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Methodological aspects of toe blood pressure measurements for evaluation of arterial insufficiency in patients with diabetes

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Abstract

Diabetes mellitus (DM) is a growing health problem in the aging population of the western world with prevalence figures around 6-7%, which are increasing fast. Treatment of this disease is complex and expensive for society. One of the most feared DM complications is foot ulcerations. As a causative factor for this complication peripheral arterial disease (PAD) is second only to neuropathy in importance, and screening for PAD is vital to prevent amputation. Toe blood pressure (TBP) measurements are often used for this purpose and this method is also included in the definition of critical leg ischemia. Unfortunately, TBP measurements are affected by a high variability and obtained pressures can be of limited value because of this. Accordingly, the aim of this thesis was to evaluate, standardise and optimise TBP measurements.

Study I: Photopletysmography (PPG) has been the standard for blood flow detection during TBP assessment. Laser Doppler (LD) has some theoretical advantage over PPG due to its higher sensitivity. The aim of this study was to evaluate LD for TBP measurements. It included 36 patients referred for PAD screening, who were examined both with PPG and LD. The correlation between pressures obtained with the two methods was high (r=0.99). Significantly ($p< .005$) higher values were obtained with LD. In a second part of the study TBP measured with LD were able to categorize 40 patients and control subjects according to the Fontaine classification. In 10 of the patients who had DM, ankle and TBP values were compared, and two of them had falsely elevated ankle blood pressures (ABP).

Study II: This study aimed to evaluate the importance of vessel wall compliance on blood pressure measurements with cuff at the ankle and toe level using the hydrostatic pressure (pole test) as gold standard. Twenty-five legs in 23 patients with DM were examined. Due to persistent signal at maximal leg elevation, it was impossible obtain pole test pressures in all patients, and only 11 at ankle and 19 at toe level could be assessed. The ABP values were higher if measured with cuff than with pole test ($p< .01$), but no difference was found in the toe. The conclusion was that falsely elevated blood pressure in patients with DM probably is of less importance for TBP than ABP.

Study III: The focus of this study was to examine if the cuff size influences TBP values. Eleven patients with DM without a history of PAD were investigated and compared to six healthy control subjects. In the entire cohort of subjects (patients and controls) TBP values were significantly higher ($p< .01$) if measured with a narrow as compared to a wider cuff. There was no difference between patients and controls, but this could be due to a limited cohort size. Differences in hallux sizes were one of the determinants of the TBP variability. The conclusion was that the cuff width does influence the obtained TBP values and needs to be considered when using TBP to screen for PAD.

Study IV: The objective of the study was to determine the optimal cuff width for measuring TBP. Twenty PAD patients with and without DM were recruited at two centers. Four cuff sizes, 1.5, 2.0, 2.5 and 3.0 cm were used to measure TBP in the cohort and the values compared with pole test recordings, which were considered to represent the true TBP value. Values were overestimated when 1.5 and 2.0 cm ($p<.001$) wide cuffs were used, while 2.5 and 3.0 cm wide cuff use produced values more in line with the real TBP. The results from this study suggested that a 2.5 cm wide cuff should be used for all TBP measurements in clinical praxis.

Study V: In this study the general variability of the TBP method was investigated and an automated device's ability to measure TBP was evaluated. TBP was recorded in 23 legs of 16 diabetic patients and pressures assessed manually from perfusion-pressure graphs and by an automated algorithm. The inter- and intra-observer variability of TBP was less than 10% of the recorded pressure. Automatic assessments of TBP was acceptably accurate for measurement of TBP <45 mm Hg (SD 4.0 mm Hg), but not for higher pressures. Adjustment of the automatic algorithm to manage the problem of biphasic wave patterns reduced the difference compared to visual readings from 5.9 to -2.9 mm Hg (SD from 16.9 to 8.9).

Conclusion

Stiff arteries do not affect TBP measurements as much as ABP in patients with DM, but examinations have to be standardised to be a reliable. LD can be used as blood flow detector when measuring TBP and is probably the best alternative when TBP is low. To reflect the hydrostatic pressure the toe cuff width should be 2.5-3 cm or 30% of the toe circumference. Standardized TBP measurements for skin temperature, flow detector and cuff size produce pressures with variability below 10%, which probably is less than for ABP. An automated TBP device is reliable for identification of diabetic patients with foot ulcerations with a risk of having critical limb ischemia.

Key words: *lower limb ischemia, diabetes, foot ulcerations, toe blood pressure, ankle blood pressure, biphasic curve pattern, cuff width, laser Doppler, automatic pressure measurements*

List of Publications

This thesis is based on the following papers, which are referred to in the text by their Arabian numerals:

1. Toe blood pressure measurements with laser Doppler.
H-I Påhlsson, E Wahlberg, S Z Wei, G Jörneskog, J Swedenborg, P Olofsson and B Fagrell
Journal of Vascular Investigation (1996) **2:2** 82-86

2. The Toe Pole Test for Evaluation of Arterial Insufficiency in Diabetic Patients.
H-I Påhlsson, E Wahlberg, P Olofsson and J Swedenborg,
European Journal of Vascular and Endovascular Surgery (1999); **18**:133-137

3. The cuff width influences the toe blood pressure value.
H-I Påhlsson, G Jörneskog and E Wahlberg,
VASA (2004); **33**: 215-218

4. The Optimal Cuff width for Measuring Toe Blood Pressure.
H-I Påhlsson, T Jogestrand, C Laskar, K Stark, A Andersson and E Wahlberg
Angiology (Accepted for publication, May 2006)

5. The validity and reliability of automated and manually measured toe blood pressure in ischemic legs of diabetic patients.
HI Påhlsson, K Lund, G Jörneskog, R Gush and E Wahlberg
Submitted *Diabetes Care*

A brief description of the formation of atheroma and

Contents

List of abbreviations

ABI= Ankle brachial index

ABP = Ankle blood pressure

ABP_{cuff} = Ankle blood pressure measured with a sphygomanometer cuff around the ankle

ABP_{pole} = Ankle blood pressure measured with the pole test method

CLI= Critical leg ischemia

CW-Doppler = continuous wave pen-Doppler

DM= Diabetes mellitus

LD = Laser Doppler

PTA = Percutaneous transluminal angioplasty

PAD = Peripheral arterial disease

PPG= Photopletysmography

SMC= Smooth muscle cell

SPP= Skin perfusion pressure

TBI= Toe brachial index

TBP = Toe blood pressure

TBP_{cuff} = Toe blood pressure measured with a sphygomanometer cuff around the base of hallux.

TBP_{pole} = Toe blood pressure measured with the pole test method

TBP_{aut}= Toe blood pressure measured with automatic equipment

$TcPo_2$ = Transcutaneous oxygen tension measurement

General Background

Historical perspective

The word DM has been associated with the Greek physician Aretaeus that lived in the second century AD, but the first known description of the disease is 1500 years older. Aretaeus described the clinical features of DM. In 1886 Minkowski and von Mering were able to show the role of the endocrine pancreas for the genesis of DM by performing pancreatectomy in a dog. Thirty-six years later Banting and Best 1922 isolated insulin.
The special form of non-atheromatous arteriosclerosis with focal calcification of the media layer of the arterial wall, typical for diabetics, was described by Mönckeberg in 1904[1] and Bowen et al suggested 1924 that arteriosclerosis in the lower extremities in diabetic patients could be diagnosed using plain x-ray because of this [2].
TBP was first mentioned in the scientific literature by Formijne who published a report 1934 in which the systolic TBP was evaluated in patients with PAD[3].
One of the fundamental steps for treatment of PAD in DM patients and for reconstructive vascular surgery was taken in 1948 when the French surgeon Kunlin performed the first free venous transplantation from the femoral artery to the popliteal artery[4].

Epidemiology

In Sweden 3-4% of the population suffer from DM, which means 300 000 people. Furthermore, the prevalence in the population over 80 years is almost 20%[5]. Type 1 DM constitutes 10-15%, which is approximately 0.5 % of the population. It is predominately Type 2 DM that is a growing epidemic worldwide. In Ontario, Canada, for instance, the age- and sex-adjusted DM prevalence increased by 69% between 1995 and 2005[6]. Because of the late complications DM consumes 8% of the direct medical and health care costs in Sweden[7].

Foot ulcerations are a very common DM complication. It has a reported prevalence of 5-10 % of patients with DM[8]. In Sweden 90 % of all amputations are caused by vascular disease and almost half are performed on patients with DM[9]. There are studies indicating a decreasing amputation frequency[10] [11]. Still, the amputation incidence is estimated to be 2-8 per 1000 patients per year[12]. These figures are just approximations due to unreliable reports on both the actual number of amputations and the uncertainties of the DM diagnosis[13].
Most of these diabetic patients have at some point required medical attention because of foot ulcerations. At least 70% of the 1400 amputations performed in Sweden every year on patients with DM are preceded by non-healing foot ulcerations[14]. In US 75 000 amputations were performed on patients with DM during 2003[15].
According to the annual report of all vascular surgical procedures in Sweden from 2006, the Swedish Vascular Surgery Registry, Swedvasc, 60 % of the 40 000 vascular procedures performed between 1996-2005 was indicated by leg ischemia, including patients with DM and foot ulcerations[16]. Of all procedures performed in leg were 50 % indicated by CLI and 15 % by acute leg ischemia.

During these ten years the number of vascular procedures performed for acute ischemia and CLI was roughly constant while procedures performed for claudicatio were decreasing.
Only 60% of the patients treated for acute leg ischemia and 75 % treated for CLI during the period 1996-2005 were still alive one year after the operation. Furthermore 5% had one of their legs amputated within one year after the operation. In the year 2005 19% of the patients undergoing revascularization for CLI had a failing graft early after their procedure, emphasising the need for better patient selection methods to surgery.

Classification of DM

Table 1: Classification of DM according to WHO classification from 1998[17]

DM type	Name	Cause
A.	Type 1-DM:	Autoimmune and non-autoimmune beta-cell destruction.
B.	Type 2-DM	Varying degrees of insulin resistance and insulin hyposecretion
C.	Gestational-DM	
D.	Other types:	DM with known cause e.g. alcohol induced pancreatitis, DM caused by corticosteroid treatment etc.

Characteristics of dyslipidemia in patients with DM type 2[18]

Several theories have been proposed to explain the specific patophysiology causing atheroma development in patients with DM. One of the most studied and clinically important ones concerns the lipid metabolism.

The elevated risk for vascular diseases in patients with Type 2 DM is associated with alterations in the plasma lipoprotein profiles (Table 2).

Table 2. Characteristics of dyslipidmia in patients with DM

s-TG	↑
s-HDL	↓
sdLDL	↑
sdHDL	↑
postprandiell hyperlipidemi	

Triglycerid rich lipoproteins (TRLs) in plasma are derived from intestine (chylomicrons) and liver (VLDL). The plasma clearance are depending on two enzymes: lipoprotein lipase (LPL) which is

responsible for conversion of VLDL to intermediate density lipoprotein (IDL) and hepatic lipase (HL) which is responsible for the next step, IDL to LDL. In patients with DM type 2 the level of LPL is low while the level of HL is high which enhance the conversion of IDL to LDL.
VLDL particles can be separated in two subtypes: $VLDL_1$ and $VLDL_2$. $VLDL_1$ is the lager type and typical for patients with DM type 2 especially in the postprandial phase were insulin fails to suppress $VLDL_1$ release.
The long circulation time of TRLs in the circulation facilitates triglyceride enrichment of both LDL and HDL particles mediated by cholesterol ester transfer protein (CETP). These triglyceride rich particles are converted to small dense particles by HL enhanced in patients with DM type 2. These small dense LDL particles constitute a risk factor for coronary heart disease (CHD)[19].

Small particle size facilitates the penetration of the intima layer of the arterial wall and small LDL size has a higher affinity to intimal proteoglycans than larger LDL particles. It has also been proposed that patients with DM type 2 have an increased trans-vascular transport of LDL. The result is an increased deposition of LDL in the vascular wall. Oxidized LDL in the intima is the trigger for the processes ending up in macrophage foam cells and plaque formation.

A brief description of the formation of atheroma and plaques in the vessel wall[20]

Monocytes binds to damaged endothelial cells and migrate into the intima where they are transformed into macrophages. Within the intima, LDL particles are incorporated in the macrophages, forming foam cells. Fatty streaks are then apparent macroscopically when vessel walls are inspected. The next step in the process is proliferation of SMCs and their migration into the intima. Some SMC are transformed fibroblasts, a fibrous cap is formed, making the intima layer thicker than normal. Cytokines and metalloproteinases are secreted from the foam cells into the matrix resulting in digestion of collagen and the vessel wall structure. The consequence is erosion of the endothelium and plaque rupture. Inflammation is important in all stages of atherogenesis. For example during the early stage inflammatory mediators are expressed that guides monocytes to the injured area, which adhere to the intima. During later stages inflammatory cells is crucial when necrosis and ulcerations are developed[21].

LONG TERM COMPLICATIONS

Vascular complications in respect to organ system

- Nephropathy: Vascular changes in glomeruli, arterioli and the renal artery and its branches are common lesions in kidneys of diabetic patients[22]. Presence of nephropathy is associated with a high mortality and is the most common etiologic factor of uremia in need of dialysis in Sweden[7].

- Retinopathy: DM retinopathy is the most common cause of blindness in people younger than 65 years in the western world[7]. -The pathologic findings can be separated into two categories; background and proliferative type. The background type is characterised by progressive capillary destruction and the proliferative lesions by new vessel formation, angiogenesis, and scarring[22].

- Cardiovascular disease: Atherosclerosis causes 80 % of all deaths in patients with DM and roughly 75% of these are attributed to coronary artery disease[23]. Compared with patients without DM the prevalence for myocardial infarction is two times higher in men and four times higher in women[7]. The risk for myocardial infarction in people with type 2 DM is as high as for people without DM who already have had a cardiac event previously[24].

- Cerebrovascular disease: A Swedish study found the mortality from stroke in a diabetic population to be 4-5 times higher than in the non-diabetic population[25].

- Peripheral arterial disease: The high incidence of PAD associated with DM was clearly demonstrated in the Framingham study[26]. In female patients with DM there were an excess of non palpable pedal pulses with 50 % compared with patients without DM. Lack of pedal pulses was associated with a two fold increase of risk for having coronary heart disease and stroke. This emphasizes the role of DM for the development of atherosclerosis.

Neuropathic complications

- Neuropathy has a high prevalence among DM patients and is associated with the duration of DM. In an English multicentre study from 1993[27] on patients attending hospital clinics, neuropathy was present in 20% of DM patients with less than 5 years of diabetes duration. It could be compared with 35% in patients with more than 10 years duration. The presence of neuropathy also increased with age and in patients with DM type 2 over 60 years old the prevalence was more than 50 %.

The diabetic foot

Neuropathy and PAD are the main etiologic factors making the diabetic foot vulnerable to minor trauma and ulceration. These ulcerations will, often after infection, progress to deeper ulcerations and finally to gangrene (Figure 1)[28].
In the foot, somatic neuropathy affects sensory nerves which results in symptoms i.e. reduction of nerve response to pain, temperature and vibration.
Also motor nerves are affected, which leads to waste of small muscles and produces an imbalance between flexor and extensor muscles. This muscle imbalance gives rise to symptoms like the claw toe deformity and prominence of metatarsal heads with secondary callus formation. The biomechanical imbalance results in increased vertical and horizontal forces on the plantar tissue, which leads to mechanical stress[29].
Most important is the engagement of the autonomic nerves. Reduced sweating results in dry skin, which is prone to form fissures. Arteriovenous shunting leads in absence of PAD to a warm foot[30], which may reduce the patients´ awareness of the disease and make them less interested in ulceration preventive measures.
The association between the lower leg amputation and neuropathy is clear. In a study from the US almost 80% of lower leg amputees had impaired sensibility compared with 18 % in a comparable group who not had undergone amputation[31].

