nerves of the finger skin have been demonstrated in pRP and sRP, and in vibration syndromes, suggesting its role in RP [9].

The vasodilator neurokinin A and substance P can also be involved in the RP pathomechanism [10]. Neurokinin A and substance P induce an increase of cAMP,

followed by SMC relaxation, and endothelial cyclic guanosine monophosphate (cGMP)-dependent nitric oxide accumulation, which strongly sustains the SMC relaxation already triggered by SMC-cAMP [11, 12].

The vasodilator vasoactive intestinal peptide (VIP), besides controlling a number of neurological functions, heart contractility and glycogenolysis, relaxes the SMC of the trachea, stomach, gallbladder and vessels. Terenghi et al. [13] observed a decreased VIP level in RP.

During local or whole body cooling, blood flow to the skin is reduced to prevent excessive heat loss. This effect is mediated by the reflex action of the sympathetic nervous system and appears to be abnormally augmented in RP. Based on pharmacological, biochemical and molecular approaches, three classes of α_2 -adrenoreceptors, α_{2A} , α_{2B} , and α_{2C} , have been described, each with a distinct distribution and function [14]. Chotani et al. [15] demonstrated that the α_{2C} adrenoreceptor is the most important of the three in thermoregulation. It has been shown that the expression of these receptors on SMC of cutaneous arterioles is markedly increased following exposure to cold, inducing potent vasoconstriction, even in the presence of physiological levels of the α_{2C} receptor agonist [16].

The role of the vasoconstrictor nerve growth factor (NGF) is intriguing. It shows elevated serum levels in SSc patients, but attempts to demonstrate a role in the generation of vasospasm in RP have not been successful [17–19].

Functional and structural vessel changes

In pRP, the vascular abnormalities are probably functional. By contrast, in sRP defective function and structural vascular abnormalities combine to compromise blood flow. Functional alterations of blood vessel tone can be mediated by endothelium-dependent and endothelium-independent factors. Among endothelium-dependent factors, decreased levels of the pleiotropic vasodilators nitric oxide (NO) and prostaglandin I₂ (PGI₂; prostacyclin) probably contribute to vasoconstriction in RP [20].

Nitric oxide, a pleiotropic mediator of inflammation, was discovered as a factor released from endothelial cells that caused vasodilatation by relaxing vascular smooth muscle and was therefore called endothelium-derived relaxing factor. Nitric oxide plays a critical role in the control of vascular tone generated constitutively by the endothelium as a messenger molecule which acts as an antithrombotic and cytoprotective agent, and also can cause tissue damage [21]. The impairment of constitutive NO synthesis and release as a consequence of vascular endothelium injury leads to

dysregulation of vascular tone control and progressive disorganization of the vascular architecture [6, 20].

The role of NO in rheumatic diseases may be protective and anti-inflammatory or cytotoxic depending on the amount of NO produced and the targets available for action [22, 23].

Nitric oxide plays an important role in the vascular and cellular components of inflammatory responses. Nitric oxide is a potent vasodilator by virtue of its action on vascular smooth muscle. Nitric oxide is an inhibitor of the migratory and proliferative activities of vascular smooth muscle cells. In addition, NO reduces platelet aggregation and adhesion, inhibits several features of mast cell-induced inflammation, and serves as an endogenous regulator of leukocyte recruitment. Blocking NO production under normal conditions promotes leukocyte recruitment. Thus, production of NO is an endogenous compensatory mechanism that reduces inflammatory responses. Abnormalities in endothelial production of NO occur in atherosclerosis, diabetes, and hypertension. Nitric oxide and its derivatives are microbicidal, and thus NO is also a mediator of host defense against infection. Nitric oxide also causes tissue damage.

Small amounts of NO released by vascular endothelium regulate relaxation of adjacent smooth muscle and prevent leukocyte and platelet adhesion, but large amounts of NO released in response to cytokines can be cytotoxic [22, 23].

The endothelium also produces vasoconstrictors, such as endothelin-1 (ET-1), one of the most potent endogenous vasoconstrictors, which also causes vascular remodeling [24]. Release of ET-1 seems to be mediated by interleukin-6 (IL-6) and other pro-inflammatory cytokines [25]. Rajagopalan et al. [20] found significantly higher serum levels of ET-1 in patients with secondary Raynaud's disease. Moreover, overexpression of ET-1 has been reported in SSc-RP patients [26].

Angiotensin II is another vasoactive mediator (endothelial independent factor) which might contribute to RP. The vasoconstrictor angiotensin II (ATII) – an α adrenergic receptor agonist – can also promote structural vessel wall changes, including fibrosis [27]. Kawaguchi et al. [28] showed that serum ATII levels in patients with diffuse cutaneous SSc were significantly higher than in patients with limited cutaneous systemic sclerosis and healthy donors. ATII may promote tissue fibrosis by increasing synthesis of extracellular matrix components by fibroblasts [28].

