Comparison of vascular and neurological parameters between diabetic subjects without diabetic foot ulceration or amputation and those with either foot ulceration or a lower extremity amputation: a pilot study.

BY

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DECLARATION

I hereby declare that this dissertation presented to the University of Pretoria for the degree Masters of Science in Clinical Epidemiology, is my own work and has not been presented previously to any other tertiary institution for any purpose.

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Authorship and Co-workers

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ABSTRACT

Comparison of vascular and neurological parameters between diabetic subjects without diabetic foot ulceration or amputation and those with either foot ulceration or a lower extremity amputation: a pilot study.

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Background: It is likely that lower limb ulceration, lower limb amputation, or their absence in diabetic subjects, indicate varying degrees of long-term diabetes and its complications, and that measures of atherosclerosis and neuropathy would reflect these differences.

Objectives: To determine feasibility and, based on our results, make sample size estimates for future study: By comparing peripheral and central vasculature between diabetic subjects with lower extremity ulcers, diabetic subjects with lower extremity amputation and a group of diabetics without these complications — through evaluating toe blood pressure (TBP), toe-brachial index (TBI) and pulse wave velocity (PWV); also, by comparing peripheral and autonomic nervous system integrity between these groups — through sensory, nerve conduction, needle-examination and autonomic function assessment.

Study design: A cross-sectional, descriptive and comparative pilot study.

Setting: Pretoria Academic Hospital.

Participants: Three groups of ten patients consecutively selected from diabetes and diabetic foot clinics — ten with chronic lower extremity ulcers, ten with healed lower extremity amputations and ten diabetic controls.

Methods: Assessment of peripheral and autonomic neuropathy included evaluation of 5.07/10-g monofilament sensation, vibration perception (using a 128Hz tuning fork), nerve conduction and electromyography, cutaneous autonomic response and heart rate variability (expressed as

an Expiration: Inspiration (E:I)-ratio). For evaluation of vascular status, we obtained the photo-plethysmographically-derived TBI and assessed carotid-femoral (CF) and carotid-radial (CR) PWV. Sample sizes for future studies were calculated through a nomogram for three-group comparisons, ANOVA, simulation and log-transformation of non-parametric data.

Results: Absence of vibration perception in at least one leg, with significant p-values of 0.000 at toe-, and 0.027 at medial malleolus- level, occurred more frequently in the amputation, than in the control group. For the total bilateral monofilament count a statistically significant difference between groups was demonstrated (p-value 0.043). Peripheral neuropathy based on abnormality of at least one conduction attribute in at least two distinct nerves, the E:I-ratio, assessment of cutaneous autonomic responses and TBI, by worsening across groups, seemed to display a correlation with severity of lower limb complications, but without statistically significant results. For CF- and CR PWV, the lowest values were observed in the amputation group. Sample size calculations based on our TBP, TBI, vibration and monofilament results, lead to a proposed equal group size of between 34 and 103 for future three-group comparisons using these outcomes measures. Should PWV be included, the group size would have to be between 160 and 222.

Conclusions: This study confirmed the usefulness of monofilament sensation and vibration perception assessment in identifying diabetic patients with differing degrees of lower extremity risk. Also, due to the large differences between groups, it demonstrated the effectiveness of these measures to display differences between groups, even in the event of very small sample sizes. The tendencies to worsen across the three groups, of the E:I -ratio, peripheral neuropathy based on nerve conduction, and the TBI, will have to be re-examined in a study with larger sample size. In order to demonstrate statistically significant CF- and CR PWV results, a larger sample size may also be required.

ABSTRAK

Vergelyking van vaskulêre en neurologiese parameters tussen diabete sonder diabetiese voet-ulkusse of amputasies, en diè met òf ‘n ulkus òf ‘n amputasie van ‘n onderste ledemaat: ‘n Loods-studie.

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Agtergrond: Dit sou redelik wees om te vermoed dat, in diabete, die teenwoordigheid of die afwesigheid van onderbeen-ulkusse en onderste ledemaat-amputasies ‘n weerspieëling is van wisselende grade van langtermyn diabetes en diabetes-komplikasies, en dat maatstawwe van aterosklerose en neuropatie tussen die groepe progressief sal versleg.

Doelwitte: Om uitvoerbaarheid te ondersoek en moontlike toekomstige studie-grootte berekenings te maak, gebaseer op ons resultate: D.m.v. die vergelyking van perifere en sentrale arteriële vattoestand tussen diabetiese pasiënte met onderbeen-ulkusse, diabetiese pasiënte met onderste ledemaat-amputasies, en ‘n groep diabete sonder sodanige komplikasies — m.b.v. die evaluasie van toon-bloeddruk (TBD), toon-brachiale indekse (TBI) en polsgolf-snelheid (PGS); verder, om perifere en outonome senuweestelsel-integriteit tussen die groepe te beoordeel — m.b.v. sensoriese, senuwee-geleiding, naald-ondersoek en outonome funksie-evaluasie.

Studie-ontwerp: ‘n Vergelykende, beskrywende deursnit-studie.

Milieu: Pretoria Akademiese Hospitaal

Deelnemers: Drie groepe van tien opeenvolgend-geskikte pasiënte is gewerf vanuit die diabetes kliniek en diabetiese voet-kliniek — tien met chroniese onderbeen-ulkusse, tien met geneesde onderste ledemaat-amputasies en tien kontrole- diabetiese pasiënte.

Metodes: Ondersoeke t.o.v. perifere en outonome neuropatie het ingesluit: Evaluasie van 5.07/ 10-g monofilament-sensasie, vibrasie-persepsie (m.b.v ‘n 128Hz stemvurk), senuwee-geleiding en naald-ondersoeke, kutane outonome respons en harttempo-varieerbaarheid (uitgedruk as ‘n Ekspirasie: Inspirasie (E:I)-verhouding). Vir die beoordeling van vaskulêre status is die TBI d.m.v. foto-pletismografie verkry en karotis-femorale (KF) en karotis-radiale (KR) PGS geëvalueer. Toekomstige studie-groottes is bereken d.m.v. ‘n nomogram vir drie-groep vergelykings, Anova, simulasie en log-transformasie van non-parametriese data.

Resultate: Afwesigheid van vibrasie-persepsie in ten minste een been (met betekenisvolle p-waardes van 0.000 op toon-, en 0.027 op mediale malleolus-vlak) het meer dikwels in die amputasie-groep voorgekom, as in die kontrole-groep. Vir die totale, bilaterale monofilament-telling, is ‘n statisties betekenisvolle verskil tussen groepe gedemonstreer, met ‘n p-waarde van 0.043. Deurdat dit van groep tot groep vererger het, het perifere neuropatie gebaseer op die abnormaliteit van ten minste een geleidings-eienskap in ten minste twee anatomies aparte senuwees, die E:I-verhouding, evaluasie van kutane outonome response en TBI, geblyk korrelasie te toon (hoewel sònder statisties betekenisvolle resultate) met onderste ledemaat komplikasies. Vir KF- en KR PGS is die laagste waardes in die amputasie-groep aangetoon. Studie-grootte berekeninge gebaseer op ons TBD, TBI, vibrasie- en monofilament resultate, het gelei tot ‘n voorgestelde gelyke studie-groep-grootte van tussen 34 en 103 vir drie-groep-vergelykings, sou dieselfde uitkomste gebruik word. Indien PGS ook ingesluit sou word, sal groep-groottes tussen 160 en 222 benodig word.

Gevolgtrekkings: Die studie bevestig die bruikbaarheid van monofilament-sensasie en vibrasie-persepsie evaluasie in die identifisering van diabetiese pasiënte met wisselende grade van onderste ledemaat-risiko. Verder het die groot verskille tussen groepe die effektiwiteit van die metings gedemonstreer t.o.v. die aanduiding van verskille selfs in die geval van baie klein pasiënt-getalle. ‘n Groter studie sal uitgevoer moet word ten einde, met die oog op die aantoon van betekenisvolle verskille, tendense van verslegting (gedemonstreer deur die E:I-verhouding, perifere neuropatie gebaseer op senuwee-geleidings-abnormaliteite en TBI) tussen die drie groepe diabete te evalueer. Ook t.o.v. CF- en CR PGS, mag groter pasiënt-getalle statisties betekenisvolle resultate tot gevolg hê.

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INTRODUCTION

1.1 BACKGROUND & LITERATURE OVERVIEW

The background and literature overview aims to present the reader with a description of the impact of diabetes and its peripheral vascular and neuropathic complications both globally and locally. It then proceeds to focus on the general importance of diabetic peripheral neuropathy and peripheral vascular disease in the development of ulceration of the lower extremity, and in necessitating amputation, as displayed by numerous overseas, and some South African studies. Next, different measures available and validated for the assessment of peripheral vascular impairment are presented, followed by their application in diabetic patients with peripheral vascular complications. Neuropathy and the methods available for its evaluation are subsequently being considered. Finally, in a brief discussion, and with implications for foot care in mind, the above is put into the context of the state of current diabetes care in Southern Africa.

1.1.1 Impact of Diabetes Mellitus and its Microvascular and Neuropathic Complications Worldwide, in Africa, and in South Africa

Globally, in developed and developing countries alike, Diabetes Mellitus has become a major problem,[1, 2] having already reached epidemic proportions in several populations.[3, 4, 5, 6, 7] The high prevalence of diabetes in developing countries sometimes even exceeds that of developed nations.[1, 2, 3, 4, 5, 6]

Ranking fourth on the WHO list of underlying causes of death,[8] the prevalence of this chronic, non-communicable disease is predicted to dramatically increase during the 21st century:[9] In 1997, it was estimated that diabetes mellitus was affecting approximately 124 million people worldwide; this figure is expected to double to 220 million by 2010,[9, 10] and to reach 300 million by the year 2025. [8, 11]

In Africa, there has until recent years, according to a review by Motala, et al in 2003, been a paucity of data on the epidemiology of diabetes mellitus**.** They however found that, with regard to Type 2 diabetes, information on its prevalence in Africa has increased over the past decade, with reported rates varying from low (in some rural areas) to moderate (some countries in North and North East Africa and in South Africa) and high (among urban communities in Cairo, and a population of mixed Egyptian ancestry in Northern Sudan). Their review indicated a lack of adequate data on Type 1 diabetes in Sub-Saharan Africa, with the limited available data suggesting a low prevalence and later age of onset than in the western world.[12]

Over-all diabetes prevalence in Africa, during the period 1990 to 2000, has however been reported to have increased by 30%. This was due mainly to urbanization and the adapting of a more westernized lifestyle[11,13] (characterized by changes in type of food, quantity and energy content of food consumed, as well as by altered levels of physical activity[14]) and the subsequent increase of obesity.[13] For Sub-Saharan Africa, where approximately 90% of patients with diabetes belong to the Type 2 diabetes category,[3] the prevalence of Type 2 diabetes is expected to increase by 18% (from 1.1 to 1.3%) from 1995 to 2025, with a corresponding increase in numbers from approximately 3 million, to 8 million.[12]

As in the rest of the world and the rest of Africa, Diabetes Mellitus is common in **South Africa**, with an estimated 2.4 to 3.2 million sufferers[13] — of whom it is estimated that more than 1 million are currently still undiagnosed, and therefore untreated — and with an estimated prevalence of between 5.3% and 8.0% among urbanized populations.[11] In 1998, Kalk, Pick and Sayed [15] estimated that among South African women, diabetes accounted for 18.2% of deaths in Asians (or Indians), 7.1% in Coloured patients, 4.3% in Blacks and 3.0% in Whites. For men, diabetes mortality in Black, Coloured and White populations ranged from 2.0 to 2.5%; amongst Asians it reached 4.9%.[11] This disease, however, not only significantly contributes to morbidity and mortality (of non-communicable diseases — after ischaemic heart disease and cancer — ranking third in South Africa), but as a result, also accounts for a substantial amount of South African health care expenditure.[16] Furthermore, over the course of the last 40 years, the disease has been increasingly affecting individuals of both sexes and across all population groups:

In South Africa, a survey in 1969, investigating a representative sample of the **White population** in the Cape Town area, yielded an over-15-years age-corrected prevalence for the general White population of 3.7% (males 4%, females 2.5%), while prevalence over the age of 55 years was found to be 11.8%. It was estimated that approximately 100 000 white South Africans were unaware of the fact that they had the disease. Prevalence of 'discovered diabetes' was 1.9%, and of known diabetes at all ages 0.8%.[17] This figure, at the time, according to Jackson et al,[17] was in agreement with the over-all prevalence of 'already known' diabetes reported in White communities in several countries, including Britain, the USA, Canada, Norway and Sweden.[18, 19, 20] (A study 25 years later of 396 urban Whites in Durban with age range 15 – 69 years, yielded a similar prevalence of 3.0%.)[21] Subsequent studies worldwide have, however, shown a marked increase in the prevalence of diabetes mellitus in White populations.[22]

In 1971, a representative community of Cape **Coloured** people was surveyed to assess the prevalence of diabetes and related variables.[23] A prevalence of 'known diabetes' of 1.1% was found – at the time comparable to that of both the White and the Black communities of Cape Town.[17, 24] For 'discovered diabetes', an over-all diabetes prevalence of 6.1% was reported. Age-corrected total diabetes prevalence for Coloureds over the age of 15, was 10.6%. A more recent urban study of Cape Town residents, aimed at determining diabetes prevalence among

200 non-institutionalized, elderly Coloured subjects (aged 65 years and older), and of which the results were published in 1997, reported a diabetes prevalence of 28.7% in this population.[25]

Two cross-sectional studies among **Indians** living in Durban (in which WHO definitions were applied), one published in 1985 and investigating diabetes prevalence among a study group of 866, and another, a 1988 community survey assessing the prevalence of both diabetes and hypertension among 1 064 subjects, have established that South African Indians have a high prevalence of diabetes. Figures of 11% and 9.8% respectively, were reported.[26, 27] A more recent study of 2 479 urban Indians living in Durban, reported a prevalence in patients with age over 15 years of 13.0 %. [21] Two decades earlier the prevalence reported for a Transvaal Indian population was about 16 %,[28] and among Cape Indians 8.4 %, for all ages over 10 years, and 24 % over the age of 55.[29] (The difference between the late 1960's and the mid-to-late 1980's results, could possibly be explained by the application of less stringent criteria, prior to the implementation of the 1980/1985 WHO Criteria for the diagnosis of Diabetes.[12, 30]) However, results of a subsequent prospective Indian community study published in 2003, demonstrated an equally high prevalence of Type 2 diabetes in this population, of 16.2 %.[31] The crude cumulative incidence over the 10 years of follow-up was 9.5%.

A study in 1992, assessing diabetes prevalence among urban **Blacks** of predominantly Xhosa origin living in Cape Town, reported an age-adjusted prevalence of 8%.[32] Not only was this figure twice as high as that reported nearly 20 years earlier,[1, 24, 33] but it was also higher than average prevalences reported in the rest of Africa and Europe.[4, 30, 34] Two similar studies investigating diabetes prevalence in other ethnic groups in South Africa, conducted among urban black populations and published at roughly the same time (Zulus living in Durban, during 1993 and the mainly Sesotho population of Mangaung in Bloemfontein in the Orange Free State, in 1995), reported age-adjusted prevalences of 5.3% and 6.0% respectively,[1, 35] while a study of a rural Sesotho population in Qua-Qua, yielded an age-adjusted prevalence of 4.8%.[36]

A study conducted in 2001 among a Xhosa community in Umtata (previously the capital of the former homeland of Transkei), which was aimed at determining the prevalence of diabetes mellitus and IGT (impaired glucose tolerance) in a group of peri-urban black South Africans, reported diabetes prevalences of 4.8% in the age group 40 – 49 years, and 8.1% in the group

50 – 59 years of age.[1] These results are in agreement with the findings of similar, earlier studies mentioned above, which were published after introduction of the 1980/1985 WHO Criteria.

A 1992 audit of public sector primary diabetes care in Cape Town, South Africa, demonstrated a high prevalence of diabetes complications among the 300 black patients investigated (10% of which belonged to the Type 1, and 90% to the Type 2 diabetes categories). Amongst others, prevalences were reported for 'any grade' retinopathy (55.4%), proliferative and pre-proliferative retinopathy (15.6 %), persistent proteinuria (5.3%), peripheral neuropathy (27.6%), absent foot pulses (8.2%) and amputations (1.4%). The mean duration of diabetes was 8 years.[34]

A 1995 study conducted among patients in Soweto, South Africa, of 64 Type 1 diabetics with mean duration of disease of 13.6 years, reported equally high (in some instances, even higher) diabetes microvascular complication prevalences: nephropathy 28%, retinopathy 52% and peripheral neuropathy 42%.[37, 38] Likewise, a study published in 2001, of South African Black and Indian patients (78.5% of whom were Type 2 diabetics), with long-duration (>10 years) diabetes mellitus, reported a high prevalence of microvascular complications in the form of retinopathy (53.2% among Type 1, and 64.5% among Type 2 patients) and nephropathy (based on persistent proteinuria, in 23.4% Type 1 and in 25% of Type 2's).[39] Similarly, a study in 2002 of 253 diabetic subjects (of which 92.9% were Type 2 patients) under care of public sector clinics in rural KwaZulu-Natal, with mean duration of disease of 4.2 years (range 6 weeks to 60 years), reported high prevalences of any grade retinopathy (40.3%), microalbuminuria (46.4%), absent foot pulses (16.6%) and diabetic foot ulceration or cellulitis (6%). [40]

1.1.2 The General Importance of Peripheral Neuropathy, Peripheral Vascular Disease, Foot Ulceration and Amputation in Diabetes

People suffering from diabetes mellitus are 2 – 3 times more likely to develop peripheral vascular disease, than the general population.[41, 42] Eight percent of diabetics, at the time of their diagnosis, may be affected. By 20 years duration, this figure may rise to 45%.[41, 43] It is agreed that peripheral nerve disorders, likewise, are important late complications of diabetes mellitus.[44] However, Pirart,[45] already in 1978, reported evidence of neuropathy (by clinical examination alone) in 8% of 4, 400 diabetic outpatients already at the time of diagnosis, increasing to 50% after 25 years of disease.[44]

In diabetics with neuropathy and accompanying large- or small-vessel disease, undetected painless trauma (and infection, which impairs wound healing), results in chronic ulceration.[44] Subsequently, lower extremity ulcers and amputations are frequent, major complications of diabetes, posing a persistent threat to individuals suffering from this disease:

Diabetic foot ulcers are common, and estimated to affect approximately 15% of all diabetic individuals during their lifetimes.[46]

Data from the American1983 -1990 National Hospital Discharge Surveys (NHDS) indicate that 6% of hospitalizations listing diabetes on discharge records also listed a lower extremity ulcer condition. Chronic ulcers were present in 2.7% of all hospitalizations that listed diabetes. The average length of stay for diabetes discharges wíth ulcer conditions was 59% longer than for diabetes discharges without them.[47]

In the population-based Wisconsin Epidemiological Study of Diabetic Retinopathy, the annual incidence of foot ulcers was 2.4% in insulin-taking younger-onset diabetic patients (i.e. diagnosed at age < 30 years, primarily Type 1 DM) and 2.6% in older-onset diabetic patients (diagnosed at age > 30 years, primarily Type 2 DM).[48]

In a population-based study of diabetic individuals age 15 - 50 years in Umea, Sweden, annual incidence of foot ulcers was 3% in patients with Type 1 DM. In this study, foot ulcer prevalence for Type 1 DM, Type 2 DM and non-diabetic patients was 10%, 9%, and 0%, respectively. In addition, higher frequencies of non-ulcerative pathology (hammertoes, callosities, fissures, and dry feet) were observed in diabetic, than in non-diabetic patients.[49]

In British studies, diabetic foot ulcer incidence was reported to be 1%, while prevalence in two community-based studies ranged from 5.3% - 7.4%.[50, 51] One, the study with 5.3% prevalence of current or previous foot ulceration, and investigating Type 2 Diabetic outpatients, reported a 41.6% neuropathy prevalence, and a prevalence of 11% for peripheral vascular disease (PVD).[50] The other (which recorded a prevalence of past or present foot ulceration of 7.4%) was a study undertaken to identify all diabetic patients with foot disease in a defined population representative of that of the U.K. In this study, of the ulcers found on examination, 39.4% were

neuropathic, 24.2% vascular, and 36.4% mixed.[51] These estimations confirm earlier,[52, 53, 54] as well as subsequent, more recent reports,[43, 55] that in the diabetic lower extremity, a combination of ischaemia and neuropathy is often seen at work to advance the evolution of trophic ulcers – the prevalence of which has been estimated to be between 3 and 8%,[56, 57, 58] and which is still and rapidly rising.[149]

In studies of diabetic outpatients, depending on ulcer severity, 6% - 43% of patients with diabetic foot ulcers ultimately have the most severe diabetic foot outcome, amputation. [52, 59, 60]

Clinical epidemiological studies estimated that foot ulcers precede approximately 85% of non-traumatic lower extremity amputations in individuals with diabetes:[47, 57]

Of NHDS discharges listing diabetes and an amputation, 40% also listed a foot ulcer condition. However, in all likelihood, there is underreporting of foot ulcer conditions in the NHDS, since this is less than half the frequency reported in two clinical epidemiological studies that systematically assessed neuropathy, ulceration, ischaemia, and other factors before amputation.[61, 62, 63] (One of these, a prospective study of 558 consecutive diabetic patients presenting with foot ulcers, found that ulcers deteriorating to deep infection and gangrene, precipitated more than 85% of lower extremity amputations performed in diabetic patients.[63] The other, a case-control study investigating causal pathways to amputation in 80 American diabetic male veterans, attributed 46% of amputations to ischaemia, 61% to neuropathy and 84% to ulceration occurring somewhere along the multi-component pathway.[61, 62]

It has been reported that 50% of major non-traumatic lower extremity amputations are performed in diabetic patients.[56, 58] Furthermore, it has been estimated that two-thirds of these amputees die within 5 years of their amputation.[56, 64, 65] In the United States, more than half of lower limb amputations occur in people with diagnosed diabetes, who represent only 5% of the U.S. population.[47, 61] NHDS data indicate that there were approximately 54,000 diabetic individuals who underwent non-traumatic lower extremity amputations in 1990. Not only were lower-level amputations (toe, foot, and ankle) more common in individuals with diabetes than without diabetes, but the more disabling above-knee amputations were also performed with greater frequency in non-diabetic individuals. Reported amputation rates are greater with

increasing age, in males compared with females, and among members of racial and ethnic minorities, compared with whites.[47, 66]

Data from several states indicated that 9% - 20% of diabetic individuals experienced a new (ipsi-lateral) or second leg (contra-lateral) amputation during a separate hospitalization within 12 months after an amputation. Five years following an initial amputation, 28% - 51% of diabetic amputees had undergone a second leg amputation. Peri-operative mortality among diabetic amputees averaged 5.8% in 1989 - 1992, according to NHDS data. Five-year mortality of 39% - 68%, following amputation, was confirmed in various studies.[47]

A report in 1976 of the US Dept of Health, Education and Welfare National Commission on Diabetes, estimated the lifetime risk of a lower extremity amputation among diabetic individuals, between 5 and 15% —15 times that of the non-diabetic population. [48, 67]

1.1.3 Non-Invasive Measures of Vascular Impairment

1.1.3.1 Ankle-brachial Index (ABI)

The ankle-brachial index, a peripheral measure of vascular function, has been shown to be predictive of major vascular events, as well as of future cardiovascular and overall mortality:[68, 69, 70, 71]

Systolic blood pressure in the legs, under normal conditions, is equal to, or slightly higher than systolic blood pressure in the upper limbs. However, in the event of the presence of arterial stenosis, a reduction in pressure distal to the lesion occurs.[72]

The resting ankle-brachial pressure index (or in short: ankle-brachial index, or ABI), which is the ratio of tibial artery systolic blood pressure to brachial artery systolic pressure,[73] is a non-invasive measurement used to assess the patency of the lower extremity arterial system, and to

screen for the presence of occlusive peripheral arterial disease. [74, 75, 76] The method, in principle, as was wéll documented by Carter[77] and Thulesius,[78] is based on the pressure-flow relationship in the peripheral vascular bed. [74]

The doppler-derived ABI, when compared with arteriography of the distal aorta and arteries of the lower extremities, was demonstrated by Yao and colleagues,[76] to be a valuable and sensitive method of assessment of occlusive arterial disease.[79]

A low ankle-brachial index (an ABI of ≤ 0.9[43] being considered abnormal, and ≥ 1 as normal[72]) is therefore a measure of peripheral artery disease in the lower limb.[73, 80]According to Donnelly and colleagues,[72] patients with claudication tend to have ABI's in the range 0.5 – 0.9, whereas those with critical ischaemia usually present with an index of < 0.5. Low ABI is also a marker for other cardiovascular events:[73] It has been shown in epidemiological, as well as in clinical studies,[74] to be associated with prevalent coronary and carotid artery disease;[81, 82, 83, 84] also with the presence of cardiovascular risk factors.[80, 82, 85, 86, 87, 88, 89, 90, 91, 92, 93] For example, in a report from the Atherosclerosis Risk in Communities Study,[73] ABI was inversely associated with prevalent clinical coronary heart disease (CHD), with prevalent stroke, as well as with pre-clinical atherosclerosis of the carotid and popliteal arteries.[73]

A study by Shinozaki, et al,[94] which investigated the use of ABI as a screening method, not only confirmed the wide applicability of this test, but also found that, when right and left measurements were compared in the same person, ABI showed a relatively good and significant correlation: $r = 0.65$; $p < 0.01$.

