LASER DOPPLER FLOWMETRY IN MICROVASCULAR SURGERY

LASER DOPPLER FLOWMETRIE IN DE MICROVASCULAIRE CHIRURGIE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. C.J. RIJNVOS
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 4 NOVEMBER 1992 OM 15.45 UUR

DOOR

LEONARDUS NICOLAAS ANTHONIUS VAN ADRICHEM

geboren te Schiedam, Nederland

universiteits drukkerij

PROMOTIECOMMISSIE:

Promotoren:	Prof. Dr. J.C. van der Meulen
	Prof. R. van Strik
Leden:	Prof. Drs. J. Steketee
	Prof. Dr. H. van Urk

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Adrichem, Leonardus Nicolaas Anthonius van

Laser Doppler flowmetry in microvascular surgery /
Leonardus Nicolaas Anthonius van Adrichem. - [S.l:s.n.]. - I11.
Proefschrift Rotterdam. - Met lit. opg. - Met samenvatting in het Nederlands.
ISBN 90-9005454-5
Trefw.:laser Doppler flow-meting; vaatchirurgie.

De publicatie van dit proefschrift is financieel mogelijk gemaakt door;

Nederlandse Hartstichting

Perimed AB, Sweden

Aan mijn ouders

en

Aimée

CONTENTS

CHAPTER I: INTRODUCTION

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

CHAPTER III: CIGARETTE SMOKING AND MICROCIRCULATION

CHAPTER I: INTRODUCTION

A: The necessity for monitoring microcirculatory blood flow

A.1 Microcirculatory flow in plastic and reconstructive surgery

In modern plastic and reconstructive surgery a great variety of tissues is transposed, transplanted or replanted. Since the success of these operations is determined to a great extent by the circulation of the tissues, the anatomical details of blood supply are the basis of flap design and replantation surgery. Blood, passing through capillaries, delivers nutrients to and removes metabolic end-products from the basic living unit of the body - the cell - via the interstitial fluid, thereby determining tissue viability.

Assessment of blood flow and more specifically of microcirculation is of great clinical importance and is therefore a challenging clinical and research goal.

A.2 Establishing blood flow

Clinical signs of microcirculation were described by Hippocrates when he noticed calor and rubor as signs of hypercirculation due to inflammation. Because clinical signs are subjective and qualitative or at best semi-quantitative, they did not satisfy in practice. For this reason other, more specific methods were developed to assess blood flow. Flow assessment in the microcirculation began over 300 years ago when Antonie van Leeuwenhoek observed moving erythrocytes in the arteriolar network and capillaries of the tail of the eel. In addition to describing the vascular morphology he measured the velocity of individual red cells, which he found to be about 2 mm/sec in the arterioles. For these evaluations van Leeuwenhoek utilized a grain of sand as his unit of distance and the time required to pronounce a four-syllable word as his unit of time.[1]

Since the 17th century and especially during the last decades a great variety of methods to objectively measure blood flow have been developed. A number of these will be described, with special emphasis on laser Doppler flowmetry.

A.3 Nomenclature

Frequently used terms are summarized.

Flow

The amount of fluid that moves through a specified area in a specified time (m^3/s).

Blood flow

The amount of blood that flows through a specified area of tissue in a specified time.

Perfusion

The passage of a fluid through the vessels of a specific organ or tissue.

Vasculature

Architecture of vessels in the body or any part of it.

Microvasculature

The portion of the vasculature of the body comprising the finer vessels, sometimes described as including all vessels with an internal diameter of 100 microns or less.

Circulation

Movement in a regular or circuitous course, as the movement of blood through the heart and blood vessels.

Microcirculation

Movement of blood through the entire system of finer vessels (microvasculature).

Flux

The number of particles that moves through a specified area in a specified time (particles/s).

Flap

Tissue (skin, dermis, fascia, muscle, bone, tendon, nerve, omentum) that is transfered from one site to another site. This tissue is perfused with blood via small vessels without a special pattern (at random vascularised) or via a main artery and vein (island flap).

Pedicled flap

A flap that keeps a connection to the body. The blood is supplied to the flap via vessels in the connecting part (pedicle).

Free Flap

An island flap of which the vascular pedicle is transected and connected to vessels at the acceptor site.

Biological zero

The laser Doppler flow value in non–perfused tissue.

Brownian movements

Small random oscillations of particles in a medium, without displacement of this medium in a certain direction.

Perfusion Units

Unit introduced by the Perimed company to give a value to the measurements of the microcirculation by the Periflux^{R} laser Doppler flowmeters. This unit is arbitrary, but is related to microcirculatory flow.

CHAPTER I: INTRODUCTION

B: The laser Doppler technique to assess microcirculatory flow

B.1 History

B.1.1 The Doppler effect

Christian Johann Doppler (born on 29 November 1803 in Salzburg, Austria; died on 17 March 1853 in Venice, Italy) was the son of a noted master stonemason. Poor health restrained him from the same career as his father and (fortunately) he became a physicist. He noted that a whistle from a railroad train had a higher pitch when the train was approaching him and the pitch dropped when the train passed and moved away. Similarly he stated that light from a star, when observed from earth, shifts towards the red end of the spectrum (lower frequency) if the star recedes, and towards the violet (higher frequency) if it approaches. In 1842 he described this frequency change due to movement in his article "Ueber das farbige Licht der Doppelsterne und einiger anderer Gestirne des Himmels" and this phenomenon became known as the Doppler effect or Doppler shift. The first experimental verification of the acoustic Doppler effect was by Buys Ballot in Utrecht in 1845, using a locomotive drawing an open car with trumpeters.

The optical Doppler effect has been used in astronomy to determine the motion of celestial objects with high velocities (in the order of 100 km/s and faster). On a microscopic scale information about the thermal motion of atoms and molecules has been obtained using Doppler broadening of spectral lines, but only particles having velocities of at least 10,000 m/s were detected.[2]

B.1.2 The laser as a device to measure velocity

Introduction of the laser made it possible to detect slowly moving particles. The word laser is an acronym derived from "light amplification by stimulated emission of radiation". A laser device emits monochromatic light (light of one frequency) in

an almost not diverging (collimated) beam and the frequency depends on the type of laser. The energy emitted differs for every type of device.

It was already demonstrated that frequency changes in light caused by slow moving particles could be detected by the optical heterodyne (or photomixing) technique. When light, that is reflected from a moving target and therefore containing Doppler shifted frequencies (figure 1), is photomixed with light from its source beam, a "beating" frequency is created proportional to the Doppler shifted frequency. The beating frequency phenomenon of sounds is better known and is used by musicians to tune their instruments; the more equal the frequencies of two sounds are, the lower beating frequency arises (figure 2).

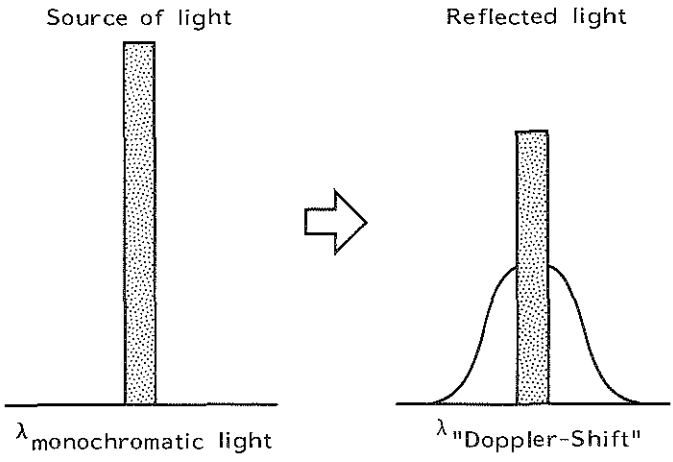


Figure 1. When laser light (monochromatic) is reflected by static structures the frequency is unchanged, but when particles are moving a Doppler shift occurs. A part of the emitted light is absorbed and therefore the intensity of reflected light is reduced.

The photomixing technique however was an imprecise method to establish the velocity of slowly moving particles, because the signal-to-noise ratio (i.e. the ratio of the authentic part of the obtained value and the part caused by disturbing factors) was very low.[3] When laser was used the signal-to-noise ratio improved significantly, because its light is monochromatic.

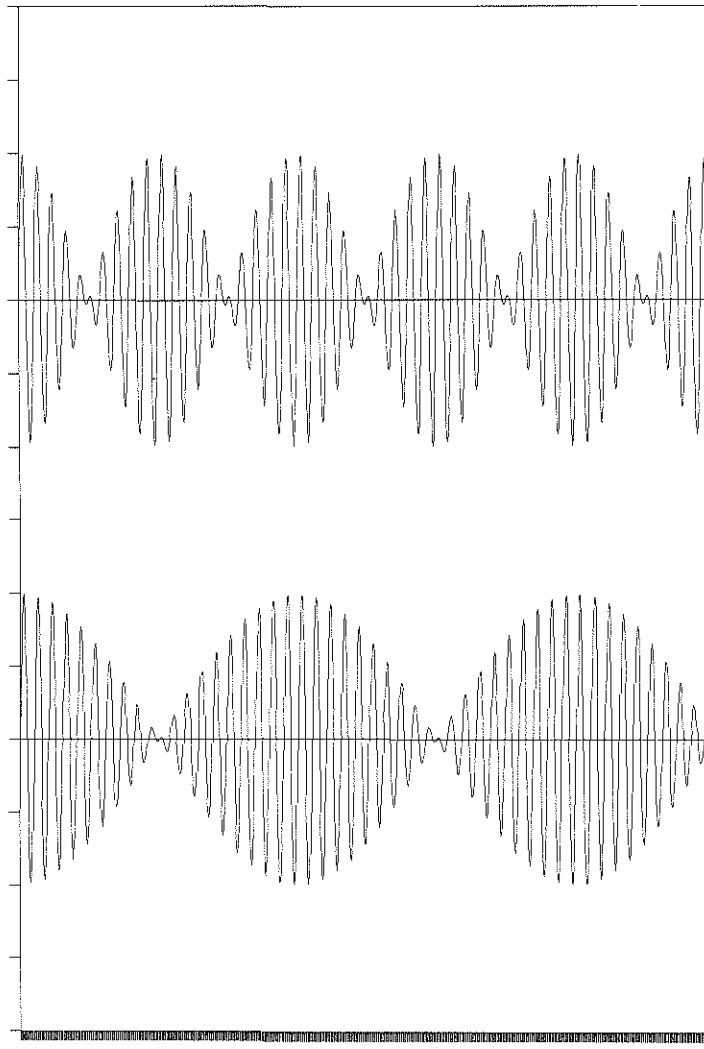


Figure 2. If two waves with a small difference in frequency are mixed a beating frequency arises. The more equal the frequencies are, the lower beating frequency arises. The upper figure shows a mixture of a 45 Hz and 50 Hz wave with a beating frequency of 5 Hz. The lower figure is a mixture of a 47.5 Hz and 50 Hz wave with a beating frequency 2.5 Hz.

B.1.3 The first laser Doppler

In 1964 Yeh and Cummins evaluated water velocity profiles in a transparant tube by the laser Doppler method, using polystyrene spheres with a diameter of 557 nm as light scatterers and velocities between 0.004 cm/s and 0.05 cm/s could be measured.[4] Lanz et al. reported that even measurements of pulsatile flow could be obtained by using the laser Doppler technique.[5]

B.1.4 The first biological application of the laser Doppler technique

The first biological application of the laser Doppler technique was by Riva et al., who investigated blood flow through retinal vessels in rabbits.[6] They demonstrated a linear relationship between the shifted frequency and the observed flow. Later Tanaka et al. studied the flow in single human retinal vessels.[7] Stern et al. demonstrated that the laser Doppler technique could objectivate cutaneous microcirculation: in the forearm skin they found a close correlation with the ^{133}Xe clearance method.[8,9] They also demonstrated a good correlation between the outcome of the laser Doppler technique and microsphere uptake in measurements of microcirculation of kidney cortex.[10]

B.1.5 The first commercially available laser Doppler (LD5000^{R})

Holloway et al. introduced fiberoptics additionally to the laser Doppler technique, using one fiberoptic cable to carry the light from a 5 mW He–Ne (632.8 nm) laser to the skin and one fiberoptic cable to send the reflected light to the photodetector (commercialized as the LD5000 Laser Doppler perfusion monitor^{R}, Med Pacific

Corporation, U.S.A.). They also found a close correlation between results of the laser Doppler technique and the ^{133}Xe clearance technique in assessing cutaneous flow.[11]

B.1.6 Introduction of a double photodetector system (Periflux^{R})

Nilsson et al. improved the signal–to–noise ratio by introducing a double photodetector system combined with a difference amplifier.[12] This apparatus used a 2 mW He–Ne (632.8 nm) laser and became commercially available as the Periflux^{R} laser Doppler flowmeter (Perimed AB, Sweden). Fluid model studies showed a linear relationship between the laser Doppler flow signal and fluid velocity x red cell volume fraction. In Europe this laser Doppler was the first to be sold in larger numbers and it became the most widely used laser Doppler perfusion monitor.

B.1.7 Introduction of the laser diode

The Laserflo BPM^{R} (TSI Inc, St. Paul, MN, USA; Vincent Medical, UK) was the first device with a laser diode as light source. The light from the 5 mW laser diode with an infrared wavelength of 780 nm has a diverging output beam, so a lens system is used to couple light to the optical fiber. This device uses a single photodetection system. The advantages of the laser diode are its lower cost and small size while it does not need high power voltage. The disadvantages are a less stable emission spectrum, dependency on temperature of the diode while the output of light can be influenced by backscattering.[13]

Jentink designed a new laser Doppler flowmeter with a diode laser (3 mW, 790 nm) and two detectors integrated into a small probe which can be attached to the

tissue.[14] This prevents distortion of measurements by movement of the commonly used optical fibers conducting light to and from the tissue. The main disadvantage is the high weight of the probe, which creates artefacts due to movement of the probe in relation to the examined tissue. The commercially available device is called Diodopp^R (Applied Laser Technology, the Netherlands).

The Moor laser Doppler flow monitor^R (Moor Instruments, UK) works with a 3 mW semiconductor laser diode (780–810 nm) and a single photodetector system in combination with optic fibers to conduct light to and from the tissue. It is the first dual–channel device, which means that two laser doppler flowmeters are united in one apparatus. Comparison of the different laser Doppler devices is described in more detail in chapter I.B.6 (Necessity for standardisation).

B.2 What is examined by the laser Doppler technique

B.2.1 Moving particles

The laser Doppler technique assesses moving particles, so it actually establishes flux. Particles can be shifted by Brownian movements or by flow. Movements of the measuring probe or fiberoptics cause artefacts.

Ever since its introduction the laser Doppler technique has been used to evaluate different types of movement. Blood flow through the cardiac ventricles and large vessels was assessed (laser Doppler anemometry) as well as the movements of particles in electrophoresis (laser Doppler spectroscopy). Furthermore sperm and cell motility, protoplasm streams and tympanic movements were established. The laser Doppler technique was also used to register vibrations (laser Doppler vibrometer) and to assess microcirculation. Microcirculation was examined in bone, brain, cochlea, esophagus, eye, gingiva, intestines, kidney, ligament, liver, lung, meniscus, mesenterium, mucosa, muscle, nerve, pancreas, salivary gland, skin,

stomach, testis, tongue, tooth, trachea and tumors. It is possible to measure microcirculation because the moving particles in the analysed tissue are mainly erythrocytes. Skin flow is most extensively investigated by laser Doppler flowmetry and it will be discussed in more detail, because some knowledge of vasculature and red cell movement is required to understand the working of the laser Doppler flowmeter. Unfortunately skin data cannot be extrapolated to other tissues, because every tissue has its own microcirculatory characteristics.

B.2.2 Anatomical and functional aspects of skin blood flow

The anatomy of cutaneous circulation shows a great variability due to development and aging, regional differences and effects of even minor pathology. There is however a general pattern which is characteristic for most skin areas (figure 3). The epidermis constitutes for most parts of the body of a 40 to 50 um

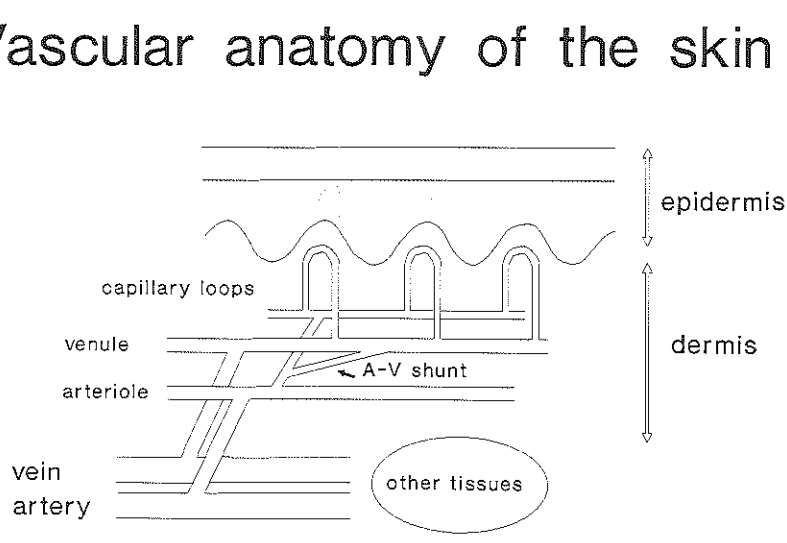


Figure 3. The vascular anatomy of the skin is described in more detail in chapter I.B.2.2.

thick keratin layer, free from blood vessels. In fingertips however the epidermal layer often reaches a thickness of 200–400 um. Underneath the epidermal layer the skin is supplied with a profuse system of capillaries which rise from the papillae of the corium and return to the subpapillary venous plexus. The length of a capillary loop is 0.2–0.4 mm and one capillary loop supplies on average 0.04–0.27 mm^2 of skin surface. The average capillary diameter is 10 um. The capillary layer is supplied by about 50 um diameter arterioles, which in turn originate from the arteries entering the lower dermis. The skin is drained by numerous venules, with diameters ranging from 40 to 60 um, in the upper and middermis and by thicker veins in the deeper tissue. Most of the larger vessels in the lower dermis run in a direction parallel to the skin surface. Arterio–venous anastomoses innervated by sympathetic fibers are mainly found in the dermis of the palms of the hands, the soles of the feet, the nose and in the ears. The diameters of these rather short shunting vessels with thick muscular walls range from 20 to 70 um in dilated state. The primary function of these vessels is to regulate the skin blood flow in relation to homeostatic control of body temperature. Blood cells in nailfold capillaries have an average velocity of about 0.5 mm/s with spontaneous periodic fluctuations. Areas, rich in anastomoses, show a great variability in blood flow, depending on the state of vasoconstriction. Total skin blood flow (per m^2 skin), which is determined by local, nervous and humoral control, was reported to be ranging from 1.3 to 13 ml/m^2*s. Other studies recorded values ranging from 2 to 6 ml/m^2*s for the forearm skin and from 12 to 60 ml/m^2*s for fingers under normal conditions.[15–24]

Insufficient blood flow can originate from the inflow, outflow or microcirculatory level. Lack of inflow is mainly caused by a (relative) deficiency of circulating blood volume, cardiac problems and arterial occlusion, stenosis or compression. Outflow may be hindered by cardiac problems and by venous occlusion, stenosis or compression. A variety of diseases impair microcirculation, for instance diabetes mellitus, scleroderma, sickle cell syndrome and thromboangiitis obliterans.

B.2.3 The examined skin volume

The interaction between light and tissue involves the process of absorption and multiple scattering. Detailed knowledge of the scattering process is however extremely difficult to obtain. The effective penetration depth, which depends on factors such as pigmentation, blood volume and degree of haemoglobin oxygenation, is also influenced by the colour of the laser light and by the distance of the emitting and receiving fiber. Jakobsson and Nilsson stated that the blood content of the skin is not of great influence on the measuring depth.[25]

The penetration depths in Caucasian skin are estimated to be 0.55 mm for light with a wavelength of 600 nm, 1.2 mm for 800 nm and 2.2 for 1200 nm.[14] Green light (543 nm) has a tissue penetration of about two thirds that of red light (633 nm) and theoretically only investigates nutritional capillaries. The absorption however is about 20 times greater and this makes clinical application difficult.[26] It is demonstrated that further fiber separation increased the penetration depth. Fiber separation over 3 mm relates to blood flow in deeper dermal tissue. Using red light and fiber separation less than 2 mm (He–Ne laser Doppler flowmeters) the capillary loops and vessels of the subpapillary plexus are mainly measured.

Functional studies indicate that the laser Doppler flowmeters assess flow in the nutritional capillaries and in deeper vessels including arterio–venous thermoregulatory shunts.[27-34] Unfortunately the examined volume cannot be defined with accuracy, because it has no sharp borders. Furthermore the results are presented in Perfusion Units (P.U.) which are directly related to microcirculatory flow, but no absolute flow values (expressed in milliliters per minute) are obtained. As expected spatial and temporal variations were detected in human skin flow by laser Doppler flowmetry.[35-39]

B.3 The laser Doppler technique in establishing tissue viability

In several studies a good correlation was found between laser Doppler flowmetry and tissue viability.[40–46] Lynch et al. reported a sensitivity of 85%.[47] Failure in the microcirculation when detected by the laser Doppler technique occurs well in advance of any noticeable physical change.[48] In full thickness burns a low laser Doppler value correlated with a microscopic picture of destroyed and obstructed blood vessels.[49]

Discrepancies were also found. Larrabee et al. studied random pattern rectangular skin flaps in piglets: the laser Doppler technique initially slightly underestimated the length of flap that survived. If the readings were taken 24 hours postoperatively however the laser Doppler technique predicted the length of the surviving flap exactly.[50]

It was demonstrated that tissues could survive periods without flow as established by the laser Doppler flowmeter, whereas apparently perfused areas later became non-viable.[51] It should be stressed however that flow can change in time, so a measurement at one time is not always representative for other periods. Furthermore tissues can endure a limited period of ischemia. Unfortunately the laser Doppler flowmeter not only measures nutritional flow in the capillaries, but also non-nutritional blood flow in deeper vessels including arterio-venous thermoregulatory shunts. For that reason the laser Doppler flowmeter can over-estimate nutritional blood flow and therefore tissue survival. Brownian movements of blood cells aggravate this condition.

In the postoperative monitoring of replantations and free vascularized tissue transplantations laser Doppler flowmetry generally recognized vascular occlusions, although some false predictions were made.[52–59]

B.4 Studies comparing laser Doppler flowmeters

Only a few studies have been performed to compare the different laser Doppler flowmeters. As expected a high correlation, but also some discrepancies were found.[60,61] Laser Doppler flowmetry systems using near infrared light (780 nm) semiconductor diodes sample larger volumes of tissue than those using He–Ne lasers (632.8 nm).[62] Some non–linearity appeared to occur at higher perfusion.[63] So data from different laser Doppler flowmeters have to be compared with caution, because differences in laser source, signal processing, probe design and calibration procedures influence the laser Doppler flow value. Comparison of the different laser Doppler devices is described in more detail in chapter I.B.6 (Necessity for standardisation).

B.5 Pitfalls in laser Doppler flowmetry

Laser Doppler assesses moving particles, so any movement will cause artefacts. Sources of these artefacts are fiber line movements, movement of probe–tip in relation to tissue and internal tissue changes. The influence of these sources of artefacts can be effectively suppressed by careful design and meticulous experimental setup. Movements such as respiration, heart beat and muscle fibrillations however cannot always be eliminated.

If the laser Doppler probe is placed at some distance to the tissue, the measuring characteristics change, so care has to be taken that the probe is just not in touch with the tissue by using the probe–holder properly.

Presence of blood between the probe and tissue influences the assessment (less or even no flow), so the interface has to be clean.

A laser Doppler flow value of (almost) zero does not directly indicate that the

tissue is dead, because tissue can overcome a period of minimal or no circulation, for instance due to vasospasm. On the other hand non-vital tissue can still have laser Doppler flow values above zero, probably due to Brownian movement of the particles (oscillations of blood particles).

Unfortunately differences exist between the various laser Doppler flowmeters and probes, so care has to be taken when attempting to compare data.

Damage to the fiberoptics causes false readings.

B.6 Necessity for standardisation

Results of laser Doppler studies are not always exchangeable, because of lack of consensus on a number of subjects.

There is no general agreement on the **nomenclature**. Indexing key words like "laser Doppler flowmetry", "laser Doppler fluxmetry" and "laser Doppler velocimetry" are used. The technique actually measures flux (movement of particles), which is directly related to microcirculatory flow. Laser Doppler flowmetry is the most frequently used term.

Signal processing differs in equipments from the various producers and even in various versions of flowmeters from the same manufacturer. Different **calibration** procedures are used. Differences in **probe design** cause variation in examined tissue volume and therefore values obtained with one probe cannot be compared to values recorded with other probes. Microvascular flow shows spatial and temporal variations. Single measurements are poorly reproducible; therefore mean values of **repeated measurements** have to be used.[64]

Although laser Doppler flowmetry undoubtedly has wide potentials for routine clinical use, the method can easily fall into disrepute because of the above mentioned lack of standardisation. Research workers and manufacturers have an

obligation to reach consensus on nomenclature, calibration, signal processing and probe characteristics and to give guide-lines for application in different tissues.

It should be stressed that it is impossible to select one of the laser Doppler devices as the best available. Every apparatus has its own characteristics and therefore its own advantages and disadvantages. The first laser Doppler devices were equiped with gas lasers (He-Ne) and they laid the foundations of experimental and clinical laser Doppler research and so they became the golden standard. Diode lasers, in contrast with gas lasers, need only a low operating voltage, have a low power consumption and a small size, but they emit their light in a complicated beam shape and light intensity as well as the emitted wavelength is dependent on the temperature of the diode laser. The diode laser Doppler flowmeters overcame these disadvantages by special engineering, resulting in flowmeters with a lower price than the gas laser Doppler flowmeters. The wavelength of the emitted light of the diode laser Doppler flowmeters is larger than in He-Ne laser Doppler flowmeters and therefore the diode lasers penetrate the skin deeper and they investigate nutritional capillaries less specifically. Double photodetection systems eliminate more disturbances than single photodetection systems. The laser Doppler device with a diode laser and two detectors integrated into a small probe which can be attached to the tissue, lacks movement artefacts due to the commonly used optical fibers. The main disadvantage, however, is the high weight of the probe, which influences the microcirculation and which causes more artefacts due to movement of the probe in relation to the examined tissue.

If a study on microcirculation is performed by using laser Doppler flowmetry, selection depends for instance on the device used in previous studies, on the desired penetration of the examined tissue, on the needed signal to noise ratio, on agreed standardisation and last but not least on the available finances.

B.7 Summary of the laser Doppler flowmeter characteristics

The laser Doppler technique is rather easy, objective, quantitative, non–invasive, it measures microcirculation without influencing it obviously while continuous monitoring for several days is possible. It reacts quickly to changes in microcirculation. The equipment however is expensive and the examined tissue volume cannot be defined with accuracy, because it has no sharp borders. Furthermore the results are presented in Perfusion Units (P.U.) which are directly related to microcirculatory flow, but no absolute flow values (expressed in milliliters per minute) are obtained.

The laser Doppler device can also be used as a photoplethysmograph. This application will be discussed in chapter I.C.4.2.

B.8 The laser Doppler flowmeter used in our studies

The Periflux^{R} laser Doppler flowmeter was used in our studies and will be described in detail to illustrate the laser Doppler flowmetry principle (figure 4). The Periflux^{R} 2a and 2b laser Doppler flowmeter uses a 2 mW He–Ne–laser (632.8 nm) as light source. The emitted light is conducted via a fiber optic cable to the tissue surface (the fibers of the Periflux^{R} 2b are producing less artefacts by movements). The end of the fiber optic cable is fixed in a probe, which is placed in a probe holder, that is fixed to the tissue to be examined. When the laser light enters the tissue, part of it is absorbed and a part is reflected. A portion of the reflected light from adjacent but still separate scattering areas of tissue is gathered by two fiber optic cables, transmitting the light to the surface of two photodetectors. Light being reflected by static structures keeps the same frequency, but when it is reflected by moving particles (in this specific case erythrocytes) a Doppler shift occurs.

Photomixing creates the beat signals in the detectors and these signals are high-pass filtered and normalized in the same way in both channels. The difference in output of the photodetectors is taken, thereby suppressing common components such as noise from the laser and the environment. The beat signal produced by the random fashion in which the red cells pass through the scattering areas can be considered as independent realizations of the same stochastic process. Consequently, the overall blood flow related output signal is selected in the difference amplifier. This laser Doppler output signal is expressed in perfusion units (P.U.) and is linearly related to microcirculatory blood flow.

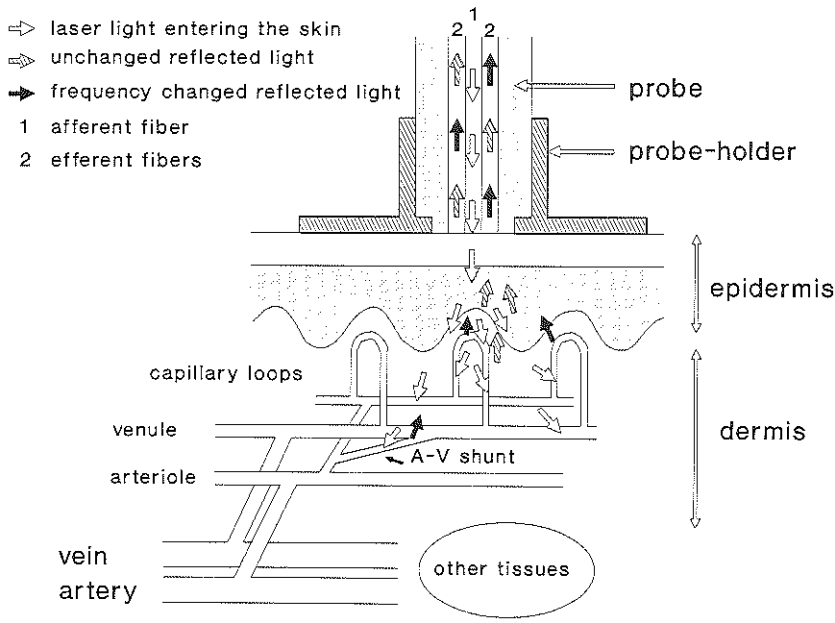


Figure 4. The laser Doppler flowmeter in relation to the skin (see text).

CHAPTER I: INTRODUCTION

C: Other methods to establish blood flow

C Other methods to establish blood flow

A subdivision is made into clinical examination, methods assessing arterial blood flow, methods using chemical or radioactive substances and instrumental methods evaluating local blood flow (figure 5 and table 1). Results of studies in which these methods were compared with laser Doppler flowmetry will be discussed.

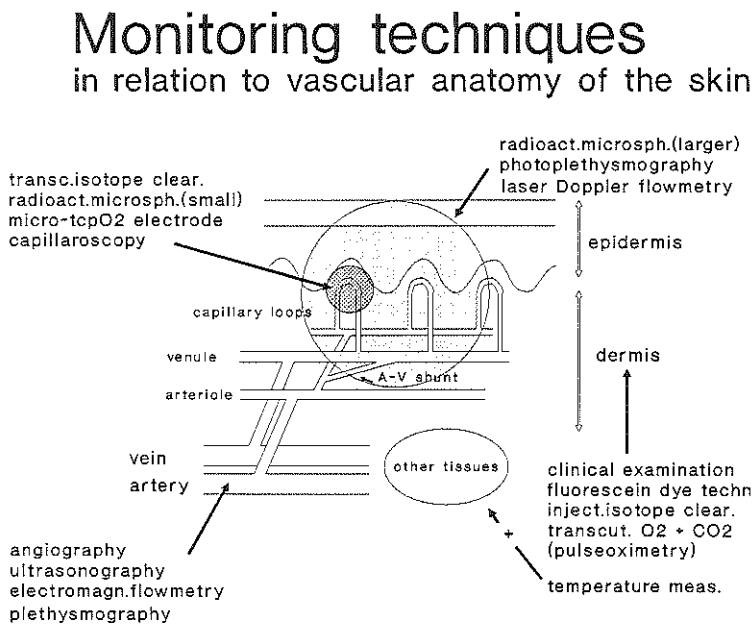


Figure 5. Schematic representation of various monitoring techniques in relation to the vascular anatomy of the skin.

C.1 Clinical examination

Clinical signs related to blood flow being indirect reflections of microcirculation are colour, capillary refill, bleeding characteristics and temperature. Colour is preferably observed on non-coloured tissues where a pink colour demonstrates a

normal circulation. A pale colour may indicate an inflow insufficiency, a bluish colour an outflow insufficiency or an inflow insufficiency with venous back-flow. Colour judgement is an easy, non-invasive method and can be monitored continuously. It is however subjective and qualitative.

Capillary refill can also be best observed on non-coloured tissues. Blood is removed by local pressure on the tissue and the time that it takes to refill is measured. A slow or absent refill may indicate an inflow problem, a rapid refill indicates an increased microcirculation or an outflow problem. It is a rather qualitative method, but by standardization it becomes more or less quantitative. The other characteristics resemble those of colour judgement.

Bleeding characteristics are studied by making a small injury preferably with a needle. No or very slow bleeding may indicate an inflow problem and normal or quick release of dark-red blood indicates an outflow problem. It is an easy method. It is however invasive, subjective, qualitative and cannot be monitored continuously.

Subjective temperature assessment is less accurate than objective instrumental monitoring, that will be described later.

C.2 Methods assessing arterial blood flow

C.2.1 Angiography

After injection of a radiopaque substance into an artery its intraluminal morphology can be visualized by X-ray. The passage time of the dye gives a semi-quantitative impression of blood flow in that specific vessel. Using digital subtraction angiography visualization of arteries is possible after intravenous injection of the radiopaque substance. Angiography is objective. It is however invasive, expensive, not easy, qualitative or semi-quantitative, it cannot be performed continuously and it does not establish local microcirculatory flow.

