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**INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR  
UNIVERSIDADE DO PORTO**

**OBJECTIVE ASSESSMENT OF  
ACUPUNCTURE EFFECTS BY  
MICROCIRCULATION-RELATED  
PARAMETERS IN PATIENTS WITH  
PROXIMAL FEMUR FRACTURE**

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Dissertação de Mestrado em Medicina Tradicional Chinesa

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Dissertação de Candidatura ao grau de Mestre em Medicina Tradicional Chinesa submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

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## DEDICATION

*For my parents for being my Anker, and to Nuno for  
all the love and support*





*Traditional Chinese Medicine is a system of sensations and findings  
designed to establish a functional vegetative state.*

Prof. Henry Johannes Greten

*"A doctor that only knows medicine not even knows medicine".*

Dr. Abel Salazar

*"Good it is to fight with determination, embrace life and live with  
passion, lose with class and win with boldness,  
For the triumph belongs to those who dare ....  
And life is too large to be negligible. "*

Charles Chaplin



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As Prof. Nuno Grande always likes to refer to a line from his mentor and friend, Dr. Abel Salazar: *“A DOCTOR THAT ONLY KNOWS MEDICINE NOT EVEN MEDICINE KNOWS”*.

## RESUMO

**INTRODUÇÃO:** A fractura proximal do fémur é uma causa comum e importante de incapacidade funcional, imobilização e um aumento geral de morbidade em pacientes idosos<sup>1,2,3</sup>. A recuperação pós-operatória é muitas vezes complicada devido a lesões múltiplas, altas taxas de necrose avascular da cabeça femoral devido ao fornecimento de sangue interrompido e desunião.

- Na Medicina Tradicional Chinesa (MTC) a acupunctura é utilizada para reduzir as complicações e condições acima mencionadas. Na verdade, a acupunctura pode levar a um aumento da força muscular com uma melhoria mensurável na marcha em pacientes geriátricos durante a reabilitação<sup>4</sup>. Segundo a teoria da MTC, eles estão relacionados com o diagnóstico de algor/"frio" que, de acordo com o modelo Heidelberg (HD) da MTC, pode ser considerado como uma falta de microcirculação (MC).
- Pontos de acupunctura são seleccionados de acordo com o diagnóstico funcional da MTC, o qual é considerado como um estado vegetativo funcional. Infelizmente um diagnóstico ocidental pode representar uma variedade de diagnósticos na MTC resultando numa variedade de conceitos de tratamento (um grande problema para a padronização da pesquisa de acupunctura). Como o diagnóstico de MTC é considerado como um estado vegetativo funcional, parametrização do estado de pré-tratamento vegetativa pode levar a melhor padronização dos pacientes pelos critérios de inclusão respectivos.

**OBJECTIVO:** O objectivo deste estudo é:

1. Demonstrar se é possível avaliar objectivamente os efeitos da acupunctura com parâmetros relacionados com MC neste cenário clínico;
2. Avaliar o papel da linha base de pré-tratamento dos parâmetros relacionados com a MC como um critério de inclusão para a comparabilidade e significância estatística em futuros estudos de acupunctura;

3. Se os parâmetros relacionados com a MC podem um dia vir a ter um papel na parametrização do diagnóstico em MTC como um estado vegetativo funcional.

**MÉTODOS:** Este estudo prospectivo, não controlado, não cego de ensaio preliminar incluiu 32 pacientes idosos com alteração da marcha após tratamento cirúrgico de fracturas do fémur. Os sujeitos foram recrutados por um profissional médico da ala de reabilitação de Bethanien Krankenhaus (Heidelberg, Alemanha), de acordo com os critérios de inclusão e exclusão.

Para o tratamento de acupunctura, os pacientes receberam acupunctura, com técnica leopard spot, no Conduto Estomacal no ponto S34 em posição supina. Medições de dois parâmetros de MC - velocidade (V) e conteúdo de hemoglobina regional (rHb) - foram continuamente tomados no início e após o tratamento com acupunctura no segundo dedo do pé a cada 2 segundos durante 30 segundos. As medições da MC foram realizadas a uma profundidade de 3mm e de 6mm com um dispositivo O2C usando espectroscopia de luz branca e laser Doppler para determinar a diferença entre uma linha de base (pré-tratamento) e valores pós-intervenção. Para análise estatística foi utilizado o teste Wilcoxon signed-rank.

**Crítérios de inclusão:** pacientes geriátricos com fractura proximal do fémur, na ala de reabilitação, o relatório de alteração da marcha, estado típico da microcirculação (medida pelo O2C).

**Os critérios de exclusão:** demência (Mini Mental State Examination (MMSE) <24), patologia na microcirculação (medida pelo O2C), tratamento de anticoagulação.

#### **RESULTADOS:**

- 1) A análise dos dados revelou que os valores da linha base de pré-tratamento de MC dos pacientes podem ser divididos em dois grupos:
  - a) 26 pacientes apresentaram valores de pré-tratamento comparáveis variando dentro de dois desvios-padrão da média (grupo de perfusão baixa (LPG)) dentro deste cenário clínico);
  - b) 6 pacientes apresentaram 6 a 17 vezes mais elevados em comparação com a média (grupo de perfusão alta (HPG)).

- 2) No LPG a análise da eficácia revelou um aumento estatisticamente significativo no fluxo da velocidade após intervenção de acupuntura em 17,6% ( $p < 0,001$ ) a 3mm de profundidade e 11,9% ( $p = 0,006$ ) a 6 mm. Mudanças na rHb não alcançaram significância estatística.
- 3) No HPG a eficácia da análise não conseguiu mostrar mudanças estatisticamente significativas.
- 4) A análise estatística independentemente da linha base MC não resultou em alterações significativas de velocidade ou rHb após a acupuntura.

## DISCUSSÃO:

- 1) Dois grupos de pacientes podem ser definidos pelos valores relacionados com a MC que são grupo de perfusão normal ou com excesso de perfusão (LPG e HPG). Segundo a teoria TCM as queixas dentro deste cenário são principalmente devido a padrões algor/"frio". O Modelo HD afirma que estes se relacionam com MC local prejudicada. Os pacientes com excesso de perfusão podem ser interpretados como uma expressão de padrões de CALOR/"calor", conforme descrito pelo modelo de HD.
- 2) O ponto S34 é conhecido por ser adequado para padrões de algor/"frio". Como o LPG representa padrões de algor/"frio", as mudanças na MC podem reflectir:
  - A adequação deste ponto para esta condição;
  - A parametrização deste padrão de algor/"frio" por valores relacionados à MC pode ser possível dentro do cenário escolhido
- 3) O excesso de perfusão nestes pacientes pode indicar padrões de calor de acordo com o modelo de HD, como não há mudanças significativas, isso pode mostrar que o ponto é inútil em padrões CALOR/"calor"
- 4) Pacientes com excesso de perfusão e perfusão normal de acordo com o modelo HD podem representar pacientes com mistura de CALOR/"calor" e algor/"frio". Isto significaria definir os critérios de inclusão apenas pelo diagnóstico ocidental independentemente da dada variedade de diagnósticos da MTC (vegetativo padrões funcionais).

- Como isso resulta em esconder a eficácia da acupuntura mostrado no ponto 2) que demonstra um efeito frequente em estudos de acupuntura que falham em provar a sua eficácia ao definir os seus pacientes independentemente do diagnóstico da MTC.
- Critérios de inclusão nos estudos de acupuntura devem ser fundamentados em critérios de diagnósticos ocidentais e chineses.
- Obviamente padrões de CALOR/"calor" e algor/"frio" podem ser distinguidos por valores relacionados da MC.

**PALAVRES CHAVE:** Acupuntura, microcirculação, fractura colo do fémur, Medicina Tradicional Chinesa, MTC, Velocidade do Sangue, Conteúdo Hemoglobina (Hb)

## ABSTRACT

**INTRODUCTION:** Fractures of the proximal femur is a common and important cause of functional impairment, immobilization and a general increase of morbidity in elderly patients<sup>1,2,3</sup>. Postoperative recovery is often complicated by multiple injuries, high rates of avascular necrosis of the femoral head due to disrupted blood supply and nonunion.

- In Traditional Chinese Medicine (TCM) acupuncture is used to reduce the aforementioned complications and complaints. In fact, acupuncture may lead to an increase in muscular strength with a measurable gait improvement within this scenario<sup>4</sup>. According to TCM theory, they are related to the diagnosis of "algia"/"cold" which, according to the Heidelberg (HD) Model of TCM, may be regarded as a lack of microcirculation (MC).
- Acupoints are selected according to the functional diagnosis of TCM which is regarded as a vegetative functional status. Unfortunately one western diagnosis may represent a variety of TCM diagnoses resulting in a variety of treatment concepts (a major problem for the standardization of acupuncture research). As the TCM diagnosis is regarded as a vegetative functional status, parameterization of the vegetative pre-treatment status may lead to better standardization of patients by the respective inclusion criteria.

**OBJECTIVE:** This aim of this study is:

1. To demonstrate if it is possible to objectively assess the effects of acupuncture by MC-related parameters in the given clinical scenario;
2. To evaluate the role of baseline pre-treatment MC-related parameters as an inclusion criterion for the comparability and the statistical significance in future acupuncture studies;
3. Whether MC-related parameters may one day play a role in parameterizing the TCM diagnosis as a vegetative functional state.

**METHODS:** This prospective, uncontrolled, unblinded preliminary trial included 32 elderly patients with gait impairment after surgical treatment of femoral fractures. The subjects were recruited by a medical professional from the rehabilitation ward of Bethanien Krankenhaus (Heidelberg, Germany) according to the inclusion and exclusion criteria.

For acupuncture treatment the patients received acupuncture with “leopard spot” technique on the stomachal conduit, on the point S34, while resting in a supine position. Measurements of two MC parameters - velocity (V) and regional hemoglobin (rHb) - were continuously taken at baseline and after acupuncture treatment on the 2<sup>nd</sup> toe every 2 seconds for 30 seconds. MC measurements were carried out at a depth of 3mm and 6mm with a O2C device using white light spectroscopy and laser Doppler to determine the difference between baseline (pre-treatment) and post-intervention values. For statistical analysis we used the Wilcoxon signed-rank test.

**Inclusion criteria:** Geriatric patients with proximal fracture of femur, at rehabilitation ward, report of gait impairment, typical microcirculatory status (as measured by O2C).

**Exclusion criteria:** Dementia (MMSE < 24), microcirculatory disease (as measured by O2C), patients on anticoagulative therapy were also excluded from the study to minimise the risk of secondary hemorrhage after blood-letting acupuncture.

## **RESULTS:**

- 1) The analysis of MC data revealed that the pre-treatment baseline values the patients can be divided up into two groups:
  - a) 26 patients showed comparable pre-treatment values ranging within two standard deviations from the mean (low perfusion group (LPG) within this clinical scenario);
  - b) 6 patients showed a 6- to 17-fold elevation as compared to the mean (high-perfusion group (HPG)).
- 2) In the LPG the efficacy analysis revealed a statistically significant increase in flow velocity following acupuncture intervention by 17.6% (p

< 0.001) at a depth of 3mm and 11.9% (p = 0.006) at 6 mm. Changes in rHb failed to reach statistical significance.

- 3) In the HPG the efficacy analysis failed to show statistically significant changes.
- 4) The statistical analysis regardless of baseline MC resulted in no significant alterations of velocity or rHb after acupuncture.

**DISCUSSION:** As to:

- 1) Two groups of patients can be defined by MC-related values which are normally or over-perfused (LPG and HPG). According to TCM theory the complaints within this scenario are mostly due to algor/"cold" patterns. The HD Model states that these correlate with impaired local MC. Over-perfused patients may be interpreted as an expression of calor/"heat" patterns as described by the HD Model.
- 2) The point S34 is known to be suitable for algor/"cold" patterns. As the LPG represents algor/"cold" patterns, the changes in MC may reflect
  - the suitability of this point for this condition
  - parameterizing this algor/"cold" pattern by MC-related values may be possible within the chosen scenario
- 3) High perfusion of patients may indicate heat patterns according to the HD Model, As there are no significant changes, this may show that the point is useless in calor/"heat" patterns
- 4) patients with over-perfusion and normal perfusion according to the HD Model may represent mixing of calor/"heat" and algor/"cold" patients. This would mean to define the inclusion criteria only by the western diagnosis regardless of the given variety of TCM diagnoses (vegetative functional patterns).
  - As this results in hiding the efficacy of acupuncture shown under point 2), it demonstrates a frequent effect in acupuncture studies failing to prove efficacy by defining the clientele regardless of the TCM diagnosis.

- Inclusion criteria in acupuncture studies should be founded on western *and* Chinese diagnostic criteria.
- Obviously calor/"heat" and algor/"cold" patterns can be distinguished by MC-related values.

**Keywords:** Acupuncture, microcirculation, femoral fracture, Traditional Chinese Medicine, TCM, blood velocity, hemoglobin (Hb) content.

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## LIST OF ABBREVIATIONS

MTC - Medicina Tradicional Chinesa

MC- Microcirculação/  
Microcirculation

V - Velocidade/Velocity

rHb - conteúdo de Hemoglobina  
regional/Regional hemoglobina  
content

MMSE - Mini Mental State  
Examination

LPG - Grupo de perfusão baixa/Low  
perfusion group

HPG - Grupo de perfusão alta/High  
perfusion group

TCM - Traditional Chinese Medicine

Hb - Hemoglobin

NO - Nitric oxide

GC - Guiding Criteria

RAAS - Renin-Angiotensin-  
Aldosterone-System

PAD - Peripheral Arterial Disease

CNS - Central Nervous System

ON - Osteonecrosis

ALT - Algor Laedens Theory

DGTCM - German Society of  
Traditional Chinese Medicine

O2C - Oxygen-to-see Device

AU - Arbitrary Units

SD - Standard deviation

VelovAob - velocity at baseline at  
3mm-depth

VelonAob - velocity after  
acupuncture at 3mm-depth

VelovAtief - velocity at baseline at  
6mm-depth

VelonAtief - velocity after  
acupuncture at 6mm-depth

rHbvAob - rHb at baseline at 3mm-  
depth

rHbnAob - rHb after acupuncture at  
3mm-depth

rHbvAtief - rHb at baseline at 6mm-  
depth

rHbnAtief - rHb after acupuncture  
at 6mm-depth



# CHAPTER ONE



# Introduction

## 1. THEORETICAL BACKGROUND ON MICROCIRCULATION (MC)

### 1.1. The role of MC in terms of Western physiology

Microcirculation is defined as a network of small vessels (arterioles, capillaries and venules) with diameter less than  $100\ \mu\text{m}$ <sup>5</sup>. The microcirculation has the vital function of providing oxygen and other essential substrates to cells and also to remove their products from cellular metabolism<sup>6</sup> (Fig. 1). It has also other physiological functions<sup>7</sup>, controlling the vascular resistance, blood clotting, inflammatory processes and immunological barrier<sup>7</sup>. One can intuitively consider that the organ failure is related to the microcirculatory dysfunction. In fact, the degree of microvascular alterations correlate with organ dysfunction and mortality in different disease states<sup>8,9,10,11</sup>.

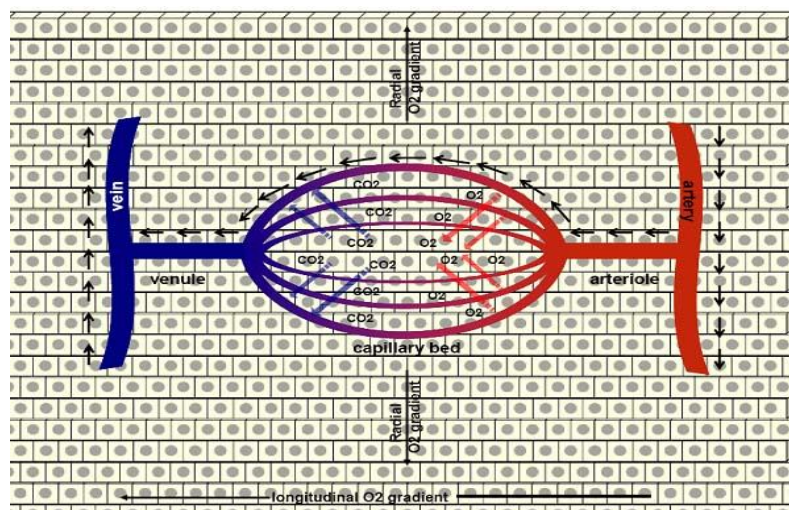


Fig.1 - Schematic representation of the network microcirculation

### 1.2. Major regulatory mechanisms of microcirculation

The regulation of capillary blood flow, and consequently, tissue perfusion, is a complex phenomenon that includes capillary perfusion pressure, arteriolar tone, hemorheology and capillary permeability<sup>5</sup>. Endothelial cells have the ability to perceive mechanical forces (shear force) and blood flow produced by local stimuli such as pH, lactate, concentration of tissue O<sub>2</sub> and CO<sub>2</sub>, and neuro-transmitter substances<sup>12,13</sup>.

An imbalance between the effects of vasodilator and vasoconstrictor agents such as nitric oxide (NO) and catecholamines, angiotensin II and endothelin-1 may have a role in regulating capillary blood flow and exacerbate a diversion of that area<sup>14</sup>. In addition, the glycocalyx, a thin layer of glycolipids and proteoglycans lining the luminal surface of endothelial cells has important functions such as control of vascular permeability, blood flow resistance, adhesion of leukocytes and platelet activation. The glycocalyx has gained wide attention recently after some reports of rupture and endothelial dysfunction in sepsis and other inflammatory diseases<sup>15,16</sup>. Finally, and still controversial, mitochondrial dysfunction may be associated with signs of microcirculatory failure, but it is a cause or consequence of the deficiency of oxygen extraction is still an issue to be discussed, although experimental data suggest that the change of microcirculation precede (and might mislead) mitochondrial dysfunction<sup>17</sup>.