A limitation of ABI, is that it can be (falsely) elevated, i.e. > 1.3,[43] or > 1.5.[82] This phenomenon has been found to be suggestive of calcification of the media of the arterial wall,[43] the arterial rigidity being the factor preventing arterial occlusion.[82] An ankle systolic blood pressure (SBP) of > 300 mmHg, or ≥ 75 mm Hg higher than that of the arm, is considered indicative of an incompressible ankle artery --- the presence of arterial wall media calcification can therefore be presumed.[43] (One study referred to in the ADA/AHA document mentioned above — that by Orchard[43] — found that a difference of 75 mmHg between ankle and arm SBP, gave a positive predictive value (PPV) of 100% for the presence of arterial calcification on X-ray.)

Therefore, in the event of arterial wall media calcification, systolic blood pressure in the lower limbs cannot, due to vessel incompressibility, be measured reliably;[72] as a result, the measurement of ABI to determine the presence of lower extremity arterial disease (LEAD), is compromised, and non-ABI methods need to be used.[43, 95]

1.1.3.2 Toe Blood Pressure (TBP) and Toe-brachial Index (TBI)

As indicated above, when vessels are calcified and incompressible, systolic blood pressure in the lower limbs cannot be measured reliably,[72] and, alternatively, non-ABI methods need to be used.[43, 95] One approach, is to measure toe systolic blood pressure:[72, 96]

Toe systolic blood pressure measurement is based on evaluation of the condition of the wall (and, subsequently, the lumen) of the small, predominantly muscular, peripheral arteries of the toes.[97]

Two studies referred to in the 1992 ADA/AHA document (those of, respectively, Drs. Orchard and Cavanagh), demonstrated that arteries at the level of the ankle and in the dorsum of the foot (i.e. the posterior or anterior tibial arteries), and arteries at metatarsal level, were more frequently calcified, than arteries at the level of the toe. To address the problem of calcified and incompressible vessels, the ADA & AHA therefore recommended the use of toe systolic blood pressure (TBP), a measurement with repeatability of ± 17%, which is similar to that of ABI measurement (coefficients of variation between 10 – 15% have been reported), but capable of overcoming the false elevation of ankle pressures from calcification.[43]

According to Donnelly, et al[72] normal toe systolic pressure ranges from 90 – 100 mm Hg, representing 80 – 90 % of brachial systolic pressure, while an absolute pressure of < 30 mm Hg, indicates critical ischaemia. This is in agreement with the conclusions of the authors of the ADA/AHA-document mentioned above:[43] They suggested that, when pressure measurements are made at the level of the toes and an absolute pressure of ≤ 30 mm Hg is found, healing in the event of ulceration, is unlikely to occur. If, however, toe pressure exceeds 30 mmHg, it has been reported by various authors that, in a large majority of cases, spontaneous healing occurs.[98, 99, 100, 101, 102] Furthermore, the ADA/AHA-group considered another cut-off to be

clinically equally valuable: They proposed that, for screening purposes, a toe-brachial index (TBI) of > 0.60 ± 17%, should be taken as normal.[43]

1.1.3.3 Pulse Wave Velocity (PWV)

In adults, vessels stiffen with increasing age, and diseased arteries stiffen more than healthy arteries.[103] According to Blacher, et al,[104, 105] in addition to age,[106, 107] arterial stiffness is known to increase with hypertension,[108] diabetes mellitus,[109] atherosclerosis[110, 111] and end-stage renal disease.[112, 113]

As a consequence of arterial stiffening, systolic blood pressure becomes higher and diastolic blood pressure lower, therefore pulse pressure is increased.[114] In turn, increased systolic pressure results in increased left ventricular after-load, while reduced diastolic pressure results in altered (reduced) coronary perfusion. [79, 105, 115, 116] In the general population, high systolic blood pressure and high pulse pressure,[117, 118, 119] low diastolic blood pressure, as well as left ventricular hypertrophy, have been identified as independent predictors of cardiovascular morbidity and mortality.[105, 120, 121, 122, 123]

Measurement of the velocity at which the pulse wave travels a given distance between 2 sites of the arterial system[103, 105, 114] (i.e. the pulse wave velocity, or 'PWV'), is a simple, reproducible, indirect and non-invasive method of evaluating stiffness of regional arteries:[104] It reflects arterial stiffness based on the analysis of 2 arterial curves detected at the same time in large arteries, thereby — when determined from the foot-to-foot transit time in the specific large artery — producing results which are independent of wave reflection,[111, 124] but which critically depend on precise measurements of both the pulse-transit time and the path length (i.e. the length of the vascular segment).[103, 104] PWV is a parameter integrating arterial geometry and intrinsic elastic properties.[125, 126] According to the Moens-Korteweg ($PWV^2 = Eh/2r\rho$, where *'h'* = vessel wall thickness; *'r'* = arterial inside radius; *'ρ'* = blood density; *'E'* = Young's modulus of elasticity of wall material[126]) and Bramwell-Hill equations ($PWV = \sqrt{(\Delta PV/\Delta V\rho)} = \sqrt{(1/\rho D)}$, where 'Δ' = change in pressure; 'ΔV ' = change in volume, 'D' = distensibility[126]), PWV is related to the square root of the 'elastic modulus', as well as to the 'thickness / radius ratio'; therefore the PWV rises in stiffer arteries.[111]

Aortic PWV, as a marker of aortic stiffness, has been shown — in older subjects over 80 years of age; in patients with end-stage renal disease (ESRD) on hemodialysis; and in patients with essential hypertension — to be a strong independent predictor of both all-cause mortality and cardiovascular risk[125] (mainly myocardial infarction and stroke[127]). Furthermore, it has been shown to be a significant independent determinant of atherosclerosis (or atherosclerotic alterations):[111]

Significant correlations and powerful interactions between PWV and the so-called 'major' cardiovascular risk factors (such as age, gender, hypertension, diabetes, and smoking) have been demonstrated in several studies performed in various populations. Similarly, significant interactions have been established between PWV and the so-called 'minor' cardiovascular risk factors, including pulse pressure, heart rate, left ventricular hypertrophy, waist circumference and waist/hip ratio, micro-albuminuria, homocysteine, and sedentariness. Most of these associations between PWV and the abovementioned cardiovascular risk factors have either been described independently, or they persist after adjustment for the other factors.[128, 129] PWV is therefore also useful as a surrogate marker of vascular disease:[125]

Bortolotto, Blacher, et al, [111] in a study on a large hypertensive population of 524, evaluated the influencing factors of vascular compliance by measuring PWV, using a Complior® device. Those patients with atherosclerotic alterations (AA) — the latter defined on the basis of clinical events such as coronary heart disease, stroke, peripheral vascular disease, and abdominal aorta aneurysm — were found to present a significantly higher PWV (14.9 ± 4 metres/ second, with $p < 0.0001$), than those without AA (12.4 ± 2 m/s). Likewise, in patients > 60 yrs of age, there was a significant difference between PWV-values of patients wíth AA (15.6 ± 0.3 m/s, with $p < 0.01$), compared to those without AA (13.7 ± 0.2 m/s). Even in younger patients < 60 yrs, PWV was significantly ($p < 0.01$) higher in patients wíth AA (13.1 ± 0.5 m/s), than in patients without AA (11.5 ± 0.2 m/s).

Similarly, Blacher, Asmar, and colleagues [105] studied a cohort of 710 patients with essential hypertension, in whom atherosclerotic alterations were defined on the basis of clinical events. By use of Framingham equations, calculation of cardiovascular risks was performed in subjects without AA. Even after adjustments on confounding factors (blood pressure, tobacco consumption, gender, lipid profile, diabetes mellitus, and left ventricular hypertrophy on ECG),

PWV was significantly higher (with $p < 0.0001$) in the presence of AA, than when AA was absent (14.9 ± 4.0 m/sec vs.12.4 ± 2.6 m/sec). PWV, with a highly significant p-value of < 0.0001, emerged as first determinant of the extent of atherosclerosis (which was assessed according to the number of atherosclerotic sites). In patients without AA, all cardiovascular risks constantly increased with increasing PWV. PWV furthermore proved to be the best predictor of cardiovascular mortality at a given age. Finally, the presence of a PWV > 13 m/s, taken alone, seemed, with high performance values (62% sensitivity, 67% specificity, 39% positive predictive value, 84% negative predictive value), a strong predictor of cardiovascular mortality.

A study by Van Popele[130] — an epidemiological study which investigated the causes and consequences of arterial stiffness in an elderly population and which formed part of a large Dutch population-based study (the Rotterdam Study) — expressed its results in terms of 1 m/s increases of PWV (so-called 'increments of aortic stiffness').

A study by Laurent S,[131] et al identified carotid-femoral PWV as independent predictor of both cardiovascular and all-cause mortality in hypertensive patients.[97]The odds ratio reported for an increment of 5 m/s in PWV, was 1.34 for all-cause and 1.51 for cardiovascular mortality. PWV in this study ranged from 9 to 13 m/s — indicating a relatively large change. Values of carotid-femoral (CF) PWV in healthy individuals (average age 24 to 62 years), reported more recently by O'Rourke MF, et al,[132] range from around 6 to 10 m/s.

Lim and colleagues[133]assessed aorto-femoral PWV in 326 patients undergoing coronary angiography for suspected coronary artery disease (CAD). Their findings not only suggested that PWV is an independent marker for CAD, but also indicated a strong association with severity of CAD. These findings were recently confirmed in a study by Sakuragi et al,[134] who measured brachial-ankle PWV (BA PWV) and evaluated its relationship with left ventricular function, in patients with (n=170) and without CAD (n=81). Brachial-ankle PWV in the CAD-group was significantly higher than in the non-CAD group. They concluded that BA PWV increases with CAD severity and correlates with left ventricular function, independent of CAD severity.

Apart from the aorta, another artery that has been widely studied for the evaluation of arterial thickness and stiffness is the radial artery. Since this is a cylindrical, straight and superficial

artery, it serves as a model for peripheral muscular arteries. It has therefore been recommended (by Safar, Blacher, et al[127]) that, in order to measure peripheral (or carotid-radial) PWV, it should, like the aorta (which yields aortic, carotid-femoral or so-called 'trunk' PWV[135]), also be investigated. In this regard, what Asmar[136] reported in 1999, should be kept in mind, namely that, based on numerous population studies early in this century, "the velocity of the radial pulse wave is consistently higher than that of the aortic pulse wave, indicating that the transmission time of the pulse wave is more rapid through the medium-sized vessels than through the larger arteries". Results of studies by Avolio, et al,[137, 138] Hallock[139] and Benetos, et al,[140] however indicated that, in subjects aged over 60 years, non-invasive PWV measurements tend to approach an equivalent value in the central and peripheral arteries.[141]

With regard to the variability of PWV results (i.e. standard deviations of PWV means) reported in published studies: As part of the Rotterdam Study[142]— specifically during its third examination phase — 3818 elderly participants were investigated to evaluate the association between arterial stiffness and prevalent cardiovascular disease (myocardial infarction and stroke). One of the measures of arterial stiffness used, was carotid-femoral pulse wave velocity. Mean PWV of subjects without MI or Stroke, was 13.4 m/s, with a standard deviation (SD) of 3.0. For subjects with MI, mean PWV was14.8 (SD 3.3), and for subjects with Stroke 14.6 (SD 3.1) m/s. Another study that reported a similar SD, was a cross-sectional study which investigated aortic PWV as a marker of cardiovascular risk in a cohort of 1087 patients with essential hypertension: a mean PWV of 12.63, and a SD of 3.10 was reported.[143] On the other hand, the Complior® Study (Asmar, et al, 2001), a large-population clinical trial (on more than 2000 patients from 69 centres in 19 countries), which was designed to evaluate the ability of an ACE-inhibition based anti-hypertensive therapy to improve arterial stiffness as assessed by Complior®-determined aortic pulse wave velocity, yielded a mean baseline carotid-femoral PWV of 11.6, with a SD of 2.4 m/s. (The decrease in PWV from baseline was reported as 1.1, with SD of 1.4 m/s).[144] Another double-blind cross-over study by Asmar, et al,[145] on 16 patients with mild to moderate hypertension, reported carotid-femoral PWV at baseline of 10.9, with a standard deviation, equally, of 2.0 m/s.

1.1.4 Application of Vascular Measurements in Patients with Ulcers or Previous Amputations, and in Patients with Diabetes

In subjects with prior amputation or current foot ulcers, very little data exist regarding the application of peripheral arterial measurements like the ankle- or toe- brachial index, or more general measures such as the pulse wave velocity.

1.1.4.1 Application of Ankle- or Toe Blood Pressures or Indices in Patients with Ulcers or Previous Amputations

Khammash and Obeidat [56] conducted a study in which they set out to determine the prevalence of lower limb ischaemia in patients with diabetic foot infection, by prospectively measuring the ankle-brachial pressure index (ABI). Over a 21-month period, 60 patients were treated in the general surgical ward of Princess Basma Teaching Hospital. Ischaemia was present in 35 of the 60 patients (58.4%). Among them 27 had moderate ischaemia (ABI 0.5 – 0.9) and were treated successfully before further vascular workup. The other 8 patients had severe ischaemia (ABI < 0.5) and required below-knee amputation because their feet were severely infected and not salvageable. Their study confirmed the recommendation for early detection of lower limb ischaemia in diabetics, especially those with foot infection, as it should improve the outcome of treatment.

In a retrospective study by Matzke and colleagues,[146] 110 consecutive patients with 145 critically ischaemic legs were assessed. The predictive values for leg survival, of the ankle and toe pressure measurements were determined, based on a three-month follow-up. Twenty five percent of the legs were eventually amputated. They reported that, although considerable overlapping was observed, ankle and toe pressures, as well as the ankle-brachial index (ABI), were lower in the amputated than in the non-amputated group. Mean values for ankle pressures were 32 and 42 mmHg ($p < 0.05$) respectively, for toe pressures 16 and 20 mmHg ($p < 0.05$) and for the ABI 0.26 and 0.32 ($p < 0.01$). Their results indicated that a single ankle-, toe- or ABI measurement had no predictive value regarding the risk of amputation. They were, however, of

the opinion that these measures could be applied as adjuncts supplementing the clinical examination.

Carter and Tate[147] examined whether the presence of low amplitude of pulse waves recorded from the toes was related to the risk of subsequent amputation and death in patients with skin ulcers or gangrene and peripheral arterial disease, and how the risk of low wave amplitude relates to the risk associated with low peripheral pressures. They evaluated a total of 309 patients with 346 limbs with skin lesions and arterial disease, who were followed up for an average of 5 years. Measurements were carried out to obtain ankle and toe pressures, pressure indices, and toe pulse wave amplitude. These variables were related to the risks of major amputation and total and cardiovascular death by means of the Cox proportional hazards model. They found that low toe pulse wave amplitude (≤ 4 mm) was associated with increased risk of amputation (relative risks 4.20 in all limbs and 2.63 in those with toe pressure ≤ 30 mm Hg; $p < 0.01$). Wave amplitude remained significantly associated with increased risk of amputation after controlling for each pressure variable ($p < 0.01$). Both low pulse wave amplitude and toe-brachial index were associated with increased risks of total and cardiovascular death in all patients (relative risks ranged from 1.43 – 1.73; $p < 0.05$), as well as with toe pressure of 30 mm Hg or less (relative risks 1.56 – 1.90; $p < 0.05$).

1.1.4.2 Application of Ankle- or Toe Blood Pressures or Indices in Diabetic Patients

Rheeder, et al [41] undertook a study in which ankle- and toe blood pressure measurements were performed on a cross-sectional sample of 85 female Type 2 diabetic patients. They demonstrated, with a wide prediction interval, a linear relationship between ABI and TBI, below an ABI value of 1.3. Furthermore, in agreement with the results of Brooks and colleagues,[148] who previously had found a mean difference between TBI and ABI, of about 40 % (0.37, with SD 0.15) in diabetic subjects, Rheeder, et al reported a mean difference of 0.36 (with 95%CI 0.32 – 0.41), if subjects with an ABI ≥ 1.3 were excluded. In patients with both pedal pulses absent on palpation, both ABI and TBI were significantly decreased.

In a longitudinal study, evaluating a screening program for lower extremity arterial disease in diabetic patients, Sahli and colleagues[149] focused on the value of toe blood pressure

assessment. They found it beneficial to include assessment of toe blood pressure and toe-arm blood pressure index to detect early LEAD in diabetic patients. Ankle blood pressure and ankle pressure indices on their own were found to be less efficient.

The finding of increased rigidity of the tibial or peroneal arteries due to medial arterial calcification (which results in 'incompressibility of the walls, and which, subsequently, gives rise to falsely high ankle pressure values), occur among many patients, but especially those with diabetes mellitus — according to Carter and Tate.[98, 100, 101, 150, 151] Since toe pressure reflects the overall obstruction in the arterial tree proximal to the digits, and therefore appears not to be affected by proximal incompressibility,[98, 100, 150, 152, 153] some authors have advocated the use of toe systolic blood pressure indices[43] (and others, toe blood pressure per se[154, 155]) as important methods to assess peripheral arterial disease in subjects with diabetes. Furthermore, the European Society of Vascular Surgeons prefers the absolute blood pressures of the ankle and the toe, instead of the ABI,[41, 156] while the recent Trans-Atlantic Inter-Society Consensus document[157] emphasizes the use of toe blood pressure when screening and evaluating patients with diabetes.[154]

1.1.4.3 Application of Pulse Wave Velocity in Diabetes Mellitus

Several studies have indicated decreased distensibility of the large arteries of patients with diabetes.[158, 159, 160, 161]

Paillole, et al[162] for instance, demonstrated, by comparison to healthy controls, higher aortic PWV in diabetic adults with good glycaemic control and without previous micro-angiopathy or heart disease. [163] The results of Lehmann, et al,[109] who analyzed aortic compliance in Type 2 diabetics using PWV measurements, showed that Type 2 diabetic patients have significantly stiffer aortas than age-and sex-matched non-diabetic controls. [163]

Taniwaki, et al [158] undertook a study in which they evaluated aortic distensibility (using carotid-femoral PWV) and carotid intima-media thickness (IMT) in 271 patients with type 2 diabetes, and in 258 age-matched controls. In all age groups, both aortic (i.e. carotid-femoral) PWV — with values of 9.02 ± 1.92, vs. 7.19 ± 1.05m — and carotid IMT, were significantly higher in the

patients than in the control subjects. In the diabetic patients, the independent risk factors associated with aortic PWV, were age and duration of diabetes.

Cruickshank, et al[164] found that, in a population with diabetes and impaired glucose tolerance aortic PWV was a powerful independent predictor of mortality in both the diabetes and IGT groups. They felt that in displacing systolic blood pressure as a prognostic factor, aortic PWV is probably further along the causal pathway for arterial disease and may represent a useful integrated index of vascular status, and hence cardiovascular risk.

Takegoshi, et al[165] reported faster PWV in diabetics with micro-albuminuria, than in those without this complication. They furthermore demonstrated a significant correlation between PWV and micro-albuminuria.[166]

Suzuki and colleagues[167] assessed PWV in the lower extremities of 60 Type 2 diabetics without a history or symptoms of lower extremity arterial disease (and with normal ABI's at the time of the study — the so-called non-PAD group), as well as in 20 non-diabetic controls. Their results demonstrated an abnormally higher PWV in the non-PAD group, compared to that of the non-diabetic control group ($p < 0.001$).

Similarly, Yokoyama, et al[168] measured brachial-ankle pulse wave velocity in 102 Type 2 diabetic patients (including those with PAD), as well as in 101 healthy controls. They found that brachial-ankle PWV was increased in diabetic patients, but decreased in the affected legs of diabetic patients with PAD In another study, evaluating brachial-ankle pulse wave velocity in 346 Type 2 diabetic patients, Yokoyama and colleagues[169] demonstrated an elevation of PWV values obtained in diabetic subjects with incipient nephropathy.