Table 1

	direct	easy	object	quant	invas	high price	continu	for longer period	influences microcirc
colour	–	+	–	–	–	–	+	+	–
capillary refill	–	+	–	+/–	–	–	+	+	+
bleeding characteristics	–	+	+/–	–	+	–	–	–	+
subjective temperature	–	+	–	–	–	–	+	+	+
angiography	–	–	+	+/–	+	+	–	–	+
ultrasonics	–	+	+	+	–	+/–	+	+	–
electromagnetic flow	–	–	+	+	+	+/–	+	+/–	+/–
plethysmography	–	+	+	+	–	–	–	–	+
fluorescence dye	+/–	+	+/–	+/–	+	–	–	–	+/–
inj.isotope clearance	–	+/–	+	+	+	+	–	–	+
tc.isotope clearance	–	–	+	+	–	+	–	–	–
radioactive microspheres	+	–	+	+	+	+	–	–	+/–
temperature	–	+	+	+	–	–	+	+	–
temperature clearance	–	+	+	+	–	–	+	+	+
photoplethysmography	–	–	+	+	–	–	+	+	–
indir.microvasc.pressure	–	+	+	+	–	+/–	–	–	+
dir.microvasc.pressure	–	–	+	+	+	+	+	+/–	+
transcut. O2 & CO2	–	+/–	+	+	–	+/–	+	+	+
needle O2	–	–	+	+	+	+/–	+	+	+
pulse oximetry	–	+	+	+	–	+/–	+	+	–
vital microscopy	–	–	–	+/–	–	+	–	–	–
dynamic capillaroscopy	+	–	+	+	–	+	+	–	–
fluoresc.capillaroscopy	–	–	+	+	+	+	–	–	–
laser Doppler flowmetry	+	+	+	+	–	+	+	+	–

Table 1. Comparison of different monitoring techniques in the evaluation of microcirculation. The different techniques are scored for; direct measurement of microcirculation, ease, objectivity, quantitative semiquantitative or qualitative measurement, invasion of the body, price, possibility of continuous monitoring and monitoring for several days and whether the technique influences the microcirculation.

C.2.2 Ultrasonography

Using an ultrasound (usually 20 MHz) Doppler probe, blood velocities can be assessed in single (superficial) blood vessels, because a Doppler shift is caused in the ultrasound frequency by the moving blood. Ultrasound Doppler flowmetry is objective, fairly easy, quantitative, continuous and non-invasive. Direct information about local microcirculatory blood flow cannot be obtained by this method.

By means of a small implantable ultrasonic Doppler probe, blood velocities can be assessed in small arteries and veins.[65]

Evident arterial flow, as detected with ultrasonics, may correlate well with laser Doppler flow measurements of tissues supplied by that specific artery. However good ultrasonic values may be combined with low laser Doppler flow values, when obstruction of flow is more distally, for instance due to microangiopathy. For that reason the laser Doppler technique is superior to ultrasonics in determining viability.[40,47] More information is obtained when both methods are combined.[66]

C.2.3 Electromagnetic flowmetry

The electromagnetic flow probe is placed around the investigated vessel and its electromagnet creates a magnetic field. The moving column of blood induces an electric potential, which is proportional to the velocity of flow.[67] The method is objective, quantitative, not expensive and continuous. It is however invasive, not easy to handle and needs regular calibration. It analyses flow in the studied vessel, but it does not establish local microcirculatory flow.

One study showed a good correlation between laser Doppler flowmetry and electromagnetic flowmetry,[68] but another demonstrated major differences.[69] These differences may be caused by the fact that the electromagnetic technique assesses total flow, whereas the laser Doppler technique assesses microcirculatory flow in a limited area.

C.2.4 Plethysmography

In (venous occlusion) plethysmography the volume of an extremity such as the hand is evaluated during short periods in which venous drainage is arrested by a collecting cuff.[70] Provided the pressure in the collecting cuff does not interfere with arterial inflow, the rate of volume increase during arrest is equal to the total arterial inflow. Plethysmography is an easy, objective, quantitative and non-invasive method. It is however rather complex and cannot analyse continuously, it influences normal circulation and it does not establish local microcirculatory flow.

Almand et al. showed that photoplethysmography is directly related to strain gauge plethysmography and that a non-lineair correlation exists between the plethysmography methods and the laser Doppler technique.[71-73] Other studies showed differences, mainly based on the fact that plethysmography evaluates total circulation of an extremity or digit, whereas laser Doppler flowmetry assesses local microcirculatory flow.[74,75]

C.3 Chemical and radioactive methods to evaluate local blood flow

C.3.1 Fluorescein dye technique

By the injection of a fluorescent agent into an artery or vein the distribution of blood flow can be studied. Under ultra-violet light a subjective and qualitative interpretation of distribution of the fluorescein dye can be obtained at different times. By means of a microfluorometer objective and quantitative values can be obtained.[76] The technique is not expensive, easy and assesses microcirculation. It is however invasive and cannot be used continuously.

The laser Doppler technique correlated well with fluorescein dye techniques in several studies.[46,50,77,78] Some studies demonstrated that even when the fluorescence was zero the laser Doppler still indicated some flow. This may be caused by to-and-fro movements of erythrocytes in capillaries during venous occlusion,[79] or by Brownian movements.[44,80]

C.3.2 Isotope clearance

Injective isotope clearance technique

After injection of an isotope bolus (e.g. ^{133}Xe, ^{24}Na, ^{99m}Tc, ^{85}Kr and ^{131}I) the wash-out is measured. The affinity to fat which many tracers have, makes evaluation difficult.[81] In addition, the trauma of injection may possibly act as a stimulus and a source of errors.[82] It has also been claimed that the clearance of ^{24}Na and ^{85}Kr when injected intracutaneously is limited by the capillary permeability rather than by the rate of blood flow.[83] The technique is objective, not too difficult and quantitative. It is however invasive, non-continuous and it assesses local microcirculatory flow indirectly.

Transcutaneous isotope clearance technique

The radioactive isotope (^{133}Xe) can be introduced atraumatically to avoid the problems of invasive methods. The ^{133}Xe gas is introduced for about 3 minutes into a small chamber adhering to the skin, allowing minute amounts of xenon gas to diffuse into the skin in doses sufficient to permit effective counting.[84] This method however is also non-continuous and it establishes local microcirculatory flow indirectly.

Several studies found a close correlation between laser Doppler flowmetry and the injective or transcutaneous ^{133}Xe clearence techniques.[9,11,85-87] Differences could be explained by imperfection of the ^{133}Xe washout method in one study,[88] but in other studies indications were found that both techniques reflect blood flow in different parts of the vascular bed. Obviously the laser Doppler technique evaluates flow in capillaries as well as arteriovenous anastomoses while the trancutaneous ^{133}Xe clearance technique assesses only capillary flow.[27-30]

C.3.3 Radioactive microsphere technique

The radioactive microsphere technique involves the injection of small plastic spheres with radioactive labelling into a large vessel. The spheres then become lodged in the microvascular network. The radioactivity in an excised tissue sample is taken as assessment of the regional blood flow. The size of the microspheres determines at what level they become lodged. In this way a differentiation can be made between arterio-venous shunt flow and nutritional flow to the skin. Absolute objective values of blood flow can be obtained by this method. It is however a difficult, invasive method and it is impossible to obtain several successive evaluations. Furthermore it is not applicable to humans.

Two studies demonstrated a good linear correlation between the radioactive microsphere technique and laser Doppler flowmetry.[43,89] Others however could not find a significant correlation between these methods, due to problems with the microsphere technique.[90]

C.4 Instrumental methods to evaluate local blood flow

C.4.1 Temperature measurement

Direct skin temperature

The skin temperature, which can be easily established by a thermocouple connected to a thermograph, is a crude index of the skin circulation. It depends on several factors, such as the rate of blood flow in the subjacent tissues, the activity of nearby muscles, the rate of evaporation of sweat and the environmental temperature. The skin temperature per se seems to be a poor indicator of capillary flow.[91] The technique is easy, not expensive, non-invasive, objective and continuous. Invasive needle thermocouples are also available for estimation of blood flow through deeper structures.

Temperature clearance

This technique establishes how much heat has to be transfered to tissues per second to keep the temperature at a higher level (heated thermocouples),[92] or how much heat is released from an extremity that is immersed in water somewhat below that of the body core (external calorimetry).[93] The characteristics are comparable to those of the atraumatic isotope clearance technique (objective, not to difficult, quantitative and indirect), although the temperature clearance can be evaluated continuously. It influences the local microcirculatory flow and is less specific.

Mustakallio and Kolari observed in irritation studies of human skin a significant correlation between the laser Doppler findings, contact temperature and erythema.[94] Other studies confirmed the correlation between these methods.[95-98] Ninet and Fronek demonstrated that during post-occlusion reactive hyperaemia skin temperature changed significantly slower than the laser Doppler values.[99] This is due to the large heat capacitance of tissue.[99] Nilsson et al. found that changes of blood flow were detected less sensitively by thermography than by the laser Doppler technique.[74] The explanation could be that thermometry is an indirect assessment of microcirculation and that skin temperature depends on several factors.

Thermal clearance correlated well to laser Doppler flowmetry, but the latter establishes skin blood flow better.[95,100] Laser Doppler flowmetry and thermometry in the postoperative monitoring of replantations are compared in chapter II.B of this thesis.

C.4.2 Photoplethysmography

The reflection of light from tissue is dependent on the blood volume. Therefore a pulsatile change in tissue blood volume produces a corresponding change in the amount of reflected light. An absolute value of tissue blood flow cannot be obtained, but the waveform can be analysed. The waveform demonstrates the inflow and outflow characteristics of blood in the studied tissue.[101] The method is objective, quantitative, non-invasive, not expensive and continuous. Unfortunately it does not measure absolute flow.

Almand et al. showed that photoplethysmography is directly related to strain gauge plethysmography and that a non-lineair correlation exists between the plethysmography methods and the laser Doppler technique.[71-73] Guy et al. found an

excellent correlation between results of laser Doppler technique and photoplethysmography and the visual observation of erythema while methylnicotinate was absorbed in human skin.[102] Other studies showed differences, mainly based on the fact that photoplethysmography does not investigate absolute flow, but analyses the waveform demonstrating the inflow and outflow characteristics.[103,104]

The laser Doppler can be used as a photoplethysmograph. It is called the "DC-value" or Total Backscattered Light (TBL). However fibre separation in the laser Doppler flowmeter (about 1mm) is smaller than in a conventional photopletysmograph (>3mm). This reduces the signal to noise ratio, but signal averaging may be used to improve the photoplethysmograph from the 1 mm fibre separation to give a pulse essentially identical to the better signal obtained at higher separations.[71-73]

C.4.3 Microvascular pressure measurement

Indirect

Increasing external pressure is put on the examined area until flow in the capillaries arrests as is observed by microscopy (see chapter I.C.4.6: microscopic techniques). This method is objective, easy, quantitative, non-invasive and repeated measurements can be done. Unfortunately it influences microcirculatory flow, but it does not assess it.

Direct

After introducing a microcannula in a capillary, local pressure can be measured including pulsatile components.[105] This method is objective, quantitative and

continuous. It is however invasive and does not assess microcirculatory flow. The method is difficult to use in routine clinical practice.

C.4.4 Oxygen and carbon dioxide monitoring

Transcutaneous

Oxygen and carbon dioxide tensions can be established by special electrodes placed on the skin. The gases diffuse transcutaneously, but an increased skin temperature (42°C – 45°C) is needed for reliable monitoring.[106,107] It is an indirect method and is influenced by several factors such as the properties of the skin, the delivery of oxygen across the capillary wall and the composition of the microvascular bed. The method is not very sensitive for evaluation of skin microcirculation. It is objective, quantitative, non-invasive and continuous. It is however not very easy to handle and it influences the local microcirculation.

Invasive

By means of a micro-$tcpO_2$ electrode placed in a small cannula it is possible to assess the pO_2 in relation to the distance from single capillaries. It is an objective, quantitative and continuous method. It is however difficult to handle, invasive and it does not analyse microcirculatory flow directly.

In several studies a correlation was demonstrated between laser Doppler flowmetry and transcutaneous pO_2 and pCO_2 evaluations if the examined area was heated, but this relation was not linear. In poorly perfused tissue a correlation was found, but in well perfused tissue laser Doppler flow responded directly to perfusion changes, whereas the $tcpO_2$ did not respond because it had reached its top value.[80,108-111] Kvernebo et al. stated that the $tcpO_2$ measurement underestimated the

oxygen tension at low values due to consumption of oxygen by the electrode.[104] In two studies no correlation was found between the methods, probably due to the fact that they examined well perfused tissues.[112,113] Reliable $tcpO_2$ recordings can only be obtained if the skin is heated.[100,114] During the postoperative monitoring of replantations and free vascularized tissue transplantations we observed that the $tcpO_2$ values were unreliable (frequently zero) if oedema existed, whereas the laser doppler flow values were hardly influenced [unpublished data]. Oedema seems to impede the transcutaneous diffusion of oxygen.

C.4.5 Pulse oximetry

Pulse oximetry assesses the oxygen saturation of blood in the examined tissue. The method is based on the fact that the colour of haemoglobin is dependent on its saturation with oxygen. The ratio of the transillumination of two different light-frequencies is a measure for oxygen saturation. The method is easy, objective, quantitative, non-invasive and continuous. It is however more or less expensive and it does not evaluate local microcirculatory flow directly.

Evaluation of human feet before and after spinal analgesia showed a significant increase in both laser Doppler output and pulse oximeter signal amplitude, but no correlation study was done.[96]

C.4.6 Microscopic techniques

Vital capillaroscopy

By using an ordinary light microscope with a magnification of 10-60x the

papillary capillaries of the skin all over the human body can be directly and non-invasively studied.[115] Paraffin oil and a blue or green filter are needed. It is one of the most reliable methods for evaluating the risk of developing skin necrosis in patients with diabetes mellitus, arterial insufficiency or scleroderma by morphological description of the capillaries. The method is non-invasive and hardly influences the microcirculation. It is however subjective, qualitative or semiquantitative and it does not assess local microcirculation directly, because it etablishes morphological changes in the capillaries due to hypocirculation and disease.

Dynamic (videometric) capillaroscopy

With a silicon matrix TV camera mounted on an ordinary light microscope, the skin capillaries can be visualized directly on a monitor at a magnification of 250-1000x. The passage of blood cells is visualized and velocity can be determined. Only the superficial nutritional capillaries of the skin are studied. This method is non-invasive, quantitative, continuous, it measures microcirculation directly and it hardly influences the microcirculation, although the studied object has to be firmly fixed, to prevent disappearance from the microscopic field of observation. The disadvantages are that it is only applicable on intact nailfold capillaries and that in about 15% of human subjects it is technically impossible to perform this study. It is a difficult technique and it gives extensive information on one or a few capillaries rather than about the microcirculation of the tissue as a whole. The selection of the capillaries to be measured is rather subjective and may give rise to different results.[91]

Fluorescence capillaroscopy

By using ultra-violet light the movement of Na-fluorescein in the capillaries and the distribution of the dye into the skin area can be followed by using a microscope

in combination with video equipment. This technique has shown to be very useful to study the disturbed movements of small molecules from capillaries out into the skin in patients with scleroderma, diabetes mellitus, and arterial or venous insufficiency. The method is objective and quantitative. It is however difficult, invasive, expensive, not continuous and it does not measure microcirculatory flow directly.[91]

As expected a high correlation was found between laser Doppler flowmetry and dynamic capillaroscopy and a broad comparability in patterns of responses was seen, but also some significant differences were observed. The rhythmical oscillations in microcirculation were asynchronous in some studies. The observed discrepancies in microcirculatory flow probably reflect differences in the sampled vascular beds. The most plausible explanation is that dynamic capillaroscopy only records blood flow in one or a few superficial nutritional capillaries, whereas laser Doppler flowmetry additionaly measures deeper vessels, including arterio–venous thermoregulatory shunts. In fact the correlation between the laser Doppler technique and videomicroscopy improved when the tissue penetration of the former was limited to 0.3–0.4 mm. It was stated that it is unlikely that a single calibration factor can be defined to convert the laser Doppler output to actual units of blood flow. Both methods may complement each other in studying microcirculation.[31–34,116]

CHAPTER I: INTRODUCTION

D: Rationale of the experiments

D.1 Microvascular surgery

Especially in microvascular surgery information about blood flow is essential, because in up to 20% of the cases an occlusion of the microvascular anastomosis occurs. Early recognition can save the threatened tissue. Therefore a method was needed that could monitor microcirculation objectively, quantitatively, non–invasively and continuously without influencing it. Moreover continuous monitoring for several days was necessary. The method had to react quickly to changes in microcirculation and it should be rather easy to handle, so that it could be used by the nursing staff. Laser Doppler flowmetry seemed the best available method. When we started our studies in 1985 two laser Doppler flowmeters were available, namely the LD5000 Laser Doppler perfusion monitor^{R} (Med Pacific Corporation, U.S.A.) and the Periflux^{R} (Perimed KB, Sweden). The latter had a double photodetection system and therefore a better signal to noise ratio, and for that reason we used it in our studies. Continuous monitoring for several days was only reported for the skin, the most extensively investigated tissue in laser Doppler flowmetry experiments.

D.2 First experiences with the laser Doppler flowmeter

Our first experiences in 1985 with the laser Doppler flowmeter were on the monitoring of microvascular surgical procedures. During monitoring the first patient, a vascular occlusion in a replanted thumb was recognized in an early stage and this thumb could be saved. Even during surgery the laser Doppler helped in assessing the result of revascularisation. When the skin was sutured too tight the laser Doppler indicated that the vascularisation was threatened. Enthusiasm from the first experiences with the method was the basis of this thesis.

D.3 Studies performed

In the first part (chapter II) laser Doppler flowmetry is evaluated as a diagnostic method to assess tissue microcirculation after various microvascular operations. A second part (chapter III) concerns the application of laser Doppler flowmetry to investigate and to objectivate the negative effects of cigarette smoking upon the microcirculation under normal circumstances as well as after microvascular procedures. Chapter II.A describes the predictive value of the laser Doppler flowmeter for postoperative monitoring. In chapter II.B laser Doppler flowmetry is compared to thermometry, the most frequently used postoperative monitoring in microvascular surgery. In chapter II.C the usefulness of laser Doppler flowmetry to distinguish arterial and/or venous occlusions in the vascular pedicle is investigated. Little is known of the physiology of autologous tissue transplants after microsurgical revascularization and therefore postoperative microcirculatory flow patterns are described in chapter II.D. In chapter II.E the correlation is investigated between laser Doppler flow and changes in microcirculation due to rejection in an allogeneic flap transplantation model.

Smoking of cigarettes is suspected to have a negative effect on the survival of both free vascularized tissue transfers and replantations. In chapter III.A the acute effect of cigarette smoking on the microcirculation of the thumb is studied. In chapter III.B this is investigated on replanted digits. In chapter III.C the effect of cigarette smoking on the survival of free vascularized and pedicled epigastric flaps in the rat is described; the laser Doppler was used to establish microcirculatory flow in the studies described in the chapters III.A, III.B and III.C.

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

A: The predictive value of laser Doppler flowmetry for postoperative microvascular monitoring

Submitted to: Annals of Plastic Surgery

A.1 Introduction

Microvascular surgeons have been confronted with the problem of vascular occlusion ever since the first recorded replant in 1962 and the first free flap in 1967.[117,118] To prevent the loss of replanted or freely transferred tissues early recognition of vascular compromise is essential, because the effectiveness of intervention is inversely related to the time that has elapsed between suspicion of vascular compromise and reexploration.[119] Furthermore, clinical judgement can be misleading and it is impossible for the surgeon to observe the patient continuously. Consequently several methods for monitoring microcirculation or patency of microvascular anastomoses have been developed.[120] Ideally postoperative monitoring should be objective, direct, non-invasive, reliable, continuous, easy and inexpensive.

In Rotterdam the first replant was performed in 1976. From 1976 to 1990 339 parts of extremities were replanted or revascularized. In the same period 197 free vascularized tissue transfers were carried out. Of the available monitoring techniques[120] colour, capillary refill and bleeding characteristics were used as clinical parameters from the beginning. Instrumental methods applied include temperature (since 1978), transcutaneous oxygen measurements (since 1982) and laser Doppler flowmetry (since 1985). Temperature measurements are strongly influenced by changes in the environment. Transcutaneous oxygen tensions tend to be unreliable in cases of oedema. Since November 1985 we have focused our attention on laser Doppler flowmetry (LDF) for postoperative monitoring, as we were not satisfied by the aforementioned methods.[6,8,11,53] Laser Doppler flowmetry is objective and non-invasive. A direct and continuous flow measurement of the surface microcirculation can be obtained. The apparatus is easy to handle , however recordings are not always easy to interpret. The purpose of this study was to establish the predictive value of laser Doppler flowmetry in microvascular monitoring following replantations and free tissue transfers.

A.2 Material and Methods

A.2.1 Laser Doppler flowmetry (LDF)

To measure microcirculatory flow, we used the Periflux[R], Pf 2a + 2b (Perimed KB, Sweden) which uses light from a helium–neon laser (2 mW) conducted via a fibre optic cable to the tissue surface (figure 6). A probe holder was sutured to the skin of the replanted part or skin element of the free flap so that the fibre optic cable could be held continuously at a constant distance from the skin surface. The apparatus measures a Doppler shift in reflected light which is related to the number and velocity of moving particles (mainly erythrocytes) in the field of investigation (a hemisphere with a radius of approximately 1 mm). This change in frequency is converted into a laser Doppler flow signal which is expressed in perfusion units (P.U.). This signal has been shown to be linearly related to microcirculatory blood flow.[12]

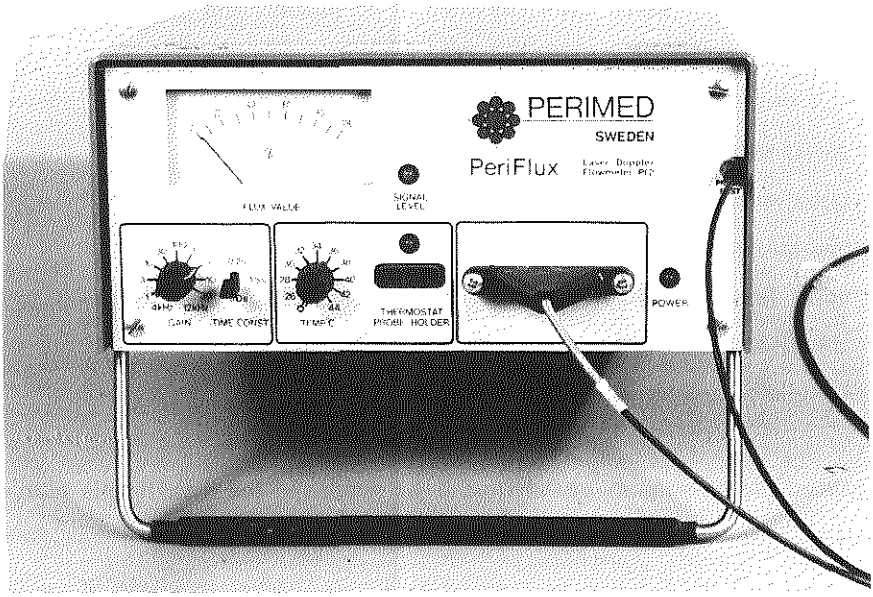


Figure 6. The Periflux[R] Pf2 laser Doppler flowmeter.

Laser Doppler flow measurements were taken continuously during the first five postoperative days unless a reexploration was necessary. Following reintervention the patient reentered the study for a further period of five days. Measurements were always taken at the same place. For replants this was the volar skin of the distal phalanx and for free tissue transfers the distal skin or the skin island.

A.2.2 Patients

During the study period Nov. 1st 1985 to Dec. 31st 1987, 176 microvascular operations were performed. Due to the lack of laser Doppler flow monitors only 79 cases could be completely monitored. Preference was always given to those cases, which on clinical ground were thought to be more likely to develop circulatory problems. The remaining 97 cases were monitored using traditional methods. This group could not be used as a control group because it had not been randomly selected. The 79 monitored cases consisted of 45 replants and revascularisations in 34 patients and 34 free tissue transfers. The latter group included:

radial forearm flap	15
latissimus dorsi flap	11
toe-to-thumb transfer	4
upper lateral arm flap	2
free fibula transplantation with skin island	1
complete upper arm flap without bone	1

The ages of the 68 patients varied from 5-62 years and the male/female ratio was 16 to 1.

A.2.3 Statistical analysis

For analysis the five days of monitoring were split into 240 half hour periods. Within every half hour period the mean value of laser Doppler flow measurements was obtained. For further analysis the lowest mean value per patient was used (figure 7). Patients were divided into the following groups; replantations and revascularisations, radial forearm flaps, latissimus dorsi flaps and other free flaps. Furthermore each group was subdivided into 3 sub-groups, according to the clinical course which was 1) uncomplicated, 2) compromised (e.g. hematoma, compression, vascular kinking etc.) and 3) complicated by an arterial or venous occlusion.

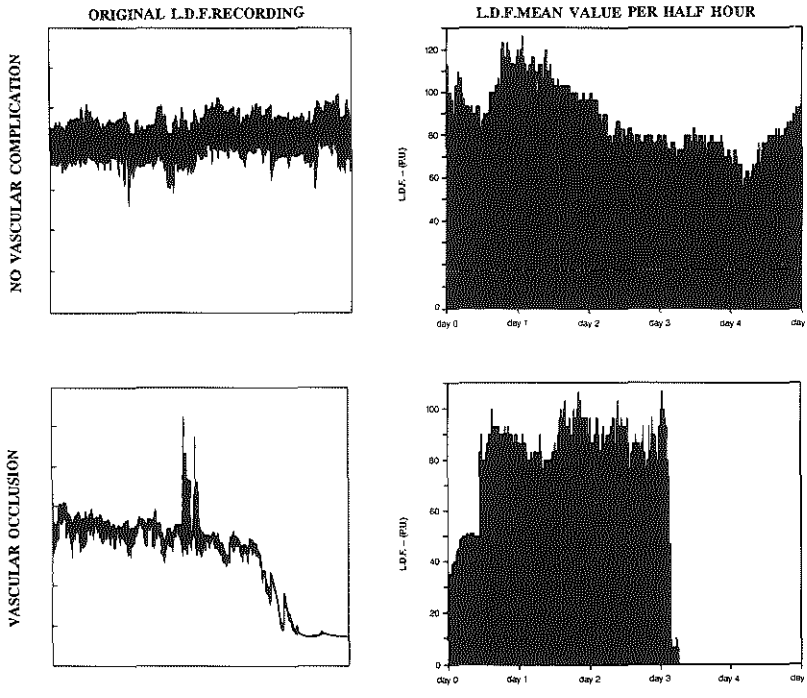


Figure 7. A part of the original L.D.F. recording (left) and the complete recording summarized in mean values per half hour (right) in a case without complications (upper) and a case with a vascular occlusion (lower).

The rank sum test according to Wilcoxon was used to assess significance of differences between the sub-groups, provided at least four patients were available in a sub-group.

In addition an analysis of sensitivity and specificity was done using the rankit score method to estimate the distribution of the laser Doppler flow measurements within each sub-group.

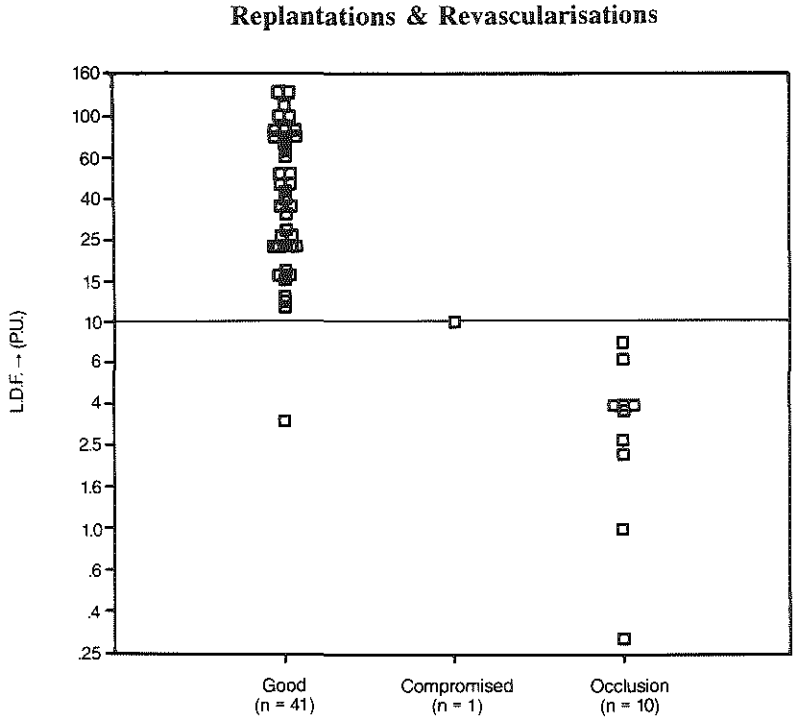


Figure 8. The lowest mean half hour L.D.F. value of every replantation and revascularisation registration is shown in this diagram on a logarithmic scale, subdivided into 41 cases with a good course, 1 case with a compromised course and 10 cases which had a vascular occlusion postoperatively. All the good cases except 1 (L.D.F. value = 3.3 P.U.) had a L.D.F. value of 12.0 P.U. and higher, the compromised case a L.D.F. value of 10.0 P.U. and the vascular occlusion cases a L.D.F. value of 8.0 P.U. and lower. An alarm value of 10.0 P.U. can be drawn.

A.3 Results

A.3.1 Replantations and revascularisations

In the group of 34 patients with 45 replants or revascularisations, 52 laser Doppler flow recordings were made according to the protocol. The difference in numbers between the laser Doppler flow recordings and the replants was due to re-entry of cases into the protocol following vascular compromise. In 41 recordings with an uncomplicated course the mean laser Doppler flow value was 49.1 P.U. (range 130 to 3.3 P.U.); in one recording with a compromised course the laser Doppler flow value was 10.0 P.U.; in ten recordings with a complicated postoperative course the mean laser Doppler flow value was 3.7 P.U. (range 8.0 to

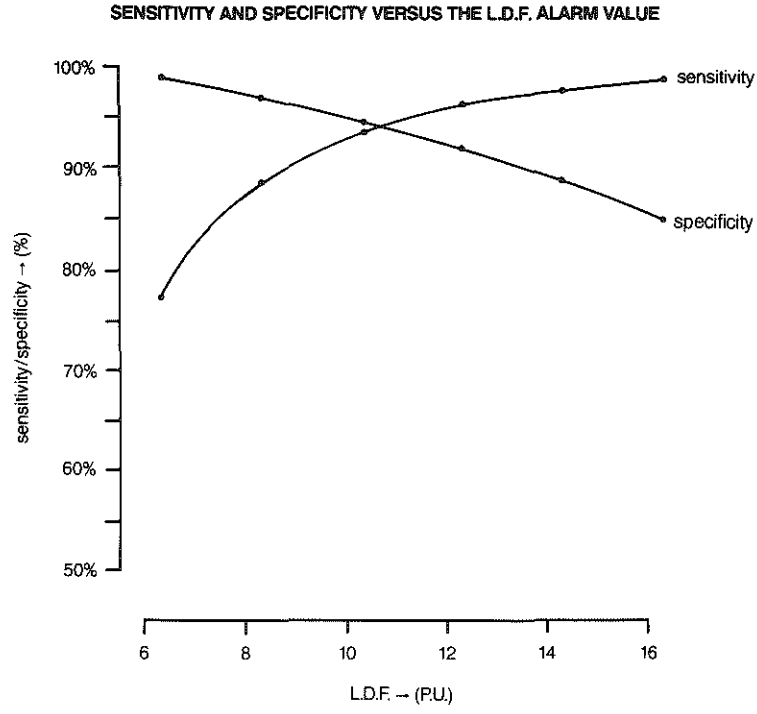


Figure 9. The sensitivity and specificity for different L.D.F. alarm values in the replantation and revascularisation group.

0.3 P.U.; figure 8). The laser Doppler flow values in the uncomplicated groups were significantly higher than the laser Doppler flow values in the complicated groups ($p < 0.001$).

It was assumed that the 41 uncomplicated cases and the 10 cases with a vascular occlusion were representative for the relevant populations. The shape of the frequency distribution of laser Doppler flow values appeared to be lognormal, for the uncomplicated group as well as in the complicated group. On the basis of these findings the values of sensitivity and specificity could be estimated for different critical (= alarm) values: 6, 8, 10, 12, 14, 16 P.U. (figure 9).

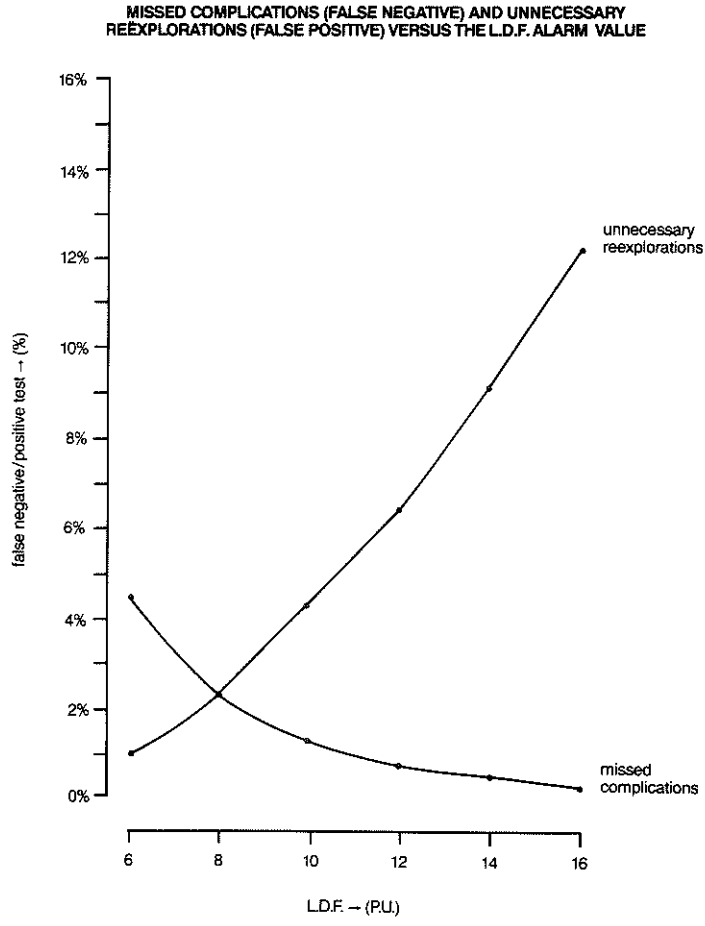


Figure 10. Missed complications and unnecessary reexplorations for different L.D.F. alarm values in the replantation and revascularisation group.