### 1.3. Hemodynamics

The heart pumps continuously at every systole, a certain volume of blood for our arteries. The blood is a certain resistance to flow, provided largely by the friction of the molecules and blood cells into the wall of a long way forward found through our blood vessels of varying diameters and numerous branches<sup>5,9,18</sup>.

Blood flow varies widely in different tissues. Certain fabrics require a much higher flow than others. Tissues such as skeletal muscle show large variations in blood flow through them in different situations: at rest the flow is relatively small, but increases significantly during labor, when the consumption of oxygen and other nutrients increases and the production of carbon dioxide and other elements also increases<sup>9,13,19,20</sup>.

Through vasodilation or vasoconstriction, at every moment, the blood flow in tissue can increase or decrease due to a greater or lesser resistance offered to it<sup>19,20,21</sup>. There are two important factors that determine the flow in a vessel which can be demonstrated by the following formula:

$$\text{FLOW} = \text{PRESSURE}/\text{RESISTANCE}$$

Given this it can be conclude that increasing the pressure, the flow increases, increasing the resistance, the flow decreases<sup>18</sup>.

The resistance to flow, in turn, depends on several other factors:

- ✓ Length of Vessel: The higher the path to be followed by blood in tissue, the greater the resistance to flow. Therefore, the greater the length of a vessel, the greater the resistance to blood flow through the vessel itself<sup>18,19,21</sup>.
- ✓ Diameter of Vessel: Vessels of different diameters also offer different resistances to flow through them. Small variations in the diameter of a vessel give large variations in flow resistance and therefore large variations in the flow. The resistance to blood flow through a vessel is inversely proportional to diameter of that vessel to the fourth<sup>18,19,21</sup>.
- ✓ Viscosity of Blood: The blood has a viscosity approximately 3 times higher than that of water. So there is about 3 times more resistance to blood flow than the flow of water through a vessel. The blood of an anemic person has lower viscosity and hence a greater flow through your veins. This can easily be checked by constant tachycardia that such people exhibit<sup>18,19,21</sup>.
- ✓ Speed of Blood in the vessels: also varies depending on vessel size; the larger the diameter of a vessel, the lower the velocity of blood so that an even flow to occur through this vessel<sup>18,19,21</sup>.

#### **1.4. Microvascular response to surgery**

Several factors may impact on the microcirculation during and after surgery: hypoperfusion related to losses of large volumes of blood during the surgery, inflammation due to incision and manipulation of the mesentery, inflammation, sympathetic stimulation (arteriolar constriction and reduction of microvascular flow). The integrity of the endothelium may be altered, leading to capillary leak, and rolling and adhesion of leukocytes increase after surgery. Sequestration of leukocytes, vasoconstriction and tissue edema in the microcirculatory beds can cause an increase in oxygen gradient leading to tissue hypoxia and organ dysfunction<sup>25</sup>.

## 2. ACUPUNCTURE AS A CLASSICAL TREATMENT METHOD OF TCM

### 2.1. Definition of Acupuncture

Acupuncture is an ancient form of Chinese medicine involving the insertion of solid filiform acupuncture needles into the skin at specific points on the body to achieve a therapeutic effect. No drug is injected. The needles alone create the beneficial effects of acupuncture. Acupuncture is used to enhance natural healing, improve mood and energy, reduce or relieve pain and improve function of affected areas of the body.

### 2.2. The system of channels or conduits

According to TCM theory the energy (Qi) flows through channels that are named as Conduit (Figure 2) that present a “connection of a group of points with effect on the clinical signs associated with a certain vegetative function, believed to serve as a conduit for the flow of qi and xue<sup>31,32</sup>. There exists a hierarchy within the conduit system. This consists of:

#### Cardinal Conduits or “Cardinales”.

There are twelve of these, the “Twelve Cardinal Conduits”. They constitute the central supporting structure of the system of conduits. They are symmetrical conduits, in other words they extend in mirrored symmetry over the right and left halves of the body- 3 sinarteria leading from the hand to the chest, and 3 sinarteria mounting from the foot to the upper body.

To these are added<sup>28</sup>:

#### Twelve Paracardinal Conduits (*sinarteriae paracardinales, jingbie*).

Eight Odd Cardinal Conduits (*cardinales impares, qijing bamo*, abbreviated to “Odd Conduits”). These cardinal conduits include the leading and responding sinarteries (*sinarteriae regens et respondens, dumo* and *renmo*) extending along the median lines of the body. They are complemented by various ramifications which – as their name indicates – produce further imbrications, netlike interconnection of the entire system.

Fifteen Reticular Conduits (*sinarteriae reticulares, luomo*)

Parareticular Conduits (“Reticular Branch Conduits”, *sinarteriae parareticulares, bieluo*)

And finally

Reticulars of the Third Generation (*reticulares parvulae, sunluo*)

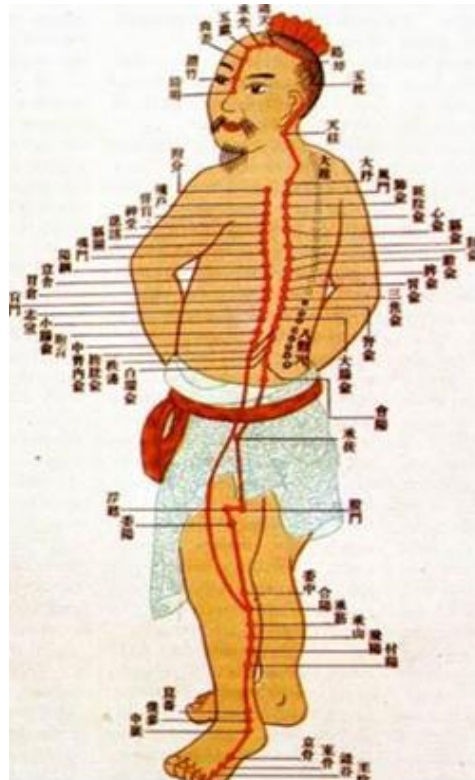


Fig.2 -Acupuncture conduits

According to traditional postulates each conduit connects to a particular “inner organ”, in other words, all conduits, as well as the reticular conduits associated with them, are considered to extend on the surface of the body within the skin and to communicate with inner parts of the body also known as the *intima*. This postulate of communication between surface and depth on the one hand explains why exogenous influences may penetrate the surface and reach the depth; or inversely, why endogenous disturbances may appear on the surface, where they can be diagnosed and directly influenced by therapeutic manipulation<sup>31,32</sup>.

## **3. CONTEMPORARY MEDICAL ACUPUNCTURE**

### **3.1. Definition**

The contemporary medical acupuncture is an evolution of traditional acupuncture, whose origins date back over 2000 years in ancient China<sup>34</sup>. Traditional Chinese Medicine has great heuristic value of concepts such as categorization of symptoms in groups of pathologies, designed according to the cultural patterns of ancient Chinese civilization, in which any event could be classified using the same criteria in a mathematics way. From the ancient Chinese doctors must say that was brilliant in creating explanations for the phenomena they observed clinical and therapeutic, even if those explanations have proven to be inconsistent with the anatomical and physiological reality<sup>35,36</sup>.

### **3.2. Acupuncture research as the basis of contemporary medical acupuncture**

Nowadays the ideas like the existence of channels through which circulates a mysterious substance, become irrelevant, given the findings of the biological mechanisms of action of acupuncture<sup>34</sup>. In fact, after large investments of time and money, research attempting to prove the existence of energy channels were abandoned. Today we seek to scrutinize the biological effects of peripheral nerve stimulation, with its psychological effects, hormonal, immune, sensory, motor and autonomic, and technological resource that has most contributed to reveal these details is functional magnetic resonance imaging of central nervous system<sup>34</sup>.

The research also showed that in these features there is no specific points to obtain systemic effects - that originate from changes in supra-segmental commands<sup>37</sup>. In practice too, this specificity is recognized. For example, in the treatment of nausea, have been successfully tested points on the median nerve, radial branches of the posterior tibial nerve branches of the cervical plexus (under the mastoid process), and points to-vertebral muscles<sup>37</sup>.

However, there is another specificity, which refers to the type of stimulated nerve fiber, and the modality of the stimulus (mechanical electrical - on different frequencies) applied, which may not be painful so as to achieve the therapeutic effects<sup>38</sup>.

### 3.3. Mechanisms of Acupuncture – what we know from acupuncture research

For millennia, it was believed that the mechanism of action of acupuncture was pure energy, or accepting only the design of the channels of Energy. However, with the spread of TCM many Western researchers began to question the participation of organic structures in the mechanism of action of acupuncture, and the development of scientific research in this area, especially in the last decade has demonstrated the close relationship between the effects of Acupuncture and central nervous system and peripheral as well as various types of neurotransmitters<sup>26,27</sup>.

This new knowledge of traditional Chinese medicine makes it possible today to accept three mechanisms to explain the action of Acupuncture: energy mechanism, humoral mechanism, neural mechanism<sup>28,29</sup>.

Energetic mechanism: This corresponds to the classical conceptions of Energy Pathways or so-called channels or conduits.

Humoral mechanism: It refers to the production of substances, usually neurotransmitters and hormones that are secreted into the blood through the action of acupuncture. This action can be demonstrated experimentally, obtaining similar analgesic effects in two animals subjected to cross-circulation, even acupuncture have been conducted only in these animals.

The humoral effect also depends indirectly on the central nervous system, which determines the release level endocrine substances found in blood.

Neural Mechanism: Recent research on the neurophysiological mechanism of the action of acupuncture analgesia have brought significant benefits to the understanding of its mechanism. This made the ancient Chinese philosophical theories started to have a scientific basis. Much of the concepts intuitively advocated that TCM can now be explained in light of the neuroanatomy and neurophysiology, allowing the physiology of the human being can be studied holistically.

Recent research aimed at understanding the mechanism of action of acupuncture<sup>30</sup>

1. Acupuncture changes the bloodstream. From the stimulating of certain points, you can change the dynamics of regional circulation from microdilations. Other points promote muscle relaxation, healing spasm, decrease inflammation and pain.

2. The stimulation of certain points promotes the release of hormones such as cortisol and endorphins, promoting analgesia

3. Acupuncture helps to increase host resistance. When there is external aggression, some organ system are affected. There is an internal regulation to provide resistance to disease. Acupuncture exacerbates these mechanisms so that the balance in less time and health to be restored. Many studies reveal the possible stimulation of the hypothalamus, pituitary and other glands that work in recovery.

4. Acupuncture regulates and normalizes body functions. The various functions in humans are interrelated. If there is any disturbance altering this interrelationship, there is the manifestation of symptoms and disease is established. Stimulating by acupuncture may stimulate and restore the previous relationship and hasten recovery.

5. Acupuncture promotes the metabolism. The metabolism is essential in maintaining life. In certain disease conditions, there are no changes in the metabolism of various organs, with consequent depletion and deficiency in the body. Acupuncture allows the recovery of this metabolism, important in the healing process.

## **4. – THE HEIDELBERG (HD) MODEL OF TCM AS A SCIENTIFIC APPROACH TO TCM**

### **4.1. Definition**

Today there exist modern approaches to TCM, integrating the fundamental principles of classical TCM theory into the current knowledge of human anatomy and physiology. The Heidelberg Model of TCM is an example. It presents a cybernetic model to systematize the diagnosis and treatment of TCM.

## 4.2. TCM Diagnose according to the HD Model

It is important to understand that TCM theory is based on a regulative perception of the body. Hence the diagnosis can be understood as the current vegetative regulative status. As a consequence, the classical terms of Phases or elements can, in cybernetic terms, be understood as parts of a circular process; in relation to the body they present vegetative functional tendencies that are manifested in so-called orbs (=group of diagnostically relevant signs indicating the functional state of a body region, which correlates with the functional properties of a conduit)<sup>31,32</sup>.

## 4.3. First step of the diagnose according to the HD model

In a first step we define the symptoms in terms of the Constitution that gives us information about the individual and functional properties of the inner nature of the patient, based on their phenotype. The Agent and pathogenic factor which is considered a power (vector) function: that changes the individual functional properties (caused by the constitution); produces clinical signs alone; and produces orbs (groups of other diagnostically relevant signs). And finally the symptoms are also defined in terms of the Orbs, groups of diagnostically relevant signs indicating the functional state of a body region, which correlates with the functional properties of a conduit.<sup>31,32</sup>

## 4.4. Second step of the diagnose according to the HD model

In this type of diagnose symptoms are interpreted in the context of global regulatory body through the guiding criteria. Guiding Criteria are, therefore, approaches to the interpretation of signs and symptoms. There are four types (repletion/depletion, calor/algors, extima/intima, yin/yang) in the context of four models of regulatory physiology. It can be said that a guiding criteria (GC) is an evaluation of clinical signs according to a regulatory model of the underlying physiology<sup>28,29,31,32</sup>.

#### 4.4.1. The 1<sup>st</sup> Guiding Criteria

The first GC, **depletion/repletion**, evaluates the clinical signs that in TCM is believed to be originated primarily from the qi and Orbs. In Western terms: a poor population of cells can be stimulated excessively vegetatively causing vegetative clinical signs as they were appointed under repletion. Therefore, a stage of near collapse with functional signs may appear similar to depletion<sup>28,29,31,32</sup>.

#### 4.4.2. The 2<sup>nd</sup> Guiding Criteria

The second GC, **calor/algor**, evaluates the signs that Chinese medicine is thought to originate from the effects of xue ("blood") which is the second functional power ("energy") of Chinese medicine. In Western terms, these signs are due to overactivation mechanisms that involve<sup>28,29,31,32</sup>.

- The microcirculation and local interdependent mechanisms of plasma, blood cells, endothelium and functional tissue of the organ
- This is an activation of body fluids, at least in some part of the body. Can evoke vegetative and systemic responses in the context of fluid distribution, supply and circulation of fluids (i.e. changes in thirst, urination, blood supply and heart rate).

In summary, clinical signs of this type may be referred to humorovegetative with respect to the origin<sup>31,32</sup>. Signs of overactivation of xue (effects on the microcirculation) are generally described as Calor, lack of signs of functional microcirculation are called Algor<sup>31,32</sup>.

#### 4.4.3. The 3<sup>rd</sup> Guiding Criteria

The Third GC, **Extima/Intima** evaluates the signs that in CM is believed to originate from the effects of a pathogenic factor (agent) that invades the body from the outside. The most common underlying pathophysiological model is the model of six stages (**Shang Han Lun**). This is the process of the agent algor damaging the body. It is therefore called **algor laedens theory** (ALT)<sup>28,29,31,32</sup>.

#### 4.4.4. The 4<sup>th</sup> Guiding Criteria

The fourth GC, **Yin/Yang**, as for the diseases with relation Yin/Yang is seen as a manifestation of excess Yin that produces Algor and their disability Calor. The transformation from one another is observed when in the maximum Algor comes

Calor and vice-versa, and Yin pathology's can become Yang. Manifestations as Algor may emerge in conditions of intense Calor. These changes can only occur when conditions for doing so, as the maturation of the internal conditions. Another condition leading to the transformation process is the time factor, in example, the conditions should be prepared for change<sup>28,29,36,41</sup>.

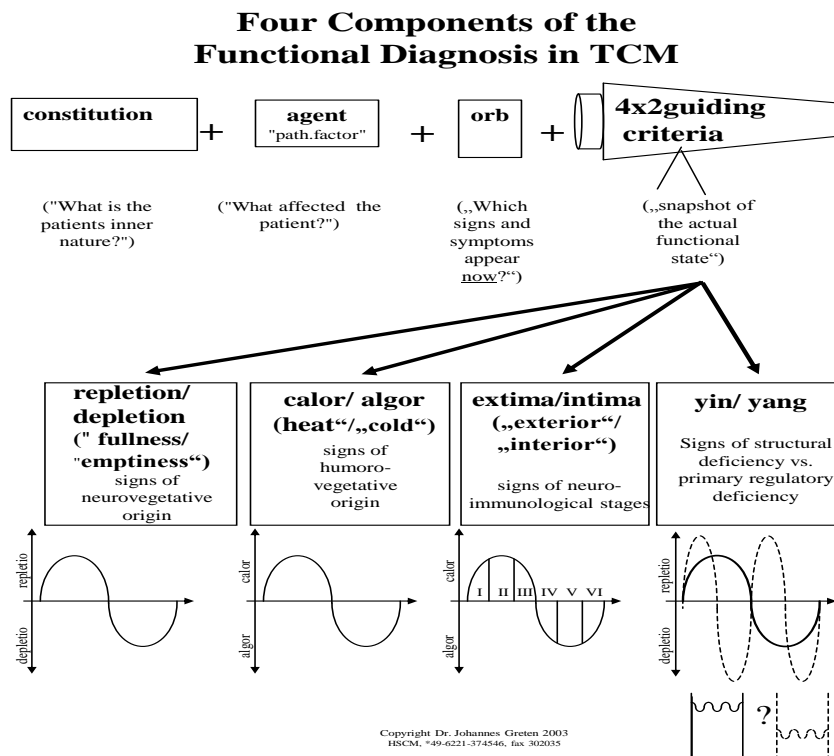


Fig.3 - TCM diagnose according to the HD model

#### 4.5. The impact of the HD Model of TCM on current acupuncture research

The HD Model of TCM revealed that the TCM diagnosis can be regarded as the current vegetative state of the body, which can be defined by key symptoms (diagnostic features). According to the HD Model acupoints elicit vegetative reflexes that have to match the current vegetative state of the subject treated in order to be clinically effective. In a clinical scenario one single western diagnosis may therefore represent a whole diversity of TCM diagnosis. For this reason it is difficult to define a homogenous study population for an acupuncture trail in which all patients show assimilable vegetative states at baseline.

