SIMPLIFYING TECHNOLOGIES FOR PERIPHERAL ARTERIAL DISEASE RECOGNITION

(STARTREC)

Thesis submitted for the degree of

Doctor of Medicine at the University of Leicester

2019

by

Ramesh Yap Kannan MBBS MRCS

Department of Cardiovascular Sciences

University of Leicester

ABSTRACT

Title : Simplifying Technologies for Peripheral Arterial Disease Recognition

Objectives : Novel diagnostics have been studied in the past to diagnose peripheral arterial disease (PAD) with variable results. These studies focused on correlation and reliability, with few comparing against an acceptable reference standard. In this study, we explore the role of novel diagnostics that measure functional parameters of peripheral circulation, for PAD diagnosis.

Materials & Methods : A review of existing technologies for PAD diagnosis was performed. Pilot tests were performed to assess the feasibility of the methodology using suitable devices. Provocation tests of limb circulatory reserve, were used to test the perfusion, oxygenation and temperature of patients and healthy controls using different devices. 150 participants were recruited.

Results: The study shows that oxygenation and temperature are suitable for diagnosis. Perfusion is too sensitive to movement and not suitable. 1-minute flexion extension (1MF) tests and the 6-minute walk test (6MWT) is better than Post occlusive reactive hyperemia as a provocation test. A temperature difference of 0.05°C, at 3 minutes after the 6MWT in the medial gastrocnemius (MG), yields a sensitivity of 62.5% and specificity of 63.16%. At the heel, 1-minute (cutoff 0.35°C), 2-minutes (cutoff 0.55°C) and 3 minutes (cutoff 0.15°C) post 6MWT, yields a sensitivity of 69.39%, 77.55% and 63.41% with a specificity of 60.98%, 53.85% and 72.97% respectively. For oxygenation, the sensitivity using baseline tissue

saturation index (bTSI), ranges from 57.94% to 73.2%, with a specificity from 72.73% to 74.42%. Difference in tissue saturation index (dTSI) post 6MWT, has a sensitivity of 61.36% to 71.43%, and specificity of 65.12% to 74.77%. Time of return to bTSI after 6MWT (T_{100}) has a sensitivity of 75.51% and specificity of 58.97%.

Conclusion : Devices that measure oxygenation or temperature have potential for diagnosing PAD in the future. Further studies should focus on these parameters.

ACKNOWLEDGEMENTS

I would like to thank Professor Robert Sayers and Professor Matthew Bown for their expert advice, supervision, continued support and encouragement throughout the period of this study. Special thanks to Professor Nick London, Professor A Ross Naylor, Mr. Mark McCarthy, Mr. Akhtar Nasim, Mr. Rob Davies, Dr. Aman Bolia, Dr. William Adair, Dr. Guy Fishwick for allowing me to recruit patients under their care.

Special thanks to the NIHR Biomedical Research Unit Leicester for providing the small research grant, which helped procure devices for this study.

To all the staff in the vascular ward, Emergency surgical assessment unit, Interventional suite and Vascular studies unit. To the staff of the Clinical simulation suite, Diagnostic development unit and University of Leicester Space Research Unit in particular to Professor Tim Coats, Mr. Karoly Keresztes, Ms. Sarina Hussain. Special thanks to Mr. Athanasios Saratzis and Mrs. Leanne Hall for statistical advice and help. Thanks to Mr.Nikesh Dattani and Mr.David Sidloff for help during the period of study.

I am very grateful to the patients who very patiently participated in the study.

Personally, I would like to thank my family for their continued support and understanding during the period of the study.

PUBLICATIONS FROM THIS THESIS

Yap Kannan R, Keresztes K, Hussain S, Coats TJ, Bown MJ. Infrared cameras are potential traceable 'fixed points' for future thermometry studies. Journal of Medical Engineering and Technology 2015;39(8):485-489.

Yap Kannan R, Dattani N, Sayers RD, Bown MJ. Survey of ankle brachial pressure index use and its perceived barriers by general practitioners in the UK. Postgraduate Medical Journal 2016; 92:322-327.