To calculate the missed complications and the unnecessary reexplorations the complication rate has to be known. According to our own figures and those of the literature the vascular occlusion rate of replantations and revascularizations is about 20% (figure 10).

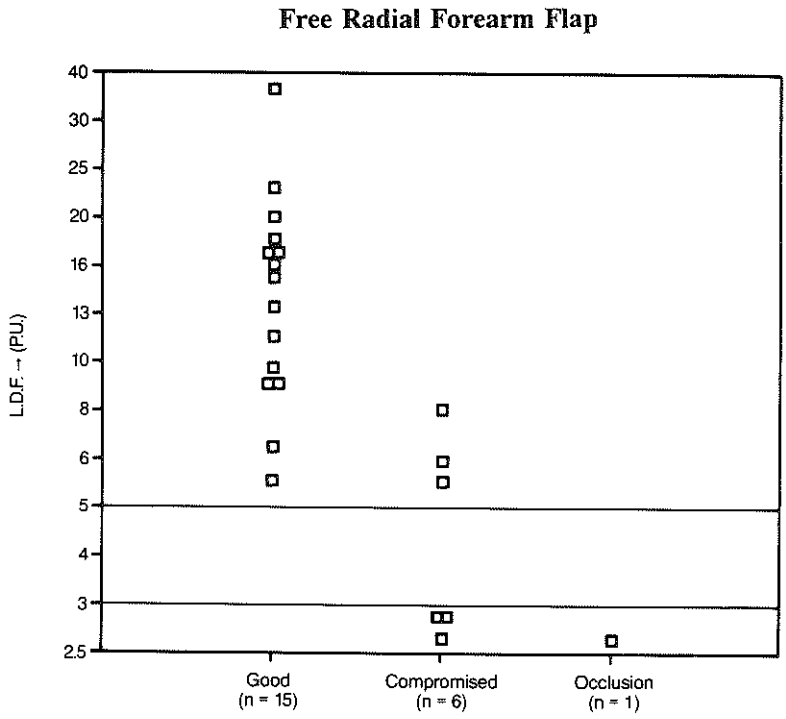


Figure 11. The lowest mean half hour value of every free radial forearm registration is shown in this diagram on a logarithmic scale, subdivided into 15 cases with a good course, 6 cases with a compromised course and 1 case which had a vascular occlusion postoperatively. All the good cases had a L.D.F. value of 5.7 P.U. and higher, the compromised cases a L.D.F. value between 2.7 and 8.0 P.U. and the vascular occlusion case a L.D.F. value of 2.7 P.U.. A L.D.F. alarm value between 2.7 and 5.7 P.U. can be estimated.

A.3.2 Radial forearm flaps

In the group of 15 patients with a free radial forearm flap, 16 flaps were performed resulting in 22 laser Doppler flow recordings according to protocol. The difference in numbers between the laser Doppler flow recordings and the flaps were again due to re-entry into the protocol following vascular compromise. In 15 recordings with an uncomplicated course the mean laser Doppler flow value was 15.1 P.U. (range 36.7 to 5.7 P.U.); in six recordings with a compromised course the mean laser Doppler flow value was 4.8 P.U. (range 8.0 to 2.7 P.U.); in one recording with a complicated course the laser Doppler flow value was 2.7 P.U. (figure 11). The laser Doppler flow values in the uncomplicated group were significantly higher than the laser Doppler flow values in the compromised group ($p < 0.01$).

A.3.3 Remaining tissue transfers

In the latissimus dorsi group no flaps were lost in 11 patients. Of the remaining 8 tissue transfers one toe-to-thumb transplantation was lost. The numbers in these last two groups were too small for statistical evaluation.

A.4 Discussion

The effectiveness of reintervention is inversely related to the time that has elapsed between the suspicion of vascular compromise and re-exploration.[119] Kerrigan et al. demonstrated in cutaneous island flaps in pigs that the secondary critical ischemic time for 90% flap survival (4.7 hours) was far less than the

primary critical ischemic time (7.0 hours).[121] Therefore reintervention in failing microvascular procedures should be as early as possible. The decision to reexplore by clinical judgement alone, even by experienced surgeons, has proven in the past to result in considerable time loss and a high secondary failure rate (up to 60%).[122] This is especially valid during the night, when most failures tend to occur and experienced staff is not always present.

The ideal monitor should have a sensitivity of 100% and a specificity of 100% i.e. no false predictions. A monitor device with these characteristics has not yet been developed, however our results suggest that laser Doppler flowmetry approaches this ideal. If for instance the laser Doppler flow alarm value of 10 P.U. is chosen for the replantations and revascularizations the estimated sensitivity is 93% and the estimated specificity is 94%. This implies that of every 100 replantations 19 out of the 20 expected vascular occlusions would be recognized, and 4 patients would be re-explored unnecessarily. Until now we have recognized all 11 vascular occlusions on 52 laser Doppler flow recordings and, if we had depended on laser Doppler flowmetry alone, we would have re-explored only one patient unnecessarily.

The critical (= alarm) value however can be adjusted to the particular surgeons choice. If the surgeon is very eager to save his replants and does not mind re-exploring 10% of his cases unnecessarily, then the critical value may be set at about 14 P.U.. If, on the other hand, the surgeon only accepts re-exploration of 1 patient out of 100 replantations unnecessarily and accepts missing 4 to 5 of the 20 expected vascular occlusions, the critical laser Doppler flow value may be chosen at about 6 P.U.. In our opinion almost all vascular occlusions should be detected and some unnecessary re-explorations have to be accepted, therefore a critical value of 10 P.U. was taken for the replants.

With regard to free tissue transfer one has to realize that the skin of each type of flap has its own vascular anatomy and capillary density, thereby influencing the

laser Doppler flow measurements. Consequently it is important to search for critical alarm values for each kind of free vascularized tissue transfer. Unfortunately the numbers included in these groups were insufficient for complete statistical analysis as described for the replant group, but there is a definite clinical impression that comparable results can be achieved.

Recently Clinton et al. demonstrated that laser Doppler flowmetry accurately identified all free flaps with compromised perfusion, resulting in an increase in salvage rates from 50 to 82.4%.[59] They also established normal ranges of perfusion for several types of free flaps.

It is our experience that laser Doppler flowmetry helps in decision making, especially when clinical judgement is difficult or when a surgeon is not present to inspect the revascularized tissue, for instance during the night. This study proves that laser Doppler flowmetry can be a very reliable postoperative monitoring device after microvascular surgery. As this study was not a prospective trial, we cannot prove that without laser Doppler flowmetry we would have had different results. It should be noted however that in the past our success rate for reexploration of replants was considerably lower. To our knowledge the only prospective trial with laser Doppler flowmetry and free tissue transfers has been performed by Walkinshaw et al..[57] They concluded that although laser Doppler flow measurements correlate with clinical observations, they did not predict them and were actually less accurate in indicating the need for clinical intervention or final outcome of the free flap procedure. However in that study short intervals of measurements were used and it is our experience that laser Doppler flow recordings are unreliable if measured during short periods, because after applying the laser Doppler flow probe vascular reactions can be seen which need time to stabilize. Furthermore we do not agree that the reference point should be the laser Doppler flow value when no circulation is present (biological zero), as there is great variation in this value. In our study alarm values did not correlate with the biological zero and thus were not used.

In using the laser Doppler flowmeter we have been twice warned of vascular compromise which was not recognized clinically. As clinical judgement was decisive for reexploration in those days we did not reoperate. Both flaps were lost due to venous occlusion. These cases indicate that there is an additional value of the laser Doppler flowmetry.

In conclusion our study shows that laser Doppler flowmetry can be a very reliable postoperative monitoring device after microvascular surgery. The high recognition rate of vascular occlusions in an early stage indicates its additional importance. A prospective trial has been started in order to validate the critical (= alarm) values which are found in this study.

A.5 Abstract

Reliable postoperative monitoring in microvascular surgery is necessary to improve the low success rate of reexploration following vascular compromise. The use of laser Doppler flowmetry has been evaluated in this study. From November 1985 to January 1988, 79 microvascular operations were monitored postoperatively. These consisted of 45 replants and revascularizations in 34 patients, as well as 34 free vascularized tissue transfers.

In the replant and revascularization group a statistically significant difference in laser Doppler flowmetry readings has been demonstrated between well vascularized and circulatory impaired cases ($p < 0.001$).

In this study a reliable critical (= alarm) value could be defined for replants and revascularizations with a sensitivity of 93% and a specificity of 94%. This critical alarm value can be adjusted according to the individual surgeon's attitude towards reexploration. Similar laser Doppler flowmetry characteristics were seen in cases of free vascularized tissue transfers, however the numbers were too small to define

reliable critical alarm values.

The laser Doppler flowmeter is recommended for the post-operative monitoring following microvascular anastomoses as it indicates vascular occlusion at an early stage so that re-exploration is worthwhile.

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

B: Comparison of laser Doppler flowmetry and thermometry in the postoperative monitoring of replantations

Submitted to: The Journal of Hand Surgery

B.1 Introduction

The most feared complication in microvascular surgery is occlusion of anastomoses. Early recognition of this condition may prevent the loss of replanted or free vascularized tissue, as the blood flow can be restored by reconstructing the vascular anastomoses. It is essential to recognize the vascular compromise early, because the effectiveness of intervention is inversely related to the time that has elapsed between suspicion of vascular compromise and reexploration.[119] Clinical judgement alone is not satisfactory, therefore several methods are developed to monitor microcirculation or to establish the patency of microvascular anastomoses.[123-125]

Postoperative monitoring should be objective, direct, non-invasive, reliable, continuous, easy and inexpensive. The tissue surface thermometry is known as an easy and inexpensive postoperative monitor and therefore it is used by many microsurgeons. Unfortunately the tissue temperature is easily influenced by the surroundings. Nevertheless several authors have demonstrated enthusiasm for surface temperature measurements as a method of monitoring following microvascular surgery.[126-129] The critical temperature, below which reexploration is indicated, should be 30 °C.[130] Leonard and Brennen stated that two thermocouples should be used, one on the revascularized tissue and one on adjacent normal skin. A difference of 2 °C between the two should indicate circulatory impairment.[131]

Because thermometry is not always the ideal monitor in microvascular surgery, other methods have been studied. The most recent reports are on laser Doppler flowmetry and promising results were presented, although false predictions were seen by some authors.[52,53,56,57,59,120,132]

Pietila et al. compared laser Doppler flowmetry and thermometry in the postoperative monitoring of replanted rabbit ears and concluded that the laser Doppler flowmeter was more sensitive to changes in capillary blood flow, but the

reproducibility of the method was worse in comparison to thermometry.[133]

The aim of this study was to compare thermometry and laser Doppler flowmetry as postoperative monitors in human replantation surgery.

B.2 Materials and methods

During the study period 45 replantations and revascularizations were monitored in 34 patients. The mean age of the patients was 34.6 years (7–62) and the male/female ratio was 32 to 2. The patients were monitored by laser Doppler flowmetry and thermometry for 5 days postoperatively unless a reexploration was necessary. Following reintervention patients reentered the study for another period of five days.

B.2.1 Laser Doppler flowmetry (LDF)

The laser Doppler flowmeter (L.D.F.; Perimed KB, Sweden) works with light from a 2 mW helium neon laser which is conducted via fiberoptics to the tissue surface. The laser beam has a penetration depth of approximately 1 mm in a hemispherical fashion. The light is partly absorbed and partly reflected.

Moving particles, mainly erythrocytes, cause a Doppler shift in the reflected light. This change in frequency is converted to a laser Doppler flow signal, which is expressed in perfusion units (P.U.). This signal has been shown to be linearly related to microcirculatory blood flow.[6,8,11,15]

LDF-measurements were taken continuously at the same site, i.e. the volar skin of the distal phalanx. A probe holder was sutured to the skin so that the fiber optic cable was held continuously at a constant distance from the skin surface.

B.2.2 Thermometry

The surface temperature was measured by a thermocouple connected to a thermograph (Y.S.I. 44 TA, Yellow Springs Ohio, U.S.A.). Temperature is an indirect measurement of skin perfusion, which is influenced by the temperature of the deeper tissues and of the surroundings. The thermocouple was fixed by medical adhesive tape to the dorsal skin of the distal phalanx of the replanted digit as well as a control digit.

B.2.3 Statistical analysis

The five days of monitoring were split into 240 half hour periods. Within every half hour period the mean value of LDF measurements was obtained as well as a single skin temperature measurement and a difference in temperature between the control and replanted digit. For further analysis of each parameter the lowest of the thus obtained values per recording was used.

Patients were divided into 3 groups, according to the clinical course which was 1) uncomplicated, 2) compromised (e.g. hematoma, compression, vascular kinking etc.) and 3) complicated by an arterial or venous occlusion.

For every parameter the two tailed Mann–Whitney rank test was used to assess differences between the uncomplicated and vascular occlusion group. The Spearman rank correlation test was used to assess the strength of relationships between the different parameters. Analysis of sensitivity and specificity was done by using the data per se. It was assumed that the 41 uncomplicated cases and the 10 cases with a vascular occlusion were representative for the relevant populations. The rankit score method was used to estimate the distributions of laser Doppler flow measurements within these relevant populations in order to calculate the sensitivity and specificity.

B.3 Results

In the 34 patients with 45 replantations or revascularizations, 52 recordings were made. The difference in numbers between the recordings and replants was due to re-entry of cases into the study following vascular compromise. During 41 recordings the course was uncomplicated, during 1 recording compromised (haematoma) and during 10 recordings a vascular occlusion occurred. The compromised replant was succesfully reexplored and after removal of the haematoma the replant survived. In 9 replants a vascular occlusion occurred. Three patients were not reexplored; one patient suffered from respiratory problems, which were a contraindication for anaesthesia and in two patients the vascular reconstruction was made on very small vessels, so that secondary reconstruction was technically impossible. In 5 patients a reexploration was done; 4 reexplorations

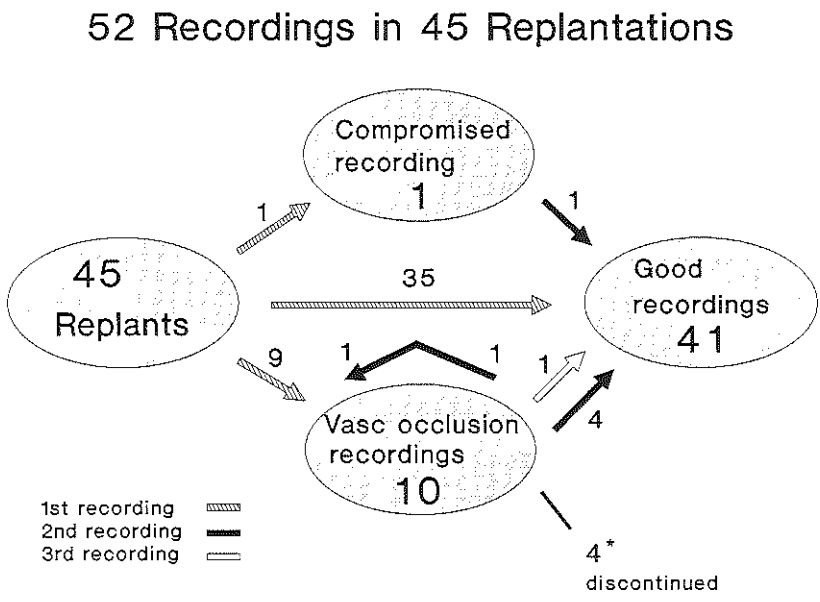


Figure 12. Fifty-two recordings in 34 patients with 45 replantations. Seven reexplorations were done and 6 of them were monitored postoperatively. Three times no reexploration was performed (see text).

were directly successful and in one patient a reocclusion occurred, but a second reexploration saved the replant. One reexplored replantation could not be monitored due to lack of apparatus (figure 12).

All 52 laser Doppler recordings were complete, 4 temperature recordings of the replants were incomplete and in 3 cases no reliable control temperature was measured.

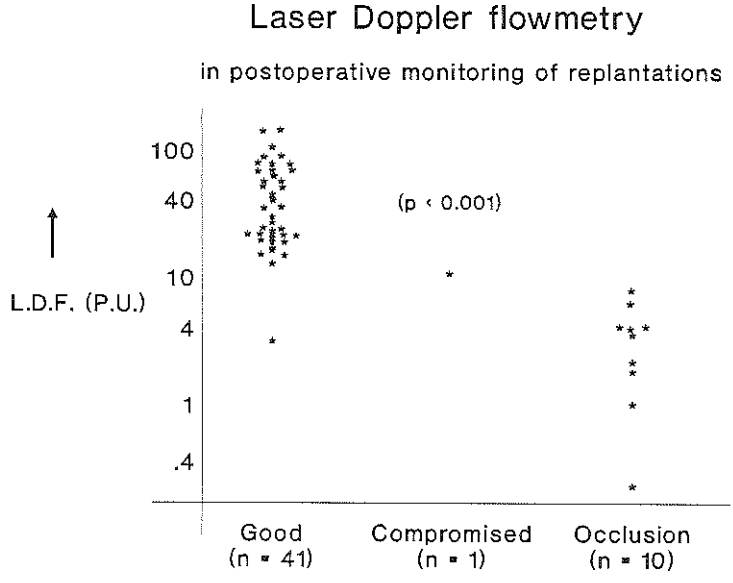


Figure 13. The lowest mean half hour L.D.F. value of every replantation and revascularisation registration is shown in this diagram on a logarithmic scale, subdivided into 41 cases with a good course, 1 case with a compromised course and 10 cases with a vascular occlusion postoperatively. The good cases ranged from 3.3 to 130 P.U. (mean, 49.1), in the compromised case the value was 10 P.U. and the vascular occlusion cases ranged from .3 to 8.0 P.U. (mean, 3.7 P.U.). The difference between the good cases and vascular occlusion cases is statistically significant ($p < 0.001$).

B.3.1 Laser Doppler flowmetry (LDF)

In 41 recordings with an uncomplicated course the mean LDF value was 49 P.U. (range 130 to 3.3); in the recording with a compromised course the LDF value was 10.0 P.U.; in 10 recordings with a complicated postoperative course the mean LDF value was 3.7 P.U. (range 8.0 to 0.3 P.U.). The LDF values in the uncomplicated

group were significantly higher than the LDF values in the complicated group ($p < 0.001$; figure 13). The sensitivity and specificity curves are shown in figures 16 and 17.

B.3.2 Thermometry

In 39 recordings with an uncomplicated course the mean lowest temperature was 31.5 °C (range 25.6 to 34.1); in the recording with a compromised course the lowest temperature was 27.5 °C; in the 8 recordings with a complicated postoperative course the mean lowest temperature was 27.2 °C (range 25.0 to 31.0). The lowest temperature in the uncomplicated group was significantly higher than those in the complicated group ($p < 0.001$; figure 14).

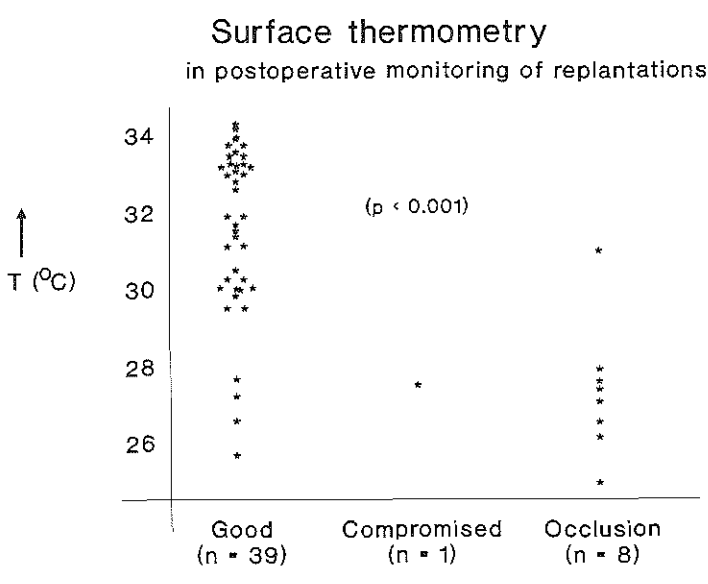


Figure 14. The lowest half hour temperature value of every replantation and revascularisation registration is shown in this diagram, subdivided into 39 cases with a good course, 1 case with a compromised course and 8 cases with a vascular occlusion postoperatively. The good cases ranged from 25.6 to 34.1 °C (mean, 31.5), in the compromised case the value was 27.5 °C and the vascular occlusion cases ranged from 25.0 to 31.0 °C (mean, 27.2). The difference between the good cases and vascular occlusion cases is statistically significant ($p < 0.001$).

In 37 recordings with an uncomplicated course the mean lowest differential temperature was −3.4 °C (range −9.1 to −0.7); in the recording with a compromised course the lowest differential temperature was − 7.7 °C; in the 7 recordings with a complicated postoperative course the mean lowest differential temperature was −6.5 °C (range −10.5 to −0.4). The lowest differential temperature in the uncomplicated group was significantly higher than those in the complicated group (p = 0.02 ; figure 15).

The sensitivity and specificity curves are shown in figures 16 and 17.

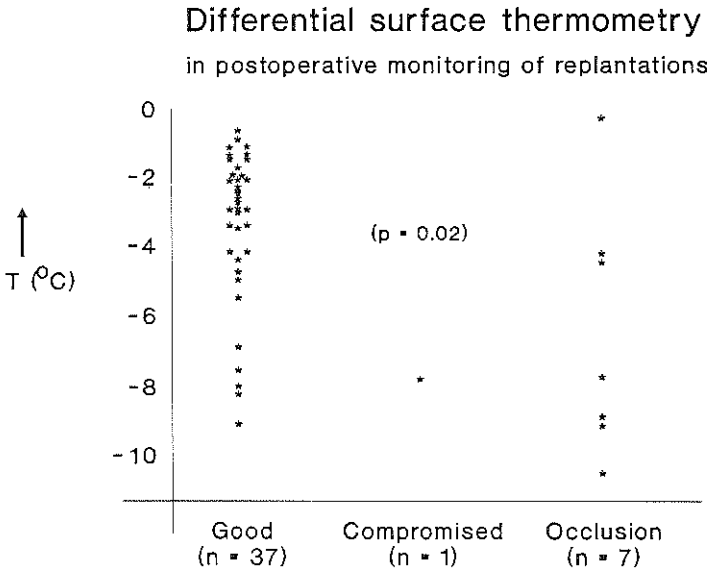


Figure 15. The lowest half hour differential temperature value of every replantation and revascularisation registration is shown in this diagram, subdivided into 37 cases with a good course, 1 case with a compromised course and 7 cases with a vascular occlusion postoperatively. The good cases ranged from −9.1 to −0.7 °C (mean, −3.4), in the compromised case the value was −7.7 °C and the vascular occlusion cases ranged from −10.5 to −0.4 °C (mean, −6.5). The difference between the good cases and vascular occlusion cases is statistically significant (p = 0.02).

B.3.3 Comparison of laser Doppler flowmetry and thermometry

Laser Doppler flowmetry and temperature were significantly correlated (rho = 0.681 ; $p < 0.0001$), as well as LDF and differential temperature (rho = 0.622 ; $p < 0.0001$). Also temperature and differential temperature showed a high correlation (rho = 0.805 ; $p < 0.0001$). The sensitivity/specificity curves were best for laser Doppler flowmetry, followed by temperature as second and differential temperature as third (figures 16 and 17).

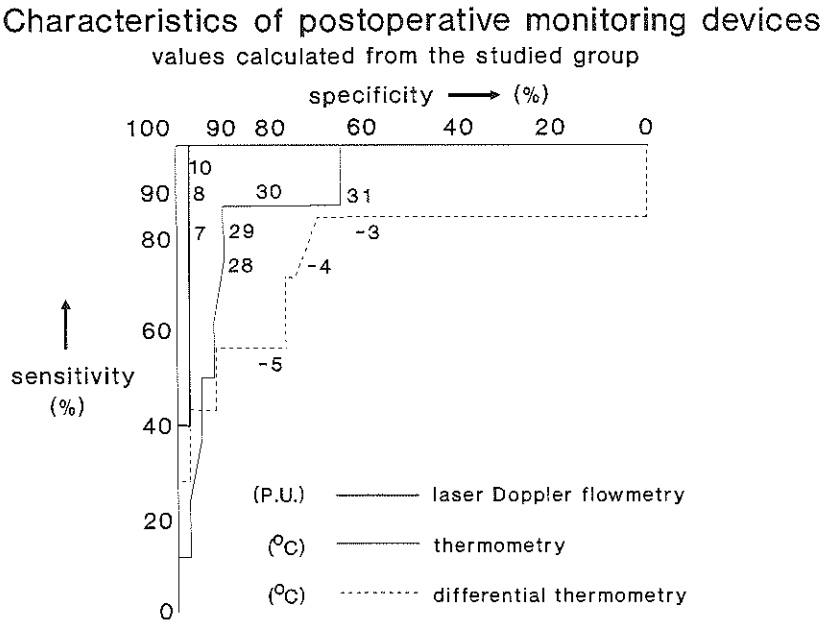


Figure 16. The sensitivity/specificity curves calculated from the studied group are shown for laser Doppler flowmetry, surface thermometry and differential surface thermometry. The sensitivity/specificity curves are best for LDF, followed by temperature as second and differential temperature as third.

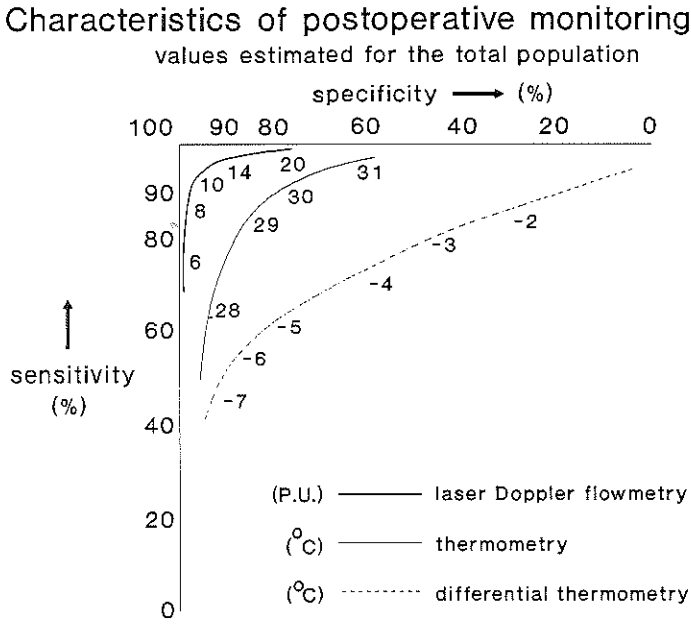


Figure 17. The sensitivity/specificity curves estimated for the total population are shown for laser Doppler flowmetry, surface thermometry and differential surface thermometry. The sensitivity/specificity curves are best for LDF, followed by temperature as second and differential temperature as third. The best alarm value for surface thermometry is 29 °C and for laser Doppler flowmetry 10 P.U..

B.4 Discussion

The effectiveness of reintervention is inversely related to the time that has elapsed between the suspicion of vascular compromise and reexploration.[119] Kerrigan et al. demonstrated in cutaneous island flaps in pigs that the secondary critical ischemic time for 90% flap survival (4.7 hours) was far less than the primary critical ischemic time (7.0 hours).[121] Therefore reintervention in clinically failing microvascular procedures should be as early as possible. The decision to

reexplore on the basis of clinical judgement alone, even by experienced surgeons, has proven to result in considerable time loss and a high secondary failure rate of up to 60%.[122] This is especially valid during the night, when most failures tend to occur and experienced staff is not always present.

Thermometry has been used by many microvascular surgeons in postoperative monitoring because it is easy, inexpensive and objective. False predictions however were made by this method and research was done for a more ideal monitor. The laser Doppler flowmetry seemed to be the most promising, although it is a rather expensive method and the microsurgeon requires knowledge of the method for proper use. This study shows that in the postoperative monitoring of replantations laser Doppler flowmetry is superior to thermometry in detecting vascular occlusions, although a false prediction was made by the laser Doppler flowmetry.

If one is not able to use laser Doppler flowmetry, thermometry is a reasonable alternative. Differential temperature measurements did not improve the sensitivity and specifity as was suggested by Leonard and Brennen,[131] it was even inferior to measuring temperature of the replant only. So if thermometry is used one should only measure the replanted digit.

The best alarm value of laser Doppler flowmetry in replantion surgery seems to be 10 P.U. with an estimated sensitivity of 93% and specificity of 94%. If however a microsurgeon is very eager to save his replants and does not mind reexploring 10% of his cases unnecessarily, then the alarm value may be 14 P.U.. If, on the other hand, the microsurgeon only accepts reexploration of 1 patient out of 100 replantations unnecessarily and accepts missing 4 to 5 of the 20 expected vascular occlusions, the alarm value may be 6 P.U.. In this study we would have recognized all 11 vascular occlusions on 52 laser Doppler flowmetry recordings and we would have reexplored 1 patient unnecessarily, if we had depended on LDF with an alarm value of 10 P.U. only.

The best alarm value of thermometry in replantation surgery seems to be 29 °C

with a sensitivity of 84% and a specificity of 86%. This value is lower than the 30 °C suggested by Stirrat et al..[130]

In the only false prediction of the LDF in this study the temperature also gave a false prediction. So we do not expect improvement of sensitivity and specificity when combining LDF with thermometry.

In conclusion this study shows that laser Doppler flowmetry is a better method than thermometry in detecting vascular occlusions in replantation surgery.

B.5 Abstract

Reliable postoperative monitoring in microvascular surgery is necessary to improve the low success rate of reexplorations following vascular compromise. The surface thermometry is known as an easy and inexpensive objective postoperative monitor and therefore it is used by many microsurgeons. The reliability however was not enough and therefore several other instrumental methods have been tested. The laser Doppler flowmetry shows the most promising results. The aim of this study was to compare laser Doppler flowmetry and thermometry in the postoperative monitoring after replantation surgery.

In 34 patients 45 replantations and revascularizations were monitored by laser Doppler flowmetry and thermometry. In this study a reliable alarm value could be defined for replantations and revascularizations of 10 P.U. with a sensitivity of 93 % and a specificity of 94 %. Thermometry showed a sensitivity of 84% and a specificity of 86% at 29 °C.

In conclusion this study shows that laser Doppler flowmetry is a better method than thermometry for postoperative monitoring of replantations and revascularisations.

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

C: Differences between arterial and venous occlusion in microvascular surgery

Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery, accepted for publication

C.1 Introduction

Occlusion of vascular anastomoses is a serious complication of replantation and free vascularized tissue transplantation. Several methods have been developed to detect this threatening condition at an early stage.[123-125,132] It is important to identify whether either the arterial or venous anastomosis or both are obstructed. Clinical signs indicating an arterial occlusion are a pale colour of the skin and no or minimal capillary refill, whereas a blue colour and a rapid capillary refill may indicate a venous problem. In experimental studies and clinical case reports it is shown that laser Doppler flowmetry can detect the difference between an arterial and venous occlusion. Svensson et al. investigated island skin flaps based on the latissimus dorsi muscle in domestic pigs using laser photometry (= Total Backscattered Light intensity of the laser Doppler flowmeter). The T.B.L. remained unchanged or was slightly increased during arterial occlusion and decreased markedly during venous occlusion.[134] In the saphenous island flap in dogs and in the human digit Fischer et al. demonstrated that the pulsatility of the laser Doppler flow signal decreased during arterial occlusion and increased during venous occlusion.[135]

In our department replantations and free vascularized tissue transfers are monitored by observation of tissue colour and capillary refill, thermometry and laser Doppler flowmetry. The aim of this retrospective study was to establish the value of these measurements in detecting differences between arterial and venous occlusions following clinical microvascular surgery.

C.2 Materials and Methods

C.2.1 Colour and capillary refill

Every 30 minutes the colour and capillary refill were clinically assessed at the

volar skin of the distal phalanx of the replanted digit and toe-to-thumb tranfer and on the distal skin or skin island of the free tissue transfer. The colour was judged according to 6 classes: blue, bluish pink, pink, pale-pink, pale or bluish pale. The capillary refill was graded as follows: very rapid, rapid, normal, slow, very slow or absent.

C.2.2 Thermometry

The skin temperature was measured by a thermocouple connected to a thermograph (Y.S.I. 44 TA, Yellow Springs Ohio, U.S.A.). The thermocouple was fixed by medical adhesive tapes on the dorsal skin of the distal phalanx of the replantated digit and toe-to-thumb transfer and on the distal skin or skin island of the free tissue transfer. The temperature was registered every 30 minutes (°C).

C.2.3 Laser Doppler flowmetry

The laser Doppler flowmeter (L.D.F.; Perimed KB, Sweden) works with light from a 2 mW helium neon laser which is conducted via fiberoptics to the tissue surface. The laser beam has a penetration depth of approximately 1 mm in a hemispherical fashion. The light is partly absorbed and partly reflected.