In current clinical acupuncture research inclusion and exclusion criteria are conventionally based on the Western diagnosis. As a consequence there is a great risk that the investigated acupuncture intervention might be suitable for some patients while it does not match the vegetative status of others. This might explain why many randomized clinical trials on acupuncture effect have not shown statistically significant superiority of the investigated acupuncture treatment to control treatment groups<sup>36</sup>. Hence, there is a clear demand for measurable parameters that help to parametrize different aspects of the current vegetative state of a patient in order to enable a more accurate definition of study populations in future acupuncture trails.

## **5. THE ROLE OF MC IN TCM THEORY (ACCORDING TO THE HD MODEL)**

### **5.1. The physiology of MC in TCM theory**

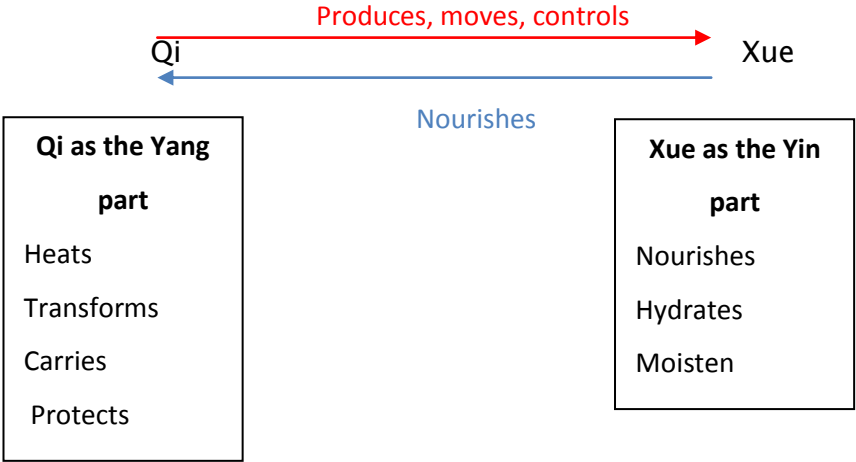
According to Porkert<sup>28,29</sup>, xue (“blood”) is the moved structivity. Xue belongs, like Qi or Shen to the untranslatable concepts of Chinese medicine, as blood in Chinese medicine and western view have quite a different functional concept. According to the auxiliary vegetative definition of the Heidelberg Model: “Xue is a form of functional capacity (“energy”) bound to body fluids with functions such as warming, moisturizing, creating Qi and nutrifying a tissue<sup>31,32</sup>.”

From a western medical view: the clinical effects of Xue can be comparable to the western concept of the effects of microcirculation, including the functional relations of microcirculation, blood cells, plasma factors, endothelium and parenchyma. From the Western point of view are clinical signs predominantly originated in the humoro-vegetative system<sup>31,32</sup>.

One aspect of Xue is that it is a substance and part of the yin and at the same time it is a form of energy, Yang. This double nature of Xue becomes obvious i.e. in the functional relation of Xue and Shen, as Xue (Yin) “checks”/or “controls” Shen (Yang)<sup>31,32</sup>.

Energy forms/functional capacities running with xue or forming divisions of xue are shen, constructive qi (ying), and fluids such as jin (passive fluids moisturizing the tissue) and ye (active fluids such as sweat)<sup>31,32</sup>.

In TCM, Xue is in itself a form of Qi and a very dense material, therefore Xue and Qi are inseparable from themselves.



The function of blood (*Xue*) in TCM is similar to that on Western Medicine, but the substance itself is different. Qi infuses into the blood and is carried by it, so that blood and Qi are inseparable<sup>28,29,31,32</sup>.

Blood is made from (fig 4) the food by the Stomachal and Lienal Orb. The fluid nature of blood means that is similar to essence, and may transform into essence in the Renal Orb. Essence in turn may transform into blood in the Hepatic Orb. Blood circulation is dominated by the Cardial Orb while the Lienal controls the blood and prevents it leaking from the blood vessels (extravasation). The Hepatic Orb stores the blood and control blood volume<sup>40</sup>.

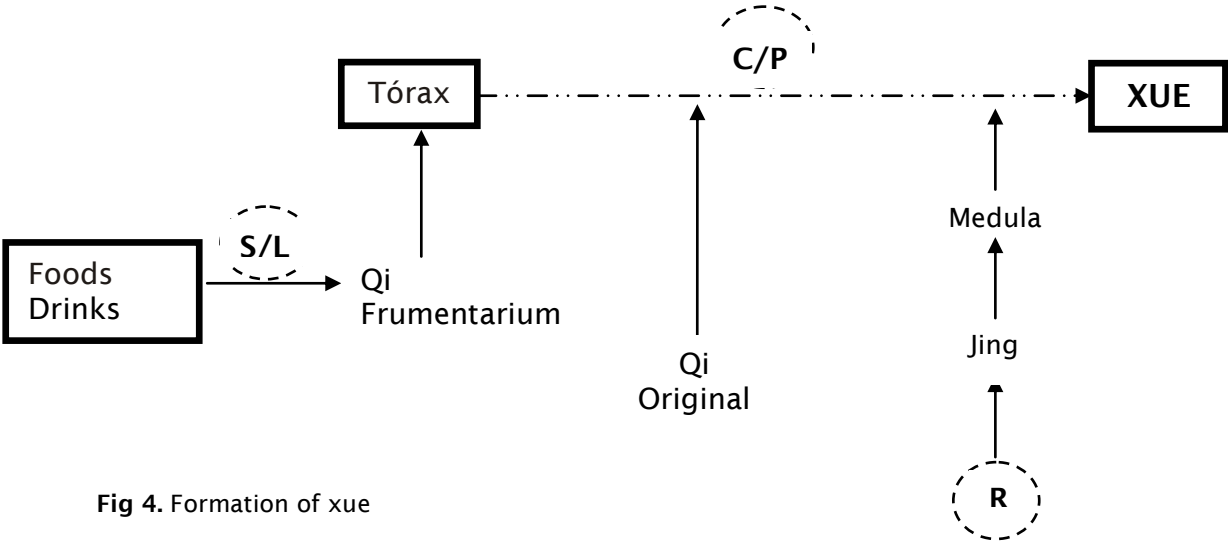


Fig 4. Formation of xue

Blood is said to nourish and moistens the body tissues, and be the foundation for mental activities. This is why deficiency of blood can give psychological effects in TCM. For example deficiency of the blood in the Cardial or in the Hepatic Orb may give insomnia and mental unrest. The inseparable nature of Qi and blood is also illustrated clinically. After heavy exertion, for example, which depletes Qi, signs of blood deficiency are seen including pallor and dizziness. Similarly after massive blood loss, Qi deficiency is seen demonstrated by sweating and cold limbs<sup>40</sup>.

The main functions of the xue are: nourish the body; hydrate; and provide material foundation for the mind. When we have a disbalance of xue it may occur because: xue deficiency (not enough manufactured, normally due to Lial deficiency); calor in the xue (calor in the hepatic) and stases of xue (movement too slow, due to stagnation of Hepatic qi or due to algor or calor)<sup>28,29,31,32,40</sup>

## 5.2. Calor/algor according to the 2<sup>nd</sup> guiding criterion (GC)

The second GC, **calor/algor**, evaluates the signs that Chinese medicine is thought to originate from the effects of xue ("blood") which is the second functional power ("energy") of Chinese medicine. Hence, one can say that it refers to humero-vegetative signs, originating from MC, local interdependent mechanisms of plasma, blood cells, endothelium and functional tissue<sup>31,32</sup>.

Calor describes a status of generally increased MC with respective signs such as increased heart rate, concentrated urine (due to the activation of the Renin-Angiotensin-Aldosterone-System (RAAS)), increased thirst, sensation of heat, reddened skin with a burning sensation, reddened and dry mucosa, hyperdynamic movements and a generally increased arousal state of the central nervous system<sup>31</sup>.

Algor, on the other hand, comprises signs connected with a generally decreased MC, leading to a sensation of cold, a low heart rate, diluted urine, little thirst, generally hypodynamic movements, pale and moist mucosa, pale and cold skin, and a generally reduced arousal of the central nervous system<sup>31,32</sup>.

## 5.3. Algor as a pathogenic factor

### 5.3.1. Definition

When we speak about xue one of the most important agent in the type of pathology that involves xue (“blood”) is algor (cold)<sup>28,29,31,32</sup>. In the HD model algor, from a humoro-vegetative perspective, defined as locally reduced MC (capillary blood flow) in a certain tissue. Clinically it is associated with signs and symptoms as if they had been exposed to cold environment, such as skin cold, stiff muscles, and excruciating pain localized to the gradual emergence<sup>31,32</sup>.

Algor is a pathogenic factor of yin nature, which leads to contraction and stagnation, an a functional deficiency of the affected tissue. Exposure to cold environments after sweating or when wearing thin clothing may lead to algor-symptoms, in classical TCM theory the algor “invades” the body, “consuming the Yang Qi of the body”. General symptoms are cold limbs, stiffness, cold pain and contractions<sup>31,32</sup>. It corresponds to the maximum strength for action, movement and change<sup>31,32</sup>. Patient show a general dislike of cold and lack of sweating. Treatment often involves moxibustion and warm needling, and reinforcing techniques, like bloodletting<sup>39</sup>.

### 5.3.2 Types of algor

There are four different types of Algor: 1) algor-algor, 2) post-traumatic algor, 3) immunological algor, and 4) depletive algor)<sup>31,32</sup>.

The algor-algor describes a local reduction of MC after exposition to cold environment due to a physiological vasospasmic reflex, which helps to keep the body core temperature constant.

Post-traumatic algor is defined as a reduced tissue perfusion in response to tissue trauma (i.e. after operation, injury, etc.) A simple explanation of this would be that the post-traumatic inflammation and remodeling processes in the tissue mechanically or by reflex mechanism lead to a permanent reduction of tissue perfusion, in a way like a "micro-scar". From a Western point of view the microvascular responses to surgery, which are outlined in Chapter 1.4 are likely to be involved in post-traumatic algor symptoms after any surgical intervention.

Immunological algor comprises regional reductions in MC due to vasoconstriction as part of an immunological response to an invading aggressor, a virus or bacterium.

The depletive type of algor comes from a lack of xue. If the warming fluid and nutrient of the body, or xue, is deficient some regions, especially the extremities, muscles, connective tissue and the conduits, may be less perfused, because xue flow is centralized to the body center, maintaining its flow in the intima (organs). Xue can be deficient for example due to loss of blood, i.e. after surgery or in women with hypermenorrhea. Lack of xue also occurs in cases of insufficient function of the stomachal and lienal orb, such as malabsorption, anorexia, chronic inflammatory bowel disease, cachexia due to consuming diseases (cancer, chronic inflammations). During lifetime, according to TCM theory, people tend to develop increasing xue deficiency. That is why elderly people tend to show symptoms of depletive algor. It is for this reason that the elderly are often heavily dressed, with long underwear even in the summer. When a doctor observes such behavior should think about the algor when the patient undresses. Obviously he/she need, unconsciously, to warm the conduits.

During lifetime people suffer a series of minor trauma to the knee, hip, back and shoulder (=post-traumatic algor). In the beginning that was not so bad, the complaints were relatively small. During the second part of their lives, when xue becomes increasingly deficient, pain of the algor type appears at the sites of former traumas, as depletive algor adds up to post-traumatic algor. Thus the regions that have been traumatized in their youth are predisposed or likely to be attacked by the depletion of xue.

#### **5.4. The Algor Laedens Theory as a diagnostic system for immuno-vegetative mechanism (3<sup>rd</sup> GC)**

In a Western understanding, the internal agents such as algor produce unbalanced reflex patterns and the body responds to these standards by counter-reactions. In Chinese medicine, this counter-reaction is called reactive Calor that can, according to Western medicine, even comprehend inflammation, increased microcirculation and sepsis<sup>28,29,31,32</sup>.

In the case of entering algor<sup>28,29,31,32</sup>.

- A lack of regional microcirculation may be caused by defensive reflexes to cold, virus (adhesion molecules, complement system, coagulation).

- The counter-reaction is a general increase in microcirculation that is itself a regulatory process.

The specific signs of each stage, are due to conflicts of functional powers, with the agent algor, producing their symptoms. When the agent attacks the skin, there is a reduction of the defensive qi, like all other powers of the functional body. The algor in Western terms, translates into a lack of circulation, or decreased MC and affects primarily the conduit that contains more xue than qi<sup>31,32</sup>.

The six energy layers comprise six different forms of energy which is technically<sup>31,32</sup>: 1) **Defensive qi** (also referred to as *Wei Qi*), which resides within the extimate outside the pipe and creates a first defensive barrier against external attacks; 2) **Qi in the conduit**, which is the qi within the conduits cardinal, since the main channels are the "conduits cardinal." When an agent blocks the flow of Qi that primarily will result in pain and functional disorders secondary of its orb. At this stage algor proceeds their invasion, causing blockages of qi, and therefore the onset of pain. At this point the body activates the xue, creating heat in the interior, to expel the algor drivers. It is in this stage that the algor is relates to pain, and calor is more reactive. If there is little reactive calor, there is an invasion of algor. 3) **Xue in the conduit**, that is guided by the Qi in the conduits and heat the conduits, while "nurture" and "moisten" the tissues. The heating effect in tissue is necessary to drive out the agent algor; 4) **Island qi body**, which is the qi in the intima, a general name for the entire interior of the body, where the functions of the orbs are generated in their respective parts of the body "islands" ("which are named according to the organs that lie approximately the same region); 5) **Island xue body**, which is a substantial part (yin) of the islands with body heat, thus activating and enhancing functional properties; 6) **Yin**, which is the functional tissue, in the western subpopulation of cells, the substrate from which the functions (yang) develop.

« ALT » algor laedens theory	stage 1 = yang major taiyang	stage 2 = splendor yang yangming	stage 3 = yang minor shaoyang	stage 4 = yin major shaoyin	stage 5 = yin flectens jueyin	stage e 6 = yin minor taiyin
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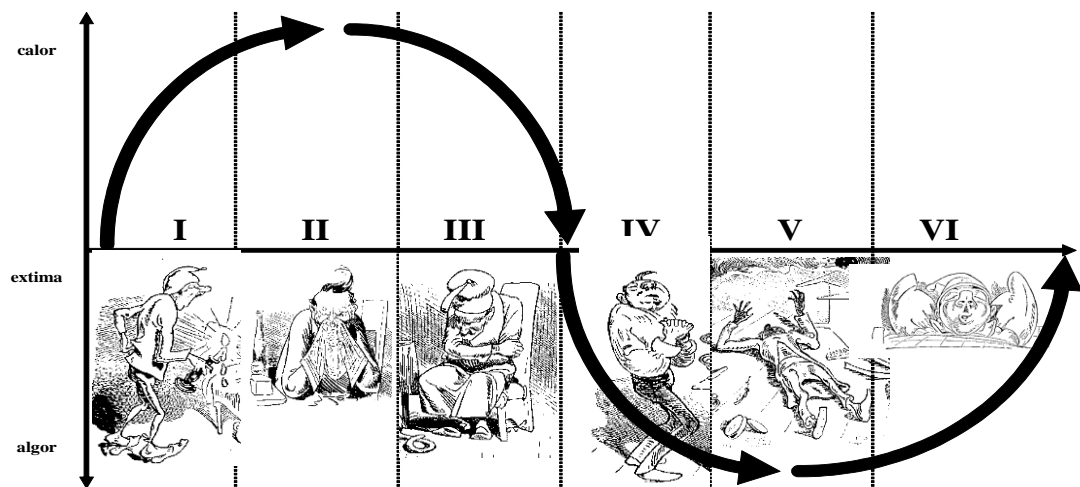


Fig.5 - ALT - Algor Laedens Theory

According to the functional analysis, the invasion of Algor (fig. 5) is the postulate of uniform within the considerations made in six phases systematic defense of Chinese medicine. The Algor is an agent Yin with functional properties of deprivation<sup>29,31,32</sup>.

Algor-induced signals and signs of orbs involved<sup>31,32</sup>, and they are categorized into 6 stages of clinical signs.

1) Yang major, when the agent invades the skin, “attacks” the defensive Qi (*Wei qi*). The defensive qi does not remain in the conduits but in the skin surrounding the conduits and in all other tissues. Symptoms of Wei Qi are varied, but the symptom clinically decisive, may be the symptom of a general feeling of chills. The Orbs that are affect are the Vesical conduit and The Tenointestinal.

2) Splendor Yang, when the agent Algor enters the conduits, this leads to a regional block of the flow of qi and Xue. The flow of qi is blocked more easily than the flow of Xue as it´s the qi that moves the Xue. It is for this reason that the phases and the orbs are more prone to this problem if they depend more on qi. In this stage the affect Orbs are the Stomachal and the Crassintestinal.

3) Minor Yang, the Qi and Xue form derive the interior and are carried through the conduits system. If the agent Algor dominate the flow of Xue in the conduit, this may lead to a reverse flow Xue to the interior. The Algor also reaches the inside causing a feeling of internal cold. Often the inside Xue (yin) will be mobilized

against the agent causing the sensation of internal heat again, or if the agent Algor is expelled out, even heat temporarily abroad, i.e. the skin (extima). Literally, it says that the agent and flow of Xue within the conduit represent the "Tom and Jerry" expelling each other repeatedly. Here we have the affection of the Felleal and Tricalorical Orbs.

