Intravital Detection of Skin Capillary Aneurysms by Videomicroscopy With Indocyanine Green in Patients With Progressive Systemic Sclerosis and Related Disorders

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Conventional capillaroscopy and infrared fluorescence videomicroscopy with indocyanine green were performed at the nailfold in 12 healthy controls and 38 patients with microangiopathy due to systemic sclerosis or related disorders. Saccular aneurysms featuring head and neck (type 1) and aneurysmatic enlargements (type 2) were defined. Microaneurysms were located at the apex or near the apex of capillary loops and were significantly more common in patients than in controls ($p<0.02$ for type 1 and $p<0.001$ for type 2). Combination of the two lesions was found only in patients and appears to be a valuable new diagnostic sign for the presence of microangiopathy. In comparison with conventional capillaroscopy, about twice as many microaneurysms were detected by videomicroscopy with indocyanine green coupling almost completely to plasma proteins. The new technique allows visualization of capillary aneurysms even when filled only by plasma. (Circulation 1991;83:546–551)

A suitable intravital technique for assessing capillary aneurysms in human skin is lacking. Although sodium fluorescein has been used for evaluating dynamics of human skin microcirculation, the dye diffuses rapidly through the skin capillary wall, which is usually not visualized.1,2

Indocyanine green (ICG) was recently introduced into the study of human skin microcirculation,3 and it can be used to detect capillary aneurysms. It binds almost completely to plasma proteins4 and delineates the inner surface of the capillary wall.3,5

The present investigation was designed to evaluate the ability of infrared fluorescence videomicroscopy with ICG to visualize capillary aneurysms in 12 healthy controls and in 38 patients who showed typical microangiopathy at the nailfold by conventional capillaroscopy.6–8 Most of the patients had progressive systemic sclerosis or related disorders.

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Methods

The nailfold capillaries were visualized by conventional capillaroscopy and by fluorescence videomicroscopy after intravenous bolus injection of ICG. The latter technique was first introduced for use in choroidal angiography9 and was later adapted to studies of skin microcirculation.3,5

The setup has been described previously in detail.3 Essentially, it consists of an incident light fluorescence microscope (Wild-Leitz, Zürich, Switzerland), an infrared sensitive microchip videocamera (Krantz, Taunusstein, FRG), a television monitor (Philips) with a time and scale marker (For-A Company), and a tape recorder (BK 204, Grundig).

ICG fluoresces in the near infrared portion of the spectrum3,9 and has a peak emission at about 835 nm. A special filter set (Ditric Optics, Marlboro, Mass.) is required for intravascular detection of the tracer.9 The vital dye binds almost completely to plasma proteins.4,10 Plasma clearance is biphasic, showing a rapid initial phase with a half-time of 3–4 minutes and a secondary phase with a half-time of more than 1 hour at low concentrations not relevant for intravital fluorescence videomicroscopy.10 ICG is eliminated by the enterohepatic route and is only minimally reabsorbed by the gastrointestinal tract.10
Study Population

Twelve healthy volunteers made up the control group. Six were men, and six were women. The mean age was 36.3 years (range, 22–58 years). Blood pressures, blood glucose levels, and creatinine levels were within normal limits.

The patient group consisted of 38 patients with a mean age of 44.7 years (range, 17–73 years). Twelve were men, and 26 were women. They suffered from collagen vascular disease or related disorders. Progressive systemic sclerosis was diagnosed in 17 patients (criterion of the American Rheumatism Association), dermatomyositis in two, lupus erythematosus in one, livedoid vasculitis in two, Sjögren’s syndrome in one, and cryoglobulinemia in one. The remaining 14 patients exhibited clear-cut microangiopathy at the nailfold according to the criteria established by conventional capillaroscopy \(^2,5-9\) and fluorescence videomicroscopy with sodium fluorescein. \(^2\) All patients had Raynaud’s phenomenon or arthralgias. Their disease could not be definitively classified, although incipient collagen vascular disease was suspected. Mean disease duration in all patients with microangiopathy was 4.3 years (1–25 years). Diabetics, hypertensives, and patients with elevated levels of blood creatinine were excluded.

