Linköping University Medical Dissertation No. 1732

To See or Not to See

A Study on Capillary Refill

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Till Charlotte, Leopold och Ester – mina älskade, kloka, toleranta och fördragsamma barn

“With the senses man measures perceptible things, with the intellect he measures intelligible things, and he attains unto supra-intelligible things transcendently.”

Nicholas Cusanus (1401-1465)

Abstract

Background: Assessment of the critically ill is traditionally based on vital signs (blood pressure, pulse, respiratory rate, temperature and level of consciousness). Altered vital signs are, however, late indicators of deranged hemodynamics pointing to a need for additional, more sensitive markers of circulatory compromise. In the beginning of the 20th century, the capillary refill (CR) time evolved as a possible, non-invasive adjunct to early prediction of outcome in the critically ill. The manoeuvre entails application of blanching pressure on the skin of e.g. the finger pulp or sternum for 5 seconds. After release of the pressure, the observer estimates time in seconds for the skin to return to original colour. This time is hypothesized to reflect the dynamics of the microcirculation and its possible connection with hemodynamics. In the 1980s the “normal capillary refill time” was set to < 2 seconds and later extended to 3 seconds, without a clear scientific foundation. Naked-eye estimations of CR time met increasing scepticism in the 1990s due to subjectivity and poor prognostic value for shock or death. Several basic traits, such as age and sex, as well as ambient temperature, were also shown to independently influence the CR time. Various methods have evolved with the capability to measure CR time quantitatively, one of which is Polarisation Spectroscopy Imaging (PSI). PSI measures the Red Blood Cell (RBC) concentration in tissue (e.g. the skin) and can be used to measure CR time.

Objectives: The purpose of this study was to establish basic characteristics for quantitative Capillary Refill (qCR) time, identify possible influencing factors in healthy subjects and to investigate how this relates to current practice. We also sought to identify technical demands for transfer of the technique into clinical studies. In paper I we analysed the qCR time characteristics at 5 different skin sites (forehead, sternum, volar forearm, finger pulp and dorsum finger).

The objective of paper II was to investigate the inter- and intra-observer variability of naked eye CR assessments of different professions, nurses, doctors and secretaries (representing laymen).

In paper III we observed the effect of low ambient temperature on the qCR time in different skin sites.

In paper IV, we transferred the equipment from a laboratory to a clinical setting in the Emergency Department (ED) for application in the potentially critically ill. In this study we evaluated the most important factors determining a reliable data collection and influencing the amount of data possible to analyse.

Methods: qCR time was measured in a total of 38 volunteers and 10 patients in different skin sites (2-5 skin sites) at different ambient temperatures. An embodiment of PSI (TiVi 600 and 700, WheelsBridge AB, Linköping, Sweden) was used to determine the rapid temporal changes in RBC concentration in skin during the CR manoeuvre. Films using a range of the first measurements from paper I were shown for assessment to 48 observers working in the ED.

Results: In paper I we could delineate qCR curves and suggest 2 possible equivalents to the naked-eye observed CR time which we named *Time to Return to Baseline 1* (tRtB1) and *Time to Peak* (t_{pk}). We demonstrated differences in qCR-curves depending on skin site and possibly

due to skin temperature. In paper II we confirmed a poor inter- and intra-observer reproducibility in visually estimating the CR time regardless of profession (clinicians or laymen). Paper III demonstrated a rapid effect of low ambient temperature on qCR time in peripheral skin sites such as finger pulp. The forehead, regarded as a more central skin site, was the most temperature stable site and showed least variability in qCR time as determined using tRtB1. Paper IV, a study on patients in an ED setting, yielded assayable data in 80% of the measurements. We identified critical performance parameters to address in the further development of a more robust, easy-to-use device for future validation of the possible relevance of qCR in patient triage and monitoring.

Conclusions: CR time can be quantified using PSI. Quantified CR time demonstrated a large variability between different skin sites, specifically, skin temperature was shown to be an important factor influencing qCR time, particularly at the fingertip. Naked-eye estimates of CR time were highly variable, both within and between observers. Agreement between quantified CR time and naked-eye estimates was poor. The prototypic PSI technique was feasible in a clinical setting and, with further improvements, clinical evaluation of qCR in relation to relevant patient outcomes will be possible.

Sammanfattning

Mikrocirkulationen omfattar bl a de minsta kärlen i kroppens alla vävnader och där sker det livsviktiga syrgasutbytet med hjälp av de röda blodkropparna. Därför är det viktigt att kunna utvärdera mikrocirkulatoriska parametrar såsom röda blodkroppar, deras koncentration och hastighet.

Kapillär återfyllnads (KÅ) tid (capillary refill – CR – time) som är ett mått på mikrocirkulationens funktion i huden nämndes första gången i artiklar i slutet av 1800-talet. KÅ tid bedöms genom att undersökaren trycker på huden tills den vitnar, snabbt släpper och därefter skattar tiden tills huden återfått sin ursprungsfärg. Detta görs oftast av en doktor eller sjuksköterska som skattar tiden intuitivt (räknar sekunder) eller ibland med hjälp av ett stoppur. Under 1900-talets två världskrig ökade behovet av snabb, non-invasiv bedömning och triagering (prioritering) av patienter på slagfältet för att avgöra vilka skadade som var i störst behov av sjukvård. 1947 inkluderades KÅ tid i bedömningsalgoritmer för prioritering av svårt skadade patienter i behov av kirurgisk åtgärd och tiden klassificerades som "normal, definite slowing or very sluggish". 1980 infördes en tidsgräns och kapillär återfyllnadstid på <2 sekunder ansågs som normal. Tidsangivelsen hade dock inte baserats på vetenskapliga studier utan på ett antagande. I slutet av 80-talet kunde man visa att KÅ tid oberoende av hälsotillstånd varierar med kön, ålder och temperatur, där äldre personer kunde ha en KÅ tid på upp till 4,5 sekunder och samtidigt vara friska. Därefter har metoden ifrågasatts alltmer, både på grund av dess subjektivitet och tveksamma relevans för det vi tror oss mäta (speglar mikrocirkulationen verkligen det övergripandet (sjukdoms)tillståndet i kroppen?). Metoden lärs trots detta ut i flera internationella kurser för läkare och sjuksköterskor i traumavård (Acute Trauma Life Support ATLS) och akutsjukvård för barn och vuxna (Basic Emergency Medicine and Pediatric Life Support) som en del i den initiala bedömningen av den svårt sjuka patienten. KÅ tid mäts på akutmottagningar över hela världen.

Idag finns det flera bildgivande metoder som kan registrera och mäta små kärl och mikrocirkulation i huden. Polarisation Spectroscopy Imaging (PSI) är en sådan metod som kan mäta koncentrationen av röda blodkroppar i huden över en större yta, utan direktkontakt och med god tidsupplösning, dvs förändringar av blodkoncentrationen kan registreras med upp till 50 bilder/sekund. Denna teknik lämpar sig därför väl i studier där man vill kvantifiera KÅ (quantified CR – qCR).

Avhandlingen består av fyra delarbeten där kvantifiering av KÅ processen utgör grunden för att på sikt kunna utvärdera dess relevans i bedömning och övervakning av svårt sjuka patienter.

Målet med det första delarbetet var att undersöka förändringen av koncentrationen av röda blodkroppar i huden under KÅ processen vilket skapade kurvor samt identifiera ekvivalenter i kurvan till den KÅ tid som man uppskattar med blotta ögat. Vi mätte i 5 hudområden: panna, bröstben, underarm, fingerblomma samt fingerrygg på 23 friska frivilliga. 2 ekvivalenter till subjektivt skattad KÅ tid bedömdes kunna vara Time to Return to Baseline (tRtB1) samt Time

to Peak (t_{pk}). Kurvor och qCR tid skiljde sig åt emellan perifera hudområden (fingerblomma) och centrala hudområden (panna och bröstben).

Det andra delarbetet undersökte hur väl sjukvårdspersonal och lekmän (totalt 48) subjektivt kan skatta KÅ tid genom att titta på 15 filmer där KÅ genomförs på ett finger. Ingen skillnad påvisades i skattningsförmåga emellan grupperna, däremot sågs en stor variabilitet emellan individer samt inom samma individ som (ovetandes) såg samma film två gånger. De subjektivt skattade tiderna skiljde sig signifikant ifrån qCR tid.