The intensity of the reflected light is called the Total Backscattered Light (T.B.L.) signal and is expressed in percentages (%). Moving particles, mainly erythrocytes, cause a Doppler shift in the reflected light. This change in frequency is converted to a laser Doppler flow signal, which is linearly related to microcirculatory blood flow.[6,8,11,15] Values are presented in perfusion units (P.U.). The measurements were always done at the same place after suturing the laser

Doppler probe holder at the area to be measured. For the replantations and the toe-to-thumb transfers this was the volar skin of the distal phalanx and for free tissue transfers the distal part of the transplanted skin.

The T.B.L. and the L.D.F. (12 kHz; time constant = 3 s.) were recorded on a paper chart (speed 6 cm/h). From the L.D.F. recordings the mean flow and the band width (= vasomotion) were used for analysis.

C.2.4 Patients

From November 1985 to November 1988 150 replantations and revascularizations were performed in 105 patients, and 62 free vascularized tissue transfers in 59 patients. Twenty-six vascular occlusions (17 replantations and 9 free tissue transfers), that occurred postoperatively were monitored by clinical examination, thermometry and laser Doppler flowmetry. Re-operation revealed that in 11 cases only the artery, in 10 cases only the vein and in 5 cases both artery and vein were occluded.

C.2.5 Statistical analysis

Comparisons were made between two groups namely arterial occlusions (n = 11) and venous occlusions (n = 10). The third group, the combination of arterial and venous occlusions (n = 5) was only summarized with mean and standard deviation. The **skin colour** following occlusion of the anastomosis was scored in the following way; 1: blue, 2: bluish pink, 3: pink, 4: pink-pale, 5: pale and 6: bluish pale. The **capillary refill** assessed by close observation was scored as follows; 1: very rapid, 2: rapid, 3: normal, 4: slow and 5: minimal or none. The measured skin temperature

was recorded in °C. Parameters obtained from the laser Doppler recordings were **mean L.D.Flow** after occurrence of the vascular occlusion, **T.B.L. change** by subtracting the mean post-occlusion value from the pre-occlusion value (1 hour) as well as **band width** (= vasomotion) of the laser Doppler flow recording before and after the vascular occlusion. The band width was scored into 4 categories: 1) < 1 P.U., 2) 1 - 2 P.U., 3) 3 - 5 P.U. and 4) > 5 P.U.. For every parameter the two tailed Mann-Whitney rank test was used to evaluate the significance of observed differences between the two groups.

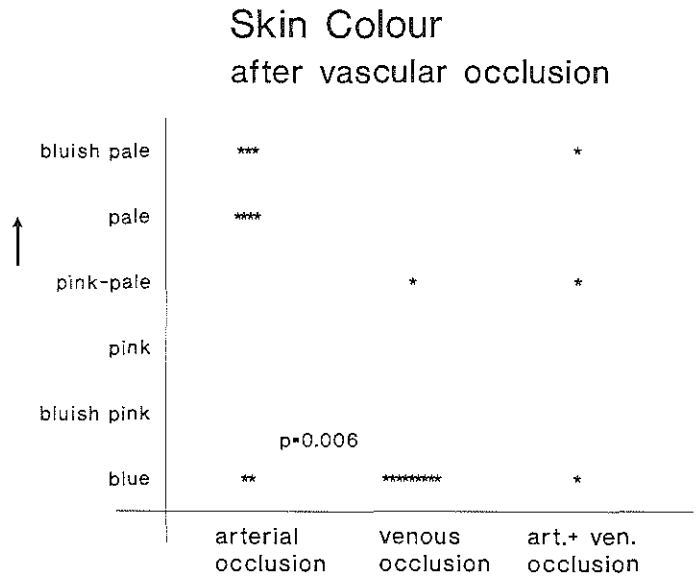


Figure 18. The skin colour after vascular occlusion showed a significant difference (p = 0.006) between the arterial occlusion and venous occlusion group.

C.3 Results

C.3.1 Colour and capillary refill (figure 18,19)

The mean colour score for arterial occlusions was 4.4 (n = 9 ; s.d.= 2.0), for venous occlusions 1.3 (n = 10 ; s.d.= 0.9) and for the combination of arterial and venous occlusions 3.7 (n = 3 ; s.d.= 2.5). The difference between arterial and

venous occlusions is statistically significant (p = 0.006).

The mean capillary refill score for arterial occlusions was 4.5 (n = 10 ; s.d.= 0.7), for venous occlusions 2.2 (n = 8 ; s.d.= 1.5) and for the combination of arterial and venous occlusions 2.25 (n = 4 ; s.d.= 1.3). The difference between arterial and venous occlusions is statistically significant (p = 0.007).

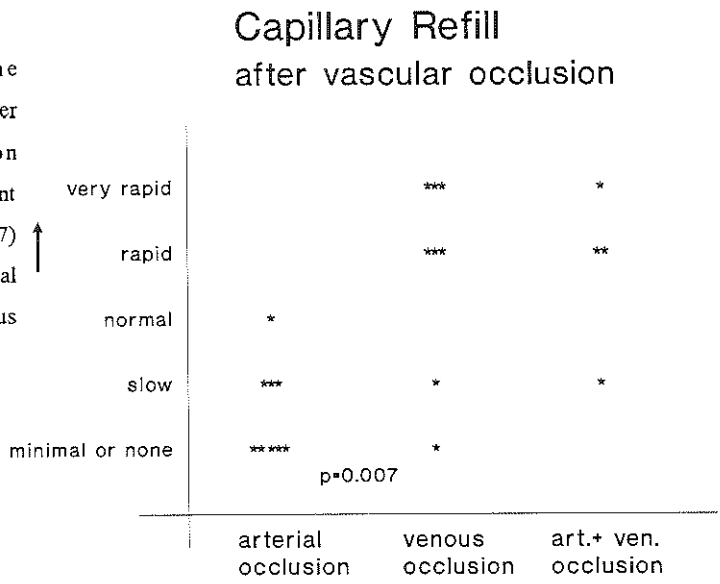


Figure 19. The capillary refill after vascular occlusion showed a significant difference (p = 0.007) between the arterial occlusion and venous occlusion group.

C.3.2 Thermometry (figure 20)

The mean temperature for arterial occlusions was 26.9 °C (n = 9 ; s.d.= 2.5 °C), for venous occlusions 28.5 °C (n = 9 ; s.d.= 2.4 °C) and for the combination of arterial and venous occlusions 30.3 °C (n = 5 ; s.d.= 2.2 °C). The difference between arterial and venous occlusions is not statistically significant (p > 0.05).

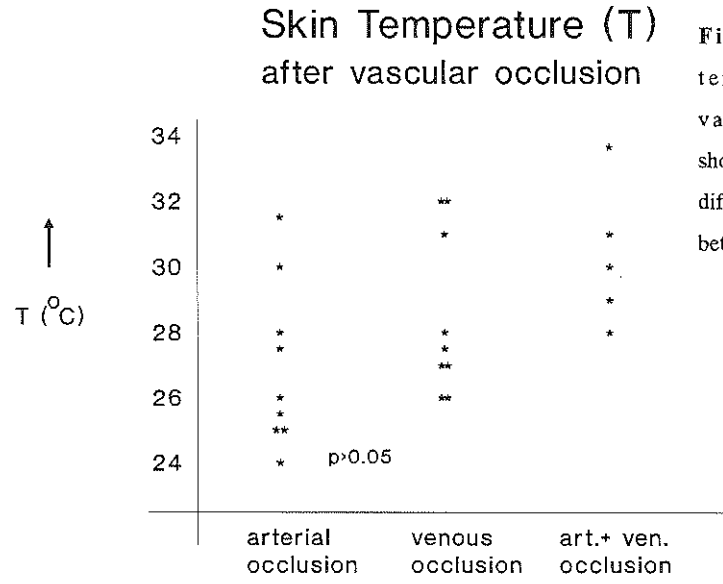


Figure 20. Skin temperature after vascular occlusion showed no significant differences ($p > 0.05$) between the groups.

C.3.3 Laser Doppler flowmetry (figure 21,22,23)

The mean laser Doppler flow for arterial occlusions was 2.7 P.U. (n = 11 ; s.d.= 1.6 P.U.), for venous occlusions 5.5 P.U. (n = 10 ; s.d.= 2.7 P.U.) and for the combination of arterial and venous occlusion 4.8 P.U. (n = 5 ; s.d.= 3.5 P.U.). The difference between arterial and venous occlusions is statistically significant (p = 0.02).

The mean TBL difference for arterial occlusions was −1.2 % (n = 6 ; s.d.= 8.0 %), for venous occlusions 0.3 % (n = 8 ; s.d.= 3.9 %) and for the combination of arterial and venous occlusions 4.5 % (n = 4 ; s.d.= 3.3 %). The difference between arterial and venous occlusions is not statistically significant (p > 0.05). The replantations and free flap transplantations showed the same results.

The mean postocclusion band width score for arterial occlusions was 1.7 (n = 10

; s.d.= 0.8), for venous occlusions 2.1 (n = 8 ; s.d.= 0.6) and for the combination of arterial and venous occlusions 1.6 (n = 5 ; s.d.= 0.9). The difference between arterial and venous occlusions is not statistically significant (p > 0.05).

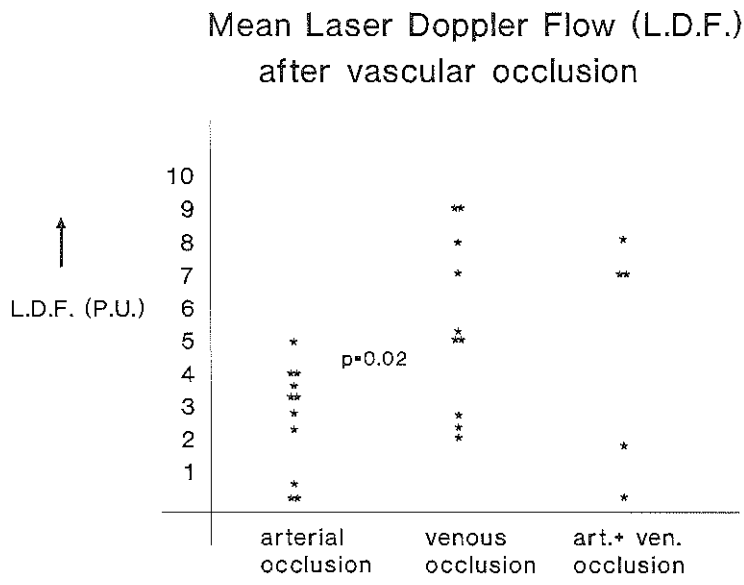


Figure 21. Laser Doppler flow after vascular occlusion showed a significant difference (p = 0.02) between the arterial occlusion and venous occlusion group.

C.4 Discussion

This study demonstrates that clinical signs i.e. tissue colour and capillary refill are the best parameters to discriminate between an arterial and venous occlusion following replantation or free vascularized tissue transfer surgery. Even though clinical signs correlated to arterial or venous occlusion some discrepancies still existed. These discrepancies were two cases with a venous occlusion and a slow capillary refill as well as one case with a venous occlusion and a pink–pale colour. This may be explained by an accompanying arterial vasospasm. Similarly in two cases with an arterial occlusion tissues had a blue colour, probably due to a venous return of blood.

Skin temperature measurements could not differentiate between arterial and

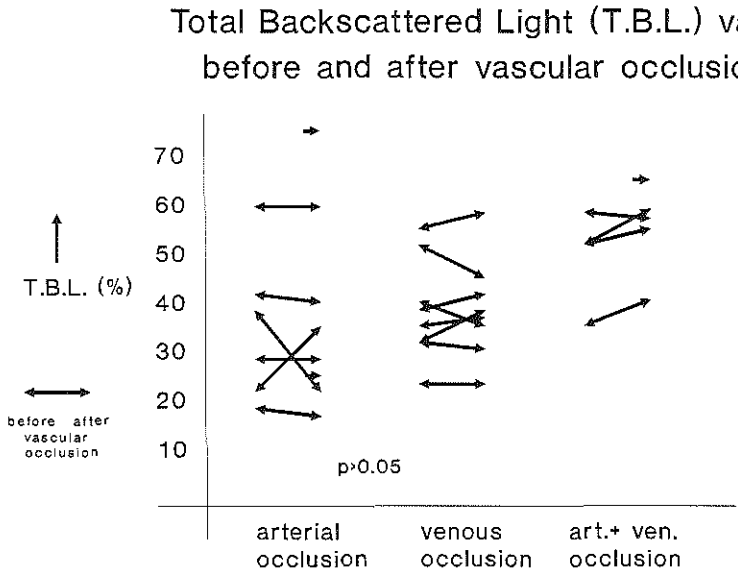


Figure 22. The total backscattered light of the laser Doppler flowmeter showed no significant differences ($p > 0.05$) between the groups.

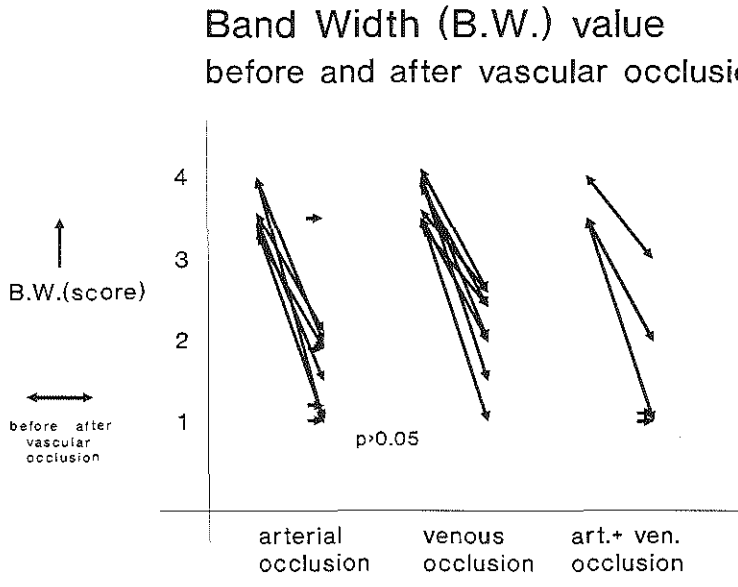


Figure 23. The band width of the laser Doppler flowmeter showed no significant differences ($p > 0.05$) between the groups.

venous occlusion. It seems that only normal circulation created by inflow and outflow of blood via different channels effectively delivers heat to tissues.

In laser Doppler measurements only the flow was statistically significantly different between arterial and venous occlusion. These laser Doppler flow values however showed a wide overlap, making the predictive value of an arterial or venous occlusion less accurate than the clinical signs. The fact that the mean laser Doppler flow value was higher in venous occlusion may be explained by to–and–fro movements of blood cells in the microvessels. These to–and–fro movements are not present in arterial occlusion. The specific total backscattered light (T.B.L.) intensity changes as described by Svensson et al.[134] were not found in this clinical study, making this measurement ineffective as a discriminator in the clinical settings. The difference in results described by Svensson and by us could not be explained by the fact that they studied flaps only and we studied both flaps and replantations. Furthermore Svensson et al. studied normally vascularized flaps that were acutely occluded, whereas in the clinical situation the flaps may start with a relatively low but acceptable flow, followed by a vascular occlusion that may occur relatively slowly. Our results, however, did not indicate such an explanation for the differences in results. The band width, which is caused by vasomotion (= rhytmical flow changes), also failed to separate the arterial occlusions from the venous occlusions. A decrease of the band width, however, may be useful in detecting vascular occlusions per se. Unfortunately the pulsatility of the laser Doppler flow signal to the heart beats could not be analysed in this retrospective study, because this pulsatility was not registered. The value of the pulsatility of the laser Doppler flow signal for discriminating between an arterial or venous occlusion in clinical microvascular surgery will be investigated in a prospective study.[135]

Differentiation between an arterial and venous occlusion is complicated by the fact that both the artery and vein may be occluded. The values of all the measured parameters in the combined arterial and venous occlusion cases turned out to be like

those of a venous occlusion or an arterial occlusion or somewhere in between. Therefore it is impossible to identify the combined arterial and venous occlusion by the studied parameters.

If a vascular occlusion occurs, colour and capillary refill predict best whether the artery or vein is occluded. None of the present parameters is decisive however. It is advised to reexplore firstly the vascular anastomosis, that is suspected to be occluded. If this anastomosis turns out to be open, the other anastomosis should be reexplored. If the vein is firstly reexplored and turns out to be occluded, the flow from the vein of replantation or free vascularized tissue transplantation has to be checked. If the flow is normal, the arterial anastomosis is open. If no or minimal bloodflow exists, the arterial anastomosis has to be reexplored too. If the artery is firstly reexplored and turns out to be occluded, the flow in the replantation or free vascularized transplantation should be normal following revision of the anastomosis. If the flow seems insufficient (by clinical signs, laser Doppler flowmetry[132] and temperature), the venous anastomosis has to be reexplored too.

C.5 Abstract

A serious complication of replantation and free vascularized tissue transfer surgery is occlusion of the vascular anastomoses. Between November 1985 and November 1988 in 11 patients an arterial occlusion occurred, in 10 patients a venous occlusion and in 5 patients a combined arterial and venous occlusion. It was studied in retrospect whether colour and capillary refill, thermometry or laser Doppler measurements could discriminate between an arterial or venous occlusion.

Skin thermometry as well as total backscattered light intensity change and flow band width (laser Doppler measurements) were not statistically significantly different ($p > 0.05$) when comparing the arterial and the venous occlusion cases. A

statistically significant difference however was found for colour ($p = 0.006$), capillary refill ($p = 0.007$) and laser Doppler flow ($p = 0.02$). For all the measured parameters the values obtained from cases with combined arterial and venous occlusion turned out to be either like those of a venous occlusion or an arterial occlusion or somewhere in between.

Although none of the parameters turned out to be decisive, it is advised to check the suspected vascular anastomosis firstly during reoperation. Even if an occluded anastomosis is found, one should pay attention to the other anastomosis because occlusion of both artery and vein cannot be reliably detected.

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

D: Postoperative microcirculatory flow patterns in microsurgically revascularized tissues

Submitted to: British Journal of Plastic Surgery

D.1 Introduction

Clinical microvascular surgery has proven to be essential in reconstructive surgery ever since the first recorded replantation in 1962 and the first free vascularized tissue transfer in 1967.[117,118] Occlusion of microvascular anastomoses gave cause for concern and methods were developed to detect this condition in an early stage.[123-125,132] Little is known however of the behaviour of microsurgically revascularized tissues. Studies on flap physiology were summarized by Sloan and Reinisch but only considered pedicled flaps.[136] The aim of this study was to investigate the postoperative flowpatterns in different microsurgically revascularized tissues in humans by means of laser Doppler flowmetry.

D.2 Materials and methods

D.2.1 The microsurgically revascularized tissues

From November 1985 until November 1988 34 replantations and revascularizations, 19 free radial forearm flaps, 13 free latissimus dorsi flaps and 6 toe-to-thumb transfers were studied. Clinically they all had an uncomplicated course, which means they all survived without reexploration for vascular occlusion or vascular compression and they were continuously monitored by laser Doppler flowmetry (replantations for 4 days, free flaps for 5 days and toe-to-thumb transfers for 3 days). The free vascularized tissue transfers were also monitored as a pedicled flap during surgery, just before transsection of the vascular pedicle. This measurement could not be done on the replants, due to the traumatic separation of the vascular pedicle.

All microsurgical cases which were reexplored for microvascular problems and the cases which had not been monitored by laser Doppler flowmetry or monitored only for a short period were excluded from the study. Only the uncomplicated cases

were studied, assuming them to be representative for normal postoperative flow patterns.

D.2.2 Laser Doppler flowmetry

The laser Doppler flowmeter (L.D.F.; Perimed KB, Sweden) works with light from a 2 mW helium neon laser which is conducted via fiberoptics to the tissue surface. The laser beam has a penetration depth of approximately 1 mm in a hemispherical fashion. The light is partly absorbed and partly reflected.

Moving particles, mainly erythrocytes, in the measured area cause a Doppler shift in the reflected light. This change in frequency is converted to a laser Doppler flow signal, which is linearly related to microcirculatory blood flow.[6,8,11,15] Values are presented in perfusion units (P.U.). The measurements were always done at the same place after suturing the laser Doppler probe holder at the area to be measured. For the replantations and the toe-to-thumb transfers this was the volar skin of the distal phalanx and for free tissue transfers the distal skin or the skin island.

D.2.3 Statistical analysis

In our analysis the monitoring time on each individual was split into half hour periods and within every half hour period the mean value of the laser Doppler flow measurements was obtained. For each of the 4 groups the resulting flow patterns were statistically analysed by means of a repeated measurements analysis of variance with polynomial contrasts (SPSS), in order to assess the polynomial degree (linear, quadratic, cubic etc. component) which will descibe the curve sufficiently close. The half hour mean values of all cases within a group were averaged and plotted against time in order to show the existence of linear and non-linear trends.

D.3 Results

D.3.1 Replantations and revascularizations (figure 24)

The mean first half hour laser Doppler flow value was 98 P.U., followed by a gradual increase (hyperaemia). The maximum mean value of 140 P.U. was reached at 27.5 hours. Subsequently the laser Doppler flow gradually decreased over the second day and stabilized on the third and fourth day around 100 P.U.. The polynomial analysis showed that the quadratic and cubic component were both statistically significant ($p < 0.001$) and that the other components were not significant. This means that the flow pattern of a hyperaemic period followed by a stabilization at the level of its starting value (a third degree polynomial) is statistically significant.

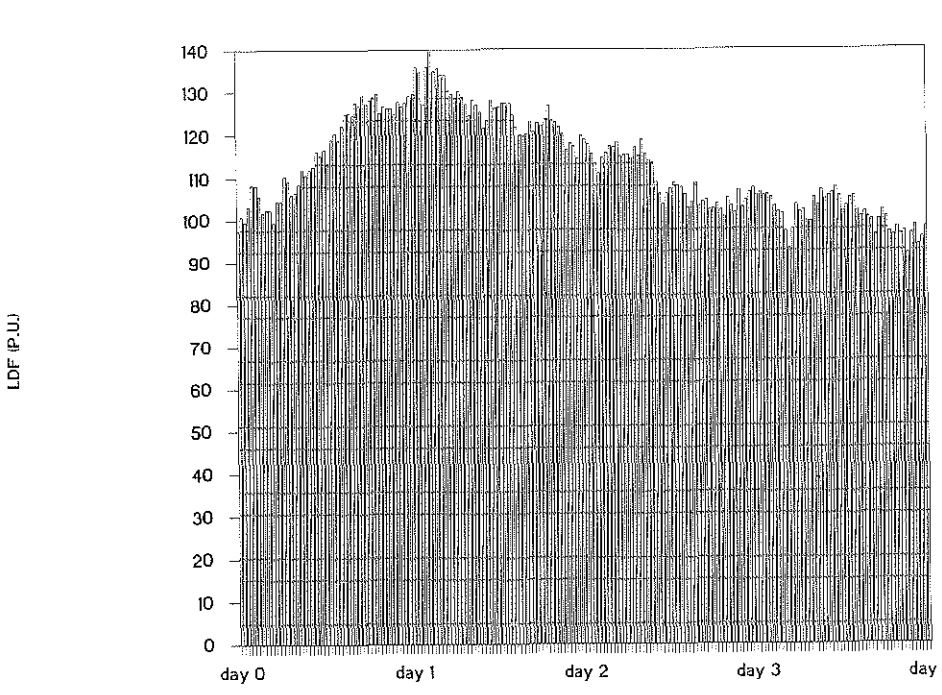


Figure 24. Mean laser Doppler flow values in the postoperative monitoring of 34 replantations. An initial high mean L.D.F. value was followed by a hyperaemic period and returned to a stable level comparable to the starting value.

D.3.2 Free radial forearm flaps (figure 25)

The mean first half hour laser Doppler flow value was 16 P.U., followed by a rapid increase over the first 2.5 days and a slow increase over the second 2.5 days. The maximum mean value of 53 P.U. was reached at the end of the fifth day. The mean value of the radial forearm flaps before transsection of their vascular pedicle was 55 P.U..

The linear component was statistically significant ($p < 0.0005$) and none of the other components were significant. This means that the flow pattern shows a significant gradual increase (a first degree polynomial).

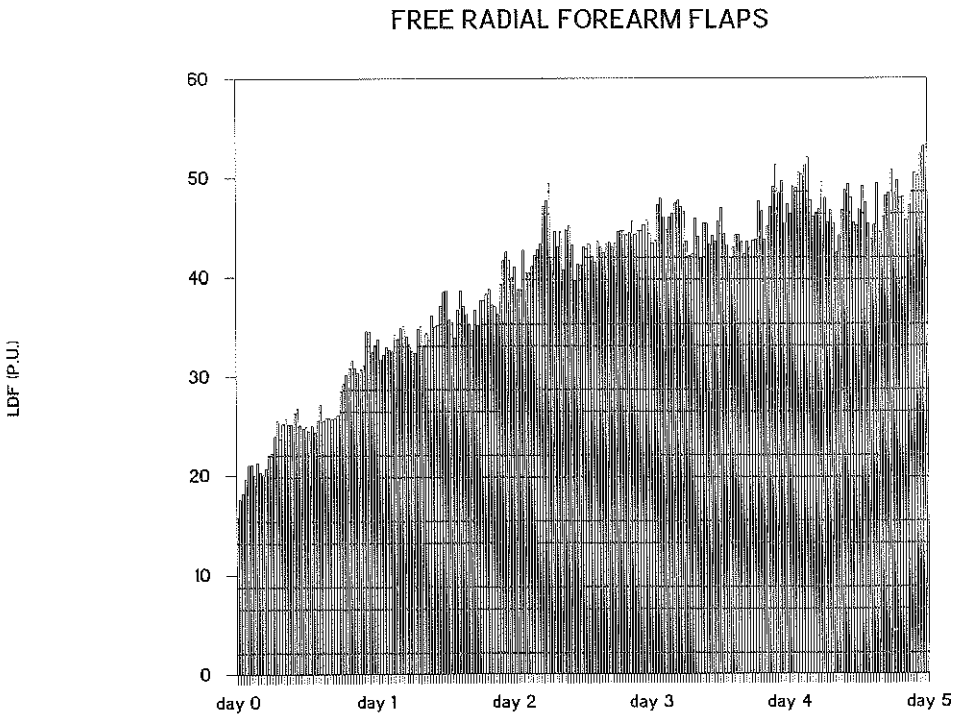


Figure 25. Mean laser Doppler flow values in the postoperative monitoring of 19 free radial forearm flaps. An initial low mean L.D.F. value was followed by a gradual increase over 5 days.

D.3.3 Free latissimus dorsi flaps (figure 26)

The mean first half hour laser Doppler flow value was 9.1 P.U., followed by an increase over the first 2.5 days and a stabilization during the second 2.5 days. The maximum mean value of 17 P.U. was reached at 2 days and 9 hours and at 4 days and 6.5 hours. The mean value of the latissimus dorsi flaps before dissection of their vascular pedicle was 32 P.U..

The linear and quadratic components were statistically significant ($p = 0.021$; $p = 0.002$ respectively) and none of the higher degrees were significant. This means that the flow pattern shows a significant increase followed by a stabilization (a second degree polynomial).

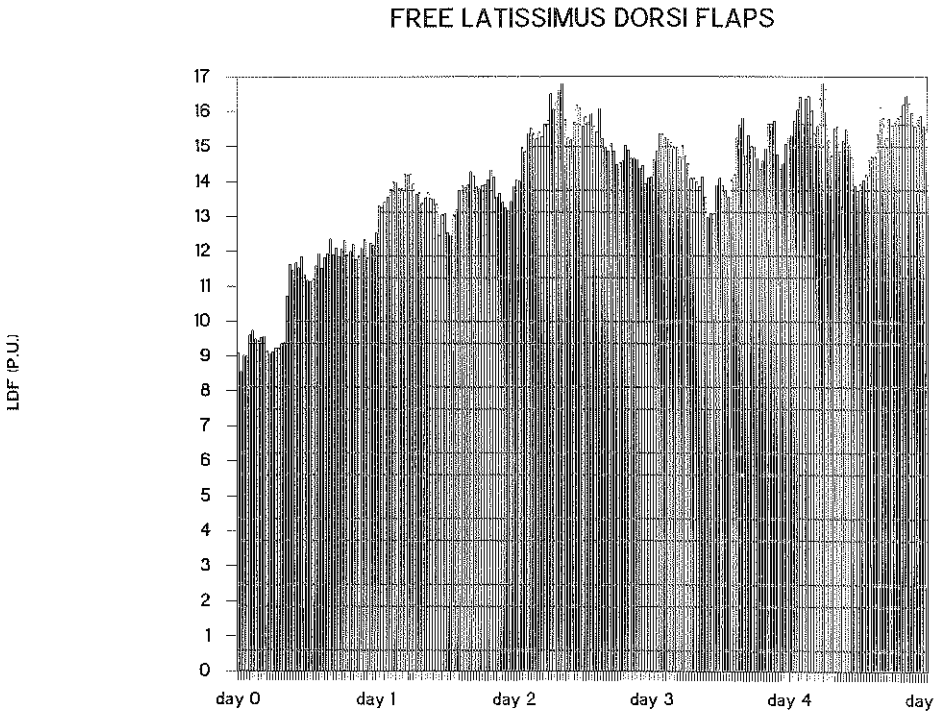


Figure 26. Mean laser Doppler flow values in the postoperative monitoring of 13 free latissimus dorsi flaps. An initial low mean L.D.F. value was followed by a gradual increase during the first 2.5 days and a stabilization on the second 2.5 days.

D.3.4 Toe–to–thumb transfers (figure 27)

The mean first half hour laser Doppler flow value was 87.7 P.U.. Statistical analysis showed that none of the polynomial components were statistically significant. This means that the pattern seems to follow a course that is somewhat erratic, but cannot be described well by a polynomial curve of moderate degree. The mean laser Doppler flow value reached a maximum mean value of 108 P.U. at 1 day and 18.5 hours. The mean value of the toes before transsection of their vascular pedicle was 82 P.U..

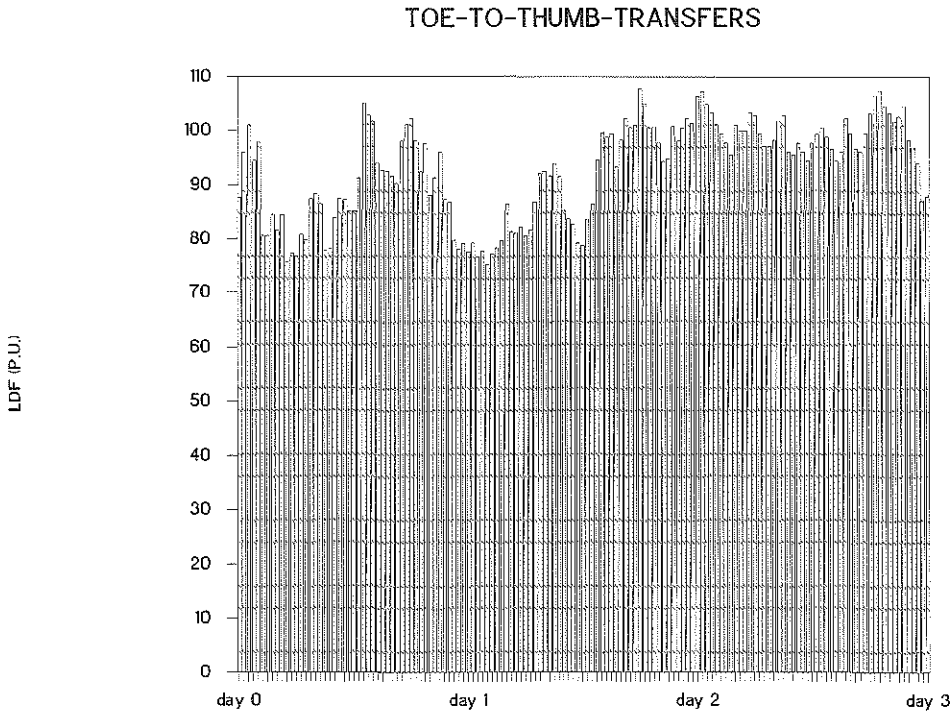


Figure 27. Mean laser Doppler flow values in the postoperative monitoring of 6 toe–to–thumb transfers. An initial high mean L.D.F. value remained stable during the whole monitoring period.

D.4 Discussion

This study shows that replantations and revascularizations, toe–to–thumb transfers, free vascularized radial forearm flaps and latissimus dorsi flaps all have different postoperative flow patterns, even though they all were dissected completely from the body, lacked any kind of innervation and lymphatic drainage and were revascularized by microvascular arterial and venous anastomoses. Unfortunately no conclusions can be drawn from the absolute laser Doppler flow values, because the skin in different sites has its own vascular anatomy and capillary density, thereby influencing the skin blood flow. It is also impossible to obtain true controls, because the inflow and outflow of blood in the skin for the measured sites is changed by surgery, but the measurements just before transsection of the vascular pedicle are the best available.

Regarding the flow patterns, division into two groups was made. The first group consisted of the replantations and revascularisations combined with the toe–to–thumb transfers. They started at a laser Doppler flow (L.D.F.) value, which was approximately equal to the L.D.F. value at the end of the registration. The replantations and revascularizations however showed a hyperaemic period on the first and second day in contrast to the toe–to–thumb transfers. This could be caused by the longer ischaemic period of the replantations and revascularizations. The second group consisted of the free vascularized radial forearm flaps and latissimus dorsi flaps. They started at an L.D.F. value which was much lower then the L.D.F. value at the end of the registration, followed by a gradual increase.

Some differences between these two groups could explain the distinction of the postoperative flow patterns. The replantations and revascularizations and toe–to–thumb transfers are primary vascular end–organs, they were fixed to the receptor site steadily by means of a bony fixation, they have relative small wound surfaces and their skin is in its normal anatomical position. The free vascularized radial

forearm flaps and latissimus dorsi flaps however are secondary vascular end organs, they were fixed to the receptor site rather flaccidily by means of soft tissue stitches, they have relative large wound surfaces and their skin is out of the original anatomical and physiological position. A clear explanation however could not be given and further research on the physiology of microsurgically revascularized tissues in the human being has to be done.