Procedure

Conventional capillaroscopy with white light was first performed at the nailfold of fingers II–V of both hands to obtain an overview of capillary morphology and to select a finger with optimal skin transparency (usually the ring or small finger). The finger was embedded and fixed in plasticine. A drop of paraffin oil rendered the skin more transparent.

Capillary distribution, shape, diameter, and number were assessed first at a low magnification of \(\times 137\) on the television monitor (plan objective, 2.5/0.08) and afterward at a high magnification of \(\times 540\) (plan fluorotar objective, 10.0/0.30). A television video recording documented skin capillary morphology in the capillaries selected for study with ICG. Care was taken to include representative microvessels well visible at the end row of the nailfold. Room temperature varied between 22° and 24°C.

After the patients had rested for at least 20 minutes in the supine position and after a small catheter had been placed in an antecubital vein, ICG was dissolved in 10 ml physiological saline and was injected as a bolus within 5–10 seconds (50 mg/l blood volume). Mean concentration of ICG in the bolus was 232 mg/10 ml saline solution. The individual blood volume was estimated according to the method of Dagher and coworkers\(^{11}\) by using a nomogram that adjusts for age and weight. The capillaries selected for study were visualized again and were focused on before switching to the fluorescent filter set. The high magnification described above was used. ICG filling the nailfold capillary loops was detected on the television screen by the infrared sensitive videocamera. The capillaries were focused on again. The time between the end of injection and first appearance of the dye was recorded. Continuous video recording lasted for 3–5 minutes. One to four capillary loops were analyzed in the field of observation. Optimal dye filling (maximal fluorescent light intensity in the capillary loops) within the preselected area was the criterion for inclusion regardless of loop size and presence of suspected aneurysms.

Evaluation

The different elements for diagnosing microangiopathy\(^2,6-8\) were assessed during conventional capillaroscopy. Diameters were determined on single frames of the television video recording by drawing the capillary contours on transparent paper. The measurements were made on an axis crossing the arterial and venous limbs of the capillary loop at a distance of 33 µm from the apex. The observer looked for any erythrocytes moving outside the streamline of the capillary loops (red blood cells whirling in microaneurysms).

Diameter measurements after ICG filling of the capillaries were obtained as previously described on the same capillary loop. The time of best dye filling was selected for measurements and potential visualization of capillary aneurysms.

Definitions

During preliminary work, two different types of capillary aneurysms were defined. Type 1 aneurysms were defined as having a smaller neck and a larger head (saccular aneurysms); type 2 aneurysms were defined as having a diameter of any visible part of the capillary loop that is at least three times the diameter of the smallest loop segment. When both type 1 and 2 aneurysms were present in the same patient, a combination of the two conditions was diagnosed. By conventional capillaroscopy, type 1 aneurysms were suspected when whirling erythrocytes were observed outside the center stream, and type 2 aneurysms were suspected when the maximal width of the erythrocyte column met the criterion of the previous definition.

For comparing the ability of conventional capillaroscopy and the ICG technique to diagnose skin capillary aneurysms, we selected for study the capillaries with optimal dye filling. One to four well-depicted loops from each subject were included for comparative evaluation. The number of identical capillaries studied by both methods was 29 from the 12 controls and 114 from the 38 patients with microangiopathy. In each control and patient, a diagnosis was made regarding the presence of type 1, type 2, or both types of aneurysms.

Statistical Analysis

The Mann–Whitney test was used to compare the times between injection and appearance of the fluorescent dye in the nailfold region and to compare the diameters of capillary including the plasma layer and of red blood cell column in controls and patients.
Possible differences between controls and patients in prevalence of type 1 and 2 aneurysms were evaluated by the χ² test.

The study protocol has been approved by the ethical committee of the University Hospital. Informed consent was obtained from each participant.

### Results

#### Conventional Capillaroscopy

According to the inclusion criteria, capillary morphology at the nailfold was normal in all the controls and was definitely pathological in the patients. Presence of many enlarged or even giant capillaries was observed in all patients. Rarefaction of loops was found in 30 (79%) patients.