Nailfold videocapillaroscopy (NVC) has been proposed as a first-line investigation in the early differential diagnosis between primary and secondary RP [29, 30]. Moreover, NVC is very helpful as a screening method for detecting patients with RP at high risk for the devel-

opment of connective tissue disease [31, 32]. A critical, and possibly early event, often associated with a lymphomonocyte infiltration, is endothelial injury. Then, SMC abnormal migration in subendothelial regions and pericyte activation occur, contributing to vessel fibrosis and inducing abnormal synthesis of extracellular matrix components with intimal and perivascular fibrosis [33–35]. Endothelial proliferation, intimal thickening with progressive lumen obliteration and abnormal and ineffective neo-angiogenesis lead to vascular remodeling [34, 36, 37]. Similar vascular changes, with SMC hypertrophy and perivascular fibrosis but without intimal fibrosis, have been found in vibration syndrome RP [38].

Angiogenesis

Many studies have concentrated on the role of angiogenesis and microvascular endothelial injury in the pathogenesis of RP [4]. Among the angiogenesis inducers, vascular endothelial growth factor (VEGF) has been characterized as a key molecule. Vascular endothelial growth factor is involved in several steps of physiological and pathological angiogenesis including proliferation, survival and migration of endothelial cells [39].

Angiopoietin (Ang-1) is constitutively expressed in periendothelial cells (pericytes and smooth muscle cells). Under physiological conditions, Ang-1 has vasoprotective and anti-inflammatory actions, mediates vessel maturation and maintains vessel integrity by the recruitment of periendothelial cells [40].

Another important factor in endothelial activation is angiopoietin-2 (Ang-2), which causes vascular destabilization, thereby rendering the endothelium responsive to stimulation by inflammatory and angiogenic cytokines [41]. Angiopoietin-2 is stored and rapidly released by endothelial Weibel-Palade bodies upon stimulation by proinflammatory stimuli, hypoxia, shear stress and VEGF.

Angiopoietin-1 and Ang-2 have opposing effects. Ang-1 is required to maintain the endothelium in a quiescent state, whereas Ang-2 is a destabilizing factor considered to be pro-inflammatory. Angiopoietin-2 acts as a vessel-destabilizing cytokine, playing an essential role in vascular remodeling. Angiopoietin-2 and VEGF coordinately regulate endothelial behavior. In the presence of VEGF, Ang-2 enables migration and proliferation of endothelial cells and the sprouting of new blood vessels, but initiates endothelial cell death and vessel regression in the absence of proangiogenic activity [42]. Several studies have shown increased Ang-2 concentrations in vascular diseases, including SSc, compared to healthy volunteers [43]. Kuryliszyn-Moskal et al. [44] have suggested that Multiwave Locked System (MLS) laser therapy showed beneficial clinical effects and the tendency of

normalization of Ang-2 concentration in the serum of RP patients. The authors suggested that Ang-2 serum level may be a useful marker of microvascular abnormalities in RP patients treated with MLS therapy [44].

Intravascular disturbances

Increased platelet aggregation and activation have been described in both systemic sclerosis and primary RP [45]. Molecules released by activated platelets include thromboxane A₂, β -thromboglobulin, serotonin, platelet-derived microparticles, and platelet-derived growth factor. Augmented synthesis of platelet thromboxane A₂, a potent vasoconstrictor and a platelet aggregator, has been reported in patients with RP, suggesting a possible mechanism [46]. Serotonin, another circulating vasoconstrictor released from platelets, has also been reported to be significantly elevated in patients with RP [47]. Fibrinolysis is normal in patients with primary RP, but in systemic sclerosis, reduced activity of the fibrinolytic system has been described [48, 49]. Impaired fibrinolysis documented by the increased serum levels of tissue plasminogen activator and elevated levels of von Willebrand factor are reported in secondary RP – mainly in patients with systemic sclerosis [45].

Oxidative stress has been implicated in the pathogenesis of RP. Repeated episodes of vasospasm followed by reperfusion are responsible for an intravascular accumulation of free radicals or reactive oxygen species (ROS) [50]. These substances mediate tissue damage and endothelial activation. Generated ROS might contribute to endothelium damage through peroxidation of cell membrane lipids in SScRP, pRP and hand-arm vibration syndrome [50, 51].

The erythrocyte membrane is another substrate of intravascular ROS. Reduced red blood cell plasticity has been observed in SSc, but not in pRP [52].

Leukocyte activation has been reported in patients with SSc, pRP and hand-arm vibration syndrome and may contribute to oxidative stress [53].