I det tredje arbetet undersökte vi effekten av kyla på qCR tid genom att lägga 15 friska frivilliga i ett kylrum i 30 min med upprepade KÅ mätningar under tiden på 3 olika hudområden (panna, bröstben och fingerblomma). Vi påvisade en stark inverkan av hudtemperatur på qCR tiden med en samvariation av sjunkande hudtemperatur och förlängd KÅ tid i fingerblomma. Pannan var mest temperaturstabil och påvisade korta kvantifierade KÅ tider. Pannans och bröstbenens kvantifierade KÅ tider var kortare än de internationella riktlinjer på 2-3 sekunder som anges.

I det sista delarbetet använde vi teknik och utrustning ifrån laboratorieverksamheten för att mäta qCR i klinisk verklighet på potentiellt allvarligt sjuka patienter på akutmottagningen på Universitetssjukhuset i Linköping. Vi gjorde upprepade qCR mätningar på 10 potentiellt kritiskt sjuka patienter som behandlades på akutmottagningen (vätska, syrgastillförsel, inhalationer etc). Syftet var att mäta vilka faktorer som var avgörande för att få tillförlitliga data. Vi kunde analysera data ifrån 80% av mätningarna. Utrustningen och tekniken behöver göras mer robust och användarvänlig för att kunna samla in och presentera data momentant (nu analyserades data vid separat tillfälle) innan qCR tid kan utvärderas avseende dess relevans och prognostiska värde i bedömningen av en kritiskt sjuk patient.

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List of original papers

This thesis is a summary and discussion of the results obtained in the following papers:

Paper I

Rani Toll John, Joakim Henricson, Gert E Nilsson, Daniel B Wilhelms, Chris D Anderson.
Reflectance spectroscopy: to shed new light on the capillary refill test.
J Biophotonics. 2018 Jan;11(1). Epub 2017 May 24.

Paper II

Rani Toll John, Joakim Henricson, Gert E Nilsson, Chris D Anderson, Daniel B Wilhelms.
Man versus machine: comparison of naked-eye estimation and quantified capillary refill.
Emerg Med J. 2019 Aug;36(8):465-471.

Paper III

Rani Toll John, Joakim Henricson, Johan Junker, Carl-Oscar Jonson, Gert E Nilsson, Daniel B Wilhelms, Chris D Anderson
A cool response-The influence of ambient temperature on capillary refill time.
J Biophotonics. 2018 Jun;11(6). Epub 2018 Mar 5.

Paper IV

Rani Toll John, Joakim Henricson, Gert E Nilsson, Chris D Anderson, Daniel B Wilhelms
Quantitative capillary refill testing in ED patients: a pragmatic approach to define the demands on equipment for clinical use.
Manuscript

Publications by the author related to but not included in this thesis.

PM McNamara, Jim O'Doherty, ML O'Connell, BW Fitzgerald, Chris D Anderson, Gert E Nilsson, Rani Toll, Michael Leahy.
Tissue viability (TiVi) imaging: temporal effects of local occlusion studies in the volar forearm.
J Biophotonics. 2010 Jan;3(1-2):66-74.

Joakim Henricson, Rani Toll John, Gert E Nilsson, Chris D Anderson, Daniel B Wilhelms
Diffuse Reflectance Spectroscopy: Getting the Capillary Refill Test Under One's Thumb.
J Vis Exp. 2017 Dec 2;(130).

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Abbreviations

AU – Arbitrary Units

AVAs – Arteriovenous Anastomoses

BP - Blood Pressure

CR – Capillary Refill

ED – Emergency Department

HSI – Hyperspectral Imaging

LDF – Laser Doppler Flowmetry

LDPI – Laser Doppler Perfusion Imaging

LED - Light Emitting Diode

MELSCI – Multi Exposure Laser Speckle Contrast Imaging

PSI - Polarisation Spectroscopy Imaging

qCR – Quantified Capillary Refill

RBC – Red Blood Cell

ROI - Region of Interest

RR - Respiratory Rate

SD – Standard Deviations

SDF – Sidestream Dark Field Imaging

TiVi - Tissue Viability Imaging

t_{pk} – Time to peak

tRtB1 – Time to Return to Baseline 1

tRtB2 – Time to Return to Baseline 2

VS - Vital Signs

1 Introduction

Determining Capillary Refill (CR) time in skin has been taught as a standard measure of assessing a patient's circulatory status in the initial stages of shock of various aetiologies¹. Following 5 seconds of cutaneous pressure by the examiner's finger on the e.g. finger pulp or sternum, CR should occur within 2-3 seconds in the supine patient²⁻⁴. A slower refill time indicates reduced skin perfusion. The concept for performance and interpretation was introduced by Beecher et al in 1947 defining CR time as "normal", "definite slowing" or "very sluggish", correlating it with no, slight/moderate and severe shock⁵. In 1980 a "normal" CR time was defined to be less than 2 s by Champion et al, although this was not based on clinical evidence but arbitrarily chosen⁶. No consistent "criterion standard" of CR test has been agreed upon concerning skin site, pressure and pressure time. The CR time is determined by the examiner's subjective naked-eye assessment of the time required for the blanched skin to reperfuse after pressure removal. The blanching pressure and pressure area are not "standardized" with respect to the variable manual pressure of different examiners. This causes difficulties in reproducibility and reliability and thus raises questions on the validity of the method⁷⁻¹². Additionally, CR time has been shown to be age and sex dependent⁴. The ambient temperature and patient's skin temperature also influence the CR time^{3,13,14}. Clinically as well as in research, CR time is often measured peripherally on soles, digits, nail beds etc, without consideration of the skin temperature differences between central and peripheral sites³.

As a result, the measure of CR time in the emergency department (ED) is diminishing among clinicians in Northern Europe. Clinicians of today mainly rely on vital signs (VS) such as respiratory rate (RR), oxygen saturation, pulse and blood pressure (BP) in the assessment of the critically ill patient. Changes in these signs, however, often occur late in the course of deterioration. Shock as a result of haemorrhage starts with tachycardia > 100 beats/min only when > 15% blood has been lost. A decrease in BP begins when between 30 and 40% of the blood volume has been lost¹⁵. The BP depends chiefly on the cardiac output and the peripheral resistance, as well as the type of blood vessel in which the pressure is measured. A major determinant of the peripheral resistance lies in the smaller but abundant vessels, particularly the arterioles. The main purpose of resuscitation is to restore an adequate blood perfusion in the capillary system to ensure delivery of oxygen and nutrients to the tissues in central organs. Recent studies indicate the importance of coherence between micro- and macrocirculation during resuscitation as well as a possible prognostic value of measuring CR time, over measurements of lactate¹⁶⁻¹⁹.

The skin is the largest vascularised organ of the body and is easily accessible for measurements of the microcirculation when investigating coherence to the macrocirculation. The skin's innate responses to external stimuli depend on physical, chemical and cellular properties specific for the individual's sex, age and condition of health²⁰.

Establishing methods to estimate the functionality of the microcirculation in clinical settings are of clear relevance and interest. CR time is one of several options to measure microcirculation but would be improved by the use of objective quantification techniques rather than naked-eye measurement. Quantification is necessary in order to reliably evaluate the pros and cons of CR in assessing and monitoring the microcirculation in patients. Due consideration of the physiological characteristics of the skin is mandatory in any attempt to standardize the measurement of the quantified CR (qCR) process.

2 Background

2.1 General Skin Function and Physiology

The skin is responsible for several essential functions related to its location as the body's outer surface, thereby being a sensory organ as well as protecting organ. The skin maintains a barrier function against physical, chemical and biological agents from the external environment. The integument also preserves the constancy of the internal environment by regulating body temperature, water loss and electrolyte balance, thus sustaining a homeostatic balance. The ability to concatenate information of the external environment to more central parts of the organism is an important sensory function. The skin is also an immunological organ, containing memory-lymphocytes and the antigen-presenting Langerhans cells as well as secretory IgA excreted into the surface film by the glandulae²¹.