The fraction of purely ischemic foot ulcerations is less than 15% of all. Much more common are the so called neuroischemic feet. They account for approximately 50% of all patients presenting at specialised foot clinics[32,33]. Atherosclerotic disease in the lower leg among patients with DM is

confined to the tibial and peroneal arteries [34,35]. Some early investigators have reported that foot arteries of DM patients were less involved in the atherosclerotic process than in non-diabetic patients[36], an important finding when choosing between surgical reconstruction methods. However, this observation has been questioned by later angiographic studies[37].

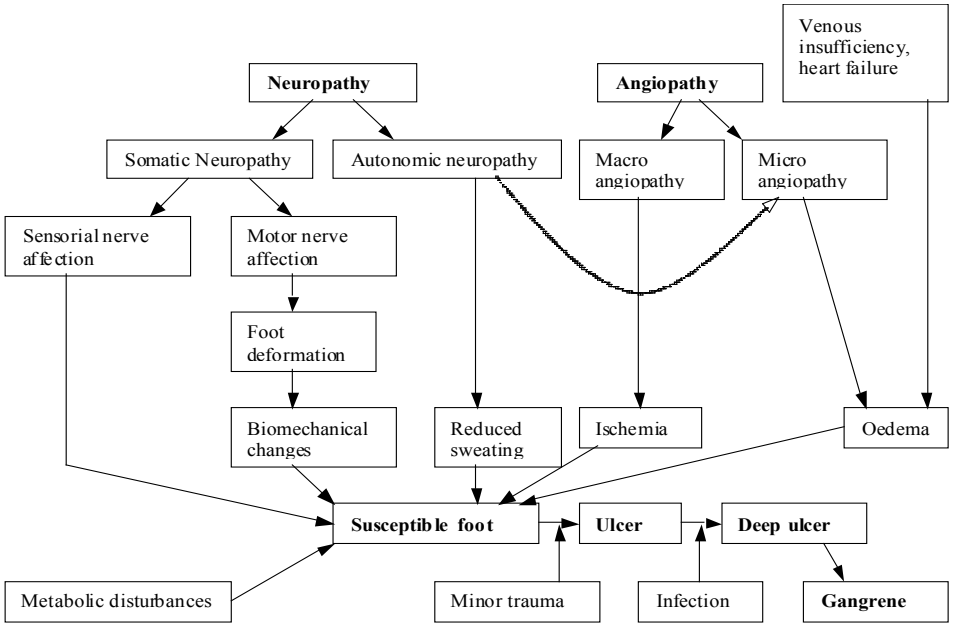

Fig. 1. Schematic pathway for the breakdown of the diabetic foot (based on an idea by A.J.M. Boulton[28]).

Pathophysiological background to leg ischemia in diabetics

Macroangiopathy

There are two types of calcifications in the wall of leg arteries in patients with DM. The calcification of the innermost part of the arterial wall, the intima, is similar to atherosclerosis seen in non-DM patients (the atheroma formation is described above). Radiologically it is characterised by patchy lesions that narrows the lumen and finally may occlude it.
The other type of morphologic change, arteriosclerosis, is a calcification of the media layer of the arterial wall. This type of calcification is predominantly seen in patients with DM and end-stage renal disease[38]. It is highly correlated to the duration of DM. The media sclerosis does not narrow or occlude the arterial lumen[39], but makes the wall stiff and fragile. This characteristic makes the usefulness of ankle blood pressure measurements, which is dependent on high wall compliance, disputable in patients with DM. It is also probable that this type of macroangiopathy is responsible for the disturbance of vessel autoregulation and blood pressure control common in patients with DM.

Microangiopathy

The cutaneous microcirculation contains two main components, thermoregulatory arteriovenous shunts and nutritive capillaries and its pathophysiology in DM patients is characterised by microvascular sclerosis. The main histological findings observed are hyalinosis of feeding arterioles and capillary basal membrane thickening. Occlusive lesions, however, are not more common in the microcirculation of patients with DM than patients without[40].

The autonomic neuropathy associated with DM involves the sympathetic fibres, which influence the thermoregulatory shunts. This disturbance cause an increase in blood flow through the shunts leading to an elevated skin temperature. It is not entirely clear, however, if a consequence of this shunting, results in ”steal” from the nutritive capillaries into the shunts[41]. There are reports focusing on the toe skin microcirculation in patients with early Type I DM showing showed a reduced capillary blood velocity (CBV) together with an elevated laser Doppler flux (LDF)[42]. Since LDF measures perfusion predominately in the arteriovenous shunts this finding suggests that “steal” actually occurs in DM. It has for long been known that neuropathy also causes an impaired postural vasoconstriction with an increase of the microvascular blood flow in the feet when the patients are sitting or standing[43].
A consequence of the disturbance in the microcirculation is a tendency to fluid extravasation and oedema. This produces an increase of the diffusion distance for oxygen between the capillaries and surrounding cells[43], reducing ulcers’ capability to heal. Another effect is the foot swelling that makes shoes fit badly, increasing the risk for ulcers.

Methods to manage the diabetic foot

Preventive measures

In Sweden the majority of the patients with DM, especially with Type 2, are managed by the primary health care system. Under supervision of general practitioners, teams involving specially trained nurses, dieticians, and podiatrists have worked together to prevent foot ulcers. These teams have achieved good results in reducing the number of amputations. Patient education is emphasised as an important factor both in Swedish and international studies for achieving this goal [44,45].

Non invasive measures

When an ulcer has developed there is a need for multidisciplinary collaboration to reduce the number of foot ulcers. If not treated optimally it may progress to deep infections and gangrene[12,32,46]. The task for the team is to identify limiting factors for ulcer healing and to deal with these factors. There are two principal management strategies; medical and invasive.
The medical management includes local treatment of the ulcer, eradication of infection, and optimised glucose control[47]. Other examples are treatment of oedema and anti-smoking measures. Another important therapy is reduction of pressures against the ulcer area, called offloading methods, which includes measures such as total-contact casting and local padding. In patients not suitable for invasive measures such as revascularization but with a predominately ischemic aetiology, agents like prostacyclin analoges may improve ulcer healing[48]. Occasional reports supporting use of growth factors that stimulates keratinocyte growth to promote wound healing have also been published[49]. Bio-engineered skin has also been proposed in order to facilitate healing. Such methods may be included in the therapeutic arsenal in the future [50].

Percutaneous methods

Percutaneous transluminal angioplasty (PTA), a balloon catheter procedure, is often regarded as the first option to reduce arterial stenoses[51,52] that are obstructing blood flow causing distal ischemia in the foot. Ideal for this method are proximally located short stenoses, but also lesions located in the distal calf can be treated successfully[53]. In DM patients with CLI and foot ulcerations where PTA was feasible the outcome was the same as in non-DM patients with 70% ulcer healing one year after the procedure[54]. Another recently adapted method is subintimal angioplasty in which a new vessel lumen is created in the subintimal space along the native artery.
Both these methods have the advantage of being less invasive than open bypass surgery, but have a less favourable outcome. An advantage, however, is that some patients not offered an open procedure due to high risk, can be treated this way.

Open surgical methods

A variety of open revascularization procedures to facilitate healing are feasible for patients with foot ulcerations and severe leg ischemia. The technical aspects and the type of vascular reconstructions appropriate are similar to patients without DM. As the atherosclerotic disease often is confined to calf arteries there is need for more distal bypass surgery. This includes bypass procedures landing in the distal peroneal or tibial arteries or down to the dorsal pedal artery in the foot The results of such distal procedures have improved considerably during the last decades, which may have increased the limb salvage rate[40,55,56,]. Overall, however, the patency and limb salvage rate one year after the procedure is performed is around 40 % and 90 % respectively for such revascularizations[57].

Methods to identify and select patients for invasive vascular procedures

Ankle blood pressure

The conventional way to estimate ABP is by compressing the ankle arteries with a cuff placed proximal to the malleoli. The pulsations in the ankle arteries are insonated with a CW-Doppler. Identification of the cuff pressure when reappearance of audible flow signals occur during slow release of an insufflated cuff is a measure of the blood pressure. A disadvantage with the method is that it only gives adequate approximation of the intraarterial blood pressure when the vessel wall compliance is high. Unfortunately a substantial part of patients with DM do not have compliant vessels. For instance, in insulin treated patients referred for evaluation of PAD, stiff arteries have been reported to occur in 9-18 % of the cases[58,59]. The day-to day variability of ABP measurements has been assessed to 11 mm Hg and the total observer variability to 10 mm Hg (Table 4).

The pole test

To overcome the problem with poor elasticity in the arterial wall of DM patients, the hydrostatic pressure induced by leg elevation can be used to assess the blood pressure. The principal concept of the pole test has been employed in clinical praxis for a long time as the “elevation test” or Ratchoff’s test. It is performed by elevating the extremity during continuous blood flow detection. The level over the heart where the blood flow not longer can be detected is measured with a pole from which the method has got its name. When using the simple “elevation test”, skin colour as a measure of perfusion is assessed instead of blood flow detection, but it is often quite difficult to

Table 3. Studies evaluating TBP, ABP and TcpO2 for predicting outcome in patients with foot ulcerations and DM.

Author	Year	TBP method	DM/non-DM	Inclusion criteria	Healing rate TBP	Healing rate ABP	Sensitivity	Specificity
Bone et al[62]	1980	Photo-pletysmography on the toe nearest amputation site	24/3	Intention to perform toe or metatarsal amputation	0 % TBP<45 mm Hg 75% TBP 45-55 mm Hg 100% TBP >55 mm Hg	No ABP difference healed vs amputated/ dead		
Ramsey et al[63]	1983	Photo-pletysmography/ cuff size not reported	31/39	Rest pain or ulceration	86% TBP>30mm Hg	55% ABP>80mm Hg	85%TBP<30mmHg 70%ABP<80mmHg (rest pain/ ulceration)	94% TBP<30mmHg (rest pain/ ulceration)
Apelqvist et al[87]	1989	Strain-gauge/ Doppler/ ”individually fitting cuffs”/ toe 1-4	314/0	Foot ulceration	85% TBP>45mm Hg vs 36%TBP≤45mm Hg	8% ABP<40 mm Hg 43% ABP=40-79 mm Hg 53% ABP=80-119 mmHg		
Apelqvist et al[94]	1992	Strain gauge/ Doppler	208/0	Foot ulceration and TBP< 45 mm Hg	28% TBP<30 mm Hg 56% TBP >30 mm Hg	No ABP difference healed vs amputated/ dead		
Kalani et al[70]	1999	Laser Doppler/cuff width and heating not defined	50/0	Chronic foot ulcerations >2 months			15% TBP< 30 mm Hg 92% $TcpO_2$<25 mm Hg	97% TBP ≤30 mm Hg 85% $TcpO_2$<25 mm Hg

Table 4. Studies assessing reproducibility and variation of ABP, TBP and TcpO2 in patients referred for vascular examination.

Author	Year	TBP detector	DM/non-DM	Inclusion criteria	Reproducibility (ICC)		Variation		
Nielsen[69]	1973	Strain gauge	20 legs DM?						Day-to-day SD(mm Hg) ABP=8-9 TBP=8-9
Arveschoug[111]	1998	Strain gauge	30 legs DM?	Patients referred to vascular laboratory			Intraobserver SD (mm Hg) ABP=4.89 TBP=2.71	Interobserver SD(mm Hg) ABP=5.65 TBP=3.47	Day-to-day SD(mm Hg) ABP=10.98 TBP=9.86
De Graaff[70]	2000	LD and PPG	86 legs 36% DM	Patients referred to vascular laboratory	1 week intra-observer variability Brachial BP = 0.72 ABP=0.85 TBP(PPG)= 0.92 TBP(LD)= 0.88				
De Graaff[71]	2001	PPG	107 legs 36% DM	Patients referred to vascular laboratory	1 day intra-observer variability ABP=0.99 ABI=0.98 TBP=0.99(hallux) TBP=0.98(dig2) $TcpO_2$=0.89	1 week inter-observer variability ABP=0.85 ABI=0.87 TBP=0.85(hallux) TBP=0.68(dig2) $TcpO_2$=0.62			

record the level over the heart when perfusion ceases. Therefore, measuring ABP using an objective method to assess blood flow is preferable.

In 1994 Smith et al found a much higher correspondence between the intraarterial blood pressure and ABP measured with the pole test method (ABP_{pole}) than with sphygomanometric cuff (ABP_{cuff}) measurements[60].

Also the compressibility in the ankle arteries can be calculated with the pole test. With a cuff around the ankle, blood pressure measurements are performed at different levels above the heart. The effective negative hydrostatic pressure superimposed on the blood pressure can thus be calculated. The ankle/brachial pressure index for cuff pressure and hydrostatic pressure is calculated separately and regressed on each other. If the arterial wall makes no resistance to the cuff compression, the pole test pressure and the cuff pressure will be the same and the regression line –1[61].

Indices

The ratio of ankle systolic pressure to brachial systolic pressure (ABI) is used as a pressure index. In healthy individuals this ABI is usually >>1. Absence of palpable ankle pulses and an ABI <<1 suggests the presence of PAD[52]. In limbs with a single complete occlusion in the popliteal artery, or more proximally, the index is usually between 0.5 and 0.8[62]. However, these statements are valid only if a falsely elevated blood pressure can be ruled out. Patients with exclusively proximal disease, i.e. in distal aorta and common iliac arteries may also have normal ABI in spite of significant disease.

A method to enhance the diagnostic value of ABI is to perform the blood pressure measurements after standardised exercise. Unfortunately this method is best suited for patients without DM because of problems with stiff arteries[63].
It is the absolute pressure, however, that provides the driving pressure and today many investigators find the absolute pressure more relevant than the index for identifying patients with severe disease and CLI who need invasive treatment[62]. Indicies can also be used in the toe level and is then called TBI.

Toe blood pressure

In 1980 it was shown that estimation of TBP was superior to ankle blood pressure in predicting healing after forefoot amputations[64]. Subsequent studies showed that TBP is more reliable than ankle pressure in assessing the degree of leg ischemia (Table 3)[65]. A low TBP is one of the strongest risk factors for the development of gangrene in the lower limb, and eventually amputation, among patients with DM[66]. There seems to be no major difference in blood pressure between the hallux and the second toe while the reproducibility of TBP in the second toe is worse [67,71].

Several methods for flow detection have been used to estimate the TBP. One method, which was more common previously, is based on volume changes of the toe. In principle, a mercury strain gauge is placed around the base of the toe. The increase in toe volume caused by the arterial inflow can then be detected during slow release of the arterial occlusion induced by a pneumatic cuff[69].
Today it is more common to register perfusion as a measure of blood flow and to apply the detector at the toe pulp. With a cuff around the toe base, the blood flow is halted and then slowly released. The blood pressure level at which the blood flow reappears is considered to be the TBP. PPG, and

more recently also LD are additional methods used for blood flow detection. The PPG consists of a light emitting diode and a phototransistor detector. It registers changes in the reflected light induced by the blood content[64]. The principles for LD are described below.

TBP assessments are presently performed at vascular laboratories because of the equipment required and the need to interpret and read the pressure-flow graphs to obtain a TBP value. In contrast, ABP measurements are easily performed by health care personal outside hospitals[68].

