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 nucleoribo-nucleoprotein (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 an expression of the disease (Chapter I), or as a consequence of treatment (6). This is an aspect which ought to be reviewed.

Patients with systemic sclerosis showed a special pattern in this study. Nevertheless, these patients were comparable to both healthy controls and patients with SLE referred to our clinic. It was the long visible loops (6). This is an aspect which needs further clarification.

The low prevalence of capillary abnormalities in the group of scleroderma patients is in line with the course of the disease in these patients. In about 40% of the patients with more progressive forms of scleroderma, the disease show a higher prevalence of capillary abnormalities. This may be related to other criteria for disease activity.

In Chapter IV, nailfold capillary microscopy is applied to clinical findings in chronic venous insufficiency, Raynaud's phenomenon, ulcers, tuft resorption, and disease activity in chronic venous insufficiency. The frequency of bushy patterns is comparable to those in rheumatoid arthritis and systemic lupus erythematosus. These symptoms may be used as indicators of disease activity.

Decreased capillary density is a frequent sign of systemic involvement. In particular, in patients with Raynaud's phenomenon, the relationship between capillary density and the course of Raynaud's phenomenon is of importance.

Besides several practical applications, the nailfold is an ideal model for the study of blood vessel abnormalities in the microcirculation. By referring patterns to specific diseases, capillary abnormalities are described, which are characteristic for each and every disease.
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of number of giant loops.
the presence of bushy
local paucities. It is
medical practice, the
microscopy, since
were not evaluable.
visualization of nailfold

Applying the seven most
scores for all digits is
results in distinguishing
primary RP did not differ
striking RP did not differ
CREST and MCTD,
giant loops especially
the nailfold was deduced.
Since a similar
conclusions in patients with
out of capillaries in the
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comparison of a healthy control
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maceration in microvessels
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CTD (5). Probably bushy patterns in healthy subjects and primary RP
may be considered as either a normal variation of a meandering loop
(Chapter I), or as a consequence of local trauma (Chapter III).

Patients with systemic lupus erythematosus (SLE), were not included
in this study. Nevertheless, we found a normal capillary density (com-
parable to both healthy controls and patients with primary RP) in pa-
tients with SLE referred to our department. The most striking finding
was the long visible length of the capillaries, also reported by others
(6). This is an aspecific feature possibly due to inactivity (Chapter I)
or to treatment with corticosteroids, as we have seen in patients with
MCTD. Thus, nailfold capillary microscopy does not seem to be an
useful aid in the diagnosis of SLE.

The low prevalence of abnormal nailfold capillary findings in our
group of scleroderma patients may be attributed to the rather benign
course of the disease in these patients. Additional data in scleroderma
patients with more progressive disease than those originally studied
show a higher prevalence of capillary abnormalities and may confirm
this statement (Addendum Chapter III).

In Chapter IV, nailfold capillary density has been studied in relation
to clinical findings in patients with RP.

Capillary density was decreased in patients with sclerodactyly, digital
ulcers, tuft resorption, and telangiectasia, compared to patients without
these symptoms. The capillographic findings in scleroderma are quite
comparable to those in atrophy blanche (7). Areas of atrophy blanche
in chronic venous insufficiency as well as the fingers in scleroderma
are predilection sites for ulceration. Thus, ulceration of the skin is
related to obliteration of microvessels.

Decreased capillary density was found to be inversely related to organ
system involvement. Decreased capillary density was observed, in part-
icular, 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 de-
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 superimposed 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 and 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 whe-
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terized by the nailfold during serial

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cular function by non-

whether these components interact with each other or whether they are

that his disease is associat-

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its severity. This test and warming up is a

changes in connective tissue diseases, such as the presence of autoanti-

observed in secondary

increased levels of circulating immune complexes, increased

transmission video micro-

is reduced capillary drop

narcotic seen in serial micro-

disease are noted in patients with minor activ-

nRNP antibodies, increased levels of acute phase reactants, were each associated with a decreased

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

the presence of Sm positive sera (as characterized

levels of acute phase reactants, were each associated with a decreased

creased levels of acute phase reactants, were each associated with a decreased

antinuclear antibodies obtained

the immunofluorescence method, the ELISA method is prefe-

neuro-ribonucleoprotein (nRNP). Antibodies to nRNP are a hallmark of CTD which ultimately
developes 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 an-
tigens, such as nRNP. Chapter VI presents a solid phase ELISA for

quantitation of antibodies to nRNP. nRNP was purified from commer-

cially available rabbit thymus preparation. Positive results in this ELISA

are obtained only in anti-nRNP or anti-Sm positive sera (as characterized

were both implicated

are both implicated

not known whe-
patient, precedes a major flare whereas anti-nRNP levels decreased in relation to clinical improvement and concomittant immunosuppressive 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 polyclonal 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.