D.5 Abstract

Thirty-four replantations and revascularizations, 19 free vascularized radial forearm flaps, 13 free vascularized latissimus dorsi flaps and 6 toe-to-thumb transfers with a succesfull postoperative course were monitored postoperatively by means of the laser Doppler flowmetry to study the physiology of microsurgically revascularized tissues in the human situation.

Different flowpatterns were seen for each group. The replantations and revascularizations and the toe-to-thumb transfers started at a value, closely to the mean of the total monitoring period, whereas the free vascularized flaps started at a low value followed by a gradual increase. The flow patterns were statistically significant.

The difference in postoperative flow patterns could be caused by difference in being primary or secondary vascular end-organs, in steadiness of fixation, in area of wound surface and in the relation to its normal anatomical position.

CHAPTER II: EVALUATING L.D.F. AS DIAGNOSTIC METHOD

E: Postoperative monitoring of allogeneic limb transplantation in rats

Annals of Plastic Surgery, 1988;21:559–65

E.1 Introduction

Without immunosuppressive drugs the life expectancy of allogeneicly transplanted limbs in rats in general does not exceed three weeks.[137–139] With the strong immunosuppressive drug cyclosporine A however, transplanted limbs remain vital for as long as the drug is given. Due to the side–effects of immunosuppressive drugs it is important to keep the dosage as low as possible without rejection and loss of the allogeneic transplant. Information about the initial onset of rejection is therefore essential. The earlier the onset of rejection is detected the earlier an adaptation of the immunosuppressive medication scheme can be initiated to prevent possible severe damage to vital tissues in the transplant.[140]

Clinical signs of rejection are not always easy to interpret and when skin changes become evident, rejection is already advanced. Frequent histological examination to detect rejection is in practice difficult.

The purpose of this study is to investigate whether laser Doppler flow changes correlate to histological changes, especially of the microvessels, during rejection and whether it can detect initial onset of rejection. Laser Doppler flowmetry was used to assess microcirculation of the skin.[53,141]

Since we were unable to obtain histology from transplanted limbs repeatedly, all measurements were done in each rat only once on the random day of sacrifice. A pilot study had been performed to obtain technical experience with the model. In this pilot study none of the allogeneic limb transplants survived over two weeks, therefore we performed all our experiments between days 2 and 14 after transplantation.

E.2 Materials and methods

E.2.1 Experimental design

Allogeneic limb transplantations were performed in 20 rats. Immunosuppresive drugs were not given. Inbred specific pathogen–free BN/Bi rats were used as donors and Wag/Rij rats were used as acceptors. This combination represents a very strong mismatch. In the control group 6 isologeous limb transplantations were performed on Wag/Rij rats. The weights of the rats ranged between 315 and 400 grams. They were not used for other experiments.

The planned day of post mortem study was determined for each rat in a random fashion, with the restriction of an even distribution of the rats over the relevant period. In the allogeneic group all experiments were performed between days 2 and 14 and in the control group between days 7 and 304. On the day of obduction all animals were subsequently subjected to: clinical examination, biopsies for histological examination and laser Doppler flowmetry in the transplanted limb as well as in the contralateral limb.

The following clinical signs were scored: edema, colour, hair loss, epidermolysis, crust formation and exudation.

Histological examination was performed on the skin, the subcutaneous soft tissue and the bone at the height of the proximal part of the foot. Histological grading was scored as no signs of rejection; epidermal changes and inflammatory infiltration; mononuclear infiltration; obstructive vascular changes and diffuse mononuclear infiltration; hemorrhage and necrosis. All gradings were done by the same pathologist in one session.

Laser Doppler flow was assessed in all animals during 5 minutes on three locations, i.e. distal and proximal volar aspect as well as the dorsum of the foot. The obtained graphic representation was divided into 5 blocks of 1 minute. The median of each block was taken from the values taken at 6, 18, 30, 42 and 54 seconds. The medians of each block were combined to achieve a mean value for

every location. We used the mean value of the three locations to obtain an overall mean value as a representative laser Doppler flow measurement for every limb.

E.2.2 Surgical technique

We used the same procedure as described by Fritz et al. in 1984.[137] After induction and maintenance of anesthesia with an ether gauze, the vessels of the donor limb were mobilized through an inguinal approach. The femoral vessels were dissected inferiorly in continuity with the saphenous vessels which run superficially along the medial aspect of the leg.

All other branches, including the profunda femoris and the popliteal vessels were ligated with bipolar coagulation. The saphenous vessels were dissected free to the level of the midcalf, where amputation was performed leaving the leg attached to the long vascular pedicle. Heparine solution, 1 ml (50 IU/ml), was given intravenously. At this point the recipient rat was prepared by mobilizing the femoral vessels to the level of the superficial epigastric vessels. A subcutaneous tunnel was formed by blunt dissection from the inguinal region to the dorsum. A 2 cm dorsal midline incision was made at the end of the subcutaneous tunnel. The donor and recipient femoral vessels were then clamped and divided. The limb was pulled through the subcutaneous tunnel with the toes first. Care was taken not to twist the long vascular pedicle. The limb was sutured to the dorsum of the recipient rat. After anastomosis of the femoral veins and arteries with 10–0 nylon, under the operating microscope, the clamps were released and blood flow observed.

A few drops of xylocaïn 2% were applied on the anastomoses. The operation was completed by the closure of the inguinal incision. At the end of the operation the recipient rat was given 2 ml 0,9% sodium chloride intravenously as infusion and recovered under heat lamps at 37°C. Time of ischemia was 45 to 60 minutes. Total operation time was 2 to 3 hours.

E.2.3 Statistical analysis

For all parameters their measured values were related to time elapse. The degree of histological rejection was compared with laser Doppler flow values. The ratio of values measured simultaneously in transplanted and contralateral limbs was taken as outcome in order to minimize surrounding influences (e.g. room temperature, depth of anesthesia etc.). For statistical analysis the rank correlation test according to Spearman was used. This test is known to be hardly influenced by extreme values. The one sided test was used because the direction of the correlation under the alternative hypothesis was known a priori.

E.3 Results

In the study vascularised transplants were performed in 34 animals. Eight animals were excluded from the experiment, i.e. two rats were lost during anaesthesia, five transplants were lost within 48 hours due to vascular thrombosis, one rat in the allogeneic group was excluded on day 12 following severe rejection and loss of the transplant.

E.3.1 Clinical signs

In the control group (6 animals) limbs were transplanted from one Wag/Rij rat to another inbred Wag/Rij rat. The transplanted limbs showed moderate edema, which resolved within a week. The limbs appeared healthy for as long as 10 months after operation, except for the toes of the denervated limb which were nibbled two to three months postoperatively.

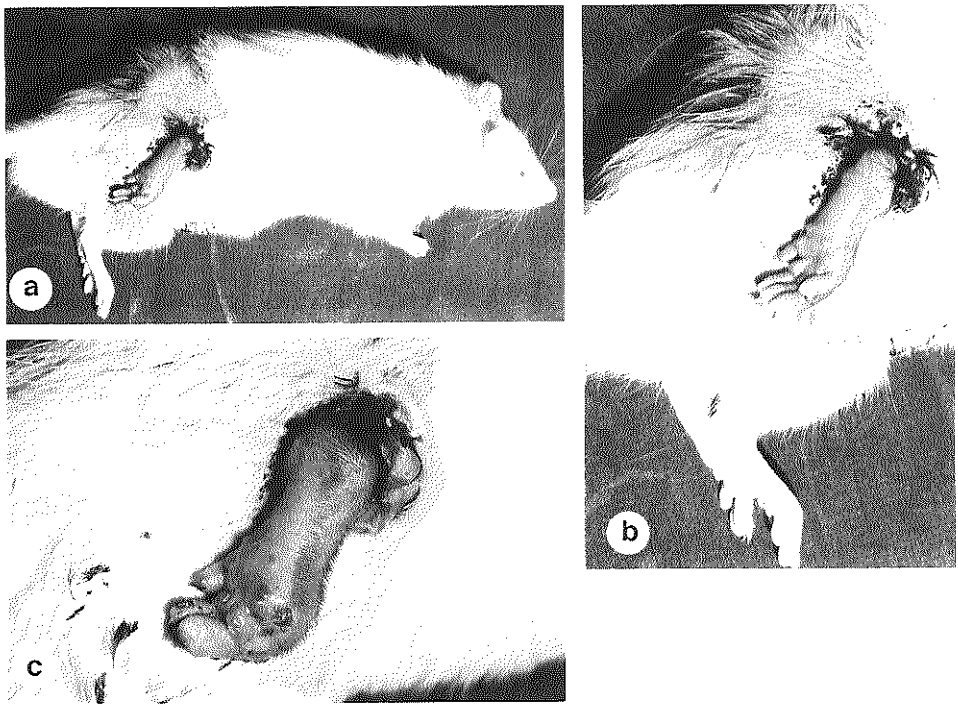


Figure 28. Allogeneic limb transplantation postoperatively;
(A) = day 2; (B) = day 7; (C) = day 12.

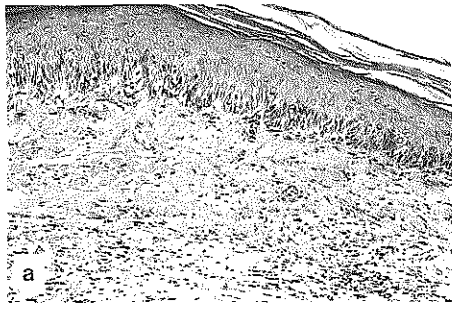
In the allogeneic group (20 animals) limbs were transplanted from BN/Bi rats to Wag/Rij rats (figure 28). The transplanted limbs showed progressive edema. Colour changes were difficult to assess due to brown donor limbs. Hair loss was seen in 5 rats after the 10th postoperative day. Epidermolysis, crust formation, exudation and leathery skin were progressively seen from the seventh postoperative day onwards.

E.3.2 Histology

The earliest morphological changes in the allotransplanted limbs became manifest on day 4 after surgery (figure 29a). The changes consisted of focal areas of

moderate epidermal spongiosis and dyskeratosis, and they were accompanied by a polymorphous leucocytic inflammatory infiltration of the papillary and the reticular dermis. The capillary blood vessels were strikingly dilated and filled with red blood cells. At the fifth and the seventh day a mononuclear inflammatory infiltration was distinctly observed in the dermal layers and in the subcutis. The infiltration had a patchy distribution mostly arround the capillaries and the small venules. The cell population was mostly lymphocytic, but some large mononuclear cells with vesicular nuclei and eosinophylic cytoplasma were seen.

The infiltration became more dens and was diffusely distributed on day 8 (figure 29b). Occlusive lesions of the dermal capillaries and small sized vessels, accompanied by endothelial damage and followed by disintegration of the vessel wall were the most prominent vascular alterations at that time.



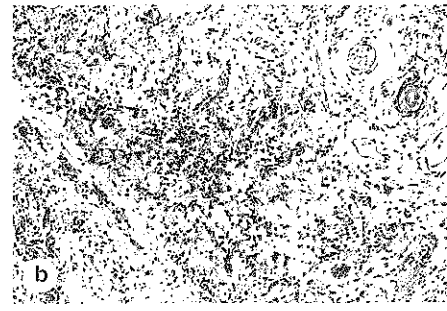


Figure 29a. Day 4. The epidermis shows a slight damage with spongiosis, nuclear pycnosis and lymphocytic infiltration. In the upper parts of the reticular dermis there is a mild mononuclear inflammatory infiltration (H&E, original magnification x 180). **Figure 29b.** Day 8. A dense diffusely distributed inflammatory infiltration in the dermis (H&E, original magnification x 180). **Figure 29c.** Day 12. Severe epidermal changes consisting of spongiosis, cell vacuolation and nuclear pycnosis. The papillary dermis shows severe diffuse edema, inflammatory infiltration and occlusion of the capillary blood vessels (H&E, original magnification x240).

The progressive obliterations of the dermal vessels resulted in ischemic necrosis of the transplants. It was evident by days 12 to 14 (figure 29c). The rejection of the transplanted limb for soft tissue in relation to time was significant ($p = 0.02$).

E.3.3 Laser Doppler flowmetry

In 16 rats of the allogeneic group a reliable graphic representation of the laser Doppler flow was obtained. This was also achieved in all rats of the control group (6 rats). In the allogeneic group the laser Doppler flow values of the contralateral limb were not statistically significant related to time elapse ($p > 0,05$). This of course is as expected, but the variation in absolute values however are substantial. The laser Doppler flow of the transplanted limb and the ratio of transplanted and contralateral limb were statistically significant in relation to time ($p < 0,001$ and $p < 0,0001$, respectively; figure 30).

Soft tissue rejection correlated well to laser Doppler flow of the transplanted limb ($p < 0,05$) as well as to the ratio transplanted and contralateral limb ($p < 0,01$; figure 31).

E.4 Discussion

Laser Doppler flow of an allogeneic transplant showed a continuous decline postoperatively ($p < 0.001$). From the 7th postoperative day the values were consistently low (at most 55% of the contralateral limb). Before the 6th postoperative day all values were above 55% of the contralateral limb. A significant correlation with histological rejection was found ($p < 0.01$). Occlusive lesions of the dermal capillaries and small sized vessels were seen on the 8th postoperative day

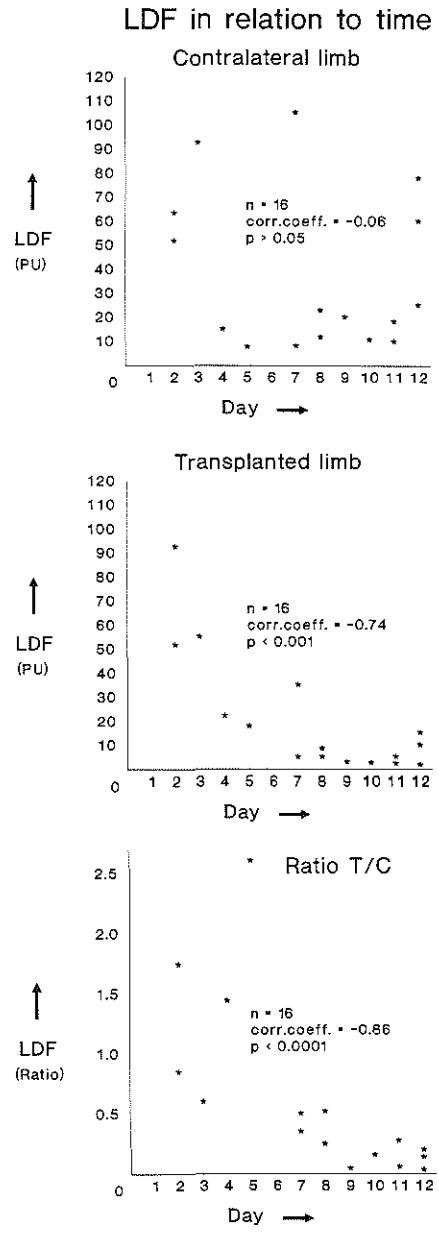


Figure 30. Laser Doppler Flowmetry (LDF) in the allogeneic group related to time (Ratio T/C = Ratio of transplanted limb and contralateral limb).

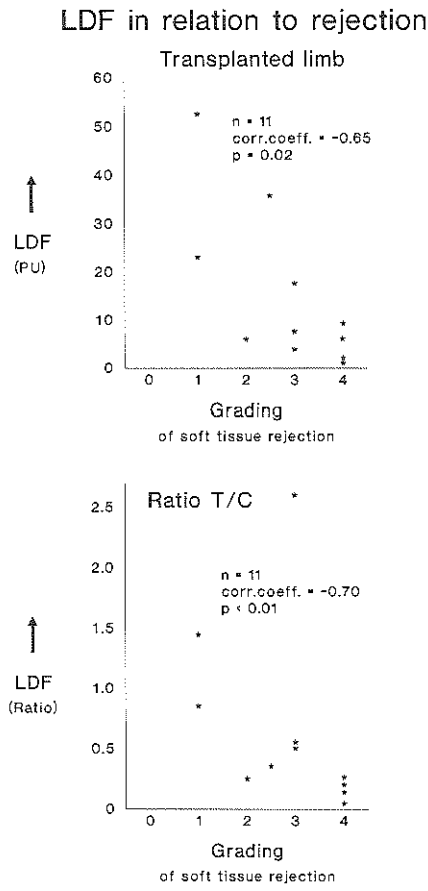


Figure 31. Rejection correlated to laser Doppler flow (LDF) in the transplanted limb. (Grading of soft tissue rejection: 0 = no signs of rejection; 1 = epidermal changes and inflammatory infiltration; 2 = mononuclear infiltration; 3 = obstructive vascular changes and diffuse mononuclear infiltration; 4 = hemorrhage and necrosis. T/C = ratio of transplanted limb and contralateral limb).

and they progressed the following days; this correlates to low laser Doppler flow values and as such the laser Doppler flow is a good parameter in establishing rejection.

Early detection of transplant rejection is of great importance because adaptation of immunosuppressive drugs dosage and/or scheme can still be administered to save the transplant. In our model however laser Doppler flowmetry did not detect rejection earlier than clinical judgement, because epidermolysis as a strong clinical

evidence of rejection was also seen from the 7th postoperative day onwards. A clear turning point of the laser Doppler flow before the 7th postoperative day could not be detected. In search for this turning point 7 rats were followed nearly daily with LDF measurements. Due to a great variety of values before the 7th postoperative day this point could not be produced. To reduce the great variety of values and to produce a turning point continuous measurements are preferred, as we are accustomed to do in the clinical patient. Unfortunately this is not possible in the rat model.

In conclusion in the allogeneic limb transplantation model in the rat clinical and histologic examination as well as laser Doppler flow measurements ($p < 0,0001$) are good parameters to determine rejection and a good correlation is seen between the methods ($p < 0.01$). In this model however laser Doppler flow values cannot predict an initial onset of the rejection before clinical signs are evident, presumably due to the impossibility of continuous measurements. Daniel et al. could reverse severe skin changes together with edema, as seen in our model from the 7th postoperative day, by changing the immunosuppressive treatment,[140] demonstrating that clinical signs, histological examination and laser Doppler flowmetry can be valuable parameters in saving allogeneic transplants.

E.5 Abstract

Allogeneic limb transplantation with and without immunosuppression has been successfully reported. The experimental model described by W.D. Fritz, et al. was used to investigate whether laser Doppler flow is related to histological changes, especially of the microvessels, during rejection and whether it can detect initial onset of rejection.

Twenty rats of inbred strains sustained an allogeneic limb transplantation without

immunosuppression. In 6 rats an isologous limb transplantation was performed as a control group.

Parameters investigated in the transplanted limbs were clinical signs, histology and laser Doppler flowmetry. All parameters were compared with the normal contralateral limb of the rat. All rats were sacrificed between the 2nd and 14th postoperative day.

In the allogeneic limb transplantation model in the rat clinical and histologic examination as well as laser doppler flow measurements ($p < 0,0001$) are good parameters for rejection. A significant correlation was found between laser Doppler flowmetry and histology ($p < 0.01$) and occlusive lesions of the dermal capillaries and small sized vessels correlated to low laser Doppler flow values.

In this model however laser Doppler flow values cannot predict an initial onset of the rejection before clinical signs are evident, presumably due to the impossibility of continuous monitoring.

CHAPTER III: CIGARETTE SMOKING AND MICROCIRCULATION

A: Acute effects of cigarette smoking on microcirculation of the thumb

British Journal of Plastic Surgery, 1992;45:9–11

A.1 Introduction

In our department patients are strongly advised to refrain from smoking before and after flap surgery to optimise wound healing conditions. Experimental and clinical studies on the effect of cigarette smoking on random vascularised pedicled skin flaps showed an increase of partial flap necrosis in smokers.[142] This negative effect of smoking is probably caused by an increased platelet aggregation,[143] lower oxygen transport due to carbon monoxide–haemoglobin (COHb) formation and a diminished microcirculatory flow due to the combination of macroangiopathy, microangiopathy and vasoconstriction.[144] Several studies indicated decreased microcirculatory flow during smoking by means of indirect measurements.[145–148]

Intravital microscopy and laser Doppler flowmetry are two direct methods that can monitor the cutaneous microcirculation continuously and non–invasively.[12,149] Continuous monitoring of the capillary flow during smoking is only described in an experimental animal study.[150]

The aim of this study was to establish the acute effects of cigarette smoking on the microcirculation in the human skin directly and continuously.

A.2 Materials and methods

A.2.1 Laser Doppler flowmetry

The laser Doppler flowmeter (L.D.F.; Perimed KB, Sweden) has been extensively described previously.[11,12] Values are presented in perfusion units (P.U.).

A.2.2 Experimental design

Thirty–two healthy volunteers entered the study. Twenty–two were smokers, 10

were non-smokers. The mean age of the smokers was 28.8 years (range 18 - 47) and of the non-smokers 28.3 years (range 19 - 47). Fifteen volunteers were males and 17 females, equally distributed among the two groups. The smoking volunteers habitually smoked 7 - 25 cigarettes per day (mean = 18); the nicotin content varied from 0.1 - 1.5 mg per cigarette (mean = 1.1 mg).

Following acclimatisation to the environment for 30 minutes at a surrounding temperature of 23°C each volunteer sat at a table with the dominant arm placed at heart level. The hand rested on a special splint with the forearm in pronation. The laser Doppler probe was placed on the volar skin of the distal phalanx of the thumb by means of a custom-made probe holder using double-adhesive tape (figure 32).

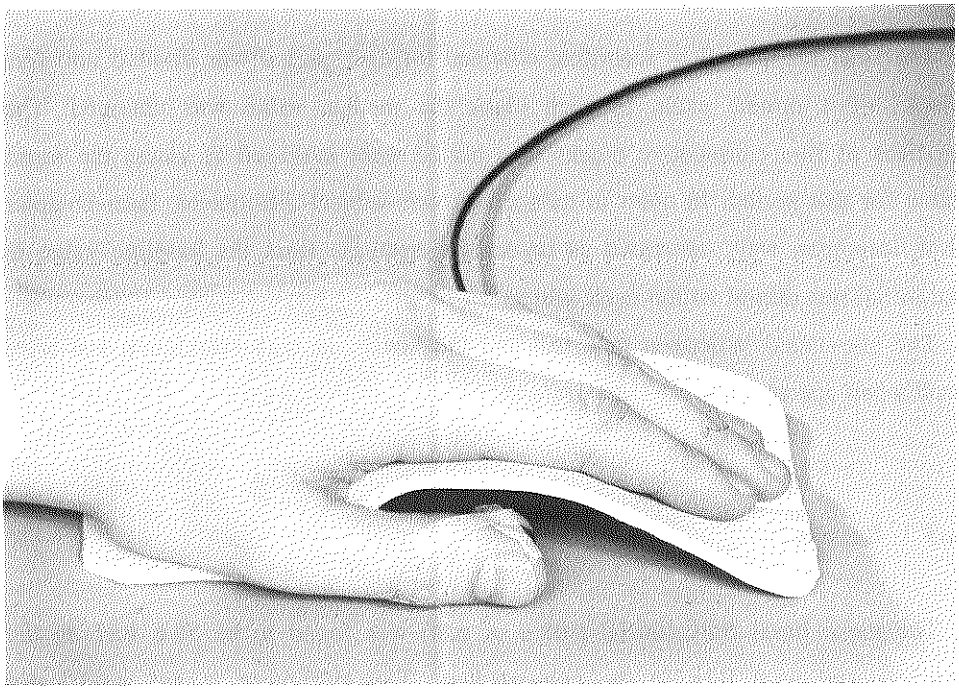


Figure 32. During laser Doppler flow measurements the hand rested on a special splint.

During the test the laser Doppler flow was monitored and recorded continuously during 25 minutes. The experiment consisted of 5 periods of 5 minutes. The smokers started with a period of rest, whereafter in the second and third period 2 own brand cigarettes were smoked, followed by 2 rest periods (4 + 5). The non-smokers had the same regime, but they inhaled through a pipe of glass instead of smoking, thereby exerting the same efforts, without inhaling cigarette smoke. All volunteers were instructed to inhale well. The first rest period was to obtain a basal flow value, the fourth and fifth period were the recovery phase.

A.2.3 Statistical analysis

The laser Doppler flow recording obtained for each period was divided into five blocks of 1 minute each. In each block the median value was obtained. The medians of five consecutive blocks in a period were averaged to assess the mean value for that period. In order to reduce between-subject differences, laser Doppler flow responses were expressed as percentage decrease from the baseline value in the initial rest period within each subject.

The one-tailed Wilcoxon rank test and the Spearman rank correlation test were used when appropriate.

A.3 Results

The mean basal flow (initial period of rest) was found to be 39.6 P.U. in the smokers and 45.0 P.U. in the non-smokers. This difference is not statistically significant ($p = .12$). During smoking of the first and second cigarette respectively, the laser Doppler flow decreased significantly (-23.8%, -29.0%). This fall had

already become evident during the first two minutes of smoking (–20.3%). The mean laser Doppler flow stayed stable during the first recovery period (–28.4%) and started to return to the basal level in the second recovery period (–20.5%), but even during the last two minutes of the fifth period the recovery was only partial (–15.6%). In the non–smokers group the mean laser Doppler flow did not show major variations. The differences in percentage decrease of the laser Doppler flow between smokers and non–smokers are statistically significant (figure 33,34).

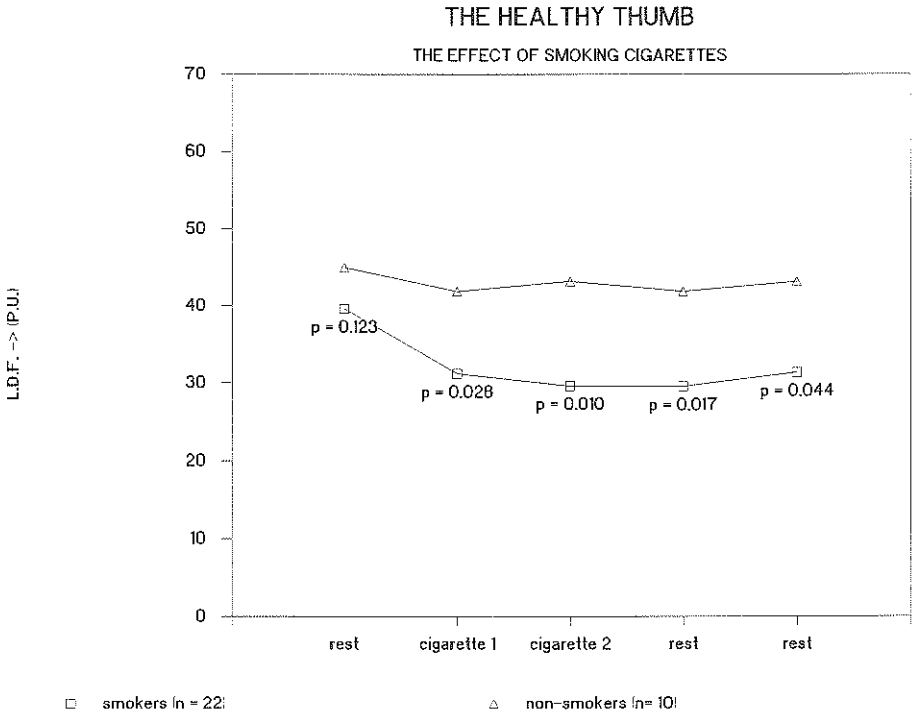


Figure 33. The effect of cigarette smoking on the microcirculation of a healthy thumb compared to the effect of sham–smoking on the microcirculation of non–smokers. The mean basal flow is lower in the smokers than the non–smokers, but this difference is not statistically significant (p = 0.12). During smoking two cigarettes the mean laser Doppler flow decreased from 39.6 P.U. to 29.6 P.U.. Ten minutes after smoking this decrease was recovered by half. The non–smokers did not show major variations.

No significant correlation was found between the nicotin content of the smoked cigarettes and the laser Doppler flow change during smoking. In conclusion, a significant decrease in the mean laser Doppler flow was seen during smoking of the first and second cigarette (–23.8 % and –29.0 %), which recovered by half after ten minutes of rest.

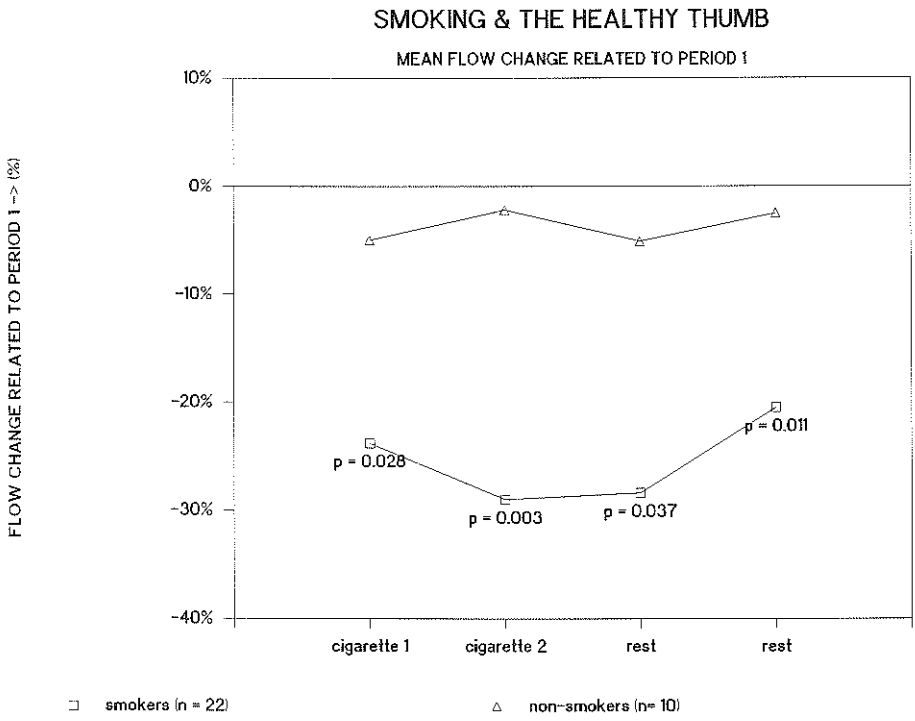


Figure 34. The effect of cigarette smoking on the microcirculation of a healthy thumb in relation to the first period of rest. During smoking the laser Doppler flow decreased 29 %. Ten minutes after smoking this decrease had recovered by half. The laser Doppler flow of the non–smokers did not show major variations.

A.4 Discussion

This study shows that during smoking of cigarettes the microcirculatory flow of the thumb skin decreases considerably and only recovers by half after 10 minutes of rest. It is to be expected that this flow decrease is detrimental to woundhealing and flap survival. By refraining from smoking this acute negative effect will be eliminated only slowly. It is unlikely that stopping smoking for the postoperative period only would be sufficient to remove all the negative effects of smoking. During the preoperative phase smoking should be abandoned, but how long before operation is uncertain. More clinical research is needed to establish the effects on wound healing of terminating smoking before as well as after surgery. Until now all research has shown only the negative effects of smoking itself, and not the beneficial effects of stopping.

It is debatable whether these experimental results can be extrapolated to microvascular surgical practice. The free vascularized tissue transplant and the replanted digit lack autonomous innervation, an important vasoregulatory mechanism. However, case reports indicate that cigarette smoking can be damaging to replanted digits.[151]

Considering the literature and our test results we advise patients to stop smoking cigarettes before and after undergoing flap surgery, especially when the circulation might be critical.

A.5 Abstract

The acute effect of smoking on the microcirculation of the skin of the thumb was investigated in healthy volunteers. Twenty–two were smokers and 10 were non–smokers. The flow was assessed by means of the laser Doppler flowmetry. The

smokers inhaled two cigarettes. During smoking of their first and second cigarette respectively, a mean decrease in laser Doppler flow of 23.8 % and 29.0 % was seen ($p = 0.03$; $p = 0.01$). Ten minutes after smoking this decrease was recovered by half.

This experiment confirms that one should prohibit smoking of cigarettes pre– and postoperatively for optimal wound healing conditions.

CHAPTER III: CIGARETTE SMOKING AND MICROCIRCULATION

B: The acute effect of cigarette smoking on the microcirculation of a replanted digit

The Journal of Hand Surgery, 1992;17:230–4

B.1 Introduction

Microsurgeons suspect that cigarette smoking has a negative effect on the success of replantation surgery. Except for a case report of the damaging effect of smoking on digital revascularization, little is known about the effect of smoking on microvascular surgery.[151] Experimental and clinical studies of the effect of cigarette smoking on random vascularized pedicled skin flaps, however, showed an increase in partial flap necrosis in smokers.[142,152-156] This negative effect is explained by an increased platelet aggregation,[143,157,158] a decreased oxygen transport due to carbon monoxide–hemoglobin formation, and a diminished microcirculatory flow due to the combination of macroangiopathy, microangiopathy and vasoconstriction.[144,159] Several studies indicated impaired microcirculatory flow during smoking by means of indirect[145-148,160] and direct flow measurements.[149,150,161] Not all these results can be extended to replantation surgery, however, because after digital replantation the regulation of microvascular flow is disturbed (e.g., as a result of complete denervation).

The aim of this study was to establish by means of laser Doppler flowmetry the effect of cigarette smoking on the microcirculation of replanted digits.

B.2 Materials and methods

B.2.1 Laser Doppler flowmetry

The laser Doppler flowmeter (Perimed KB, Sweden) works with light from a 2 mW helium neon laser, which is conducted by means of fiberoptics to the tissue surface. The laser beam has a penetration depth of approximately 1 mm in a hemispherical fashion. The light is partly absorbed and partly reflected.
Moving particles, mainly erythrocytes, cause a Doppler shift in the reflected light.

This change in frequency is converted to a laser Doppler flow signal, which is linearly related to microcirculatory blood flow.[6,8,11,15] Values are presented in perfusion units (P.U.).