Transcutaneous oxygen measurement

This method uses a $TcpO_2$ electrode on the skin surface to register the amount of free O_2 that diffuses from the capillary bed to the surface. Accordingly, it reflects not only the cutaneous blood flow but also the metabolic activity and oxygen diffusion capability through the tissues[72]. In cases where $TcpO_2$ is very low, or not possible to register at all, it should not be interpreted as no oxygen at all reaches the tissue, but rather that all is consumed and no free oxygen reaches the surface. This explains a $TcpO_2$ reading of zero obtained in the presence of a persistent blood flow registered by LD[88]. Not only local factors influence the estimated $TcpO_2$ values but also the patients' cardiovascular and pulmonary status and $TcpO_2$ is dependent on the oxygen saturation of the blood. Patients with hypoxia have a lower $TcpO_2$. For comparison, in clinical trials for instance, a reference electrode is often used to avoid this problem.
$TcpO_2$ in the foot skin has been reported to have a variability of up to 34% during repeated measurements during 24 hours on the same patients suffering from claudicatio intermittens[73]. A study with patients suffering from PAD found the variability for $TcpO_2$ higher compared to TBP (using PPG as detection method)[71](Table 4). Regardless, $TcpO_2$ has been used to prospectively grade the severity of foot ischemia in patients with DM and to select patients to appropriate treatment[74]. A recent published report indicate that the probability for ulceration healing is low when $TcpO_2$ <25 mm Hg and is an even better indicator for ulceration healing in DM patients than TBP[75].

Pulse Oximetry

Pulse oximeters measure the O_2 saturation in peripheral blood. A reduced blood flow in an extremity also reduces the O_2 saturation. This phenomenon can be used to assess perfusion and a difference >2% in SaO_2 between finger and toe readings or a decrease >2% in SaO_2 during leg rise can be a sign of leg ischemia. This method has been proposed to be a screening tool for leg ischemia in non-symptomatic patients with DM[76].

Other Methods

Vital Microscopy

The nutritional capillary bed can directly be investigated with a light microscope [77]. With this method it is possible to quantify parameters such as capillary blood pressure, vessel dimensions and, the one most commonly used, capillary blood flow velocity[78]. It can also be combined with a TV-video system, which facilitates measurements of the blood flow velocity[79]. DM patients, without macroangiopathy and a normal total microcirculation as evaluated with LD, have a reduced capillary blood flow of nutritive capillaries[80]. This method is rarely used for screening purposes for CLI in DM patients, and should be regarded a primarily a research tool.

Plain x-ray

The possibility to identify vessel calcification with x-ray has been known since the childhood of diagnostic radiology. The method has successfully been used also in modern time. In one study on diabetic patients with severe PAD, the severity of vascular disease was better predicted by examining the amount of calcification on plain x-ray than using ABI [81]. Though, it cannot be used as a screening tool for severe ischemia in general.

Angiography

The percutaneous catheter technique has been used to visualise arteries through the direct injection of water-soluble contrast media since the fifties. The method is the standard imaging modality for preoperative evaluation of disease and is sometimes still used for diagnosis but should not be used for diagnosing PAD in DM patients. Previously the technique was plagued by allergic and toxic reactions to the contrast agents but development of less harmful contrast media has made severe reactions rare. Intravascular contrast media may still have a nephrotoxic effects, especially in patients with DM having impaired renal function. If these patients are also treated with the anti-diabetic drug metformin, lactic acidosis may occur[82]. There is also a small risk for bleeding complications due to the arterial puncture. Since angiography also is time consuming and expensive it has been challenged by other techniques e.g. colour-coded duplex imaging that could serve as an alternative to angiography in the vast majority of the cases with PAD[83]. Recently developed methods, e.g. CAT-angiography (CAT) and MR-angiography (MRA), constitute further alternatives. Even if angiography can be used to stratify patients for different treatment strategies to heal ulcerations[84] the above mentioned problems and a poor risk-benefit ratio for screening purposes favours non-invasive methods.

Measurement of skin perfusion pressure with isotope clearance technique

The skin perfusion pressure can in diabetic patients with lower leg ischemia be estimated with a spot clearance technique. The procedure includes intra-dermal injection of 99mTc-pertechnetate on the dorsal aspect of the foot. An external pressure is applied over the injection spot and the gamma emission is counted with a detector. The external pressure is elevated until the clearance of the pertechnetate ceases. At an SPP lower than 40 mm Hg no wound healing can be expected in patients with DM[85]. This technique, however, has not been widely used to evaluate PAD in diabetic limbs. The explanation is probably its high cost, and because measurements are lengthy and uncomfortable for the patients. Instead LD has been used for skin perfusion measurements and found to correlate well with the radionuclide clearance[86].

LD fluxmetry

A low power light beam from a He-Ne laser illuminates a tissue i.e. the skin. The light scatters on moving red cells up to a depth of 1 mm, and undergoes a frequency shift according to the Doppler effect. A photo detector collects some of the back scattered light. By signal processing the noise from external sources can be suppressed and the portion, originated from the moving red cells, can be enhanced. However, when the detector is held in contact with almost any tissue it will register a low flux value although there is no perfusion at all. This baseline level is called biological zero.
There is an almost linear correlation between the flux value and a low to moderate volume of moving blood cells, a fact used when designing flow meters[87].
Because LD values includes non-nutritional subpapillary vessels in the skin, the resting flux cannot be used for diagnosing PAD. The spatial and temporal variation is considerable. Therefore, the skin should be warmed to overcome local vascular resistance and to achieve a better correlation to skin perfusion [88]. A special heated probe has been invented for this purpose. Even with the heated

probe, however, resting LD values has not been evaluated properly for diagnosing PAD in DM patients.

A special application for the LD device is to use it as a flow detector when performing TBP measurements during slow release of a pneumatic cuff around the base of the toe[89]. Together with physiologic tests like such as reactive hyperemia it can be used to classify the severity of PAD.

Diagnosing critical limb ischemia in patients with DM

According to the European consensus document from 1992[51], CLI, in both diabetic and non-diabetic patients is defined by either of two criteria:

- Ischemic rest pain requiring regular adequate analgesia for more than 2 weeks, with an ankle systolic pressure ≤ 50 mm Hg and/or a toe pressure of ≤ 30 mm Hg.

- Ulceration or gangrene of the foot or toes with an ankle systolic pressure ≤ 50 mm Hg and/or a toe pressure of ≤ 30 mm Hg.

For patients with DM it was proposed that also microcirculatory parameters as $TcpO_2$ should be included in the definition. Microcirculatory parameters together with TBP would enhance the prognostic value of the examinations[90].

Consequently, in June 2000 the Trans-Atlantic Inter-society Consensus Document on Management of Peripheral Arterial Disease (TASC)[91] included $TcpO_2$ in their recommendations for trial and reporting standard definition of critical limb ischemia as absolute pressure of either:

- Ankle pressure < 50-70 mm Hg or

- Reduced toe pressure < 30-50 mm Hg or

- Reduced $TcpO_2$ < 30-50 mm Hg

In January 2007 the Trans-Atlantic Inter-society Consensus Document on Management of Peripheral Arterial Disease (TASC II) was published[92]. In this document, as compared to TASC I, more focus is on patients with DM and PAD. It is stated that CLI is a clinical diagnosis but should be supported by objective tests. For patients with DM this should include TBP measurements. The critical level is defined as <50 mm Hg.

Most management guidelines like TASC II strongly support the use of TBP for evaluation of ischemia in DM patients. In order to increase the validity of TBP measurements, the method needs standardisation and scrutiny. This was the aim for this thesis

Aims of the study

The overall aim of this thesis was to evaluate, standardise and optimise TBP measurements. The specific aims were:

- To evaluate the LD technique for measuring TBP by comparing pressures obtained with LD and PPG, and the ability of TBP with LD to diagnose the severity of disease in patients with PAD with and without DM (Study 1).

- To evaluate if the pole test at the toe level can be used for assessment of arterial insufficiency in patients with DM (Study 2).

- To evaluate the influence of cuff width on indirect TBP measurements (Study 3).

- To determine the optimal cuff width for toe TBP measurements in patients with PAD (Study 4).

- To evaluate the validity of an automatic TBP measuring device by comparing the automatic LD perfusion graph readings with the standard procedure of visual assessment of the Laser Doppler perfusion graphs (Study 5).

Methodology

Patients

Study 1: Ten healthy subjects and 76 patients with PAD were included in the study. The study was divided into two parts. In the first part 36 patients with different stages of PAD referred to vascular laboratory were included. In the second part 10 healthy subjects were compared with 30 patients with different symptoms of PAD and to 10 patients with DM.

Study 2: Twenty-five legs in 23 patients with DM and PAD were included.

Study 3: Eleven patients with DM without a history of atherosclerotic events or symptoms and six age-matched healthy control subjects were examined.

Study 4: A total of 20 patients, 8 with DM and 12 without DM, were recruited into the study. Thirteen of these had been referred for vascular examination at the Department of Clinical Physiology, Karolinska Hospital, Huddinge, Sweden. Seven patients were admitted to the ward for revascularization at the Department of Vascular Surgery, Karolinska Hospital, Solna, Sweden. All patients were recruited consecutively.

Study 5: Twenty-three legs in 16 patients with DM and PAD were included in the study. The patients were recruited at the outpatient clinic at the Department of Endocrinology, Metabolism and Diabetes, at Karolinska University Hospital, Solna (16 legs in 10 patients) and at Department of Medicine, Danderyd University Hospital, Sweden (7 legs in 6 patients).

Methods and procedures

The brachial blood pressure (Study 1, 2, 3 and 4)

The arm blood pressure was derived from the brachial artery by placing a 12 cm (Study 1) or 15 cm (Study 2) wide pneumatic cuff around the arm immediately above the elbow.
In Study 3 the circumference of the upper arm was measured before starting the blood pressure measurements. Two cuff widths, 12 and 15 cm were available. The cuff dimension closest to 40% of the arm circumference was used[93].
In both studies the Korotkoff´s sounds were detected with a stethoscope placed over the brachial artery in the cubital region. The procedure was repeated once, (twice in Study 3) and the mean (Study 1 and 2) or median value (Study 3) was used for the analysis. In study 4 a CW-Doppler was attached to the wrist to detect blood velocity in the radial artery.

Ankle blood pressure estimated with cuff (ABP_{cuff}, Study 1, 2 and 3)

A 12 cm (Study 1) or 15 cm (Study 2) wide sphygomanometer was placed just proximal to the malleoli. In Study 3 the circumference of the lower leg was measured. Three cuff dimensions were available 15, 12 and 9. The one closest to 40% of the circumference was used. In Study 2 the CW-Doppler was of the model 811-B (Parks Medical Electronics Inc. Aloha, OR, U.S.A).

The cuff was inflated until the Doppler signal disappeared. It was slowly deflated (2-3 mm Hg per 5 seconds) and the pressure in the cuff when the signal reappeared was registered as the systolic ABP, ABP_{cuff}.
The pressures in the three ankle arteries were all measured and the one with the highest pressure was considered to be the main contributor to the foot circulation. The pressure in the main artery was measured two times (three times in Study 3) and the mean value (Study 1, 2) or median value (Study 3) was used for analysis.

Toe blood pressure estimated with cuff (TBP_{cuff}, Study 1-5)

A pneumatic cuff was wrapped around the base of the toe and the cuff was inflated. Microvascular perfusion was monitored with a LD probe (Study 1, MBF 3 D, Moor Instruments, UK and Pfld®, Perimed AB, Sweden, Study 2, MBF 3D, Moor Instruments England, Study 3 and 4 PeriFlux 5000 and 5001, Perimed AB, Sweden, Study 5 Moor Instruments, Ltd PresTo v 2.0) attached to the hallux pulp skin. The cuff was then slowly deflated (2-3 mm Hg per 5 sec) and the value when the flux signal reappeared or increased more than 10 % above the occlusion value, was recorded as TBP_{cuff}.
In Study 5 results were also presented after a change in the algorithm used to automatically record TBP. The automatic algorithm was changed to depend on the rise of flux from the occlusive value without appearance of spikes only if the blood pressure was <45 mm Hg.

In Study 1 the skin circulation was also recorded with a PPG instrument (Electromedicin AB, Sweden AB) during TBP measurements.

The cuff widths used were 2.0 cm (Study 1 and 2), 2.0 and 2.5 cm (Study 3), 1.5, 2.0, 2.5, 3.0 cm (Study 4) and 2.5 cm (Study 5).

Skin temperature (Study 1, 3 and 4)

The skin temperature was measured in Study 1 with a termistor probe (Exacon®, Denmark) and in Study 5 using a (Digital Thermometer, KM330, Kane-May, Taiwan), or standardised to 34°C (Study 3 and 4) with a thermostatic probe (PF 5020, Perimed AB, Sweden).

The pole test (Study 2 and 4)

When ABP_{pole} was evaluated the ankle artery with highest pressure was assessed as dominant and was insonated with CW- Doppler during leg rise (Figure 2). When the Doppler signal ceased, the leg was slowly lowered again until an audible Doppler signal reappeared. This height, registered as the level over the heart's left ventricle, represented the systolic ABP_{pole}. The distance obtained from the pole test, measured in cm, were converted to mm Hg by assuming the density of blood to be 1.055 g/cm^3 and mercury to be 13.54 g/cm3[94]. Accordingly, the pressure unit 1 cm blood above the heart corresponded to 0.78 mm Hg (10 mm x 1.055/ 13.54 = 0.78 mm Hg).

LD was used to assess perfusion for the pole test at the toe level (TBP_{pole}). It was measured by elevating the leg until the LD signal dropped to biological zero. The leg was then slowly lowered again. The level above the heart where the flux signal reappeared or increased to more than 10% above the biological zero value was defined as TBP_{pole}.

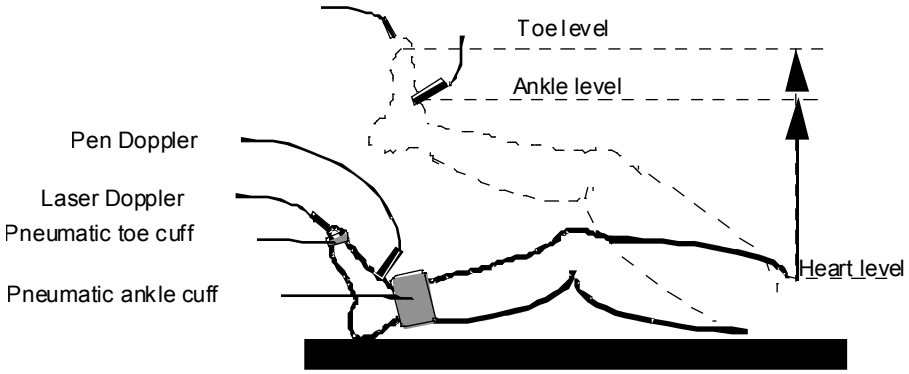

Figure 2. Schematic drawing of the study set up in Study 2.

Automatic toe blood pressure measurement (Study 5)

Two identical automatic TBP devices (Moor Instruments Ltd, PresTo v 2.0) were used and calibrated against each other (Figure 3). An LD probe was attached to the hallux toe pulp and a 2.5 cm wide toe cuff was used to compress the toe base during inflation. A computer software processed the signals from the LD probe and air pressure units. From that data the software algorithm calculated the blood pressure value (TBP_{aut}), which was displayed digitally on the front of the device. One measurement of TBP took around one minute to complete with the device. The LD and pressure signals were also recorded on a connected PC to enable visual assessment of TBP later.
Two operators, one specially trained nurse and one vascular laboratory technician, performed all examinations. These persons were trained together to align the procedures as much as possible. The feet were warmed for ten minutes with a heating blanket wrapped round the foot.

All TBP graphs recorded were distributed, in a randomised order, to three independent examiners. They individually assessed the graphs and read the resulting TBP values from each graph at two separate occasions with 4 weeks in-between.

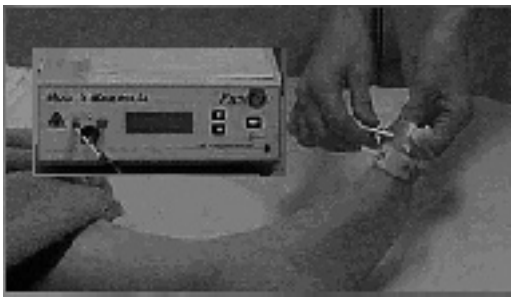

Figure 3. Device for automatic TBP measurement.