CONTENTS

METHODS

Chapter 3. Methods

RESULTS

Chapter 4. Precision studies

Chapter 5. Accuracy study: Oxygenation

Chapter 6. Accuracy study: Thermometry

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ABPI	Ankle Brachial Pressure Index
AUC	Area Under the Curve
AV shunts	Arterio-venous shunts
COPD	Chronic Obstructive Pulmonary Disease
CTA	Computed Tomography Angiography
CVA	Cerebrovascular accident
CVC	Cutaneous Vascular Conductance
CWA	Continuous wave doppler Waveform Analysis
DCS	Diffuse Correlation Spectroscopy
DCS-NIRS	Diffuse Correlation Spectroscopy and Near-infrared Spectroscopy combined device
DRS	Diffuse Reflectance Spectroscopy
DSA	Digital Subtraction Angiography
DTA	Diagnostic Test Accuracy studies
eTC1	Exercise induced Temperature Change at 1 minute
eTC2	Exercise induced Temperature Change at 2 minute
eTC3	Exercise induced Temperature Change at 3 minute
EQUATOR	Enhancing the QUAlity and Transparency Of health Research
FFT	Fast Fourier Transformation
FPPG	Functional Photoplethysmography
GPs	General Practitioners
GRRAS	Guidelines for Reporting Reliability and Agreement Studies
ICC	Intraclass correlation
IRTh	Infrared Thermometry

IRTg	Infrared Thermography
LDA	Laser Doppler Anemometer
LDF	Laser Doppler Flowmetry
LDI	Laser Doppler Imaging
LM	Lateral Malleolus
LSCI	Laser Speckle Contrast Imaging
MAP	Mean Arterial Pressure
MG	Medial Gastrocnemius
MM	Medial Malleolus
MRA	Magnetic Resonance Angiography
NIRS	Near-infrared Spectroscopy
NPA	Negative Percent Agreement
NPV	Negative Predictive Value
PAD	Peripheral Arterial Disease
PICO	Population, Index test, Comparator and Outcome
PORH	Post Occlusive Reactive Hyperemia
PPA	Positive Percent Agreement
PPG	Photoplethysmography
PPV	Positive Predictive Value
PU	Perfusion Unit
PVR	Pulse Volume Recordings
QAREL	Quality Appraisal tool for studies of diagnostic RELiability
QUADAS	QUality Assessment tool for Diagnostic Accuracy Studies
REC	Regional Ethics Committee
$RmVO_2$	Resting muscle oxygen consumption

ROC	Receiver Operating Curve
RPI	Regional Perfusion Index
SBP	Systolic Blood Pressure
SD	Standard Deviations
STARD	Standard for Reporting Diagnostic accuracy studies
StO_2	Tissue oxygen saturation
$TcpO_2$	Transcutaneous Oximetry
TFI	Transfer Function Index
TIA	Transient Ishaemic Attack
TSI	Tissue Saturation Index
$TMRO_2$	Tissue Metabolic Rate of Oxygen consumption
T_{100}	Time to complete recovery of the baseline TSI value
T_{50}	Time to recovery to half of the baseline TSI value
6MWT	6-Minute Walk Test
1MF	1-Minute Flexion extension test

Introduction

Chapter 1: Peripheral Arterial Disease

Anatomy of peripheral arteries

The peripheral arteries are either nutritional of thermoregulatory in nature. In the lower limb, there are 21 vascular territories that give rise to approximately 93 +/- 26 perforators (Morris *et al.*, 2006). The dermal capillaries arise from these perforators. These are divided into a deep cutaneous and a sub-papillary horizontal plexus of capillaries, that communicate via vessels vertically traversing the dermis either between muscle fibers (septocutaneous perforators) or through the muscles directly (musculocutaneous perforators) (Figure 1). A vessel loop arises from the sub-papillary plexus to every dermal papilla. Arterioles and venules are connected by arteriovenous shunts (AV shunts) (Figure 2). These shunts play an important role in the thermoregulation of the skin and are controlled by systemic, humoral and neurogenic factors (Venus, M. Waterman, J. McNab, I, 2011). The shunts are densely located in non-hairy (glabrous) parts of the skin (Walløe, 2015; Abularrage *et al.*, 2005) and can cause large fluctuations in blood flow (Saad *et al.*, 2001).