The skin is made of three layers: the **epidermis**, composed of closely packed epithelial cells, and the **dermis**, composed of dense, irregular connective tissue that contains blood vessels, hair follicles, sweat glands, and other structures²². Beneath the dermis lies the hypodermis or **subcutis**, composed mainly of loose connective and fatty tissues of various amounts. Epidermis, 0.05 to 1 mm thick, is a non-vascular tissue and contains keratinocytes (>90%), melanocytes, Langerhans cells, lipids and free fatty acids²³⁻²⁵. Dermis, 1-10 mm thick, consisting mainly of connective tissue is composed of two layers, the stratum papillare and the stratum reticulare²⁴. The more superficial stratum papillare contains the superficial (nutritive) vessel plexus that serves but does not enter the epidermis. Dermal papillae, finger-like connective tissue protrusions project into the tissue beneath the epidermis, thus enhancing the attachment of the epidermis to dermis by an increased interface between the two tissues. The more deeply situated stratum reticulare contains a deeper vessel plexus at the border to the subcutis and both horizontal vessel plexuses are connected by vessels traversing dermis vertically²⁵.

Figure 1. Schematic view of skin and some appendages. Image by Charlotte John.

2.2 Cutaneous Vascular Anatomy and Physiology

The microvasculature of the skin consists of vessels with diameters of less than 100 μm , comprising arterioles, capillaries and venules²⁶ and arteriovenous anastomoses (AVAs)²⁷. Arterioles are responsible for maintaining the vascular tonus and, consequently, for control of the pressure gradient between the proximal and distal capillaries²⁸. They promote local blood flow control, according to the tissue's metabolic demand^{29 30}. Blood is distributed from the more deeply lying arteries to the dermis by superficial and deep vascular plexuses.

Figure 2. An overview of the different cutaneous vessels and their characteristics. Image modified with permission from Dr Güllü³¹, *Hyperbaric Oxygen Treatment in Research and Clinical Practice - Mechanisms of Action in Focus*. Diameters from Fritsch et al.²¹

In 1961 Winkelmann and Saunders showed that the apparently separate plexuses are really interconnected by vessels of different sizes at all levels of the dermis. Additionally, parts of the smallest vessels are by-passed with arteriovenous anastomoses (AVA)^{32 33}. AVAs also enable blood to pass directly from arteries to veins and have a marked effect on blood flow and temperature^{34 35}. They are most frequent in the reticular dermis in glabrous skin of hands, toes, nose, ears and buttocks although their presence elsewhere in the skin varies^{35 36}. Opinions on whether they exist in forehead, cheek and sternum differ³⁷. Sympathetic noradrenergic C-fibres with vasoconstrictive effect innervate AVAs and most arterioles and are purported to contribute to the skin's thermoregulatory effect³⁸ although paradoxically, C-fibres also provide cholinergic innervation for the stimulation and vasodilation of AVAs³⁹. The presence of myelinated fibres (A δ) also supplying AVAs suggests that their main afferent innervation are fast conducting fibres, able to rapidly detect changing hemodynamic conditions. AVAs are sensitive to sensory and psychological stimuli⁴⁰. Arteries and arterioles contain smooth muscle cells to allow control of blood flow²². The capillaries contain pericytes⁴¹ and these cells have different functions in different tissues⁴². This results in a better contractility and mechanical resistance in the cutaneous vessels than e.g. in the more internally protected visceral capillary vessels⁴³. The major purpose of the

cutaneous vascular network is to regulate temperature and blood pressure^{44 45}. Different body tissues have different vascular resistance contributing to varying organ sensitivity to changes in systemic blood pressure/flow⁴⁶.

The distinction between perfusion (amount of blood delivered to the capillary beds of a block of tissue during a certain time period, in millilitres of blood per 100 g of tissue per minute) and flow (the volume of blood passing per time unit or length of vessel that blood passes per time unit) is important in the understanding of the function of AVAs⁴⁷. The constriction of AVAs diminishes the blood flow to capacitance vessels such as venules and affects the perfusion pressure of the capillaries. Regarding the relevance for tissues of whether capillaries are open or closed, August Krogh showed in elegant experiments 100 years ago, that the distance in the tissues between perfused capillaries was decisive for the oxygen delivery to the tissue and that a lower pO₂-tension meant a lower oxygen delivery along the capillary. With increasing oxygen demand in the tissue more capillaries need to be perfused⁴⁸.

2.3 Vessel Density and Perfusion

The density of vessels differs greatly between body areas, e.g. it is higher in the scalp and upper extremities compared to the torso⁴⁹. The number of capillaries ranges from >650/cm² (in toes), to >2000/cm² (in hands)⁵⁰. The capillary density decreases by 65-75% in older age (60-75 yrs compared to 20-29 yrs)⁵⁰ contributing to the more pale and uneven skin texture of older people. There are few AVAs in arterioles from the temporal and thoracic arteries supplying the skin of the trunk³⁶. The forehead and trunk skin arterioles have a lower vasoconstrictive tonus with different reactions to cold and heat exposure compared to nose and lip^{51 52} contributing to the higher temperature stability of the forehead skin⁵³. During vasodilation the cutaneous perfusion is much higher in fingers, palms, face, ears than in e.g. torso or lower extremities⁵⁴. Of the cardiac output, a variable 5 to 70% is distributed to the vasculature of the skin^{55 56}, which emphasizes the importance of skin vasculature to overall hemodynamics^{57 58}.

*Table 1. Summary of capillary and AVA density in skin at different anatomical sites^{35 36 50 59-61}.
Capillary density in neonates⁵⁹.

Skin site	Capillaries/cm ²	AVAs/Cm ²
Forehead	>900	
Sternum	1130*	
Forearm	>1300	
Hand	>2000	
Finger pulp		>200
Nailfold	700-1000	>600
Toe	>650	

Table 2. Average diameter, cross-sectional area and blood flow velocity for different vessel types at rest^{62 63}.

Vessel type	Diameter (mm)	Cross sectional Area (cm²)	Velocity (mm/s)
Aorta	20-30	3-5	400
Small arteries	0.1-10	20	40
Arterioles	0.01-0.1	40	20
Capillaries	0.004-0.01	2500-6000	0.1
Venules	0.01-0.1	250	3.3
Small veins	0.1-20	80	10
Vena Cava	20-35	14	150

2.4 Capillaries – Morphology and Function

Capillaries originate from arterioles and are lined by a single layer of endothelial cells. It is chiefly in the capillaries that the exchange of oxygen and carbon dioxide and nutrients between the intravascular and adjacent tissue²⁸ take place. In resting conditions, it is considered that only 20 to 30% of the capillaries are “open” i.e. actively participating in tissue perfusion^{29 64 65}. In tissue hypoxia, capillary recruitment (“opening”) occurs rapidly due to the loss of vasoconstrictor tone and following local relaxation of the capillaries under the influence of the reduced blood flow, local reflexes and endothelial mediators (e.g. nitric oxide) with vasodilating effect⁴³. This recruitment enables the maintenance of a dynamic environment for gas exchange and supplies nutrients to the tissue^{66 67}. Venules play an important role, due to their degree of distensibility and high capacitance, in storage and mobilization of large amounts of blood^{68 69}. Skin blood perfusion shows spatial heterogeneity, of which more than 70% arises from the capillary network rather than from venules and the arterioles⁷⁰. Thus, Red Blood Cell (RBC) perfusion continuously redistributes among capillaries. Haemoglobin is the strongest chromophore in the RBCs and provides the tissue with oxygen by diffusion from arterioles and capillaries⁷¹.

Functional capillary density can be defined as the length of red blood cell-perfused capillaries/cm² and can be used as an indicator of the quality of tissue perfusion. Actual blood flow in the microcirculation depends to a large extent on blood viscosity⁷⁰. Thus, blood pressure and blood rheology are responsible for the amount of movement affecting not only the flow pattern but also the functional capillary density although vascular tone, controlled by the endothelium, can also change regional blood flow dramatically⁷². The skin vasculature contain gender differences; the more peripheral arteries have a significantly larger diameter, blood flow and total blood viscosity in men than in women⁷³.