Results

Study 1: This study, which aimed to evaluate LD as a flow detection method for TBP measurements, compared LD and PPG in its first part. It showed that TBP values recorded with PPG were significantly lower than those obtained with LD ($P< 0.01$), although the correlation between values from the two methods was highly significant (Figure 4).
In the second part of the study the capability of TBP measured with LD for grading severity of disease was studied. The results showed that in patients without DM the worse the PAD state according to Fontaine, the lower was the mean TBP and TBI. There was, however, a substantial overlap between different Fontaine states between the values. In the patients with DM the TBP was a better detector of PAD than ABP in 3 out of 10 patients (Figure 5).
Accordingly, this study provided some evidence that LD can be used to assess TBP and that TBP measured this way has the capability to evaluate different PAD stages.

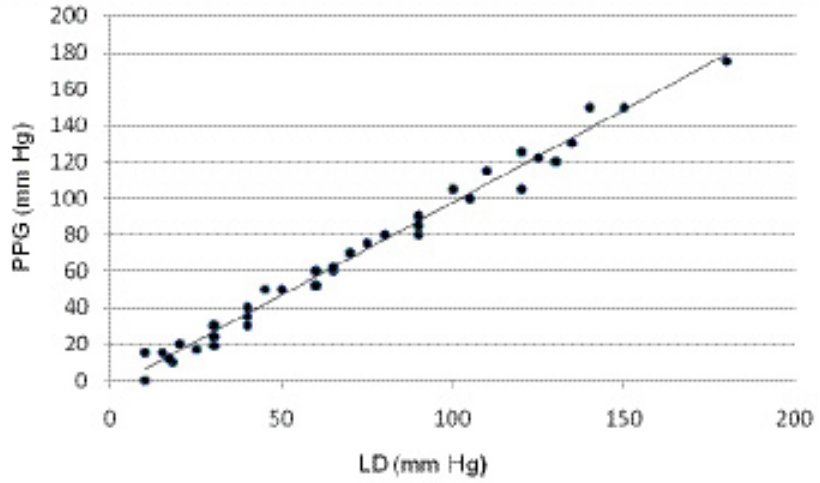

Figure 4. TBP measured with PPG and LD in 36 patients referred for vascular evaluation (r=0.99)

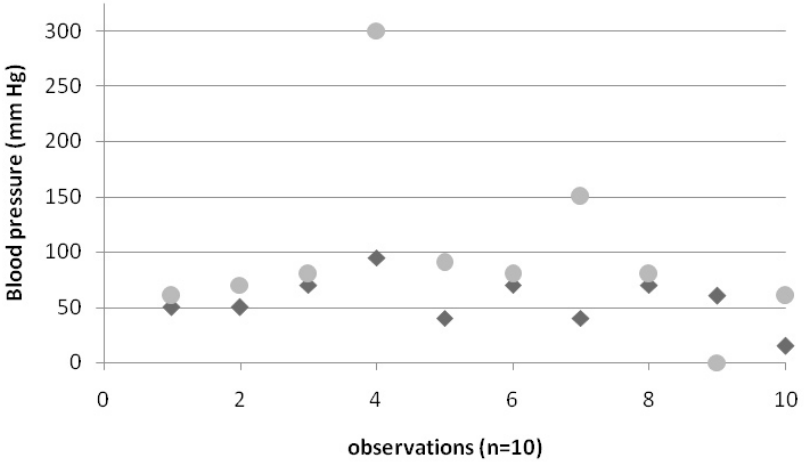

Figure 5. TBP (boxes) and ABP (circles) in 10 patients with DM. In two cases ABP was falsely elevated and in one case the blood flow could not be traced at ankle level.

Study 2: Whether TBP is a good reflection of the "true" blood pressure or not was evaluated in this study. Furthermore, it also examined the characteristics of the pole test at the toe level. The results showed that in the ankle it was possible to compare ABP_{pole} with ABP_{cuff} in only 44% of the patients. In 13 legs the blood flow was still detectable in one or more of the ankle arteries when the leg was maximally elevated. One leg had incompressible ankle arteries. In the legs in which it was possible to compare the two methods, ABP_{cuff} was significantly higher than ABP_{pole} ($P<0.01$) (Figure 6). Accordingly, ABP_{cuff} was a poor method for assessing the "true" ABP in the ankle level, and ABP_{pole} has limitations in clinical use because it can only measure very low pressures.
In the toe, on the other hand, the pole test seemed to be slightly more useful. In 76% of the legs it was possible to measure TBP_{pole}. In four legs there was no detectable perfusion and two legs still had persistent microcirculation when the leg was maximally elevated. The highest pressure possible to record with TBP_{pole} was 69 mm Hg. However, in the two patients with remaining perfusion at maximal leg elevation it was just possible to elevate the leg 65 and 74 cm corresponding to a mean of 54 mm Hg.
In the legs where both way to measure TBP could be compared there was no significant difference between the pressures recorded with cuff and pole test ($P=0.18$). This suggests that the cuff pressure is a better reflection of the true blood pressure at the toe level than in the ankle (Figure 7).

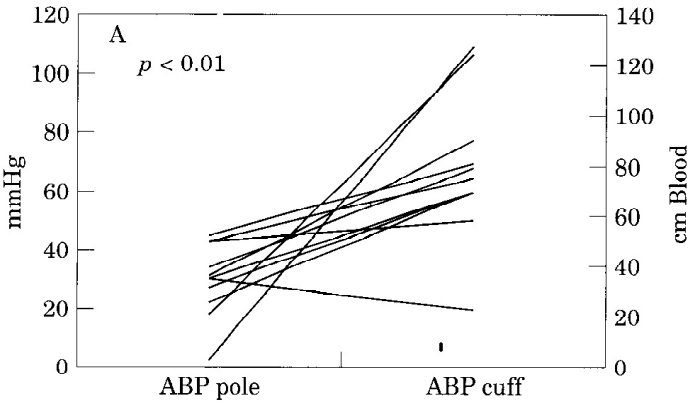

Figure 6. Blood pressures that could be measured with cuff and the pole test at ankle level in the same patient (N=11).

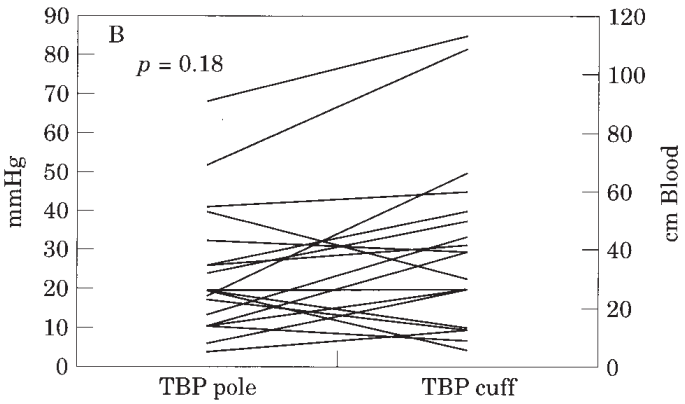

Figure 7. Blood pressures that could be measured with cuff and the pole test at toe level in the same patient (N=19).

Study 3: The possible impact of cuff size for TBP measurements was evaluated in this study and the included DM patients without PAD had mean TBP of 158 mm Hg (95% CI 9.4) and 143 mm Hg (12.0) with the 2.0 and 2.5 cm cuffs, respectively (Figure 8). The difference was significant (P=0.05). Control subjects also tended to have lower TBP values (P=0.09) with the 2.5 cm cuff, 128 (7.3), as compared to the 2.0 cm cuff, 151 (9.9). When both groups were analysed together the blood pressure difference between the two cuff sizes was 18 mm Hg (P<0.01). The difference in TBI values had the same relations as the absolute values, as the arm blood pressure was not dependent on cuff sized used.

A temporal variability test was also performed with the two cuff sizes. The difference between TBP values within the same subject was consistently small. There was no significant difference between the three measurements performed, and the average SD ranged from 2.0 to 6.4 mm Hg. Also in control subjects with the same toe size the variation was small, when measuring at two different occasions. The SD for three measurements day 1 and 2 was similar between the cuffs and ranged from 1.5 to 7.8 mm Hg (mean SD 3.9 mm Hg). TBP values correlated to the toe circumference (Figure 9) for the 2.0 cm cuff with r-values of 0.625 (P<0.05) and tended to do so also with the 2.5 cm cuff, r = 0.530 (P=0.09). Discrepancies between the two cuffs were observed primarily in patients with smaller hallux sizes.

In summary, this study showed that choosing the correct cuff width is important for obtaining a valid TBP. This is particularly important for patients with very large or small hallux sizes.

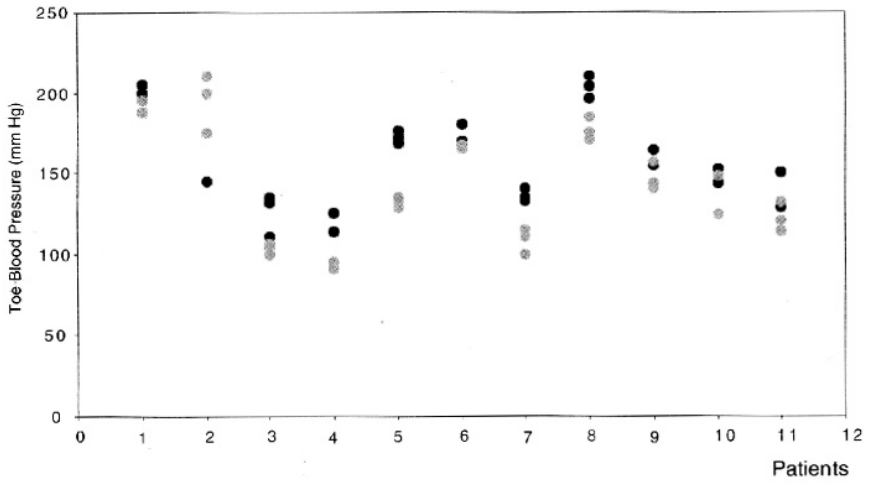

Figure 8. TBP measured with a 2.0 cm cuff (black circles) and 2.5 cm cuff (grey circles) in 11 DM patients.

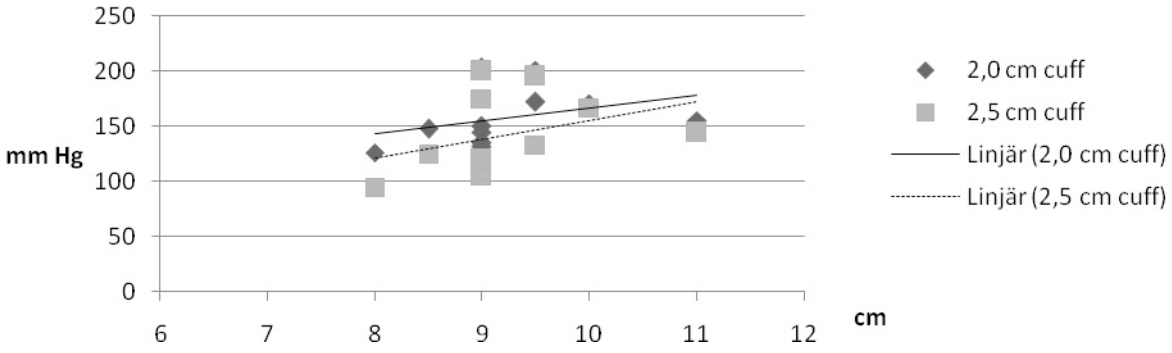

Figure 9. TBP values correlated to toe circumference in 11 patients with DM.

Study 4: The optimal cuff width for TBP measurements was determined in this study. The patients in the study had quite severe PAD and the mean TBP for them was 28 mm Hg (range 6-55 mm Hg, measured with the 2.5 cm cuff, Figure 10). As observed in Study 3 the difference between cuff sizes was clear also in these patients with PAD. For instance, TBP measured with the 2.0 cm cuff was 15.6 mm Hg higher (mean, CI 8-23, P<0.01) than the pole-test value, which was considered to reflect the "true" TBP. This difference was consistent when separately analyzing the groups recruited at the two hospital sites (P<0.01 and P=0.05, respectively) and in patients without DM (P< 0.01). No difference was found in patients with DM because a low number of patients. TBP assessed with cuff tended to be higher than pole test values also when the 2.5 cm cuff was used (4.5 mm Hg, 18%, CI 0-9, P= 0.07). In seven patients in whom additional cuffs were used no difference was found between TBP measured with a 3.0 cm cuff and pole test values (-2.0 mm Hg, CI -11-+8, P= 0.68), but the values were 27.0 (CI 13-43) mm Hg higher when using the 1.5 cm cuff (P=0.02). In the examined patients the TBP values differed significantly between the four cuff sizes (P< 0.01, Figure 11).

No correlation was found between the toe circumference and the difference in values between cuff and pole test TBP for any of the cuff sizes. Using TBI for the analysis did not influence the results.

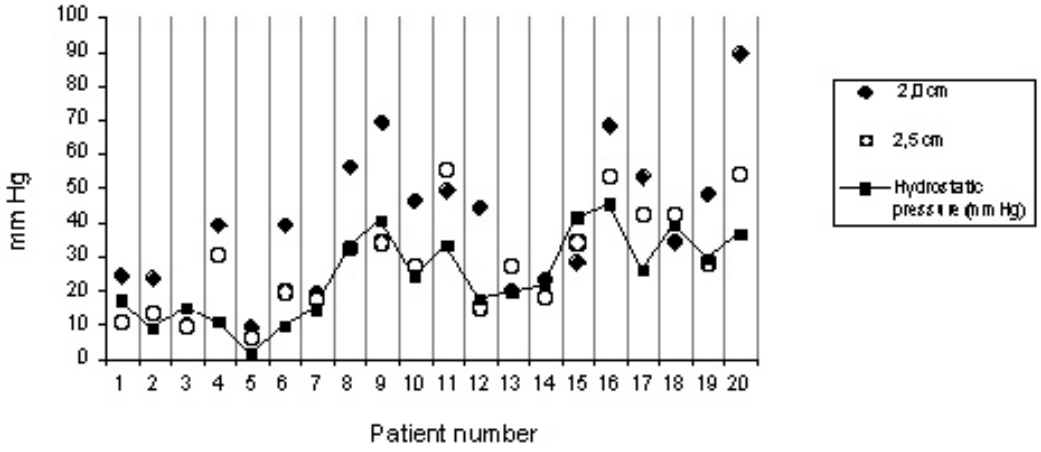

Figure 10. TBP in 20 patients with leg ischemia examined with 2.0 and 2.5 cm cuffs compared with hydrostatic pressure values assessed with the pole test.

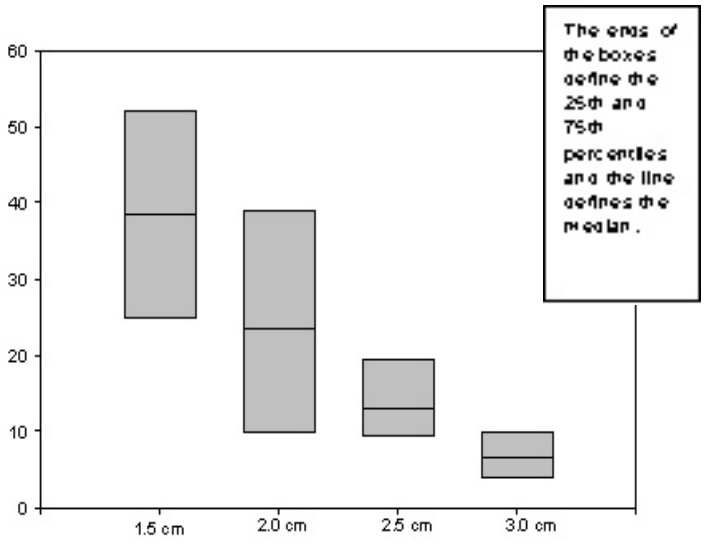

Figure 11. TBP results from 7 patients admitted for revascularisation at Karolinska Hospital, Solna, Sweden measured with four different cuff sizes at the same occasion. The difference between the cuff sizes was significant (P= 0.001).

