The use of microcirculatory techniques in the assessment of pathophysiology, diagnosis and management of critical limb ischemia

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Chapter 7

The feasibility and reliability of capillary blood pressure measurements in the finger nail fold

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Abstract

Introduction Capillary blood pressure is an essential parameter in the study of the (patho-) physiology of the microvascular perfusion. Presently, capillary pressure measurements in humans are performed using a servonulling micropressure system containing a oil-water interface, which suffers some drawbacks. In addition, the effect of the preparation of the skin and the presence of the tip of the pipette in the capillary during the measurement on microcirculatory perfusion has never been described. Therefore, we assessed the feasibility of capillary pressure measurements using an alternative micropressure system using an air-water interface (900A, WPI), as well as the effect of the measurement on local microcirculation.

Methods In 19 healthy male volunteers the apex of capillaries in the eponychium of the fourth finger was punctured, after skin peeling, by a micropipette connected to a servo-nulling micropressure system. Red blood cell velocity (RBCV) was assessed after peeling during the measurement and at an adjacent area.

Results Mean capillary pressure (in 16/19 volunteers) was 20.5 ± 3.7 mm Hg (systolic: 26.2 ± 5.6 mm Hg, diastolic 17.6 ± 3.9 mm Hg). RBCV was not significantly different between before (0.52 mm/s) and during the measurement (0.51 mm/s), and at an adjacent area (0.51 mm/s).

Conclusions Capillary pressure can be measured well with the alternative set-up used without hampering capillary flow, while the pressures obtained are in agreement with the results reported earlier by other investigators.
Introduction

Capillary microcirculation is essential for the nutrition of the skin, based on transcapillary exchange of fluids and solutes. In this process capillary blood pressure is a crucial factor, since the fluid exchange across the capillary membrane of a single, short section of a capillary, as initially proposed by Starling,\(^1\) is dependent on the transmural pressure gradient.\(^2\) Furthermore, the capillary pressure parameter may play an important role in microvascular regulatory mechanisms upon a change in posture, in peripheral ischaemia, and to investigate therapeutic effects of vasoactive drugs or vascular interventions.

Capillary pressure can be estimated by indirect functional measurements but also by direct cannulation.\(^3,4\) In the direct method, the pressure is measured by direct cannulation of the capillary. In 1930 Landis cannulated the capillaries with a micropipette communicating with a manometer, the height of which was adjusted to balance the blood pressure under observation.\(^5\) However, the size of the tip of the pipette is too small to allow fast intermittent flow in order for the manometer to trace heart beats.\(^6\) Therefore Wiederhielm et al.\(^7\) designed a dynamic, servo-nulling micropressure system. In this system the micropipette, filled with a 2M NaCl solution, is used as a ultra-low compliance transducer. The servo-nulling system balances the change in impedance, caused by an influx of blood into the pipette tip, by a counteracting pressure equal to the capillary pressure. In the commercially available system by Intaglia et al. this counteracting pressure is generated by a magnetic driving unit and transmitted to the pipette through a system filled with oil.\(^5,8\) This system has been used in humans in the finger nail fold by, among others, Mahler et al.,\(^9\) and Tooke and Shore et al.\(^4,10\)

Some practical drawbacks of the oil-filled servo-nulling system used thus far in humans are that the system needs to be absolutely free of air, which makes a pipette change a delicate procedure. In addition, measurements have thus far been performed through a layer of water, which reduces the visualisation of the capillaries with time.\(^11\) Recently, another servo-nulling micropressure system has become available using an air-water interface as described by Fein.\(^12\) The system has been used in many animal models but has never been applied in humans.

Furthermore, the disadvantage of any direct method is that the finger nail fold has to be prepared by paring away the cuticle and upper layer of the stratum corneum. In addition, the flow and, consequently, the pressure can be disturbed by the relatively large pipette tip (Ø approx. 4 mm) inserted in the tiny capillary (Ø approx. 8 mm). To date, the effect of skin preparation and presence of the tip of the pipette in the capillary on capillary perfusion has not been described.

