Microvascular and Immunological studies in Raynaud's phenomenon.
Houtman, Pieternella

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1985

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Houtman, P. M. (1985). Microvascular and immunological studies in Raynaud's phenomenon. s.n.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Summary and general discussion

Raynaud's phenomenon (RP) may present as an idiopathic or primary phenomenon or as part of an underlying disorder, in particular of connective tissue diseases (secondary RP). The differential diagnosis between the fully developed scleroderma and primary RP is clinically not difficult. However, in early stages it may present a problem especially if one considers that RP may precede connective tissue disease (CTD) by many years. An early diagnosis of CTD is of clinical importance because inflammatory pathologic changes in internal organs, especially the lung, can be inhibited in the early phase of the disease by immunosuppressive therapy.

The purpose of this thesis was to investigate the diagnostic significance of microvascular abnormalities - as observed in the nailfold - in patients with RP with respect to the presence or development of a connective tissue disease. In addition, we investigated whether the observed abnormalities were an expression of widespread microvascular organ disease. Finally, we studied the relationship between antibodies to nucleoribonucleoprotein (nRNP) and disease activity in a particular group of patients with RP characterized by the presence of these antibodies, both from a diagnostic and an immunoregulatory point of view.

In vivo, vascular abnormalities can be studied easily and non-invasively at the microcirculatory level by microscopy of the nailfold. CTDs underlying RP, especially scleroderma, are characterized by structural microvascular abnormalities. The first (microvascular) part of this thesis deals with the diagnostic significance of abnormalities in the nailfold capillaries of patients with RP.

Chapter I is a general introduction to nailfold capillary microscopy. The combined equipment of microscope and photomicrographing system has the practical advantage that all fingers of the subject can be examined and photographed under the same conditions.

Chapter II deals with the methodology for quantitative evaluation of capillary distribution and morphology. The distal row of nailfold capillaries was studied in 115 patients with RP (with and without CTD) and in 55 healthy subjects by using a stereozoom microscope. All ten fingers were observed and of each an area of 5 mm was photographed. Photos were coded and evaluated according to a protocol by two independent observers not informed about the clinical data of the subjects. The
inter-rater concordance was high for the scores of the total number of capillaries, the number of enlarged loops and the number of giant loops. The inter-rater concordance was also high for the presence of bushy patterns, coiled balls and enlarged loops bordering local paucities. It is of importance to mention that for the routine medical practice, the fourth finger proved to be most suitable for nailfold microscopy, since it yielded the lowest percentage of photos that were not evaluable. Warming up of the hands resulted in an optimum visualization of nailfold capillaries.

In chapter III, a discriminant analysis embodying the seven most reproducible capillary patterns as well as their scores for all digits is described. The fourth digit again yielded the best results in distinguishing primary and secondary RP. Patients with primary RP did not differ from healthy controls in their capillary distribution and morphology. Extravasates were observed most frequently in CREST and MCTD, bushy patterns in scleroderma and MCTD, and giant loops especially in CREST. In our studies the capillary density of the nailfold was deduced from the number of capillary loops in the distal row. Since a similar distribution of nailfold capillaries in serial examinations in patients with CTD was seen, we conclude that capillary drop out of capillaries in the nailfold is not due to changes in vascular tone in these patients, but is really a reflection of structural changes of the microvasculature. The contribution of rheological abnormalities to capillary drop out and enlargements can not be excluded. Although nailfold biopsies ought to be performed to validate our definition of nailfold capillary density in vivo (1,2), capillary density was the most discriminative feature in distinguishing primary RP and secondary RP. None of the capillary configurations was specific for any of the connective tissue diseases studied.