Likewise, a study by Ogawa, et al[170] evaluated 1066 patients with Type 2 diabetes and found that the brachial-ankle pulse wave velocity was elevated in the 86 individuals who had evidence of cerebral infarction.

Interestingly, it has also been reported that, consistent with increased muscular artery stiffness, the carotid-radial pulse wave velocity is increased in the healthy offspring of patients with Type 2 diabetes.[171]

Finally, the results of a study by Sengstock, et al[172] indicated that in hypertensive, non-diabetic, older adults, insulin resistance is, independent of glucose tolerance status, associated with arterial stiffness (which was assessed by means of aortic PWV measurement).

1.1.5 Neuropathy and its Evaluation in Diabetic Patients

Neuropathy, one of the most commonly occurring, and therefore, most important of diabetes complications, is characterized by a highly variable clinical picture.[173] Most common of the major neuropathic syndromes occurring in diabetes, is a distal symmetrical polyneuropathy, which, in most cases, involves a combination of sensory, motor, and autonomic nerve fibre abnormalities.[44, 174] Further complications may include focal neuropathies (such as median nerve entrapment — the so-called 'Carpal tunnel syndrome'), or other mono-neuropathies, poly-radiculopathies, or autonomic neuropathy.[175] However, according to The Diabetes Control and Complications Trial Research Group, advanced distal sensory, motor and autonomic deficits underlie most foot ulcers and amputations in patients with diabetes.[175, 176]

It has been reported that, of diabetic patients with foot lesions, the presence of peripheral neuropathy has been demonstrated in over 80 %.[62, 177, 178] (Cavanagh PR, et al,[179] in 1994, demonstrated a statistically significant association between lower extremity atherosclerosis (depicted by the presence of medial arterial wall calcification), and peripheral sensory neuropathy, by comparing the weight-bearing radiographs of 94 patients with diabetic sensory neuropathy, to those of 43 diabetic patients without neuropathy, and those of 50 age-matched non-diabetic controls.)[43]

Estimates of polyneuropathy (PNP) prevalence in diabetic populations depends on the specific definition used,[175, 180] and have been reported to range from 0 to 93% [44, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190] — the wide variation resulting from a variety of factors, including patient selection

criteria, investigators' criteria for neuropathy diagnosis,[191] and the respective sensitivities of the various methods of detection.[44] However, a high prevalence of diabetic neuropathy is always associated with significant morbidity, including foot ulcerations, infections, and subsequent amputations. [191]

As has been alluded to above, many different approaches are followed in the assessment of patients regarding diabetic neuropathy. However, there is no gold standard available for diagnosing PNP,[192, 193] since, what evaluation scheme should best be applied, has not been established.[173] The first comprehensive set of diagnostic criteria for diabetic neuropathy (DN) was introduced in 1985 by Dyck, et al,[194] at the Mayo Clinic in Rochester, Minnesota.[191] It involved completing the Neuropathy Symptom Profile (NSP), clinical evaluations using the Neuropathy Disability Score (NDS), vibration thresholds, nerve conduction tests, and autonomic function testing, and resulted in classification of patients according to stages of neuropathy severity. Subsequently, the ADA & AAN San Antonio consensus panel, in 1988, recommended that, among five diagnostic categories, namely symptom scoring, physical examination scoring, quantitative sensory testing (QST), cardiovascular autonomic function testing (cAFT), and electro-diagnostic studies (EDS), at least one measurement should be performed.[193, 195] However, both the Mayo Clinic and San Antonio testing criteria require experienced personnel using specialized equipment. And although both proposals have proven effective for diagnosing DN — as well as for distinguishing it from other forms of neuropathy — they were intended for research purposes,[191] are elaborate and time-consuming,[174] and are therefore less practical in many routine clinical settings, where simpler assessment is often required for the rapid screening of large numbers of patients.[191]

Between 2000 and 2002, Meijer and associates[196] introduced, respectively, the DNS (Diabetic Neuropathy Symptom) score (a four-item validated symptom score, with high predictive value to screen for polyneuropathy in diabetes) and the DNE (Diabetic Neuropathy Examination) score (which has been described as a sensitive and validated hierarchical scoring system allowing discrimination between patients with and without diabetic PNP).[193]

Gryz, et al, [173] in their 2004 literature review regarding the diagnosis and assessment of diabetic neuropathy, concluded that, apart from the physical examination, the most valid methods are the determination of vibration sense and assessment of nerve conduction velocity. They

suggested that, in addition, autonomic functions tests should be utilized to diagnose autonomic neuropathy.

1.1.5.1 Monofilament Testing

During recent years, regarding the use of simple screening methods for diabetic polyneuropathy, much attention has been focused on the use of the Semmes-Weinstein 5.07/10-g monofilament[197] as a measure of touch perception.[191] Since it has been identified by various studies as a valid and sensitive method for the prediction of foot ulceration and/ or amputation by detecting neuropathy,[198, 199, 200] this instrument has become a useful adjunct to and valuable extension of the physical examination in patients with suspected diabetic neuropathy:

In diabetic patients, in most cases, ulceration is a consequence of the loss of protective sensation, or, in other words, the loss of the awareness of trauma that could cause the breakdown of skin.[179, 201, 202] Monofilament testing allows for a simple, rapid, practical and inexpensive means[179, 197] of identifying patients who have lost protective sensation, [203] and are therefore prone to ulceration: A designated 5.07, nylon monofilament is pressed against the skin to the point of buckling; if patients are unable to perceive the monofilament, they are at risk for ulceration.[191, 179, 203] This test has been described as being as effective, but less time-consuming, than tests of thermal sensation[179] (usually performed as part of the routine physical examination on a patient suspected to have peripheral neuropathy[175]) and tests of vibration.[179, 197, 202] Also, the monofilament has been demonstrated to detect more abnormalities than the cotton wool test for light touch and the pin-prick test for evaluation of superficial pain sensation.[198] It is furthermore portable, easy to administer, painless, and thus acceptable to patients.[200, 204]

Monofilament sensation is well established as a prognostic risk factor for complications of neuropathy.[197]The Semmes-Weinstein Monofilament Examination (SWME), as a means of Quantitative Sensory Testing (QST),[195] therefore is currently the best choice for screening clinically significant neuropathy:[200, 204] When performed applying the 'instrument' repeatedly, but in an arrhythmic manner[197] to a pre-designated number of non-callused sites on the feet, it provides a method for the assessment of protective sensation, which is not only calibrated and

reproducible,[197, 205, 206] but which also renders good predictive ability for the risk of the development of diabetic foot complications. [193, 200, 204, 207]

1.1.5.2 Vibration Perception Testing

Decreased vibratory perception occurs in the limbs in most cases of diabetic polyneuropathy:[44] In a population-based study in the UK, for instance, 66% of patients with diabetes had a decline in vibratory sensation in the feet on repeated testing over time.[191, 208]

Different approaches exist, as to the evaluation of vibratory perception: Various studies simply tested the presence or absence of vibration sense, by applying a 128Hz tuning fork repeatedly on one or more of the bony prominences in the lower extremity.[190, 198]

For quantitative vibration testing, a variety of methods have been used. The so-called 'on-off method', evaluates vibration sensibility by requiring of patients to report perception of both the start of the vibration sensation, and of cessation of vibration on dampening.[197] Vibration testing by the 'timed method', involves recording of the time (in seconds) at which vibration sensation diminishes beyond the examiner's perception, past the point of imperceptibility, as reported by the patient.[197]

Vibration perception threshold (VPT) testing, one of the five different diagnostic categories recommended by the ADA & AAN San Antonio consensus panel,[193, 195] provides a means of quantitative sensory testing[209, 210] with predictive value regarding the development of diabetic foot complications. [193, 211] VPT testing can be conducted by making use of a vibrating stylus or probe in which voltage is gradually increased, until the point of perception of vibration by the patient.[183, 192] Similarly, the 'two-alternative forced-choice method', provides a means of evaluation of the vibration threshold on, for instance,[191] the plantar aspect of the great toe, or the tip of the index finger:[212] Patients are required to indicate which of two rods (as randomly determined by the examiner), is vibrating; upon each correct response, voltage is decreased by 10%, until the patient errs.

The available methods of vibratory sensation evaluation clearly differ with regard to complexity, time and equipment required, and information yielded. However, whatever method is used,

vibration sensitivity has been reported to correlate well with both electroneurographic examination, and with the presence of peripheral neuropathy.[185, 190, 213]

1.1.5.3 Nerve Conduction

Not only are electrophysiologic (or 'electroneurographic',[185] or 'electro-diagnostic'[195]) studies of peripheral nerve function (which include needle electromyography and electric stimulation[214]), considered sensitive, objective, reliable[215] and reproducible,[216, 217] but nerve conduction studies, nerve conduction velocities in particular, are also considered one of the most sensitive indices of the severity of neuropathy.[218, 219] They are, therefore, highly regarded for the detection and characterization of peripheral neuropathy.[215] Moreover, evaluation of nerve conduction parameters in the limbs has been shown to be a valid, sensitive and reliable method of detecting and assessing the severity of diabetic polyneuropathy.[192, 215, 220, 221, 222]

In Type 2 diabetic patients, decreased nerve conduction velocity (NCV) probably is one of the earliest abnormalities present. It has also been reported previously, to be present in the neurologically asymptomatic diabetic subject.[218, 223] Abnormal NCV in the form of decreased motor conduction velocity has, furthermore, recently been demonstrated in subjects with impaired glucose tolerance (IGT).[218]

Decreased NCV, therefore, is often present even at diagnosis.[218] Slowing of NCV, thereafter, generally progresses at a steady rate of approximately 1m/s per year. NCV has also been demonstrated to correlate with diabetes duration.[218, 224]

Interestingly, Larsen and colleagues, in 2003,[216] in their study of 39 Type 1 diabetic patients with diabetes duration of 30 years, demonstrated a significant association between mean HbA1c over 18 years, and nerve conduction velocity and nerve action potential amplitude in the lower limbs — indicating that in patients with Type 1 diabetes, physiological peripheral nerve function is predicted by long-term blood glucose concentration.

Electromyography is even more sensitive to early neuropathic changes than are conduction velocity measurements,[44, 225, 226] and has detected denervation abnormalities in diabetics before

slowing of the fastest motor velocities,[44, 227] however, needle examination of nerves suffers from the disadvantage of invasiveness and patient discomfort, and is hence less preferable.

It has been recommend, that, because the test is objective, sensitive and repeatable, nerve conduction should be used to set minimal criteria for neuropathy.[215] Abnormal nerve conduction, therefore, as defined by the Diabetes Control and Complications Trial Research Group,[175] and based on the Mayo Clinic staging criteria,[191, 215] requires the abnormality of at least one conduction attribute (amplitude, distal latency, or conduction velocity[191, 228]) on each of at least two anatomically distinct peripheral nerves.[175]

However, even though electro-diagnostic studies have been described as the most sensitive diagnostic tool for diabetic PNP, its prognostic value has not been clearly established,[193] since subtle abnormalities of nerve conduction, according to Dyck,[215] do imply nerve dysfunction, but do not necessarily indicate symptomatic degrees of peripheral neuropathy.

1.1.5.4 Autonomic Function Assessment

Several large studies have reported abnormalities of autonomic function to be present in between 20 to 40% of all diabetics.[229, 230, 231, 232] Autonomic neuropathy is therefore a frequent complication of diabetes; moreover, it often carries a poor prognosis in both Type 1 and Type 2 DM patients.[233, 234, 235]Even when sub-clinical, diabetic autonomic dysfunction is associated with a high risk of mortality.[236, 237, 238] When the condition has become symptomatic, autonomic neuropathy has been demonstrated to carry a worse prognosis than any other complication of diabetes:[229, 234] Diabetics with autonomic neuropathy are subject to an increased incidence of cardiac (or cardio-respiratory) arrest and sudden and unexpected death,[44, 239, 240, 241] especially (but not exclusively) during and after surgery.[229, 242]

In 1980, a 5-year mortality of 53% was reported by Ewing, et al, [234] in diabetic patients with autonomic symptoms and abnormal autonomic tests, while O'Brien, et al,[243] in 1991, demonstrated a five-fold increase in cumulative 5-year mortality in insulin dependent diabetic subjects with autonomic neuropathy, compared to those with normal autonomic function.[233]

An observation by Keen[244] in 1959, which he ascribed to decreased vagal tone, namely that diabetics often had tachycardia at rest, was confirmed by Wheeler and Watkins[245] in 1973, who reported that, in diabetics with autonomic neuropathy, normal variations in heart frequency were diminished or abolished, likely due to vagal denervation of the heart. [242, 246]

Cardiovascular autonomic function testing in general, has important prognostic value for the prediction of not only mortality due to cardiovascular problems,[193, 247] but also for diabetic foot complications.[248, 249, 250] However, cardiac autonomic neuropathy, partly due to its frequently asymptomatic presentation, and partly due to non-utilization of the tests available for its diagnosis, has been described as grossly under-diagnosed.[251]

Several tests for evaluation of the cardiovascular system with regard to autonomic functioning (both sympathetic, and parasympathetic) have been described,[246] including: recording of heart rate variations,[233, 242, 245, 252, 253, 254, 255] valsalva manoeuvre,[256] cold pressor,[253, 257] sustained handgrip,[233, 258]simulated diving (the so-called 'cold face' test[259]), and orthostatic tests (e.g. the immediate heart rate response to standing,[233, 260] and the blood pressure response to standing,[233] or to tilting[261]). However, some of the abovementioned tests, are complex — due partly to the fact that they require a certain degree of patient co-operation,[246] (which may, in turn, affect autonomic functions, and therefore, the interpretation of results), but also to the need, in some instances, for special equipment.

In the natural course of diabetic autonomic neuropathy, parasympathetic damage occurs before sympathetic damage. [234, 229] One simple to perform (since requiring very little patient co-operation), objective [229] and reliable, and therefore, frequently applied method [242, 245, 246, 253, 254]of cardiac autonomic function evaluation, is the beat-to-beat heart rate variation test, which resulted[242, 246] from the abovementioned observations by Wheeler and Watkins.[245] Their original method has since been modified by Sunkvist, et al,[242] and by Hilsted and Jensen,[231] to measure heart rate variation (maximum and minimum heart rates) during one minute of deep breathing, using an ECG.[252] It has subsequently been studied by several authors and in various age groups,[246, 255] in healthy subjects, as well as in patients with diabetes and polyneuropathy, both with and without autonomic dysfunction.[246] The test, which reflects parasympathetic nerve function,[246] is based on the fact that, in normal subjects at rest, respiration induces a physiological variation in heart rate, expressed in a shortening of R-R intervals on the

electrocardiographic recording during inspiration, and prolongation of the intervals during expiration. This variation is more pronounced in younger patients,[245, 246] at slow heart rates, and during deep breathing and, as stated above,[242] can be noticeably reduced, or completely absent in diabetics with autonomic neuropathy.[229, 242, 262, 263] The difference between inspiratory (I) and expiratory (E) heart rate can be calculated as a ratio (the E:I-ratio), taking into consideration the mean of the longest R-R intervals during expiration, and the mean of the shortest R-R intervals during inspiration. Autonomic dysfunction is considered present, when a difference of 10% or less (E:I-ratio < 1.10) is demonstrated between inspiratory and expiratory heart rate.[242]

Measurement of heart rate variation (HRV) has been demonstrated to be reliable, reproducible,[231, 252] and sensitive,[251] and has therefore long been generally accepted as a valid test of cardiac parasympathetic function.[252] Heart rate variability, reflecting abnormalities of autonomic activity,[236] is strongly associated with both an increased risk for cardiac events,[264, 265] and overall mortality.[264, 266]

Previous findings regarding the clinical utility of HRV, and its role in the surveillance of diabetics,[267, 268] were confirmed and extended,[236] when recently, in a population-based investigation, 1 990 males and females (normal subjects, subjects with impaired glucose tolerance, and with diabetes mellitus) from the Framingham Offspring Study underwent HRV testing. Results demonstrated that HRV is inversely associated with plasma glucose levels, and is reduced in both diabetics, and in subjects with impaired fasting glucose levels.[236]

1.1.6 Current Diabetes Care in Southern Africa — Implications for Foot Care

A study published fairly recently (2004),[11] reporting on the quality of diabetes care in the South African government sponsored hospital, Kalafong (a tertiary teaching facility affiliated to the University of Pretoria), found that glycaemic control of patients in the intervention group compared well with results obtained in a study of diabetic patients in a large urban hospital in the USA: In the South African study, 36.8% of patients were found to have uncontrolled blood glucose levels (defined as HbA1c > 9.5%), and in the USA study, 31 – 43%.[269] In the Kalafong Hospital study, a number of process measures were utilized in the investigation, (including foot- and eye examinations, tests for micro-albuminuria and HbA1c, a lipid profile, and a dietician

visit), but no specific mention is made of measures of evaluation of neuropathy, or of peripheral vascular function.[11]

Rotchford and Rotchford, in their study published in 2002, investigated 253 diabetic patients (73.1% female) attending public health sector clinics in a rural district of KwaZulu-Natal, South Africa, and found that acceptable glycaemic control (defined as HbA1c < 2% above the normal population range) was present in only 15.7% of subjects, and that complications were common and mostly undiagnosed. Peripheral neuropathy was not assessed in this study, but a 6% prevalence of 'foot abnormalities attributable to diabetes' (ulceration, sepsis and surgical amputation – in 1 patient) was reported, and dorsalis pedis pulses were absent in 16.6%.[40]

A study by Mollentze and colleagues (abstract published in 2000), investigated the prevalence of foot ulceration and peripheral neuropathy in 120 diabetics attending hospital diabetes clinics. (Two thirds were female, and 60.8% Type 2 diabetics.) The mean HbA1c concentration for this group was 9.2%. For foot ulceration a prevalence of 5.8% was reported. Peripheral vascular integrity was only assessed clinically, and the presence of sensory deficits by evaluating light touch, monofilament and vibration sense (the latter by means of a 128Hz tuning fork). Abnormality — reported in 74.2% of subjects — was defined as either a decrease or an absence of one or more of these modalities of sensation.[270]

Erasmus, et al in 1999, assessed glycaemic control in 708 peri-urban black Type 2 diabetic patients attending the diabetes clinic at the Umtata hospital, a referral center for the former homeland of Transkei. Target values of HbA1c — in this study an HbA1c of < 7% was required for acceptable control — were reportedly achieved in only 20.1% of patients.[1] (Peripheral neuropathy and peripheral vascular disease were not assessed.)

Likewise, another South African study undertaken between 1992 – 1997, by Levitt, et al (the audit among Blacks attending primary care public sector clinics in Cape Town, mentioned earlier), indicated the presence of a high prevalence of sub-optimal glycaemic control: Here acceptable glycaemic control (HbA1c < 10%) was reported to be present in only 49.4% of patients.[34] In this study, peripheral neuropathy (prevalence 27.6%) was defined as the presence of two of: bilaterally absent ankle reflexes, sensory symptoms, or sensory signs, (absent fine

touch, pinprick or vibration sense). Peripheral vascular disease (prevalence 8.2%) was defined simply by the absence of dorsalis pedis pulses.

Still earlier, Gill and colleagues followed up sixty-four Type1 diabetic patients from 1982 – 1992 at the Baragwanath Hospital Diabetic Clinic, in Soweto, South Africa. Peripheral neuropathy was defined as any definite sensory or motor loss with no other explanation (excluding isolated absent ankle reflexes). It affected 42% of patients, and significantly increased over the 10-year assessment period. Autonomic neuropathy (assessed by electro-cardiographic R-R measurements during deep breathing) was also common, affecting 47% of patients. One patient only, had had a minor amputation (of a toe, because of neuropathic ulceration with underlying osteitis).[38]

Southern African data, specifically on the prevalence and assessment of diabetic peripheral neuropathy, peripheral vascular function impairment, lower extremity ulceration and amputation in diabetics, clearly, are sparse. Apart from the studies mentioned above and two fairly recent studies by Rheeder, et al (discussed below), no other studies exist in South Africa that have recently investigated or reported on the complicated lower leg in the diabetic patient:

Against the background of the lack of published data on the prevalence of peripheral arterial disease or medial arterial calcification in an unselected group of black South Africans with diabetes mellitus, Rheeder and colleagues, during 2003, at a community hospital serving mainly as a primary health care facility for the urban black community of Mamelodi, investigated a convenience sample of 85 previously diagnosed Type 2 diabetics (all female, due to the fact that a relatively small number of men and Type 1 subjects attended the clinic). Subjects underwent a series of peripheral vascular assessments, including pedal pulse palpation, assessment of ankle- and toe- blood pressure indices, (Doppler- and photo-plethysmographically -derived, respectively) and antero-posterior radiographs of the feet. Both the ABI and the TBI were significantly lower in patients who had had pedal pulses absent bilaterally. But the prevalence of PAD (as defined by an ABI < 0.9), as well as the prevalence of medial arterial calcification, based on radiological evidence (but not correlating with an ABI >1.3), were low: 0-4.7% and 9.9%, respectively.[41]

In another study at the same community hospital diabetes clinic, Rheeder, et al assessed 89 female diabetic subjects for neuropathy, using monofilament, cotton wool, pinprick, and vibration sense evaluations, and a verbal questionnaire to determine symptoms. Neuropathy was present in 26%, 3%, 6%, 8% and 75% respectively, depending on the method used. The authors concluded that more abnormalities had been detected by using the monofilament, than by using cotton wool or the pinprick; also that there was poor concordance between symptoms and clinically detected neuropathy, and that the number of sites to be evaluated, remained undecided.[198]

Not only is it clear that data on current diabetes care in South Africa — in general, as well as with specific reference to the epidemiology and assessment of the diabetic foot — is limited, but the lack of standardization of methods of assessment of the complicated lower extremity, makes it difficult to compare the results of the few studies published. Therefore, although the studies mentioned above, give an indication of the extent of diabetic neuropathy and peripheral vascular impairment in South Africa, a clear and representative picture of the current status of South African diabetes care — specifically with regard to the evaluation of neuropathy and peripheral vascular impairment — does not exist.

1.2 MOTIVATION FOR THIS STUDY

There are no studies that compare measures of vascular function, such as the above, between diabetic patients with different degrees of lower extremity complications (foot ulcers or amputation), and diabetic patients without such lesions. It stands to reason, that such patients reflect varying degrees of long-term diabetes and that the measures of atherosclerosis could be expected to worsen across these 3 groups (namely diabetic subjects without amputation or ulceration, diabetics with foot ulceration, and diabetics with evidence of a lower limb amputation).