4) Yin major, from this stage forward, the main symptoms don't come from the conduits, but more frequently from the interior, the "body island" and its respective functions. When an agent affects the qi of the "body island", the Lienal and the Pulmonary Orbs are the most affected, because the center and the metal are sensitive to the decrease of the activity of qi.

5) Yin flectens, when the Algor affects Xue in the inside, the functions of the Orbs of which depend on Xue are more easily affected than others. Since the Hepatic Orb is considered the *Mare Xue* ("sea of blood"), the functions of the Hepatic Orb may be weakened. Signs may also appear with symptoms such as from the Pericardial orb.

6) Yin minor, the Yin is the structural condition to the yang development. Consequently, also the yin (functional tissue) is a technical form of energy. When this energy is affected by Algor, renal symptoms are more likely. Since the Yang results from the Yin, a similar process is the development of the ascendent Yang Qi that is in connection with the Cardial Qi. This connection is also expressed in terms of a Reno-Cardial axis, in which the pole yin is the Renal Orb and the pole Yang is the Cardial Orb<sup>31,32</sup>.

## 6. ACUPUNCTURE AND MICROCIRCULATION

Several studies indicate that acupuncture can be used as "complementary medicine" in the treatment of hypertension<sup>47,48</sup>. The effect of lowering blood pressure, obtained by acupuncture in hypertensive patients may be at least partly due to a reduced secretion of renin-aldosterone, a decreased activity of angiotensin II<sup>49,50</sup> or activity of the sympathetic nervous system<sup>51</sup>.

### 6.1. Acupuncture and Peripheral Circulation

Peripheral Arterial Disease (PAD) affects about 8 million to 12 million Americans and is associated with significant morbidity and mortality and its prevalence increases dramatically with age<sup>52</sup>. Although there is high prevalence of PAD, with implications for cardiovascular risk, only 25% of patients with PAD are treated, resulting in critical lower limb ischemia. The prevalence of intermittent claudication is around 15% for patients over 50 years, and 1% of present critical ischemia<sup>53</sup>.

PAD usually affects the arteries that supply the legs and is mainly caused by arteriosclerosis. The restriction of blood flow due to arterial stenosis or occlusion, it also causes the patient, muscle pain when walking - intermittent claudication<sup>54</sup>. A study of type "double blind" randomized trial showed that acupuncture can determine an objective of the radial artery vasodilation in patients regularly exposed to acupuncture, compared with patients not exposed to the same technique<sup>55</sup>. The study group Sandberg demonstrated that acupuncture can induce increased muscle blood flow and skin blood flow<sup>56,57,58</sup>. Currently rigorous clinical studies are very encouraging, suggesting that acupuncture is effective for patients with peripheral vascular disease.

In addition to regulation by the central nervous system by modulating the activity level of the peripheral autonomic nervous system, several local mechanisms are also involved in the regulation of vascular resistance within which can be highlight<sup>59</sup>:

- a) pressure-sensitive myogenic mechanism, which correlates inversely pressure and vessel diameter;
- b) metabolic vascular regulation, which through changes in vascular resistance, the blood supply adapts to the needs of different tissues.

## **6.2. Physiological Mechanisms of Reflex regulation of circulation by the action of acupuncture**

The explanation of the physiological effects of acupuncture are believed to result in vasodilation and decreased blood pressure, obtained by the response to stimulation of both needles at specific points or acupuncture, due to the reflex regulation of circulation by the neuronal pathways in the brainstem baroreflex.

The neural control of movement has been systematically studied for more than a century, but little is known about the structures of the central nervous system (CNS) pathways involved in cardiovascular reflexes. Due to the lack of precision in defining, with some degree of security, the different areas of the brainstem involved in generation and control of sympathetic and parasympathetic autonomic activity are apparent difficulty in understanding how these areas and how to intercommunicate neurotransmission occurs between different neural systems involved in reflex regulation of circulation<sup>63</sup>.

### 6.3. Acupuncture, with the Leopard spot technique, as a special technique to influence xue

Acupuncture, as leopard spot technique, is a method of therapy that is difficult to explain in modern terms. Aside from the traditional theoretical basis for these treatments in letting out calor and excess factors, a key issue is whether it actually produces the claimed effects. Many Western acupuncturists have stated informally that they get dramatic results from this treatment method, but, unfortunately, there is no evidence presented to support such contentions. Despite the frequent mention of treating peripheral points by this technique in both ancient and modern Chinese medical texts, there is little reference to it in Chinese medical journal reports. Very few articles focus specifically on use of this technique. Further, descriptions of therapies for the disorders that peripheral acupuncture is supposed to successfully treat rarely include that method. Instead, standard acupuncture techniques without leopard spot technique, as well as herbal therapies, are described<sup>30,42</sup>.

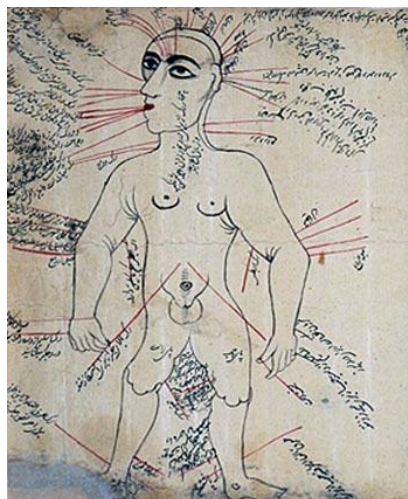


Fig 6. Old picture of the Leopard Spot technique

Letting out blood is among the oldest of acupuncture techniques. Indeed, it has been speculated that acupuncture started as a method of pricking boils, then expanded to letting out "bad blood" that was generated by injuries or fevers, and finally allowing invisible evil spirits and perverse atmospheric qi (most notably "ventus") escape from the body<sup>53</sup>. Only later, perhaps as the needles became more refined and as scholars developed of a more subtle theoretical framework, were thin filiform needles used as the primary acupuncture tools for the purpose of adjusting the flow of qi and blood, without necessarily releasing something from the body<sup>30</sup>.

For excess type syndromes, bleeding is recommended because it can drain the excess, alleviate congestion and stasis, and remove the pathogens. The function of this therapy is "to drain calor or "quicken" the xue and qi and relieve local congestion"<sup>43</sup>.

One of the claims commonly made by Western acupuncturists is that, acupuncture by the leopard spot technique at the *jing*-well points or at the ear can rapidly decrease blood pressure. Acupuncture at the ear apex was mentioned only in passing as one ear acupuncture technique in the book *Traditional Chinese Treatment of Hypertension*<sup>44</sup>, but was reported to be highly effective for hypertension in a single case report<sup>45</sup>.

While standard acupuncture therapy is depicted as being effective, in part, by releasing various transmitter substances (i.e., endorphins), by stimulating local blood flow (i.e., by dilating vessels), and by producing changes in the brain that may have both systemic and highly specific effects, letting out a small amount of blood (usually just a few drops) remains without a suitable explanation for the potent effects claimed. The technique used to let out the blood is one of quick and light pricking to pierce the skin and vein. The Leopard Spot technique has four major therapeutic aims that are useful in the clinical arena<sup>46</sup>:

1. It can invigorate the smooth flow of qi and xue, thereby picking up and facilitating its flow when the qi and xue need invigoration. An example of this scenario occurs when a patient presents with a wiry pulse and mild feelings of stagnation that indicate qi stagnation. Improving circulation and preventing xue from remaining stagnated;
2. It disperses qi and xue stasis, as in cases of backache or spider veins;

3. It can drain excess, calor and ardor. Such excess includes pathogenic factors as in an invasion of Calor-Venti in the Pulmonary conduit that produces a fever and extremely sore throat;
4. Finally, bleeding can bring down yang rising, as in the varieties of high blood pressure due to Hepatic yang rising.

## 7. FEMORAL FRACTURE IN ELDERLY PEOPLE

### 7.1. Background on incidence and potential complications

One of the most common orthopedic problems in the elderly is a hip fracture, or more accurately, fracture of the most proximal portion of the femur in the joint area. The incidence of this type of fracture increases with age<sup>64</sup>. There is a great increase in the incidence of these fractures in the age group above 65 years which is mainly due the installation of the osteoporosis (condition associated with aging, weak bones and commonly affects the hip)<sup>65,66</sup>, as well as to the largest incidence of falls in this age group.

Fractures of the proximal femur are often associated with multiple injuries and high rates of avascular necrosis and nonunion. The post-operative outcome of the affected femur depends on (1) the extent of injury (i.e., amount of displacement, amount of comminution, whether circulation has been disturbed), (2) the adequacy of the reduction, and (3) the adequacy of fixation. Recognition of the disabling complications of femoral neck fractures requires meticulous attention to detail in their management<sup>75</sup>. Osteonecrosis (ON) of femoral head is believed to be the result an interruption in blood supply to the femur head<sup>78</sup>. The incidence of ON depends of fracture type, time until reduction and accuracy of reduction, procedure type, fixation type and surgical technique<sup>65,66</sup>.

General complications, as in any injury or surgery in the elderly, are likely to occur here too, especially: gait impairment, peripheral arterial disease, venous thrombosis, pulmonary embolism, pneumonia and decubitus bedsores. Most of these patients have serious medical conditions and over thirty percent die within two years following the fracture<sup>65,76,77</sup>.

The social and economic costs of fracture of femur rises further by the fact that after a variable period of time hospitalized elderly patients face high mortality rates,

requiring intensive medical care and rehabilitation programs for long periods. In recent studies, senior patients with a fracture of the proximal femur reached the previous capacity to accomplish the daily tasks in only 17% of the cases after 4 months, and only 43% reacquired the previous walking capacity<sup>68</sup>.

Besides the social damage caused by a femoral fracture, the elderly have a reduced functional reservation along with a large number of chronic diseases which are associated with a compromised microcirculation, about 70% of patients have at least two other diseases at the moment of fracture<sup>68</sup> and are therefore much more prone to postoperative complications, both immediate and delayed, with an average of three complications, which in 26% of cases are severe, leading to an increased risk of death<sup>68</sup>.

## **7.2. Connection between age and post-operative mortality**

The mortality rate for the geriatric patients found on the studies of Fransen et al and Meyer et al show that there really is an important influence of the fracture of the proximal femur on the survival of these patients, mainly if compared with the indexes found in the population without fractures. The mortality rate in the control group at six months of follow-up is of 3,2%, at the end of a year 5,2% and after two years 9,6%<sup>69</sup> much smaller than found in the patients with fractures, 10,8%, 19,2% and 24,9% respectively<sup>69,70</sup>. The high age as decisive factor for the greater mortality in geriatric patients with a fracture of the femur is mentioned by several authors. While Alarcón et al<sup>64</sup> conclude that patients with more than 90 years do not present a mortality rate larger than usually found in the literature, Forster et al<sup>71</sup>, when evaluating only patients over 100 years of age, show that 56% of the patients die at the end of one year (this rate is 20% larger than the expected mortality for this age, for people without fractures of the femur). In this group, the incidence of death (31%) is also significantly larger than in the general geriatric group, emphasizing the need of intense medical care during hospitalization

## **7.3. The significance of gait impairment in this clinical scenario**

According to several authors the gait impairment previous to the patient's fracture is directly related to the post-operative mortality<sup>67,70,72</sup>. Those elderly patients

that are able to walk out of house and have a fracture of the femur, present the same death risk as those that do not have a fracture<sup>70</sup>.

#### **7.4. The significance of Algor as a pathogenic factor in this clinical scenario**

According to the HD model of TCM elderly patients with gait impairment after surgery for fracture of the femur are likely to be affected by post-traumatic algor and depletive algor (due to the age-related extent of xue-deficiency and due to surgery-associated losses of blood) which leads to local pain and decreased muscular strength, resulting in general gait impairment.

Post-traumatic algor is induced by tissue trauma due to the initial injury and the surgical intervention. Due to the aforementioned mechanisms (see Chapter 1.2 and 1.4) the tissue MC will be reduced mechanically and by reflex mechanisms (also see Chapter 5.3.2).

The extent of depletive algor depends on the pre-existing xue deficiency (medical history of anemia or diseases associated with compromised MC) as well as surgery-associated blood losses. The depletive algor correlates with a generally reduced functional reservation of MC making the body more susceptible for post-traumatic algor and other complications. Accordingly, Gruson et al<sup>73</sup> have shown the mortality rate at six and twelve months after femoral fracture is directly proportional to the degree of anemia. An elderly with a fracture of the femur and serious anemia has five times more risks of dying than one without anemia.

The relevancy of algor as a pathogenic factor in this clinical scenario automatically implies the application of the ALT as the 3<sup>rd</sup> GC. There are several signs that indicate the presence of a splendor yang state (stage II ALT) such as gait impairment due to post-operative pain in the region of the femur (location of the felleal and stomachal conduit) and reduced muscular strength especially of the musculus quadriceps and the musculus tibialis anterior (stomachal conduit). Furthermore, in this post-operative state patients show a considerable accumulation of humor/pituita with swellings and edemas due to a algor-induced deficiency of drainage through the stomach orb, or, in Western terms due to capillary leak (see Chapter 1.4).

## 7.5. The role of calor/algor as the 2<sup>nd</sup> GC in this clinical scenario

Unlike algor as a pathogenic factor, which relates to a locally reduced MC, the terms of calor and algor as the 2<sup>nd</sup> GC refer to the general state of MC in a body (also see Chapter 5.2 and 5.3).

According to the ALT a lack of microcirculation induced by the agent algor provokes a humero-vegetative reactions with a general increase in MC, also known as "reactive calor" in terms of the 2<sup>nd</sup> GC. This correlates with typical pathophysiological changes as part of what is known as the post-operative inflammatory response in Western medicine<sup>28,29,30,31,32,36,41</sup>.

## 8. OBJECTIVE OF THIS STUDY

The fracture of the proximal femur presents a common and important cause of functional impairment, immobilization and a general increase of morbidity in elderly patients. Acupuncture as one therapeutical method of TCM might accelerate postoperative functional recovery as it has been shown to increase muscular strength, improve gait performance, and influence MC. As outlined in Chapter 4.5 acupuncture research depends on a vegetatively homogeneous study groups in order to reveal significant acupuncture effects. Hence, measurable parameters are needed to help parametrising the TCM diagnosis for future acupuncture studies.

The aim of this study is:

- 1) to demonstrate if it is possible to objectively assess the effects of acupuncture by MC-related parameters in the given clinical scenario
- 2) to evaluate the role of baseline pre-treatment MC-related parameters as an inclusion criterion for the comparability and the statistical significance in future acupuncture studies, and
- 3) to assess the suitability of MC-related parameters for parametrising the TCM diagnosis as vegetative functional state, especially those aspects related to xue (calor/algor).

## CHAPTER TWO



# Material and Methods

## 1. Study design

The study was designed as a prospective, uncontrolled, unblinded clinical trial and was carried out according to the guidelines specified in the Declaration of Helsinki 2008<sup>79</sup>. The ethical committee endorsed the study protocol prior to patient acquisition and informed consent was obtained from every patient.

## 2. Organisational structure

### 2.1 General aspects

The development of this research project occurred as part of the Master Program in Traditional Chinese Medicine and the clinical research activities of the Bethanien Krankenhaus, Heidelberg (Germany), in collaboration with the University of Heidelberg. The research topic (acupuncture in microcirculation) and clinical research protocol is shared with another medical professional Dr. Christoph Doenitz, Internal Doctor of the Bethanien Krankenhaus. The study had no external funding.

### 2.2 Research team

#### 2.2.1 Main investigators

##### **Ana Alexandra Anjos**

- Physiotherapist in the Institut für Chinesische Medizin, Heidelberg-Germany.
- Master student of Traditional Chinese Medicine at Abel Salazar Institute for Biomedical Sciences

##### **Christoph Doenitz**

- Medical Doctor in the Bethanian Krankenhaus, Heidelberg-Germany
- Medical Doctor in the Institut für Chinesische Medizin, Heidelberg-Germany

## 2.2.2 Research supervisors

**Main supervisor:** Prof. Doctor Henry Johannes Greten. Head of the Heidelberg School of Traditional Chinese Medicine; President of the German Society of Traditional Chinese Medicine (DGTCM), Heidelberg, Germany.

### **Co-supervisors:**

Medical Doctor Anna Maimer. Medical Doctor in the Institut für Chinesische Medizin, Heidelberg-Germany. Assistant Specializing in Internal Medicine and Endocrinology. Prof. Assistant in the Heidelberg School of Traditional Chinese Medicine.

Prof. Doctor Anibal António Braga de Albuquerque, Cardiologist at the Centro Hospitalar de Vila Nova de Gaia/Espinho EPE, visiting Assistant Professor in the Abel Salazar Institute for Biomedical Sciences

**2.2.3 Statistical analyses:** statistical analyses were conducted in cooperation with the Institut für Chinesische Medizin, Heidelberg-Germany

## 3. Study objective

The aim of this study is, firstly, to objectively assess the effects of acupuncture on MC-related parameters in patients with gait impairment after surgery for fracture of the proximal femur; Secondly, to evaluate the role of baseline pre-treatment MC-related parameters as an inclusion criterion for the comparability and the statistical significance in future acupuncture studies; Thirdly, to investigate whether MC-related parameters may one day play a role in parametrising the TCM diagnosis as a vegetative functional state, with respect to the diagnosis of algor as an pathogenic factor (local lack of MC) as well as calor/algor as the second guiding criterion (generally increased or reduced MC within the body).