B.2.2 Experimental design

Thirty-one patients who had undergone digital replantation or revascularization volunteered to participate in this study. At the time of the study 14 patients were smokers and 17 were non-smokers. The mean age of the smokers was 40 years (range, 21 to 63) and that of the non-smokers 39 years (range, 19 to 66). All patients were men, with the exception of two non-smoking women. The smoking patients habitually smoked 4 to 40 cigarettes per day (mean, 18), the nicotine content varied from 1.0 to 1.5 mg per cigarette (mean, 1.3 mg). The mean time between the replantation and this study was 26 months in the smokers (range, 5 to 48) and 31 months in the non-smokers (range, 7 to 52).

Volunteers were acclimatized to the environment for 30 minutes. During this period they neither drank coffee nor smoked cigarettes. The surrounding temperature was 23°C. The volunteers were sitting during the experiment, and the forearm with the replanted digit was placed on a table at heart level (figure 35). The hand rested on a special splint with the arm in pronation. The laser Doppler probe was placed on the palmar skin of the distal phalanx of the replanted digit by means of a homemade laser Doppler probe holder and double-adhesive tape (figure 36). The laser Doppler flow was monitored continuously for 25 minutes and recorded on a paper chart. The experiment consisted of five periods of 5 minutes. The smokers started with a period of rest, after which in the second and third periods they smoked two cigarettes of their own brand; this was followed by two rest periods (periods four and five). The non-smokers had the same regimen, but they inhaled

through a glass pipe instead of smoking cigarettes, thereby exerting the same efforts without inhaling cigarette smoke. All volunteers were instructed to inhale well. During the first period a basal flow value was obtained; the fourth and fifth periods constituded the recovery phase.

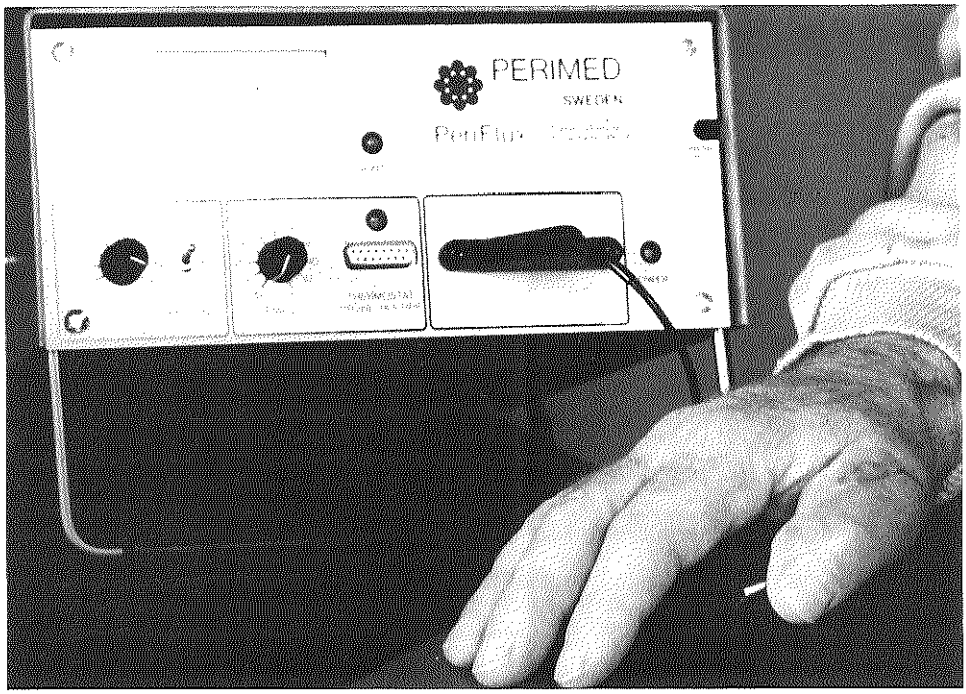


Figure 35. The skin blood flow of a replanted thumb monitored by a laser Doppler flowmeter.

B.2.3 Statistical analysis

The laser Doppler flow recordings were analysed in the following way. The graphic representation obtained for each period was divided into five blocks of 1 minute each. In each block the median of the values taken at 6, 18, 30, 42 and 54 seconds was obtained as a robust estimate of the block level. The medians of five consecutive blocks in a period were averaged to assess the mean value for that

period, which was regarded as a representative laser Doppler flow value for the period. To reduce between-subject differences, laser Doppler flowmeter responses were expressed as percentage decrease from the baseline value in the first rest period within each subject.

Within every period the two tailed Wilcoxon rank sum test was used to compare the average responses of smokers and non-smokers. The Spearman rank correlation test was used to evaluate the relation between the time that had passed between the replantation and this study and the flow changes during smoking.

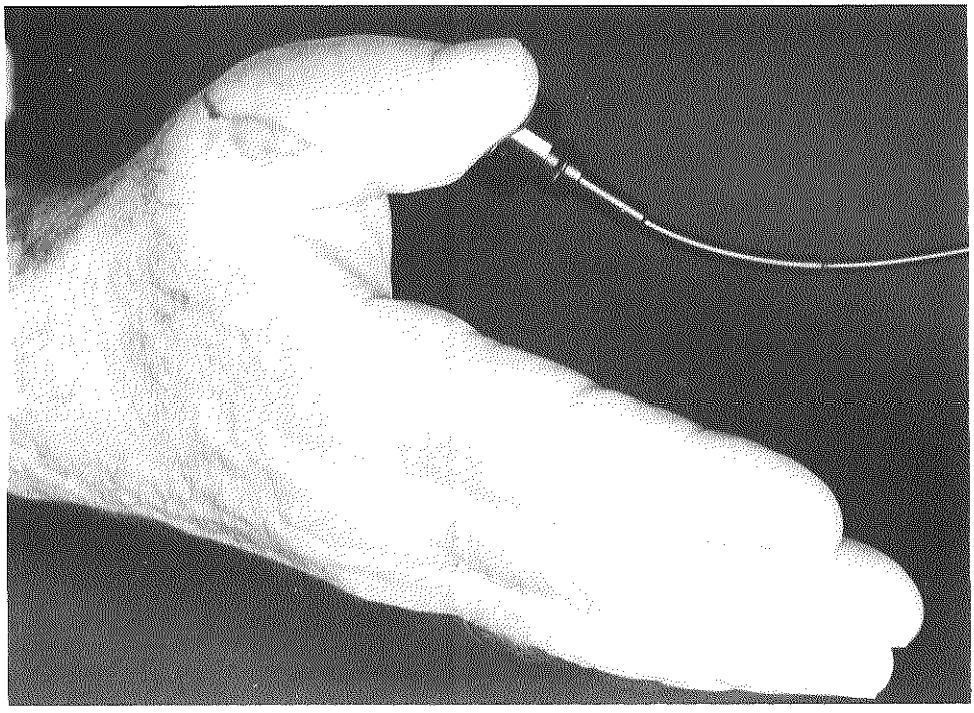


Figure 36. The laser Doppler probe placed on a replanted digit by means of a homemade laser Doppler probe holder with double-adhesive tape.

B.3 Results

The mean basal flow (first period of rest) was found to be 44 P.U. in the smokers

and 59 P.U. in the non-smokers. Thus the laser Doppler flow was lower for the smokers than for the non-smokers ($p < 0.05$). During smoking of the first and second cigarette the laser Doppler flow decreased significantly (-8%, -19%). This flow decrease maintained during the first and second recovery period (-18%, -18%). In the non-smokers the mean laser Doppler flow did not show major variation. The differences between the smokers and the non-smokers are statistically significant (figure 37,38).

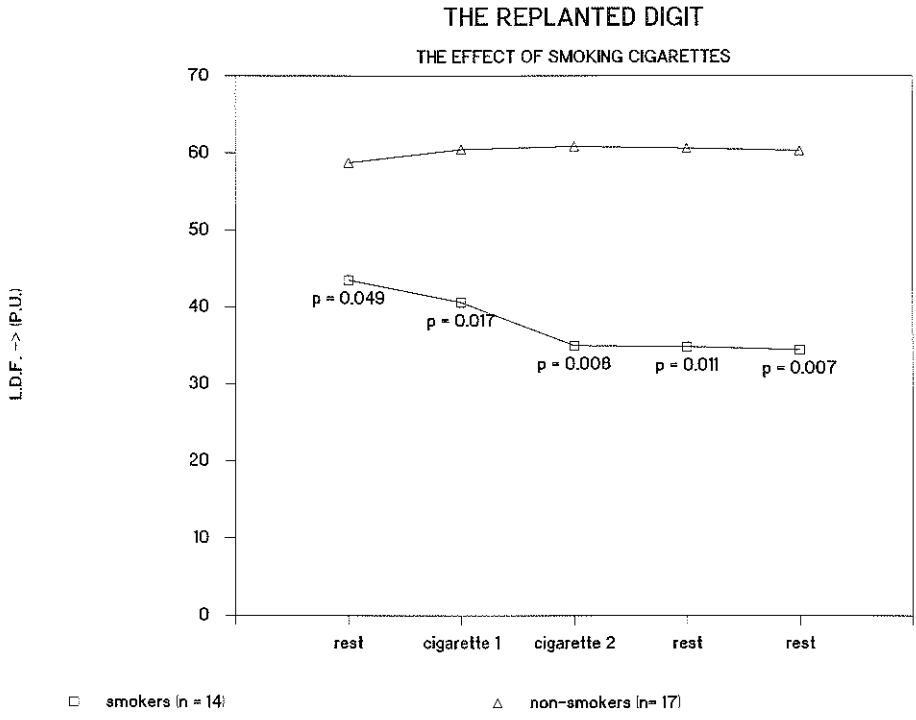


Figure 37. The effect of cigarette smoking on the microcirculation of a replanted digit compared to the effect of sham-smoking on the microcirculation of non-smokers. The mean basal flow is significantly lower in the smokers than in the non-smokers. During smoking two cigarettes the mean laser Doppler flow decreased from 44 P.U. to 35 P.U. and stayed stable after stopping smoking. The non-smokers did not show major variations.

The negative effect of smoking on the microcirculation in replanted digits proved to be more pronounced in patients operated upon more recently (Spearman's rho = 0.57, p = 0.03, figure 39). As expected, no such correlation was seen in the non-smokers (Spearman's rho = 0.08, p = 0.75).

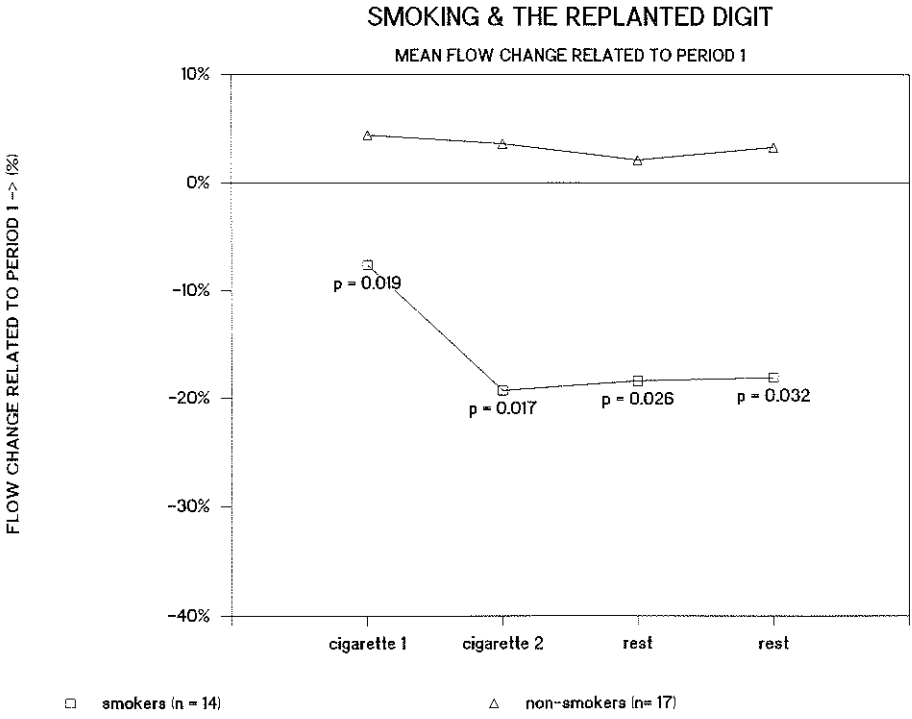


Figure 38 The effect of cigarette smoking on the microcirculation of a replanted digit in relation to the first period of rest. During smoking the laser Doppler flow decreased by 19%. After cessation of smoking no significant recovery was seen. The laser Doppler flow of the non-smokers did not show major variations.

B.4 Discussion

There are clear indications that the smoking of cigarettes decreases the

microcirculatory flow in replanted digits considerably. This effect is comparable to that seen in healthy digits, as we have shown in a previous study,[161] although some differences exist. In healthy digits smoking caused a rapid decrease in 1 to 2 minutes, whereas the replanted digits showed a slower response of about 5 minutes. The healthy digits also recovered more quickly (a 46% recovery after 10 minutes) than the replanted digits, which showed almost no signs of recovery after 10 minutes. Replanted digits seem to react slowly to the vasoconstrictive effect of cigarette smoking, but this effect is more prolonged, a phenomenon that resembles the sensitivity of replanted digits to cold.

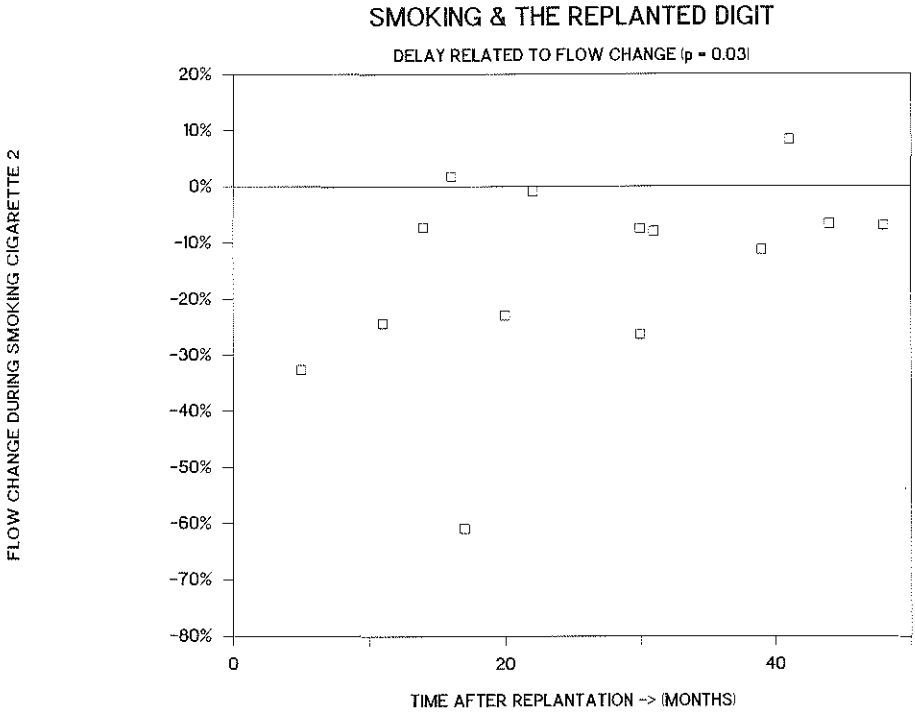


Figure 39. The negative effect of smoking on the microcirculation in the replanted digits was more pronounced in the patients operated on more recently (p = 0.03).

The vasoconstrictive effect of smoking was demonstrated to be more pronounced when the replantation had taken place more recently. Unfortunately, we could not investigate the effect of smoking on digits immediately after surgery because we strongly advise patients to stop smoking after replantations, and during this study all patients who had undergone a replantation, reported that they had not smoked in the early postoperative period.

Only postoperative smoking behaviour after replantation surgery can be influenced, and our study confirms that it is advisable to stop cigarette smoking after this kind of surgery. It is expected that smoking will decrease the survival of replanted digits.

B.5 Abstract

Thirty-one patients, who had undergone a digital replantation or revascularization volunteered to participate in a study of the acute effect of smoking on the microcirculation of the skin of replanted fingers. Fourteen were smokers and 17 were non-smokers at the time of the study. Blood flow was assessed by means of the laser Doppler flowmeter under standard conditions. Each smoker inhaled 2 cigarettes. During smoking of the first and second cigarettes a mean decrease in laser Doppler flow of 8% and 19% respectively was found, whereas the non-smokers showed a slight increase of 4% and 4% respectively ($p = 0.019$; $p = 0.017$). Ten minutes after the last cigarette almost no recovery could be detected. The negative effect of smoking on the microcirculation in replanted digits proved to be more pronounced in the patients operated on more recently (Spearman's rho = 0.568, $p = 0.03$).

This experiment confirms that smoking after replantation surgery should be prohibited to guarantee optimal circulation.

CHAPTER III: CIGARETTE SMOKING AND MICROCIRCULATION

C: The effect of cigarette smoking on the survival of free vascularized and pedicled epigastric flaps in the rat

Submitted to: Journal of Plastic and Reconstructive Surgery

C.1 Introduction

Microsurgeons suspect cigarette smoking to have a negative effect on the survival of both free vascularized tissue transfers and replantations. In macrovascular surgery it was found that the patency rates of arterial reconstructions decreased when cigarettes were smoked.[162,163] Besides a case report concerning the damaging effect of smoking on digital revascularisation,[151] little is known however about the effect of smoking on the succes rate of microvascular surgery. Experimental and clinical studies concerning the effect of cigarette smoking on at random vascularized pedicled skin flaps showed an increase of partial flap necrosis in smokers.[142,152-156] This negative effect is explained by an increased platelet aggregation,[143,157,158] a decreased oxygen transport due to COHb formation and an impaired microcirculatory flow due to the combination of macro- and microangiopathy and vasoconstriction.[144,159] Several studies indicated impaired microcirculatory flow during smoking in healthy tissues[145-150,160,164] and replanted digits.[165]

The aim of this experimental study was to investigate the effects of cigarette smoking on the survival of free vascularized and pedicled tissue transfers.

C.2 Materials and methods

C.2.1 Experimental design

Experiments were carried out on 60 healthy male wistar rats weighing 250 to 300 grams. Epigastric flaps, described by Petry and Wortham as an axial flap, were used as a free vascularized flap model.[166] In 30 rats a free flap and in 30 a pedicled flap were performed. A distally based dorsal skin flap of 3 to about 10 cm was also created in all rats, representing an at random vascularized skin flap. Of each group 10 rats were smoked during 6 weeks preoperatively and 2 weeks postoperatively, 10

rats only during 6 weeks preoperatively while 10 rats underwent only the sham smoking procedure (figure 40). The sequence by which the rats were entered into the study was formally randomized. Three rats died preoperatively, 2 being smoked and 1 being sham smoked, so 63 rats entered the study and 60 rats were operated.

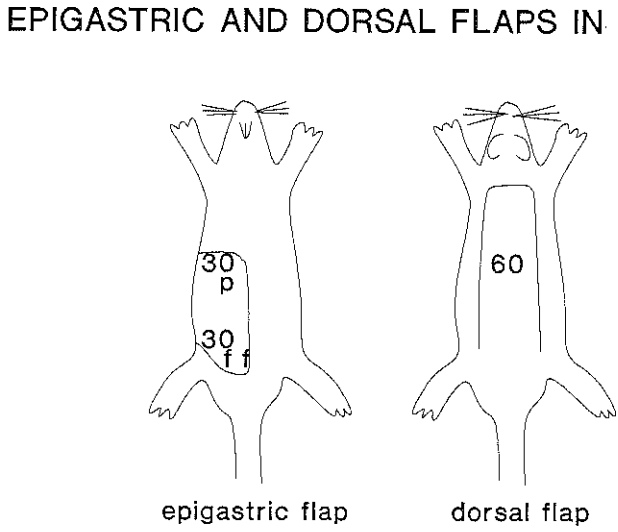


Figure 40. In 60 rats 60 dorsal flaps were created as well as 30 pedicled epigastric flaps (P) and 30 free vascularized epigastric flaps (FF).

Laser Doppler flow and temperature were assessed before, during and after operation to study the microcirculation of the epigastric flaps. Nicotine, cotinine and carbon monoxide–hemoglobin were measured to objectivate the smoking procedure pre– and postoperatively. Two weeks after operation the epigastric and dorsal flaps were studied by clinical parameters, measurement of flap size and by fluorescein staining. Patency of the vessels was established by clinical parameters and histology (figure 41).

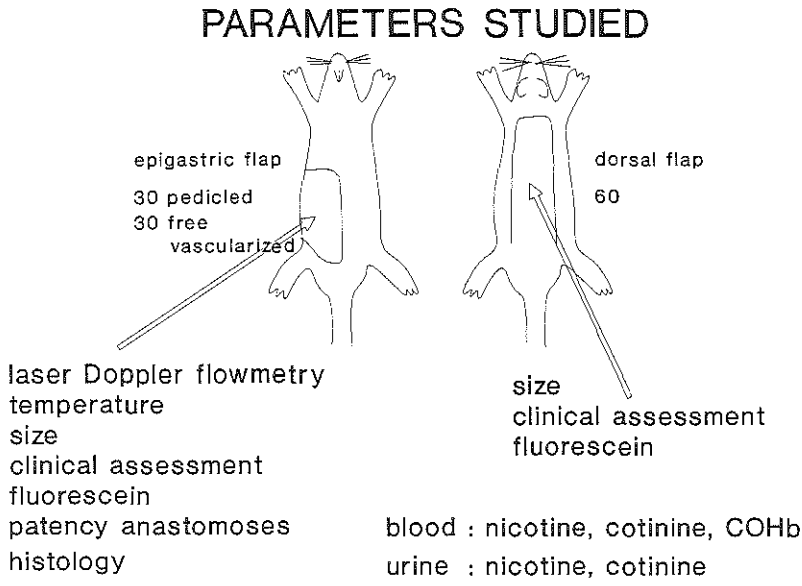


Figure 41. Flap and blood parameters.

C.2.2 Smoking procedure

The rats were daily exposed to cigarette smoke in the Hamburg II smoking apparatus for laboratory animals (Heinr. Borgwaldt, Hamburg, Germany) during 3 periods of 20 minutes with a 4 hour interval. The animals were placed in plexiglass cylinders, with their faces in a smoking room of 525 ml. Every 2 seconds 35 ml cigarette smoke (Caballero: 1.5 mg nicotine and 18 mg tar per sigarette) diluted with 700 ml air (1:20) was blown into the smoking room, containing 20.8 % oxygen, 0.3 % carbon dioxide and 200 ppm carbon monoxide.

In the sham smoking procedure (= the same procedure without cigarette smoke) the animals were placed during 20 minutes 3 times daily in the plexiglass cylinders, putting their faces into normal air. On the day of operation the rats were not smoked.

During three days before the smoking procedure started, the rats were acclimatized to the cylinders.

C.2.3 Surgical technique

After induction and maintenance of anaesthesia with an ether gauze the skin was shaved by an electric dry-shaver. The rat was placed on a heating mattress. Primarily a modified McFarlane dorsal skin flap was cut and resutured with Vicryl 5-0 FS1 (Ethicon).[167] The flap was caudally based, 3 cm wide and reached from the hip joints to the inferior angles of the scapulae (length = approximately 10 cm) and included the panniculus carnosus. Secondly a right sided epigastric flap was designed. Its medial margin was the midline of the abdomen, its cranial margin the xyphoid process of the sternum, its caudal margin the os pubis and the lateral margin was approximately 7 mm lateral to the lateral branch of the epigastric vessels. The flap was raised from the abdominal wall musculature and completely isolated on its epigastric vessels, which are about 3 cm long. These vessels are too small (diameter about 0.3 mm) to perform reliable microvascular anastomoses, so subsequently the femoral artery and vein were dissected. Distally of the epigastric vessels the femoral vessels were ligated and transsected. The saphenous branches and the branches to the muscles were also ligated and cut. If the pedicled flap was created the flap was resutured to its original place. When the free vascularized flap was performed the femoral artery and vein were cut and end-to-end anastomoses were made by 8 ethilon 11-0 BV8 (Ethicon) sutures using the operating microscope. Subsequently the flap was resutured in its original place using vicryl 5-0 FS1 (Ethicon). In the free vascularized flap procedure the time of ischemia was 1 to 1.5 hours and the total operation time 4.5 hours. In the pedicled flap procedure operation time was approximately 3 hours. To prevent autocannibalization of the

flap, all animals were dressed in a vest made of an X-ray film strip as described by Westin and Heden.[168]

C.2.4 Carbon monoxide-hemoglobin (COHb), nicotine and cotinine

On day 14 before operation and on day 14 after operation 24 hour urine was collected and a blood sample was taken from the retroocular venous plexus approximately 5 minutes after the third smoking procedure. The COHb content was measured by means of spectrophotometry. The nicotine and cotinine content of the urine and plasma were assessed by means of high performance liquid chromatography.

C.2.5 Laser Doppler flowmetry

The laser Doppler flowmeter (L.D.F.; Perimed KB, Sweden) works with laser light. Reflection from moving erythrocytes causes a Doppler shift, which is representing microcirculatory blood flow.[6,8,11,15] Values are presented in perfusion units (P.U.). The laser Doppler probeholder was fixed by medical adhesive tape to the center of the epigastric flap. Measurements were obtained under ether anaesthesia on days 42, 39, 36, 7, 4, 1 before operation and on the day of operation at the end of the procedure and on days 1, 3, 7 and 13 postoperatively. On every day 3 values were obtained by 3 short interval (10 s) measurements approximately 5 minutes after stopping the third smoking or sham-smoking procedure.

C.2.6 Thermometry

The temperature was measured by a thermocouple connected to a thermograph (Y.S.I. 44 TA, Yellow Springs Ohio, U.S.A.). Temperature is an indirect measurement of skin perfusion, which is influenced by the temperature of the deeper tissues and of the surroundings. The thermocouple was also fixed by medical adhesive tape to the center of the epigastric flap. Temperature was assessed at the same time as the laser Doppler flowmetry.

C.2.7 Establishing flap viability and patency of the microvascular anastomoses

On day 14 the viability of the epigastric flap was established clinically: a viable flap shows a normal skin color, is pliable and soft with hair growth, whereas an avital flap is contracted to a crust. In the same way the viable and necrotic parts of the dorsal flap were determined. Also by injection of 60 mg/kg fluoresceine intravenously the viability of the flaps was assessed using UV-light. The viable and non-viable parts of the flaps were drawn on tracing-paper. The areas were assessed in square cm by a computerized system. Flap-areas were also assessed on the day of operation as reference.

The patency of the microvascular anastomoses was established on day 14 after operation. After dissecting the anastomoses the filling of the artery distal to the anastomosis and of the vein proximal to the anastomosis was determined by Acland's filling test, without touching the anastomoses. The femoral vein and artery were cut distally to the anastomoses and it was observed whether blood passed the anastomosis. Futhermore the microvascular anastomoses were examined histologically.

C.2.8 Statistical analysis

Of all laser Doppler flow and temperature measurements mean values of the sixth and first preoperative week respectively were calculated for every rat, serving as baseline values. The results in smoking rats were compared to non–smoking rats (controls) by using the Mann Whitney rank test (two tailed). This test was also used for comparisons with respect to COHb, nicotine and cotinine measurements. The viability of the epigastric flap and the patency of the vascular anastomoses were statistically evaluated using the chi square test comparing three or more subgroups and the Fisher exact test for comparing two specific subgroups. The ratios of area measurements on the day of operation and on day 14 were analysed using the Kruskal Wallis test comparing all subgroups and using the Mann Whitney rank test (two tailed) when comparing two specific subgroups.

Postoperatively rats were divided into 12 subgroups depending on the smoking procedure (none; only preoperatively; pre– and postoperatively), the type of epigastric flap (free vascularized or pedicled) and the survival of the epigastric flap (survival or necrosis). For every rat daily mean values of the laser Doppler flow and temperature measurements were calculated. A repeated measurements analysis was performed, taking into account the multivariate intercorrelation between measurements on consecutive days.

C.3 Results

C.3.1 Carbon monoxide–hemoglobin (COHb), nicotine and cotinine

The mean results are summarized in table 2.

Table 2

CARBON MONOXIDE–HEMOGLOBIN (COHb), NICOTINE, COTININE

	smoking rats			non–smoking rats		
Day –14 (before operation)	Mean	SD	(n)	Mean	SD	(n)
plasma nicotine (ng/ml)	273	109	(38)	0.0	0.0	(18)[a]
plasma cotinine (ng/ml)	567	304	(38)	0.0	0.0	(18)[a]
COHb (%)	17.9	5.9	(40)	3.3	2.7	(18)[a]
urine nicot.(* 10^{-6}g/24 h)	62	40	(40)	0.5	1.2	(18)[a]
urine cotin.(* 10^{-6}g/24 h)	55	30	(40)	0.0	0.0	(18)[a]
Day 14 (after operation)						
plasma nicotine (ng/ml)	366	176	(20)	0.0	0.0	(39)[a]
plasma cotinine (ng/ml)	733	314	(20)	0.0	0.0	(39)[a]
COHb (%)	22.4	6.4	(19)	1.4	3.1	(35)[a]
urine nicot.(* 10^{-6}g/24 h)	128	61	(19)	0.0	0.0	(38)[a]
urine cotin.(* 10^{-6}g/24 h)	94	45	(19)	0.0	0.0	(38)[a]

[a] $p < 0.000005$ (two tailed Mann Whitney rank test)

Table 2. Carbon monoxide–hemoglobin (COHb), nicotine and cotinine in plasma and urine 14 days before and 14 days after operation for smoking and non–smoking rats.

C.3.2 Laser Doppler flowmetry

The overall mean laser Doppler flow value before operation was 34 P.U. (s.d. = 10). Neither in the sixth week nor in the first week preoperatively, subgroup means differed significantly ($p > 0.05$, Mann–Whitney two tailed).

On the day of operation just after finishing the flap procedure no significant differences were observed between subgroups ($p > 0.05$, Kruskal–Wallis); the mean L.D.F.value ($n = 60$) was 11.5 P.U. (s.d. = 4.8).

In the postoperative period within any of the treatment groups no significant differences were observed between day 1, 3, 7 and 13 (repeated measurement analysis, $p > 0.05$). Clearly significant differences in mean L.D.F. values were established between surviving and non-surviving flaps, irrespective of the time of measurement, flap procedure and smoking procedure ($p < 0.00005$). For surviving flaps no significant differences were observed neither between free and pedicled flaps nor between smoking categories ($p > 0.05$). On every postoperative day all individual flaps that survived had a laser Doppler flow value of 20 P.U. or more and all flaps that necrosed a value below 20 P.U. except 4 flaps on the first postoperative day (values 29.3 P.U., 42.0 P.U., 45.3 P.U. and 34.0 P.U.) and 1 flap on the third postoperative day (25.3 P.U.).

C.3.3 Thermometry

During the first and sixth preoperative week the mean temperature was significantly lower in the smokers (31.2 °C) in comparison to the controls (32.8 °C; both $p < 0.000005$; Mann-Whitney two tailed).

On the day of operation just after finishing the flap procedure no significant differences were observed between subgroups ($p > 0.05$, Kruskal-Wallis); the mean temperature value ($n = 60$) was 30.8 °C (s.d. = 0.8).

Postoperative temperature measurements showed a statistically significant difference between all subgroups on all days (day 1, $p = 0.02$; day 3, $p = 0.0002$; day 7, $p = 0.0001$; day 13, $p < 0.00005$; Kruskal-Wallis). Multivariate analysis showed that it was caused by the difference in smoking procedure on every postoperative day (lower temperature for rats being smoked) and by the difference in flap survival on day 7 and day 13 (lower temperature for necrosed flaps).

C.3.4 Survival of epigastric flaps

The survival of the free vascularized epigastric flaps, established by clinical judgement and by fluoresceine examination, is shown in figure 42 for each of the 6 treatment groups. All pedicled epigastric flaps survived except 1 in the non-smokers group, partial necrosis was not seen. With respect to free vascularized

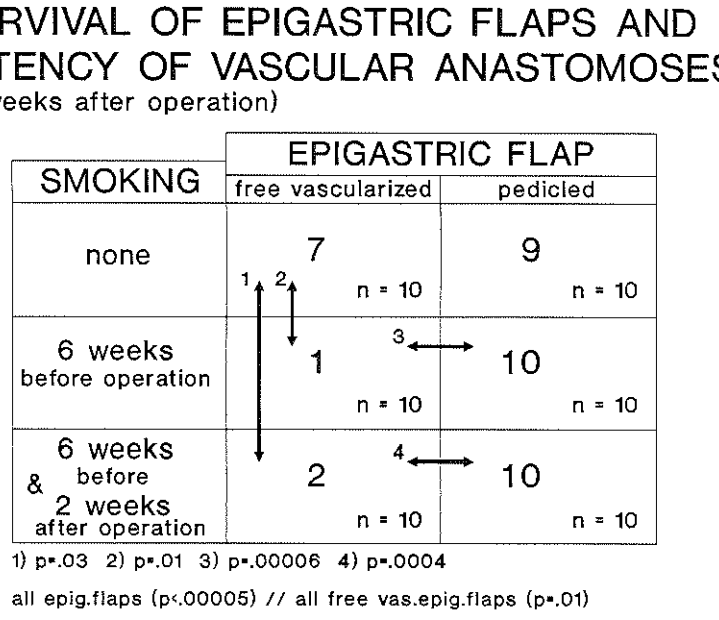
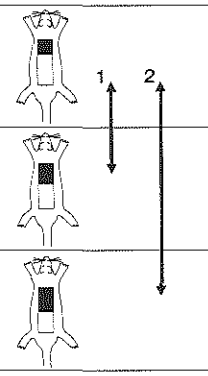


Figure 42. Survival of the epigastric flaps and patency of the vascular anastomoses 2 weeks after operation. For the free vascularized flaps the differences of survival and patency of all the smoking groups ($p < .00005$) and between the non-smokers and the preoperative smokers and between the non-smokers and pre- and postoperative smokers ($p = .01$ resp. $p = .03$) were significant. No significant difference was seen in the pedicled flaps ($p > .05$). The differences between the free vascularized and pedicled flaps were significant for the preoperative smokers and for the pre- and postoperative smokers ($p = .00006$ resp. $p = .0004$).

epigastric flaps the proportion survival differed significantly between smoking categories; p = 0.01 (chi-sqaure test between 3 groups) and p = 0.03 (preoperative smoking) and p = 0.01 (preoperative and postoperative smoking) respectively (Fisher's exact test comparing each treated group with the control group). With respect to the pedicled epigastric flaps no such difference was observed between the 3 groups (p > 0.05). With respect to the pedicled and free flaps the difference between the two control groups was not significant (p > 0.05), but between the smoking groups differences were highly significant (p = 0.00006 for preoperative smoking and p = 0.0004 for preoperative and postoperative smoking).