Likewise, it can be expected, that peripheral and autonomic nervous function testing will produce results that will differ between diabetic patients with a complicated lower limb and diabetic patients without this complication.

The aim of this study, therefore, is to investigate among the 3 groups, the magnitude of absolute differences in results obtained from these respective measures, in order to plan sample size estimates for future studies on diabetes complications, using these measures.

1.3 STUDY OBJECTIVES

Thus, the objectives of this study are, firstly, to compare the photo-plethysmographically derived toe-brachial index and pulse wave velocity between diabetic subjects with lower extremity ulcers, diabetic subjects with lower extremity amputation and a group of diabetics without these complications; secondly, to compare peripheral and autonomic nervous system integrity between these groups, by sensory, nerve conduction, needle examination, as well as autonomic function assessment; and finally, to calculate sample sizes for future comparisons, using these measures.

METHODOLOGY

2.1 STUDY DESIGN

The design of this pilot study is cross-sectional, descriptive and comparative:

Similar to a dose-finding study in a drug trial, this study serves as a pilot study for the purpose of establishing mean (or median) values and standard deviations (or inter-quartile ranges) — for application in sample size calculations for future studies — of certain vascular and neurological measurements, as well as for investigating any trends towards differences between the 3 groups being evaluated.

2.2 SETTING

The Pretoria Academic Hospital (PAH), a tertiary care teaching hospital affiliated to the University of Pretoria (UP), Faculty of Health Sciences.

2.3 SAMPLE SIZE, SAMPLING METHOD AND PATIENT SELECTION

No formal ‘a priori’ sample size calculations have been done, due to uncertainty as to what differences in measurement results could be expected between our 3 subject groups: Only one

South African study exist (performed in only one group of unselected diabetics), that have previously reported on TBI.[41] No comparison was made in that study, with diabetics exhibiting lower extremity complications. No South African studies exist, reporting on PWV. No comparisons of three groups with differing degrees of lower extremity complications, applying all of the outcomes measures utilized in this study, were available for obtaining of means and standard deviations for sample size calculations.

Due to recruitment difficulties, which included incomplete diabetes and diabetic foot clinic patient lists, lack of (and often outdated) patient contact information, unwillingness of eligible patients to be included (resulting mainly from a combination of transport-problems and a reluctance to take two days' leave from work), a convenience sample consisting of 3 groups of ten consecutively eligible and available diabetic patients were selected, over a period of fifteen months, from the diabetes clinic and diabetic foot clinic: Ten patients with ulcers on their lower extremities, ten with healed lower extremity amputations (above-knee or below-knee) and ten control diabetic patients without these complications.

2.4 ETHICAL CONSIDERATIONS

The project had been submitted to and approved by the Ethics Committee of the Faculty of Health Sciences, University of Pretoria.

2.5 OBTAINING OF INFORMED CONSENT

Before signing it, the document was read through to and with patients in a language that they understood. Where necessary, staff members were used as translators. The content and meaning of the information contained in the consent document, was explained, and patients were given adequate opportunity to ask questions and receive satisfying answers. It was emphasized that participation would be voluntary. The implications and subsequent management of positive evidence of vascular disease, or neurological lower extremity disease, or of evidence of renal function impairment, were furthermore explained.

2.6 IN- AND EXCLUSION CRITERIA

Diabetic patients of all race and age groups were eligible for inclusion.

The following patients were excluded: Those with -

▪ a persistent documented urinary tract infection on the day of urine-albumin and vascular evaluation; or

▪ severe congestive heart failure (NYHA grade iii, iv) — Congestive heart failure is known to be associated with impaired arterial compliance and distensibility (as demonstrated by an increase in PWV, accentuated with worsening severity of heart failure[271]); or

▪ chronic renal failure or nephritis, or on dialysis — PWV, independent of age, gender and blood pressure levels, is increased at the level of the aorta, lower limb, and to a lesser extent, the upper limb;[272]

▪ uncontrolled hypo- or hyperthyroidism — Interactions exist between thyroid hormones and cardiovascular hemodynamics, which may influence PWV in patients with thyroid dysfunction; [273] or with

▪ known malignancies, or receiving chemotherapy; were excluded.

Patients who were unwilling or unable to comply with all study requirements (which resulted in failure to provide written informed consent) were also excluded.

2.7 METHODS OF INVESTIGATION

Patients at the Diabetes and Diabetes Foot Clinics, who were considered eligible for inclusion by tending clinicians (who, in turn, had been duly informed with regard to the study protocol — specifically in- and exclusion criteria), were referred to the Clinical Epidemiology Unit researcher. After confirmation of diabetes diagnosis — based on patient history and clinical records — and of lower extremity condition, patients were extensively informed as to the requirements and the implications of the study. Consenting patients were then again, approximately two weeks after initial screening, seen at the Clinical Epidemiology Unit and evaluated in the following manner:

2.7.1 Laboratory Investigations

In order to firstly rule out the presence of a urinary tract infection (which could influence albumin excretion), patients, during the initial screening consultation, underwent routine dipstix testing of a mid-stream urine specimen (using Multistix® 5 test strips, 2308A, Bayer Diagnostics Division, NY 10591 – 5097, USA). Where indicated (i.e. if urinalysis was positive for leucocytes, nitrates or blood), this was followed by delivery of the specimen to the UP PAH microbiology laboratory for microscopy, culture and sensitivity (MC&S) examination, while at the same time, commencing empiric Bactrim (Trimethroprim, Sulfamethoxazole) treatment for 5 days. MC&S results were followed up and treatment adapted accordingly, where required.

Urinary albumin is used to classify diabetic nephropathy according to accepted guidelines. Micro-albuminuria, furthermore, is a potent marker of atherosclerotic disease in diabetes mellitus.[274, 275, 276] Therefore after proper instruction during the screening visit, patients were required to collect and provide us with two successive overnight urine samples for determination of urinary albumin. This was to coincide with obtaining of the remaining fasting blood specimens for chemical and hematological analysis, thus, one sample was to be collected two nights before, and the other during the night immediately preceding their neurovascular evaluation visit.

Upon arrival of patients for the vascular and clinical neurological investigation visit (two weeks post-screening), in order to establish the absence of a current urinary tract infection, another freshly obtained mid-stream urine specimen was first of all screened for leucocytes, nitrates and blood. If positive, patients were to be excluded from albumin measurements. If negative, the

overnight urine samples collected by the patient during the preceding two nights, were delivered to the UP PAH microbiology laboratory, where overnight urine-albumin excretion was determined, using a Beckman IMMAGE®.

Provided that patients had adhered to an overnight fast of at least 10 hours, standard venepunction techniques were used to obtain venous specimens for determination of serum lipids — by requesting a fasting lipogram, which included serum-total cholesterol, LDL (low density lipoprotein)-cholesterol, HDL (high density lipoprotein)-cholesterol and triglycerides — as well as for HbA1c (glycosylated hemoglobin — as an index of average blood glucose levels during the preceding two to three months) and serum-creatinine determination (as an index of kidney function). Specimens for fasting lipogram and s-creatinine determination were centrifuged for 5 minutes, upon which they were kept in a cooler box with the remaining blood specimens, until delivery, within 4 hours to the UP Dept. of Chemical Pathology at the PAH, where analyses were performed on a Beckman LX20®.

2.7.2 Questionnaire

The questionnaire was completed before initiation of vascular and neurological evaluation. Apart from patient demographics, information obtained comprised race, gender, age, ulcer or amputation history, and diabetes diagnosis, treatment and complications history. The questionnaire furthermore included sections pertaining to known cardiovascular risk factors; history (treatment for, symptoms or investigations) of peripheral arterial impairment (e.g. intermittent claudication, resting lower limb pain), or of cardiac (angina, MI, coronary angiogram, etc.) or cerebrovascular ischaemia (stroke or TIA); and known diabetic eye or renal complications. Finally, other known previous or present illnesses and medication use were noted.

2.7.3 Clinical Parameters

Clinical evaluation by the researcher was directed at recording of the following general baseline information: height, mass, body-mass index (BMI), waist circumference, hip circumference, radial pulse rate and rhythm, right upper mid-arm circumference and brachial blood pressure, as

well as at establishing the presence or absence of peripheral pulses, femoral bruits, chronic ulcers of the lower limb, and evidence of lower limb amputation.

(1) Mass was determined to the nearest 0.1 kg, with the patient standing barefoot in light clothing on a calibrated electronic scale (Tanita®).

(2) Where possible, **height** (to the nearest 0.1 cm) was determined using a rigid tape measure attached to the wall.

(3) Body mass index (BMI) was calculated as mass to height squared, and expressed in terms of kg/m².

(4) Waist circumference was measured in centimetres, using the standard, soft, flexible tape measure included in the Colson® Complior Pulse Wave Velocity kit. It was determined with the patient in the standing, upright position (with weight evenly distributed on both feet, and the feet about 25 – 30 cm apart). The tape measure was fitted snugly — but not so tightly as to compress underlying soft tissues — at the smallest diameter between the xiphisternum and the umbilicus.[277] Measurement was recorded at the end of a normal, mild expiration and to the nearest 0.1cm.[278]

(5) Hip circumference was measured with the patient in the standing position (wearing only non-restrictive underwear), to the nearest 0.1 cm, using the same flexible tape measure, and at the level of maximal protrusion of gluteus maximus muscles posteriorly and the symphysis pubis anteriorly.[277]

->For both the waist and the hip circumference, two measurements were taken, and, in the event of a difference of more than 2 cm between the two, a third was added. The mean, either of the two measurements, or of the closest two in the case of three measurements, was used.[278]

Five patients with amputations proximal to the foot underwent mass and height measurements, while wearing their limb prostheses. Where indicated, corrections were made in recorded values for the added weight and length of these prostheses.

The two patients with only sub-total (mid-) foot unilateral amputations underwent anthropometric measurements per protocol, as described above.

Two more patients with more proximal unilateral amputations, balanced by slightly leaning against the wall for height and hip measurements, and against the bed for determination of the waist circumference.

One patient with bilateral amputations was unable to stand. His mass was estimated from that of other patients with similar build, while his height was recorded from (his own) memory of previous measurements, and confirmed using a flexible tape (with the patient lying down flat, measuring from the perpendicularly held heel of the foot with sub-total resection, to the vertex).

In one patient with bilateral amputations (on one side, with a sub-total foot amputation), waist circumference was measured in the supine position, at a level midway between the lower rib margin and the iliac crest, at the end of a mild expiration and to the nearest 0.1cm.

(6) Waist hip ratio was subsequently calculated.

(7) Right upper mid-arm circumference was measured in centimetres, using the standard, soft, flexible tape measure included in the Colson® Complior pulse wave velocity kit.

(8) Pulse rate was obtained at the right radial arterial site, after at least 5 minutes of rest, counting for 60 seconds and prior to blood pressure measurement.

(9) Brachial blood pressure was measured in the supine position, after at least 10 minutes of rest, with the right* arm resting on the bed, and the standing model Mercury Baumanometer on the bedside trolley, according to published guidelines.[279] For an arm circumference of 33cm or greater, a large cuff (i.e. with a 15 cm rubber bladder) was used.[280] Two measurements were taken; intervals of at least two minutes between measurements were observed. In the event of a difference of more than 5 mmHg between readings, a third reading was obtained. The mean of the two closest measurements was then used to determine the patient's mean blood pressure, to the nearest 1 mmHg.

(* The Colson® Complior System, by convention, measures PWV on the patient's right side; mean BP is therefore based on right-sided measurements.)

(10) Peripheral pulses were palpated, noted, and graded as being 'present', 'reduced', or 'absent'. Apart from the Radial Artery, the following peripheral pulses were also palpated bilaterally: A. Femoralis, A. Poplitealis, A. Tibialis Posterior and A. Dorsalis Pedis. The absence of both foot pulses (A. Tibialis Posterior and A. Dorsalis Pedis) in at least one leg was considered as diagnostic of peripheral arterial disease.[149]

(11) Auscultation for the presence of **arterial bruits** was done over the femoral arteries, bilaterally, by means of a stethoscope with double-sided head.

(12) Lower limbs were examined for the presence of chronic ulcers and evidence of previous amputations.

2.7.4 Vascular Investigations

Only one observer (the primary researcher, MCD-B, clinical research assistant in the Division of Clinical Epidemiology) performed all measurements.

2.7.4.1 Pulse Wave Velocity (PWV) Evaluation

Pulse wave velocity was assessed using the Colson Complior® II system (Createch Industries, Paris, France; ref. no. Comp. 2, 12 – 98; serial no. 0002), which is a portable, automatic device, with software installed on a Pentium II personal computer.

Asmar and colleagues tested this device, analyzing its accuracy (in comparison to the manual method) and the reproducibility of its time delay and PWV determination. The correlation between the two methods was linear, with r = 0.99, indicating good agreement between the

manual method (gold standard) and the automatic device. Furthermore, both inter- and intra-observer repeatability coefficients for PWV measurements, using these two methods, were high: > 0.90.[281, 282]

The Colson Complior® system makes use of three pressure transducers to determine the pulse waveform. Velocity is calculated as distance (mm) divided by time (ms), and expressed in metres per second (m/s). The system therefore requires distance measurements between the carotid and femoral arteries (i.e. the CF measurement), as well as between the carotid and radial arteries (i.e. the CR measurement), in order to calculate the respective carotid-femoral (CF) and carotid-radial (CR) pulse wave velocities.

Regarding measurement of the distances, several methods have been suggested in the literature.[283] We made use of the following simplified distance measurement method, as considered compatible with clinical practice, and proposed by the manufacturers of the Complior® device: The carotid artery pulse was found by following out the superior right border of the lamina of the thyroid cartilage to its very end. The radial artery pulse was located by palpating next to the right styloid process of the radius. The femoral artery pulse was located by palpating, with the leg in slight external rotation, in the area of the right inguinal ligament. These sites were marked; so too, was the sternal notch. (The three pressure transducers – carotid, radial and femoral — were later positioned over the respective pulse sites.) Using the flexible Colson tape measure, the covered distances were estimated by direct superficial measurements in a straight line over the skin: The distance from the carotid pulse marking to the sternal notch (carotid-sternal, or CS), was firstly obtained, followed by the distances between the sternal notch and the radial pulse marking (radio-sternal, or RS) and the sternal notch and the femoral pulse marking (femoro-sternal, or FS). Finally, to calculate the required CR and CF distances, the CS distance was subtracted[104] from the RS and FS distances respectively. This method compensates for the pulse wave traveling in opposite directions in the arterial tree.

The pulse waveform is recognized by the Complior® software as the first vertical deviation of a significant amplitude, from the baseline. The software calculates two sets of pulse wave velocities: the CR and CF PWV. In order to cover a complete respiratory cycle, each dataset

consists of at least 10 successive measurements, the average of which is used in the analyses.[111, 284]

Provided that the correlation in each data-set was not less than 30%— in which case it needed to be discarded, and a new set of PWV readings obtained — the datasets were saved in a database until statistical analyses were to be performed. Two sequences of measurements were performed in each subject, and their mean considered for analysis.[124]

Preparations made and precautions taken, included the following:

- Patients were examined after an over-night fast of at least 10 hours (Klip,[285] who analyzed the influence of meals on PWV in young men, noticed, after a meal, a significant increase in PWV of peripheral vessels, whereas in the aorta, a tendency toward decreased PWV was noted. Asmar,[286] in 1999, reported that this finding might possibly be the result of coinciding post-prandial peripheral vasoconstriction and splanchnic vessel dilatation.)

- Patients were required to have refrained from smoking overnight for the duration of the 12 hours preceding the evaluation;[287]

- and to have been supine for at least 10 minutes prior to initiation of PWV measurements.

- Care was taken to measure the carotid-femoral distance betwéén the breasts, as measuring óver the breasts would result in an over-estimation of the distance.

2.7.4.2 Toe Blood Pressure (TBP) and Toe-brachial Index (TBI) Evaluation

Photo-plethysmography (PPG) was used to measure toe systolic blood pressure:[288]

The method involves attaching a photo-sensor to the distal part of the pulp space of the first toe to record pulse reflections. A miniature pneumatic blood pressure cuff is placed at the base of the toe, encircling the proximal phalanx. On inflation of the miniature cuff above systolic

pressure, pulsations disappear on the recording device. During controlled, slow deflation of the cuff, the pulse waveform reappears, coinciding with the toe systolic pressure.

Before the two actual measurements were taken, on the left and on the right hallux, one measurement was taken as a reference.[41] A mean systolic toe pressure was calculated by averaging the values obtained on the left and right toes respectively. In the event of absence of either the right or left hallux, two values were obtained in the remaining hallux, and averaged. Where the hallux, due to amputation, was absent bilaterally, ankle pressure was measured placing the same blood pressure cuff as that used for brachial pressure measurement, just above the ankle,[149] and utilizing a hand-held pen Doppler to detect arterial pulsation,[289] on whichever of the dorsalis pedis, or tibialis posterior artery, was best audible on prior auscultation.

For calculation of the toe-brachial index (TBI), the mean of two brachial systolic blood pressure readings was used. (In the event of a more than 5 mmHg difference, a third brachial systolic pressure measurement was obtained, and the two closest of the three readings were used in calculation of the mean.)

A **toe pressure** of ≤ 30 mmHg [98-102, 288] was taken to indicate critical ischaemia, while a **toe pressure** of < 80mmHg [149, 288, 289] and a **toe-brachial index** of ≤ 0.75 [149] (in agreement with Orchard, et al's proposed screening cut-off of 0.60 ± 17% [43] was used to define impaired peripheral circulation.

Instrumentation utilized, included:

- a photo-plethysmograph, which was used as sensor;
- mercury Baumanometer®;
- miniature pneumatic blood pressure cuff with a standard 3-way connector piece;
- Tele-thermometer, for assessing skin temperature of the toes;
- thermometer, for determining room temperature; and a
- foot spa, for warming of feet, where indicated.

Instrument specifications were as follows:

- Photo-plethysmograph: Hokanson® MD6 RP; serial no. 04249849; with Hokanson® chart recorder MD6R; serial no. 02229926.
  (D. E. Hokanson, Inc; Bellevue, Washington 98005, USA)
- Toe blood pressure cuff: 2.5 cm wide by 10 cm long. Hokanson® PC 2.5.
  (D. E. Hokanson, Inc; Bellevue, Washington 98005, USA)
- Mercury Baumanometer®: desk model. U.S. patent nos. 1594039 & 1821902; serial no. BH4119.
  (W. A. Baum Co. Inc.; New York, USA)
- Tele-thermometer: YSI; model 44TC; serial no. 5067.
  (Yellow Springs Instrument Company, Inc; Yellow Springs, Ohio, USA)
- Wall-attached mercury thermometer: Degrees Celsius indicated.
  (TFA design.)
- Foot spa: Sunbeam®; model no. SUN0050; serial no. 051299
  (Nu World Ind. (Pty.) LTD; JHB 2000, RSA)

In order to obtain useful and reproducible results, the following *preparations were made and precautions taken,* based on the recommendations of Orchard TJ, and associates:[43]

- Room temperature was comfortable, not below 22° C, so that patients would not feel cold.
- Patients had to have been resting, quiet and supine for at least 5 minutes prior to measurement of the pressures.
- Because temperature-dependent vasoconstriction could lead to false readings, skin temperature of the toes was measured, and had to be at least 25°C. Where necessary, the feet were warmed gently for 10 minutes in warm water (37 – 39°C), using a foot bath. (In case of a skin lesion, a protective plastic bag was drawn over the foot.)
- In order to ensure a clearly defined curve, the toe was squeezed gently between the fingers of the examiner, before inflation of the toe blood pressure cuff, thereby emptying the toe vessels of blood.
- The cuff was deflated stepwise (at 2 mmHg/ second), so as to facilitate reading of the tracings at very low pressures)

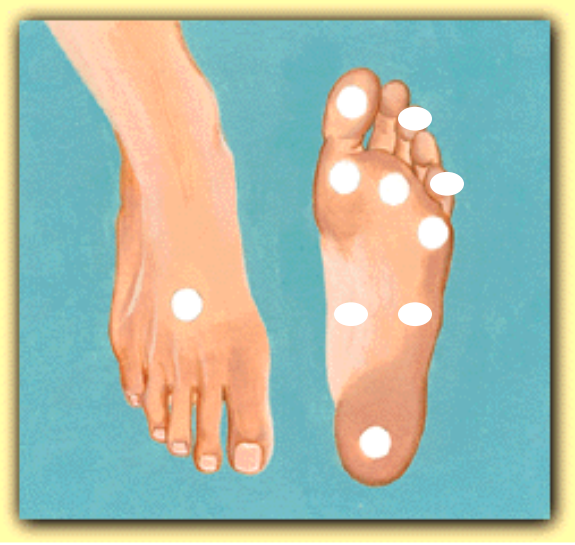
2.7.5 Neurological Assessment:

This part of the evaluations comprised monofilament, vibration sense, R-R variation to deep breathing and cutaneous autonomic response assessment, as well as nerve conduction and needle-examination studies:

2.7.5.1 Clinical Neurological Evaluation

2.7.5.1.1 Vibration sense was tested using a 128Hz tuning fork (ref 871210, Ragg Tuning Forks; Granton Ragg Ltd, England S1 3BE). Subjects were asked what they felt; a response indicating vibration was acceptable. The first metatarsal head (bony prominence) was first of all tested; if not present, proceeding to the medial malleolus; if still not present, to the tuber tibiae. The forehead was used as a reference of normal vibratory sense.

2.7.5.1.2 Monofilament perception was evaluated applying the 5.07/ 10-g-instrument (STS 1-888-2899293, Sensory Testing Systems; Baton Rouge, LA 70806, USA) at ten specified sites on each foot.[290, 291] The forehead was used as a reference for normal sensory perception.



Neuropathy, according to the Armstrong and Lavery classification, [290] was defined as absence of monofilament sensation in four or more sites on either, or both of the right and left feet. Prior to testing on the feet, all subjects had the monofilament applied to their hand (right hand dorsum, 1st web space), so that they were familiar with the procedure. Subjects were instructed to close their eyes and to indicate when and on which foot they felt the monofilament. The monofilament was placed on the skin, held at a right angle. The total time of skin contact was 1.5 seconds. Where callus, scarring, ulceration or

necrotic tissue was present at a particular site, the closest unaffected area was tested. Repeated skin contact, as well as sliding of the filament over or along the skin, was avoided. The sites were examined in random order.