Blood flow to the skin is controlled by an active vasodilator and a noradrenergic driven vasoconstrictor system (Crandall *et al.*, 1995). Glabrous skin blood flow is controlled by the vasoconstrictor system only (Johnson *et al.*, 1995). Saad et al quantified blood flow through the AV shunts as cutaneous vascular conductance (CVC). CVC is the ratio of the perfusion units, measured by laser doppler flowmetry (LDF) and the mean arterial pressure (MAP). By comparing CVC between glabrous (palm and sole) against non-glabrous skin (forearm and ventral leg), before and after isometric exercise, he demonstrated that the skin blood flow is significantly

reduced, in glabrous skin but not in non-glabrous skin. This was postulated to be due to prolonged vasoconstrictor tone in the vessels of the glabrous skin. This makes the glabrous parts of the skin more prone to sequelae of peripheral arterial disease (PAD) (Saad *et al.*, 2001) .

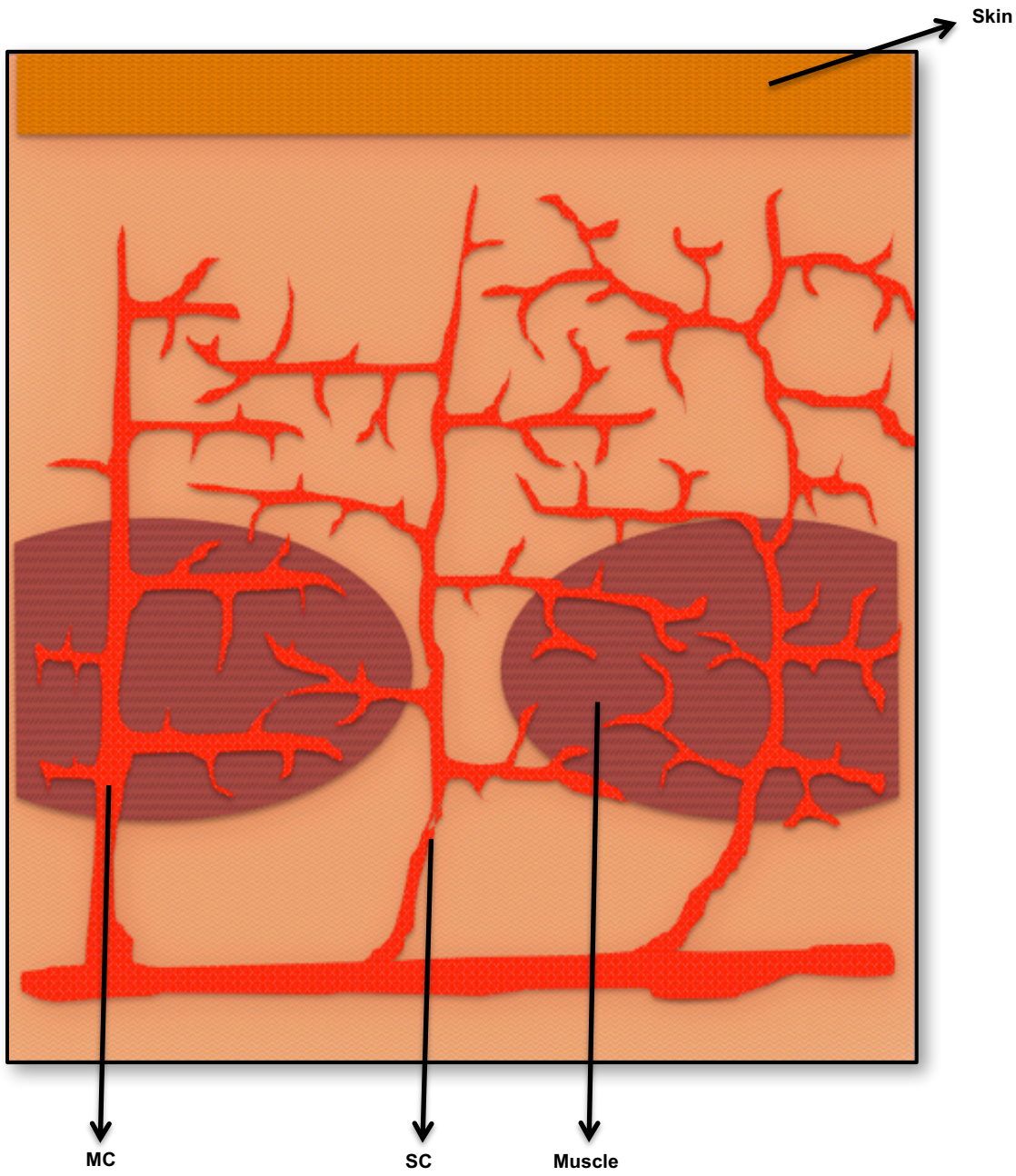


Figure 1: Image depicting the Musculocutaneous artery (MC) and the Septocutaneous artery (SC).