VITAL AREA OF AT RANDOM DORSAL FLAPS
judged on day 14 postoperatively,
as a percentage of the area on day of operation

SMOKING	ALL 60 RATS (n = 20 for every group)
none	65.4% (sd = 10.1)
6 weeks before operation	52.7% (sd = 11.2)
6 weeks & before 2 weeks after operation	47.5% (sd = 6.3)



* 1: p=.002 / 2: p=.002 / all:p=.00001
* After subdivision of groups into free and pedicled epigastric flaps the results differed < 1%

Figure 43. The differences between the vital areas of at random vascularized dorsal flaps 2 weeks after operation were significant for all groups (p = .00001), between the non-smokers and preoperative smokers (p = .002) and between the non-smokers and pre- and postoperative smokers (p = .002). After subdivision of groups into free vascularized and pedicled epigastric flaps the results differed less than 1%.

C.3.5 Vital area of dorsal flaps

The non–necrotic area of the dorsal flaps on day 14 for each rat was expressed as a percentage of the area at the day of operation. The results are shown in figure 43 for each treatment group. As might be expected within each smoking category no significant differences were observed between the two epigastric flap groups ($p < 0.05$, Mann Whitney two tailed). So the results were pooled by smoking category. With respect to smoking category the differences turned out to be significant between the 3 categories ($p = 0.00001$, Kruskal–Wallis test). Both treated groups differed significantly from the control group ($p = 0.002$, Mann Whitney two tailed) but not among themselves ($p > 0.05$, Mann Whitney two tailed).

C.3.6 Area measurement of epigastric flaps

The area of the epigastric flaps on day 14 for each rat was expressed as a percentage of the area at the day of operation. The results for the surviving flaps are shown in figure 44 for each treatment group. On the day of operation the mean area of all 60 epigastric flaps was 34.7 cm^2 (s.d. = 2.6 cm^2); as might be expected no significant differences were observed between the 6 treatment groups ($p > 0.05$, Kruskal–Wallis).

The mean contraction of the non–surviving flaps was at least twofold as compared to surviving flaps ($p < 0.05$, combined Mann–Whitney test two tailed). With respect to the mean contraction in the surviving pedicled flaps, highly significant differences were observed between the 3 smoking categories ($p < 0.00005$, Kruskal Wallis); the differences between the two smoking groups and the control group were clearly significant ($p = 0.0002$, Mann Whitney two tailed) as well as among the two smoking groups ($p = 0.03$), showing a downward trend from

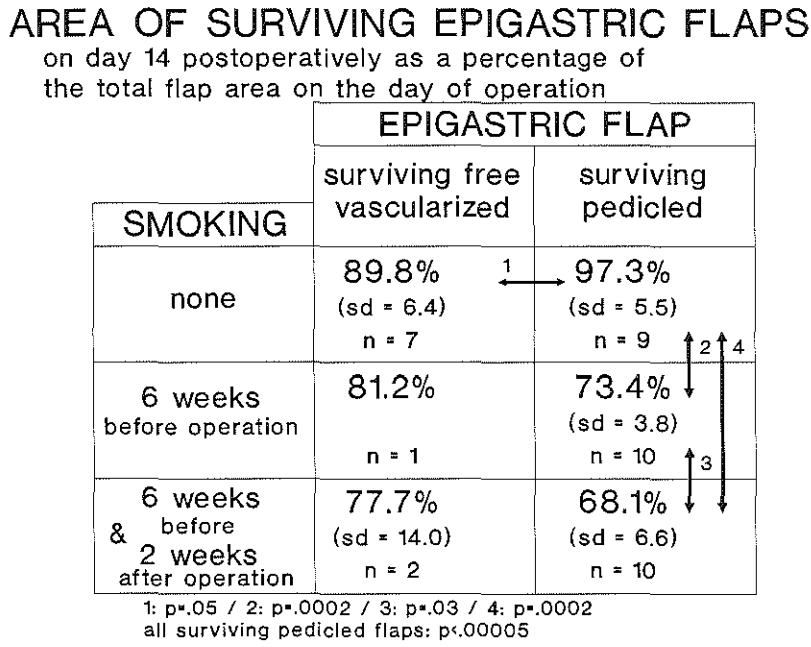


Figure 44. The area of surviving epigastric flaps 2 weeks after operation. For the pedicled flaps the differences of area of all the smoking groups ($p < .00005$) and between the non–smokers and the preoperative smokers, between the non–smokers and pre– and postoperative smokers and between the two smoking groups ($p = .0002$, $p = .0002$ and $p = .03$ resp.) were significant. The difference between the free vascularized and pedicled flaps were significant for the non–smokers ($p = 0.05$).

non–smoking, only preoperative smoking to preoperative and postoperative smoking. A similar pattern of the mean contraction was found in the surviving free epigastric flaps; the numbers are too small however to attain statistical significance of differences. Concerning the two control groups as to surviving pedicled and free vascularized flaps respectively, the difference in mean contraction was significant ($p = 0.05$, Mann Whitney two tailed).

C.3.7 Patency of vascular anastomoses

The arterial and venous microvascular anastomoses, judged by the filling test and the cut test, were patent in all flaps that survived and occluded in all flaps that necrosed. Histological examination supported these findings. The numbers cited in figure 42 and the p–values mentioned in the previous section apply therefore also to this outcome.

C.4 Discussion

This study demonstrated that smoking of cigarettes is detrimental to the survival of the free vascularized epigastric flap in the rat. Preoperative smoking proved to be most damaging. The at random vascularized dorsal flap also survived less when rats were smoked preoperatively, in addition postoperative smoking further decreased the surviving part of flaps. The survival of the pedicled epigastric flap was not influenced by the smoking procedure. So smoking is detrimental to survival of the poorly vascularized distal parts of at random vascularized flaps. According to Petry and Wortham the epigastric flap of the rat is an axial pattern vascularized flap without at random vascularized parts,[166] this could explain why the epigastric flap survived completely in all rats and not partially. The complete necrosis of flaps is most likely to be caused by cessation of the bloodsupply via its vascular pedicle. The pathogenesis of the epigastric flap necrosis might be as follows. After performing the epigastric flap procedure the flap is poorly or even not vascularized as is shown by extremely low laser Doppler flow values. This is probably caused by vasospasm of the vascular pedicle. If the vessels are not damaged (i.e the pedicled epigastric flap) the flap can overcome this period and the flap will survive due to restoration of the blood flow. If however the vessels are injured by a microvascular

anastomosis this low flow state may cause thrombosis. If the rats are smoked it is more likely that this thrombosis will occur, because smoking causes an increased platelet aggregation[143,157,158] and macro- and microangiopathy. Pitillo et al. demonstrated endothelial changes following six weeks of smoking.[169]

Some experimental studies on the effects of cigarette smoking on the results of microvascular surgery however did not show damaging effects. Perioperative intraperitoneal nicotine injections[170] and 6 days preoperative and 12 days postoperative smoking[171] did not show a significant decrease of the patency of femoral artery anastomoses in animals. It can be explained that Rao et al.[170] did not see this thrombosis because they only injected nicotine, whereas cigarettes contain numerous other substances[172] nor did Yaffe et al.,[171] as they only smoked the rats for a very brief period. Another possible explanation could be that the vasospasm is less vigorous when only an arterial anastomosis is made.

Pedicled epigastric flaps showed more contraction when rats are smoked. This probably indicates the detrimental effects of cigarette smoking to wound healing, even when no necrosis occurs. The pedicled epigastric flap hardly contracted in the non-smokers, whereas the free vascularized flap in the non-smokers contracted to about 90 % of the original size; this could indicate slightly impaired wound healing in the free flap. It is possible that during the period of ischemia irreversible tissue damage develops.

The nicotine, cotinine and COHb measurements demonstrate that the used smoking procedure is comparable to heavy cigarette smoking. Six weeks of smoking can not be compared to chronic cigarette smoking, which causes more macro- and microangiopathy. Unfortunately it is very hard to smoke rats for at least one year. For practical reasons we have chosen 6 weeks of preoperative smoking, because at that time endothelial changes have been seen.[169]

The preoperative laser Doppler flow and temperature measurements about 5 minutes after stopping smoking gave different results. The laser Doppler flow of the

normal skin was equal in smoking and in non-smoking rats, whereas the skin temperature was lower in the smokers. It is possible that vasoconstriction caused by cigarette smoking has already disappeared after 5 minutes, while the lower temperature is still present, however such a quick release of vasoconstriction is unlikely. A second explanation is that a central temperature decrease is caused by smoking, giving an indirect temperature fall of the skin, while no vasoconstriction is present. Postoperatively the same discrepancy was found.

Laser Doppler flowmetry proved to be a good viability monitor in the postoperative period. All flaps that survived had a laser Doppler flow value of 20 P.U. or more, whereas all flaps that died were below this level except 4 flaps on the first postoperative day and 1 flap on the third postoperative day. Those flaps may have been alive at that time however and necrosed later. It was also seen that the temperature of the dead flaps was lower on day 7 and 13 after operation. At that time the flaps were transformed to crusts, which conduct heat poorly.

This study proves that cigarette smoking is detrimental to microvascular surgery. It is not known how long a non-smoking period is needed to recover from the damaging effects of smoking. It is unlikely that a full recovery will ever occur, because some changes (e.g. macroangiopathy) are hardly reversable.

C.5 Abstract

Microsurgeons suspect cigarette smoking to have a negative effect on the survival of both free vascularized flaps and replantations, but this has never been proven. This experimental study was performed to investigate the effect of smoking on free flap survival.

The fasciocutaneous epigastric flap was used in 30 rats as a free flap and in 30 rats as a pedicled flap. Of each group 10 rats were smoked 6 weeks preoperatively

and 2 weeks postoperatively, 10 rats only 6 weeks preoperatively and 10 rats underwent the sham smoking procedure. Also a distally based dorsal skin flap was created in all rats, representing an at random vascularized skin flap. Fourteen days after the operation the vitality and area-change of the epigastric and dorsal flaps were established as well as the patency of the vascular anastomoses. The epigastric flaps were monitored by laser Doppler flowmetry and thermometry during the experiment.

The survival of the free vascularized epigastric flaps was significantly lower in the smoking rats. All pedicled flaps exept 1 survived. Only complete necrosis or survival of the epigastric flap was seen, which correlated completely to the patency of the vascular anastomoses.

The mean surviving area of the dorsal flaps was inversely related to the extent of the smoking procedure. The differences were statistically significant.

The postoperative laser Doppler flowmetry values showed a statistically significant difference between the surviving and non-surviving flaps. This affirms the value of laser Doppler flowmetry for postoperative monitoring of microvascular surgery.

In conclusion this study proves that smoking of cigarettes is detrimental to the survival of free vascularized flaps.

CHAPTER IV: DISCUSSION & SUMMARY

In the first part of this thesis, describing clinical and experimental studies, laser Doppler flowmetry is evaluated as diagnostic tool to assess tissue microcirculation after various microvascular operations. The second part concerns the application of laser Doppler flowmetry to investigate and to objectivate the negative effects of cigarette smoking upon the microcirculation under normal circumstances as well as after microvascular operative procedures.

Success of plastic and reconstructive operations, especially of microvascular surgery, is determined to a great extent by circulation of the tissues. Reliable assessment of blood flow and more specifically of microcirculation, which determines tissue viability is of great clinical importance.

In the first chapter (the introduction) the history and the principles of laser Doppler flowmetry are summarized. Anatomical and functional aspects of skin blood flow in relation to laser Doppler flowmetry are discussed in more detail. Establishment of tissue viability by laser Doppler flowmetry is highlighted, while pitfalls are described and the necessity for standardisation is stressed. The characteristics, advantages and disadvantages of different laser Doppler flowmeters are described and it is concluded that it is impossible to select one device as the best available. Several other current methods to establish blood flow are summarized. Subdivision is made into clinical examination, methods assessing arterial blood flow, techniques using chemical or radioactive substances and instrumental methods evaluating local blood flow. Results of published studies in which these methods were compared with laser Doppler flowmetry are discussed.

In the postoperative monitoring of replants and revascularizations (chapter II.A) laser Doppler flowmetry readings were significantly different in cases with a sufficient circulation in comparison to cases with a failing blood flow. A reliable critical (= alarm) value of 10 perfusion units could be defined for the replants and revascularizations with a sensitivity of 93% and a specificity of 94%. It was shown that this critical alarm value could be adjusted according to an individual surgeon's

attitude towards re-exploration. Similar laser Doppler flowmetry characteristics were seen in cases of free vascularized tissue transfers, however their numbers were too small to define reliable critical (= alarm) values. The laser Doppler flowmeter is recommended for the postoperative evaluation of tissue viability, following microvascular anastomoses as it reliably indicates vascular occlusion at an early stage, when re-exploration is still worthwhile.

A comparison between laser Doppler flowmetry and thermometry in the postoperative monitoring after replantation surgery is described in chapter II.B. The surface thermometry is well known as an easy and inexpensive objective postoperative monitor and therefore it is used by many microsurgeons. In general practice however the reliability is not always satisfactory, the reason why several other instrumental methods have been tested. In our hands thermometry was less sensitive and specific than laser Doppler flowmetry and it is concluded that laser Doppler flowmetry is preferable to thermometry for postoperative monitoring of replantations and revascularizations.

The relevance of laser Doppler flowmetry to discriminate arterial and/or venous occlusions after microsurgical anastomoses was evaluated. Results were compared with assessment of colour, capillary refill as well as with thermometry (chapter II.C). Skin thermometry as well as total backscattered light intensity change and flow band width (laser Doppler measurements) were not significantly different when comparing arterial and venous occlusion. A significant difference between arterial and venous occlusion was found for colour, capillary refill and laser Doppler flow. The clinical parameters were more discriminating than results of laser Doppler flowmetry. For all the measured parameters however the values obtained in cases of combined arterial and venous occlusion turned out to be either like those of a venous occlusion or of an arterial occlusion or somewhere in between. Although none of the parameters turned out to be decisive, it is advised to check the suspected vascular anastomosis first during reoperation. Even if an occluded

anastomosis is found, one should also pay attention to the other anastomosis because simultaneous occlusion of both artery and vein cannot be reliably detected and predicted.

The circulatory physiology of microsurgically revascularized human tissues was examined by laser Doppler flowmetry (chapter II.D). After replantations and revascularizations initially a high mean laser Doppler flow value was found followed by a hyperaemic phase and returning to a stable level comparable to the starting value. Free vascularized radial forearm flaps initially showed a low mean laser Doppler flow value followed by a gradual increase over 5 days. The free vascularized latissimus dorsi flaps started with a low mean laser Doppler flow value, followed by a gradual increase during the first 2.5 days and a stabilization on the following days. The toe-to-thumb transfers started with a high mean value and did not show a systematic pattern in the subsequent monitoring period. According to a repeated measurements analysis of variance with polynomial contrasts (SPSS), the flow patterns proved to be statistically significant. The difference in postoperative flow patterns after various types of surgery may be explained by the fact that finger replantations or revascularizations and toe-to-thumb transfers concern primary vascular end-organs, which are fixed to the receptor site firmly by means of a bony fixation. In these cases wound surfaces are relatively small and the skin is in its normal anatomical position, creating a rather stable microvasculature. In contrast the free vascularized radial forearm flaps and latissimus dorsi flaps are secondary vascular end organs, which are fixed to the receptor site rather flaccidily by means of soft tissue stitches. These free flaps have relatively large wound surfaces while the overlying skin is not in the original anatomical position and demonstrates signs of collapse. Laser Doppler flowmetry is found to be a valuable technique in examining the circulatory physiology of microsurgically revascularized tissues.

Laser Doppler flowmetry, clinical signs and histology were compared in the rejection process of allogeneic transplanted limbs of rats (chapter II.E). Clinical and

histological examination as well as laser Doppler flow measurements are good parameters for rejection. A significant correlation was found between results of laser Doppler flowmetry and histology, and occlusive lesions of the dermal capillaries and small sized vessels correlated well to low laser Doppler flow values. In this model however laser Doppler flow values could not predict an initial onset of the rejection before clinical signs were evident, presumably due to the impossibility of continuous monitoring.

Smoking of cigarettes is suspected to have negative effects on microcirculation and therefore on the survival of free vascularized tissue transfers. Acute effects of smoking on the microcirculation of the skin of the thumb were investigated by means of laser Doppler flowmetry in healthy volunteers (chapter III.A). During smoking of two cigarettes a mean decrease in laser Doppler flow of 29% was seen, whereas the non-smokers showed no significant changes. Ten minutes after smoking the decrease was recovered only by half. This experiment confirms that one should prohibit smoking of cigarettes perioperatively for optimal woundhealing conditions.

Acute effects of smoking on the skin microcirculation of replanted fingers was studied by laser Doppler flowmetry (chapter III.B). During smoking two cigarettes a mean decrease in laser Doppler flow of 19% was found, whereas the non-smokers showed no significant changes. Ten minutes after the last cigarette almost no recovery could be detected. The negative effect of smoking on the microcirculation in replanted digits proved to be more pronounced in the more recently operated cases. This experiment confirms that smoking after replantation surgery should be prohibited to guarantee optimal circulation.

An experimental study in rats was performed to investigate the effect of smoking on free flap survival (chapter III.C). Created epigastric flaps were monitored by laser Doppler flowmetry and thermometry. Twenty rats were exposed to cigarette smoke before and after the operation via an inhalation apparatus 3 times a day for

20 minutes, 20 rats only before the operation and 20 rats underwent the procedure without cigarette smoke. The survival of the free vascularized epigastric flaps was significantly lower in the smoking rats. All pedicled flaps except one survived. Only complete necrosis or survival of the epigastric flap was found, which correlated to the patency of the vascular anastomoses. The mean surviving area of at random vascularized pedicled dorsal flaps was inversely related to the duration of the smoking procedure. These effects were statistically significant. The postoperative laser Doppler flowmetry values were significantly different between the surviving and non-surviving flaps. This confirms the value of laser Doppler flowmetry in postoperative monitoring after microvascular surgery. This study proves that smoking of cigarettes is detrimental to the survival of free vascularized flaps.

Laser Doppler flowmetry is a rather easy, objective, quantitative and non-invasive technique to assess microcirculation. It measures microcirculatory blood flow continuously without influencing it while permanent monitoring for several days is possible. The method is sensitive because it reacts promptly to changes in microcirculation. The apparatus however is rather expensive and the examined tissue volumes cannot be defined with accuracy, because it has no sharp borders. Futhermore the results are presented in perfusion units (P.U.) which are directly related to microcirculatory flow, but are no absolute flow values (expressed in milliliters per minute). If its limitations and pitfalls are taken into account, laser Doppler flowmetry is a valuable technique to examine microcirculation in clinical and experimental studies.

HOOFDSTUK V: DISCUSSIE & SAMENVATTING

In het eerste deel van dit proefschrift, dat klinische en experimentele studies beschrijft, wordt de diagnostische waarde van laser Doppler flowmetrie onderzocht bij het meten van de microcirculatie na microvasculaire ingrepen. Het tweede deel heeft betrekking op de toepassing van laser Doppler flowmetrie om de nadelige effecten van het roken van sigaretten op de microcirculatie, zowel onder normale omstandigheden als na microvasculaire ingrepen, te onderzoeken en te objectiveren.

Het succes van plastische en reconstructieve operaties, met name van microvasculaire chirurgie, wordt voor een groot deel bepaald door de doorbloeding van de betreffende weefsels. Het betrouwbaar beoordelen van de doorbloeding en met name van de microcirculatie, die de overleving van weefsels bepaalt, is van groot klinisch belang.

In hoofdstuk I, de inleiding, worden de ontwikkeling en de grondbeginselen van de laser Doppler flowmetrie samengevat. Anatomische en functionele aspecten van huiddoorbloeding in samenhang met laser Doppler flowmetrie worden besproken. Het vaststellen van de levensvatbaarheid van weefsels met behulp van laser Doppler flowmetrie krijgt extra aandacht, terwijl de valkuilen beschreven worden en de noodzaak tot standaardisatie wordt benadrukt. De kenmerken, voordelen en nadelen van verschillende laser Doppler flowmeters worden besproken en de conclusie wordt getrokken, dat het onmogelijk is om een apparaat tot de beste uit te roepen. Andere veel gebruikte methoden om doorbloeding vast te stellen, worden kort besproken. Er wordt onderscheid gemaakt tussen klinisch onderzoek, methoden om arteriele doorbloeding te beoordelen, technieken die gebruik maken van chemische of radioactive stoffen en instrumentele methoden om locale doorbloeding te bepalen. Resultaten van gepubliceerde studies, waarin deze methoden worden vergeleken met laser Doppler flowmetrie, passeren de revue.

Bij de postoperatieve bewaking van replantaties en revascularisaties (hoofdstuk II.A) verschilden de laser Doppler flowmetingen in gevallen met voldoende doorbloeding significant met gevallen waarbij de doorbloeding faalde. Een

betrouwbare kritische (= alarm) waarde van 10 perfusion units kon gevonden worden voor de replantaties en revascularisaties met een sensitiviteit van 93% en een specificiteit van 94%. Vergelijkbare laser Doppler flow karakteristieken werden gevonden bij de vrij gevasculariseerde weefseltransplantaties, echter de aantallen waren te klein om betrouwbare kritische (= alarm) waarden vast te stellen. De laser Doppler flowmeter wordt aangeraden voor het bepalen van de levensvatbaarheid van weefsels na het maken van microvaatanastomosen, omdat het optreden van een vaatafsluiting in een vroege fase betrouwbaar herkend wordt, op een moment waarop reinterventie nog zinvol is.

Een vergelijking van laser Doppler flowmetrie met thermometrie tijdens postoperatieve bewaking van replantaties en revascularisaties wordt beschreven in hoofdstuk II.B. Oppervlakte thermometrie is bekend als een eenvoudige, goedkope en objectieve postoperatieve bewakingsmethode en wordt daarom door vele microchirurgen gebruikt. In de dagelijkse praktijk is de betrouwbaarheid niet altijd voldoende, reden waarom andere instrumentele methoden zijn beproefd. In onze handen bleek thermometrie minder sensitief en specifiek dan laser Doppler flowmetrie en de conclusie is dan ook, dat laser Doppler flowmetrie de voorkeur verdient voor de postoperatieve bewaking van replantaties en revascularisaties.

De waarde van laser Doppler flowmetry in het onderscheiden van arteriele en/of veneuze afsluitingen van microvasculaire anastomoses wordt in hoofdstuk II.C besproken. De resultaten worden vergeleken met die van bepaling van kleur of capillaire refill en thermometrie. Bepaling van huidtemperatuur evenals de total backscattered light en bandbreedte (laser Doppler metingen) gaven geen significant verschil tussen arteriele en veneuze afsluitingen. Wel werd er een significant verschil gevonden tussen arteriele en veneuze afsluitingen bij bepaling van kleur, capillaire refill en laser Doppler flow. De klinische parameters toonden een groter onderscheidingsvermogen dan de laser Doppler flowmetrie. Bij een combinatie van een arteriele en veneuze afsluiting bleken alle parameters daarentegen een waarde te

hebben passend bij een arteriele of een veneuze afsluiting, dan wel een tussenliggende waarde te hebben. Omdat geen van de parameters een volledig betrouwbare voorspelling gaf, is het raadzaam eerst de "verdachte" vasculaire anastomose te exploreren. Zelfs als daar een afsluiting gevonden wordt, dient ook de andere anastomose gecontroleerd te worden, omdat een gelijktijdige afsluiting van arterie en vene niet als zodanig herkend en voorspeld kan worden.

Bestudering van de doorbloeding van microchirurgisch gerevasculariseerde menselijke weefsels met laser Doppler flowmetrie wordt beschreven in hoofdstuk II.D. Na replantaties en revascularisaties werd een hoge gemiddelde laser Doppler flow waarde gezien gevolgd door een hyperaemische periode, waarna de flow terugkeerde naar een stabiel niveau vergelijkbaar met de beginwaarde. Vrij gevascularisseerde radiale onderarmslappen vertoonden aanvankelijk een lage gemiddelde laser Doppler flow gevolgd door een geleidelijke stijging gedurende 5 dagen. Vrij gevascularisseerde latissimus dorsi lappen hadden in het begin een lage gemiddelde laser Doppler flow, die geleidelijk steeg gedurende de eerste 2.5 dag en zich daarna stabiliseerde gedurende de daaropvolgende dagen. De teen-duim transplantaties begonnen met een hoge gemiddelde laser Doppler flow waarde, die nagenoeg gelijk bleef tijdens de gehele duur van de observatie. Volgens een repeated measurements analysis of variance with polynomial contrasts (SPSS) bleken de flow-patronen statistisch significant. De verschillen in de postoperatieve flow-patronen na de diverse microvasculaire ingrepen kunnen mogelijk verklaard worden door het feit dat replantaties en revascularisaties evenals teen-duim transplantaties oorspronkelijk vasculaire eind-organen betreffen, die stevig op de ontvangersplaats worden bevestigd door middel van een benige fixatie. In die gevallen zijn de wondoppervlakten relatief klein en de huid is in zijn oorspronkelijke anatomische positie, hetgeen een redelijke stabiele microvaatstructuur geeft. Daarentegen zijn de vrij gevascularisseerde radiale onderarmslappen en latissimus dorsi lappen secundaire vasculaire eind-organen, die

tamelijk los bevestigd zijn met enkele weke delen hechtingen. Deze vrije lappen hebben relatief grote wondoppervlakten, terwijl het huid–gedeelte zich niet in haar oorspronkelijke positie bevindt en deels samengevallen is. Laser Doppler flowmetrie blijkt een waardevolle methode om de doorbloedingsfysiologie van microchirurgisch gerevasculariseerde weefsels te bestuderen.

Uitkomsten van laser Doppler flowmetrie, klinisch onderzoek en histologisch onderzoek worden vergeleken tijdens het afstotingsproces van allogeen getransplanteerde extremiteiten bij de rat in hoofdstuk II.E. Klinisch en histologisch onderzoek evenals laser Doppler flowmetrie zijn goede parameters ter beoordeling van afstoting. Een significante correlatie werd gevonden tussen de resultaten van laser Doppler flowmetrie en histologie. Occluderende beschadigingen van de huid–capillairen en microvaten correleerden goed met de gevonden laser Doppler flow waarden. In het onderzoek blijkt met laser Doppler flowmetrie een begin van de afstoting niet vroegtijdig herkend te hunnen worden, dus nog voordat klinische tekenen duidelijk zijn. Mogelijk is dit een gevolg van het feit dat continue bewaking onmogelijk was.

Er wordt verondersteld dat het roken van sigaretten nadelige invloeden heeft op microcirculatie en daarmee ook op de "overleving" van vrij gevascullariseerde weefsel transplantaties. Onderzoek naar acute effecten van het roken op de microcirculatie van de duimhuid bij gezonde vrijwilligers met behulp van laser Doppler flowmetrie wordt beschreven in hoofdstuk III.A. Tijdens het roken van twee sigaretten werd een gemiddelde daling van de laser Doppler flow gezien van 29%, terwijl bij de niet–rokers geen significante veranderingen werden gevonden. Tien minuten na het stoppen met roken was de daling slechts voor de helft hersteld. Dit onderzoek bevestigt dat het beter is om perioperatief het roken van sigaretten te staken om optimale wondgenezingsomstandigheden te scheppen.

Onderzoek naar de acute invloeden van het roken van sigaretten op de microcirculatie van gereplanteerde vingers met behulp van laser Doppler flowmetrie

wordt beschreven in hoofdstuk III.B. Tijdens het roken van twee sigaretten trad er een gemiddelde daling op van de laser Doppler flow van 19%, terwijl bij de niet-rokers geen significante veranderingen optraden. Tien minuten na de laatste sigaret waren er vrijwel geen tekenen van herstel. De nadelige invloed van het roken op de microcirculatie van gereplanteerde vingers bleek meer uitgesproken bij meer recent geopereerde patienten. Dit onderzoek bevestigt, dat het roken na replantatie-chirurgie gestaakt zou moeten worden om optimale doorbloeding te garanderen.

In hoofdstuk III.C. wordt een experimenteel onderzoek naar het effect van roken op de overleving van vrije lappen bij ratten beschreven. Van epigastrische lappen werden de laser Doppler flow en temperatuur bepaald. Twintig ratten werden bloodgesteld aan sigarettenrook voor en na de operatie in een berokingsmachine en wel 3 maal daags gedurende 20 minuten. Twintig ratten werden alleen voor de operatie berookt en 20 ratten ondergingen dezelfde procedure zonder sigarettenrook. De overleving van de vrij gevascullariseerde epigastrische lappen was significant lager bij de rokende ratten. Alle gesteelde lappen overleefden behalve een. De epigastrische lappen overleefden of necrotiseerden volledig, afhankelijk van de doorgankelijkheid van de vaatanastomosen. De gemiddelde oppervlakte van "at random" gevascullariseerde ruglappen die overleefde was omgekeerd evenredig met de duur van de berokingsprocedure. Dit verschil was statistisch significant. De postoperatieve laser Doppler flow waarden waren duidelijk verschillend bij overlevende en niet overlevende lappen. Dit bevestigt de waarde van laser Doppler flowmetrie bij de postoperatieve bewaking van microvaatchirurgie. Deze studie bewijst dat het roken van sigaretten schadelijk is voor de overleving van vrij gevascullariseerde weefsel transplantaten.

Laser Doppler flowmetrie is een betrekkelijk eenvoudige, objectieve, quantitatieve en niet-invasieve techniek om microcirculatie van de huid vast te stellen. Microvasculaire bloeddoorstroming wordt continu gemeten, zonder dat deze beinvloed wordt terwijl permanente bewaking gedurende enkele dagen mogelijk is.

De methode is sensitief omdat het snel reageert op veranderingen van de microcirculatie. De apparatuur is echter tamelijk duur, en de bestudeerde weefselinhouden kunnen niet nauwkeurig gedefinieerd worden, omdat er geen scherpe grenzen bestaan. De resultaten worden weergegeven in perfusion units (P.U.), die weliswaar direct gerelateerd zijn aan de microvasculaire flow, maar geen absolute flowwaarden zijn (uitgedrukt in milliliters per minuut). Als genoemde beperkingen in acht worden genomen, is laser Doppler flowmetrie een waardevolle methode om microcirculatie te beoordelen in klinische en experimentele studies.