In this study we investigated the feasibility and reliability of capillary pressure measurements of the nail fold (eponychium) in humans at heart level, by using this alternative micropressure system, and assessed the effect of skin preparation, presence of the tip of the pipette and pressure measurements on capillary red blood cell perfusion.
Subjects and methods

Nineteen healthy men (mean age: 28 ± 5 yr.) were investigated after a 30 min acclimatization period in a temperature controlled environment (25±1°C). Volunteers refrained from smoking and caffeine containing drinks for at least four hours before the measurement. All measurements were performed in the supine position with the hand at heart level. The investigation protocol was approved by the local medical ethical committee and conforms with the principles outlined in the declaration of Helsinki. Written informed consent was obtained from all volunteers.

Intracapillary blood pressure measurements were performed in the fourth finger of the non-dominant hand. Simultaneously, total skin perfusion was investigated by means of laser Doppler perfusion measurements. The blood pressure of the middle finger was measured using a Finapres BP Monitor (2300, Ohmeda, Louisville, CO, USA). Also, ECG and skin temperature (monitor 78342A, Hewlett Packard, USA) were monitored (figure 1). All measurements were sampled on-line and analysed off-line by means of a data acquisition system (AcqKnowledge III and MP 100WSW, Biopac System, Inc., Santa Barbara, CA, USA). The power supply of the whole system was electrically isolated for subject safety. During the same session the brachial systolic and diastolic blood pressures (Dinamap Plus, Criticon, Tampa, Fl, USA) were measured.

Figure 1. Schematic drawing of the application of the equipment as used in this study. The capillary blood pressure is measured with a servo-nulling micropressure system by direct cannulation, while visualized with a standard capillary microscope connected to a monitor. Simultaneously, the skin temperature, skin perfusion (laser Doppler), continuous finger blood pressure and ECG are monitored and stored in a computerized data acquisition system.
intravital capillaroscopy

The capillaries of the finger nail fold were visualised by means of a capillary microscope with motor focusing in combination with a video circuitry as described before. In short: The hand was fixed with a mass of clay on a cross table. A light of 100 W AC mercury arc, with two heat absorption filters (Leitz Ploemopak 2.1, Germany) and a polarizing filter in a POL-cube (Leitz, Germany) achieved incident illumination. In the POL-cube a 50% mirror is positioned at a 45-degree angle. The crossed analyzer in the POL-cube cancels the light reflected directly from lenses and skin, while the light from deeper tissues is transmitted. Capillaries were punctured while visualized using a 10x objective (PL Fluotar, 10/0.30 Leitz Wetzlar Germany), and a digital camera (Tm-6CN Pulnix America Inc., Sunnyvale, Ca, USA), giving a total magnification on a monitor (PM 931, Ikegami, Korea) of about 3110x (screen: 180 x 136 mm = 0.58 x 0.44 mm skin area). The images were stored on videotape for off-line analysis of the capillary red blood cell velocity (RBCV).

Capillary pressure measurements, circuit description

Capillary pressure was measured by direct puncturing of the capillaries by a micropipette filled with a 2M NaCl solution, connected to the servo-nulling micro pressure system (900A World Precision Instruments, Sarasota, FL), which has been described in detail by Fein. The apparatus contains an electrical circuit (located in the control unit and probe) and an air circuit (located in the pressure pod) which regulates pressure inside the pipette so that it equals the pressure outside the tip (figure 2). The electrical circuit is formed by a Wein bridge oscillator, which generates a 1000 Hz (sinusoidal voltage) constant carrier current through the microelectrode. A pressure control driver will automatically adjust the microelectrode tip resistance to a change in pressure outside the tip of the pipette. A buffer amplifier between the detector and the pressure control driver compensates for the inherent lag in propagating the pressure changes transmitted to the micropipette. The lag would normally cause the system to oscillate. A damping knob sets the amplitude of the buffer amplifier. Furthermore, a sensitivity knob regulates the sensitivity of the measurement.