The presence of type 1 capillary aneurysms was suspected in one of 12 controls. Some erythrocytes seemed to be localized outside the streamline of the capillary loop. In three additional controls, type 2 aneurysms were diagnosed. Nine patients with microangiopathy appeared to have type 1 aneurysms, and 17 appeared to have type 2 aneurysms.

Mean diameters of the red blood cell column at the arterial, venous, and apical parts of the capillary loop are given in Table 1. In the patients, mean diameters of the three loop segments were significantly (p<0.05 and 0.01) larger than those of the controls.

#### Infrared Fluorescence Videomicroscopy With ICG

The time elapsing between the end of intravenous injection of ICG and dye appearance at the nailfold averaged 49±23 seconds in the controls and 51±26 seconds in the patients with microangiopathy. The difference was not significant. Maximal fluorescent light intensity emanating from intracapillary ICG occurred 30–120 seconds after dye arrival.

The diameters of the capillaries including red blood cell column and plasma layer are listed in Table 1. In the patients, mean diameters of the arterial, venous, and apical parts of the capillary loop were significantly (p<0.05 and 0.01) larger than those of the controls.

Saccular aneurysms with head and neck (type 1) were present in 21 patients (55%), whereas type 1 aneurysms were diagnosed in only two controls. These aneurysms were exclusively located at the apex or near the apex of the capillary loop. A typical example is shown in Figure 1. In most cases, the fluorescence emanating from the aneurysmatic part of the capillary was more extensive than the fluorescence observed emanating from the arterial or venous limb of the loop.

Aneurysmatic dilatations of type 2 (Figure 2) were detected in 30 patients (79%) and in three controls. The dilatations either involved the apical part of the loop or the adjacent ascending or descending limb.

A combination of type 1 and 2 aneurysms was found in all 21 patients (55%) exhibiting type 1 aneurysms. In other words, the two aneurysmatic

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**Table 1. Mean Diameters of Red Blood Cell Column (by Conventional Capillaroscopy) and of Full Capillary (by Indocyanine Green) in Controls and Patients**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Red blood cell column</th>
<th>Capillary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial side</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>12.1±3.0</td>
<td>17.8±3.9</td>
</tr>
<tr>
<td>Patients</td>
<td>19.8±8.8*</td>
<td>25.9±9.7*</td>
</tr>
<tr>
<td>Venous side</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>13.7±4.1</td>
<td>20.1±4.4</td>
</tr>
<tr>
<td>Patients</td>
<td>22.9±9.0*</td>
<td>32.6±12.0*</td>
</tr>
<tr>
<td>Apex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>19.2±7.2</td>
<td>32.3±7.5</td>
</tr>
<tr>
<td>Patients</td>
<td>35.5±17.3†</td>
<td>50.7±21.4†</td>
</tr>
</tbody>
</table>

Values in μm are mean±SD; n=12 for controls, and n=38 for patients.

The values for the controls were published previously.3

*p<0.05, †p<0.01 vs. controls.

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**Figure 1. Type 1 capillary aneurysms in nailfold loops of a patient with mixed connective tissue disease. Left panel: Conventional epi-illumination capillaroscopy does not depict microaneurysms. Right panel: Infrared fluorescence videomicroscopy with indocyanine green reveals two type 1 aneurysms at the apex of both loops. Most intensive fluorescence is emitted by aneurysms.**
changes were most often combined. Only in nine patients were type 2 aneurysms diagnosed without
the presence of type 1 changes. No control subject
had a combination of both forms of aneurysms.

The prevalence of type 1 capillary aneurysms was
significantly higher in the patients with microangi-
opathy than in the controls ($p<0.02$). Also, the
significance was higher for type 2 microaneurysms
($p<0.001$) and for the combination of the two
changes ($p<0.001$).

Comparison of Conventional Capillaroscopy and ICG
Technique for Detection of Microaneurysms
In Figure 3, diagnosis of type 1 and 2 aneurysms is
illustrated for single capillaries of controls and pa-
tients according to conventional capillaroscopy
and the ICG technique. More aneurysmatic lesions
were diagnosed by fluorescence videomicroscopy than by
capillaroscopy. In 13 patients with microangiopathy,
type 1 aneurysms were detected only by the ICG
technique, in eight patients by both procedures, and
in one patient suspected of having lesions by conven-
tional capillaroscopy but not confirmed by the ICG
technique. Similar results were obtained for type 2
lesions (Figure 3).