REFERENCES

1. **Munro PAG.** Advances in optical and electron microscopy. *Barer R and Cosslet VE. New York, Academic Press 1966:1–40*

2. **Foreman JW, George EW, Jetton JL, Lewis RD, Thornton JR, Watson HJ.** Fluid flow measurements with a laser Doppler velocimeter. *IEEE J Quantum Electron 1966;2:260–6*

3. **Forrester AT, Gudmundsen RA, Johnson PO.** Photoelectric mixing of coherent light. *Phys Rev 1955;99:1691–700*

4. **Yeh Y, Cummins HZ.** Localized fluid flow measurements with an He–Ne laser spectrometer. *Appl Phys Lett 1964;4:176–8*

5. **Lanz O, Johnson C, Morikawa S.** High resolution laser Doppler velocity measurements of bidirectional pulsatile fluid flow. *Appl Phys Lett 1970;17:523–5*

6. **Riva C, Ross B, Benedek GB.** Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. *Invest Ophthalmol 1972;11:936–44*

7. **Tanaka T, Riva C, Ben-Sira I.** Blood velocity measurements in human retinal vessels. *Science 1974;186:830–1*

8. **Stern MD.** In vivo evaluation of microcirculation by coherent light scattering. *Nature 1975;254:56–8*

9. **Stern MD, Lappe DL, Bowen PD, Chjimosky JE, Holloway GA, Keiser HR, Bowman RL.** Continuous measurement of tissue flow by laser Doppler spectroscopy. *Am J Physiol 1977;232:H441–8*

10. **Stern MD, Bowen PD, Parma R, Osgood RW, Bowman RL, Stein JH.** Measurement of renal cortical and medullary blood flow by laser Doppler spectroscopy in the rat. *Am J Physiol 1979;236:F80–7*

11. **Holloway GA, Watkins DW.** Laser Doppler measurement of cutaneous blood flow. *J Invest Dermatol 1977;69:306–9*

12. **Nilsson GE, Tenland T, Oberg PA.** A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng 1980;27:12–9*

13. **Haumschield DJ.** An overview of laser Doppler flowmetry. *Biomed Sci Instrum 1986;22:35–40*

14. **Jentink HW.** Laser Doppler velocimetry using diode lasers. *Thesis, University of Twente, the Netherlands 1989*

15. **Nilsson GE, Tenland T, Oberg PA.** Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *IEEE Trans Biomed Eng 1980;27:597–604*

16. **Greenfield ADM.** The circulation through the skin. *Handbook of physiology – circulation II, Hamilton WF. Washington DC; Am Physiol Society 1963;1325–51*

17. **Ryan TJ.** Structure and shape of blood vessels of skin. *The physiology and pathophysiology of the skin, Jarrett. London, England; Academic 1973;619–25*

18. **Sparks HV.** Skin and muscle. *Peripheral circulation, Johnson PD. New York; Wiley 1978;193–230*

19. **Whitton JT, Everall JD.** The thickness of the epidermis. *Br J Derm 1973;89:467–76*

20. **Le-Cong P, Zweifach BW.** In vivo and in vitro velocity measurements in microvasculature with a laser. *Microvasc Res 1979;17:131–41*

21. **Fagrell B, Fronek A, Intaglietta M.** A microscope–television system for studying flow velocity in human skin capillaries. *Am J Physiol 1977;233:318–21*

22. **Altman PL, Dittmer DS.** Respiration and circulation. *Bethesda MD, Fed of Am Soc for Exp Biol 1971;383–437*

23. **Hertzman AB, Randall WC, Jochim KE.** Relations between cutaneous blood flow and blood content in the finger pad, forearm and forehead. *Am J Physiol 1947;150:122–32*

24. **Hertzman AB, Randall WC.** Regional differences in the basal and maximum rates of blood flow in the skin. *J Appl Physiol 1948;1:234–41*

25. **Jakobsson A, Nilsson GE.** Estimation of laser Doppler measuring depth by Monte Carlo modelling. *Presented at The 1st European laser Doppler users' meeting, Oxford UK, 24 & 25–3–1991*

26. **Gush RJ, King TA, Jayson MIV.** Aspects of laser light scattering from skin tissue with application to laser Doppler blood flow measurement. *Phys Med Biol 1984;29:1463–76*

27. **Engelhart M, Kristensen JK.** Evaluation of cutaneous blood flow responses by ^{133}Xenon washout and a laser Doppler flowmeter. *J Invest Dermatol 1983;80:12–5*

28. **Engelhart M, Petersen LJ, Kristensen JK.** The local regulation of blood flow evaluated simultaneously by 133–Xenon washout and laser Doppler flowmetry. *J Invest Dermatol 1988;91:451–3*

29. **Klemp P, Staberg B.** The effects of antipsoriatic treatment on cutaneous blood flow in psoriasis measured by ^{133}Xe washout method and laser Doppler velocimetry. *J Invest Dermatol 1985;85;259–63*

30. **Olsson P.** A comparison between the ^{133}Xe washout and laser Doppler techniques for estimation of nasal mucosal blood flow in humans. *Acta Otolarynol 1986;102:106–12*

31. **Tooke JE, Ostergren J, Fagrell B.** Synchronous assessment of human skin microcirculation by laser Doppler flowmetry and dynamic capillaroscopy. *Int J Microcirc Clin Exp 1983;2:277–84*

32. **Tyml K, Ellis CG.** Simultaneous assessment of red cell perfusion in skeletal muscle by laser Doppler flowmetry and video microscopy. *Int J Microcirc Clin Exp 1985;4:397–406*

33. **Larouere MJ, Sillman JS, Nuttall AL, Miller JM.** A comparison of laser Doppler and intravital microscopic measures of cochlear blood flow. *Otolaryngol Head Neck Surg 1989;101:375–84*

34. **Bongerd O, Fagrell B.** Discrepancies between total and nutritional skin microcirculation in patients with peripheral arterial occlusive disease (PAOD). *Vasa 1990;19:105–11*

35. **Tenland T, Salerud EG, Nilsson GE, Oberg PA.** Spatial and temperal variations in human skin blood flow. *Int J Microcirc: Clin Exp 1983;2:81–90*

36. **Salerud EG, Tenland T, Nilsson GE, Oberg PA.** Rhythmical variations in human skin blood flow. *Int J Microcirc: Clin Exp 1983;2:91–102*

37. **Low PA, Neumann LPN, Dyck PJ, Fealey RD, Tuck RR.** Evaluation of skin vasomotor reflexes by using laser Doppler velocimetry. *Mayo Clin Proc 1983;58:583–92*

38. **Tur E, Tur M, Maibach HI, Guy RH.** Basal perfusion of the cutaneous microcirculation: measurements as function of anatomic position. *J Invest Dermatol 1983;81:442–6*

39. **Sundberg.** Acute effects and long-term variations in skin blood flow measured with laser Doppler flowmetry. *Scand J Clin Lab Invest 1984;44:341–5*

40. **Rotering RH, Dixon JA, Holloway GA, McCloskey DW.** A comparison of the He–Ne laser and ultrasound Doppler systems in the determination of viability of ischemic canine intestine. *Ann Surg 1982;196:705–8*

41. **Larrabee WF, Holloway GA, Trachy RE, Sutton D.** Skin flap tension and wound slough: correlation with laser Doppler velocimetry. *Otolaryngol Head Neck Surg 1982;90:185–7*

42. **Larrabee WF, Holloway GA, Sutton D.** Wound tension and blood flow in skin flaps. *Ann Otol Rhinol Laryngol 1984;93:112–5*

43. **Holmstrom A, Lewis DH.** Regional blood flow in skeletal muscle after high-energy trauma. An experimental study in pigs, using a new laser Doppler technique and radioactive microspheres. *Acta Chir Scand 1983;149:453–8*

44. **Cummings CW, Trachy RE, Richardson MA, Patterson HC.** Prognostification of myocutaneous flap viability using laser Doppler velocimetry and fluorescein microfluorometry. *Otolaryngol Head Neck Surg 1984;92:559–63*

45. **Cummings CW, Trachy RE.** Measurements of alternative blood flow in the porcine panniculus carnosus myocutaneous flap. *Arch Otolaryngol 1985;111:598–600*

46. **Heden P, Jurell G, Arnander C.** Prediction of skin flap necrosis: a comparative study between laser Doppler flowmetry and fluorescein test in a rat model. *Ann Plast Surg 1986;17:485–8*

47. **Lynch TG, Hobson RW, Kerr JC, Brousseau DA, Silverman DG, Reilly CA, Tseng H.** Doppler ultrasound, laser Doppler, and perfusion fluorometry in bowel ischemia. *Arch Surg 1988;123:483–6*

48. **Knight KR, Collopy PA, O'Brien BM.** Correlation of viability and laser Doppler flowmetry in ischemic flaps. *J Surg Res 1987;43:444–51*

49. **Rosenberg L, Molcho J, Dotan Y, Baruchin A, Mahler D.** Use of the Doppler effect in visible laser light to assess tissue viability by capillary blood flow. *Ann Plast Surg 1982;8:206–12*

50. **Larrabee WF, Sutton GD, Holloway GA, Tolentino G.** Laser Doppler velocimetry and fluorescein dye in the prediction of skin flap viability. A comparison. *Arch Otolaryngol 1983;109:454–6*

51. **Marks MJ, Trachy RE, Cummings CW.** Dynamic variations in blood flow as measured by laser Doppler velocimetry: a study in rat skin flaps. *Plast Reconstr Surg 1984;73:804–10*

52. **Svensson H, Pettersson H, Svedman P.** Laser Doppler flowmetry and laser photometry for monitoring free flaps. *Scand J Plast Reconstr Surg 1985;19:245–9*

53. **Heden PG, Hamilton R, Arnander C, Jurell G.** Laser Doppler surveillance of the circulation of free flaps and replanted digits. *Microsurgery 1985;6:11–9*

54. **Jones BM.** Predicting the fate of free tissue transfers. *Ann R Coll Surg Engl 1985;67:63–70*

55. **Jenkins SD, Sepka RS, Barwick WJ, Serafin D, Klitzman B.** Routine clinical use of laser Doppler flowmeter to monitor free tissue transfer: preliminary results. *J Reconstr Microsurg 1987;3:281–3*

56. **Jenkins SD, Sepka RS, Barwick WJ.** Routine use of laser Doppler flowmetry for monitoring autologous tissue transplants. *Ann Plast Surg 1988;21:423–6*

57. **Walkinshaw M, Holloway A, Bulkley A, Engrav LH.** Clinical evaluation of laser Doppler blood flow measurements in free flaps. *Ann Plast Surg 1987;18:212–7*

58. **Lowdon IM, Toby EB, Ecker J, Seaber AV, Urbaniak JR.** Laser Doppler monitoring of replants using a small prism probe. *Microsurgery 1989;10:175–7*

59. **Clinton MS, Sepka RS, Bristol D, Pederson WC, Barwick WJ, Serafin D, Klitzman B.** Establishment of normal ranges of laser Doppler blood flow in autologous tissue transplants. *Plast Reconstr Surg 1991;87:299–309*

60. **Fischer JC, Parker PM, Shaw WW.** Comparison of two laser Doppler flowmeters for the monitoring of dermal blood flow. *Microsurgery 1983;4:164–70*

61. **Barnett NJ, Dougherty G, Pettinger SJ.** Comparative study of two laser Doppler blood flowmeters. *J Med Engineer Technol 1990;14:243–9*

62. **Obeid AN, Dougherty G, Pettinger SJ.** In vivo comparison of a twin wavelenth laser Doppler flowmeter using He–Ne and laser diode sources. *J Med Eng Technol 1990;14:102–10*

63. **Douglas SLE, de Trafford JC, Roberts VC.** Comparing the performance of three commercially available laser Doppler flowmeters. *Presented at the 3rd UK meeting on laser Doppler flowmetry, Manchester UK, 8–4–1988*

64. **Kvernebo K.** Standardisation of laser Doppler flowmetry measurements; a prerequisite for routine clinical applications. *Presented at The 1st European laser Doppler users' meeting, Oxford UK, 24 & 25–3–1991*

65. **Swartz WM, Jones NF, Cherup L, Klein A.** Direct monitoring of microvascular anastomoses with the 20–MHz ultrasonic Doppler probe: an experimental and clinical study. *Plast Reconstr Surg 1988;81:149–61*

66. **Cochrane T, Fail T, Sherriff SB.** Comparison of laser Doppler and Doppler ultrasound in lower limb vascular diagnosis. *Clin Phys Physiol Meas 1987;8:231–8*

67. **Denison AB, Spencer MP, Green HD.** A square wave electromagnetic flowmeter for application to intact blood vessels. *Circ Res 1955;3:39*

68. **Pershing LK, Huether S, Conklin RL, Krueger GG.** Cutaneous blood flow and percutaneous absorption: a quantitative analysis using a laser Doppler velocimeter and a blood flow meter. *J Invest Dermatol 1989;92:355–9*

69. **Heden P, Eriksson E.** Skin flap circulation. Simultaneous monitoring with laser Doppler and electromagnetic flowmeters in the pig island buttock flap. *Scand J Plast Reconstr Surg Hand Surg 1989;23:1–9*

70. **Brodie TG, Russel AE.** On the determination of the rate of blood flow through an organ. *J Physiol London 1905;32:47–9*

71. **Almond NE, Jones DP, Cooke ED.** High quality photoplethysmograph signals from a laser Doppler flowmeter: preliminary studies of two simultaneous outputs from the finger. *J Biomed Eng 1988;10:458–62*

72. **Almond NE, Jones DP, Cooke ED.** Noninvasive measurement of the human peripheral circulation: relationship between laser Doppler flowmeter and photoplethysmograph signals from the finger. *Angiology 1988;39:819–29*

73. **Almond NE, Cooke ED.** Observations on the photoplethysmograph pulse derived from a laser Doppler flowmeter. *Clin Phys Physiol Meas 1989;10:137–45*

74. **Nilsson G, Lindblom P, Palmer B, Vernersson E, Aberg M.** The effect of triglycyl–lysine–vasopressin (terlipressin INN, Glycopressin) on skin blood flow, measured with laser Doppler flowmetry, thermography and plethysmography. A dose–response study. *Scand J Plast Reconstr Surg Hand Surg 1987;21:149–57*

75. **Yamamoto Y, Oberg PA.** Measurement of digital blood flow using the laser Doppler, impedance and strain-gauge methods. *Med Biol Eng Comp 1990;28:113–8*

76. **Lange K, Krewer SE.** The dermofluorometer. *J Lab Clin Med 1943;28:1746–51*

77. **Lanzafane RJ, Zeiler DZ, Naim JO, Rogers DW, Hishaw JR.** A comparison of cutaneous perfusion as determined by laser Doppler velocimetry or sodium fluorescein. *Curr Surg 1984;41:271–4*

78. **Perbeck L, Lindquist K, Proano E, Liljeqvist L.** Correlation between fluorescein flowmetry and laser Doppler flowmetry. A study in the intestine (ileoanal pouch) in man. *Scand J Gastroenterol 1990;25:520–4*

79. **Liu AJ, Cummings CW, Trachy RE.** Venous outflow obstruction in myocutaneous flaps: changes in microcirculation detected by perfusion fluorometer and laser Doppler. *Otolaryngol Head Neck Surg 1986;94:164–8*

80. **Silverman DG, Weinstock BS, Brousseau DA, Norton KJ, Kniffin SA, Smith G.** Comparative assessment of blood flow to canine island flaps. *Arch Otolaryngol 1985;111:677–81*

81. **Holti G, Mitchell KW.** Estimation of the nutrient skin blood flow using a segmental thermal clearance probe. *Clin Exp Dermatol 1978;3:189–98*

82. **Holloway GA.** Cutaneous blood flow responses to injection trauma measured by laser Doppler velocimetry. *J Invest Dermatol 1980;74:1–4*

83. **Sejrsen P.** Diffusion processes invalidating the intraarterial krypton-85 beta-particle clearance method for measurement of skin blood flow in man. *Circ Res 1967;21:281–95*

84. **Sejrsen P.** Atraumatic local labeling of skin by inert gas: epicutaneous application of Xenon 133. *J Appl Physiol 1968;24:570–2*

85. **Nicholson CD, Schmitt RM, Wilke R.** The effect of acute and chronic femoral artery ligation on the blood flow through the gastrocnemius muscle of the rat examined using laser Doppler flowmetry and Xenon-133 clearance. *Int J Microcirc Clin Exp 1985;4:157–71*

86. **Kim S, Liu M, Markowitz K, Bilotto G, Dorscher-Kim J.** Comparison of pulpal blood flow in dog canine teeth determined by the laser Doppler and the 133Xenon washout methods. *Arch Oral Biol 1990;35:411–3*

87. **Monteiro AA, Svensson H, Bornmyr S, Arborelius M, Kopp S.** Comparison of ^{133}Xe clearance and laser Doppler flowmetry in assessment of blood flow changes in human masseter muscle induced by isometric contraction. *Arch Oral Biol 1989;34:779–86*

88. **Hellem S, Jacobsson LS, Nilsson GE, Lewis DH.** Measurements of microvascular blood flow in cancellous bone using laser Doppler flowmetry and ^{133}Xe-clearance. *Int J Oral Surg 1983;12:165–77*

89. **Eyre JA, Essex TJ, Flecknell PA, Bartholomew PH, Sinclair JI.** A comparison of measurements of cerebral blood flow in the rabbit using laser Doppler spectroscopy and radionuclide labelled microspheres. *Clin Phys Physiol Meas 1988;9:65–74*

90. **Swiontkowski MF, Tepic S, Perren SM, Moor R, Ganz R, Rahn BA.** Laser Doppler flowmetry for bone flow measurement: correlation with microsphere estimates and evaluation of the effect of intracapsular pressure on femoral head flow. *J Orthop Res 1986;4:362–71*

91. **Fagrell B.** Skin preparations: advantages and disadvantages. *Baker CH and Nastuk WL, Microcirculatory technology. Orlando, Academic Press 1986:19–42*

92. **Hensel H, Bender F.** Fortlaufende Bestimmung der Hautdurchblutung am Menschen mit einem electrischen Waermeleitmesser. *Pflurgers Arch Gesamte Physiol Menschen Tiere 1956;263:603–14*

93. **Cooper KE, Edholm OG, Mottram RF.** The blood flow in skin and muscle of the human forearm. *J Physiol London 1955;128:258–68*

94. **Mustakallio KK, Kolari PJ.** Irritation and staining by dithranol (anthralin) and related compounds. IV. Visual estimation of erythema compared with contact thermometry and laser Doppler flowmetry. *Acta Derm Venereol (Stockh) 1983;63:531–8*

95. **Nilsson G.** Blood flow, temperature, and heat loss of skin exposed to local radiative and convective cooling. *J Invest Dermatol 1987;88:586–93*

96. **Vegfors M, Tryggvason B, Sjoberg F, Lennmarken C.** Assessment of peripheral blood flow using a pulse oximeter. *J Clin Monit 1990;6:1–4*

97. **Heden P, Sollevi A, Hamberger B.** Early circulatory and metabolic events in island skin flaps of the pig. *Plast Reconstr Surg 1989;84:468–74*

98. **Toshitani S, Nakayama J, Yahata T, Yasuda M, Urabe H.** A new apparatus for hair regrowth in male-pattern baldness. *J Dermatol 1990;17:240–6*

99. **Ninet J, Fronek A.** Cutaneous postocclusive reactive hyperemia monitored by laser Doppler flux metering and skin temperature. *Microvasc Res 1985;30:125–32*

100. **Enkema L, Holloway GA, Piraino DW, Harry D, Zick GL, Kenny MA.** Laser Doppler velocimetry vs heater power as indicators of skin perfusion during transcutaneous O_2 monitoring. *Clin Chem 1981;27:391–6*

101. **Hertzman AB.** Photoelectric plethysmography of the nasal septum in man. *Proc Soc Exp Biol Med 1938;37:290*

102. **Guy RH, Wester RC, Tur E, Maibach HI.** Noninvasive assessments of the percutaneous absorption of methyl nicotinate in humans. *J Pharm Sci 1983;72:1077–9*

103. **Wester RC, Maibach HI, Guy RH, Novak E.** Minoxidil stimulates cutaneous blood flow in human balding scalps: pharmodynamics measured by laser Doppler velocimetry and photopulse plethysmography. *J Invest Dermatol 1984;82:515–7*

104. **Kvernebo K, Megerman J, Hamilton G, Abbott WM.** Response of skin photopletysmography, laser Doppler flowmetry and transcutaneous oxygen tensiometry to stenosis-induced reductions in limb blood flow. *Eur J Vasc Surg 1989;3:113–20*

105. **Intaglietta M, Pawula RF, Tompkins WR.** Pressure measurements in the mammalian microvasculature. *Microvasc Res 1970;2:212–20*

106. **Severinghaus JW, Stafford M, Thunstrom AM.** Estimation of skin metabolism and blood flow with $tcPO_2$ and $tcPCO_2$ electrodes by cuff occlusion of the circulation. *Acta Anaesthesiol Scand 1978;(suppl) 68:9–15*

107. **Smith AR, Sonneveld GJ, Kort WJ, van der Meulen JC.** Clinical application of transcutaneous oxygen measurements in replantation surgery and free tissue transfer. *J Hand Surg 1983;8:139–45*

108. **Allen PI, Goldman M.** Skin blood flow: a comparison of transcutaneous oximetry and laser Doppler flowmetry. Eur *J Vasc Surg 1987;1:315–8*

109. **Ewald U, Huch A, Huch R, Rooth G.** Skin reactive hyperemia recorded by a combined $TcpO_2$ and laser Doppler sensor. *Adv Exp Med Biol 1987;220:231–4*

110. **Smits TM, Aarnoudse JG, Zijlstra WG.** Scalp blood flow, measured by laser Doppler flowmetry, transcutaneous pO_2 and pCO_2 in the lamb. *Pediatr Res 1990;27:442–4*

111. **Smits TM, Aarnoudse JG, Zijlstra WG.** Fetal scalp blood flow as recorded by laser Doppler flowmetry and transcutaneous pO_2 during labour. *Early Hum Dev 1989;20:109–24*

112. **Svensson H, Svedman P, Holmberg J, Jacobsson S.** Laser Doppler flowmetry and transcutaneously measured carbon dioxyde tension for observing changes of skin blood flow in fingers. *Scand J Plast Reconstr Surg 1983;17:183–6*

113. **Nove-Josserand R, Ninet J, Bachet P.** Etude comparative de la pression d'oxygen transcutanee et du flux laser Doppler au niveau du pied chez 15 sujets sains. *J Mal Vasc 1988;13:328–34*

114. **Matsen FA, Wyss CR, Robertson CL, Oberg PA, Holloway GA.** The relationship of transcutaneous pO_2 and laser Doppler measurements in a human model of local arterial insufficiency. *Surg Gynecol Obstet 1984;159:418–22*

115. **Gilje O.** Capillary microscopy in the differential diagnosis of skin diseases. *Acta Derm Venereol 1953;33:303–17*

116. **Damber JE, Bergh A, Fagrell B, Lindahl O, Rooth P.** Testicular microcirculation in the rat studied by videophotometric capillaroscopy, fluorescence microscopy and laser Doppler flowmetry. *Acta Physiol Scand 1986;126:371–6*

117. **Malt RA, McKhann CF.** Replantation of severed arms. *JAMA 1964;189:716–22*

118. **Antia NH, Buch VI.** Transfer of an abdominal dermo–fat graft by direct anastomosis of blood vessels. *Br J Plast Surg 1971;24:15–9*

119. **Goodstein WA, Buncke HJ.** Patterns of vascular island anastomoses v.s. success of free groin flap transfers. *Plast Reconstr Surg 1979;64:37–40*

120. **Jones MB, Mayou BJ.** The laser Doppler flowmeter for microvascular monitoring: a preliminary report. *Br J Plast Surg 1982;35:147–9*

121. **Kerrigan CL, Zelft RG, Daniel RK.** Secondary critical ischemia time of experimental skin flaps. *Plast Reconstr Surg 1984;74:522–4*

122. **Shaw WW.** A general study of free flaps. *Presented at "Clinical Frontiers in Reconstructive Surgery", California, June 1981*

123. **Jones BM.** Monitors for the cutaneous microcirculation. *Plast Reconstr Surg 1984;73:843–50*

124. **Harrison DH, Girling M, Mott G.** Experience in monitoring the circulation in free–flap transfers. *Plast Reconstr Surg 1981;68:543–53*

125. **Sloan GM, Sasaki GH.** Noninvasive monitoring of tissue viability. *Clin Plast Surg 1985;12:185–95*

126. **Ackland RD.** Experience in monitoring the circulation in free flap transfers (Discussion). *Plast Reconstr Surg 1976;57:577*

127. **Lister GD, Kleinert HE.** Replantation. *In WC Grabb and JW Smith (Eds), Plastic Surgery, 3rd Ed. Boston: Little, Brown 1979:697–715*

128. **Lister GD, Kalisman M, Tsai TM.** Reconstruction of the hand with free microneurovascular toe–to–hand transfer. Experience with 54 toe transfers. *Plast Reconstr Surg 1983;71:372–86*

129. **Okutsu I, Ninomiya, Takatori Y, Kuroshima N.** Monitoring skin temperature in microvascular anastomosis. *Orthoped Rev 1982;11:39–42*

130. **Stirrat CR, Seaber AV, Urbaniak JR, Bright DS.** Temperature monitoring in digital replantation. *J Hand Surg 1978;3:342–7*

131. **Leonard AG, Brennen MD, Colville J.** The use of continuous temperature monitoring in the postoperative management of microvascular cases. *Br J Plast Surg 1982;35:337–42*

132. **Hovius SER, van Adrichem LNA, Mulder HD, van Strik R, van der Meulen JC.** Postoperative monitoring in microvascular surgery. *Proceedings of the first European congress of hand surgery. Taranto, Italy 1989;299–304*

133. **Pietila J, v Smitten K, Sundell B.** The effect of perfusion on postoperative viability in the replanted rabbit ear: measured by laser Doppler flowmetry and skin temperature. *Scand J Plast Reconstr Surg 1985;19:251–4*

134. **Svensson H, Svedman P, Holmberg J, Jacobsson S.** Detecting arterial and venous obstruction in flaps. *Ann Plast Surg 1985;14:20–3*

135. **Fischer JC, Parker PM, Shaw WW.** Waveform analysis applied to laser Doppler flowmetry. *Microsurgery 1986;7:67–71*

136. **Sloan GM, Reinisch JF.** Flap physiology and the prediction of flap viability. *Hand Clinics 1985;1:609–19*

137. **Fritz WD, Swartz WM, Rose S, Futrell JW, Klein E.** Limb allografts in rats immunosuppressed with Cyclosporin A. *Ann Surg 1984;199:211–5*

138. **Furnas DW, Black KS, Hewitt CW, Fraser LA, Achauer BM.** Cyclosporine and long-term survival of composite tissue allografts (limb transplants) in rats (with historical notes on the role of plastic surgeons in allotransplantation). *Transplant Proc 1983;15:3063–8*

139. **Kim SK, Aziz S, Oyer P, Hentz VR.** Use of cyclosporin A in allotransplantation of rat limbs. *Ann Plast Surg 1984;12:249–55*

140. **Daniel RK, Egerszegi EP, Samulack DD, Skanes SE, Dykes RW, Rennie WRJ.** Tissue transplants in primates for upper extremity reconstruction: a preliminary report. *J Hand Surg [Am] 1986;11:1–8*

141. **Svensson H, Svedman P.** Continuous monitoring of the circulation in flaps. *VIII international congres of plastic surgery, Montreal, 26–6 / 1–7–1983*

142. **Lawrence WT, Murphy RC, Robson MC, Heggers JP.** The detrimental effect of cigarette smoking on flap survival: an experimental study in the rat. *Br J Plast Surg 1984;37:216–9*

143. **Levine PH.** An acute effect of cigarette smoking on platelet function: A possible link between smoking and arterial thrombosis. *Circulation 1973;48:619–23*

144. **Strong JP, Richards ML.** Cigarette smoking and atherosclerosis in autopsied men. *Atherosclerosis 1976;23:451–76*

145. **Bruce JW, Miller JR, Hooker DR.** The effect of smoking upon the bloodpressures and upon the volume of the hand. *Am J Physiol 1909;24:104–16*

146. **Maddock WG, Coller FA.** Peripheral vasoconstriction by tobacco demonstrated by skin temperature changes. *Proc Soc Exp Biol Med 1932;29:487–8*

147. **Saumet JL, Leftheriotis G, Dittmar A, Delhomme G.** Relationship between pulse amplitude and thermal exchange in the finger: the effect of smoking. *Clin Physiol 1986;6:139–46*

148. **Sarin CL, Austin JC, Nickel WO.** Effects of smoking on digital blood flow velocity. *JAMA 1974;229:1327–8*

149. **Richardson D.** Effects of tobacco smoke inhalation on capillary blood flow in human skin. *Arch Environm Health 1987;42:19–25*

150. **Reus WF, Robson MC, Zachary L, Heggers JP.** Acute effects of tobacco smoking on blood flow in the cutaneous microcirculation. *Br J Plast Surg 1984;37:213–5*

151. **Wilson GR, Jones BM.** The damaging effect of smoking on digital revascularisation: two further case reports. *Br J Plast Surg 1984;37:613–4*

152. **Nolan J, Jenkins RA, Kurihara K, Schultz RC.** The acute effects of cigarette smoke exposure on experimental skin flaps. *Plast Reconstr Surg 1985;75:544–9*

153. **Craig S, Rees TD.** The effects of smoking on experimental skin flaps in hamsters. *Plast Reconstr Surg 1985;75:842–6*

154. **Kaufman T, Eichenlaub EH, Levin M, Hurwitz DJ, Klain M.** Tobacco smoking: impairment of experimental flap survival. *Ann Plast Surg 1984;13:468–72*

155. **Rees TD, Liverett DM, Guy CL.** The effect of cigarette smoking on skin-flap survival in the face lift patient. *Plast Reconstr Surg 1984;73:911–5*

156. **Riefkohl R, Wolfe JA, Cox EB, McCarty KS.** Association between cutaneous occlusive vascular disease, cigarette smoking and skin slough after rhytidectomy. *Plast Reconstr Surg 1986;77:592–5*

157. **Davis JW, Davis RF.** Acute effect of tobacco cigarette smoking on the platelet aggregate ratio. *Am J Med Sciences 1979;278:139–43*

158. **Hawkins RI.** Smoking, platelets and thrombosis. *Nature 1972;236:450–2*

159. **Sackett DL, Gibson RW, Bross IDJ, Pickren JW.** Relation between aortic atherosclerosis and the use of cigarettes and alcohol: an autopsy study. *N Engl J Med 1968;279:1413–20*

160. **Roth GM, McDonald JB, Sheard C.** The effect of smoking cigarettes and of intravenous administration of nicotine on the electrocardiogram, basal metabolic rate, cutaneous temperature, blood pressure and pulse rate of normal persons. *JAMA 1944;125:761–7*

161. **van Adrichem LNA, Hovius SER.** Het acute effect van het roken van sigaretten op de microcirculatie van de duim. *Ned Tijdschr Geneeskd 1989;133:703*

162. **Myers KA, King RB, Scott DF, Johnson N, Morris PJ.** The effect of smoking on the late patency of arterial reconstructions in legs. *Br J Surg 1978;65:267–71*

163. **Wray R, DePalma RG, Hubay CH.** Late occlusion of aortofemoral bypass grafts: influence of smoking. *Surgery 1971;70:969–73*

164. **van Adrichem LNA, Hovius SER, van Strik R, van der Meulen JC.** Acute effects of cigarette smoking on microcirculation of the thumb. *Br J Plast Surg 1992;45:9–11*

165. **van Adrichem LNA, Hovius SER, van Strik R, van der Meulen JC.** The acute effect of cigarette smoking on the microcirculation of a replanted digit. *J Hand Surg 1992;17:230–4*

166. **Petry JJ, Wortham KA.** The anatomy of the epigastric flap in the experimental rat. *Plast Reconstr Surg 1984;74:410–3*

167. **McFarlane RM, DeYoung G, Henry RA.** The design of a pedicle flap in the rat to study necrosis and its prevention. *Plast Reconstr Surg 1965;35:177–82*

168. **Westin M, Heden P.** Calcitonin gene-related peptide delays the no-reflow phenomenon in the rat island flap. *Ann Plast Surg 1988;21:329–34*

169. **Pitillo RM, Mackie IJ, Rowles PM, Machin SJ, Woolf N.** Effects of cigarette smoking on the ultrastructure of rat thoracic aorta and its ability to produce prostacyclin. *Thromb Haemostas 1982;48:173–6*

170. **Rao VK, Morrison WA, O'Brien BMcC.** Effect of nicotine on bloodflow and patency of experimental microvascular anastomosis. *Ann Plast Surg 1983;11:206–9*

171. **Yaffe B, Cushin BJ, Strauch B.** Effect of cigarette smoking on experimental microvascular anastomoses. *Microsurgery 1984;5:70–2*

172. **Wynder EL, Hoffman D.** Tabacco and tabacco smoke. *New York: Academic Press, 1967*

ACKNOWLEDGEMENTS

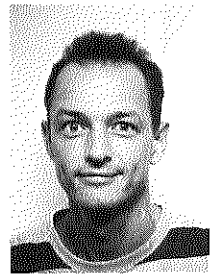
Thanks !

Prof.Dr. J.C. van der Meulen
Prof. R van Strik
Prof.Drs. J. Steketee
Pauline Alers
Prof. Dr. H. van Urk
John van der Werff
Peter Don Griot
Dorien Mulder
Ismail Gultuna
Paul van der Heijden
Arien Hofman
Dr. V.D. Vuzevski
Ruud Hoegen
Dr. Th.A. Plomp
Dr. Rombout
Chris van der Werken
P.M. Gilbert
Wil Kort
& Co-workers
Afdeling 7M

Annerieke
& Steven
Hovius

Aimée

CURRICULUM VITAE



October 2nd 1961	Born in Schiedam, the Netherlands
1974 – 1980	Gymnasium–ß, "Spieringshoek" Schiedam
1980 – 1987	Medical School, Erasmus University, Rotterdam
1983	Bachelors (cum laude)
1985	Masters
1987	M.D. degree
1985 – 1987	Student–assistent department of plastic and reconstructive surgery Dijkzigt university hospital Rotterdam (head: Prof.Dr.J.C.van der Meulen)
1987 – 1989	Military service Medical Officers training OCMGD, Hilversum & Detached at Dijkzigt university hospital Rotterdam department of plastic and reconstructive surgery (head: Prof.Dr.J.C.van der Meulen)

1989	Research fellow Erasmus University Rotterdam department of plastic and reconstructive surgery (head: Prof.Dr.J.C.van der Meulen) Financially supported by the Dutch Heart Foundation (Grant 88.229)
Since 1989	Training General Surgery (first part of the training in plastic surgery) St. Elisabeth hospital Tilburg (Head Dr. Chr.van der Werken)

PUBLICATIONS USED FOR THIS THESIS

Hovius SER, van Adrichem LNA, Mulder HD, van Strik R, van der Meulen JC. The predictive value of the laser Doppler flowmeter for postoperative microvascular monitoring. *Submitted to Ann Plast Surg*

Hovius SER, van Adrichem LNA, Mulder HD, van Strik R, van der Meulen JC. Comparison of laser Doppler flowmetry and thermometry in the postoperative monitoring of replantations. *Submitted to J Hand Surg*

van Adrichem LNA, Gultuna I, Mulder HD, Hovius SER, van Strik R, van der Meulen JC. Differences between arterial and venous occlusion in microvascular surgery. *Accepted for Scand J Plast Reconstr Hand Surg*

van Adrichem LNA, van der Werff JFA, Gultuna I, Hovius SER, van Strik R, van der Meulen JC. Postoperative microcirculatory flow patterns in microsurgically revascularized tissues. *Submitted to Br J Plast Surg*

Hovius SER, van Adrichem LNA, van der Heijden PMA, Vuzevski VD, van Strik R, van der Meulen JC. Postoperative monitoring in allogeneic limb transplantation in rats. *Ann Plast Surg 1988;21:559–65*

van Adrichem LNA, Hovius SER, van Strik R, van der Meulen JC. Acute effects of cigarette smoking on microcirculation of the thumb. *Br J Plast Surg 1992;45:9–11*

van Adrichem LNA, Hovius SER, van Strik R, van der Meulen JC. The acute effect of cigarette smoking on the microcirculation of a replanted digit. *J Hand Surg 1992;17:230–4*

van Adrichem LNA, Hoegen R, Hovius SER, Kort WJ, van Strik R, Plomp ThA, Vuzevski VD, van der Meulen JC. The effect of cigarette smoking on the survival of free vascularized and pedicled epigastric flaps in the rat. *Submitted to Plast Reconstr Surg*