Study 5: This study strived to determine the general variability of TBP measurements and also to evaluate an automatic device. The studied cohort had a mean TBP, based on visual assessment of the TBP graphs readings, of 51.0 mm Hg (range 22.4-95.7). The intra-observer variability was 2.3 mm Hg (mean) or 0.9 mm Hg (median) and 5.5 mm Hg (SD) and the inter-observer variability was 3.6 mm Hg (mean), 1.5 mm Hg (median) and 5.9 mm Hg (SD), which is displayed in Figure 12. Overall this corresponded to a variability of 4 to 9% of the measured values.

When the same TBP graphs were assessed with the automatic algorithm the TBP was estimated to be 56.4 mm Hg (range 20.5- 92.2), as compared to 51.0 mm Hg when visually read. Accordingly the TBP_{aut} values were 5.4 mmHg (mean) or 0.64 mm Hg (median) higher with TBP_{aut} than when visually assessed. The difference between the methods was 7.5 mm Hg (mean), 1.8 (median) with an SD of 11.4.
The discrepancy between the two methods was lowest in examinations where TBP was lower than 45 mm Hg. In this group the mean TBP_{aut} was 35.6 mm Hg and when visually analysed 37.1 mm Hg. In the examinations with TBP >45 mm Hg the mean TBP_{aut} was 69.1 mm Hg, as compared to a visually analysed TBP of 59.2 mm Hg. The difference in SD between these two groups was significant ($P<0.01$, Figure 13).

The two-minute variability (SD) of TBP_{aut} was 8.1 mm Hg (mean) or 5.0 mmHg (median). The corresponding data when graphs were read visually were 4.9 and 4.1, respectively. The first and the last of the three TBP_{aut} examinations did not differ significantly ($P=0.36$).

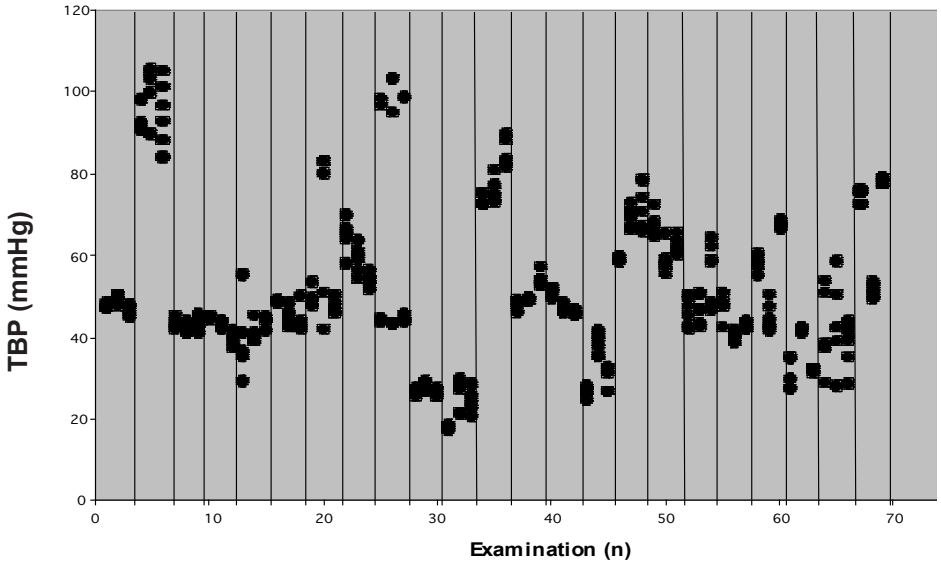

Figure 12. Visually read TBP examinations (N=69) from 23 legs (separated by bars) of patients with DM and PAD. LD was used as blood flow detector and the LD-flux/blood pressure graphs were visually assessed two times by three independent examiners. SD of the six visual assessments of every graph was 3.9 mm Hg (mean) and 1.9 mm Hg (median).

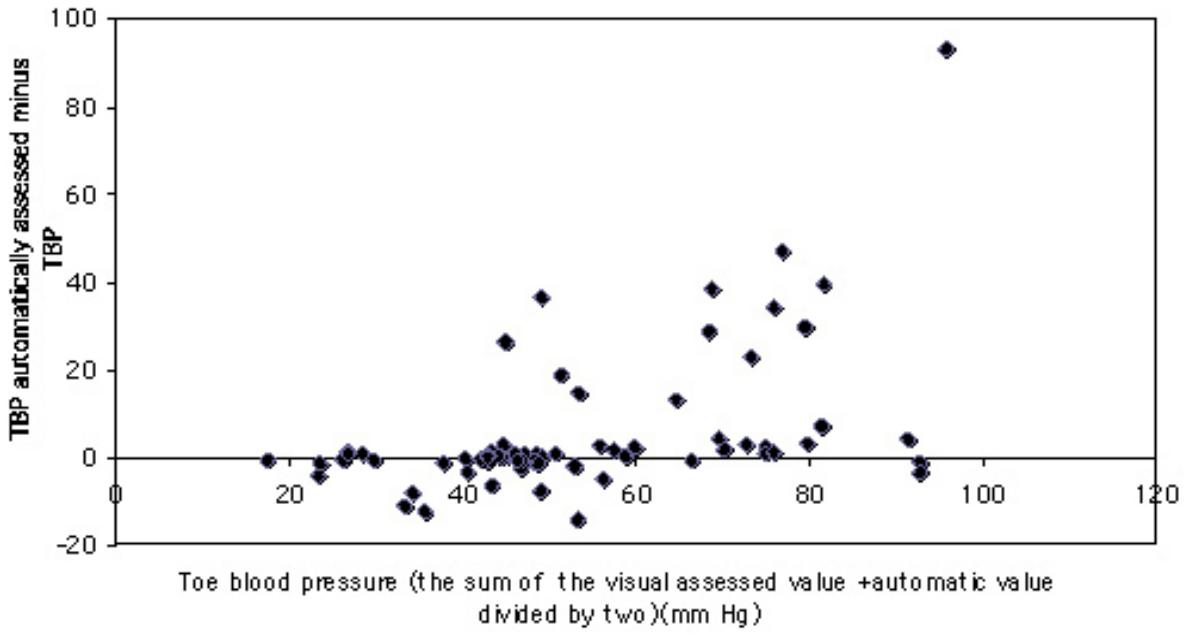

Figure 13. The difference between automatically and visually assessed TBP graphs in 69 examinations correlated to the average TBP. In 48 (70%) of the examinations the difference was <5 mm Hg, and in 10 out of the 69 (14%) the difference was >20 mm Hg. In the 25 examinations with TBP < 45 mm Hg the variability was significantly lower than in the examinations with TBP ≥ 45 mm Hg ($P<0.01$).

Statistics

(paper 1-5)

Groups are described with mean, median, range, standard deviation (SD) and 95% confidence intervals (CI). For paired comparisons, when the material was normally distributed, two-tailed paired t-test is used (Study 1 part A, 5). For non-paired comparisons between normally distributed independent groups, the independent t-test is used (Study 5). When the data is paired but not can be assumed to be normally distributed, Wilcoxon signed rank sum test is used (Study 2,3,4). For comparisons between two independent groups of non-paired data, which not can be assumed to be normally distributed, the non-parametric Mann-Whitney *U* test is used (Study 1 part B, 2). The non-parametric test used for comparing several groups of data was the Kruskal-Wallis test (Study 4). The non-parametric test used for correlation between two sets of data, depending on rank, is Spearman´s rank correlation coefficient (Study 1 part A, 3, 4). A two way non-parametric analysis of variance was used for comparing observations repeated in the same object, Friedman´s test (Study 3)

A Bland-Altman plot was made to expose a possible relation between the difference between two measurements and their mean value[95] (Study 5).

Discussion

This thesis focuses on one important method used for identifying DM patients with foot ulcers in need of more aggressive management such as revascularization to prevent amputation. The reason why the attention is entirely on TBP and not on other methods is primarily because TBP seem to have many promising features suggesting that it could be used more extensively for screening purposes. Examples are its non-invasiveness, the relative simplicity to interpret the values obtained and that it already is incorporated in treatment guidelines. The main disadvantages are that TBP measurements are poorly standardised, that the scientific support is limited for the notion that widespread use does reduce amputations and that TBP is difficult to measure outside vascular laboratories. By evaluating a current TBP method this thesis have tried to address two of these major disadvantages.

Identifying DM patients with severe PAD for invasive management

Amputation constitutes a major threat to patients with DM. The amputation incidence is 2-8 per 1000 patients with DM in Western countries[96,97]. The prevalence in Sweden for amputation above ankle in DM patients was 1-2 %, 2005[5]. In United States alone nearly 80 000 amputations are performed on patients with DM each year[98]. Accordingly, amputation is an enormous problem for society and DM patients with foot ulcers and emphasise the need for better management.
It is also important that aggressive treatment such as revascularisations not are performed in vain and that the right patients are selected for surgery[99]. In Sweden 2005 the 30-day mortality after operation for CLI was 4 % for non-DM and DM patients, and the amputation rate was 5% during that time. The one-year mortality 2004 was 23 % when operated for CLI and 35 % for acute ischemia and the amputation risk was about 4% for both groups. Revascularisation are not successful unanimously, but better selection may improve outcome. The failure to find the patients who will benefit the most from vascular procedures may have two different explanations. The wrong parameters are perhaps gathered for patient selection, and the methods used to measure these parameters may not be accurate enough.

Distal blood pressure assessment is an important part of this management process in patients with DM because neuropathy masks pain as a symptom of severe ischemia and CLI. The blood pressure at distal locations is directly correlated to the severity of disease. Blood pressure measurement for evaluating patients with PAD was first established in patients without DM and it can be questioned if the same parameters and methods used in patients with PAD without DM can be applied on patients with DM. New methods like laser Doppler heated probe fluxmetry, $TcPo_2$ and vital microscopy have made studies of the haemodynamic situation closer to the capillaries and the symptomatic organ – the skin - possible [74,78, 79,87]. None of these, with the possible exception for $TcPo_2$, has so far been generally used in clinical practice, and blood pressure measurement is still the method used for patient evaluation in most hospitals and clinics. The reasons for this are probably that distal pressure methods are well-established and measurements inexpensive to execute. The different methods have their own specific advantages and drawbacks in predicting healing of ulcerations and no method can perfectly predict outcome. For example, Apelquist et al showed that no patient with DM and an ulcer healed if they had an ABP <40 mm Hg but an upper limit above

which amputations was not required could not be defined. Most patients healed with a TBP >30 mm Hg, but still a TBP >45 mm Hg was needed for a primary healing rate of 85%[100] in that study. Moreover, Kalani found the positive predictive value for ulceration healing using $TcpO_2$ and TBP to be79% and 67%, respectively (Table 3).

Is TBP the method of choice?

In patients with DM the clinically most useful distal pressure to assess is TBP. This is because patients with DM often have lesions that obstruct blood flow located distally in the leg and, as mentioned previously, that the arteries often are affected by wall stiffness. In contrast to ABP, TBP can in ischemic patients with DM and foot ulcerations, quite well predict primary wound healing, but there are no safe blood pressure levels[101]. Even though TBP is recommended in the management guidelines such as the TASC documents, little attention has been directed to the methodological problems with this technique. Factors such as cuff size and the blood flow detection method used, influence the measurements[102,103]. The skin temperature is a third important factor that influence TBP values and should be kept constant during measurements. Several investigators have underlined this observation. In healthy young subjects there is a decrease in TBP during body heating compared to cooling[104]. In a study on non-DM patients with intermittent claudication, TBI was lower after body heating but not after cooling, compared to room temperature in examinations using PPG for perfusion assessment during the measurements[105]. It has been speculated if this reaction could depend on lower resistance in the vascular bed during body warming. Interestingly, the mean TBP was significantly higher when assessed with a heated LD-probe compared to unheated PPG in patients referred for evaluation at vascular laboratory in another study. When an unheated LD-probe was used the TBP values did not differ from unheated PPG[106]. These somewhat contradictory findings of the temperature's influence are difficult to interpret.

It has also been argued that presence of diabetic neuropathy influences the TBP values. In one study that used ABI as reference method it was proposed that it may cause falsely low TBP values. Since the population studied were old and had long diabetes duration this finding can be questioned. ABP based parameters in populations like this one is hardly optimal for reference considering its problems with stiff arteries[107].

The variability of TBP measurements appears to be quite low, at least in the controlled situation of research studies. For instance, the inter- and intra-observer variability, when measuring TBP with strain gauge technique, is slightly lower than for ABP measurements[111] (Table 4). Also when using LD for flow detection during measurements the variability seems to be quite reasonable, in line with the findings with strain gauge (Study 5). Together these data further support the use of TBP for PAD assessment in DM patients.

The impact of the flow or perfusion assessment method for TBP measurements

It has recently been demonstrated that ABI changes when different flow detection methods are used to measure the brachial artery blood pressure[102]. In Study 1 we observed that TBP values recorded with PPG and LD are very similar and highly correlated. In the lower pressure range, however, when PPG was used the values obtained tended to be lower than for LD based TBP values. This finding has to be interpreted with caution because it is based on few observations. In spite of this it seems that TBP is rather constant regardless if PPG or LD is used for flow detection during measurements. This is supported in more recent study than ours that come to similar results. In this study the intraclass correlation coefficient (ICC) was >0.95. Furthermore, the advantage noted in

our study (Study 1) of using LD compared to PPG for examination of patients with TBP < 25 mm Hg due to higher sensitivity was reinforced in that study[70]. Unfortunately, there is no study available that compares strain gauge with neither PPG nor LD for TBP assessment.

The impact of stiff arteries on the TBP value

One reason for advocating TBP instead of ABP when evaluating PAD in patients with DM is the assumption that calcified arteries is less of a problem in digital arteries than in calf arteries. It is based on the clinical experience that a substantial number of DM patients have incompressible arteries when measuring ABP, and a few clinical studies[58,59]. It was unknown if TBP values also are affected by stiff arteries.

Considering the lack of an appropriate number of cohort studies evaluating the predictive potential of TBP values for ulcer healing, intra-arterial blood pressure values can be regarded as the golden standard to which all other methods should be compared for accuracy. Our use of pole test as an alternative standard method is based on results from Smith et al[60]. In that study a good correlation between pole test values measured in the ankle and intra-arterial pressures recorded during surgery was reported. This is also the main support for choosing the pole test as the reference method for Study 4.

A few patients with a suspected falsely elevated ABP was identified in Study 1, but in Study 2 it was confirmed that erroneously high ABP values is an obvious problem when the blood pressure is assessed with a cuff in patients with DM and PAD. The difference between simultaneously performed recordings in the same patient of ABP in the cohort was 33 mm Hg, 68 with cuff and 35 with pole test. In the toe the difference was substantially less, only 5 mm Hg. Because the data was based on few patients the results have to be interpreted with some caution but the problem with stiff arteries is probably less in the toe.

It was also possible to conclude from that study (Study 3) that the pole test readily can be used for blood pressure measurements at toe level in patients suffering from PAD, at least when the pressure is lower than 60-70 mm Hg. Above that limit the length of the leg and the mobility of the hip joint restrict measurements. However, in a study from last year the ability of pole test to detect CLI in a patient cohort that included 50% with DM was examined. If ≤50 mm Hg was used as cut off value the sensitivity was 95% and the specificity 73 %[108]. The main problem, besides the inability to measure pressures in the higher range, is that the measurements are rather cumbersome to perform. Determining the level over the heart where perfusion reappears requires persistence from the examiner with strong attention to detail and some endurance from the patient.