Discussion
In previous work, diameters of nailfold capillaries
have been measured by the ICG method.\textsuperscript{3,5} Because
the dye couples almost completely to plasma pro-
teins,\textsuperscript{4,10} it depicts the full capillary diameter includ-
ing the plasma layer on both sides of the red blood
cell column. By subtracting the width of the erythro-
cyte column from the full capillary diameter, we
obtained the width of the plasma layer. Two facts
suggest that the real capillary diameter is determined
by measuring the thickness of the column depicted by
ICG. In contrast to sodium fluorescein,\textsuperscript{1,2} ICG does
not fill the pericapillary area of the interstitial space
by crossing the microvascular wall. The diameter of
the full capillary does not increase after first dye
appearance. However, coupling of ICG to the glyco-
calix tapering the endothelial cells and to the small
endothelial cells themselves cannot be excluded be-
cause of insufficient optical resolution.

The diameter of the flowing red blood cell col-
umn in nailfold capillaries is well known in healthy
controls\textsuperscript{1–3,12,13} and in patients with progressive
systemic sclerosis.\textsuperscript{2,5,12} The values measured in this
study are within the range described in the litera-

![Figure 2. Type 2 capillary aneurysms in a nailfold loop of a patient with progressive systemic sclerosis. Left panel: Conventional epi-illumination capillaroscopy. Right panel: Fluorescence videomicroscopy with indocyanine green.](http://circ.ahajournals.org/lookup/doi/10.1161/01.CIR.91.10.549)

<table>
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<tr>
<th>Prevalence of microaneurysms in healthy controls</th>
<th>Prevalence of microaneurysms in patients with microangiopathy</th>
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<td><strong>ICG - technique</strong></td>
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![Figure 3. Prevalence of capillary aneurysms with epi-illumination capillaroscopy and indocyanine green technique. Left panel: Chart for healthy controls. Right panel: Chart for patients with microangiopathy.](http://circ.ahajournals.org/lookup/doi/10.1161/01.CIR.91.10.549)
ture. In studies involving only patients with advanced systemic sclerosis and not a larger spectrum of collagen vascular disorders, capillary enlargement is still more pronounced. Of note, normal nailfold capillaries are wider than capillaries in most other areas of the body.

The width of the plasma layer has previously been determined by the ICG technique in healthy controls and in patients with progressive systemic sclerosis. An alternative method able to assess plasma layer width and, possibly, capillary aneurysms has been described by Mahler and coworkers, who injected FITC-labeled (fluorescein isothiocyanate) human albumin into the cubital artery of healthy volunteers and observed the passage of the fluorescent compound through nailfold capillaries. Their mean value for the width of the plasma layer on both sides of the erythrocyte column was 10.8 ± 3.0 μm (compared with 12.1 ± 3.0 μm for the controls in the present study). On one side of the red blood cell column, the plasma layer reaches approximately half that value.

The mean width of the plasma layer on one side of the erythrocyte column (Table 1) is lowest at the arterial side of the capillary (2.9 μm in controls and 3.1 μm in patients) and largest at the apex of the hairpin-shaped loop (6.6 μm in controls and 7.6 μm in patients). This difference is best explained by the increased diameter of the capillary in the curved section near the loop apex. Much lower values are observed in the straight parts of the ascending and descending limbs of the loop in patients and controls.

The time normally elapsing from the end of intracubital injection of sodium fluorescein to first dye appearance at the nailfold is about 30 seconds. Two factors may explain the longer circulation times in the present study. First, each group contained one subject with a particularly long dye appearance time (80 seconds); second, ICG that has been dissolved in 10 ml physiological saline is a more viscous fluid than the solution of sodium fluorescein administered in a smaller volume of 1–2 ml. Injection requires 5–10 seconds for ICG and 1–2 seconds for sodium fluorescein. Most probably, the ICG bolus starts with a lower initial flow velocity than does the sodium fluorescein bolus. In agreement with earlier findings obtained by sodium fluorescein, mean circulation time in the present study was not significantly different in controls and in patients with connective tissue disease.