Before each pressure measurement each new pipette was balanced and calibrated against atmospheric pressure in a drop of 0.9% NaCl solution, embedded in a ring of grease (High vacuum grease, Dow Corning Corp., Midland, MI, USA), at an adjacent site of the finger measured. At first each pipette was balanced in the open loop mode. The resulting Voltage change, which is caused by the variation of the individual pipette, is compensated in the follower circuit by advancing the electrode resistance dial so that the balance null is achieved (detected by a null detector). Thereafter the electrode resistance dial is somewhat increased (until the null detector reads 50 micro amperes). Then the system is switched to the measuring mode. The pressure control driver will then automatically adjust the microelectrode resistance to the higher value of resistance by drawing some of the external dilute solution into the tip. When a the proper resistance is reached the null detector needle turns to 0 microamperes. Now the interface between the 2 M NaCl solution and the 0.9% NaCl solution is located somewhere inside the pipette. The pressure required to balance the system is called the calibration pressure and is subtracted from the subsequent pressure reading.
The air circuit is located in the pressure pod, which is connected to a pressure sink (-300 mm Hg) and pressure source (+400 mm Hg) source. In the pressure pod the counteracting pressure is balanced by an equilibrium between a constant outflow of air to the vacuum and a adjustable inflow of pressure by a piezo-electric valve (figure 2). This counteracting pressure is conducted via a small flexible tube (length 20 cm) and a pipette holder to the base of the pipette. To increase the response time we minimized the volume of the connecting tubes. The pipette holder (MTO-AMC, Amsterdam, The Netherlands) allows for the electrical and air circuit between the pipette and the micropressure system (figure 2). The interface between the 2M NaCl solution and air is located in the pipette, while the electrical contact between the apparatus and the 2M NaCl solution is achieved by an Ag/AgCl wire coming from the pipette holder and extending into the pipette.

**Capillary pressure, investigation protocol**

The micropipettes, with a tip diameter varying between 3 and 4 µm, were pulled beforehand by means of a micropipette puller (Narishige PB-7, Sea Cliff, NY, USA) from a borosilicate glass tube (inner diameter: 0.73/0.75mm, outer diameter: 0.96/0.98 mm, MTO-AMC, Amsterdam, the Netherlands) with an omega dot to stiffen the tip and to facilitate filling. The pipettes were filled with a 2M NaCl solution with 10 E/ml heparin to prevent plugging. Typically, the resistance varied between 0.2 and 0.5 Meg Ohms. The pipette was manoeuvred into the apex of a capillary at an angle of approx. 40° to the skin by means of a micromanipulator (model M, Leitz, Wetzlar, Germany). Here the micromanipulator was mounted on a statue with an angle of 30°.

Firstly, the cuticle and the upper layer of the stratum corneum of the epidermis were removed with a surgical banana-shaped knife, a small gush, and/or tweezers to facilitate the puncture of the capillaries.

Volunteers were grounded to the micro-pressure system using an monitoring electrode (Red Dot Ag/AgCl nr 2255, 3M Health Care, Borken, Germany). Thereafter each new pipette was calibrated, and the sensitivity and damping were adjusted until an optimal signal without noise was achieved.

Subsequently, the capillaries were punctured while the servo-nulling system was in the open loop mode. The capillaries were punctured gently and the influx of some erythrocytes into the pipette indicated that the tip of the pipette was located in the capillary. Then the system was promptly switched to the measuring mode, through which the erythrocytes were flushed out of the pipette. During the measurement the capillary and finger pressure waveform was evaluated on a monitor (7803b, Hewlett-Packard GMBH, Boeblingen Germany). The correct position of the tip of the pipette could be checked by temporarily switching the system to the open loop mode, which caused an influx of blood in the tip of the pipette. Furthermore, the position of the tip of the pipette was adjusted so that flow through the capillary was visually unobstructed. A measurement was regarded valid when the capillary pulse pressure waves were in phase with the waveforms of the ECG, finger pressure and laser Doppler, whilst capillary flow was unobstructed for at least 5 s. The mean systolic and diastolic and mean pressures were derived from the valid interval. After the investigation any remaining shards were removed by wiping with a paper tissue. The puncture area was disinfected.

Separately, the accuracy of the system was evaluated every other 5 procedures by calibrating the system against a reference pressure system in a test set-up. The response time of the system was evaluated by applying a steep pressure
Figure 2. Schematic drawing of the principle of the micropressure system as described by Fein.12 The pipette, filled with a 2M NaCl solution is used as an ultra-low compliance transducer, of which the impedance is assessed continuously by the servo-nulling system. As soon as the pipette enters the capillary, the higher capillary pressure urges blood to enter the pipette. This results in an inward displacement of the interface at the pipette tip between 2M NaCl solution (with a relatively low resistance) and blood (having a higher resistance) from the capillary. An influx of blood into the pipette increases the impedance and vice versa. This change in impedance is detected by the system, which in turn generates counteracting pressure that readjust the starting impedance and with that the interface. This counteracting pressure reflects the capillary pressure and is measured with a pressure transducer. This counteracting pressure is generated by the equilibrium of a continuous airflow from a pressure source (+400 mm Hg) to a pressure sink (-300 mm Hg), of which the inflow can be regulated by a piezoelectric valve. The pipette holder constitutes the interface between the 2 M and the electrical and air circuit which is located in the pipette.
increase and decrease generated by a modified Finapres (BMI, TNO Amsterdam) to our test set-up. The reliability of the measurements was assessed by duplicate measurements at an adjacent capillary of the same fingers in 10 volunteers.