2.7.5.2 Autonomic Responses

2.7.5.2.1 The ECG-derived heart-rate-variability-response to six deep breaths/min (5 s of inspiration and 5 s of expiration) was recorded for 1 min on a continuous electrocardiogram trace[191] (Cardiofax®, Nihon Kohden, Tokyo), using 4 standard and 3 pre-cordial leads, to minimize interference.[252] A marker was used to indicate the onset of each inspiration and expiration. The maximum and minimum R-R intervals during each breathing cycle were measured with a ruler[231] and converted to heart rate (beats/min): [191, 229]

$$\text{Heart rate} = \frac{300}{\text{(R-R in mm / 5)}}$$

The difference between the maximum and minimum heart rate for each breathing cycle was determined next, followed by the mean difference between maximum and minimum heart rates for the six breathing cycles, in beats/min:[229, 252]

$$\text{Mean (Maximum – Minimum) Heart rate} = \frac{\Sigma\ (\text{max} - \text{min})}{6}$$

Differences larger than 15 beats/min were considered normal, under 10 abnormal, and values in between as borderline.[229, 233]

Finally, the R-R ratio was determined by calculating the mean of the six maximum expiratory R-R intervals and the mean of the six minimum inspiratory R-R-intervals (obtained during the 6 breathing cycles of the one minute sample period), and expressing these as a so-called E:I (Expiration: Inspiration)-ratio:[242, 292]

$$\text{E:I} = \frac{\text{Mean value for longest R-R interval during each of 6 expirations}}{\text{Mean value for shortest R-R interval during each of 6 inspirations}}$$

An E:I-ratio of < 1.10 (representing a difference of 10% or less between inspiratory and expiratory heart rate) was considered abnormal and indicative of the presence of (parasympathetic)[243] autonomic dysfunction.[242]

The following *preparations and precautions were applied:*

- Each patient was given a training period of deep breathing at 6 breaths min (5 s inspiration and 5 s expiration) prior to formal testing;[252]
- Testing was only initiated after a supine resting period of 20 minutes.[191]

2.7.5.2.2 Cutaneous autonomic response assessment was conducted for assessment of peripheral cholinergic sympathetic function,[293] by specialist clinicians of the Neurology Dept, in the Neurophysiology Laboratory of the PAH:

Utilizing — depending on availability — either the MS 25 EMG machine (Medelec, Old Woking, UK), or the Nihon Kohden Evoked Potential and/ EMG Measuring System (MEB9104K 00001AA, Nihon Kohden, Tokyo, Japan), the so-called Sympathetic Skin Response, or SSR, was performed in one upper (palmar SSR) and one lower limb (plantar SSR),[294] and measured from the skin surface, by means of differential bipolar recording: [293]

A pair of standard surface silver - silver chloride [293, 294] electromyogram disc electrodes were positioned on the hand (active recording electrodes on the mid-palmar aspect,[295] and reference electrodes on the dorsal aspect, immediately opposite the palmar electrode), as well as on the foot (reference electrodes on the mid-tarsal part of the dorsum of the foot, and active recording electrodes on the sole, immediately opposite the dorsal electrode).[293]

[The cutaneous response is mediated by a sympathetic axon reflex and (due to sudomotor/ sweat-driven activity) leads to a change in voltage.[293] Hence, when the active recording electrodes (placed on the sole of the foot and the palm of the hand, respectively), are connected to the negative input of the amplifier, recording with the first negative deflection, is permitted.[293] Absence of a negative deflection, therefore, indicates absence of the cutaneous autonomic response.]

An electric stimulus with a 40 mA intensity and initial duration of 0.2 msec,[293, 294, 295] was applied to the skin of the wrist[294, 295] and the lower limb respectively.[295] Care was taken not to apply the stimulus close to the natural course of any nerve. Stimuli were delivered twice on each limb, and separated by at least one minute,[293, 295] to avoid habituation of the response.

Responses to electrical stimulation (negative baseline deflection) were noted as present or absent.

SSR tests were performed with al patients lying comfortably in a semi-darkened room with the ambient temperature maintained between 21 and 23 °C.[228] Limb temperature, likewise, was controlled during testing: Hand temperature was maintained between 32° and 34°C and the temperature of the feet between 31° and 33° C.[294]

2.7.5.3 Electrophysiology

Electro-diagnostic studies were performed in all patients — except for one patient, who had passed away, due to a complication during elective, routine surgery, prior to his scheduled EMG appointment — and were conducted by two specialists experienced in neurophysiology, of the Neurology Dept, in the Neurophysiology Laboratory, PAH, applying the same methods, and using (depending on availability), either the MS 25 (Medelec, Old Woking, UK), or the Nihon Kohden EP/ EMG Measuring System (model MEB9104K , serial no. 00001AA; Nishiochiai Shinjukuku, Tokyo, Japan) electromyogram apparatus. For skin temperature assessment, a Tele-thermometer (YSI, model 44TC, serial no. 5067; Yellow Springs Instrument Company, Inc; Yellow Springs, Ohio, USA) was utilized.

2.7.5.3.1 Nerve conduction studies

Nerve conduction studies (NCS) were performed according to accepted principles and following a standardized protocol, based on conventional methods, which included using standard filter settings[193] on the electromyography apparatus, employing standard surface stimulation and recording techniques[193] (i.e. recording of well-defined and artifact-free responses, [218, 223] and using surface electrodes[292]at standard recording sites [216]), applying fixed distances (where

indicated, i.e. for sural and median nerves), or careful distance[218, 223] and length of nerve measurements,[292]and temperature control:[197]

NCS included evaluation of motor (peroneal, tibial, median, and ulnar) and sensory (sural, median, and ulnar) nerves. All measurements were performed in a warm room with ambient temperature between 21° and 23°C. [228] After warming of the fore-arm and lower leg in hot water (about 38°C[193]) for 10 to 15 min,[193] skin temperature was recorded,[292] and during examination, was maintained at ≥ 30.5°C. Measurements of latencies and nerve action potential[216] amplitudes were performed in a standard fashion: Initial positive (if present)-to negative peak measurements were conducted for sensory responses;[197] for the determination of motor nerve conduction velocity, onset latencies (in ms)[197] and peak-peak amplitudes (in mV) were used.[193] Conduction velocities, calculated for motor and sensory nerves,[197] were expressed in m/s.[192] Mayo Clinic Electromyography and Clinical Neurophysiology Laboratory reference values were used. [296]

In patients with mild or moderate symptoms[174, 191] or signs, the more involved extremity (of the arm, or the leg) was assessed firstly, followed by the less involved limb; while in patients with severe symptoms or signs, assessment was initiated on the less involved limb.

In the leg, if the arm had been tested first, at least one motor conduction and one sensory conduction had to be performed. Otherwise, testing was conducted according to the following protocol:

Motor fibres of the peroneal nerve, innervating the extensor digitorum brevis muscle (MEDB), were tested by shocking at the knee and ankle (i.e. at the head of the fibula, and midway between the malleoli on the anterior surface of the limb[292]), and by recording action potentials[292] at the muscle (MEDB). Posterior tibial nerve motor fibres, innervating the abductor hallucis muscle, were stimulated at the knee and ankle, and recorded at the muscle. In the absence of a response, peroneal nerve motor fibres were tested more proximally, by recording at the anterior tibial muscle, after stimulating at the fibula and knee. F-waves were generated for all motor nerves, and minimal, reproducible latencies were measured.[197]

Sensory fibres of the sural nerve, innervating the ankle, were stimulated proximal to the ankle, lateral of the Achilles tendon,[193] and recorded antidromically[192]at the ankle. If no response could be elicited, sensory fibres of the superficial peroneal nerve, innervating the dorsum of the foot, were stimulated and recorded at the ankle. In patients aged less than 55, and if clinically indicated, sensory fibres of the medial plantar branch of the tibial nerve, innervating the sole of the foot, were tested by stimulating at the sole and recording at the ankle.

In the event of abnormality of any of the above, or if upper extremities were symptomatic, the arm was assessed next, according to the following protocol:

Sensory fibres of the median nerve were assessed by shocking at the wrist and elbow and by recording at the 2ndor index- finger, using ring electrodes[193] placed around the index finger. In the absence of antidromic conduction, or if Carpal Tunnel Syndrome was present, median nerve sensory fibres were tested by shocking at the palm and recording at the wrist, proximal to the palm. If responses in distal sensory nerves were absent, more proximal nerve testing — for instance, of the ante-brachial cutaneous nerve — was performed.

If symptoms were appropriate, or if median nerve examination proved abnormal, sensory fibres of the ulnar nerve were tested by stimulating at the wrist and elbow, and recording at the 5th finger.

[In the presence of compression syndromes affecting the median or ulnar nerves (e.g. Carpal Tunnel Syndrome), radial sensory nerve fibres (which innervate the dorsum of the hand), were tested by stimulating at the snuffbox (i.e. over the abductor pollicis longus muscle tendon), and recording over the dorsal aspect of the forearm.]

Motor fibres of the ulnar nerve, innervating the hypothenar muscle, were assessed by delivering electrical stimuli at the elbow and wrist, while recording over the hypothenar muscle. Only in the event of a significant drop in amplitude, was four point ulnar testing performed.

Additional motor nerves (e.g. the median nerve, innervating the thenar muscle[193]) were examined, only if findings were equivocal. Again F-waves were generated for all motor nerves, and minimal, reproducible latencies were measured.[197]

If, having followed the above testing sequence, no responses could be elicited, or if poly-radiculopathy was suspected, more proximal nerve conduction studies were performed (e.g. of the musculo-cutaneous nerve, innervating and recorded at the biceps muscle), or sensory evoked potential testing of the limbs (arm and/or leg) was conducted, or the blink reflex was assessed.

In agreement with Diabetes Control and Complications Trial Neurologic End Point Definitions,[175] and based on Mayo Clinic staging criteria,[215] and Mayo Clinic reference values,[296] nerve conduction was considered abnormal in the event of abnormality of at least one conduction attribute (amplitude, distal latency, or conduction velocity) — not due to a disease other than diabetes mellitus, faulty electrode placement, nerve cross-over, or low nerve temperature[215] — in each of at least two anatomically distinct peripheral nerves.[191] (For this purpose, median motor and median sensory were considered as separate nerves.[215])

2.7.5.3.2 Needle electromyography

Invasive muscle fibre examination was performed at rest, by means of needle electrodes, in the tibialis anterior muscle, the medial gastrocnemius, the lumbar para-spinal, and the first dorsal interosseus muscles, in all patients. If findings in abovementioned muscles were normal, the evaluation was extended to include examination of the muscles of the foot, more distally. If however, findings were abnormal, the opposite extremity as well as the proximal muscles of the leg (and arm), was examined.

Motor evoked potentials were assessed by directly stimulating muscle fibres in the distal part of the muscle by a short monopolar needle electrode (cathode), using a surface electrode as anode. The resulting muscle fibre action potentials were detected at a known distance by a small concentric needle electrode. With this technique, action potentials supposed to represent individual muscle fibres, were identified. The presence of fibrillation or fassiculation on insertion of the needle into the muscle during rest, and the presence of either reduced activation during recruitment, polyphasia, prolonged duration, or increased amplitude of action potentials were considered abnormal.[193] A muscle was considered abnormal in case of either abnormal insertional activity (fibrillation or fassiculation), or the presence of abnormality (based on Mayo

Clinic normative values),[296] of at least 2 of the motor evoked potential attributes (duration, amplitude or polyphasia) and recruitment activation.

2.8 PERSONNEL

Apart from electro-diagnostic studies — nerve conduction, needle examination and cutaneous autonomic response testing — which, as mentioned above, were performed by two experienced Neurologists, all tasks related to the conducting and completion of this project, were the responsibility of a single investigator (the primary researcher, MCD-B, clinical research assistant in the Division of Clinical Epidemiology, University of Pretoria, Pretoria Academic Hospital). These included: finalization of the study protocol, design and duplication of clinical record forms, patient screening and recruitment, obtaining of informed consent, data collection, extraction and recording, statistical analyses and report; also, obtaining from patients and delivery to the laboratory of urine and blood specimens, performing the general clinical examination, clinical neurological evaluations (monofilament and vibration sense testing and R-R variation), as well as vascular evaluations (PWV and TBP).

2.9 DATA HANDLING

2.9.1 Data Collection

Data were collected on standardized record forms. (Refer Appendices)

2.9.2 Statistical Analyses

Data analyses were performed using STATA software (version 8.2: StataCorp LP, College Station, Texas).

Due to the small sample size, and, subsequently, skew distribution of data, groups were compared using appropriate nonparametric tests, namely the Fischer's Exact Test for comparison of categorical variables, and the Kruskall-Wallis Test for analysis of continuous numerical variables.

A p- value of 0.05 or less was considered statistically significant.

Where, for continuous numerical variables, significant differences between groups were found, p-values were adjusted for post-hoc comparisons.

Data are presented as medians with inter-quartile ranges and minimum and maximum values.

Based on the results obtained from this pilot study (for TBI, TBP, PWV, vibration sense absence and monofilament perception absence), and in order to plan for future studies, the Day & Graham Difference Parameter and Nomogram[297] and the nQuery Advisor program (Version 4.0, Statistical Solutions, Cork Ireland) — as well as simulation and data-transformation techniques, were employed for calculation and comparison of possible future study sample sizes. (Both the Day & Graham Difference Parameter and Nomogram[297] and nQuery ANOVA are applicable in the case of the comparison of more than two groups with normally distributed data. Simulation and data-transformation techniques are applicable in the event of non-parametric continuous data — where, as in our study, the assumptions of normality of data and equality of variances cannot be met.):

With regard to categorical variables, sample sizes for three-group comparisons were determined based on the proportions observed in the three groups in our study, using nQuery.

The continuous data in our study (TBI, TBP and PWV) — after evaluating the effect of log-transformation on the distribution — were, firstly, log-transformed. The log-transformed means and standard deviations were then used for future study sample size calculations, using the nomogram and nQuery Advisor.

Secondly, realizing that in the event of large samples, data-transformation would not be necessary, and also, that log-transformed results are difficult to interpret clinically, the medians

and inter-quartile ranges of our study were used as estimates of non-transformed means and standard deviations for determination of future study sample sizes.

These calculations (repeated thrice for the three different inter-quartile ranges yielded by the three groups in our study) were performed for the comparison between three groups with equal sample sizes and equal variances, but with three unequal means, at the 5% significance level, with 90% power.

Next, calculations were repeated based on either the means and the standard deviations (SD's), or the proportions reported in some published studies that, with regard to study groups and outcomes (TBI, TBP, PWV, vibration sense absence or monofilament perception absence), were deemed comparable to our study.

Finally, we evaluated the effect on the p-value of using unequal standard deviations (obtained from the three groups in our study), by applying simulation, using 'simanova' in STATA — a technique for evaluation of the robustness of standard ANOVA in calculating sample sizes based on skew data with unequal variances, as found in our study. Five thousand repetitions were used, and p-values and 95% confidence intervals yielded, for comparison.

RESULTS

3.1 CLASSIFICATION OF PATIENTS

Of the 10 patients in the ulcer group, 9 had non-healing ulcers at the time of inclusion, while the remaining patient had a history of a chronic lower limb ulcer that had healed less than a month before. Reported ulcer duration ranged from 1 month to 5 years. One patient in the ulcer group, had also undergone an amputation 17 years prior to the study, but that had been traumatic (through the left hip, due to a motor vehicle accident injury), and had not been ascribed to peripheral arterial disease or neuropathy.

Three of the ten patients in the amputation group also had non-healing foot ulcers at the time of inclusion. Two patients in the amputation group had undergone amputations bilaterally. In both patients, one of the resections had, however, been sub-total (namely mid-foot or MP — metatarso-phalangeal — level amputations), while the amputations in the contra-lateral leg had been either below-knee or upper leg resections. Another 2 patients in this group had had unilateral mid-foot amputations. The remaining 6 patients in this group had all undergone unilateral below knee amputations. In none of the 10 patients classified in the amputation group, could resections have been ascribed to acute trauma.

3.2 SUBJECT CHARACTERISTICS

Relevant study group characteristics, including gender, race, age, DM and hypertension type, presence and duration, risk factor history and therapy, as well as metabolic variables are depicted in Table 1.

Of the 30 patients 21 were male: 6, 7, and 8 respectively, in the control, lower extremity ulcer, and amputation groups. Distribution of the 9 females among the three study groups was inversed: 4, 3, and 2 in the control, ulcer and amputation groups respectively (difference not statistically significant).

In all of the three study groups, at least three-fifths of the subjects were White, with the remaining Black, Coloured and Asian patients distributed approximately evenly among the groups. Subject age was about the same in the control and ulcer groups, but patients in these groups were plus-minus 7 years younger than those in the amputation group. However, this difference was not statistically significant.

WHR, with slight increments, was the lowest among controls, higher in the ulcer group and highest in patients with amputations. BMI was the highest for controls, and the lowest for amputations. These differences were not statistically significant.

In both the control and amputation groups, 6 of the 10 patients belonged to the DM Type 2 category, while the ulcer group had 8 Type 2 diabetics. Median diabetes duration was the longest in the ulcer group (4 years longer than in the amputation group), with median duration in the amputation group, in turn, 1 year longer than that in the control group (thus longer in the 2 groups with lower extremity complications, than in the group without/ the control group). Differences were not statistically significant. The amputation, ulcer and control groups, respectively, had 7, 8 and 9 patients with a history of hypertension. Subjects in the control group had a longer median known duration of hypertension (by 4.5 years) than those in the ulcer group (whose hypertension duration, in turn, was 1 year longer than that of subjects in the amputation group). However, in the ulcer group, duration of hypertension was not known in two patients with a history of hypertension, and in the amputation group, in one patient. Again, the differences between groups were statistically insignificant.

Despite the fact that the history of hypertension gradually increased across groups from group 1 (controls), through group 2 (ulcers), to 3 (amputations), use of ACE-inhibitor therapy at the time of inclusion, was equally common in the three groups— occurring in 7/10 subjects. Five out of ten control and amputation group patients, and 3/10 patients with ulcers, reported a history of hyperlipidemia. Likewise, in both the control and amputation groups, 3/5 patients, with reported hyperlipidemia, were on lipid-lowering agents (Statins/ Fibrates) at the time, while among patients with ulcers, the proportion was only 3/10. Similarly, but this time with a statistically significant difference between groups, the proportion of current smokers in the ulcer group (7/10), by far exceeded that in both the control (2/10) and the amputation groups; the amputation group containing the smallest proportion (1/10) of current smokers (p=0.023). Half of the subjects in the amputation group were, however, ex-smokers, in comparison to the 3/10 subjects in the control and only 1/10 in the ulcer group (difference not significant).

Once-a-day aspirin use was more common among subjects in the amputation and ulcer groups — reported by 4/10 and 3/10, respectively, but by only 1 patient in the control group (difference not significant). As elicited through specific enquiry during administering of the questionnaire (and as could be expected, considering the groups under study), all patients in the amputation and ulcer groups, with a significant p-value of 0.000, had a positive peripheral vascular disease history, and none in the control group. Likewise, no controls had a history of IHD or stroke, while 4 ulcer patients and 3 amputees (patients belonging to the amputation group) affirmed an IHD history, and 1 ulcer patient and 2 amputees a history of stroke.

Previous laser therapy of the eyes was reported much more frequently in subjects belonging to the control and amputation groups (i.e. occurring in 4/10 in both groups), than in ulcer patients (reported in only 1 patient In all groups, at least 60% of patients had a previous history of macro-albuminuria: The prevalence was the highest in the amputation group (9/10), followed by the control group (8/10) and the ulcer group (6/10). (Differences were not significant.)

Regarding metabolic investigation variables: In the 2 groups with lower extremity complications (particularly and markedly in the amputation group), the overnight urinary albumin excretion (UAE, Fig.1.1) was higher than in the control group. Despite large variability (probably due to normal biological variability) in the results of the 2 groups with complicated lower limbs — who

had inter-quartile ranges more than twice the median value for the amputees, and more than 5 times the median value for the ulcer group — the difference between groups was significant (p=0.003), and persisted with adjustment of the level of significance to 0.0083, when it was demonstrated to exist (with a p-value of 0.0004) between specifically groups 1 and 3 (i.e. the control and amputation groups). Micro-albuminuria also, occurred in the 2 groups with lower extremity complications — in 3/10 patients, in both groups — but not at all in control subjects, while macro-albuminuria was detected only among patients in the amputation group (in 4/10, with p=0.023).

Fig.1.1

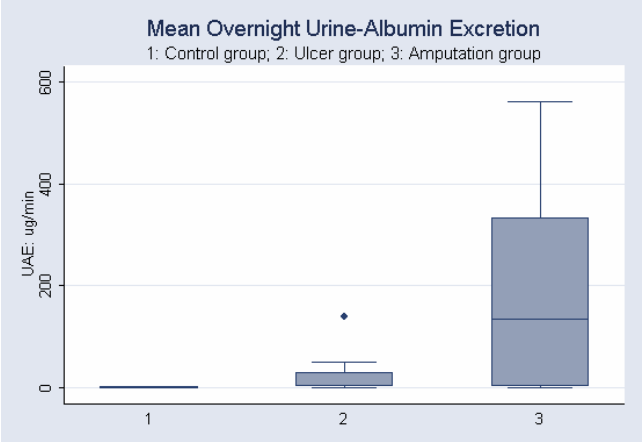
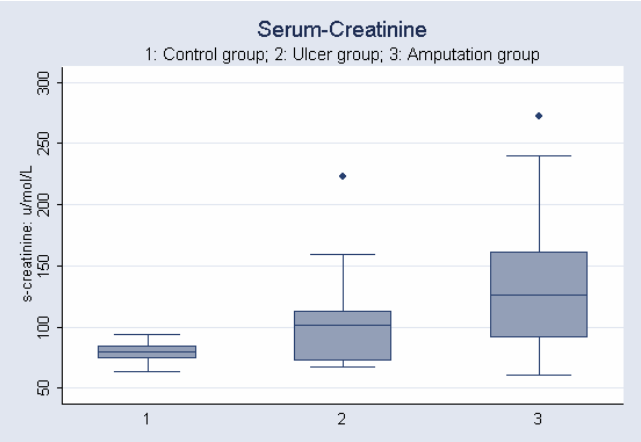


Fig.1.2



Serum-Creatinine levels (Fig.1.2), similarly, increased from Group 1 (controls), through Group 2 (patients with ulcers) to Group 3 (amputees). Again, the significant difference between the 3 groups (p=0.011) persisted when the level of significance was adjusted to examine individual differences between groups — the difference was demonstrated to specifically exist between groups 1 and 3 (p-value 0.0013). Serum-Creatinine was elevated in 2/10 subjects with ulcers and in 6/10 amputees, but no elevation of levels was found in the control group (p=0.011).

HbA1c-levels gradually, but slightly, increased with increasing severity of lower limb complications: from the controls, through the ulcer group, to the group with amputations. Elevated HbA1c-levels, similarly, were most frequent in the amputation group (occurring in 10/10 patients), and least frequent among controls (recorded in 7/10). These differences between groups were, however, not significant.