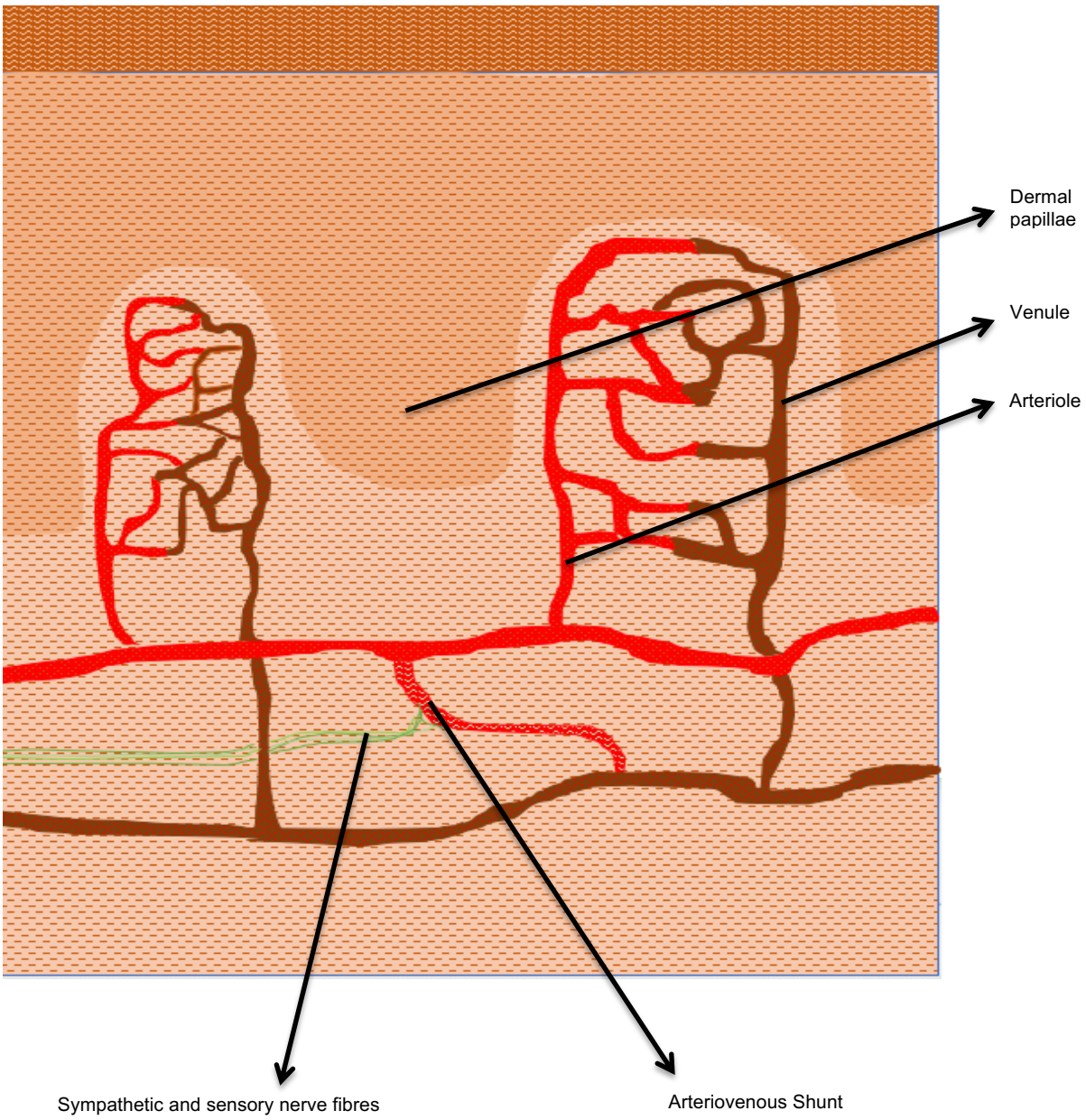


Figure 2: Arteriovenous shunt. Sympathetic fibers close the shunts when it is hot, whereas sensory fibers save heat in cold conditions.

Physiology of PAD

In the resting state, the body is able to maintain blood supply to the peripheries despite the presence of stenosis (Hiatt, William R. *et al.*, 2014) . The rate at which the flow is provided though may vary depending on the degree of stenosis as per

Poiseuille's law (Figure 3). This law defines the association between flow (Q), pressure (P), and resistance (R) in the vessel. This is important because, the radius of the vessel lumen, length of stenosis, blood viscosity and pressure changes effects flow.

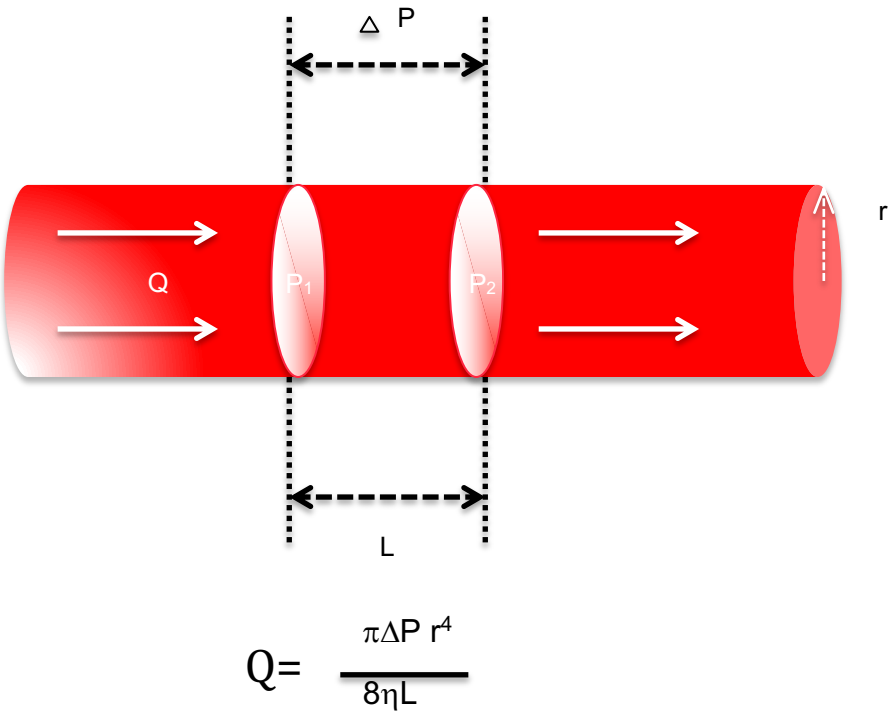


Figure 3: Poiseuille's law explaining the relationship between flow (Q) with pressure (P), radius (r), viscosity (η) and length (L)

Another important law applicable to hemodynamics is the Ohm's law, where

$$V = I \times R$$

or

Mean Arterial Pressure = Cardiac Output x Systemic Vascular Resistance

Using this principle, during a state of exercise, the mean cardiac output increases as a result of increased stroke volume and heart rate. As cardiac output increases,

systemic vascular resistance reduces, which is manifested as peripheral vasodilation. When peripheral arteries are normal, this vasodilation would mean an increase in the flow to this region by Poiseulle's law and an increase in mean arterial pressure. This should be reflected in the parameters that study flow (LDF), pressure (ABPI, TBPI, TFI, plethysmography). Oxygen and temperature changes of the skin are more physiological changes that are affected by additional factors such as environmental and cardiorespiratory function.