**Total skin perfusion**
Local skin perfusion was assessed simultaneously by means of a laser Doppler (Periflux 4001, Perimed, Sweden), which was attached adjacent to the puncture place at the nail fold using an unheated small probe (PF 407), a probe holder (PF PH 07-4) and double sided adhesive tape (Double-Stick Discs, 3M Health Care, St. Paul, MN). The filter time was set at 0.03 s to trace heartbeats. Laser Doppler is a simple non-invasive technique to assess total cutaneous blood flow, as it not only measures flow in the capillaries, but also in the subpapillary venular and arteriolar plexus and arteriovenous shunts.16

**Arterial blood pressure measurements**
Continuous finger blood pressure measurements were performed using a Finapres instrument (Finapres BP monitor, Omeda, Louisville, CO) on the middle finger of the target hand. The method is based on the development of the dynamic (pulsatile) unloading of the finger arterial walls using an inflatable cuff with built-in photo-electric plethysmograph.17

**Capillary red blood cell velocity**
From the recorded images the mean capillary red blood cell velocity (RBCV, in mm/s) was assessed in three capillaries in a peeled skin area and in another three capillaries of an untouched, adjacent skin area on the same finger. RBCV was also assessed during the pressure measurement to investigate the effect of the presence of the tip of the pipette in the capillary during the measurement on capillary perfusion. RBCV was measured by means of the Cap-Image software (Zeintl, Biomedical Engineering, Heidelberg, Germany), using the flying spot method.

**Statistics**
The results are expressed in means with standard deviations (±) and ranges, after testing for skewness. Possible differences in RBCV between just before and during the capillary pressure measurement were evaluated by the paired Student t-test, while the comparison of the RBCV in a peeled skin area with an untouched, adjacent area was performed using the unpaired Student t-test. The reliability of the capillary pressure measurements is expressed as the standard deviation of the differences between two repeated measurements in the same finger.

**Results**
In 16 out of the 19 (84%) volunteers one, and in 10/16 more acceptable measurements could be obtained. Generally, 1-15 pipettes were needed for a successful measurement. The resistance was routinely set at 0.4 Meg Ohms and usually only a marginal adjustment of sensitivity and damping was necessary to achieve a stable signal. The average total investigation time was 1½ hour per volunteer (range: 1-3 hours), including set-up of the equipment (15 min) and preparation of the skin (15 min). Penetration of the capillary by the tip of the pipette could easily be observed by an influx of some erythrocytes before the
system was switched on. If the flow through the capillary was visually unobstructed the pulse contour of the pressure could be visualised clearly, showing a steep upstroke in phase with the other measurements, whereas obstructing the flow caused irregularities or an increase in the pressure recording (figure 3). The duration of one continuous pressure recording varied from 5 s. to 2 min, and was typically stable over time (figure 4).

Mean capillary pressure was 20.5 ± 3.7 mm Hg (range 15.0 - 29.6 mm Hg). Mean systolic and diastolic capillary pressures were 26.2 ± 5.6 (range 17.0 - 37.9) mm Hg and 17.6 ± 3.9 (range 12.9 - 25.5) mm Hg, respectively. Mean systolic and diastolic blood pressures of the adjacent middle finger were 136 ± 17 mm Hg and 70 ± 8 mm Hg, respectively.