The frequency of bushy patterns is remarkably high amongst healthy controls and primary RP. The definition of bushy patterns has to be renewed. The major difference in the bushy pattern of a healthy control compared to that of a patient with CTD is that the control pattern remains unchanged (3). Furthermore, since bushy patterns in CTD appear to be associated with local paucities, their presence suggests capillary neoformation as an expression of an active microvascular process. Although the basal rate of endothelial replication in microvessels is extremely low in vivo (4), disappearance and regrowth of capillaries is seen in repeated examinations in follow up studies of patients with CTD (5). Probably bushy patterns may be considered as a sign of activity (Chapter I), or as a compensation to treatment with immunosuppressants (6). This is an aspect to refer to treatment with steroids in CTD. Thus, nailfold capillaroscopy proved to be a useful aid in the diagnosis of CTD.

The low prevalence of bushy patterns in a group of scleroderma patients with diffuse disease course of the disease probably reflects the fact that patients with more progressive disease do show a higher prevalence of these symptoms. The low prevalence of these symptoms is comparable to those in a long term group of patients with chronic venous insufficiency, who are predilection sites for ulcerations, tuft resorption, etc., related to obliteration of the deep system involvement. In particular, in patients with subacute exacerbation the relationship between capillary density and ulcers is promising.

Besides several practical aspects, the nailfold is an ideal objective means to follow on the relationship between capillary density and the symptoms. The capillaries are described by referring patterns to a typical capillary pattern and the term, defined CTD. Even slight changes in capillary density or in individual capillary length may be registered in serial examinations.
of the total number of number of giant loops. The presence of bushy local paucities. It is medical practice, the nailfold microscopy, since were not evaluable. Visualization of nailfold

The prevalence of bushy patterns is

although the seven most scores for all digits is that results in distinguishing

CTD and MCTD, giant loops especially the nailfold was deduced row. Since a similar situations in patients with out of capillaries in the these patients, but is microvasculature. The capillary drop out and en-

high amongst healthy primary RP did not differ and capillary density in native feature in dis-

found to be inversely related to organ system involvement. Decreased capillary density was observed, in particular, in patients with esophageal hypomotility. Longitudinal studies on the relationship between pulmonary diffusion capacity and capillary density are promising.

Besides several practical reasons (as compared to the conjunctiva) the nailfold is an ideal site for observation of capillary changes, since the location of capillary patterns in follow up studies is easy to ascertain by referring patterns to both lateral nailfold edges. In Chapter V, nailfold capillaries are described in one patient presenting with RP who developed CTD. Even slight capillary abnormalities at initial presentation may
point to the development of scleroderma-like disease. More importantly, rapid changes of microvascular patterns in the nailfold during serial observations in the same patient may indicate that his disease is associated with progressive organ involvement. The study of prognostic significance of capillary patterns needs to be extended in future.

Photoelectric plethysmography during cooling and warming up is a functional test registering RP and quantitating its severity. This test does not discriminate between vasospasm per se and vasospasm superposed upon luminal narrowing of the arterioles as observed in secondary RP. An inverse relationship was found in patients with CTD between the severity of RP, assessed by photoelectric plethysmography, at first presentation and capillary density some years later (Chapter IV). Although the foregoing results do not yet allow for definite statements, the position of the primary location of the “local fault” (8) appears to be merely the arteriola than the capillary.

Enlarged loops were seen at microscopy in all groups of patients. The enlarged loops appear to be distributed at random in the nailfold, but may occur in clusters. Teleangiectatic lesions are a frequent finding in scleroderma, CREST an MCTD (9-11), and are in fact enlarged capillary loops (5). It is important to determine whether the enlarged capillary is primarily damaged or merely dilated. Increased permeability may lead to enlargements of capillary loops. Fluorescence video microscopic studies have demonstrated an increased leakage of fluorescent dye in scleroderma (12). The inverse relationship between capillary density and the number of enlarged loops as well as longitudinal observations suggested that enlargement of capillaries precedes capillary drop out (Chapter V). In this respect the findings of Maricq in serial microscopic evaluations of nailfold capillary patterns after nailfold biopsy in patients with scleroderma are very interesting (13). She found normal microvascular patterns early after the biopsy whereas enlarged loops and drop out of capillary loops were observed a half year and two years respectively after the biopsy. In future a combination of nailfold capillary examinations with simultaneous recording of arteriolar function by non-invasive tests (for instance digital plethysmography) and possibly a study of arteriolar morphology (angiography) will yield interesting information.