2.5 Skin Site Temperature and Physiological Differences

Studies show that skin temperature is related to the blood flow of the skin⁷⁴. Skin blood flow plays an important role in thermoregulation processes of the human body as a whole⁵⁷, enabling the maintenance of a relatively constant average body temperature despite changes in the temperature of either body or environment^{75 76}. Some specialised organs have high metabolic rates (e.g. brain, liver, heart, and kidneys) and are the primary heat sources of the body. Less-active tissues behave as short-term heat sinks (e.g. bone, adipose tissue, and skin), particularly during states of altered heat storage⁷⁷. Moreover, even under steady-state

conditions, there is a continuous flow and redistribution of thermal energy throughout the body *via* convective (heat transfer by the movement of molecules within a fluid) and conductive (movement of heat through a substance – movements of molecules colliding) mechanisms, before the excess is dissipated to the environment through radiation of heat from the skin. The skin's ability to regulate body temperature through dissipation of excess thermal energy may vary with external factors such as wind chill, sun radiation, changes in ambient temperature and humidity, as well as with internal mechanisms such as sweating and changes in local skin blood flow. Body core temperature is therefore not static or uniform but varies depending on which inner organ is measured⁷⁷, with the brain being hottest⁷⁸. Here, forehead skin, as a site for qCR, deserves a special consideration. Through convective and conductive heat transfer, forehead temperature is 'affected' by the temperature of the underlying metabolically active brain, as the effective heat transfer coefficient for the forehead is higher than for the rest of the body⁷⁹. As shown by previous studies, forehead skin temperature ($34.7 \pm 0.6^\circ\text{C}$)⁸⁰ and rectal temperature⁸¹ are less variable than sternum ($32\text{--}34^\circ\text{C}$)⁸² and finger skin temperature ($22\text{--}35^\circ\text{C}$)⁸³.

3 Non-invasive Measures of Skin Microcirculation

There are several available techniques to measure circulation in skin vasculature. Both morphological and functional studies are possible. Morphological parameters measurable with imaging methods include vessel density, proportion of perfused vessels and vessel diameter in addition to dynamic measurements of vascular blood velocity and RBC concentration⁸⁴. Functional studies, especially targeting microvascular endothelial function, include pharmacological tests with vasoactive drugs and post-occlusive reactive hyperaemia test^{85 86}. Further, microvascular evaluation can be performed with both single-point measurements and imaging methods, giving a full-field image of the microcirculation at a tissue surface. The use of imaging methods is preferable due to the known spatial heterogeneity of perfusion in microvascular beds. Imaging methods measure larger areas as compared to methods with single-point measurements which gives a better reproducibility⁸⁷. Microcirculation can be measured intermittently or continuously, depending on the temporal resolution of the technique used. Continuous measurement is preferred, especially in studies where changes appear within a short time span, e.g. after the release of blanching pressure during the CR test. A disadvantage of continuous measurements is that they often require larger data storage capacity.

There have been several techniques developed for measuring skin blood flow of which perhaps photoplethysmography has become most known, due to its use in pulse oximeters since the 1970s⁸⁸. Another early method of measuring microcirculation in skin was Laser Doppler Flowmetry (LDF) emerging in the 1970s⁸⁹. In the 1980s, Laser Doppler Perfusion Imaging (LDPI), a development of LDF became the gold standard of blood perfusion measurement in experimental settings for many years. Recent advances in technologies as well as increased computational capacity have brought a broader spectrum of measurement technologies, such as various laser based methods and polarised visible light reflectance spectroscopy in various variants with increasing temporal capacity of data collation⁹⁰. In this thesis we have used polarisation spectroscopy imaging (PSI) to capture and quantify the CR process, because a higher temporal resolution is required to record the rapid increase in RBC concentration during the reperfusion phase.

3.1 Polarisation Spectroscopy Imaging

The progress of this research has been in close parallel to the development of a measurement technology called Tissue Viability Imaging (TiVi) based on PSI⁹¹. The TiVi system consists of a camera equipped with an external ring of Light Emitting Diodes (LEDs) and linear polarizing filters perpendicularly placed in front of the LEDs and camera detector, respectively. The LEDs emit a broad-spectrum white light that, upon passing the first filter in front of the LEDs becomes linearly polarised. When this broad-spectrum light reaches the surface of the skin, a part of it is directly reflected while the remaining light penetrates the tissue. The light directly reflected, retains its original polarisation state and thus cannot pass the second filter placed in front of the detector. A larger part of the light, however, continues into the tissue. This “sub-surface” light successively becomes randomly scattered and during its re-emission to the surface, a part of it has become depolarised and can pass the second polarisation filter located in front of the camera lens. The TiVi system is capable of both single photo and continuous measurements. In the continuous mode the system can capture videos with a frame rate of up to 50 frames/second.

Figure 3.1

Quantification of the red blood cell concentration in the cutaneous microcirculation by use of the TiVi system. Broad spectrum, visible light from the light emitting diodes (LEDs) is linearly polarized (LP) by a filter (1). A proportion of the polarized light is directly reflected from the surface of the skin and retains its polarisation. This polarised light reflected in the skin surface is inhibited by the second filter (2) in front of the lens to reach the detector array. A part of the light penetrates the tissue and is randomly scattered. When re-emitted through the skin surface it has become randomly polarised (RP) and some of it can pass through the second filter. The images created by this light is analysed using a specific software algorithm for quantification of spatial and/or temporal variations in red blood cell concentration in either the full image or in up to 12 specific regions of interest (ROI).

The video sequences are separated into individual frames by a built-in feature of the software. With a frame rate of 50 frames per second, the temporal resolution in the generated reperfusion curves becomes 20 milliseconds. A Region of Interest (ROI) within the blanched area is selected for every series of images recorded during the CR process. The microcirculation contains RBCs prone to absorb light in the green wave-length region (500-600 nm) to a much higher extent than light in the red wave-length region (600-700 nm). The surrounding tissue components of the dermis, in comparison, absorb green and red light to approximately the same amount. The TiVi-technique utilizes this difference in absorption by separating the images into their three different colour coded planes of red, green and blue. A mathematical calculation on each photograph is then performed with an algorithm that subtracts the value of each picture element in the green colour matrix from the colour in the red colour matrix. The result is divided by a signal proportional to the total light intensity within the actual wave-length region. The obtained values (one for each pixel) for the resulting matrix are referred to as TiVi values and are reported in arbitrary units (a.u.). The TiVi value is linearly proportional to the momentary concentration of red blood cells in the actual illuminated tissue volume⁹¹⁹². A limitation of the technique is that it measures in arbitrary units but to its advantage this

technique is relatively insensitive to movements, ambient temperature and light, as well as images partly out of focus since the measurements are based on the RBC concentration in skin^{91 92}. TiVi does not need calibration since it does not have components which change over time⁹².

4 Alternative Skin Imaging Techniques

4.1 Laser Techniques for Measuring Tissue Perfusion

Change in blood perfusion and blood flow has often been measured with laser-based techniques^{93 94} mainly Laser Doppler Flowmetry⁹⁵.

Laser Doppler Flowmetry (LDF) uses the Doppler effect to estimate the velocity of erythrocytes in the skin vasculature⁹⁶. When a narrow and monochromatic light beam illuminates a tissue surface, photons penetrate the tissue to a depth determined by its optical properties. In the presence of moving blood cells, the incident monochromatic light becomes Doppler-shifted to a degree determined by the concentration and velocity of the moving RBCs. A fraction of the back-scattered and doppler-broadened light is received by a photodetector and converted to an electrical signal proportionate to the doppler shift⁹⁵. Clinical use of this technique is limited by the fact that the measurements are given in arbitrary units (a.u.) or relative units; unknown measurement volumes and non-linearity at increased blood tissue fractions^{97 98}. Consistency is secured by calibrating the device. In general, a near infrared wavelength is used since this has better properties for skin penetration and is more specific for erythrocytes than other moving components (e.g. proteins or water)⁹⁸. Laser Doppler Flowmetry measures a small volume of about 1 mm³ is sensitive for movements and requires direct skin contact. This increases the risk of sampling error and has thus not been ideal for clinical measurements.

Laser Doppler Perfusion Imaging (LDPI) uses LDF, but rather than measuring a single-point it combines multiple single measurements over a tissue surface to create an image of microcirculation of the tissue^{96 99}. The standard LDPI apparatus uses a movable mirror that directs the laser beam onto the different measurement points, capable of scanning tissue surfaces of an area up to 50×50 cm⁹⁸. The time to acquire an image with modern equipment is 2-10 seconds⁹⁰.