Cuff size is important for TBP measurements

There are also other potential problems associated with the TBP measurements. One such is the size of the cuff. It has been known for decades that using too narrow cuffs during arm blood pressure measurements gives higher values than those obtained with wider cuffs[103]. Limited scientific efforts have addressed this problem in the toe and only one study form the 1970-ies have published data indicating the cuff size's importance in small digits[104]. In Study 3 we observed that in patients with DM but without PAD the TBP values estimated with 2.0 cm cuffs were significantly higher compared with 2.5 cm cuffs. The TBP values obtained were also related to the circumference of the toe and discrepancies between the two cuffs were found predominately in smaller halluxes. The findings are in accordance with the previously study that compared different cuffs in the thumb[109]. We were not able to confirm this finding in Study 4, where TBP values seemed to be independent

of the toe's circumference. In that study, however, hallux sizes were similar and neither as large, nor as small digits as in Study 3. Overall, it is probable that cuff width appears to be an important factor affecting the obtained TBP value that needs to be considered when evaluating patients with DM.

The profound influence of cuff width on the estimated TBP value observed resulted in a new question. Which cuff width could be used in clinical practice? This was the main focus in Study 4, its study design being based on our toe-pole test experiences from Study 2, the report from Smith et al[60], and the findings in Study 3.
There are several theoretical anatomical and technical explanations why the cuff width could influence the TBP value. First, frictional forces increase the pressure drop if a larger part of the artery is compressed (Poiseuille-Hagen formula, Figure 14). Second, due to relatively higher grade of dissipation of cuff pressure into the surrounding tissue a narrow cuff has to be insufflated more than a larger one. Third, most of a narrow cuff is applied over the diaphysis of the phalang. At this location the artery is often embedded in soft tissue more than at the epiphysis. Accordingly, the cuff has to be chosen carefully considering vessel anatomy, circumference and toe length.

$$\Phi = \frac{dV}{dt} = v\pi R^2 = \frac{\pi R^4}{8\eta}\left(\frac{-\Delta P}{\Delta x}\right) = \frac{\pi R^4}{8\eta}\frac{|\Delta P|}{L}$$

Figure 14. The Poiseuille-Hagen formula regarding the laminar stationary flow Φ of incompressible uniform viscous liquid through a cylindrical tube. It is defined by: *V* is a volume of the liquid, poured in the time unit *t*, *v* the median fluid velocity along the length of the tube, *x* the direction of flow, *R* the internal radius of the tube, *P* the pressure difference between the two ends, η the dynamic fluid viscosity, and *L* the total length of the tube in the *x* direction.

In Study 3 the patients had no signs of PAD the included subjects and the two cuff widths were compared only against each other. In contrast, the patients in Study 4 were referred for evaluation of PAD and the estimated cuff pressures were compared with the hydrostatic pressure. This study confirmed the great influence of cuff width on the measured TBP values, and provided some information on the great overestimation of TBP that occurs if narrow cuffs are used also in severely ischemic legs. It may result in false negative examinations, and could lead to underestimation of the ischemic component obstructing ulcer healing. These observations appear to be valid regardless if the patient has DM or not.

Another major finding was that TBP_{cuff} values obtained with 2.5-3.0 cm cuffs concurred best with the hydrostatic pressure. These widths were about 30 % of the hallux circumference. This observation is slightly surprising since a cuff width of 40% of the arm circumference has been recommended for brachial blood pressure measurements[110]. If this rule was applied to patients in this study the largest cuff needed should be 4.6 cm wide. However, a cuff of that size interferes with the flow detector and is not impossible to use. To some extent this was also true for 3.0 cm wide cuffs in some digits. Our data, while not yet supported by other groups, provides evidence for use of standardised cuff size for TBP measurements. In clinical trials, for reporting standards and to enable comparison between studies we propose that a 2.5 cm wide cuff should be used. At least, the cuff size should be given to enable interpretation and comparison.

The variability of visually read pressure-flow TBP graphs

Conventional TBP measurements are depending on manually performed visual readings of pressure-perfusion (or volume changes) graphs to determine the actual pressure. When LD is used this consists of visualising the LDF value during slow release of cuff pressure from a supra systolic level. These graphs can be assessed instantly on a screen or later if saved on a computer or printed. In Study 1 the graphs were printed on paper and in Study 2, 3 and 4 they were visually assessed instantly from the screen of the LD-equipment. An important source of error is of course the individual assessor's ability to correctly evaluate the graphs. It is important that readings do not differ from time to time or from pressures determined by other assessors. In Study 5 the LD-flux and cuff pressure graphs were saved and distributed to three assessors. The inter-observer variability from these readings was 3.6 mm Hg and the intra-observer variability 2.3 mm Hg. These figures can be compared with TBP measurements using the strain-gauge technique that has an inter-observer variability of 3.5 mm Hg and an intra-observer variability of 2.7 mm Hg[111]. Overall this variability is quite low and less or well in the range of ABP measurements.

One important observation, however, is that the variability in Study 5 was not equally distributed among the examined legs. Three examinations that derived from the same leg had the highest variability of all recorded and three additional examinations obtained from another leg also had a high variability (Figure 15). Both patients had digital ulcerations. When analysed in detail it was apparent that the LD-graphs with the highest variability between visual readings had a biphasic curve pattern. The common way of determining the TBP by reading pressure-perfusion LD graphs is to identify occurrence of pulsatile spikes with subsequent elevation of the LD-flux value or a non-pulsatile elevation of LD-flux >10-20% from the biological zero value (Figures 16 and 17). However, in the graphs with a very high SD this point was poorly defined as both conditions were fulfilled but at separate blood pressure levels (Figure 18). This is probably the explanation for the high variability observed in some patients. In Table 5 a model for visual readings of TBP from pressure-LD perfusion graphs addressing the biphasic curve pattern problem is presented.

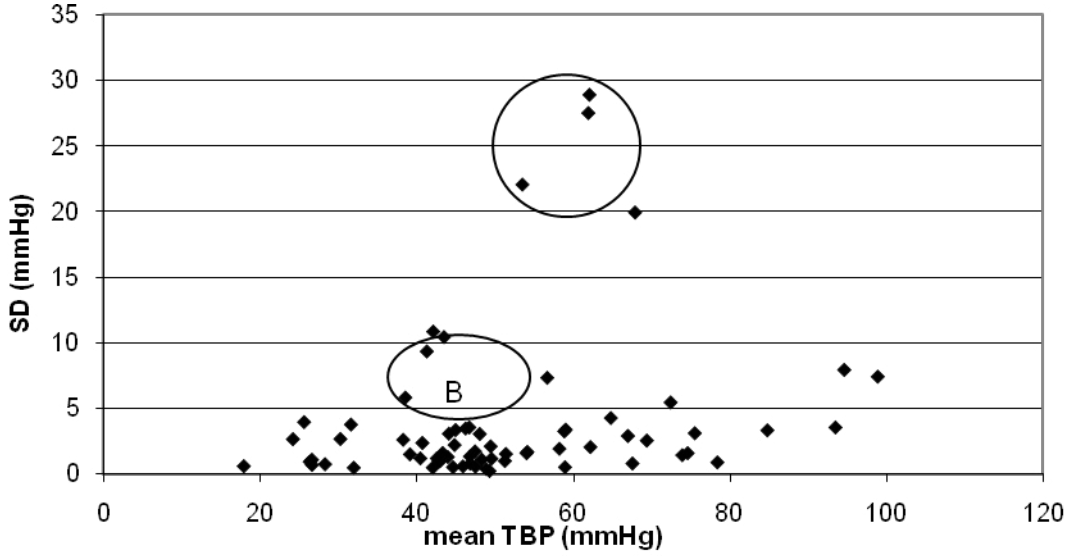

Figure 15. The SD of six TBP graphs assessments correlated to the mean TBP in 69 examinations performed on 23 ischemic legs in DM patients. In two legs the SD was remarkably high (encircled)

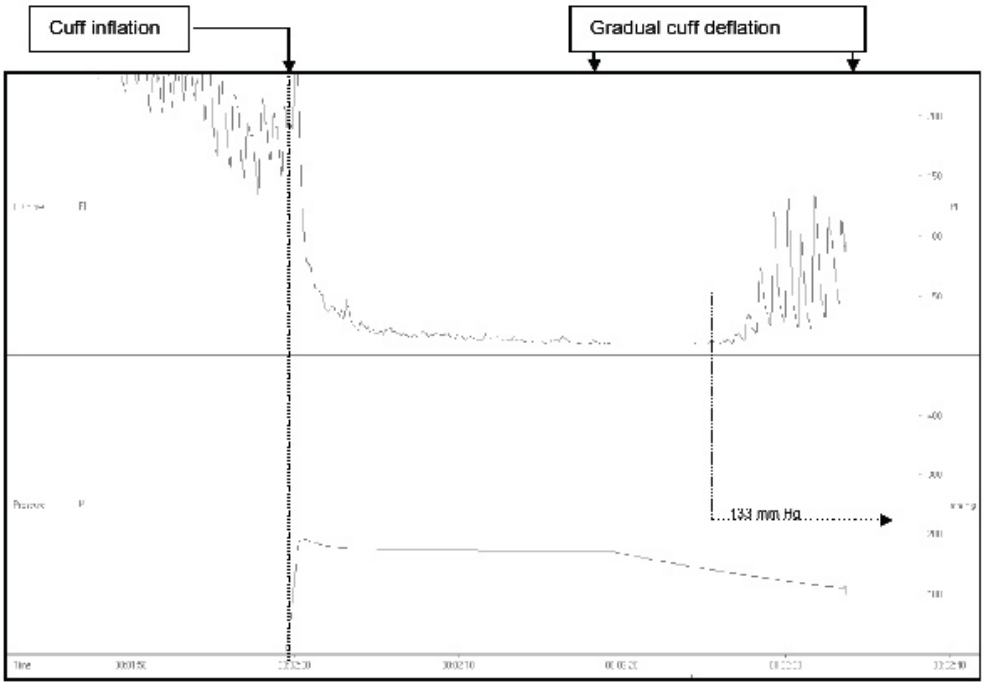

Figure 16 Screen print during automatic toe blood pressure assessment. The upper half screen displays the LD-graph and the lower the concomitant cuff pressure. This is a recording of a healthy person. It is fairly easy to decide the point at which the pulsatile blood flow returns, in this case 133 mm Hg.

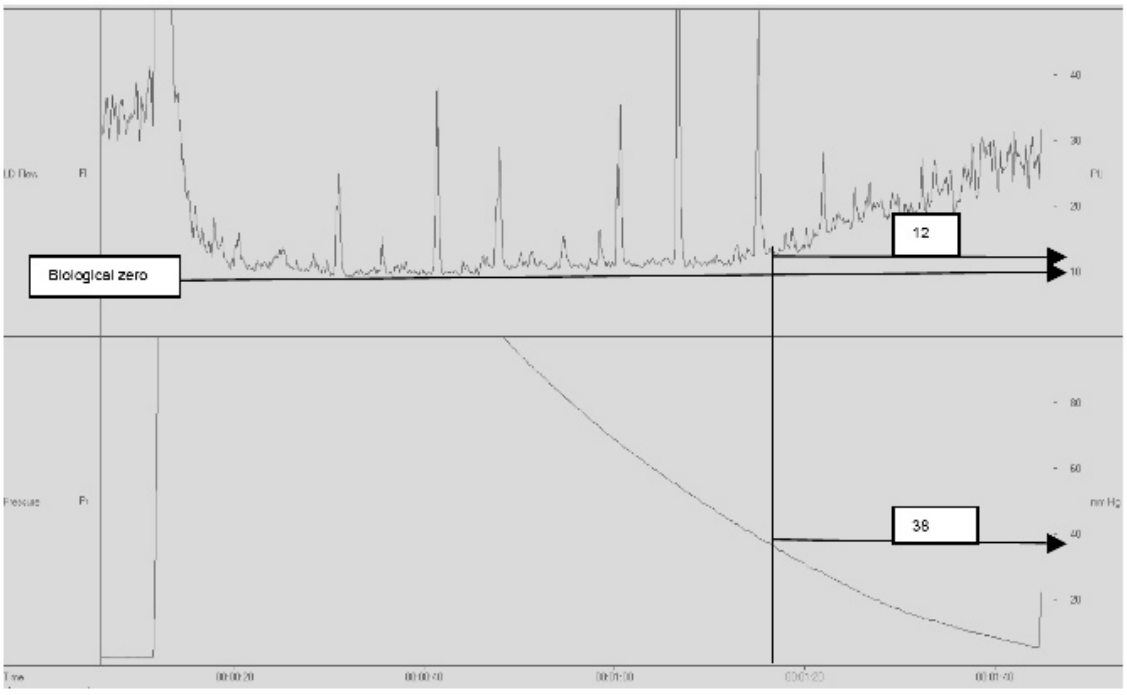

Figure 17. The upper half of the figure displays the LD-perfusion graph with a slowly elevating rise of the flux value. The blood flow is considered to have returned when the LD-flux value has elevated 20% from the biological zero value. The blood pressure read is 38 mmHg.

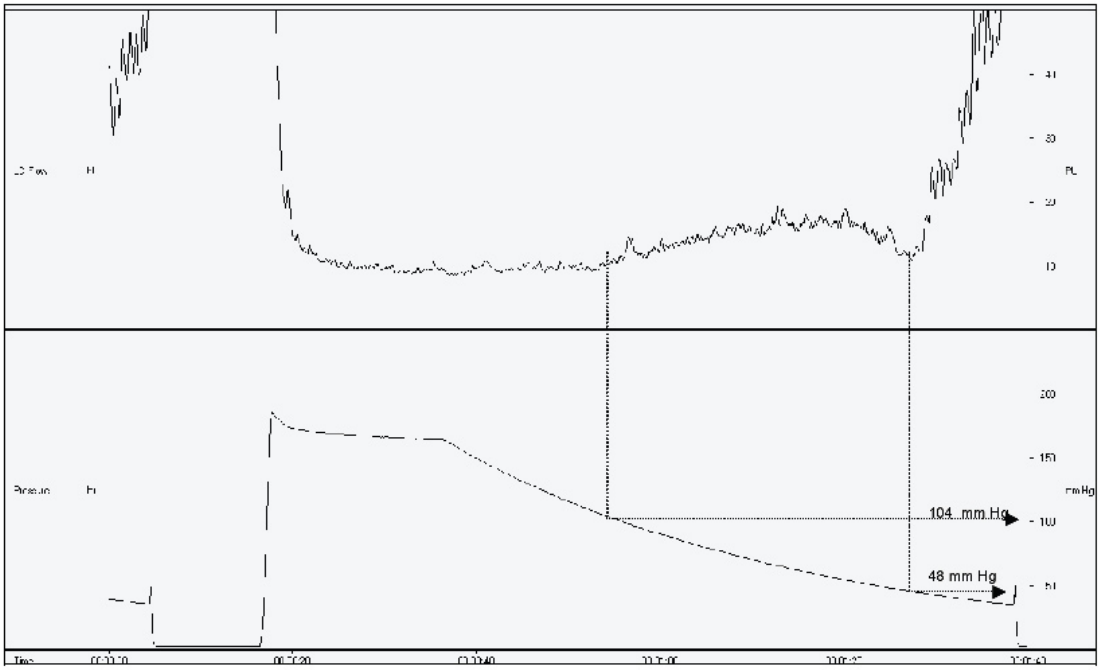

Figure 18. The upper half of this figure displays the LD-graph and the lower the concomitant cuff pressure. If the pressure is read from the beginning of the first elevation of the LD flux value the TBP is assessed to be 104 mm Hg in contrast to 48 mm Hg when read from the final elevation.