## 4. Study protocol

### 4.1. Setting & Recruitment

This study was conducted in the Bethanien Krankenhaus, Heidelberg (Germany) in cooperation with the University of Heidelberg, Heidelberg (Germany). Patient recruitment from the rehabilitation ward of the Bethanien Krankenhaus was carried out by a medical professional. Patients were selected according to the defined inclusion and exclusion criteria (see 4.4) after a physical examination and medical history review.

## **4.2 Informed consent**

Before patient enrollment the study staff guided the patient through the consent process in which patients were informed about the study design, including the use of penetrating needles, and the possible risks of acupuncture treatment (hematoma, infection and fainting).

## **4.3 Study population**

This study focused on elderly patients who had undergone surgery for proximal femur fracture and who stayed in the Bethanien Krankenhaus for postoperative rehabilitation.

## **4.4 Eligibility criteria**

### **4.4.1 General aspects**

Eligibility criteria were developed with the goal of maximizing enrollment at the local clinical setting. At the same time they served to establish a homogenous group of patients.

### **4.4.2 Inclusion criteria**

Subjects were eligible if they were geriatric patients of the rehab ward of the Bethanien Krankenhaus, aged between 65 and 90 years, who had undergone surgery for proximal fracture of femur. In addition they had to report gait impairment .

### **4.4.3 Exclusion criteria**

Subjects had to be excluded from the study if they were diagnosed dementia with an MMSE score < 24. A medical history of microcirculatory disease or signs of significantly increased microcirculation at baseline (as measured by O2C) were not

accepted. Patients on anticoagulative therapy were also excluded from the study to minimise the risk of secondary hemorrhage after blood-letting acupuncture.

## 4.5 Baseline and control assessment of MC

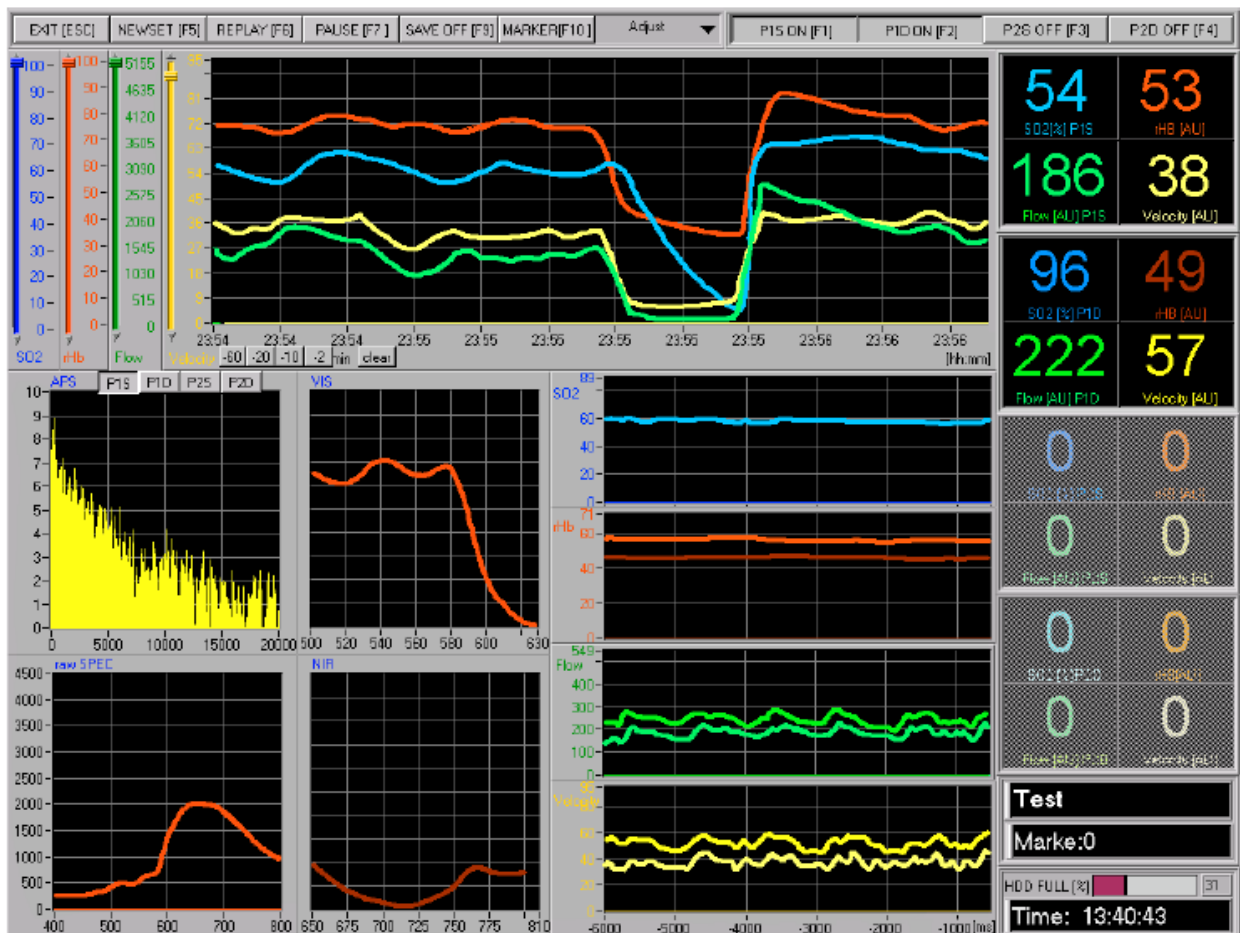
### 4.5.1 General aspects of the O2C device

In this study two aspects of local tissue microcirculation were measured by an oxygen-to-see (O2C) device. The O2C apparatus presents a non-invasive device to measure various parameters of microcirculation and local tissue oxygen saturation in real-time. Figure 7 shows the O2C apparatus. It uses a principle known as "tissue photo spectrometry" by combining white light spectroscopy and laser Doppler spectroscopy in just one device<sup>80</sup>.



Fig. 7- O2C device<sup>80</sup>

The underlying physical principle of optical measurement to determine microvascular blood flow, velocity, local blood content and oxygen saturation in real-time was first introduced in 2002<sup>80,81</sup>. Figure 8 shows the O2C display.



**Fig.8-** Display of O2C device<sup>80</sup>

One key element of the O2C device is its sensor (see Figure 9), which always consists of 6 fiber glass elements<sup>80</sup>:

- 1) fiber 1 for emission of white light
- 2) fiber 2 for detection of laser light in a defined separation (separation = distance between site of illumination and site of detection)
- 3) fiber 3 for detection of white light in a defined separation
- 4) fiber 4 for illumination of laser light
- 5) fiber 5 for detection of white light in a defined separation
- 6) fiber 6 for detection of laser light in a defined separation.



**Fig. 9-** O2C sensor containing 6 fiber glass elements<sup>80</sup>

## **4.5.2 Physical principles**

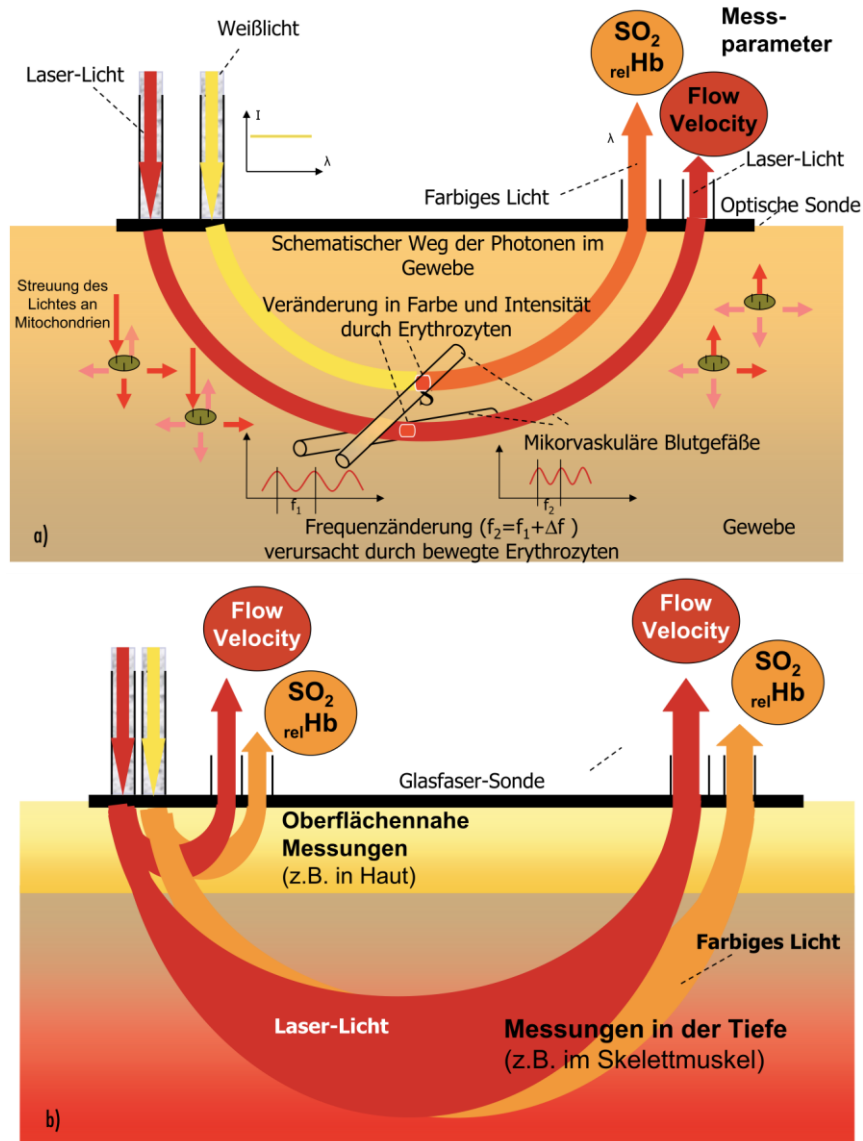
As the fundamental physical principles of the O2C device are complex the following descriptions aim to explain and outline the main processes of measurement in a simplified way.

### **4.5.2.1. Light propagation in tissue**

The O2C-device uses two different optical techniques: the emission of light of a continuous spectrum (=white light) and the application of light of one specific wave length (=laser light). In both techniques the applied light will be specifically altered depending on the characteristics of the investigated tissue<sup>80</sup>.

Irradiated light will be dispersed by mitochondria, so that its direction of propagation is altered. The dispersed light can be detected on the tissue surface by special detection fibers (fiber 2, 3, 5, 6). Through the mentioned dispersion the intensity of light will change, however, its colour will stay the same. In addition, the light will get wave-length-specifically absorbed by blood pigments (especially hemoglobin) depending on their oxygen saturation, and in this way, its colour will change. Hence, the applied light can be detected in changed colour and intensity at a site of detection. The distance between site of emission and site of detection is called separation<sup>80</sup>. By varying the separation and the wavelength range any depth of detection between 100 micrometers and 15 millimeters can be defined. In this way microcirculation in different kinds of tissue (skin, mucosa, muscle, bone) can be investigated<sup>80</sup>. Figure 10 summarizes the principles of light propagation in tissue,

light dispersion by mitochondria, change of colour in white light through absorption of certain wavelengths in blood pigments, Doppler shift phenomenon of laser light and the connection between separation and depth of target tissue.



**Fig. 10-** Propagation of light in tissue<sup>80</sup>. A) spectroscopic determination of local oxygen saturation with white light and measurement of blood flow/velocity with Doppler shift through moving erythrocytes. B) measurement in different depths through variation of separation

#### 4.5.2.2 Laser Doppler

The laser light used in the O2C device almost resembles infrared light with a wavelength of approximately 820nm<sup>80</sup>. If laser light of a specific wavelength strikes an erythrocyte in motion its wavelength will get altered in its frequency, a phenomenon called Doppler-shift. Figure 11 illustrates the fundamental principle of

the Doppler shift or Doppler effect. From the detected Doppler frequency shift the O2C device can calculate the velocity of the erythrocyte<sup>80,83</sup>.

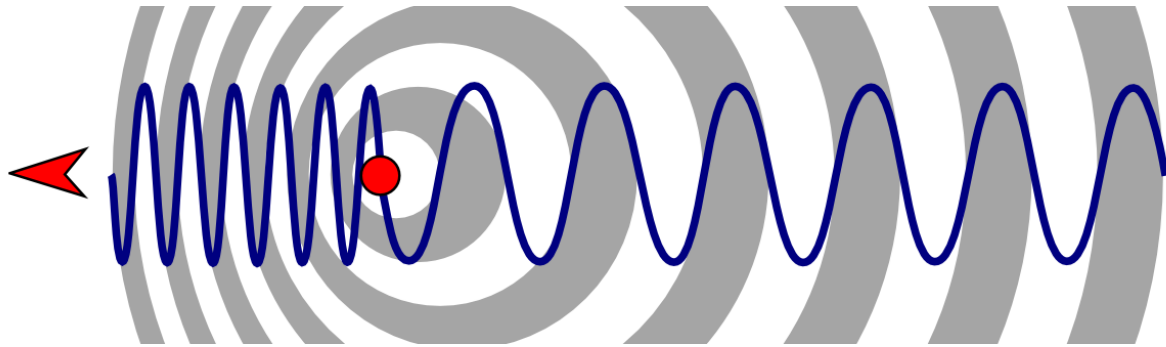


Fig. 11- Doppler shift. Change of wavelength through motion of the source

#### 4.5.2.3 Tissue spectroscopy

Tissue spectroscopy is based on white light technology. White light is characterized by a continuous spectrum of wavelengths. If it is emitted into tissue it will get dispersed by mitochondria and its colour will get altered through absorption of certain wavelengths by local blood pigments, depending on their oxygen saturation (see Figure 12) From the absorption spectrum of the detected light the O2C device can draw conclusions on the local amount of hemoglobin and its oxygen saturation in the investigated tissue through spectral analysis<sup>80,84,85</sup>.

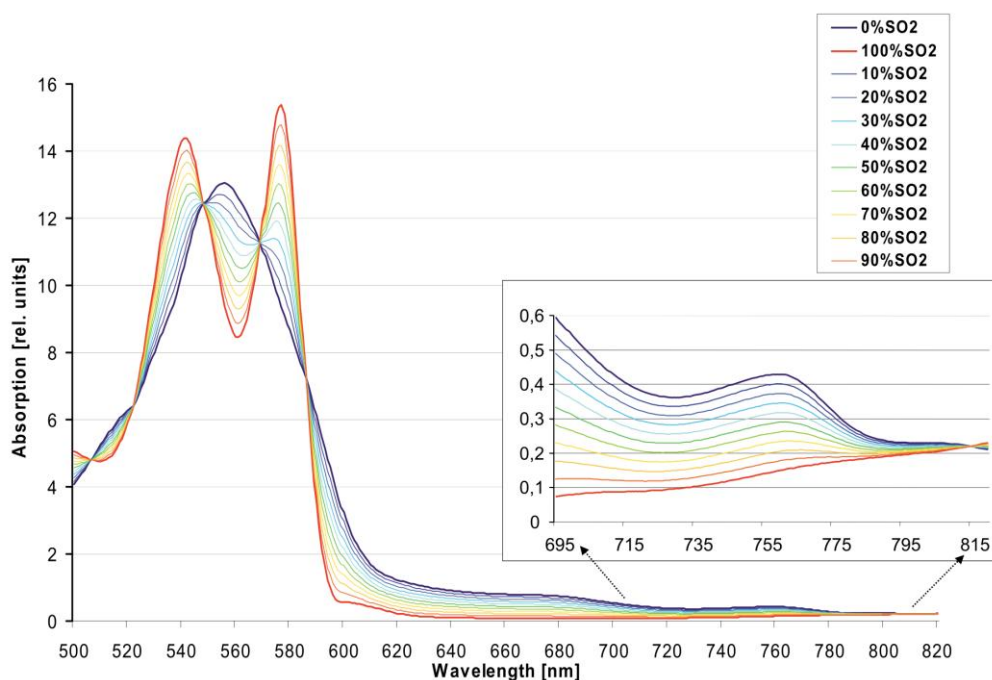


Fig. 12- Absorption spectrum of hemoglobin in relation to degrees of oxygenation (from 0 to 100% saturation in intervals of 10%)<sup>80</sup>

### 4.5.3 Measurement of blood velocity

The measurement of blood flow velocity is carried out by laser doppler. It is reported in arbitrary units (AU)<sup>80,82,86,87</sup>. From a TCM point a view it is likely to mirror – especially in the tissue of interest.

### 4.5.4 Measurement of regional hemoglobin (rHb)

The measurement of rHb is carried out by white light spectroscopy. Values of rHb are reported in arbitrary units (AU)<sup>80,88</sup>. In contrast to the systemic hemoglobin concentration the rHb presents a measurement for the local amount of blood in the microvascular system, As 75% of the rHb is located in the post-capillary part of the microcirculative system the rHb mirror the current degree of filling in the venules, and can thus be used as a marker for degree of venous stagnation or xue stasis respectively<sup>80</sup>.