Definition of PAD

Peripheral arterial disease (PAD) is the occlusive narrowing of arteries of the body. PAD manifests itself when symptoms arise. Typically, these occur during exercise. These symptoms worsen with disease progression. The initial symptoms are often intermittent claudication. This is described as buttock or leg pain on walking which resolves with rest. Critical ischemia occurs when the perfusion to the limb is insufficient even at rest leading to either rest pain and eventual tissue loss. PAD is classified based on the presence or absence of symptoms (Rutherford *et al.,* 1997; Fontaine *et al.,* 1954), as shown below (Table 1). Other classifications have emerged based on the anatomical distribution of disease, onset of symptoms and the presence of diabetes (Hardman *et al.,* 2014). Nevertheless, the Fontaine classification remains popular in clinical practice and research and is easy to apply. Globally, over 200 million people have PAD (Fowkes *et al.,* 2013). Of these patients, about 10% have typical intermittent claudication (Schirmang *et al.,* 2009; Hirsch *et al.,* 2001) The severity of PAD symptoms generally depends on the severity of the stenosis and the presence of microvasculature that can cope with the demands

(Hills *et al.*, 2009). Nevertheless, patients with total occlusion may still remain asymptomatic (Fowkes *et al.*, 1991; Premalatha *et al.*, 2002).

Grade	Symptoms
Stage 1	Asymptomatic, incomplete blood vessel obstruction
Stage 2	Mild claudication pain in limb
Stage 2a	Claudication at a distance >200m
Stage 2b	Claudication at a distance <200m
Stage 3	Rest pain, mostly in the feet
Stage 4	Necrosis and/or gangrene of the limb

Table 1: Fontaine Classification (Hardman et al. 2014)

Incidence of PAD

Epidemiological studies on the incidence of PAD require serial measurements for PAD, and the use of a sensitive diagnostic criterion. Incidence reports would vary depending on the diagnostic criteria. There are not many studies that report incidence of PAD. Of those that do, there is heterogeneity in the inclusion criteria and reporting methods.

A retrospective observational study in the UK, the incidence of symptomatic PAD in people between 50-89 years, has reduced from 38.6 to 17.3 per 10000 person years from 2000 to 2014 (Cea-Soriano *et al.*, 2018). The study was limited as it is unclear how patients were actually diagnosed with PAD, except that they were coded as PAD on the electronic software in primary care. However, it is interesting to note that patients aged between 50-59 did not demonstrate any increase or decrease in the incidence. The German getABi study was a prospective study over a 7 year period by GPs who were trained by Vascular specialists to diagnose PAD

using an ABI cutoff of 0.9, in patients over the age of 65. The ABI measurements were done at baseline, 1,3,5 and 7 years. In this study, claudicants were not considered as symptomatic PAD because of its low specificity. Patients were defined as having PAD, when the straight regression line crossed an ABI of 0.9. The prevalence of PAD at baseline was 16.6% (1145 of 6889 participants). The incidence rate of PAD was 20.3 per 1000 person-years of whom 10.4% had symptomatic disease (incidence rate of 2 per 1000 person-years) (Krause *et al.,* 2016).