Automatic assessment of TBP

The principal aim in Study 5 was to evaluate an automatic TBP measuring device. This device has been developed by our research group in collaboration with Moor Instruments Ltd and has previously been described briefly in 2002[68] (Figure 3). Its principle is also explained in the Material & Methods sections of Study 5. Its main advantage is its versatility and simplicity. It is small and easy to handle, and even unexperienced persons can perform measurements. Each recording requires only around one minute to accomplish. Up till now it has not been properly evaluated because its main innovative feature, the software algorithm, has required extensive adjustment and development.

It was clear in Study 5 that the software algorithm had the same problem as the visual observers when dealing with the problem of biphasic LD graphs. This is illustrated by one patient's pressure-perfusion graph displayed in Figure 18. In this particular patient the SD for the three examinations was 31.6 mm Hg when measured with the automatic device and only slightly lower when visually assessed. Besides this problem the automatic TBP device, relevant for higher TBPs, it appeared to work well with a variability in line with visual readings. In the 25 examinations where TBP was <45 mm Hg the mean TBP_{aut} was 35.6 mm Hg, and when visually analysed 37.1 with low SDs. Candidates for referral and aggressive management usually have a TBP in the range between 30 and 45 mm Hg. This means that the automatic device can be used to readily identify these patients, and it can be regarded as reliable for identifying patients with a possible CLI.

It would be an advantage if the algorithm also would take the problem with the biphasic curve pattern into account. The automatic device would then possibly be reliable for the entire range of TBP values.

Table 5. A model for how TBP can be assessed, using LD for flow detection considering the biphasic flux-pressure response graph.

TBP <45 mm Hg	**The occurrence of pulsatile spikes with sequently elevated LD-flux value Or**	**A non-pulsatile elevation of LD-flux value >20% from the biological zero value**
TBP>45 mm Hg	The occurrence of pulsatile spikes with sequently elevated LD-flux value.	

The principle proposed in Table 5 for TBP determination can also be used for automatic measurements. In Study 5 an evaluation of a new software algorithm is described. For example, the mean difference to visual readings was only 2.9 mm Hg as compared to 5.9 mm Hg in the previous one (Figure 19). The new algorithm has a tendency to underestimate the TBP compared to visual assessment rather than overestimating it as the old one did. The number false negatives with the new algorithm was <2% as compared to 9% for the old algorithm when a cut off value of visually determined TBP<45 mm Hg were used. If the cut off level < 50 mm Hg is used no false negatives were found. Accordingly, the performance of this new algorithm and the automtic device on the whole is most promising, but still needs some further evaluation.

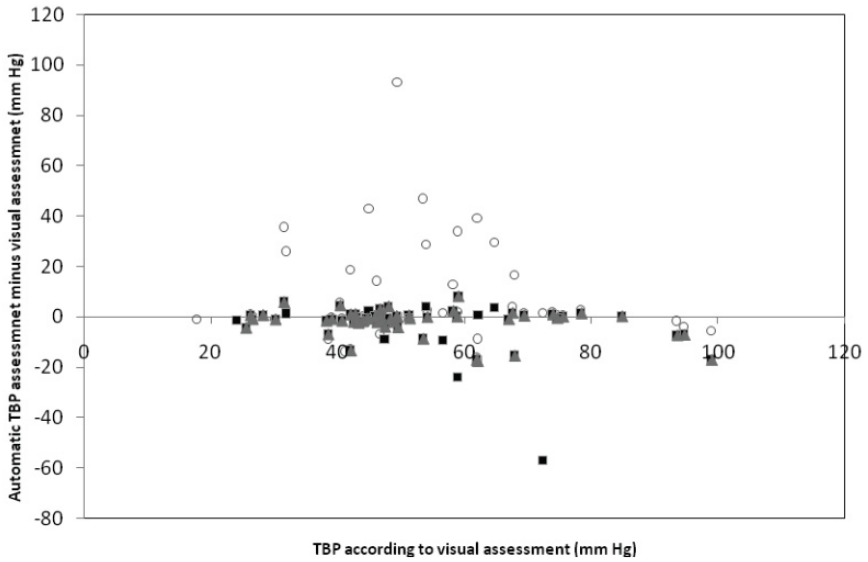

Figure 19. The result of 69 TBP measurements assessed with two different automatic algorithms and correlated to visual assessment. The original algorithm was able to produce a TBP value in 69/69 examinations(□). The upgraded algorithm, considering the biphasic LD curve, was able to assess 66/69 examinations (□). The modification reduced the number of examinations differing >20 mm Hg from visual assessment from 9 to 2 and those >10 mm Hg from 15 to 7. In 10 of the examinations the new algorithm categorized the signal as unreliable although it produced a value. If these unreliable values are excluded none of the 56 examinations left differed > 20 mm Hg and only 5 differed more than 10 mm Hg from the visual value (□).

Clinical applications of the findings in this thesis

It is certainly a relevant question whether there is a place at all for TBP or $TcpO_2$ measurements in management of severe leg ischemia and CLI. In one study, for instance, a small group of PAD patients referred to a hospital were randomised to a management strategy involving a clinical examination and ABP measurements or a strategy were the results from TBP and $TcpO_2$ measurements also guided treatment. At follow up after 18 months no signs of improved clinical outcome in the group undergoing TBP and $TcpO_2$ was observed. The proportion of patients with DM was the same in both groups (46%) and could not explain the findings. On the other hand, DM patients with incompressible arteries, 15 of the 21 with DM in the clinical examination group had TBP measured for ethical reasons, which also guided management[112]. To our knowledge, besides what is presented in Table 3, there are no further studies evaluating the outcome of foot ulcers managed by TBP guidance. Overall, in line with the TASC document statement, this means that at least for DM patients TBP is probably a valuable tool for assessment of leg ischemia.

This thesis has tried to provide some background information for more widespread and more accurate clinical use of TBP. When measuring it, our Study 1 as well as other reports, supports the use of LD for TBP measurements. As LD was found to be more sensitive than PPG for recording of lower TBPs, all patients can be assessed and smaller number with an undectable value encountered. This is helpful for long term comparisons and clinical studies. LD is also easy to make portable and a wider use outside vascular laboratories using small simple devices can be expected. The importance of cuff width standardisation for TBP assessments must also be underlined. In the literature studies have used a variety of cuff widths, ranging from 1.5 to 3 cm, and in clinical practise it can be suspected that cuffs with different widths has been used randomly. This has contributed to a distrust of the TBP method. Our proposal of a general recommendation of using a 2.5 cm wide cuff or one that is 30 % of the toe circumference needs to be communicated and discussed. Another source of high variability related solely to TBP examinations with LD are the occurrence of biphasic LD curves. The identification of this problem and the proposal on how to handle it will further reduce the variability and make the examinations more reliable. It will also facilitate reproducible automatic TBP measurements.

Future aspects

In order to further evaluate the management principles of DM patients with foot ulcers there are many issues and information gaps that still need to be addressed. As mentioned, the most important one is probably the value of additional diagnostic work up besides clinical examination and palpation of foot pulses. It is soon twenty years since Apelquist et al presented their study were patients with DM and foot ulcers underwent TBP measurements and its prognostic value was assessed ([100]Table 3). In this study TBP was measured with strain-gauge or LD with unknown cuff sizes. Skin temperature was not controlled. It would be very valuable to perform a similar new study including patients with DM and foot ulcers to evaluate the predictive value of TBP performed as optimial as possible as described in this thesis. Our group have recently initiated one such a study and have so far included almost 20 patients. In this new study the automatic TBP device is used and patients are excluded if they have palpable pulses to avoid patients with purely neuropathic ulcers.

Other areas where TBP could be of importance are for epidemiological studies of PAD. It is unknown if TBP is better or equal to ABP in predicting cardiovascular events in this DM patients. The development of the automated device has made it possible to consider TBP measurements in cohort studies on natural history of PAD patients.
Optimized TBP measurements should also be employed in clinical trials evaluating pharmacological treatment of PAD, in particular in DM patients.

There are also more technical aspects of TBP measurements that need further consideration. For example the properties of the biphasic curve patterns could be addressed to further improve TBP measurements with LD. With reference to the animal studies mentioned in study 5 it can be speculated that this wave form depend on arterial stenosis in arteria iliaca or femoralis. If this hypothesis is true it would be possible to induce it in healthy individuals by compression of arteria femoralis in the groin. To make such a study even more interesting TBP could be measured in patients with DM in whom angiography with femoral access was performed and bleeding managed with a compression device. During stepwise release of the compression applied in groin, TBP with LD could be assessed repeatedly to disclose any relation between the stenosis and the biphasic wave. To objectify the degree of the applied stenosis this could be assessed with diagnostic ultrasound.

Another technical aspect of TBP measurements is the influence of temperature. Body warming is lowering the TBI in non-DM patients while heated LD measurements has been reported to assess higher TBP values in patients referred for vascular evaluation. It should be valuable to know how body warming, foot warming and heated probes influence the assessed TBP values in patients with DM and different ischemic states.

Conclusions

In summary, TBP is a commonly used method to evaluate the severity of ischemia in patients with DM and PAD that is supported in management guidelines. In this thesis the TBP measurement technique has been evaluated and in some circumstances improved and standardised. LD can safely replace PPG as blood flow detector during measurements, and TBP does reflect a "true" blood pressure better than ABP. The introduction of the pole test at toe level has made it possible to estimate the TBP in patients with DM and PAD without any influence from stiff arteries though falsely elevated blood pressures. The optimal cuff width is important to consider and shall be 30% of the toe circumference or 2.5 cm wide. TBP assessment with automatic devices is a reliable method to screen for CLI and if the biphasic curve pattern is handled automatic devices can be used for TBP measurements all over the scale. These findings may contribute to a more accurate and widespread use of TBP in the future.

The conclusions for the more specific aims are as follows:

I. LD fluxmetry can be used for flow recordings during assessment of toe blood pressure in patients with PAD.

II. The pole test can be used at toe level for evaluation of patients with DM and severe PAD.

III. The cuff width used during TBP measurements affects the obtained TBP value and need to be considered when evaluating patients with DM.

IV. The proper cuff width to use corresponds to 30% of the toe circumference or in most cases 2.5-3.0 cm.

V. An automatic TBP measurement can be used to identify patients with a TBP< 45 mm Hg. One problem that seems to account for the high variability in TBP measurements in the higher pressure range for both visual and automatic graph readings using Laser Doppler is biphasic curve patterns. The biphasic curve pattern can be addressed with a modified algorithm. The improved algorithm increases the usefulness of TBP measurements with LD.

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References

[1] Mönckeberg J.G.: Über die reine Mediaverkalkung der Extremitätenarterien und ihr Verhaltung zur Arteriosclrose.*Virchows Atrchiv für Pathologische Anatomie und Physiologie und für Klinische Medizin* 171(1904) 141-167
[2] Bowen et al: A study of the lower extremities in diabetes as compared with non-diabetic states, from the standpoint of x-ray findings, with particular reference to the relationship of arteriosclerosis and Diabetes; *Bullentin of the Buffalo General Hospital* **2**(1924) 35-41
[3] Formijne P, Investigation of the patency of peripheral arteries. *Am Heart J* 1934; 10:1-16
[4] De Bakey, Professor Jean Kunlin, J Cardiovasc Surg, 1992, **33**:259-262
[5] Nationella Diabetes registret (NDR) Årsrapport 2006
[6] Lipscombe LL and Hux JE, Trends in Diabetes prevalence, incidence, and mortality in Ontario, Canada 1995-2005: a population-based study. *The Lancet* (2007); vol369, issue 9563, 750-756
[7] SOS(National Board of Health and Welfare in Sweden),MARS(Medical Access and Result System), Nationella riktlinjer för vård och behandling vid Diabetes mellitus,1999-09-15,(Sw.)
[8] Reiber GE, Epidemiology of foot ulcers and amputations in the diabetic foot, In:Levin and O´Neal´s The Diabetic Foot (6) 13-32 (2001)
[9] SOS(National Board of Health and Welfare in Sweden),MARS(Medical Access and Result System),State of the Art-Den diabetiska foten.1997-07-08,(Sw.)
[10] Larsson J et al, Decreasing incidens of major amputations in diabetic patients: A consequence of a multidisciplinary foot care team approach. *Diab Med.* 1995;12:770-6
[11] LoGerfo F W et al: Trends in the care of the diabetic foot. Expanded role of arterial reconstruction. Arch Surg 1992; 127(5):617-20
[12] Larsson J, Apelqvist J: Towards less amputations in diabetic patients. Incidence, causes, cost, treatment, and prevention—a review; *Acta Orthop Scand. 1995 Apr;66(2):181-92.*
[13] Reiber GE. The epidemiology of diabetic foot problems. Diabet Med 1996;13:6-11
[14] Apelqist et al;Diabetic foot; optimal prevention and treatment can halve the risk of amputation, *Läkartidningen 1999;96:37-41*
[15] Centers for Disease control and Prevention. *The Burden of Cardiovascular Disease, cancer and diabetes.* Available at : http://www.cdc.gov/Diabetes/statistics/lea/table1link.htm
[16]Vascular registry in Sweden, annual report 2006, http://www.ssvs.se/swedvasc.htm
[17] Alberti KG,Zimmet PZ: Definition and classification of Diabetes mellitus and its complications.Part1: diagnosis and classification of Diabetes mellitus provisional report of a WHO consultation.*Diabet Med* 1998 Jul;15(7):539-53
[18] Taskinen M.-R, Diabetic dyslipidaemia:from basic research to clinical practice. *Diabetologia*(2003) **46**;733-749
[19] Gardner CD, Fortmann SP, Krauss RM, Association of small low-density lipoprotein particles with the incidense of coronary artery disease in men and women. *JAMA*(1996) **276**:875-881
[20] Amar J, Perez L, Burcelin R, Chamontin B, Arteries, inflammation and insulin resistance. *J of Hypertension* (2006),**24**(suppl 5); S18-20
[21] Libby P, Inflammation in atheroscleroses. *Nature* (2002); **420**: 868-874
[22] Foster D W, Diabetes Mellitus, In: Petersdorf,Adamsx,Braunswald,Isselbacher,Martin,Wilson in Harrison`s Principles of Internal Medicine, McGraw-Hill International Book Company 1983;114:661-679
[23] Webster and Scott: What cardiologists need to know about Diabetes. *The Lancet;* **350**(suppl I): 23-28
[24] Haffner S M, Lehto S, Rönnemaa T, Pyörälä K, and Laakso M; Mortality from Coronary Heart Disease in Subjects with Type 2 Diabetes and in Non-diabetic Subjects with and without Prior Myocardial Infarction (1998) *N Engl J Med* 339: 229-234