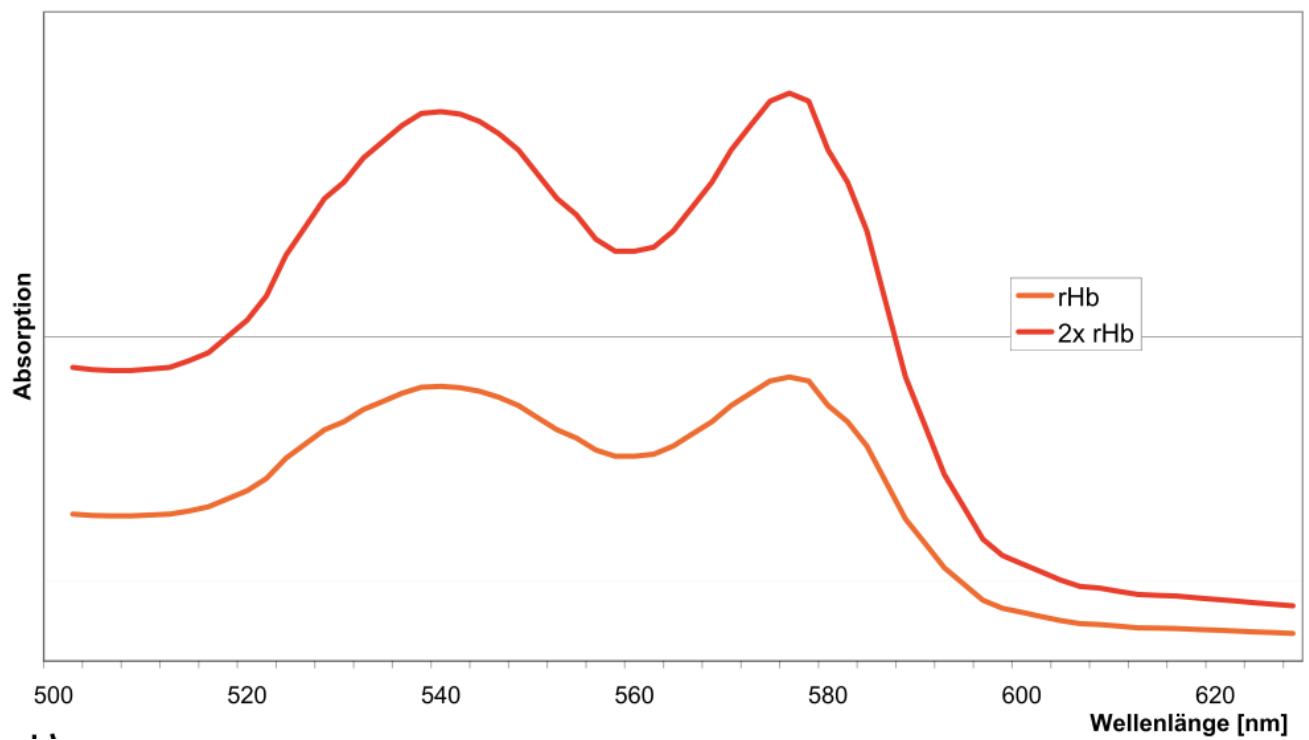


Fig. 13- Absorption spectrum of hemoglobin in relation to local amount of blood (rHb)<sup>80</sup>

### 4.5.5 Practical aspects of baseline and control measurements

Measurement of blood flow velocity and rHb was carried out at downside of the 2<sup>nd</sup> toe (=origin of stomachal conduit) at baseline and directly after acupuncture

treatment, while the patient rested in a supine position. For baseline and control measurement the O2C device automatically took measurements every 2 seconds continuously for a total of 30 seconds, in a depth of 3mm and 6mm.

#### 4.5.6 – Depth of MC measurement and expected target tissue

The skin can generally be divided into 3 layers: the epidermis, dermis and subcutis. The epidermis is the outer layer of the skin. The thickness of the epidermis varies in different types of skin. It is the thickness on the back at .05mm and the thickness on the palms and soles at 1.5mm.

The dermis also varies in thickness depending on the location of the skin. It is 0.3mm on the eyelid, 3.0mm on the back and up to 5.0mm on the soles. The dermis is composed of three types of tissue – collagen, elastic tissue and reticular fibers – that are present throughout – not in layers. The subcutaneous tissue is a layer of fat and connective tissue that houses larger blood vessels and nerves. This layer is important in the regulation of temperature of the skin itself and the body. The size of this layer varies throughout the body and from person to person.

Despite the variability of the skin thickness it can assumed that the MC measurement in 3mm depth was mainly targeting the dermis whereas the MC measurement in 6mm depth was taken from the subcutaneous tissue.

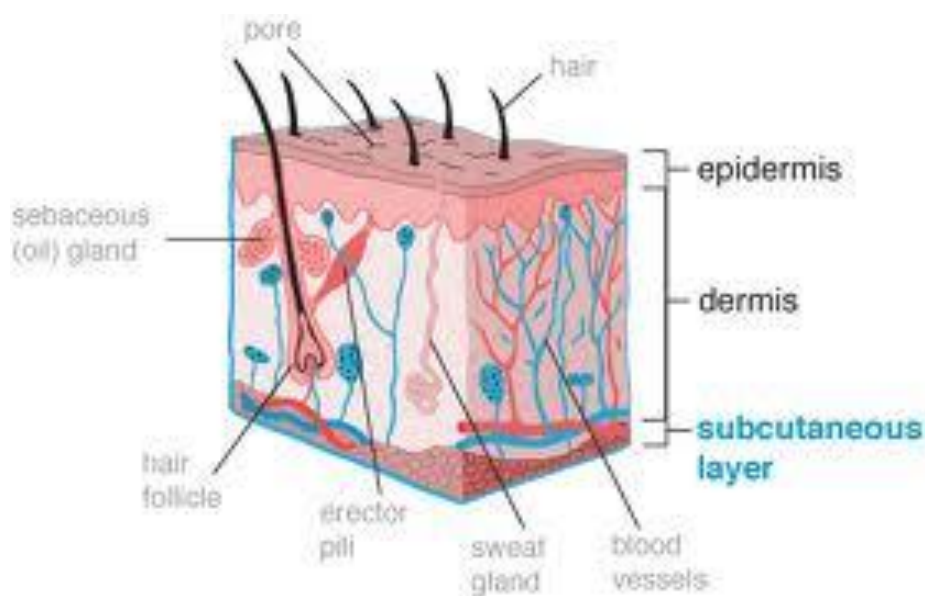
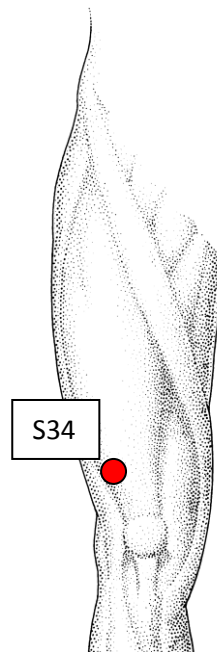


Fig.14 – Anatomy of human skin

## 4.6 Acupuncture intervention

### 4.6.1 – General descriptions

The acupuncture treatment consisted in acupuncture with the technique "Leopard spot" on the Stomachal conduit on the point S34 (see Figure 15). During acupuncture intervention subjects rested in a supine position. Acupuncture was performed using a standard insuline canule.



**Fig. 15-** Location of acupuncture with Leopard spot technique on the stomachal conduit on the point S34.

### 4.6.2 – Background on the Stomachal Conduit

The Stomachal conduit, also known as the sinarteria yang pedis, represents the connection of the 45 individual Stomachal acupoints on the body surface. It runs from the head to the 2<sup>nd</sup> toe (where baseline and control measurement of MC was carried out), passing important anatomical structures such as the breast, the abdomen, the lateral vastus of musculus quadricipes and the musculus tibialis anterior. In correspondence to the systemic down-regulative function of the Stomachal orb<sup>31</sup> the Stomachal conduit is also directed downwards. Together with the Lial Orb the Stomachal orb is responsible to direct xue-flow to the extremities<sup>31</sup>. The course of the Stomachal conduit is illustrated in Fig.12.

The acupuncture treatment in this study focused on the point S34 on the Stomachal, because this acupoint is suitable to treat algor patterns<sup>31,32</sup> (also see

Chapter 4.6.3 in Introduction.). As elderly patients who have undergone surgery for proximal femur fracture are likely to suffer from both post-traumatic algor and depletive algor these acupoints were chosen for acupuncture treatment. Furthermore, the anatomical relation to the musculus quadriceps and the musculus tibialis anterioris highlights the importance of the Stomachal conduit to muscular strength of these muscles and gait performance.

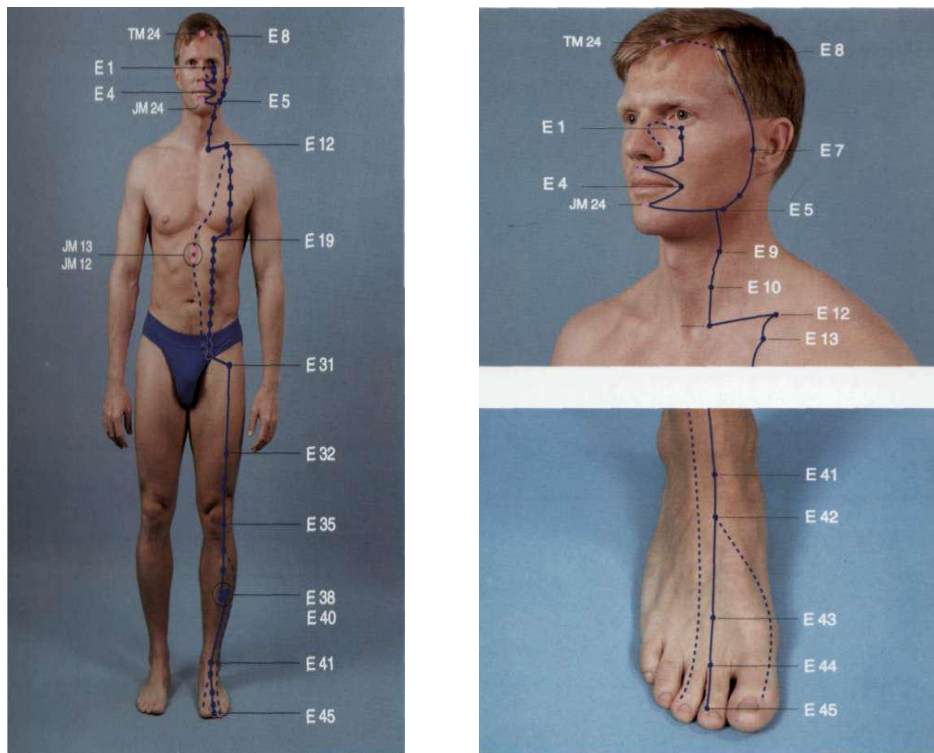


Fig 12. Stomachal conduit

#### 4.6.3 – Background on S34

##### S34<sup>89</sup>

The main action of the S34 point is to activate the conduit and alleviates pain, harmonises the Stomach and alleviates pain, moderate acute conditions and also have an important action against Algor. Its indications are swelling and pain of the knee, difficulty in flexing and extending the knee, pain of the knee and leg, gait impairment, painful obstruction by algor with numbness, cold of the legs and feet, lumbar pain.

#### 4.6.4 – Acupuncture, with Leopard spot technique, as a special acupuncture technique to influence xue

It has already been outlined in Chapter 6.3 this technique presents a special acupuncture technique that is believed to have a considerable effect on xue or MC, respectively. In the given clinical scenario it is used to reinforce xue-flow in the Stomachal conduit to treat the pathogenic factor.

## **5. Statistical analysis**

### **5.1. General aspects**

All statistical analyses were performed with SPSS (Version 17.0). Due to an asymmetric distribution of velocity and rHb non-parametric tests were used for the inductive statistical analysis. Two-sided p-values were reported in all cases and an effect was considered significant at a p-value  $\leq 0.05$ .

### **5.2. Statistical testing**

The Wilcoxon signed-rank test served as a non-parametric "paired difference test", to compare the repeated measurements (baseline measurement and post-acupuncture measurement) of the investigated study population.

### **5.3. Subgroup definition according to baseline MC status**

Baseline analysis of MC data was used to differentiate between two sets of patients:

- 1) Subjects with "lower" perfusion at baseline ("lower perfusion" group LPG))
- 2) Subjects with an elevated microcirculatory status at baseline ("high perfusion" group HPG))

As a reference "normal" baseline values of MC parameters were determined in analogy to international standards of normal lab values, defining normality as two standard deviations from the mean.

In order to investigate the connection baseline microcirculatory status and acupuncture effect on MC-related parameters further statistical analysis was performed on two samples: 1) LPG only and 2) the entire study population (LPG+HPG).



## CHAPTER THREE



# Results

## 1. Subgroup formation

At baseline six patients showed a 6- to 17-fold elevation of MC-related parameters compared to mean values and were, thus, merged to a subgroup called HPG (high-perfusion group, n = 6). The remaining patients were defined as a subgroup called LPG (lower perfusion group, n=26). To investigate the influence of baseline MC on statistical outcome the statistical analysis was performed on three groups: the entire study population (LPG + HPG), the HPG subgroup (n=6) and the LPG subgroup (n=26).

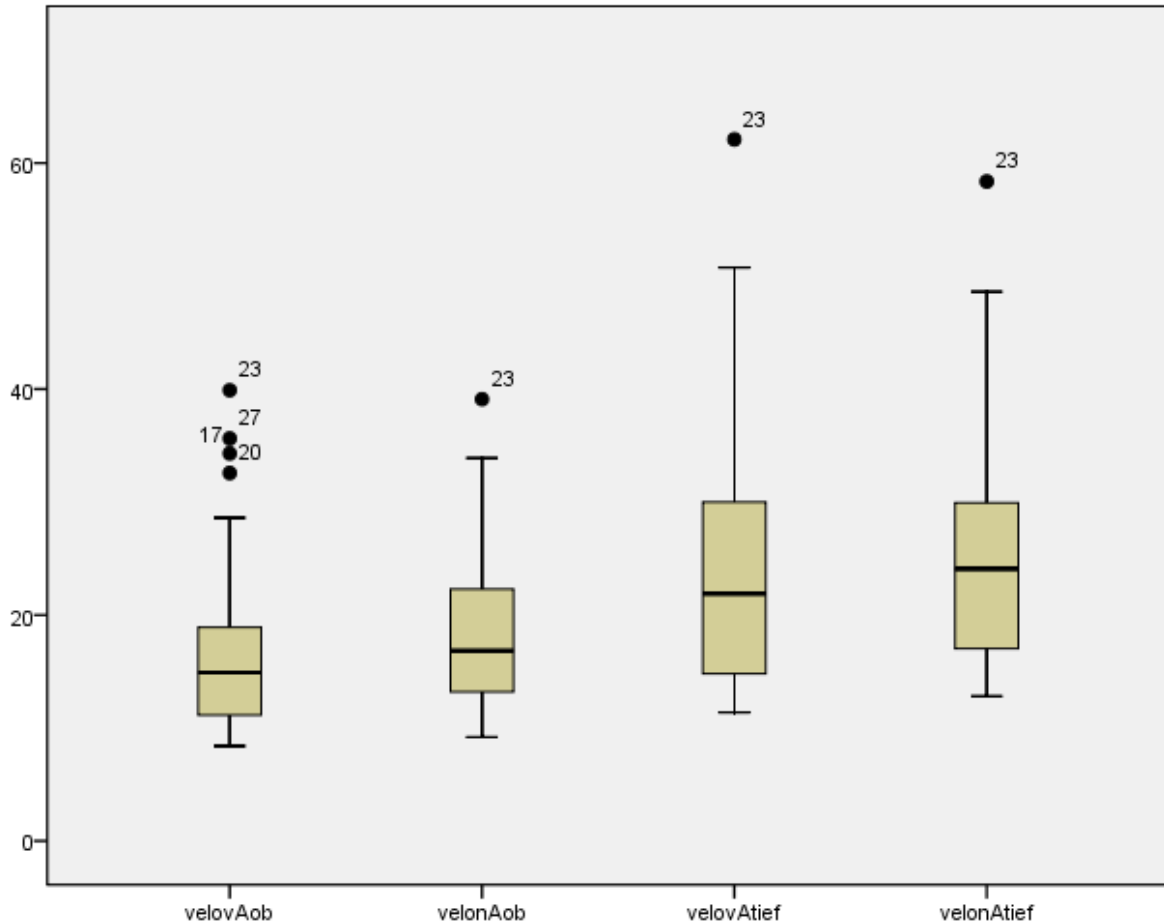
## 2. Efficacy analysis of entire study population (LPG + HPG)

### 2.1 Acupuncture effect on blood flow velocity

#### 2.1.1 Descriptive statistical analysis

Baseline assessment revealed a mean velocity of 17,05 AU (arbitrary units) (standard deviation (SD) = 8,58) at 3mm-depth and a mean velocity of 24,51 AU at 6mm-depth (SD = 12,46). The maximum baseline velocity was 39,9 AU at 3mm-depth and 62,1 AU at 6mm-depth; the minimum baseline velocity was 8,4 AU superficially (3mm depth) and 11,38 AU at 6mm-depth.

After acupuncture treatment the mean velocity increased by 9,5% at 3mm-depth, and by 5,5% at 6mm-depth. Figure 17 displays the distribution of velocity measurements at baseline and after acupuncture intervention. Table 1 summarizes the results of the descriptive statistical analysis for baseline and post-interventional velocity measurements.



**Fig. 17** Distribution of blood flow velocity (vel) at baseline (vA) and after acupuncture intervention (nA), at 3mm-depth (ob) and at 6mm-depth (tief). The Box-and-Whisker-Plots represent the lower quartile (lower line of the box), the median (horizontal line in the box) and the upper quartile (upper line of the box). The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers are defined as values that are between 1.5 and 3 times the interquartile range and are marked by dots. Extreme values are defined as values that are more than 3 times the interquartile range and are marked by asterisks.