RBCV after preparation of the skin (0.52 ± 0.11 mm/s) did not differ significantly (p=0.22) from RBCV in an untouched area of the nail fold of the same finger (0.51 ± 0.13 mm/s; n=11 fingers). The capillary RBCV was not influenced by the presence of the tip of the pipette in the capillary during the measurement: the RBCV before (0.51 ± 0.16 mm/s) and during the pressure measurement (0.53 ± 0.16 mm/s; n=36 evaluations) were not significantly different (p=0.12). The mean temperature of the investigated finger was 29.5 ± 3.3°C, which did not change during the investigation. The reliability of the pressure in two capillaries in the same finger, expressed as the standard deviation of the difference between two paired measurements (n=10), was small: 2.8 mm Hg. The variability of the system was less than 0.1 mm Hg. The response time (10 – 90%) did not exceed 10 ms and was faster during an increase than a decrease in pressure (figure 5). No complications during and after the procedure were reported.

**Figure 3.** Typical example of a recording of the laser Doppler flux, capillary and finger pressure and ECG. The pressure wave with the dicrotic notch is clearly visible in the pressure signal.
Figure 4. Typical example of the variation over a longer time period of ECG, laser Doppler, capillary and finger pressure, with a valid measurement period indicated between the arrow, proceeding a period in which the pipette is obstructing the flow (encircled) and disturbed by noise.

Discussion

With this alternative set-up capillary pressure can be measured quite well and in a relatively limited time. It furthermore overcomes some drawbacks of previous techniques. The mean apical capillary pressure as measured here is in agreement with the direct apical capillary pressure measured by other investigators in the nail fold of the finger, varying between 17.3 and 21.1 mm Hg (18.7 [15.6 - 20.7]^{18}, 19.1 [14.1 - 23.6]^{19}, 18.0 ± 2.5 (only men, mean age: 32.5, n = 20)^{20}, 19.4 ±1.0^{11}, 21.1 ± 4.9^{21}, 17.3 [9.0 - 21.6]^{10} mm Hg). The reliability of capillary pressure measured in different capillaries across the nail fold is agreement with those reported by others (5.4 ± 2.0%).^{15}

With the indirect methods only the mean capillary pressure can be estimated from a whole or part of an organ in animal models (small intestine or skeletal muscle)^{22} or a whole limb in humans, although its use remains debatable.^{3,23} Theoretical drawbacks of direct capillary pressure measurements (which applies for both the conventional and the here proposed system) might be in the first place that manipulation of the skin and capillaries may influence the reliability of the capillary pressure and velocity. However, this study showed that capillary flow is not influenced by the removal of the stratum corneum nor by the presence of the tip of the pipette in the capillary. Secondly, the measurement can sometimes be
hampered by movement artefacts. Nevertheless, direct capillary pressure measurements appear to be the only way to obtain reliable data about capillary pressure.

Minimal breakage of the pipette tip does not influence the recording. Since the bridge balance is set higher than the lowest pipette resistance the interface between blood and 2M NaCl solution is slightly inside the pipette. With a small breakage of the tip, the resistance alters slightly and the interface is displaced. As long as the interface remains inside the pipette, the system will work. The system is therefore independent of minor changes in the pipette resistance. Hypothetically, even mixture of the 2M NaCl solution with blood or 0.9% NaCl would not lead to erroneous measurements. Furthermore, small shards caused by breakage of the pipette did not caused any problems afterwards.

The advantages of the present set-up are that it is relative insensible to disturbances, e.g. the pipette can be replaced quickly without extreme prudence. Furthermore, the position of the tip of the pipette can be checked by the influx of erythrocytes at the start and during the measurement, since the erythrocytes can pass through a cylindrical tube as narrow as 2.8 µm.24 With this set-up the measuring time is not limited by a reduced visibility of the capillaries by the 0.9% NaCl solution covering the measurement surface. Since we removed the superficial dead layers of the skin the capillaries were clearly visible without oil.13 Moreover, the method presented a good accuracy and response rate, so that the capillary pressure wave form can be investigated, which can be important in the investigation of the influence of pulsatile perfusion pressure on the basal (micro-) vascular tone.20,25-27

Figure 5. Graph of the accuracy and rise-and-dive time of the micropressure system (black line) calibrated against the reference pressure (grey line) in a test set-up.
In conclusion, the micropressure system used in this study is a reliable and worthwhile method to measure capillary pressures in the human nail fold. Furthermore, this study showed that the capillary flow and pressure is not hampered by the measurement itself. The method presented appears a promising tool to further investigate the (patho-)physiology and evaluation of therapeutic interventions of various diseases affecting the microcirculation (e.g., peripheral vascular diseases, diabetes mellitus, and vasospastic disorders) in both animals and humans.

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Reference List