Vascular changes and immunologic aberrations are both implicated in the pathogenesis of scleroderma-like disorders. It is not known whether these components of separate elements of separate diseases. Several factors support changes in connective tissue antibodies, increased levels of acute phase proteins and number of nailfold capillaries.

In conclusion, markers are not specific for any of the local and systemic changes of several immunologic factors. Secondary RP seem highly heterogeneous.

The second (immunodot) assay for the estimation of nRNP antibodies develops into a serologic prominent feature of this disease. Several techniques allowed the quantitation of antibodies, such as nRNP. Furthermore, quantitation of antibodies, especially available rabbit antisera, are obtained only in a paired with the counterimmunoelectrophoresis of patient sera. This finding supports the idea and in complex with the ELISA method coupled by the immunoassay because of the antigen preparation. It may be registered in long-term follow-up.

In Chapter VII, a study is presented in a follow-up disease. Levels of an antibody to a recall-antigen, globulin beta were also noted in patients who coincide with a rise in agents are used. Inte
Several factors supposed to be involved in the pathogenesis of vascular changes in connective tissue diseases, such as the presence of autoantibodies, increased levels of circulating immune complexes, increased levels of acute phase reactants, were each associated with a decreased number of nailfold capillaries (Chapter IV).

In conclusion, morphologic changes of nailfold capillaries in CTDs are not specific for any of those diseases, but merely reflect the progress of the local and systemic microvascular disease. Pathophysiological roles of several immunologic and inflammatory factors (interrelated) in secondary RP seem highly probable, but further basic research is needed.

The second (immunological) part of this thesis describes a sensitive assay for the estimation of autoantibodies to nucleo-ribonucleoprotein (nRNP). Antibodies to nRNP are a hallmark of CTD which ultimately develops into a scleroderma-like disorder. Vascular symptoms are a prominent feature of this disease (RP, vasculitis). New biochemical techniques allowed the purification and characterization of nuclear antigens, such as nRNP. Chapter VI presents a solid phase ELISA for quantitation of antibodies to nRNP. nRNP was purified from commercially available rabbit thymus preparation. Positive results in this ELISA are obtained only in anti-nRNP or anti-Sm positive sera (as characterized with the counterimmunoelectroforesis method using reference sera). This finding supports the concept of the occurrence of Sm, both alone and in complex with nRNP. Although anti-nRNP levels as measured by ELISA method correlate with titers of antinuclear antibodies obtained by the immunofluorescence method, the ELISA method is preferable because of the more accurate reading and the highly purified antigen preparation. As a result fluctuations of anti-nRNP levels could be registered in longitudinal observations using this method.

In Chapter VII, anti-nRNP levels as quantitated by ELISA were studied in a follow up study in eleven patients with connective tissue disease. Levels of another autoantibody (IgM rheumatoid factor), an antibody to a recall-antigen (tetanustoxoid) and total amount of immunoglobulin G were also determined. No changes in anti-nRNP levels are noted in patients with minor activity of disease, but major flares coincide with a rise in anti-nRNP antibodies unless immunosuppressive agents are used. Interestingly, each rise in anti-nRNP, except in one
patient, precedes a major flare whereas anti-nRNP levels decreased in relation to clinical improvement and concomittant immunosupressive treatment. In conclusion, quantitation of anti-nRNP by ELISA may be a guide for assessing disease activity in connective tissue disease. However, all patients also showed fluctuations in rheumatoid factor and total immunoglobulin G levels. Parallel fluctuations are also seen in anti-nRNP and anti-tetanustoxoid levels except in one patient. These findings, together with the recognition of several polypeptides by anti-nRNP in immunoblotting, point to a polycional stimulation of the immune system in these patients with CTD. Future biochemical studies of structure, organization and the roles in nuclear metabolism probably will lead to more evidence of the pathogenetic significance of these antibodies.

References.