Multiexposure Laser Speckle Contrast Imaging (MELSCI) is an emerging, alternative, laser-based method of measuring skin perfusion and possibly blood flow. It uses the technique of laser speckle contrast imaging for video rate imaging of blood flow combining it with different exposure times to increase both temporal and spatial resolution¹⁰⁰. It is a non-contact equipment and measures over full field areas (30×30 cm)¹⁰⁰ enabling the study of microcirculatory heterogeneity in critically ill patients. The technique, however, is very sensitive to movements such as e.g. shivering.

4.2 Broad Spectrum Optical Techniques of Measuring Tissue Perfusion

Hyperspectral Imaging (HSI) is a technique that was originally developed to make an identification of surface materials (minerals, vegetation) on the Earth, in the form of images by remote sensing (e.g. from airplanes)¹⁰¹. HSI analyses a wide spectrum of light instead of just assigning primary colours (red, green, blue) to each pixel as in the PSI technique. The light impinging on each pixel is broken down into many different spectral bands providing more information on what is imaged. In HSI, the unique colour signature of an individual object can be detected. Unlike other optical technologies that can only scan for a single colour, HSI is able to distinguish the full colour spectrum in each pixel. Therefore, it provides spectral information in addition to 2D spatial images and can identify more substances than haemoglobin, such as water or specific proteins in a living tissue¹⁰². HSI, like PSI is not particularly sensitive to

movement, can image large areas and give a detailed analysis of tissue composition but is still under development for human research.

Sidestream Darkfield (SDF) Imaging, a development of the optical spectroscopy technique, was described first in 2004 and has been used in several studies of alteration in microcirculation of intensive care unit patients¹⁰³. It uses light-emitting diodes (LEDs) placed around the tip of the light guide with a centre core optically isolated from the outer ring. When the light guide is placed on tissue surfaces, the light from the outer ring penetrates the tissue, illuminating the microvasculature from the interior. The dark-field illumination thus avoids reflections from the tissue surface and yields a clear image of microcirculatory components, with both flowing red and white blood cells¹⁰⁴. This technique visualizes the morphology of the vessels and shows which vessels are perfused. It can only semi quantify the amount of heterogeneity in perfusion of the vessels and requires direct contact with tissue⁸⁴.

5 Aims of the Thesis

The general aim was to develop and compare quantified measures of CR time using the TiVi-technology, based on polarisation spectroscopy imaging (PSI), with naked-eye derived CR estimates and thus lay a foundation for possible determination of the relevance of qCR time in patient triage and monitoring.

The specific aims for the papers included in this thesis were:

- I. To derive quantitative estimates of the CR time using PSI and to compare qCR times at different commonly used anatomical sites.
- II. To investigate/substantiate the inter-observer variability of naked eye CR time estimation and whether it differs from qCR time.
- III. To describe the effect of low ambient temperature on qCR time in forehead, sternum and finger pulp.
- IV. To investigate the demands on equipment when measuring qCR time in patients in an emergency department setting rather than on healthy volunteers in a laboratory setting.

6 Methods

6.1 Subjects

Twenty-three (paper I) and 15 (paper III) healthy volunteers (see demographic data Table 6.1) were recruited for qCR measurements in a laboratory environment. Most subjects were free from medications (except for oral contraceptives 4, antidepressant 1, levothyroxine 1 and β -blocker 1 in paper I) and all subjects were without skin disease or disturbing tattoos. All volunteers participated after having given informed consent. All volunteers refrained from drinking coffee or tea, use of tobacco or exercise at least 6 hours prior to experiments. For paper II 48 staff volunteers of different professions (14 physicians, 15 nurses and 19 secretaries) were recruited at the Department of Emergency Medicine, Linköping University Hospital, Sweden after informed consent to watch and estimate the CR time from previously collected qCR video recordings. Video recordings of the qCR process shown to the ED staff were based on material from study I. For paper IV 11 patients were recruited at the Department of Emergency Medicine, Linköping University Hospital, Sweden, and 10 generated data that could be evaluated.

Table 6.1 Demographic data for the subjects that participated in the studies.

Paper	Participant	N	Mean age	Men	Women
I	Healthy volunteers	23	36	9	14
II	ED Staff	48	N/A	12	36
III	Healthy volunteers	15	38	9	6
IV	Patients	10	62	8	2

6.2 Equipment

PSI using a commercial TiVi system (TiVi 600 study I and TiVi 700 study III and IV, WheelsBridge AB, Linköping, Sweden) was utilized to quantify the qCR process in terms of RBC concentration changes over time. The software used to analyse data from the images was the TiVi700 Tissue Viability Analyzer from WheelsBridge AB, Linköping, Sweden.

The camera was placed 15 to 25 cm above the skin on which a measurement area was marked with 4 black dots (see Figure 3.1). The image size was set to a resolution of 1920x1080 and 1280x720 pixels, in each camera respectively (Canon EOS 550D used in paper I, Canon EOS 750D used in paper II, III and IV). The camera was controlled remotely from a laptop and the video recordings were stored on the computer's hard drive. Skin temperatures were measured using a Fluke 572 Infrared Thermometer (Fluke Corporation 2012, Germany) in all papers and VS were measured before each qCR procedure started.

Slightly different measurement setups were used for different papers. The recording rate in paper I was 25 frames/second whereas it was 50 frames/second in paper III and IV. Recording duration of the qCR process was 25 seconds in paper I and 20 seconds in paper III and IV.

6.3 Laboratory and Clinical Settings

All measurements in paper I were done by the first author, in paper III all baseline measurements (in room temperature) were done by the first author and in paper IV a third of the measurements were done by the first author the rest being done by research nurses. In paper I and III the qCR measurements were done in a laboratory with controlled room

temperature ($23\pm1^{\circ}\text{C}$), dimmed ambient light during the investigations involving polarisation spectroscopy measurements to avoid possible interference. Forehead and sternum were used throughout all investigations and in paper III also the finger pulp. The volar forearm and finger dorsum was only used in paper I. The healthy volunteers rested in a supine position for at least 20 minutes before the start of the video recordings of the CR process.

In paper IV the time interval for qCR recordings at the ED was performed with consideration to the patient's condition and the clinical need for intervention and most often in a half-supine position. Patients could be, due to their illness, sweating, shivering and tired which influenced the possibilities to follow the protocol. On average, qCR measures were taken every 10 to 30 minutes. Number of qCR measurements depended on the length of stay at the ED and on the research staff's working hours. Regular data on VS were collected according to clinical routine using standard monitoring equipment (IntelliVue MX450 Patient Monitor, Philips, Boeblingen, Germany). Core body temperature was measured with one of two different tympanic ear thermometers commonly used in the ED; Covidien Genius 2 (Covidien, Ireland) and Braun Thermoscan® PRO 6000 (Welch Allyn, Ireland). Blood samples were taken based on symptoms and suspected diagnose, according to clinical routine. Ancillary documentation on subjects was collated after the clinical course.

6.4 Inter- and Intra-Observer Variability

In paper II, three different observer groups, according to profession, watched a film with 18 video recordings of CR tests. The groups consisted of 14 ED physicians, 15 ED nurses and 19 ED secretaries, the last group representing laymen. Each observer received an oral presentation of the procedure as well as a form to fill in continuously with the time observations for each film. Three videos were shown twice without the knowledge of the observers. Observers had five seconds to deliver their estimates after viewing each video. No information was given to the doctors or nurses about guidelines and reference values of CR time prior to the assessment. Secretaries were assumed to be less familiar with the CR test, and were instructed to estimate time in seconds to when the colour of the blanched area had returned to the same colour as before the applied pressure and to give their categorical estimation of fast, normal and slow without information about our cut-off limits. All observers were shown two CR test videos as practice prior to the tests included in the analysis – questions were allowed when seeing the test videos. No questions concerning time estimates during and after the film was shown were answered. The observers were watching the film in groups of 2-4 on a wide screen monitor. The same room, lightning and monitor was used in all screenings. Each filled out their own paper under silence.