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
velovAob	17,05	14,91	8,40	39,90	73,68	8,58
velonAob	18,67	16,83	9,19	39,10	59,89	7,74
velovAtief	24,51	21,89	11,38	62,10	155,28	12,46
velonAtief	25,86	24,09	12,81	58,38	124,14	11,14

**Table 1.** Summary of descriptive statistical analysis for baseline and post-interventional velocity measurement.

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

## 2.1.2 Inductive statistical analysis

The Wilcoxon signed-rank test revealed that the observed mean increase of velocity after acupuncture intervention remained below statistical significance, with a two-sided p-value of 0,01 at 3mm-depth and 0,098 at 6mm-depth. Table 2 summarizes the results of the Wilcoxon signed-rank test.

	VelonAob – velovAob	VelonAtief – velovAtief
Z	-2,587	-1,655
Asymp. Sig. (2-tailed)	0,010	0,098

**Table 2-** Results of Wilcoxon signed-rank test for velocity measurements

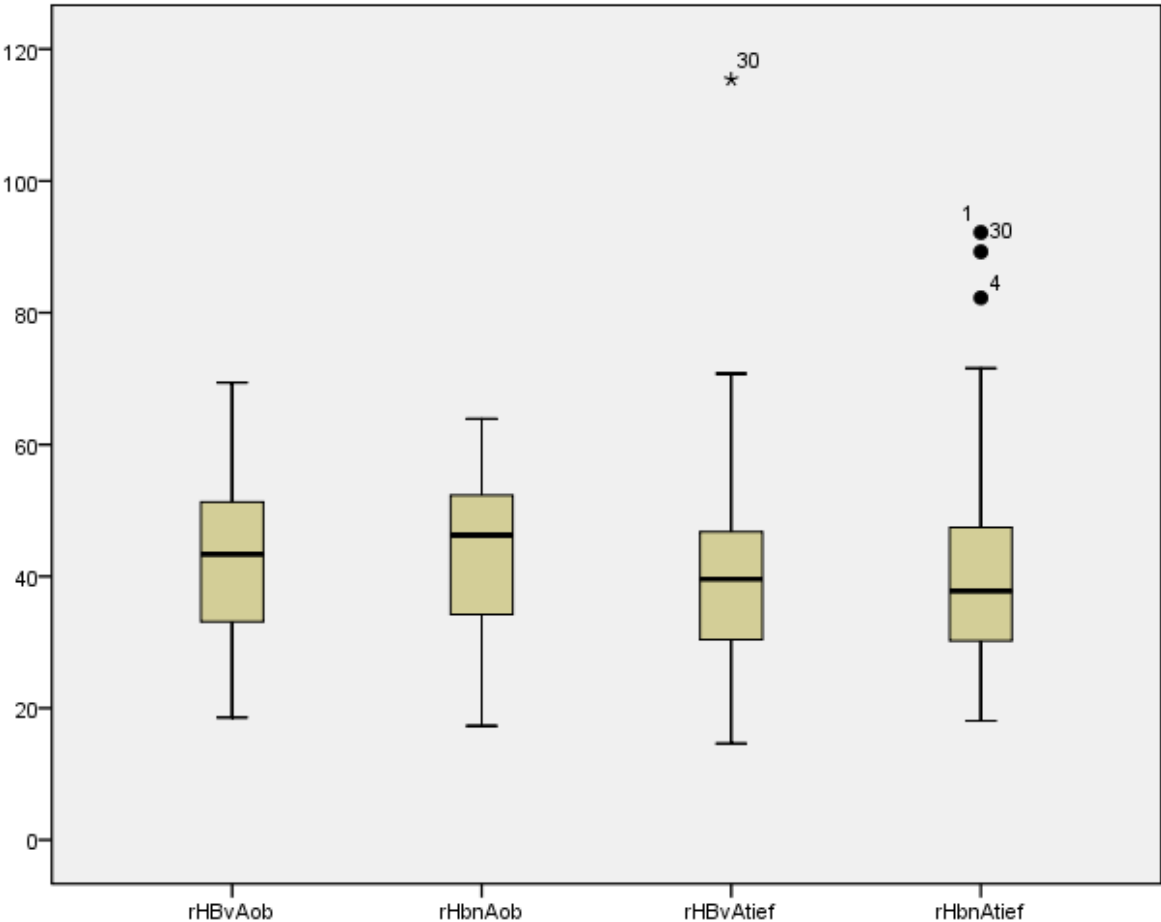
**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

## 2.2 Acupuncture effect on regional hemoglobin (rHb)

### 2.2.1 Descriptive statistical analysis

Baseline assessment revealed a mean rHb of 44,1 AU (SD = 12,48) superficially (3mm-depth) and a mean rHb of 43,56 AU at 6mm-depth (SD = 13,15). The maximum baseline rHb was 69,4 AU superficially and 115,4 AU at 6mm-depth; the minimum baseline rHb was 18,6 AU at 3mm-depth and 14,63 AU at 6mm-depth.

After acupuncture treatment the mean rHb at 3mm-depth decreased by 0,54%. In 6mm-depth the mean rHb increased by 1,35%. Figure 18 displays the distribution of rHb measurements at baseline and after acupuncture intervention. Table 3 summarizes the results of the descriptive statistical analysis for baseline and post-interventional rHb measurements.



**Fig. 18-** Distribution of regional hemoglobin (rHb) at baseline (vA) and after acupuncture intervention (nA), at 3mm-depth (ob) and at 6mm-depth (tief). The Box-and-Whisker-Plots represent the lower quartile (lower line of the box), the median (horizontal line in the box) and the upper quartile (upper line of the box). The

whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers are defined as values that are between 1.5 and 3 times the interquartile range and are marked by dots. Extreme values are defined as values that are more than 3 times the interquartile range and are marked by asterisks.

**Abbreviations:** rHBvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHBvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth).

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
rHBvAob	44,10	43,35	18,60	69,40	155,72	12,48
rHbnAob	43,56	46,25	17,30	63,90	172,83	13,15
rHBvAtief	41,74	39,59	14,63	115,40	371,61	19,28
rHbnAtief	42,02	37,78	18,06	92,19	360,68	18,99

**Table 3.** Summary of descriptive statistical analysis for baseline and post-interventional rHb measurement

**Abbreviations:** rHBvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHBvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

### 2.2.2 Inductive statistical analysis

The Wilcoxon signed-rank test revealed that changes in rHb between baseline and post-interventional measurements remained below statistical significance (p-value 3mm-depth = 0,906; p-value 6mm-depth = 0,911) Table 4 summarizes the results of the Wilcoxon signed-rank test.

	rHbnAob – rHbvAob	rHbnAtief – rHbvAtief
Z	-0,118	-0,112
Asymp. Sig. (2-tailed)	0,906	0,911

**Table 4.** Results of the Wilcoxon signed-rank test for rHb measurements

**Abbreviations:** rHbvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHbvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

### 3. Efficacy analysis of HPG Subgroup

#### 3.1 Acupuncture effect on blood flow velocity

##### 3.1.1 Descriptive statistical analysis

At baseline the mean velocity in the HPG subgroup (n=6) was 30,42 AU (SD = 7,12) at 3mm-depth and 42,37 AU at 6mm-depth (SD = 12,59). After acupuncture treatment the mean velocity decreased by 6,7% at 3mm-depth, and by 7,6% at 6mm-depth. Table 5 summarizes the results of the descriptive statistical analysis for baseline and post-interventional velocity measurements.

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
VelovAob	30,42	30,58	20,10	39,90	50,64	7,12
VelonAob	28,39	29,11	14,10	39,10	68,71	8,29
VelovAtief	42,37	40,46	26,90	62,10	158,60	12,59
VelonAtief	39,15	37,16	26,38	58,38	155,82	12,48

**Table 5.** Summary of descriptive statistical analysis for baseline and post-interventional velocity measurement in the HPG subgroup.

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

### 3.1.2 Inductive statistical analysis

According to the Wilcoxon signed-rank test the observed mean decreases in velocity after acupuncture intervention remained below statistical significance, with a two-sided p-value of 0,078 at 3mm-depth and 0,116 at 6mm-depth. Table 6 summarizes the results of the Wilcoxon signed-rank test.

	VelonAob – velovAob	VelonAtief – velovAtief
Z	-1,761	-1,572
Asymp. Sig. (2-tailed)	0,078	0,116

**Table 6.** Results of Wilcoxon signed-rank test for velocity measurements in the HPG subgroup

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

## 3.2 Acupuncture effect on regional hemoglobin (rHb)

### 3.2.1 Descriptive statistical analysis

Baseline assessment of the HPG subgroup (n=6) revealed a mean rHb of 50,23 AU (SD = 6,89) superficially (3mm-depth) and a mean rHb of 39,54 AU at 6mm-depth (SD = 13,75). After acupuncture treatment the mean rHb at 3mm-depth increased by 2,5%. In 6mm-depth the mean rHb decreased by 7,4%. Table 7 summarizes the results of the descriptive statistical analysis for baseline and post-interventional rHb measurements.

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
rHBvAob	50,23	49,75	39,60	59,70	47,54	6,89
rHbnAob	51,26	49,03	44,94	61,80	43,81	6,62
rHBvAtief	39,54	38,58	22,90	56,38	189,00	13,75
rHbnAtief	36,63	36,00	22,69	50,88	114,47	10,70

**Table 7.** Summary of descriptive statistical analysis for baseline and post-interventional rHb measurement in the HPG subgroup (n=6)

**Abbreviations:** rHBvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHBvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

### 3.2.2 Inductive statistical analysis

The Wilcoxon signed-rank test revealed that the post-interventional increase in rHb at 3mm-depth as well as the post-interventional decrease in rHb at 6mm-depth did not attain statistical significance (p-value 3mm-depth = 0,600; p-value 6mm-depth = 0,173) Table 8 summarizes the results of the Wilcoxon signed-rank test.

	rHbnAob – rHBvAob	rHbnAtief – rHBvAtief
Z	-0,524	-1,363
Asymp. Sig. (2-tailed)	0,600	0,173

**Table 8.** Results of the Wilcoxon signed-rank test for rHb measurements in the HPG subgroup

**Abbreviations:** rHBvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHBvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth),

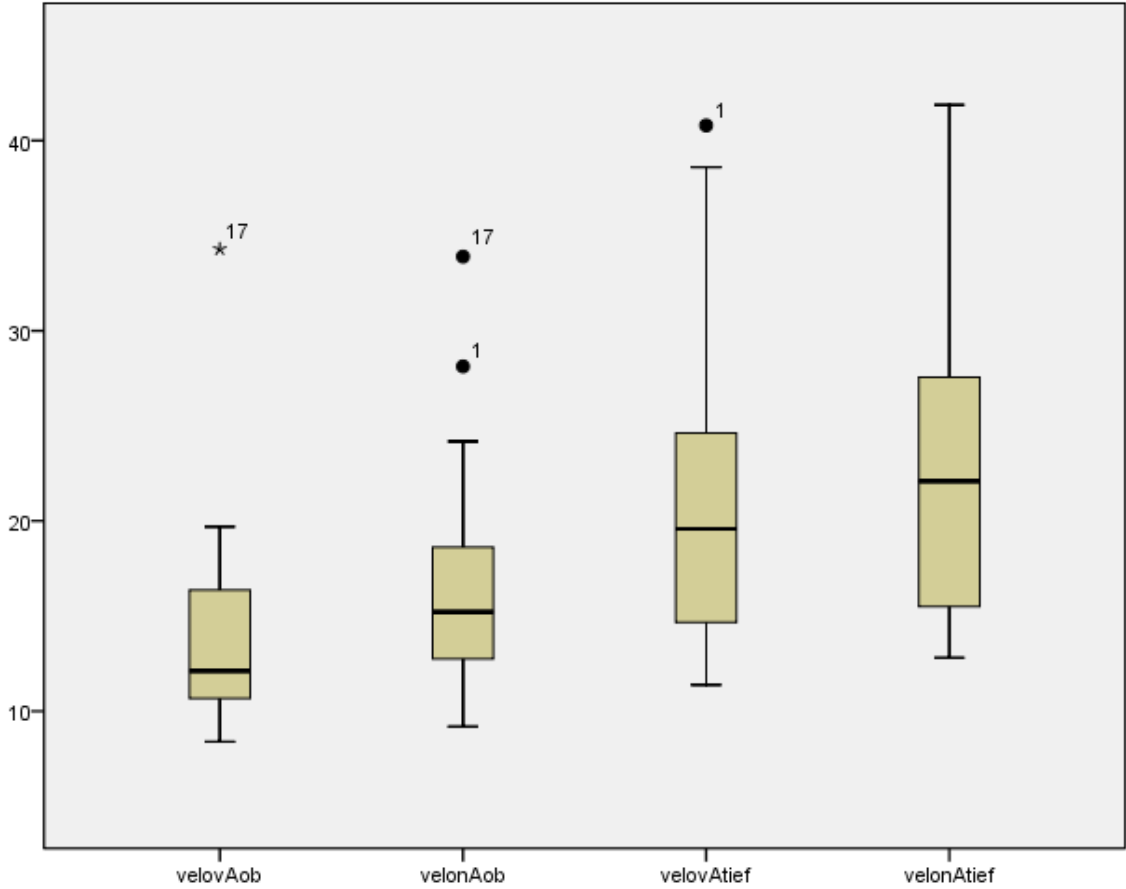
# 4. Efficacy analysis of LPG Subgroup

## 4.1 Acupuncture effect on blood flow velocity

### 4.1.1 Descriptive statistical analysis

Baseline assessment revealed a mean velocity of 13,96 AU (standard deviation (SD) = 5,33) at 3mm-depth and a mean velocity of 20,38 AU at 6mm-depth (SD = 8,16). The maximum baseline velocity was 28,38 AU at 3mm-depth and 66,53 AU at 6mm-depth; the minimum baseline velocity was 8,4 AU superficially (3mm depth) and 11,38 AU at 6mm-depth.

After acupuncture treatment the mean velocity increased by 17,6% at 3mm depth, and by 11,9% at 6mm-depth. Figure 19 displays the distribution of velocity measurements at baseline and after acupuncture intervention. Table 9 summarizes the results of the descriptive statistical analysis for baseline and post-interventional velocity measurements.



**Fig. 19** Distribution of blood flow velocity (vel) at baseline (vA) and after acupuncture intervention (nA), at 3mm-depth (ob) and at 6mm-depth (tief). The Box-

and-Whisker-Plots represent the lower quartile (lower line of the box), the median (horizontal line in the box) and the upper quartile (upper line of the box). The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers are defined as values that are between 1.5 and 3 times the interquartile range and are marked by dots. Extreme values are defined as values that are more than 3 times the interquartile range and are marked by asterisks.

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
velovAob	13,96	12,12	8,40	34,30	28,38	5,33
velonAob	16,42	15,22	9,19	33,90	32,57	5,71
velovAtief	20,38	19,58	11,38	40,80	66,53	8,16
velonAtief	22,80	22,09	12,81	41,88	70,65	8,41

**Table 9.** Summary of descriptive statistical analysis for baseline and post-interventional velocity measurement.

**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

#### 4.1.2 Inductive statistical analysis

The Wilcoxon signed-rank test revealed that the observed mean increase of velocity after acupuncture intervention attained statistical significance, with a two-sided p-value < 0,001 at 3mm-depth and 0,006 at 6mm-depth. Table 10 summarizes the results of the Wilcoxon signed-rank test.

	VelonAob – velovAob	VelonAtief – velovAtief
Z	-3,543	-2,730
Asymp. Sig. (2-tailed)	<u>0,000</u>	<u>0,006</u>

**Table 10.** Results of Wilcoxon signed-rank test for velocity measurements

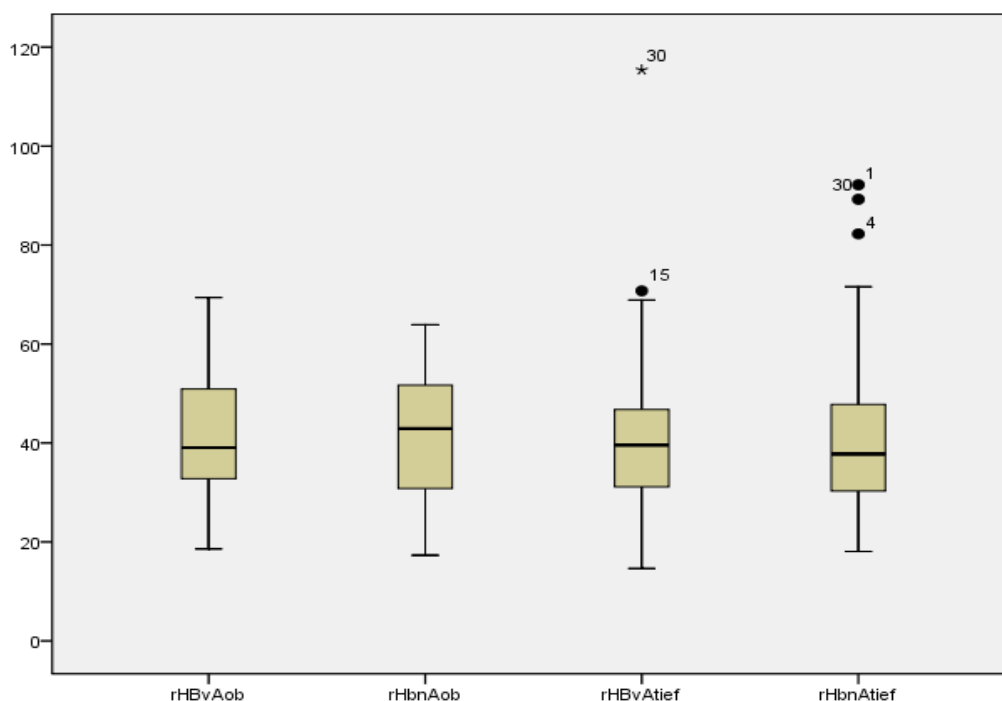
**Abbreviations:** velovAob (velocity at baseline at 3mm-depth), velonAob (velocity after acupuncture at 3mm-depth), velovAtief (velocity at baseline at 6mm-depth), velonAtief (velocity after acupuncture at 6mm-depth)

## 4.2 Acupuncture effect on regional hemoglobin (rHb)

### 4.2.1 Descriptive statistical analysis

Baseline assessment revealed a mean rHb of 42,68 AU (SD = 13,13) superficially (3mm-depth) and a mean rHb of 42,25 AU at 6mm-depth (SD = 20,53). The maximum baseline rHb was 69,4 AU superficially and 115,4 AU at 6mm-depth; the minimum baseline rHb was 18,6 AU at 3mm-depth and 14,63 AU at 6mm-depth.