TABLE 1: DESCRIPTIVE DATA [Characteristics of the study group]

VARIABLE: *(N=30)*	SAMPLE: **Median** (IQR [xv])	CONTROL GROUP: *No. of patients* **Median** (IQR) (Range)	ULCER GROUP: *No. of patients* **Median** (IQR) (Range)	AMPUTATION GROUP: *No. of patients* **Median** (IQR) (Range)	P-VALUE:	CORRECTED P-VALUE:
Gender: Male Female		*6* *4*	*7* *3*	*8* *2*	0.879	-
Race: Asian Black Coloured White		*0* *1* *1* *8*	*2* *1* *1* *6*	*0* *1* *0* *9*	0.645	-
Age (years)	**57.81** (16.48)	*10/10* **55.29** (20.04) (40.74-68.74)	*10/10* **54.72** (9.64) (42.21-75.93)	*10/10* **62.46** (13.60) (39.44-84.91)	0.520	-
BMI [i] (kg/m^2)	**29.44** (10.60)	*10/10* **30.38** (10.49) (23.38-40.29)	*10/10* **30.03** (9.94) (20.08-38.67)	*10/10* **25.06** (12.72) (20.28-36.71)	0.619	-
WHR [ii]	**0.98** (0.16)	*10/10* **0.91** (0.15) (0.77-1.07)	*10/10* **0.99** (0.14) (0.89-1.14)	*10/10* **1.00** (0.22) (0.81-1.18)	0.224	-
DM type 1 2		*4* *6*	*2* *8*	*4* *6*	0.698	-
DM [iii] **duration** (years)	**12** (11.00)	*10/10* **11** (16) (5-33)	*10/10* **16** (10) (0-30)	*10/10* **12** (11) (3-30)	0.915	-
Hypertension		*9/10*	*8/10*	*7/10*	0.847	-
HT [iv] **duration** (years) *(N=21)*	**11.00** (13.00)	*9/9* **15.00** (19.00) (1-47)	*6/8* **10.50** (6.00) (4-16)	*6/7* **9.50** (15.00) (7-26)	0.946	-
ACE [v]**-inhibitor therapy**		*7/10*	*7/10*	*7/10*	1.000	-
Hyperlipidemia history		*5/10*	*3/10*	*5/10*	0.722	-
Statin / Fibrate therapy		*3/5*	*1/3*	*3/5*	0.864	-
Current smokers		*2/10*	*7/10*	*1/10*	**0.023**	-
Ex-smokers		*3/10*	*1/10*	*5/10*	0.061	-
Disprin		*1/10*	*3/10*	*4/10*	0.450	-
PVD [vi] **history**		*0/10*	*10/10*	*10/10*	**0.000**	-
IHD [vii] **history**		*0/10*	*4/10*	*3/10*	0.151	-
Stroke history		*0/10*	*1/10*	*2/10*	0.754	-
Laser therapy eyes		*4/10*	*1/10*	*4/10*	0.297	-
Macro-albuminuria history		*8/10*	*6/10*	*9/10*	0.430	-
u-Mean Albumin /min[viii] (μg/min)	**3.50** (48.10)	*10/10* **1.58** (0.45) (0.75-1.85)	*10/10* **5.50** (26.60) (0.60-140.40)	*10/10* **134.68**(330.25) (0.50-561.75)	**0.003**	Adjusted P=0.0083: H_0 Gr1=Gr2: 0.0198 **H_0Gr1=Gr3: 0.0004** H_0 Gr2=Gr3: 0.0976
Micro-albuminuria *		*0/10*	*3/10*	*3/10*	0.195	-
Macro-albuminuria †		*0/10*	*0/10*	*4/10*	**0.023**	-
s-Creatinine [ix] (μmol/L)	**90.50** (41.00)	*10/10* **79.50** (10.00) (64.00-94.00)	*10/10* **101.50** (41.00) (67.00-223.00)	*10/10* **126.00** (70.00) (61.00-272.00)	**0.011**	Adjusted P=0.0083: H_0 Gr1=Gr2: 0.0421 **H_0Gr1=Gr3: 0.0013** H_0 Gr2=Gr3: 0.1020
s-Creatinine elevated ‡		*0/10*	*2/10*	*6/10*	**0.011**	-
HbA1c [x] (%)	**7.45** (2.70)	*10/10* **7.20** (3.90) (3.10-11.40)	*10/10* **7.85** (2.20) (5.20-11.20)	*10/10* **8.30** (3.40) (6.30-11.10)	0.838	-
HbA1c elevated §		*7/10*	*8/10*	*10/10*	0.321	-
s-LDL [xi] (mmol/L) *(N=29)*	**3.18** (1.21)	*10/10* **3.31** (0.31) (2.76-4.58)	*9/9* **2.73** (1.21) (2.1-4.66)	*10/10* **3.01** (1.39) (1.03-5.72)	0.390	-
s-HDL [xii] (mmol/L)	**1.01** (0.44)	*10/10* **1.14** (0.48) (0.69-1.62)	*10/10* **0.84** (0.50) (0.67-1.97)	*10/10* **1.01** (0.41) (0.68-1.89)	0.416	-
s-total Cholesterol (mmol/L)	**4.91** (1.10)	*10/10* **4.93** (0.81) (4.42-6.49)	*10/10* **4.81** (2.10) (3.36-6.83)	*10/10* **4.76** (1.04) (2.71-8.15)	0.617	-
s-TotChol : HDLChol [xiii] (mmol/L)	**4.00** (3.00)	*10/10* **3.50** (3.00) (3.00-6.00)	*10/10* **5.00** (4.00) (2.00-8.00)	*10/10* **4.50** (1.00) (2.00-6.00)	0.622	-
s-TG [xiv] (mmol/L)	**1.29** (0.88)	*10/10* **1.40** (0.97) (0.44-1.93)	*10/10* **1.26** (0.54) (0.59-4.87)	*10/10* **1.48** (0.89) (0.81-2.93)	0.827	-

[i] : BMI = Body-mass index
[ii] : WHR = Waist-hip ratio
[iii] : DM = Diabetes mellitus
[iv] : HT = Hypertension
[v] : ACE = Angiotensin-converting enzyme
[vi] : PVD = Peripheral vascular disease
[vii] : IHD = Ischaemic heart disease
[viii] : u = Urine
[ix] : s = Serum
[x] : HbA1C = Hemoglobin A1c
[xi] : LDL = Low density lipoprotein
[xii] : HDL = High density lipoprotein
[xiii] : Total cholesterol to high density lipoprotein cholesterol -ratio
[xiv] : s-TG = Triglyceride
[xv] : IQR = Inter-quartile range
* : Urine albumin excretion (UAE) 20 –199 μg/min
† : UAE ≥ 200 μg/min
‡ : > 115 μmol/L
§ : > 6.2 %

With regard to serum-lipid studies: The control group had the highest s-LDL (serum-low density lipoprotein) and s-Cholesterol levels, but also the best (highest) s-HDL concentration, while in the amputation group, the highest serum-Triglyceride levels were found. However, the ulcer group (i.e. the group in which lipid-lowering agent use was the least common) demonstrated the highest Total-Cholesterol-to-HDL-Cholesterol ratio, namely 5. (Due to a s-Triglyceride concentration exceeding 4.5 mmol/L, with subsequent unreliability of s-LDL evaluation, this (the s-LDL level) was not determined in one patient in the ulcer group.) No statistically significant differences between the 3 groups were found for any of the s-lipid analyses.

3.3 PERIPHERAL CIRCULATION

Results of vascular parameters and evaluations are presented in Table 2.

There was no significant difference in the pulse rate of the 3 groups, although it was slightly higher in the ulcer group, than in the other 2 groups. Systolic blood pressure was higher in the amputation group, than in the control and ulcer groups, while diastolic blood pressure was highest in the control group. However, pulse pressure was clearly (though not significantly) higher in the amputation, than in either of the other 2 groups.

The expected upward trend in pulse wave velocity results, from the control, through the ulcer, to the amputation group, did not occur. Rather, for carotid-radial (i.e. CR, or peripheral) PWV values decreased across groups with the highest median value reported in the control group (8.87 m/s), followed by 8.60 in the ulcer group, and 7.77 for the amputees. Inter-quartile ranges (in Fig. 2.1, represented by the boxes, and depicting the middle 50% of data) and ranges for the control and ulcer groups were similar and wider than those of the amputation group. In each of the ulcer and amputation groups, there was one outlier. For carotid-femoral (i.e. CF, or central) PWV, the highest values were reported in the ulcer group (median 9.84 m/s), followed by a value 1.13 lower in the control group, and the lowest value in the amputation group — 0.69 m/s less than that of the control group.

The control and amputation groups each had one outlier (values outside of the 'whiskers', i.e. not included in the 1.5 times the IQR from the 25th and the 75th percentiles respectively.) Differences were not statistically significant.

Fig. 2.1

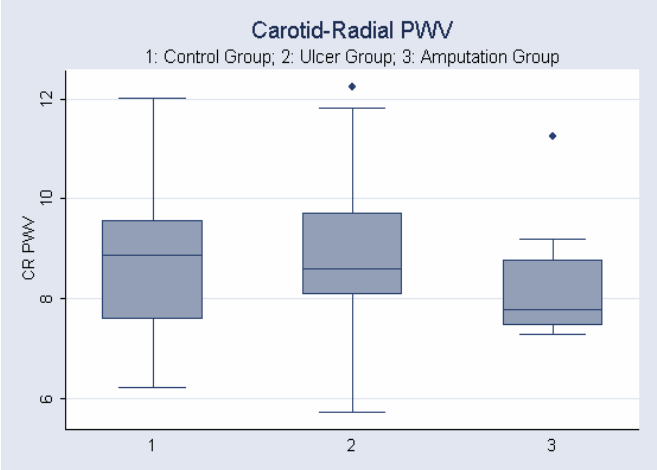
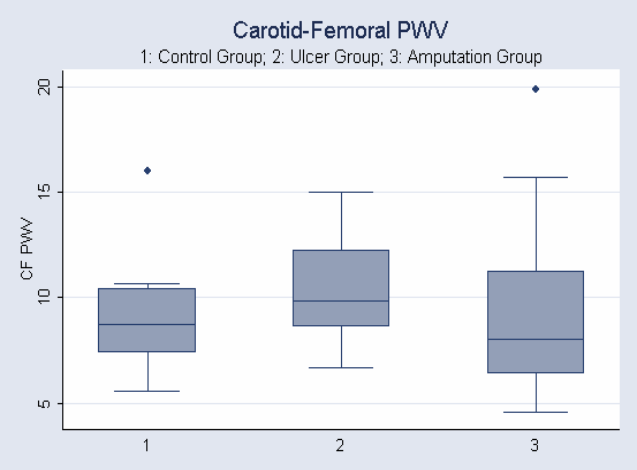


Fig. 2.2



As can be understood, due to limb amputations, palpation of foot pulses in both feet was not possible in most of the patients in the amputation group. In 2 patients in this group, however, amputations in one foot had been sub-total (distal to both the posterior tibial and dorsalis pedis arteries), thereby enabling evaluation of these pulses in both feet. In one patient included in the ulcer group, bilateral evaluation was, likewise, not possible, due to a prior unilateral traumatic — therefore not considered as fulfilling the criteria of this study for a complicated limb — amputation. In none of the patients in whom both feet could actually be evaluated, were both pulses in one foot found to be absent. (Thus the answer was 'no'— coded as '2'— for all evaluable patients with regard to this variable, resulting in, apart from 'missing data' for amputated feet, only one category for analysis. No p-value could therefore be generated for the absence of both pulses in any of a patient's feet.)

Femoral bruits were present in only one control patient, while the ulcer and amputation groups had four patients each with femoral bruits (difference not significant). One patient in the ulcer group could not be evaluated, due to prior unilateral traumatic amputation.

Since the calculation of toe blood pressure was based on obtaining the mean of 2 big toe-values per patient, in patients with unilateral amputations, two values were obtained in the contra-lateral available limb. Therefore, apart from the 2 patients in the amputation group with

bilateral amputations, mentioned earlier, it remained possible to evaluate all patients in each group. As expected, toe blood pressure (therefore assessed in only 8 of the amputees), was markedly lower in the amputation group (median 54.50 mmHg), than in the 2 other groups (Fig.2.3). However, in the control group, which was expected to yield the highest values, surprisingly, toe pressures were lower (median 74.50) than in the ulcer group, in which the highest values were reported (group median 82.35 mmHg). This difference between groups was not significant. In agreement with the above findings, mean toe blood pressures equal to or below 80 mmHg, occurred in 6/10 subjects in the control and in 6/8 patients in the amputation group (proportion 0.75) but in only 5/10 patients with ulcers. Mean toe blood pressures equal to or below 30 mmHg, were reported only in 2 of the 8 evaluable amputees, and not at all in the control and ulcer groups. Again, these differences were not statistically significant.

Fig. 2.3

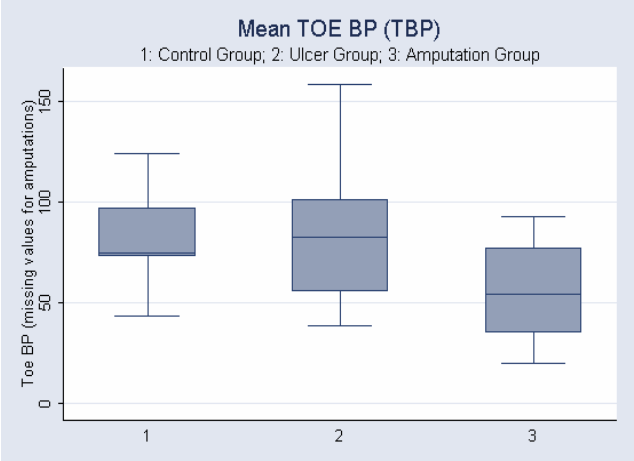
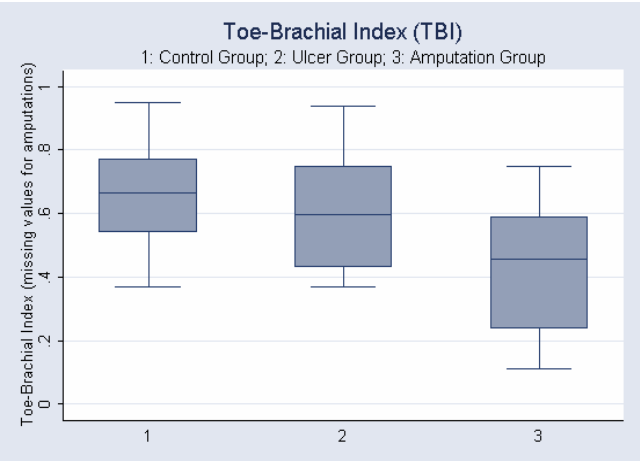


Fig. 2.4



Toe-brachial index (TBI) was generally low and, as expected, decreased across groups, starting from the control group, with the highest index of 0.67, through the ulcer group, with an index of 0.60, to the amputation group, with the lowest index of 0.46 (Fig.2.4). Similarly, a toe-brachial index equal to or below 0.75 (indicating the presence of peripheral vascular impairment) was reported in 7/10 controls, 8/10 subjects with lower extremity ulcers, and 8/8 evaluable amputees. The same trend persisted for a toe-brachial index equal to or below 0.60, (which occurred in 4/10 controls, 6/10 subjects with ulcers, and 6/8 (proportion 0.75) amputees, and indicated more pronounced peripheral arterial disease). Only in the amputation group, (in 3/8 patients, and with a significant p-value of 0.017), was a toe-brachial index equal to or below 0.33 — representing the lower third of brachial blood pressure, and therefore critical limb ischaemia — reported.

TABLE 2: VASCULAR PARAMETERS

VARIABLE: *(N=30)*	**SAMPLE:** **Median** (IQR [viii])	**CONTROL GROUP:** *No. of patients* **Median** (IQR) (Range)	**ULCER GROUP:** *No. of patients* **Median** (IQR) (Range)	**AMPUTATION GROUP:** *No. of patients* **Median** (IQR) (Range)	**P-VALUE:**
Pulse rate (bpm)	**78.00**(8.00)	***10/10*** **77.00** (6.00) (60.00-89.00)	***10/10*** **80.00**(13.00) (70.00-98.00)	***10/10*** **77.50**(10.00) (64.00-92.00)	0.278
Systolic BP [i](mmHg)	**127.00**(30.00)	***10/10*** **126.00**(40.00) (100.00-161.00)	***10/10*** **126.00**(22.00) (100.00-155.00)	***10/10*** **133.50**(54.00) (119.00-200.00)	0.238
Diastolic BP (mmHg)	**78.50**(15.00)	***10/10*** **78.50**(14.00) (50.00-95.00)	***10/10*** **77.50**(16.00) (57.00-105.00)	***10/10*** **77.50**(20.00) (55.00-96.00)	0.897
Pulse pressure (mmHg)	**51.50**(17.00)	***10/10*** **50.50**(14.00) (37.00-64.00)	***10/10*** **46.50**(15.00) (11.00-72.00)	***10/10*** **56.50**(28.00) (39.00-142.00)	0.183
Carotid-femoral PWV [ii] (m/s)	**8.91**(3.85)	***10/10*** **8.71** (3.06) (5.60-16.01)	***10/10*** **9.84** (3.67) (6.66-15.02)	***10/10*** **8.02** (4.83) (4.56-19.84)	0.406
Carotid-radial PWV (m/s)	**8.38**(1.87)	***10/10*** **8.87** (1.97) (6.22-12.02)	***10/10*** **8.60** (1.64) (5.71-12.25)	***10/10*** **7.77** (1.30) (7.27-11.24)	0.418
Foot pulses: Both absent in any foot (R[iii] and/or L[iv]) *(N=21)*		***0/10***	***0/9***	***0/2***	-
Femoral bruit: Any present *(N=29)*		***1/10***	***4/9***	***4/10***	0.228
Mean toe BP (mmHg) *(N=28)*	**73.65**(38.45)	***10/10*** **74.50**(24.00) (43.70-124.30)	***10/10*** **82.35**(46.00) (38.50-158.50)	***8/8*** **54.50**(42.35) (20.00-92.50)	0.101
Mean toe BP ≤ 80 mmHg (=>PAD [v]) *(N=28)*		***6/10***	***5/10***	***6/8***	0.563
Mean toe BP ≤ 30 mmHg (=>Critical Ischaemia) *(N=28)*		***0/10***	***0/10***	***2/8***	0.074
Toe: brachial index *(N=28)*	**0.58** (0.33)	***10/10*** **0.67** (0.23) (0.37-0.95)	***10/10*** **0.60** (0.32) (0.37-0.94)	***8/8*** **0.46** (0.36) (0.11-0.75)	0.143
Toe: brachial index ≤ 0.75 (=>Peripheral Vascular Impairment) *(N=28)*		***7/10***	***8/10***	***8/8***	0.326
Toe- or ankle: brachial index ≤ 0.75 (for TBI [vi]) or ≤ 0.9 (for ABI [vii]) (=>Peripheral Vascular Impairment)		***7/10***	***8/10***	***10/10***	0.321
Toe: brachial index ≤ 0.60 (=>PAD) *(N=28)*		***4/10***	***6/10***	***6/8***	0.321
Toe- or ankle: brachial index ≤ 0.60		***4/10***	***6/10***	***8/10***	0.248
Toe: brachial index ≤ 0.33 (lower 1/3 of brachial BP) *(N=28)*		***0/10***	***0/10***	***3/8***	**0.017**
Toe- or ankle: brachial index ≤ 0.33 (lower 1/3 of brachial BP)		***0/10***	***0/10***	***5/10***	**0.005**

[i] : BP = Blood pressure [ii] : PWV = Pulse wave velocity [iii] : R = Right [iv] : L = Left [v] : PAD = Peripheral arterial disease
[vi] : TBI = Toe-brachial index [vii] : ABI = Ankle-brachial index [viii] : IQR = Inter-quartile range

In an attempt to obtain 3 complete groups of ten patients each for evaluation of a 'lower limb-brachial index', instead of a toe-brachial index, an ankle-brachial index was calculated in the two patients with bilateral foot amputations. These results were combined with those of the remaining 28 patients in whom a proper toe-brachial index could be obtained. In both these patients, the ankle brachial-index was equal to or below 0.33, thereby resulting in lower p-values overall — significant, however, only for the toe-or-ankle-brachial index ≤ 0.33-variable, with p=0.005.

3.4 NERVE FUNCTION

The results of the tuning fork tests of vibration sensation, as well as of monofilament, perception, heart rate variation and cutaneous autonomic response, nerve conduction, and needle examination appear in Table 3.

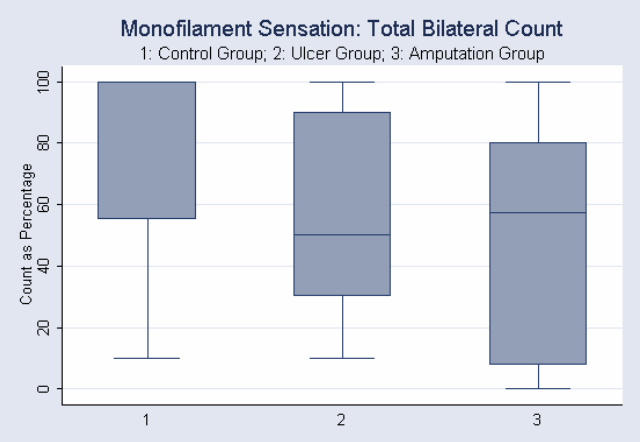
Excluding the cutaneous autonomic response, (and the ECG-derived heart rate variation, which, in one amputee was not performed, due to the presence of more than 5 supra-ventricular extra-systoles per minute), nerve function assessment was complete in 29 patients; 1 patient in the amputation group, however, died due to complications following a routine surgical procedure, prior to undergoing the electromyography (nerve conduction and needle examination) and cutaneous autonomic response evaluations.

Absence of vibration perception at the level of the toe in either of or both the limbs, was most common in the amputation group — it occurred in 7 out of 8 evaluable patients (i.e. the 8 subjects with at least one unaffected/ non-amputated big toe); followed by the ulcer group (occurring in 3/10). Among controls, vibration perception was normal in both limbs, both at the level of the toe, and at medial malleolus level. In the amputation group, it was possible to evaluate vibration sense at medial malleolus level in at least one limb in all patients. At this level, again, absence of vibration sense was most common among amputees (occurring in 5/10), followed by the group of patients with ulcers (in 1/10). Differences between groups at both levels (toe and medial malleolus), were statistically significant — toe level: p=0.000; medial malleolus level: p=0.027.