6.5 Blanching Pressure – Standardization

Normal capillary pressure ranges from 10-35 mmHg equivalent to 0.133-0.293 Newton/cm² in men and women under 50 years^{28 105 106}. Previously used pressure devices have used 4-7 N/cm² on the fingers showing increasing CR time with increased force but not with increased pressure time¹⁰⁷⁻¹⁰⁹. One study, measuring investigators' pressure on force sensors with a finger showed that in the human compression of skin, the values ranged from at least 4 to 4.6 N¹⁰⁹. Capillary pressure increases with age but interstitial tissue density as well as capillary density decreases^{110 111}. Hypertension and diabetes type 1 increase capillary pressure but not density compared to normal subjects¹¹²⁻¹¹⁴. In paper III a curved custom-made mechanical pneumatic "finger" with a surface area estimated to roughly 1 cm² and a pressure of

ca 9 N/cm² was used to achieve standardized blanching pressure. The pressure and area depended on the tissue due to the curved form of the pneumatic “finger”. In paper IV 5 Newton/cm² as standard pressure was used and applied using a dynamometer with flat tip. The pressures chosen was based on above mentioned factors affecting capillary pressure, subjective testing of finger size and force applied among the staff within the laboratory and that it was found to be not too uncomfortable for the patients¹⁰⁹.

6.6 Data Analysis

The qCR data in paper I, III and IV was analysed in a similar way. The qCR procedure was filmed with the TiVi system creating videos that could be watched in video mode or in TiVi mode (the colour coded version visualising the blanched area more clearly). The video sequences were then separated into individual frames. A ROI within the blanched area on the subject’s skin was selected in the first image of every series of images for all subjects. Each pixel within the ROI generates a TiVi value that correlates to the change of red blood cell concentration during the CR procedure (Figure 3.1). Mean TiVi value data for each ROI were generated by use of the built-in analysis feature of the software and exported to Microsoft Excel and GraphPad Prism for further calculations and analysis. The mean TiVi values from the individual ROIs (Figure 3.1) were displayed as points in the curve to assess the RBC concentration change in the responses (Figure 6.6). The shape of the TiVi value curve illustrates the main characteristics of the qCR response (Figure 6.6). From the continuous data, TiVi generates four data endpoint values (y-axis) of relevance for the qCR test as presently used: the baseline values (A) prior to the application of pressure; the lowest RBC registration (t=0 or “blood zero”) at the time point immediately after the blanching manoeuvre (B), the point where the curve crosses the baseline (C) and the peak value in the hyperaemic phase (D). Thereafter time variables in (milli)seconds can be determined (x-axis). After release of pressure (B), (t=0 s) the RBC concentration returns rapidly to the baseline level (C), tRtB1 (t=0.46 s). The time to peak value (D) is termed t_{pk} (t=1.2 s). After the hyperaemic phase, the RBC concentration returns to baseline more slowly. Using this analysis process meant, however, that no tRtB1 could be generated in curves where the baseline values were higher, due to movement artefacts or inadvertent polarisation setting changes, than the peak value after pressure release despite the fact that there was a capillary refill process.

Figure 6.6

The curve of the quantified capillary refill (qCR) from forehead during the measurement of 5 s baseline, 5 s pressure and 10 s of recording after pressure release. Time to Return to Baseline (tRtB1) is proposed to be closest to the naked eye CR time measurement. TiVi-value on the y-axis correlates to the RBC concentration in skin microvasculature. Below the curve are photos and corresponding colour coded images from three different stages of the qCR process (A = Baseline, B = Blanching and D = Time to Peak t_{pk} - Hyperaemia). The region of interest (ROI) is placed within the blanched area. The serrated pattern of the curve is most likely due to pulsations correlating to heart frequency.

In paper IV 109 measurements (video recordings) were made and analysed to extract tRtB1 as a measure of the technique's applicability in a clinical setting. In 20% of the measurements no tRtB1 could be determined. These video recordings and corresponding frames (~21000) were visually inspected to retrieve and categorize the causes of data loss.

Common causes were heterogenous blanching, inadvertent lack of polarisation, movement artefacts (the entire ROI moving out of image during recording), blankets and thermometers obscuring parts of the measured area, and error in transfer of data from camera to computer. These causes were divided into three categories; *Equipment* – data loss due to hardware or software problems, *Patient* – patients unable to remain still during measurement (with the exemption of shivering and shaking) and *Handling error* – inadvertent change of camera settings, polarization or routine management of patient interferes with data collection during video recording.

7 Statistical Analysis (Paper I-IV)

All statistical analyses were made with the aid of GraphPad Prism versions 4.0, 5.0, 6.0 and 8.0 for Windows (GraphPad Software, San Diego, California USA, (www.graphpad.com)), Excel 2010, Microsoft® Excel® for Office MSO365 Version 2001 (Microsoft, Redmond Washington USA, www.microsoft.com).

In paper I, Student's t-test was used to evaluate differences in tRtB1 at group level between men and women. One-way analysis of variance (ANOVA) test was used to evaluate differences in tRtB1 between sites.

In paper II, Kruskal-Wallis test was conducted to compare naked-eye CR time estimations and tRtB1/t_{pk} values. We assumed tRtB1 and t_{pk} to have been conducted an equal number of times to the number of naked-eye time estimates for each profession. The agreement of *categorical* assessments made by the observers (fast, normal or slow) was analysed using Cohen's Kappa (0.27, considered as "poor"¹¹⁵) and compared to the classifications based on tRtB1.

Differences between professions in naked-eye CR *time* estimation was tested with ANOVA. To calculate interobserver agreement, a modification of the Bland-Altman plot¹¹⁶, multiple observer Bland-Altman plot, was used¹¹⁷. This method retains the capacity to evaluate consistency of agreement over different magnitudes of continuous measurements using a single plot. The limits of agreement with the mean represent how different an individual observer estimate compares with the mean measurement of all observers. The differences between each observer and the overall mean for each of the 18 videos were calculated according to the profession of a given participant. Systematic differences between observers were investigated using ANOVA, calculating mean square residuals by profession prior to constructing the plot.

In paper III, time series data were analysed using the repeated measures ANOVA. Each ANOVA model was built using "time" as a within-subject effect, "temperature" as an independent factor, and "time * temperature" as the interacting term. Dependent factor (outcome) was tRtB1. A significant "time * temperature" effect was interpreted as a significant effect of cold exposure on tRtB1 over time. One-way ANOVA and Tukey's post-hoc tests were used to discern differences between sites concerning tRtB1 and temperature. tRtB1 data from the sternum and forehead were analysed by Student's t-test.

In paper IV, scatterplots of the repeated tRtB1 measurements in each of the 10 patients were made. Data losses or suspected outliers were evaluated by the author through manual inspection of the qCR video recordings and the corresponding frames, looking for lack of focus, involuntary reflections, movement artefacts etc. The derived qCR curves were also inspected in these cases to find baseline shift or curve shift leading to data loss or inadequate calculations of tRtB1.

8 Ethical approvals and Ethical Considerations

All studies (paper I-IV) were performed after approval by the Regional Ethics Committee for Human Research at Linköping University (reference numbers M200-07 and 2015-99-31).

All subjects that participated in papers I, II, III and IV gave signed informed consent. The subjects in paper I-III were recruited by convenience and had no health care relationship to the researchers. The patients in paper IV had another physician taking the main responsibility for the medical care while the author of this thesis performed the study. No change in treatments of the patients were done due to the trial measurements. The subjects and patients volunteered and were informed that they could exit the study at any moment without any explanation required. No financial compensation was given to volunteers or patients.

9 Review of the Studies (Paper I-IV)

9.1 Paper I

The aim of the study was to determine and assess qCR at 5 different anatomical skin sites. The purpose was to define the temporal changes in RBC concentration during the CR test. qCR curves of different shapes were delineated for forehead, sternum, volar forearm, dorsum of the finger and finger pulp in 23 healthy subjects (9 men). Prior to the main study, the complete time to return of RBC concentration to baseline values after peak hyperemic response, termed time to return to baseline 2 or t_{RtB2} (not shown in Figure 6.6), was determined in 5 subjects (age range 25-64). The t_{RtB2} ranged from 35 to 240 seconds depending on site. In the main study measurements were set to 25 seconds for feasibility reasons considering the future clinical time requirements. t_{RtB1} and t_{pk} were derived from the qCR curves and considered possible equivalences to the naked eye derived CR time. Skin temperature was measured at each skin site and showed increased variability at peripheral sites compared to central sites. t_{RtB1} similarly varied more in peripheral than central sites whereas t_{pk} demonstrated large variability also at central sites, see Figure 9.1.