[25] Stegmayr B,Asplund K, Diabetes as a risk factor for stroke. A population perspective. *Diabetologia* 1995 Sep;38(9):1061-8
[26] Abbott RD et al. Epidemiology of some peripheral arterial findings in diabetic men and women: experiences from the Framingham study. *Am J Med* 1990;**88**:376-381
[27] Young MJ et al, A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 1993 Feb;36(2):150-4
[28] Boulton AJM, The Pathogenesis of Diabetic foot Problems: an Overview
[29] Laing P, Diabetic Foot Ulcers, *The American Journal of Surgery*, vol 167,No. 1A(Suppl),Jan 1994
[30] Shaw JE and Boulton JM. The Pathgenesis of Diabetic Foot Problems- An Overview, *Diabetes*; vol 46,suppl.2, September 1997
[31] Reiber GE. Risk factors for amputation in patients with Diabetes mellitus: a case control study. *Ann Int Med* 1992;**117**:97-105.
[32] Edmonds ME et al, Improved survival of the diabetic foot: the role of the specialized foot clinic. *Quart J Med.* New Series 1986; 60(232): 763-71
[33] Thomson FJ et al, A team approach to diabetic foot care - the Manchester experience. *The Foot* 1991; 1:75-82
[34] Jude et al, Peripheral vascular disease(PVD) in diabetic and non-diabetic patients: a comparison (Abstract). *Diabetic Med* 13 (Suppl. 7):S47,1996
[35] Strandness DE et al, Combined clinical and pathologic study of diabetic and non diabetic peripheral arterial disease. *Diabetes* 1964;13:366-72
[36] Conrad MC. Large and small artery occlusion in diabetics and non diabetics with severe vascular disease. *Circulation* 1967;36:83-91
[37] Ciavarella A et al, Angiographic evaluation of the anatomic pattern of arterial obstructions in diabetic patients with critical limb ischaemia, *Diabetes & Metabolism*(Paris), **19,** 586-589, 1993
[38] Mackey RH,Venkitachalam L,Sutton-Tyrrell K, Calcifications, Arterial Stiffness and Atherosclerosis in: Safar ME, Frohlich ED(eds): Atherosclerosis, Large Arteries and Cardiovascular Risk. *Adv Cardiol*.Basel, Karger, (2007), vol 44, pp 234-244
[39] Neubauer B, Diabetic Macroangiopathy; Medial Calcifications, Narrowing, Rugosities, Stiffness, Norepinephrine Depletion and Reduced Blood Flow Capacity in the Leg Arteries. *Acta Med Scand,* Suppl.687:37-45
[40] FW LoGerfo and GW Gibbons, Clinical Features and Treatment of Peripheral Vascular Disease in Diabetes Mellitus. In: KGMM Alberti, P Zimmet, RA DeFronzo, H Keen (Honorary). *International Textbook of Diabetes Mellitus*,John Wiley & Sons Ltd 1997;**84**, 1623-1630
[41] Flynn MD et al, Direct measurement of capillary blood flow in the diabetic neuropathic foot. *Diabetologia* **31**: 652-656; 1988
[42] Jörneskog G: Functional microangiopathy in the digital skin of patients with diabetes mellitus, *Thesis, Karolinska Institute,Stockholm, Sweden,*1995
[43] Tooke JE and Brash PD, Microvascular Aspects of Diabetic Foot Disease, *Diab Med.* (1996); 13:S26-S29
[44] Falkenberg M: Metabolic control and amputation among diabetics in primary health care a population based intesified programme governed by patient education. *Scand J Prim Health Care 1990;8:25-9*
[45] Malone J M et al. Prevention of amputation by diabetic education. *Am J Surg 1989;159:520-24*
[46] Apelqvist J, Larsson J. The organisation of diabetic foot care In: Advances in Wound Management, Cherry GW, Leaper O, Lawrence JC, Milwould P(eds), MacMillan Magazines Ltd, London 1995, 168-170. ISBN 0 333 650 409
[47] Hehenberger K, Kratz G, Hansson A, Brismar K, Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. J Dermatol Sci 1998; 16(2): 144-51
[48] Grant SM and Goa KL, Iloprost. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in peripheral vascular disease, myocardial ischemia and extracorporeal circulation procedures. *Drugs* 1992 Jun;43(6):889-924
[49] Wang HJ, Wan HL, Yang TS,Wang DS, Chen TM, Chang DM. Acceleration of skin graft healing by growth factors. *Burns*. 1996 Feb;22(1):10-4
[50] Hogge J et al, The potential benefits of advanced therapeutic modalities in the treatment of diabetic foot

wounds, J Am Podiatr Med Assoc 2000 Feb;90
[51] Consensus Document, *Eur J Vasc Surg* **6** (Supplement A), 1-32 (1992)
[52] Edmonds M E and Watkins PJ, The Diabetic Foot. In: KGMM Alberti, P Zimmet, RA DeFronzo, H Keen (Honorary). *International Textbook of Diabetes Mellitus*,John Wiley & Sons Ltd 1997;**87**, 1657-1669
[53] Bakal C et al. Percutaneous transluminal angioplasty of the infrapopliteal arteries; results in 53 patients. *Am J Roentgenol* 1990;**154**: 171-4
[54] Jacqueminet S, Hartemann-Heurtier A, Izzillo R, Cluzel P, Golmard JL, Ha Van G,Koskas F,Grimaldi A. Percutaneous transluminal angioplasty in severe diabetic foot ischemia: outcomes and prognostic factors. *Diabetes Metab.* 2005 Sep;31(4 Pt 1):370-5
[55] Pomposelli FB et al. Dorsalis pedis arterial bypass: Durable limb salvage for foot ischemia in patients with Diabetes mellitus. J Vasc Surg 1995; 21:375-84
[56] Karacagil S et al, Comparative Analysis of Patency, Limb Salvage and Survival in Diabetic and Non-diabetic Patients Undergoing Infrainguinal Bypass Surgery. *Diabetic Med, 1995; 12: 537-541*
[57] Ubbink DT et al, Diabetes mellitus is not a contra-indication for peripheral vascular intervention in patients with leg ischemia. *Vasa* 1997;**26**(1):39-42
[58] Goss DE et al, Raised ankle/brachial pressure index in insulin-treated diabetic patients, *Diabet Med*, 6(7): 576-8, 1989 Sep-Oct
[59] Brooks B, Dean R,Patel S, Wu B, Molyneaux L, Yue DK, TBI or not TBI: that is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients? *Diabetic Medicine,* 18, 528-532
[60] Smith FCT et al. Falsely Elevated Ankle Pressures in Severe Leg Ischemia: The Pole Test-An Alternative Approach, *Eur J Vasc Surg* **8**, 408-412 (1994)
[61] Goss D E et al, Falsely Raised Ankle/Brachial Pressure Index: A Method to Determine Tibial Artery Compressibility, *Eur J Vasc Surg,* **5**,23-26, 1991
[62] Carter SA, Ankle and toe systolic pressures comparison of value and limitations in arterial occlusive disease. *Int Angiol* 1992; **11**:289-97
[63] Osmundson PJ et al. A Prospective Study of Peripheral Occlusive Arterial Disease in Diabetes. II. Vascular Laboratory Assessment, *Mayo Clin Proc* **56**:223-232, 1981
[64] Bone G E and Pomajzl M J, Toe blood pressure by photopletysmography: An index of healing in forefoot amputation. *Surgery* **116**: 80-3, 1981
[65] Ramsey D E et al, Toe blood pressure A valuable adjunct to ankle pressure measurement for assessing peripheral arterial disease, *J Cardiovasc. Surg.* **24**, 43-48, 1983
[66] Apelqvist J et al, Medical risk factors in diabetic patients with foot ulcers and severe peripheral vascular disease and their influence on outcome. *J Diabetes Complications* **6**(3): 167-74, 1992
[67] Carter S A and Lezack J D, Digital Systolic Pressures in the Lower Limb in Arterial Disease, *Circulation* Vol XLIII, June 1971
[68] Wahlberg E and Gush R, A New Automated Toe Blood Pressure Monitor for Assessment of Limb Ischemia. *Eur J Vasc Endovasc Surg* (2002); **24**, 304-308
[69] Nielsen PE, Bell G and Lassen NA, Strain Gauge Studies of Distal Blood Pressure in Normal Sunbjects and in Patients with Peripheral Arterial Disease. Analysis of Normal Variation and Reproducibility and Comparison to Intaarterial Measurements. *Scand J Clin Lab Invest*,(1973); **31**(Suppl. 128), 103-109
[70] De Graaff JC, Ubbink TH, Legemate DA, de Haan RJ, Jacobs JHM, The usefulness of a laser Doppler in the measurement of to blood pressures,*J Vasc Surg*,(2000); **32**:1172-9
[71] De Graaff JC, Ubbink D Th, Legemate DA, de Haan RJ, Jacobs M J H M, Interobserver and intraobserver reproducibility of peripheral blood and oxygen pressure measurements in the assessment of lower extremity arterial disease, *J Vasc Surg* (2001);33:1033-40
[72] Rutherford R B, Hemodynamics and Diagnosis of Arterial Disease: Basic Techniques and Applications in: *Vascular Surgery* **I**: 89-92
[73] Eickhoff JH and Engell HC, Transcutaneous oxygen tension ($tcPO_2$) measurements on the foot in normal subjects and in patients with peripheral arterial occlusive disease admitted for vascular surgery. *Scand J Clin Lab Invest* **41**:743-8, 1981
[74] Ballard J L et al, A prospective evaluation of transcutaneous oxygen measurements in the management of diabetic foot problems. *J Vasc Surg*, **22**: 485-92, 1995
[75] Kalani M et al, Transcutaneous oxygen tension and toe blood pressure as predictor for outcome of

diabetic foot ulcers. *Diabetes Care* 22(1):147-51, 1999 Jan
[76] Parameswaran G I, Brand K, Dolan J, Pulse Oximetry as a Potential Screening Tool for Lower Extremity Arterial Disease in Asymptomatic Patients With Diabetes Mellitus, *Arch Intern Med.*(2005); 165:442-446
[77] Fagrell B, Vital Microscopy – A clinical Method for Evaluating the Risk of Skin Necrosis in Patients with Occlusive Arterial Disease, 7th Europ. Conf. Microcirculation, Aberdeen 1972, Part I. Bibl. Anat., No. 11, pp. 328-333 (Karger, Basel 1973)
[78] Johnson, P.C, Renaissance in the microcirculation. *Circulation Res*, **31**: 817-823, 1972
[79] Fagrell et al., A microscope-television system for studying flow velocity in human skin capillaries, *Am J Physiol*, 233(2): H318-H321, 1977
[80] Jörneskog G et al, Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications, *Diabetologia*, **38**:474-480, 1995
[81] Emanuele M A et al, Elevated Leg Systolic Pressures and Arterial Calcification in Diabetic Occlusive Vascular Disease. *Diabetes Care*, **4**(2),1981 March-April
[82] Nawaz S et al, Clinical risk associated with contrast angiography in metformin treated patients: a clinical review. Clin Radiol, 53(5): 342-4, 1998 May
[83] Pemberrton M, Nydahl S et al, Colour-coded duplex imaging can safely replace diagnostic arteriography in patients with lower-limb arterial disease. *Br J Surg*, 83(12):1725-8, 1996 Dec
[84] Gentile AT et al, A regional pedal ischemia scoring system for decision analysis in patients with heel ulceration. *Am J Surg*, 176(2): 109-14, 1998 Aug
[85] Faris I and Duncan H, Skin perfusion pressure in the prediction of healing in diabetic patients with ulcers or gangrene of the foot, *J Vasc Surg*, **2**,536-40, 1985
[86] Malvezzi L et al, The correlation between three methods of skin perfusion pressure measurement: Radionuclide washout, laser Doppler flow, and photopletysmography, *J Vasc Surg*;**15**:823-30, 1992
[87] Öberg et al. Laser-Doppler Flowmetry Non-invasive and Continuos Method for Blood Flow Evaluation in Microvascular Studies. *Acta Med Scand, Suppl*.1983;**687**:17-24
[88] Matsen FA, Craig R W, Robertson CL, Öberg P Å, Holloway G A ~~et al~~, Relationship of transcutaneous PO_2 and laser Doppler measurements in a human model of local arterial insufficiency. *Surg Gynecol Obstet* 1984;**159**: 418-22
[89] Andersson S et al, A laser Doppler Technique for measuring distal blood-pressure: a comparision with conventional strain-gauge technique, *Clinical Physiology*(1986) **6**: 329-335
[90] Ubbink DT et al, The value of non-invasive techniques for the assessment of critical limb ischaemia. *Eur J Vasc Endovasc Surg* 1997 Mar; **13**(3):296-300
[91] Management of Peripheral Arterial Disease (PAD) TransAtlantic Inter-Society Consensus (TASC), *Eur J Vasc Endovasc Surg*, vol 19,suppl A, June 2000
[92] Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG; on behalf of the TASC II Working Group. Inter-society Consensus Document on Management of Peripheral Arterial Disease (TASC II). *J Vasc Surg* Jan;45(1 Suppl):S5-S67
[93] American Heart Association Committee Report.. Recommendations for human blood pressure determinations by sphygommanometers. *Circulation* 1980;**62**: A1146-55
[94] Van Slyke et al. Calculation of haemoglobin from blood specific gravityís. *J of biological chemistry* 1950;**183**: 349-360,
[95] Bland JM and Altman DG; Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* 1995;346:1085-87
[96] Trautner et al, Incidence of lower limb amputations and Diabetes, *Diabetes Care,*19:1006-1009, 1996
[97] Letho et al, Risk factors predicting lower extremity amputations in patients with NIDDM, *Diabetes Care* 19:607-612,1996
[98] National Hospital Discharge Survey 1995
[99] Taylor SM, Kalbaugh CA, Blackhurst DW, Kellicut DC, Langan EM 3rd, Youkey JR, A comparision of percutaneous transluminal angioplasty versus amputation for critical limb ischemia in patients unsuitable for open surgery, *J Vasc Surg.*(2007) Feb; 45(2):404-10
[100] Apelquist J et al, Prognostic Value of Systolic Ankle and Toe Blood Pressure Levels in Outcome of Diabetic Foot Ulcer, *Diabetes Care,* 1989 June, 12(6): 373-378
[101] Apelqvist J, Larsson J, Agardh CD, Medical RiskFactors in Diabetic Patients With Foot Ulcers and

Severe Peripheral Vascular Disease and Their Influence on Outcome, *J Diab Comp,*(1992),6:167-174
[102] Jeelani NU et al, Variation of method for measurement of brachial artery pressure significantly affects ankle-brachial pressure index values, *Eur J Vasc Endovasc Surg* 2000 Jul;20(1):25-8
[103] Geddes LA, Tivey R, The importance of cuff width in measurement of blood pressure indirectly, *Cardiovasc Res Cent Bull*, 1976 Jan-Mar;**14**(3):69-79
[104] Lezack JD and Carter SA, Systolic pressures in the extremities of man with special reference to the toes, *Canadian Journal of Physiology and Pharmacology* (1970), 48:469-74
[105] Carter SA et al, The effect of body heating and cooling on the ankle and toe systolic pressures in arterial disease, *J Vasc, Surg* 1992 16:2(148-153)
[106] Ubbink D Th, Toe Blood Pressure Measurements in Patients Suspected of Leg Ischemia: A New Laser Doppler Device Compared With Photopletysomgraphy, *Eur J Vasc Endovasc Surg* 27, 629-634 (2004)
[107] Uccioli L, Monticone G, Durola L, Mormilee F, Mennuni G, Menzinger G, Autonomic Neuropathy Influences Great Toe Blood Pressure,*Diabetes Care* (17) 4, 284-287
[108] Paraskevas N, Ayari R, Malikov S, Mollo M, Branchereau P, Hut F and Branchereeau A, Pole Test Measurements in Crtical Leg Ischemia, *Eur J Vasc Endovasc Surg*(2006), 31, 253-257
[109] McEwen JA et al, New finger cuffs for use with digital tourniquets. *J Hand Surg(Am)* 1988 Nov;13(6): 888-92
[110] Rastam L, Prineas RJ, Gomez-Marin O. Ratio of cuff width/arm circumference as a determinant of arm blood pressure measurements in adults. *J Intern Med* 1990; 227(4): 225-32
[111] Arveschoug AK, Revsbech P and Brøchner-Mortensen J, Sources of variation in the determionation of distal blood pressure measurement using the strain gauge technique, *Clinical physiology*(1998), 18,**4**,361-368
[112] de Graaff JC, Ubbink D TH, Legemate DA, Tijssen JGP, Jacobs M J H M, Evaluation of toe pressure and transcutaneous oxygen measurement in management of chronic crtical leg ischemia: A diagnostic randomized clinical trial *J Vasc Surg* (2003) 38:528-34