After acupuncture treatment the mean rHb showed no increase at 3mm-depth. In 6mm-depth the mean rHb increased to 43,26 AU. Figure 20 displays the distribution of rHb measurements at baseline and after acupuncture intervention. Table 11 summarizes the results of the descriptive statistical analysis for baseline and post-interventional rHb measurements.



**Fig. 20** Distribution of regional hemoglobin (rHb) at baseline (vA) and after acupuncture intervention (nA), at 3mm-depth (ob) and at 6mm-depth (tief). The Box-and-Whisker-Plots represent the lower quartile (lower line of the box), the median (horizontal line in the box) and the upper quartile (upper line of the box). The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers are defined as values that are between 1.5 and 3 times the interquartile range and are marked by dots. Extreme values are defined as values that are more than 3 times the interquartile range and are marked by asterisks.

**Abbreviations:** rHbvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHbvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

	Mean	Median	Minimum	Maximum	Variance	Standard Deviation
rHBvAob	42,68	39,05	18,60	69,40	172,46	13,13
rHbnAob	41,78	42,90	17,30	63,90	188,02	13,71
rHBvAtief	42,25	39,59	14,63	115,40	421,56	20,53
rHbnAtief	43,26	37,78	18,06	92,19	415,76	20,39

**Table 11.** Summary of descriptive statistical analysis for baseline and post-interventional rHb measurement

**Abbreviations:** rHbvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHbvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

#### 4.2.2 Inductive statistical analysis

The Wilcoxon signed-rank test revealed that changes in rHb between baseline and post-interventional measurements remained below statistical significance (p-value 3mm-depth = 0,757; p-value 6mm-depth = 0,751) Table 12 summarizes the results of the Wilcoxon signed-rank test.

	rHbnAob – rHbvAob	rHbnAtief – rHbvAtief
Z	-0,309	-0,317
Asymp. Sig. (2-tailed)	0,757	0,751

**Table 12.** Results of the Wilcoxon signed-rank test for rHb measurements

**Abbreviations:** rHbvAob (rHb at baseline at 3mm-depth), rHbnAob (rHbnAob after acupuncture at 3mm-depth), rHbvAtief (rHb at baseline at 6mm-depth), rHbnAtief (rHb after acupuncture at 6mm-depth)

## 5. Summary of results

Efficacy analysis of the entire study population (LPG + HPG) and the HPG subgroup did not reveal statistically significant changes of blood flow velocity or rHb, neither in 3mm-depth nor in 6mm-depth. After excluding the HPG to form the LPG subgroup efficacy analysis revealed a statistically significant mean increase in flow velocity by 17,6% at 3mm-depth ( $p < 0.001$ ), and a 11,9% mean increase at 6mm-depth ( $p = 0.006$ ), following acupuncture intervention. Post-treatment changes of rHb in the LPG did not attain statistical significance.



## CHAPTER FOUR



# Discussion

## Distinctive features of the study design

This clinical trial was designed as a preliminary, uncontrolled, unblinded study in order to assess new methods of visualizing acupuncture effects via MC-related parameters as well as parametrizing aspects of the TCM diagnosis that are related to current MC status of study subjects. The findings of the study serve as the basis for further controlled and more elaborated studies on this subject. Unlike many clinical acupuncture trials that are based on "soft" parameters such as pain assessment via visual analogue scales or the use of questionnaires the study focused on the real-time measurement of objective MC-related parameters leaving no space for subjectiveness. The investigated acupuncture intervention – acupuncture with the Leopard spot technique, on the stomachal conduit on the point S34 – was selected to treat algor patterns. The selection of this acupoint based on the presumption that elderly patients with gait impairment after surgery for fracture of the proximal femur are likely to have post-traumatic and depletive algor in the stomachal conduit. It is important to note that according to the HD model of TCM the existence of algor patterns is an essential pre-condition to make this treatment effective.

## Discussion of subgroup formation

One of the study objectives was to evaluate the role of baseline pre-treatment MC status on the outcome of the efficacy analysis (see Chapter 8 of Introduction). Baseline analysis of the entire study population revealed a distinct statistical spread, indicating that the study population was fairly heterogenous with respect to the MC aspect of their vegetative status. Hence, 2 subgroups, LPG and HPG, were defined in accordance to the MC status at baseline (see Chapter 5.3 of Material & Methods). In terms of the TCM diagnosis the LPG subgroup is likely to have local algor in the stomachal conduit at the site of operation and normal overall perfusion of the microcirculatory system, as their baseline MC was within two standard deviation from the mean. The HPG subgroup, on the other hand, supposedly has an overall increased MC due to an overreaching reactive calor reaction.

## **Interpretation of efficacy results**

In order to evaluate the role of baseline MC status on the study outcome efficacy analysis was performed on 3 groups: 1) the entire study population regardless of the baseline MC status, 2) the OPG subgroup and 3) the NPG subgroup.

## **The role of baseline MC on efficacy results**

The HPG subgroup as well as the entire study population failed to show statistically significant alterations of either blood flow velocity or rHb after acupuncture treatment. Only in the LPG subgroup efficacy analysis revealed a statistically significant increase of blood flow velocity in 3mm- and 6mm-depth, while rHb did not change in a statistically significant manner.

These results support the hypothesis of the HD model of TCM which says that acupoints elicit specific vegetative reflexes that have to match the current vegetative state of a patient to result in clinically significant, measurable effects.

In relation to this study the investigated acupuncture treatment, which is indicated to treat algor patterns, can only result in measurable MC changes if it is performed on patients with locally reduced MC (=algor as a pathogenic factor) and "lower" overall perfusion (=LPG subgroup).

A pre-existing state of high-perfusion (=calor as 2<sup>nd</sup> GC in the HPG subgroup) without locally reduced MC makes the chose acupoint unsuitable and thus objectively ineffective.

In summary, the observed results of the study are compatible with the theoretical background of the HD model of TCM. It is interesting to note that a heterogenous study population resulting from a general lack of parameters to better define the vegetative state (=TCM diagnosis) of interest will lead to a blurred outcome. Mixing patients with TCM diagnosis in which the point is indicated and not indicated, results in hiding objectively existing acupuncture effects. This may be a cause of unsuccessful acupuncture studies. Accordingly, in this study the efficacy

analysis of the overall study population did not show any statistically significant changes in MC.

### **Findings of acupuncture effect on MC**

The efficacy results of the LPG subgroup suggest that the investigated acupuncture treatment has a specific effect on blood flow velocity in the stomachal conduit as there have been statistically significant increases at the origin of the stomachal conduit following acupuncture treatment. As blood flow velocity measurement was taken distant from the location of acupuncture treatment it suggests that the observed velocity increases are not only caused by local release of vasoactive substances at the site of needle insertion (=non-specific effects of acupuncture). Specific vegetative reflexes elicited by the treated acupoints seem to play a role.

Studies from the Sandberg group have also demonstrated that acupuncture can induce increased muscle blood flow and skin blood flow, and the induction of increased muscle blood flow from the stimulation of acupuncture points is due to activation of nociceptors<sup>104</sup>. Sandberg et al.<sup>105</sup> also show that "deep stimulation" of the tibialis anterior muscle with needles resulted in a greater increase of blood flow in skin and muscle blood flow when compared to "Subcutaneous insertion."

### **Suitability of rHb and blood flow velocity to parametrize calor/algor**

The observed changes of blood flow velocity following acupuncture intervention in the different study groups are compatible with the pathophysiological hypothesis of calor/algor based on the HD model of TCM: if there is calor in terms of the 2<sup>nd</sup> GC at baseline (=HPG) the acupuncture treatment in the point S34 does not increase blood flow velocity. On the contrary, it even leads to a minimal, statistically insignificant reduction of velocity, correlating with the fundamental principle of up- and down-regulation of the orthopathia. If there is algor as a pathogenic factor in the stomachal conduit the acupuncture treatment leads to an increase in blood flow velocity. Hence, the measurement of blood flow velocity seems to be suitable to parametrize calor/algor patterns.

According to the efficacy results concerning post-treatment rHb-changes there was no statistically significant alteration in any of the investigated study group. As we know hemoglobin is predominantly located in the venous branch of

the microcirculatory system (also see Chapter 4.5.4 of Methods & Results), this parameter may correlate to the stagnation of xue in terms of the HD model of TCM. As this condition was not being treated with the investigated acupuncture intervention the result is compatible with the HD model. Hence, changes in rHb seem to be unsuitable to reflect effects of acupoints on algor.

### **The O2C device as a useful tool in parametrizing TCM diagnosis**

The results of this study also reveal that O2C measurements provide a useful tool to objectify physiological acupuncture effects. The effects within a depth of 3 and 6 mm can be chosen for further studies. As the O2C device enables to measure MC-related parameters that correlate with calor/algor-patterns it seems to be a useful tool for future acupuncture trails in order to further define the vegetative status of interest, in other words the TCM diagnosis of interest. In other words, it may facilitate the definition of the vegetative functional properties of target tissues to specify inclusion criteria in future studies. It can also be used to predict which patient target group will benefit from a certain acupuncture treatment.

### **Limitations of the study**

As this study was designed as a preliminary trail obvious limitations are the lack of controls and the limited number of patients. Further randomized, blinded, clinical trails with suitable control groups and a larger subject sample are needed to confirm the observed acupuncture effects. Furthermore the formation of subgroups results in multiple testing which constrains the statistical validity.

### **Impact of the findings on future acupuncture trails**

The results of this study encourage further studies aimed to better understanding the mechanisms involved in acupuncture. In these investigations the measurement of MC-related parameters such as blood flow velocity using the O2C device can contribute to the advancement in this area of research.

The findings have highlighted the importance to parametrize the TCM diagnosis in order to define homogenous study groups with assimilable baseline

vegetative status in order to produce significant efficacy results. The failure to define inclusion criteria that respect both the Western AND the TCM diagnosis of the patients might be one reason why randomized clinical trials on acupuncture frequently fail to prove efficacy of the investigated acupuncture treatment. Here, an upgrade of inclusion criteria including parameters of the vegetative regulation (such as MC status) is essential for future acupuncture trails. Inclusion criteria in acupuncture studies should be founded on western *and* Chinese diagnostic criteria as the TCM diagnosis is important for acupoint selection.

## Conclusion

Microcirculatory-related parameters maybe worthwhile:

- To measure acupuncture effects objectively;
- To characterize the vegetative functions (tissue regulations), as they are useful to define the vegetative pre-interventional state which in TCM is specified by a functional TCM diagnoses. Future studies may be directed to correlate the vegetative status as measurable by TCM vegetative parameters with the key symptoms of TCM diagnosis. Another study purpose is to use MC-related parameters as an inclusion criteria to homogenize the study groups functionally. This may drastically enhance statistical power of acupuncture studies.
- Doble- and triple-blinded studies may then be designed to exclude any doubts of clinical acupuncture effects.



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## APPENDIX



## Appendix 1.

# DECLARAÇÃO DE CONSENTIMENTO

(traduzida do alemão)

*Considerando a “Declaração de Helsínquia” da Associação Médica Mundial*

*(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)*

### **Designação do Estudo (traduzida do alemão):**

AVALIAÇÃO DOS EFEITOS DA ACUPUNTURA COM PARAMETROS RELACIONADOS COM MICROCIRCULAÇÃO EM PACIENTES COM FRATURA PROXIMAL DO FEMUR

**Eu, abaixo-assinado, (nome completo do participante)**

-----,  
compreendi a explicação que me foi fornecida, por escrito e verbalmente, da investigação que se tenciona realizar, para qual é pedida a minha participação. Foi-me dada oportunidade de fazer as perguntas que julguei necessárias, e para todas obtive resposta satisfatória.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação que me foi prestada versou os objectivos, os métodos, os benefícios previstos, os riscos potenciais e o eventual desconforto. Além disso, foi-me afirmado que tenho o direito de decidir livremente aceitar ou recusar a todo o tempo a minha participação no estudo. Sei que se recusar não haverá qualquer prejuízo na assistência que me é prestada.

Foi-me dado todo o tempo de que necessitei para reflectir sobre esta proposta de participação.

Nestas circunstâncias, decido livremente aceitar participar neste projecto de investigação, tal como me foi apresentado pelo investigador.

Data: \_\_\_\_ / \_\_\_\_\_ / 20\_\_\_\_

***Assinatura do(a) participante:***

-----

**O Investigador responsável:**

*Nome:*

-----

*Assinatura:*

-----

**Appendix 2.**  
(Original)

## Einverständniserklärung

### **NACH DER "DEKLARATION VON HELSINKI" DES WELTÄRZTEVERBANDES**

*(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)*

#### **STUDIENBESCHREIBUNG:**

BEWERTUNG DER AKPUKTURWIRKUNG AUF DIE MICRO-ZIERKULIERUNG DER PATIENTEN MIT FREMUR-FRAKTUR PROXIMAL.

**ICH, (vollständiger Name des Teilnehmers)**

-----, habe die Erklärung über die bevorstehende Forschung, bei der ich gebeten wurde teilzunehmen verstanden. Ich hatte die Möglichkeit alle Fragen zu stellen, die ich für notwendig empfand, und für alle erhielt ich eine zufriedenstellende Antwort.

Mir wurden, laut der Empfehlung der Deklaration von Helsinki, die Methoden, die zu erwartenden Vorteile, die möglichen Risiken und die eventuellen negativen Folgen auf das Wohlbefinden, mitgeteilt. Ausserdem habe ich das Recht freiwillig an der Studie teilzunehmen oder sie jederzeit abzubeheben. Ich weiss, dass ich keinerlei Nachteile in der Hilfeleistung zu befürchten habe, wenn ich die Studie ablehnen sollte.

Mir wurde die nötige Zeit gegeben über die Teilnahme an dieser Studie gründlich nachzudenken.

In diesem Zusammenhang entscheide ich mich freiwillig an diesem Projekt teilzunehmen, so wie sie mir von dem Forscher vorgelegt wurde

Datum: \_\_\_\_ / \_\_\_\_\_ / 20\_\_\_\_

**Unterschrift des Teilnehmers:**

-----

**Der verantwortliche Forscher:**

*Name:*

-----

*Unterschrift:*

-----



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR  
UNIVERSIDADE DO PORTO

### DECLARATION

I, Johannes Greten, supervisor of the Master Thesis of Ana Alexandra Pereira da Cunha Anjos, on the subject: “**Objective Assessment of Acupuncture effect by Microcirculation-related parameters in patients with proximal femur fracture**”, a scientific research preliminary study carried out within the course of Traditional Chinese Medicine, declare that it is suitable and ready to be presented in public for its defense.

Porto, 22 September 2011

Yours sincerely

The Supervisor

A handwritten signature in blue ink, consisting of a large initial 'H' followed by a cursive name.

---

Prof. Dr. med. Henry Johannes Greten

## Appendix. 4



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PD Dr. Klaus Hauer  
Bethanien-Krankenhaus – Geriatisches Zentrum  
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05.06.2008  
Str/wi

**VOTUM**  
(Zustimmende Bewertung)

**Titel:** Vergleich von Verumakupunktur nach dem Heidelberger Modell gegenüber einer Sham-Akupunktur auf das Gangbild älterer Menschen mit Gehstörungen.

**Geprüfte Unterlagen:** Schreiben vom 05.04.2008  
Zusammenfassung (undatiert)  
Erstantragsformular vom 05.04.2008  
Patienteninformation (undatiert)  
Einverständniserklärung (undatiert)  
Studienprotokoll (undatiert)  
Dokumentationsbogen (undatiert)  
CD-ROM

**Nachgereichte Unterlagen:**

Email vom 04.06.2008  
Geändertes Studienprotokoll vom 25.05.2008

**Unser Zeichen:** S-094/2008 (Bitte stets angeben)

Sehr geehrter Herr Dr. Hauer,

mit den Änderungen bzw. Ergänzungen in den o.g. Dokumenten wurden die im Votum vom 23.04.2008 genannten Empfehlungen der Ethikkommission berücksichtigt. Damit liegt die Zustimmung der Kommission zu der o.g. Untersuchung vor.

Unabhängig vom Beratungsergebnis macht die Ethikkommission Sie darauf aufmerksam, dass die ethische und rechtliche Verantwortung für die Durchführung einer Studie beim Leiter der Studie und bei allen teilnehmenden Ärzten liegt.

Wir wünschen Ihnen bei der Durchführung des Projektes viel Erfolg.

Mit freundlichen Grüßen

Prof. Dr. med. Thomas Strowitzki  
Vorsitzender der Ethikkommission

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