Conclusions: Quantification of CR time yielded qCR curves where t_{RtB1} and t_{pk} could be possible equivalents of the naked-eye assessed CR time. The variability in t_{RtB1} was smaller at central sites (forehead and sternum) compared to peripheral sites (finger pulp) probably correlating with skin temperature.

Figure 9.1
Individual variability in (A) t_{RtB1} and (B) t_{pk} values by gender at the 5 tested skin sites. Error bars show mean values and SD for the groups, $n=23$ (9 men and 14 women).

9.2 Paper II

The aims of this study were to compare naked-eye assessment with $tRtB1$ and t_{pk} to measure the intra-observer repeatability and interobserver agreement in estimations of CR time among medical and non-medical staff and to investigate the agreement between 'man' and 'machine' in categorical estimations of the CR response. The purpose was to demonstrate the necessity of quantification of the CR when discussing the role of CR time in the assessment of patients. Forty-eight observers (14 doctors, 15 nurses and 19 secretaries) observed 18 video recordings of the CR of 15 unique subjects. Three videos, CR of three subjects, were shown twice without the knowledge of the observers to investigate intra-observer variability. A significant difference between naked-eye assessment and qCR time in terms of $tRtB1$ was observed. We could not discern any difference in precision in naked-eye estimates between laymen (secretaries) and professionals (physicians and nurses). Naked-eye estimates were closer to $tRtB1$ than t_{pk} . There was a low intra-observer repeatability (ranging from 6 to 60% identical estimations) and poor interobserver agreement (the 95% limits of agreement of the mean ranged between ± 1.98 s for doctors, ± 1.6 s for nurses and ± 1.75 s for secretaries) by clinical staff in their naked-eye assessment of CR time. Categorical naked-eye estimates (fast, normal or slow) did not improve intra-observer repeatability or interobserver agreement (Cohen's Kappa 0.27, corresponding to a low agreement).

Conclusions: Naked-eye assessed CR time demonstrates poor reproducibility, even by the same observers and differs from objective measures of CR time. This emphasizes the necessity of measuring CR with an objective method rather than by naked-eye assessment.

Figure 9.2. Box plots of the naked-eye estimation of CR time in seconds, shown by profession (A-C), including the 5th to 95th percentile of the values. qCR time in tRtB1 (red circle) and t_{pk} (green square) are shown for each video. The fastest tRtB1 values are plotted to the left and the slowest to the right on the x- axis. The number of each video indicates the order in which they were shown to the observers in the film. Videos 5 and 10, 6 and 13, and 3 and 15 (marked with arrows) are, identical videos that were shown twice. CR, capillary refill; qCR, quantified CR; t_{pk} , time to peak; tRtB1, time to Return to Baseline 1.

9.3 Paper III

The aim of the study was to investigate the effect of low ambient temperature on skin temperature and qCR in terms of tRtB1 in forehead, sternum and finger pulp. The finger pulp showed a conspicuous temperature fall and prolonged qCR times (tRtB1 >10 seconds) see Figure 9.3. The qCR curves of the forehead and sternum were more comparable to control measurements at room temperature, and skin temperature falls were less marked. tRtB1 was not prolonged in forehead measurements. At the sternum, some individuals showed tRtB1 beyond naked-eye guideline recommendations despite only a small skin temperature decrease.

Conclusion: Low ambient temperature is a strong independent factor for prolonged tRtB1 at peripheral sites. Caution regarding sternum as a “central” site of measurement is needed since cold provocation produced prolonged tRtB1 in some individuals. Forehead appeared as the most thermostable concerning skin temperature and was thus proposed as the preferred site to avoid ambient temperature artefacts when measuring qCR.

Figure 9.3
qCR expressed in terms of Time to Return to Baseline 1 (tRtB1) at different skin sites in room temperature and at different exposure times in a cold room (8 °C). In finger the curve did not return to baseline within the entire post-pressure recording period of 10 seconds (7 of 15 subjects). Instead, the tRtB1 was plotted as 10 seconds for these subjects. Squares represent men and filled circles represent women. Error bars show mean and SD.

9.4 Paper IV (Manuscript)

The aims of this study were to assess the functionality of laboratory prototypic equipment for measuring qCR and to elucidate demands on the design and handling of equipment when applied on patients in an ED environment.

In a convenience sample of 10 patients triaged as “urgent” qCR was measured repeatedly during 45 - 180 minutes in parallel with standard VS monitoring as determined by the patient’s condition. Only two women were included in this convenience sample of patients. tRtB1 could be derived in 80% of 109 qCR measurements. Causes of data loss were classified as equipment-related (8%), patient associated (9%) or due to handling error (7%). We identified several areas of improvement to achieve better equipment (e.g. attachable with predetermined settings) and protocols (e.g. start measuring before intervention in ambulance). The small number of patients precluded conclusions about any relationship between tRtB1 and patient outcomes, but some general observations were noted. tRtB1 longer than guideline values (>3 seconds) in forehead were not seen in any measurement. Comparison to our own reference values from healthy subjects in paper I more often showed prolonged times in the ED patients as compared to international guideline values (see example in Figure 9.4).

Conclusion: CR data can be measured and potentially integrated with other patient data (VS). More robust, easy-to-use equipment and technique is necessary to retrieve reliable data for future validation of the possible relevance of qCR in patient triage and monitoring. The results imply the need for more refined (e.g. skin site, gender and age adjusted) quantified reference values.

Figure 9.4

Each patient's Time to Return to Baseline (tRtB1) value from forehead (squares) and sternum (circles), plotted with reference values based on naked eye derived guidelines for normal capillary refill time (solid horizontal line) and our own material of mean tRtB1 measured on healthy females and males (dotted horizontal lines). In forehead the reference tRtB1 was the same for men and women.

10 Discussion

10.1 Main Findings

The main findings of this thesis are that PSI can be used to quantify the CR procedure at a correct and much more detailed level than naked-eye estimates and that the qCR time differs from standard naked-eye estimation, as well as between sites and differing ambient conditions such as temperature.

The finding that naked-eye estimates of CR time are unreliable and not reproducible within and between observers is important since the results of most scientific reports concerning CR time are based on naked-eye estimates. Our findings indicate that such reports should be cautiously interpreted. Only by quantification of CR time and standardization of the qCR procedure may we define what normal reference values would be in a standard population. There is a current increase in the interest for modern techniques to quantify microcirculation and to correlate such readouts with clinical outcome in patients⁸⁴. However, very few studies have been conducted with methods of quantifying CR time¹¹⁸. A large recent study in patients with septic shock admitted to the ICU, on a resuscitation strategy targeting normalization of CR time used naked-eye measurements timed with a chronometer and compared to a strategy targeting serum lactate levels. CR time guided treatment did not show reduced mortality and only barely significantly less organ dysfunction was observed at 72 hours¹⁷. The study was performed on fingers where abnormal CR time was considered to be >3 seconds. The study did not take into consideration skin temperature and shows, in patients with septic shock, a clearly smaller range (2-6 s in initial measurements) of CR time as compared to our qCR time in finger pulp in healthy subjects (men 1.7 ± 3 s and women 2.2 ± 2.1 s with a range of 1-10 seconds). The microvasculature of finger pulp contains many AVAs possibly leading to prolonged CR times in patients resuscitated with vasopressors used such as norepinephrine. The low intra and inter-observer reliability are additional explanations to a poor or absent significance in outcome measures concerning mortality and organ dysfunction.

The clinical implementation of qCR requires modifications of the equipment to render it easier to use and to reduce data loss. To evaluate the role of qCR to guide treatment, qCR measures will also have to be readily available for bedside interpretation. The role of qCR time as an additional VS in assessment of the potentially critically ill thus still remains to be evaluated. The many physiological parameters affecting this seemingly simple manoeuvre also need to be further elucidated. The collation of different data (demographic, physiological) will require more advanced statistics for the interpretation and should be one of the aims for continuing studies.