Across the continent in the United States, Nehler et al performed a retrospective cohort analysis of participants who had one medical claim over a 5-year period (2003-2008). The reported annual incidences for PAD was 2.35% (Nehler *et al.,* 2014). In the Netherlands, the Limburg PAOD study used an ABPI criteria of <0.95, reporting an annual incidence of 1.7 and 5.9 per 1000 for men and women aged 40 to 54 years, respectively. The incidence was 17.8 and 22.9 per 1000 in men and women over 65 years, respectively, which is quite similar to the incidence of the getABI study. Interestingly in both age groups, the incidence of asymptomatic PAD was higher than symptomatic PAD, irrespective of gender (Hooi *et al.,* 2001; Stoffers, H. E. J. H. *et al.,* 1996). The higher ABPI threshold used for PAD diagnosis could explain this apparent higher incidence. Mohler et al demonstrated that a significant number of asymptomatic patients show a progression of PAD over 1 year. In this study, a duplex scan was performed in asymptomatic participants with an ABI between 0.6 and 0.9, at baseline and after a year. 28.7% of participants (19 of 66 legs) developed new lesions (n=2) or progressed with new lesions (n=17) on duplex (Mohler *et al.,* 2012).

Although the methodologies and reporting varies between these different studies, what these constantly show is that the incidence of PAD is higher than previously thought and that asymptomatic disease is more common than symptomatic PAD (Criqui, Michael & Aboyans, 2015). What is also evident, is that the diagnostic criteria used can influence PAD incidence studies.

Prevalence of PAD

Early prevalence studies underestimated the actual prevalence of PAD by reporting symptomatic PAD patients using questionnaires based on the symptoms of Intermittent Claudication (Rose, 1962). One of the early studies that looked at the prevalence of symptomatic and asymptomatic PAD was the Edinburgh Artery Study, using an ABPI<0.9 as being diagnostic for PAD. This study showed that patients with PAD could remain asymptomatic even in the presence of moderately severe disease (Fowkes *et al.*, 1991). Over the past decade, the prevalence of PAD has increased by 23.5% globally from 164 in 2000 to 202 million in 2010 (Fowkes *et al.*, 2013). This systematic review and meta-analysis had shown that the prevalence of PAD, using similar diagnostic criteria, has increased by 28.7% in low to middle income countries and by 13.1% in high income countries, with the vast majority of PAD patients being in the low to middle income countries (69.7% of 202 million) (Figure 4). Furthermore, the prevalence of PAD in men was consistently higher than women in high-income countries for all ages and for patients over 65 years in low to middle income countries. Unlike the west, the prevalence was higher in women below the age of 65 years in low to middle income countries (Fowkes *et al.*, 2013). More recently, there is a suggestion of a decreasing prevalence of PAD.

In the UK, PAD decreased from 3.4% in 2000 to 2.4% in 2014 in participants over the age of 50 (Cea-Soriano *et al.,* 2018). The criteria used for PAD diagnosis is unclear in this study. The Hermex study in Spain, reported a PAD prevalence of 3.7%. The study was conducted in a population of high risk for cardiovascular disease in Spain, of participants between 25 and 79 years. Using an ABPI cutoff of 0.9 and the Edinburgh questionnaire, the authors reported that the prevalence increased with age. The cumulative prevalence were 6.2%, 9.1% and 13.1% for participants aged 50, 60 and 70 respectively (Felix-Redondo *et al.,* 2012). This could be explained in part by the better preventative management in western countries.

Despite these figures, the accuracy of prevalence studies depends on the diagnostic technique used. A recent review demonstrated a high specificity (83-100%) for an ABPI<0.9 to detect >50% stenosis. However, there was a varied sensitivity (15-79%) (Dachun *et al.,* 2010). What these studies do show is that PAD is a global burden, especially in low to middle income countries and that preventative management could potentially decrease its incidence.