Because of the coupling of ICG to plasma proteins, the ICG technique visualizes capillary aneurysms more accurately than does conventional capillaroscopy. The inner contours of microvessel walls are well delineated by the dye.

Conventional capillaroscopy depicts the erythrocytes moving in microvessels but not the plasma layer. Aggregates of erythrocytes visualized by conventional capillaroscopy occur frequently with low flow velocity and may simulate the presence of aneurysmatic enlargements (type 2). Because only about half of the microaneurysms were suspected without ICG application (Figure 3), we can reasonably assume that the remaining lesions were filled by plasma alone or did contain a minor number of red blood cells difficult to recognize. By staining the plasma, ICG renders capillary aneurysms visible even in the absence of corpuscular elements. A typical example for plasma-filled type 1 aneurysms is shown in Figure 1. Probably, microvascular hemodynamics are responsible for the fact that not all the microaneurysms are filled by red blood cells. The latter tend to follow the main direction of flow governed by the main pressure gradient. In some microaneurysms, a small neck may impede the passage of corpuscular elements into the saccular enlargement containing plasma.

Probably, the diagnostic accuracy of conventional capillaroscopy for detecting capillary aneurysms is overestimated by the data presented in this study. The analysis of the video images was performed by two skilled observers who were particularly aware of the possible presence of microaneurysms (whirling of erythrocytes outside the main stream and enlarged segments of the erythrocyte column).

Although fluorescence videomicroscopy with ICG appears to be the only technique available at present to depict capillary aneurysms with accuracy, some sources of erroneous interpretation have to be considered. The fluorescent light emanating from ICG that circulates in the capillary loops is not very intense. Therefore, the edges of the capillary wall may not be sharply delineated in some instances. This limitation may be overcome in the future by novel techniques of image enhancement and by further improvement of infrared fluorescence videomicroscopy. Other possible sources of error include tortuous loops suggesting apical aneurysm formation, occasional superimposition of two capillary loops, and the subjective evaluation of whether head and neck are present (borderline cases).

Prevalence of type 1 capillary aneurysms was significantly (p < 0.02) increased in patients with microangiopathy. This increased prevalence was established in selected capillaries well filled by the dye and not in a random sample of nailfold capillaries. The unexpected finding of occasional type 1 microaneurysms in healthy controls is probably explained by trauma often involving the nailfold region.

Because widened capillaries are well known to occur frequently in systemic sclerosis and related disorders, type 2 aneurysms may be associated with abnormal loop characteristics of this disease. The significance level of increased prevalence (p < 0.001, type 2) is higher than the significance level observed for type 1 lesions (p < 0.02). The combination of the two forms of capillary enlargement offers the best differentiation between controls and patients.

Microangiopathy diagnosed in all 38 patients was due to different disease states with predominance of progressive systemic sclerosis and related disorders. The frequent occurrence of capillary aneurysms in
microangiopathy suggests that this finding represents a new diagnostic element. Because the combination of type 1 and 2 aneurysms has not been encountered in control subjects, but often in patients, it is potentially the best diagnostic criterion and supplements the established criteria of giant capillaries, rarefaction of loops,\textsuperscript{2,6–8,16} and asymmetrically enhanced transcapillary diffusion of sodium fluorescein.\textsuperscript{2,17}

Probably, microaneurysms are not specific for progressive systemic sclerosis and related disorders. They are well-recognized elements for the diagnosis of diabetic retinopathy.\textsuperscript{18–20} At the nailfold, a symmetrical increase of transcapillary diffusion (by sodium fluorescein) with respect to the capillary loops has been found in subjects with chronic diabetes.\textsuperscript{21} Whether the prevalence of capillary aneurysms is augmented in these patients as it is in patients with collagen vascular disease and microangiopathy is of future clinical interest.

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References


Key Words • progressive systemic sclerosis • capillaroscopy • videomicroscopy • microaneurysms
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