10.2 Factors such as Site and Temperature Influencing the qCR Curve

We showed that skin site and skin temperature are strong influencing factors on tRtB1 as peripheral sites demonstrated high qCR variability compared to more central sites. The skin's microvasculature differs considerably between sites concerning capillary density and is related to health/disease and age^{50 60 114 119}. The innervation, morphology and structure of the vasculature, and thus microcirculation, differs as well between sites¹²⁰. Skin areas containing more AVAs are more prone to react on sympathetic adrenergic stimulation regardless of cause (psychological and physical stress) affecting the qCR time. The forehead showed markedly less variability in temperature and qCR supposedly due to less AVAs⁵⁸. The forehead skin

demonstrates less responsivity to the local cutaneous sympathetic vasoconstrictive system but more sensitivity to the sympathetic cholinergic vasodilative system. The vasoconstriction of the forehead seems more correlated to blood pressure (BP) reductions as shown in studies on facial pallor during syncope¹²¹. Thus, measuring qCR on the forehead might be less sensitive but more specific to clinically relevant changes in hemodynamics.

Single measurements of qCR time are probably less conclusive than repeated measurements, e.g. in a monitoring situation. Additionally, there are very few studies on the effect on CR time after repeated measurements with blanching pressure in the same skin site¹²². We observed an increasing baseline redness in the ROI in subjects with sensitive, fair skin upon repeated qCR time measurements. Increased baseline redness could possibly influence the qCR time.¹²⁰ The future determination of “normal” qCR values needs to be more differentiated since capillary density and perfusion depends on sex, age⁴, hormones, as well as rheological properties of the blood, especially in disease¹²³. Further, the effect of different medications on vascular reactivity and rheology should be considered^{123 124}.

10.3 Inter- and Intra-Observer Reproducibility

The low intra/interobserver reproducibility and agreement of CR procedure – with similar performance for clinicians and laymen – demonstrated in paper II give reason to question studies claiming correlation between severity of illness and naked-eye assessed CR. However, several studies claim a quite good co-variation between CR time and other physiological parameters such as e.g. lactate or skin blood flow^{17 125}. Our set up showing a film with video recordings of the CR procedure with only a fingertip in focus, reduced the clinical evaluation to a time estimate in seconds or a categorical value. No global assessment of the subject’s general colour, appearance or movement pattern could unconsciously be included in the evaluation such as in studies of “eye-balling”¹²⁶, which could possibly influence subjective time estimates.

10.4 Demands on Equipment

The step from laboratory to real-life, clinical research is large, as shown in paper IV. Many artefacts causing data losses could have been avoided with more robust, user-friendly equipment to facilitate handling. Having research nurses with long clinical experience in the ED gave a lot of input for improvement. During resuscitation multiple interventions and measurements need to be done, often simultaneously which places demands on both equipment and protocols. These aspects need to be considered as use of qCR is developed.

10.5 Data Retrieval of RBC Concentration in Skin

The aim of this thesis was primarily to develop and compare quantified measures of CR *time* using PSI-technique. The PSI-technique, however assembles large amounts of data concerning RBC concentration in skin in addition to the temporal aspects of qCR. Optimal collation and use of the complete PSI-data (e.g. qCR time, RBC concentration in unprovoked skin, variations of RBC within ROI), needs to be considered. qCR time reflects the microcirculatory behaviour and skin reactivity upon mild provocation (blanching pressure for 5 seconds). Since critically ill patients of various aetiology demonstrate microcirculatory alterations^{127 128}, changes in unprovoked skin after treatment (such as Ringer’s or oxygen-treatment) as seen with PSI might be of additional interest. One could combine the qCR time results of repeated measurements during treatment of critically ill patients with images reflecting the

heterogeneity of unprovoked skin in the same area (baseline measurements before and during/after ED treatment). The many parameters measured in parallel with the qCR procedure in clinical routine could be analysed using multivariate analysis to possibly discern which factors are most influential on the qCR time.

11 Limitations

PSI as measured with the TiVi-system has several limitations. Some of these limitations are technical and relate to the prototypic status of our hardware but there are also limitations in our study design which need to be considered when evaluating our results.

11.1 Limitations of Polarisation Spectroscopy Imaging

PSI, as used here, measures changes in RBC concentration over time and not purely blood perfusion and/or blood flow. If we measured blood flow, we could specify this into SI-units such as ml/min. Since we want to measure the blood perfusion (ml/100g/min) non-invasively, we are looking for indirect surrogate measures, correlating to the true blood perfusion. The TiVi-technique raises some issues:

1. We do not know the exact penetration depth of the light into the tissue due to different characteristics of the tissues in different individuals and sites.
2. Blood flow does not necessarily correlate with perfusion.
3. There are other chromophores that might influence the signal (melanin).

This means that we do not really know what part of the microvasculature we measure: capillaries where perfusion is more central or arterioles/venules where blood flow is relevant. The epidermis and dermis as well as the microvasculature of the skin have different characteristics at different skin sites contributing to this problem. The optical techniques to measure tissue perfusion all depend on the specific characteristics of the tissue, complicating the data analysis and interpretation from tissues of different individuals and sites.

Measuring skin reactivity after provocation could be an approach to eliminate part of these in-built complicating factors of the human constitution since measuring temporal changes in skin would reduce the need of knowledge of exact constituents of the tissue.

Motion artefacts can also be a problem. The technique can handle small motions caused for instance by shivering as well as a slightly blurred image due to the camera objective not focusing optimally. Movements causing larger parts of the ROI to be moved during data collection will give false values depending of what light might be reflected. Movement artefacts are however much less of a problem for TiVi than for LD flowmetry, LDPI and MELSCI.

11.2 Study Related Limitations

Beginning our research on healthy volunteers using convenience samples of co-workers and later focusing on a convenience sample of ED patients resulted in suboptimal age and gender matching. In Sweden most critically ill patients, especially septic patients are elderly. In paper III the skin temperature fell so quickly in the cold room that our initially intended baseline measurement (within 5 min) had to be replaced by a measurement taken outside the cold room on a different occasion. All participants were fair-skinned and classified as Fitzpatrick skin photo type I-III¹²⁹. Very dark phototypes (V and VI) contain more melanin which attenuates the light transmitted to the deeper skin layers and may influence the backscattered light¹³⁰. Although this influences the signal, qCR measures RBC changes in skin rather than absolute RBC which should diminish the problem of light absorption of melanin in differing skin phenotypes.

There are also some data processing limitations in the studies. The ROIs were marked either by the author or one other researcher. A selection bias could therefore affect the results and should be further investigated. This could be done by having two researchers assessing the same image and independently placing their ROIs. With updates on the TiVi-system placing a ROI automatically within the blanched area could be a built-in feature.

12 Future Research

We view qCR as an overall concept that may include different variables of the microcirculation in skin using the CR manoeuvre as a mild skin provocation to demonstrate vascular skin reactivity. We believe that tRtB1 is currently the closest quantified equivalent to the naked-eye estimated CR time but further analysis may show that other time points or mathematical indicators of change derived from the form of the qCR curve are more relevant or sensitive.

Apart from standardizing the qCR manoeuvre, there are different measures of skin microcirculation, not involving skin provocation, which are possible to evaluate with PSI. The increased heterogeneity of blood perfusion in the critically ill is one such area worthy of further study.

The PSI technique can visualise and quantify the distribution of RBC concentration in unprovoked skin as well as spatial changes in larger ROIs over time. PSI can also measure e.g. oxygenated and deoxygenated RBCs in the tissue displaying absolute values as possible outcome measures of a clinical intervention or treatment.

13 Concluding Remarks

In this thesis, we showed that CR time could be quantified using PSI. The conventional naked-eye assessment of CR time has been proven to lack precision and reproducibility within and between observers. Quantified CR time demonstrated a large variability between different skin sites. We identified skin temperature as a specifically important factor influencing qCR time, particularly at the fingertip. The PSI technique was feasible in a clinical setting and, with further improvements, clinical evaluation of qCR in relation to relevant patient outcomes will be possible.

The results of this thesis imply the need for physiological studies to establish more refined reference values correlated to skin site, temperature, age, sex and procedural effect. Easily retrievable qCR may facilitate investigations of the microcirculatory changes in disease and possible redundancy in the vascular system during shock of various aetiologies. Regarding the skin as an organ, we cannot establish the ranking order of organ protection at a whole body level, as related to microvascular changes during hemodynamic instability but given the accessibility, possible sensitivity and non-invasive nature of the CR test, continued accrument of qCR data in a standardized manner is warranted.

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