The total bilateral monofilament count (Fig.3.1) — a percentage, based on the number of remaining, available monofilament assessment sites per foot, post-amputation — was calculated in all patients. The highest percentage of ‘monofilament present’ sites was found in the control group (median100%), followed by — surprisingly — the amputation group, with positive monofilament testing in 57.5% of available sites. The ulcer group displayed the lowest percentage: monofilament sensation was present in only 50% of tested sites. This difference

between groups, again, was found to be statistically significant, with a p-value = 0.043, persisting with adjustment of the level of significance, which served to demonstrate the difference existing between, specifically, groups 1 and 3 (p=0.0079).

Fig.3.1



When left and right limbs were considered together, monofilament sensation was reported absent in at least 4 out of a possible 10 sites in at least one leg, in all of the 8 patients in the amputation group with at least 1 complete foot available for evaluation. This phenomenon (absence in a minimum of 4/10 sites in at least one leg) was less common among subjects with ulcers (reported in 6/10), and even less among controls, occurring in 3/10. The difference between the groups was significant, with p=0.010.

Based on the presence of 1 or more abnormal conduction attributes in at least 2 distinct nerves, peripheral neuropathy, as expected, was present in 9/9 assessed amputees, 9/10 patients with ulcers, and in 8/10 controls.

When based on abnormal needle examination in at least two muscles, unexpectedly, peripheral neuropathy was present not only in 9/9 assessed amputees, but also in 10/10 controls, and in only 8/10 patients in the ulcer group.

When the abovementioned two categories were combined to assess the presence of peripheral neuropathy on conduction, as well as on needle examination results, interestingly, the condition was found present — with the exception of one ulcer patient — in all patients in all groups, including all controls. No statistically significant differences were, therefore, detected.

TABLE 3: NEUROLOGICAL PARAMETERS

VARIABLE: *(N=30)*	CONTROL GROUP: *No. of patients* **Median** (IQR [v]) (Range)	ULCER GROUP: *No. of patients* **Median** (IQR) (Range)	AMPUTATION GROUP: *No. of patients* **Median** (IQR) (Range)	P-VALUE:	CORRECTED P-VALUE:
Vibration sense absent at toe level R [i] +/ L [ii] *(N=28)*	***0****/10*	***3****/10*	***7****/8*	**0.000**	-
Vibration sense absent at medial malleolus level R +/ L	***0****/10*	***1****/10*	***5****/10*	**0.027**	-
Total* bilateral monofilament count *(Percentage)	***10****/10* **100.00**(45.00) (10.00-100.00)	***10****/10* **50.00** (60.00) (10.00-100.00)	***10****/10* **57.50** (72.31) (0.00-100.00)	**0.043**	Adjusted P=0.0083: H_0 Gr1=Gr2: 0.0444 **H_0Gr1=Gr3: 0.0079** H_0 Gr2=Gr3: 0.2385
Monofilament sensation absent at least at 4/10 sites: *right foot* *(N=28)*	***3****/10*	***6****/10*	***6****/8*	0.202	-
Monofilament sensation absent at least at 4/10 sites: *left foot* *(N=23)*	***2****/10*	***5****/9*	***3****/4*	0.134	-
Monofilament sensation absent at least at 4/10 sites: *R and /or L* *(N=28)*	***3****/10*	***6****/10*	***8****/8*	**0.010**	-
Peripheral neuropathy: based on presence of 1 or more abnormal **conduction attribute/s** in at least 2 distinct nerves *(N=29)*	***8****/10*	***9****/10*	***9****/9*	0.754	-
Peripheral neuropathy: based on abnormal needle examination in at least two muscles *(N=29)*	***10****/10*	***8****/10*	***9****/9*	0.310	-
Peripheral neuropathy: based on *both* the presence of 1 or more **abnormal conduction attribute/s** in at least 2 distinct nerves, ***and*** on abnormality of at least 2 distinct muscles **on needle examination** *(N=29)*	***10****/10*	***9****/10*	***9****/9*	1.000	-
Cutaneous autonomic response: Any **absent** (in a hand or a foot) *(N=20)*	***5****/7*	***5****/6*	***6****/7*	0.509	-
Heart rate variation (bpm [iii]): Mean (maximum – minimum) heart rate difference *(N=29)*	***10****/10* **6.73** (5.25) (2.40-9.29)	***10****/10* **7.37** (8.24) (1.17-13.74)	***9****/9* **2.49** (4.64) (0.00-8.78)	0.138	-
Heart rate variation decreased: Mean (maximum – minimum) heart rate difference < 10 bpm *(N=29)*	***10****/10*	***7****/10*	***9****/9*	0.089	-
Heart rate variation normal: Mean (maximum – minimum) heart rate difference > 15 bpm *(N=29)*	***0****/10*	***0****/10*	***0****/9*	-	-
E: I-ratio [iv] *(N=29)*	***10****/10* **1.10** (0.11) (1.03-1.16)	***10****/10* **1.09** (0.13) (1.02-1.22)	***9****/9* **1.03** (0.06) (1.00-1.15)	0.124	-
E: I-ratio decreased: < 1.10 *(N=29)*	***5****/10*	***5****/10*	***8****/9*	0.150	-

[i]: R = Right [ii]: L = Left [iii]: bpm = beats/min [iv]: E: I-ratio = Expiration: Inspiration-ratio [v]: IQR = Inter-quartile range

Due to a protocol violation, cutaneous autonomic response assessment did not take place in all patients. In the patients tested, as expected, the response was recorded absent in either a hand or a foot, in 6/7 (proportion 0.857) patients in the amputation group, in slightly fewer ulcer patients (5/6, proportion 0.833), and in only 5/7 (0.714 of) controls. This difference between groups was not significant.

Assessment of ECG-derived heart-rate-variability-response to six deep breaths/min (or beat-to-beat 'heart rate variation'), expressed in terms of both the mean (maximum – minimum) heart rate difference, and the E:I-ratio, yielded the following results:

Fig.3.2

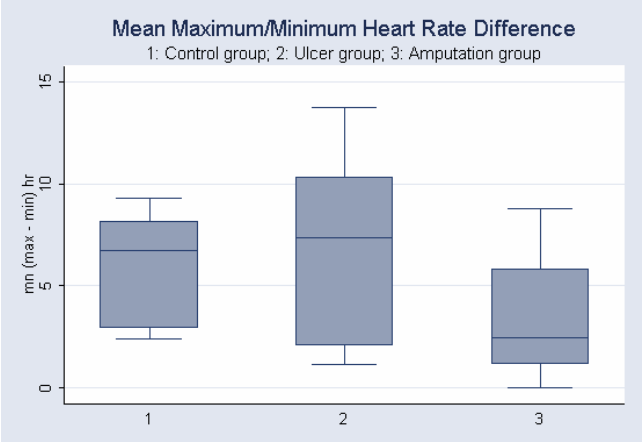
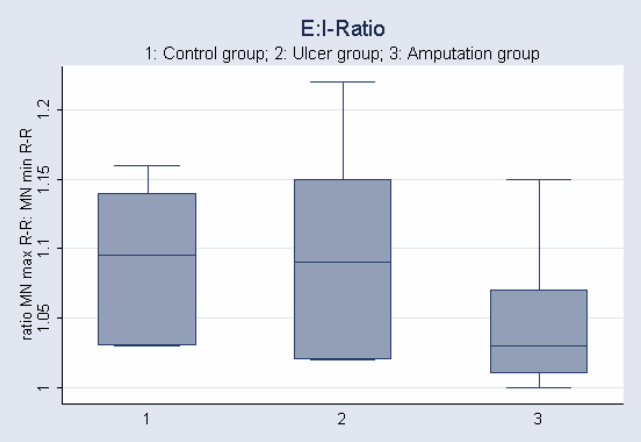


Fig.3.3



The mean maximum to minimum heart rate difference (Fig.3.2), not surprisingly, was clearly (though not significantly) lower in the amputation group, than in both the ulcer and control groups. However, it was in the ulcer group, that the slightly higher (by 0.64 beats per minute) and largest difference was demonstrated: median for group 7.37 bpm ('p' not significant). Similarly, results for decreased heart rate variation were worst for amputation and control groups and, unexpectedly, better for the subjects with ulcers: Decreased heart rate variation occurred in 9/9 assessed amputees and in 10/10 controls, while only in 7/10 patients with ulcers. No patient in any of the three study groups had normal heart rate variation (namely more than 15 bpm).

As, expected, the E:I-ratio (Fig.3.3) was highest among controls (with median value 1.10), followed closely by the ulcer group (1.09), and then the amputee group, who had the lowest ratio (1.03). Subsequently, the finding of decreased E:I-ratio was most common among subjects in the amputation group (occurring in 8/9). However, though less frequently, it was found to occur equally commonly in subjects belonging to the control and ulcer groups (among 5/10 in both groups). Differences between groups were not statistically significant.

3.5 FUTURE STUDY SAMPLE SIZE CALCULATIONS

Throughout, the results of sample-size calculations are based on three-group comparisons. For both continuous and categorical data, if two-group comparisons were to be made, much larger sample sizes would be required (data not shown).

3.5.1 Sample Size Based on Continuous Data

3.5.1.1 Based on the Log-transformed Means and Standard Deviations of Our Study (Table 4)

Using:-
3.5.1.1.1 TBP
3.5.1.1.2 TBI
3.5.1.1.3 PWV

To normalize data distribution log-transformation was performed on the continuous outcomes variables in our study (TBP, TBI and PWV). In every instance, log-transformation of data yielded chi-square-obtained p-values, for the three study groups respectively, which were wéll above 0.05 — indicating the appropriateness of applying log-transformation to normalize the distribution of these data.

TABLE 4: LOG TRANSFORMED VASCULAR PARAMETERS

VARIABLE:	**SAMPLE:** **Means** (95%CI):	**CONTROL GROUP:** ***No. of patients*** **Mean** (SD [iii])	**P-VALUE: ***	**ULCER GROUP:** ***No. of patients*** **Mean** (SD)	**P-VALUE:**	**AMPUTATION GROUP:** ***No. of patients*** **Mean** (SD)	**P-VALUE:**

Log transformed carotid-femoral PWV [i] (m/s) *(N=30)*	**2.224** (2.099 – 2.349)	***10/10*** **2.175** (0.284)	0.202	***10/10*** **2.314** (0.242)	0.999	***10/10*** **2.183** (0.457)	0.786
Log transformed mean toe BP [ii] (mmHg) *(N=28)*	**4.220** (4.047 – 4.394)	***10/10*** **4.352** (0.306)	0.722	***10/10*** **4.336** (0.412)	0.994	***8/8*** **3.910** (0.528)	0.715
Log transformed toe: brachial index *(N=28)*	**-0.654**(-0.840 – -0.469)	***10/10*** **-0.471**(0.306)	0.641	***10/10*** **-0.558** (0.330)	0.263	***8/8*** **-1.005** (0.646)	0.409

[i]: PWV = Pulse wave velocity [ii]: BP = Blood pressure [iii]: SD = Standard deviation *: P-value for log-transformation: acceptable if > 0.05

3.5.1.1.1 Using TBP

For a three-group comparison, using our log-transformed TBP means of 3.910, 4.336 and 4.352, equal study group sizes of 11, or 19, or 30 would be required, depending on the choice of SD (0.306/ 0.412/ 0.528).

3.5.1.1.2 Using TBI

For the comparison of three equal-sized groups, using our log-transformed means for TBI of –0.471, –0.558 and –1.005 (Table 4), 9, 10, or 34 subjects per group would be required, depending on the SD (0.306/ 0.330/ 0.646).

3.5.1.1.3 Using PWV

Making use of our log-transformed means of 2.175, 2.183 and 2.314 (Table 4) for PWV, required sizes for the comparison of three equal groups would be 63, 85, or 222, depending on the SD used (0.242/ 0.284/ 0.457).

3.5.1.2 Based on Means and Standard Deviations, Using the Medians and Inter-quartile Ranges of Our Study as Guide

Using:-
3.5.1.2.1 TBP
3.5.1.2.2 TBI
3.5.1.2.3 PWV

3.5.1.2.1 Using TBP

For a three-group comparison, using our TBP medians (Table 2) of 55, 75 and ≈80 mmHg as guides for mean values, equal study group sizes of 24, 59, or 92 would be required, depending on the inter-quartile-range-derived SD that was used (≈25/ ≈40/ ≈50).

3.5.1.2.2 Using TBI

For the comparison of three equal-sized groups, using means derived from our medians for TBI (Table 2) of ≈0.5, 0.6 and ≈0.7, the number of subjects required per group would be 27, 58, or 103, depending on the SD (derived from the inter-quartile ranges in our study, of ≈0.2, ≈0.3, or ≈0.4).

3.5.1.2.3 Using PWV

Using our study's medians for PWV (≈8, ≈9 and ≈10 m/s, in Table 2) as guides for mean-values, required sizes for the comparison of three equal groups would be 58, 103, or 160, depending on the SD used (≈3/ ≈4/ ≈5, based on our inter-quartile ranges).

3.5.1.3 Based on the Means and Standard Deviations Reported in Other Studies

Using:-
3.5.1.3.1 TBP
3.5.1.3.2 TBI
3.5.1.3.3 PWV

3.5.1.3.1 Using TBP

A study by Apelqvist and colleagues[52] reported means for TBP in three different diabetic study groups with a broad spectrum of foot ulcers, of 27.4, 72.2, and 82.5 — in our calculations rounded off to 30, 70 and 80, respectively — with standard deviations of 27.4 (≈ 30), 38.4 (≈ 40) and 46.9 (≈ 50). For a three-group comparison using these means, depending on the standard deviation, equal study group sizes of 10, 16, or 24 would be required.

3.5.1.3.2 Using TBI

A study by Sahli, et al,[149] reported means for TBI in three different study groups (healthy controls, Type 1 and Type 2 diabetics, but with no previous history of lower extremity arterial disease), of 0.89, 0.91 and 0.93, and standard deviations of 0.01, 0.01 and 0.02. Applying these means in calculations yielded required equal group sizes of 3, or 8 — depending on which of the two standard deviations was used.

3.5.1.3.3 Using PWV

Van Popele,[298] and colleagues, in the Rotterdam Study [79] (an epidemiological study that included 3 818 elderly participants), investigated the association between arterial stiffness and prevalent cardiovascular disease. Means reported for PWV in three different study groups (subjects without myocardial infarction or stroke, subjects with myocardial infarction, and subjects with stroke), were 13.4, 14.6 and 14.8 m/s (for the purposes of our calculations, rounded off to 13.0, 14.5 and 15 respectively), and standard deviations reported were 3.0, 3.1 and 3.3. Applying these data in calculations for three-group comparisons resulted in required equal group sizes of 54, 58,or 65, depending on the standard deviation.

3.5.1.4 Simulation Analyses to Investigate the Robustness of ANOVA in Abovementioned Calculations

Based on:-
3.5.1.4.1 TBP
3.5.1.4.2 TBI
3.5.1.4.3 PWV

3.5.1.4.1 Based on TBP

Using our log-transformed TBP data (means, SD's, Table 4) for a three-group comparison, when standard deviations were not equal, simulation p-values varied from 0.056 to 0.053, depending on the sample size (11/ 19/ 30) used.

Applying our TBP medians and inter-quartile ranges (Table 2) as guides for mean- and SD-values, simulation analyses using unequal SD's resulted in p-values ranging from 0.056, through 0.060, to 0.054, depending on the choice of sample size (24, 59, or 92).

3.5.1.4.2 Based on TBI

Making use of our log-transformed TBI data (Table 4) for a three-group comparison, when standard deviations were not equal, simulation p-values varied from 0.071 to 0.062, depending on the sample size (9/ 10/ 34) used.

Employing our TBI medians and inter-quartile ranges (Table 2) as guides for mean- and SD-values, simulation analyses using unequal SD's, resulted in p-values ranging from 0.052, through 0.056, to 0.054, depending on the choice of sample size (27, 58, or 103).

3.5.1.4.3 Based on PWV

Making use of our log-transformed PWV data for a three-group comparison, when standard deviations were not equal, simulation p-values varied from 0.052 through 0.053 to 0.056, depending on the sample size (63/ 85/ 222) used.

Applying our PWV medians and inter-quartile ranges as guides for mean- and SD-values, simulation analyses using unequal SD's resulted in p-values ranging from 0.052 to 0.054, depending on the choice of sample size (58, 103, or 160).

3.5.2 Sample Size based on Categorical Data

3.5.2.1 Based on Proportions Observed in Our Study

Using:-
3.5.2.1.1 Vibration Sense
3.5.2.1.2 Monofilament Perception

3.5.2.1.1 Using Vibration Sense

Based on differences such as found in our study, and depending on the level of vibration sense absence — i.e. at the toe, or medial malleolus — equal group sizes of 8 or 15 would be required for a comparison between three groups.

3.5.2.1.2 Using Monofilament Perception

Applying the differences observed between the three groups in our study (with proportions 0.3, 0.6 and 1 respectively), the sample size required would be 12 per group.

3.5.2.2 Based on Proportions Reported in Other Studies

Using:-
3.5.2.2.1 Vibration Sense
3.5.2.2.2 Monofilament Perception

3.5.2.2.1 Using Vibration Sense

Using the differences for absence of vibration sense at toe-level, as reported by Sahli and colleagues[149] in their study of three different study groups — healthy controls and Type 1 and Type 2 diabetics with no previous history of lower extremity arterial disease — the equal group size required (based on 5, 18≈20 and 25% absence, respectively) would be 82.

Applying their differences for absence of vibration sense at medial malleolus-level, the equal group size required (based on absence, respectively, in 0, 0, and 6% of patients), would be 111.

3.5.2.2.2 Monofilament Perception

Perkins, et al,[197] assessed monofilament sensation in reference subjects and in diabetics recruited from the community, from a diabetes clinic and from a diabetic neuropathy research clinic. They reported absence (in the 5 different broad-spectrum clinical strata their subjects had been divided into) in 16, 33, 58, 72 and 84% of patients, respectively.

For a three-group comparison using strata 2, 3 and 4 — proportions with absence 0.33, 0.58 and 0.72 respectively — the required sample size would be 41 per group. Applying the proportions of absence reported for strata 1, 3 and 5 (0.16, 0.58, 0.84), would require an equal group size of 14.

Chapter 4

DISCUSSION

As this was a small pilot study aimed at assessing differences — in terms of the results of various vascular and neurological evaluations — between 3 groups of diabetic subjects with and without differing degrees of lower extremity complications, the discussion is restricted to interpretation of the absolute differences observed.

Given the small sample size, no significant differences were expected.

Regarding vascular evaluations, we found in our study that, for PWV, the overall medians for the total sample of 3 groups, when compared to other studies,[109] were lower (even normal) for both carotid-radial PWV (8.38m/s, IQR 1.87), and carotid-femoral PWV (8.91m/s, IQR 3.85). In their longitudinal data from a cohort of diabetic patients followed up for 9 years, Lehmann ED, and colleagues,[299] had shown stiffer aortas at baseline, than what was found in our study, with aortic PWV's between 9.9 and 12.0m/s reported. [300] Rajkumar and associates,[301] among 41 Type 2 diabetes patients (21 Caucasian and 20 Afro-Caribbeans), reported CR-PWV values of 11.13 (± 0.28) and 12.10 (± 0.34) respectively, and CF-PWV values of 13.84 (± 0.28) and 13.97 (± 0.34) m/s respectively, for the 2 groups. In our study, for CF-PWV, instead of the anticipated highest values (indicating stiffer arteries), the lowest values were recorded for the amputation group (8.02, IQR 4.83). For CR-PWV, the fastest velocity was recorded among controls (8.87m/s, IQR 1.97), and the slowest in the amputation group (7.77m/s, IQR 1.30). This is contrary to the findings of, for instance, the study of Taniwaki, et al,[158] who evaluated carotid-femoral (aortic) PWV in type 2 diabetes patients and in controls. They demonstrated that aortic PWV was significantly higher in patients than in control subjects, in all age groups. Similarly, Suzuki and colleagues, [167] demonstrated an abnormally higher brachial-ankle PWV in the non-PAD diabetic group they had investigated, compared to that of their non-diabetic control group ($p < 0.001$). (PWV in the diabetic group was 16.83 (± 3.74) m/s, vs. the 12.74 (± 1.11) m/s demonstrated among non-diabetic subjects.) And, Yokoyama, et al, [168] likewise, found that

brachial-ankle PWV was increased in diabetic patients, but decreased in the affected legs of diabetic patients with PAD.

Concerning toe blood pressure (TBP), a notable observation — although in this pilot-study, without statistical significance — was that of the median (based on the mean of two pressures in each individual) having been the lowest in the amputation group (54.5 mmHg, p=0.101). This group — with a p-value not significant at 0.074 — also had the highest number of patients with mean pressures ≤ 30 mmHg (which implied critical ischaemia), and, proportionally, the highest number of patients with mean toe blood pressures ≤ 80 mmHg (indicating the presence of peripheral arterial disease).

Contrary to PWV, toe-brachial index, in our small study, although without statistical significance, behaved as expected, by not only being generally low, but also by decreasing across groups — from 0.67 among controls, through 0.6 for patients with ulcers, to 0.46 among amputees — following increasing severity of lower limb complications. Not surprisingly, TBI equal to or below 0.33 — regarded as indicative of critical limb ischaemia — was displayed only among amputees. (This difference between groups was significant, with p= 0.017.)

Another study that investigated TBI in diabetics — that by Rheeder and colleagues [41] — reported, in the 85 female type 2 diabetes patients they assessed, a mean TBI of 0.76 — 9% higher than that found in our diabetic control group, who, equally, were without evidence of lower extremity arterial disease. (However, it must be kept in mind, that the mean diabetes duration in the Rheeder, et al-study, was 6 years shorter than the median duration found in our study.)

In a study by Edmonds and colleagues[60] a mean ABI of 0.63% (± 0.26) was reported for diabetic patients with ulcers, but with absent pedal pulses (therefore with critical limb ischaemia). If we were to subtract 0.36 from this value (in accordance with the previously reported mean difference between ABI and TBI of about 0.36[41]), this would indicate a TBI of 0.27 (± 0.26) — the resulting 0.53 for the highest value agreeing with our findings among patients with amputations (0.46). In their ulcer group with pedal pulses present, Edmonds et al reported quite a high ABI — when compared to our index of 0.60 among ulcer patients — namely 1.43 ± 0.20, corresponding with a TBI of between 0.87 and 1.27.