Nailfold videocapillaroscopy

The first person to note the relationship between conjunctival inflammation and the presence of capillary disturbances, using a magnifying glass, was the Italian physician Giovanni Rasori [29]. Thanks to these descriptions, at the beginning of the 20th century Hutchinson was able to differentiate RP into a primary phenomenon and secondary phenomenon by detecting microvascular changes [54].

In 1973, Maricq and LeRoy [55] published the first paper describing the specific capillaroscopic patterns in systemic sclerosis (SSc): the extent of microangiopathy

detected by nailfold capillaroscopy has been shown to correlate with disease severity and prognosis. Abnormal capillary microscopy, the presence of certain autoantibodies such as anticentromere antibody, older age at onset of RP, severe vasospasm, and any signs of systemic disease are the predominant risk factors for the development of a connective tissue disease, predominantly SSc.

In the year 2000, Cutolo et al. [56] defined major nailfold videocapillaroscopy patterns that are useful when assessing the appearance and progression of sclerodermic microangiopathy.

Capillaroscopy is the most reliable way to distinguish between primary and secondary RP through identification of an early pattern of systemic sclerosis [57].

Morphological examination of skin capillaries is generally performed at the nailfold because this area is easily accessible for evaluation, and the major axis of the capillaries is parallel to the skin surface.

Nailfold videocapillaroscopy (NVC) is performed using a stereomicroscope. A fiber optic light source and filter provide cold illumination. The optical microscope may be connected to a color digital camera and a personal computer with a high-resolution color monitor (14 inches). Usually four consecutive fields extending over 1 mm in the middle of the nailfold are studied per finger using image analysis software. The images are taken at the time of examination and should be analyzed by the same experienced investigator without knowledge of the patient's clinical diagnosis.

Nailfold videocapillaroscopy of the fingers of both hands should be performed following a period of acclimatization lasting at least 20 minutes at a room temperature of 20–24°C. To obtain the best visibility of the microvasculature a drop of immersion oil is recommended. Only the capillaries in the distal row of the nailfold are analyzed.

The usual capillaroscopic features are 10–30 capillaries/1 mm, with typical hairpin-shaped loops arranged in parallel rows. Isolated tortuous, meandering and elongated capillaries can be found in healthy controls also.

The evaluation of morphological changes includes such parameters as: loop density, capillary length variability, percentage of loops with architectural derangement, i.e. tortuous, meandering, enlarged/giant, ramified or bushy capillaries, irregular distribution of the capillary array and the presence of extravasations into perivascular tissue. In recent studies an attempt of the classifications [30–32] of morphological changes has been proposed: dilated capillaries – microvessels with a diameter of the arterial limb wider than 0.015 mm (= 15 μ m) or a venous limb wider than 0.020 mm (= 20 μ m), giant capillary loops – homogeneously enlarged loops with a diameter wider than 0.050 mm (= 50 μ m), elongated capillaries – microvessels with

length greater than 0.500 mm (= 500 µm), microhemorrhage – presence of a dark red mass characterized by hemosiderin deposit derived from capillary injury, tortuosity – a variation of the typical hairpin capillary shape and avascular area – a distance between two adjacent capillary loops longer than 0.500 mm (= 500 µm).

In our examinations the capillaroscopic findings are graded from 0 to 3, where: score 0 indicates normal features (> 8 capillaries/mm, typical hairpin-shaped loops arranged in parallel rows), 1 indicates mild (6–8 capillaries/mm, < 33% changed capillary loops with nonhomogeneous distribution or size of loops, elongation of the loop or shortened loops, and the absence of hemorrhages), 2 indicates moderate (33–66% morphologically changed capillaries, enlarged loops, diminished loop density and microhaemorrhages), and 3 indicates severe changes (more than 66% dilated capillaries with heterogeneous features of angiogenesis with a variable capillaroscopic pattern, avascular areas and microhemorrhages). The mean score for each subject is obtained from the analysis of all ten fingers [58, 59].

Nailfold capillaroscopy has enormous potential in the assessment of digital vascular disease. Widefield microscopy is inexpensive, easy to use, and useful prognostically in patients presenting with RP [60]. Direct visualization of capillaries of different patient subgroups may raise fundamental questions regarding pathogenesis. Videocapillaroscopy is an extension of the widefield technique which allows quantitation of these abnormalities: using a video camera and digitizing system, dimensions of individual capillaries can be measured, thus offering the potential of a non-invasive technique which should allow objective assessment of microvascular disease and its progression. The technique of videocapillaroscopy facilitates the quantitation of the nailfold capillary abnormalities which have been long recognized in patients with connective tissue disease. Because images are recorded on video, images can be stored for analysis and reanalysis.

Videocapillaroscopy may allow quantitation of microvascular disease progression over time and therefore be an important research tool. This technique is useful for assessing early diagnosis and providing prognostic information, and is capable of differentiating between active and inactive forms of RP, and also of monitoring disease progression.

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