With regard to neurological evaluations, it was found in the three groups of our study, that absence of vibration perception (both at the level of the toe and at medial malleolus level) occurred most frequently among amputees — in 7/8 (87.5%) and 5/10 subjects respectively, at the two levels — followed by patients in the ulcer group (in 3/10 and 1/10). These differences were statistically significant (p=0.000 at toe-level, and 0.027 at the level of the medial malleolus), thereby demonstrating the effectiveness of the established method of assessment of vibration sense using a 128Hz tuning fork, to display differences between very small groups.

Results of other studies regarding absence of vibration perception, vary: Nielsen and associates [190] reported vibration sense absence at ankle (i.e. malleolus) -level, in 15% and 24%, respectively, of the Swedish and Saudi-Arabian Type 2 diabetic subjects that they investigated. Edmonds and colleagues,[60] in their study, reported absence of vibration sense at the level of the big toe in 78.4% of diabetic patients with ulcers in the neuropathic group (pedal pulses present) — comparing best to our ulcer group — and in 25% of diabetic patients with ulcers in the ischaemic group (i.e. in those with pedal pulses absent, probably corresponding best with our amputation group). At lateral malleolus level, results were 14% and 3% respectively, for the 2 groups mentioned.

For monofilament sensation, (as evaluated in our study by obtaining a total bilateral count, as well as by assessing absence in at least 4 sites in any leg) statistically significant differences (with absence increasing with worsening degrees of lower extremity complications) were displayed between our 3 diabetic groups — again demonstrating the effectiveness of these methods to display differences, even in the event of very small study-groups.

The results of our study regarding the assessment of peripheral neuropathy, based on the presence of 1 or more abnormal conduction attributes in at least 2 distinct nerves, seemed to follow the severity of lower limb complications (p=0.754), but — occurring in 8/10 controls, 9/10 patients with ulcers and in 9/9 assessed amputees — were fairly high when compared to other studies: Based on the same criteria (of 1 or more abnormal conduction attributes in at least 2 distinct nerves), Feldman and associates,[191] for instance, reported abnormal nerve conductions in 69% of 56 Type 1 and Type 2 diabetic outpatients.

When based on abnormal needle examination in at least two muscles, peripheral neuropathy was present not only in all of the assessed amputees (9/9) and in all 10 controls, but also in 8/10 patients in the ulcer group. Differences between the 3 groups were not statistically significant. However, in agreement with the results of previous reports,[175, 215] our findings do suggest the presence of pre-clinical peripheral neuropathy in neurologically asymptomatic diabetics.

In our subjects, based on both the mean beat-to-beat (maximum to minimum) heart rate variation, and the E:I-ratio, the worst degree of cardiac autonomic — specifically, parasympathetic nerve — dysfunction, occurred in the amputation group. Although our findings were not statistically significant, the E:I-ratio in our study, in agreement with the findings of Sundkvist, et al[242] and Carrington, et al,[292] decreased across groups, following severity of lower limb complications.

With regard to cutaneous autonomic response assessment (as indication of peripheral sympathetic function), differences were without statistical significance, although the proportion of patients with absent responses increased with worsening degrees of lower extremity complications. This finding is in agreement with the results of Cacciatori, et al, [293] who in their study, observed a progressive worsening of sympathetic peripheral function across groups, from controls, to diabetic subjects with severe neuropathic involvement and foot ulceration.

As a pilot study intended for comparing vascular and neurological parameters between diabetic subjects without diabetic foot ulceration or amputation (the diabetic control group) and those with either foot ulceration (ulcer group) or a lower extremity amputation (amputation group), this study is unique in the sense that no other studies of diabetics have been published investigating the same groups of subjects:

Studies investigating diabetic patients, in which similar (though not exactly the same) process and outcomes measures have been utilized, but with different approaches with regard to the choice of subject groups, include the following:

Rheeder and colleagues,[41] in order to assess them for lower extremity arterial disease, performed a series of vascular evaluations (including pedal pulse palpation, ABI, toe blood

pressure and TBI) on 85 female Type 2 diabetes patients. Khammash and Obeidat [56] investigated 60 patients with diabetic foot infection, by prospectively measuring the ankle-brachial pressure index (ABI), in order to determine the prevalence of lower limb ischaemia. Yokoyama, et al [168] measured brachial-ankle pulse wave velocity in 102 type 2 diabetic patients (including those with PAD), as well as in 101 healthy controls.

Suzuki and colleagues [167] assessed PWV in the lower extremities (brachial-ankle PWV), as well as motor nerve conduction velocity and cardiac autonomic function (R-R variation) in 60 type 2 diabetics without a history or symptoms of lower extremity arterial disease (and with normal ABI's at the time of the study — the so-called non-PAD group), as well as in 20 non-diabetic controls. Taniwaki, [158] et al evaluated carotid-femoral (aortic) PWV and carotid intima media thickness (CIMT) in 271 type 2 diabetes patients and in 258 age-matched controls.

Matsuto and colleagues[302] examined 33 diabetic patients with, and 33 diabetic patients without peripheral neuropathy, to assess cardiovascular autonomic neuropathy by heart rate variability, and pulse wave velocity. Okada et al[303]investigated, in 103 patients with non-insulin-dependent diabetes, the relationship between cardiac autonomic neuropathy (using RR-interval variation), diabetic micro-angiopathy (as assessed in terms diabetic nephropathy and neuropathy, the latter by means of nerve conduction velocity assessment) and macro-angiopathy (using pulse wave velocity).

Carrington and associates, [292] in their 6-year follow-up study, examined motor nerve conduction velocity, and also performed other peripheral (monofilament, vibration and temperature perception thresholds) and autonomic nerve (E:I-ratio) and vascular tests (including ABPI) as predictors for foot ulceration, amputation and mortality in diabetes. This study involved 22 non-diabetic control subjects, 51 diabetic subjects without neuropathy, 67 diabetic subjects with neuropathy, 34 diabetic subjects with a history of foot ulcers, and 17 diabetic subjects with Charcot neuropathy.

Edmonds ME, and others [60] studied 239 diabetics with foot ulcers — divided into 2 groups, namely the neuropathic group (those with palpable foot pulses) and the ischaemic group (those without palpable pedal pulses). Vascular evaluation involved performing of ankle-brachial

pressures and ABI's, whereas neurological assessment comprised evaluation of knee-and ankle reflexes, pin-prick sensation, and vibration sensation (using the 128Hz tuning fork).

Meijer JW, and colleagues, [193] to evaluate the discriminative power of 2 diabetic neuropathy scores for diagnosing diabetic polyneuropathy, as well as their relation to cardiovascular autonomic function testing, and electro-diagnostic studies, investigated 3 groups of subjects matched for age and sex: 24 diabetic patients with neuropathic foot ulcers, 24 diabetic patients without clinical neuropathy or ulcers, and 21 non-diabetic controls.

Clearly, not one study compared both the measures of vascular function used in this study (photo-plethysmographically derived toe-brachial index and pulse wave velocity), and of peripheral (monofilament, vibration perception using a tuning fork, nerve conduction and needle examination), and autonomic nervous system integrity (heart rate variation) applied here, between diabetic patients with different degrees of lower extremity complications (foot ulcers or amputation), and diabetic patients without such lesions.

Our pilot-study had limitations. Foremost among these, was the small sample size, which not only contributed to skew data (thereby necessitating the use of non-parametric tests), and to the lack of statistically significant differences between groups that was observed for certain outcomes measures, but which also restricted the drawing of conclusions or making of inferences regarding differences observed.

Selection bias may have resulted (both from the small numbers in our study, and our method of consecutive sampling), with regard to subject age and race, as well as with regard to disease duration, and, consequently, the prevalence and degree of diabetes complications (including medial arterial calcification).

Within the small sample size of this pilot study, however, it would not be appropriate to apply conventional age-adjustment techniques in an attempt to rectify this disparity between groups. With regard to race: Ideally, patients belonging to the four main South-African race groups should have been equally distributed between the 3 subject groups in our study. However, the distribution of the different races in this study is indicative of the availability (or lack of availability) of patients of a certain race group, at the time of recruitment and inclusion, which, in

turn, resulted from a number of factors mentioned earlier (including transport-problems, and differing attitudes toward research and follow-up compliance.)

Concerning diabetes duration, progression and complications, it may be possible that our diabetic clinics are attended more by complicated than by uncomplicated diabetic patients. It is, furthermore, more than likely that diabetic clinic control patients would be more diseased than the general population outside of the hospital, and that this could have lead to smaller differences between groups.

Arterial medial calcification in the lower extremity is known to be more common among men, the elderly, and patients with Type 2 diabetes mellitus, especially with long duration of disease.[304, 305, 306] The possibility of its undetected presence in some of our patients, could therefore, by causing false elevation, have introduced bias with regard to the interpretation of TBI results. However, although a value of ≥ 1.3 is commonly regarded as the cut-off for the presence of arterial medial calcification, the study by Rheeder, et al, showed very poor concordance between radiological evidence of arterial medial calcification (which is considered the gold standard), and an ABI > 1.3: it was found present in only 8 out of 81 subjects in their study.[41] Furthermore, in the Rheeder study, 4 of the 11 patients (i.e. 36%) with high ABI's, also had high TBI's, which, according to the authors, raised the possibility, in their study population, of the presence of a higher prevalence of calcification of the smaller toe vessels. This was contrary to the findings of Brooks, et al, who did not find high TBI associated with high ABI. [148] The results of the Rheeder study, however, suggest that the cut-off of 1.3 would not necessarily distinguish between all patients with and without arterial medial calcification. Having said this, it remains possible that arterial medial calcification might have influenced toe pressures in our study, and that, without radiological evidence — (which was, unfortunately, not obtained in our study) — its presence cannot be ruled out.

Due to, first of all, the obvious nature of the subjects' lower limb pathology (amputations, ulcers, or neither), and secondly, the fact that one investigator only (MCD-B), was responsible for final subject screening and inclusion, administering of the questionnaire, clinical examination (including peripheral pulses, monofilament and vibration sense assessment), as well as for all vascular evaluations, blinding to patients' status was not possible. All evaluations that normally would have involved subjective decisions (for instance obtaining the toe blood pressure, or

assessing monofilament sensation), could therefore, potentially, have been subject to prejudice (Observer's bias).

With regard to toe blood pressure, the procedure of obtaining a reference value, before recording of the actual 2 values for averaging, was employed in an attempt to minimize this effect. During monofilament sensation assessment, the maximum number of available and evaluable sites per foot, were tested. With regard to PWV, prior to this study, the investigator had undertaken 2 separate studies of intra-observer repeatability, on respectively 18 and 10 patients, all evaluated on 2 different occasions, a minimum of 2 weeks apart. The repeatability coefficients (based on 2 X the standard deviation of the difference between pairs) for carotid-femoral PWV of the 2 studies were 2.22, improving to 1.89 in the second series of patients, and for carotid-radial PWV, 3.23 and 2.67, respectively, for the 2 studies. Coefficients of variation for carotid-femoral PWV were 9.89 and 9.54, in the two studies, respectively, and for carotid-radial PWV 13.34 and 12.23.

Confounding, in the situation of our cross-sectional pilot-study — where the aim was not (as would have been the case in a large etiological study) to assess cause and effect or to point out etiological differences, but, in order to be able to plan future studies, to show the absolute differences between the three groups under investigation — does not come in to play. (Our hypothesis was plainly, that — disregarding the other risk factors present — vascular disease would be more progressed in diabetic patients with a history of lower extremity amputation, or ulcers, than in other diabetics). However, it is possible that the differences in clinical characteristics between the three groups (for instance, diabetes type) might have influenced results.

Our pilot study provided useful information for planning of future studies applying the same clinical parameters and outcomes measures. Most evaluations were feasible in our setting. However, certain difficulties were identified:

With regard to patient recruitment, quite a few obstacles had to be overcome: Computerized patient information was incomplete and outdated, thereby not only making random patient selection very difficult, but also resulting in a prolonged recruitment period. (This problem has since been addressed, by a concerted and well-structured effort to update and keep up to date

all Diabetes Clinic patient lists and records. In future studies in our setting, random selection of patients should, therefore, be much more attainable.) Furthermore, some eligible patients were lost, due to the requirement of a commitment of at least two full mornings (one for vascular evaluations and bedside neurological examinations, and the other for nerve conduction and electromyography). Based on the effectiveness of monofilament and vibration sense evaluations in our small study to point out differences between groups, it might prove better, in future studies, to restrict investigations for peripheral neuropathy to these bedside evaluations.

Although pedal pulse evaluation was not meant as one of the main outcomes measures in this study, the use of the measure of absence of both pulses in any foot, in this small study group, turned out inappropriate and ineffective, due to the fact that too few patients in the amputation group could be assessed. It may be feasible, in future studies involving peripheral vascular evaluation of amputees, to only include those patients with distal-, or sub-total foot-, or toe amputations. This, however, is likely to introduce bias through the inclusion of patients with — possibly — less progressed PAD. Another possibility, when assessing amputees, would be not to define peripheral arterial disease on the basis of the absence of both pulses in a foot, but simply to record any absence of pedal pulses.

Unfortunately, due to the design and specifications of the specific PWV apparatus we had available in our unit, performing of brachial-ankle PWV (which would have yielded results specific for the lower extremity) was not possible. We were thus limited to its carotid-femoral and carotid-radial PWV-application — elevation of which would indicate the presence of central and peripheral (but not necessarily lower limb-) atherosclerosis.

The dilemma in any process of sample size calculation, is three-fold, namely: Deciding on the magnitude of the differences in outcomes measures to be demonstrated between groups; determining the size of the variances expected in the different groups, and knowing what distribution of data to expect.

Previous studies that have compared different groups with diabetes have used sample sizes that varied from 314 (Apelqvist, et al[52]; TBP results used in our calculations), to 437 (Sahli, and colleagues;[149] TBI results used), to 3 175 (Van Popele, [298] Rotterdam Study, PWV results

used.) Although it is likely that sample sizes in theses studies had been pre-planned, no mention is made in their published methods sections of ‘a priori’ sample size calculations.

Applying the results of our study (3.5.1.1, 3.5.1.2 and 3.5.2.1) in order to plan sample sizes for future three-group comparisons, using the same outcomes measures as in this study — all of TBP /TBI, PWV, vibration sense and monofilament perception absence — indicated required equal group sizes of between 160 and 222 (i.e. depending on the data-distribution assumed). Should it be decided to leave out PWV, based on our own results —TBP /TBI, vibration and monofilament perception — equal group sizes of between 34 and 103 would be sufficient (depending on the assumed data-distribution). When using the results of other published studies — based on all of the outcomes measures applied in our study (with or without PWV) — an equal study group size of 111 would be needed.

In conclusion, we find that the smallest sample size required for significant absolute differences would be that based on TBP (30 to 92 per group, thus n = 90 to 276) and TBI (34 to 103 per group, thus n = 102 to 309), ór — if (as supported by the literature and our study) large differences were anticipated, on vibration- (15 or 111 per group; n = 45 to 333) and monofilament perception (12 to 41 per group; n = 36 to 123). Adding PWV to the outcomes measures would necessitate an up to two-fold increase in the number of subjects required (between 160 and 222 per group; n = 480 to 666).

DEFINITIONS AND REFERENCE VALUES

• **amputation:** resection of a terminal part of a limb

• **chronic foot ulcer:** any break in the cutaneous barrier, that is non-healing (i.e. present for at least one month, or healed not more than three months ago, after having been present for at least one month.)

• **peripheral arterial disease (PAD) or peripheral vascular disease (PVD):** can include the following:- claudication, either with exertion, or at rest; amputation for arterial vascular insufficiency; vascular reconstruction, bypass surgery, or percutaneous intervention to the extremities; documented aortic aneurysm; or a positive non-invasive test. [307]

• **increased PWV:** > 13 m/s [105]

• **systolic blood pressure (SBP):** at first appearance (i.e. phase I[113]) of Korotkoff sounds.

• **diastolic blood pressure (DBP):** at disappearance (i.e. phase V[113]) of Korotkoff sounds.

• **pulse pressure:** derived from subtraction of diastolic blood pressure from systolic blood pressure.[48]

• **hypertension:** SBP > 140 mmHg and/or DBP > 90 mmHg, on at least 2 occasions; *or*: current use of anti-hypertensive pharmacological therapy; *or*: history of hypertension diagnosed and treated with medication, diet, and/ or exercise.[307]

• **severe congestive heart failure:** Rales over more than 50 % of the lung fields, or evidence of new pulmonary oedema on chest radiograph (ACC),[307]

or: NYHA grade iii and iv:-

- Grade III indicates marked limitation of physical activity. Although patients are comfortable at rest, less-than-ordinary activity leads to symptoms. Patients exhibit symptoms with minimal exertion.
- Grade IV indicates the inability to carry on any physical activity without discomfort: Symptoms of congestive failure are present even at rest. With any physical activity, increased discomfort is experienced

• **smoking:** History confirming cigarette smoking in the past:-

- current — smoking cigarettes within one month of this admission;

- recent — stopped smoking cigarettes between one month and one year prior to this admission;

- former /ex — stopped smoking cigarettes greater than one year before this admission;
- never — never smoked cigarettes.[307]

• **obesity:** BMI > 30 kg/m^2.[308]

• **diabetes mellitus:** a group of heterogeneous disorders with the common elements of hyperglycaemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action, or both:-

• **a patient is considered as having diabetes mellitus if:** Fasting blood glucose is greater than 7 mmol/L (or 126 mg/dL), or random blood glucose level is > 11.1mmol/L. [WHO 1997];
or: the patient has a history of diabetes (regardless of duration of disease);
or: the patient has a need for anti-diabetic agents.[307]

• **type 1 diabetics:** diabetes patients on insulin treatment (which reflects total loss of insulin secretion) since onset / diagnosis of the disease;
or: diabetes patients who start insulin therapy within one year of diagnosis. Patients are usually young at onset of the disease, with the onset presenting typically.

• **type 2 diabetics:** diabetes patients on diet treatment or oral antihyperglycaemic agents, or both (indicating the continuation of insulin production);
or: diabetes patients on insulin therapy, but who have been on oral antihyperglycaemic agents or diet therapy for a duration of at least one year from onset / diagnosis of the disease. Patients are generally older and asymptomatic at disease onset.

• **type 2 diabetes mellitus:** diabetes diagnosed after the age of 30 years, and insulin not used within the first year of diagnosis.

• **increased plasma-glucose:** > 5.8 mmol/L (fasting) [UP lab]

• **HbA1c:** Glycosylated Hemoglobin[218]:- the fraction of hemoglobin that is used as an objective and quantitative index of blood glucose levels during preceding months. HbA1c between 4.4 and 6.4% is usually regarded as normal. However, UP lab reference values (4.4 – 6.2 %), have been adhered to in this study.

The following table (Diabetes Control and Complications Trial / DCCT Research Group) illustrates the relationship between HbA1c and average blood glucose during the preceding two to three months:-

HbA1c Average Blood Glucose (mmol/L)

4	3.3	
5	5.0	
6	6.6	
7	8.3	(A 1% change in HbA1c
8	10.0	equals a 1.7 mmol/l change
9	11.7	in average blood glucose)
10	13.3	
11	15.0	
12	16.7	

- **elevated HbA1c:** > 6.2 %. [UP lab]
- **elevated s-Creatinine:** > 115 µmol/L (reference values: 62 – 115µmol/L) [UP lab]
- **elevated s-total Cholesterol:** ≥ 5.2 mmol/L, (fasting) [UP lab]
- **elevated s-LDL:** ≥ 3.4 mmol/L (fasting) [UP lab]
- **elevated s-TGL:** ≥ 1.5 mmol/L (fasting) [UP lab]
- **decreased s-HDL:** < 0.9 mmol/L (fasting) [UP lab]
- **increased s-HDL:** > 2.0 mmol/L (fasting) [UP lab]
- **urine test strip interpretation:** Multistix® 5 (Bayer Diagnostics):
  - amount of protein: ◦ Trace : < 30 mg/dL (0.30 g/dL);
    ◦ + : ≥ 30 mg/dL (0.30 g/dL);
    ◦ ++ : ≥ 100 mg/dL (1.00 g/dL);
    ◦ +++ : ≥ 300 mg/dL (3.00 g/dL);
    ◦ ++++ : ≥ 2 000 mg/dL (20.00 g/dL);
  - leucocytes: ◦ Trace : ≥ 15 leucocytes/µL;
    ◦ + : ≥ 70 leucocytes/µL;
    ◦ ++ : ≥ 125 leucocytes/µL;
    ◦ +++ : ≥ 500 leucocytes/µL.
- **albuminuria:**[309] in two of three samples:-

Urine Albumin Excretion:	mg/24h	µg/min
Normal:	< 30	< 20
Micro-albuminuria:	30 – 299	20 – 199 (detectable by a reagent strip)
Macro-albuminuria:	≥ 300	≥ 200 (clinical albuminuria /gross proteinuria [310]/overt nephropathy[216])

• **abnormal nerve conduction:** abnormality of nerve conduction of one or more conduction attributes (amplitude, distal latency, or conduction velocity) in two or more nerves and not due to a disease other than diabetes mellitus or due to faulty electrode placement, nerve cross-over, or low nerve temperature[215] (= at least one abnormal conduction attribute on each of at least two anatomically distinct peripheral nerves)[175]

• **symptoms of neuropathy:** muscular cramps, numbness, abnormal hot or cold sensations, tingling sensation, burning sensation, aching pain and irritation in the lower legs and the feet.[218]

• **stages of neuropathy (according to the Mayo Clinic classification):** on the basis of combined clinical and electrophysiological evaluation, patients are categorized into four stages: [174, 191]

- 0/ normal: no neuropathy,
- stage 1: mild / asymptomatic neuropathy,
- stage 2: moderate / symptomatic neuropathy,
- stage 3: severe / disabling neuropathy.

• **confirmed clinical neuropathy:** a finding of definite clinical neuropathy (based on the presence of at least two of: physical symptoms, abnormalities on sensory examination, and /or absent or decreased deep tendon reflexes[175]) by physical examination and history, confirmed by unequivocal abnormality of either nerve conduction or autonomic nervous system response (e.g. mean resultant R-R variation <15.0).[175]

• **Kruskall-Wallis Test:** also called: 'equality of populations' rank test, for analysis of continuous numerical variables — for testing the hypothesis that several samples are from the same population (STATA 8.0).

• **Kruskall-Wallis2:** A 'one-way-analysis-of-variance-by-ranks'-test for deciding whether k independent samples are from different populations; the null hypothesis is that k samples come from the same population or from identical populations with the same median (STATA 8.0).

• **repeatability coefficient (RC):** based on 2 X the standard deviation of the differences between pairs.[311]

• **coefficient of variation (CV):** based on the within-subject standard deviation, divided by the mean of all values obtained in all subjects for repeated visits.[312]

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