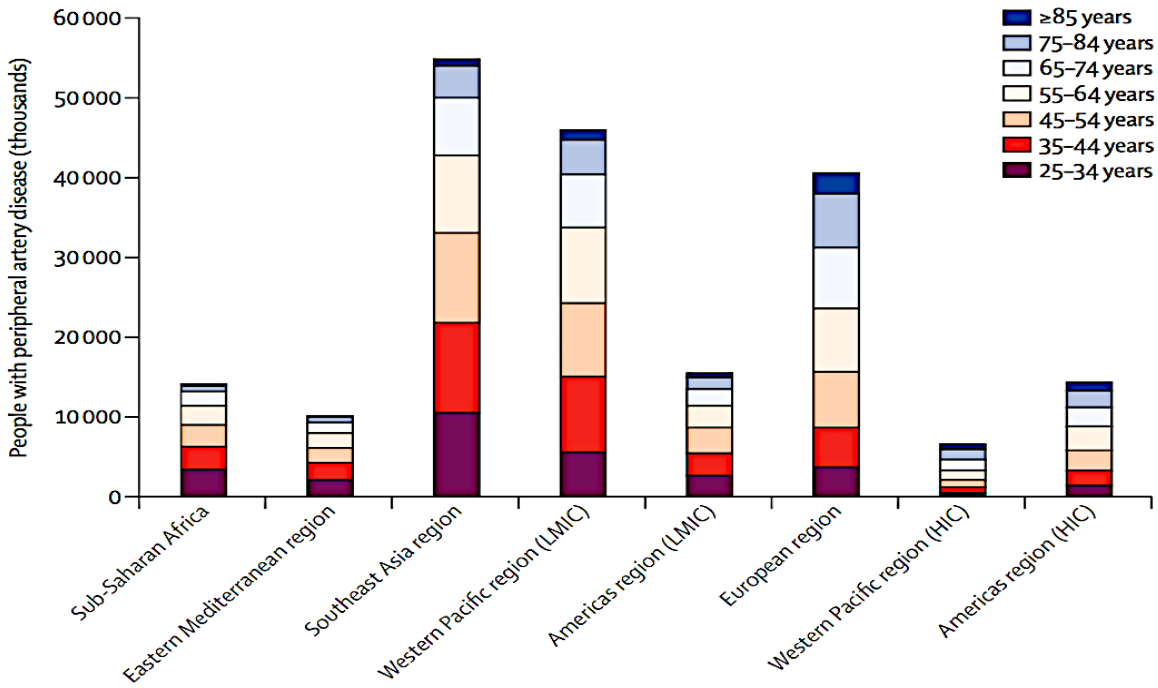


Figure 4: Estimate of the number of cases and contributing age groups in eight WHO regions in the year 2010. LMIC = low-income and middle-income countries, HIC =High-income countries (Reproduced with permission from Fowkes et al 2013)

Risk factors for PAD

PAD is a marker for systemic atherosclerosis (Manfredini *et al.*, 2013) and predicts the presence of cardiovascular and cerebrovascular disease (Criqui & Aboyans, 2015; Jager *et al.*, 1999). Smokers have been shown to have a 1.9 to 3.4 times higher odds of developing PAD (Murabito *et al.*, 2002; Murabito *et al.*, 1997; Meijer *et al.*, 2000; Allison *et al.*, 2006). This risk of developing PAD persists even after 20 years of smoking cessation (Joosten *et al.*, 2012). Patients with diabetes mellitus have a higher odds ratio (1.89 to 4.05) of developing PAD (Newman *et al.*, 1993; Murabito *et al.*, 1997; Meijer *et al.*, 2000; Allison *et al.*, 2006). The duration of diabetes mellitus, use of insulin and poor glycemic control is associated with the development of PAD (Kallio *et al.*, 2003; Katsilambros *et al.*, 1996; Tseng, 2003; Althouse *et al.*, 2014). Diabetics have a five-fold risk of amputation with a three-fold

higher risk of mortality (Jude *et al.*, 2001), with infection aggravating the risk of this (Faglia *et al.*, 2006). Diabetics develop asymptomatic PAD early (Forrest *et al.*, 2000) and if not well controlled this will increase their risk of PAD further (Adler *et al.*, 2002). People with hypertension have an odds ratio 1.32 to 2.2 of developing PAD (Criqui & Aboyans, 2015). Systolic blood pressure rather than diastolic blood pressure was associated with the development of PAD (Newman *et al.*, 1993; Meijer *et al.*, 2000). In patients with hyperlipidemia, total cholesterol to high density lipoprotein cholesterol ratio rather than total cholesterol is better at predicting PAD of the two lipid measures (Natarajan *et al.*, 2003). The evidence for obesity and alcohol consumption is divided (Criqui & Aboyans, 2015).

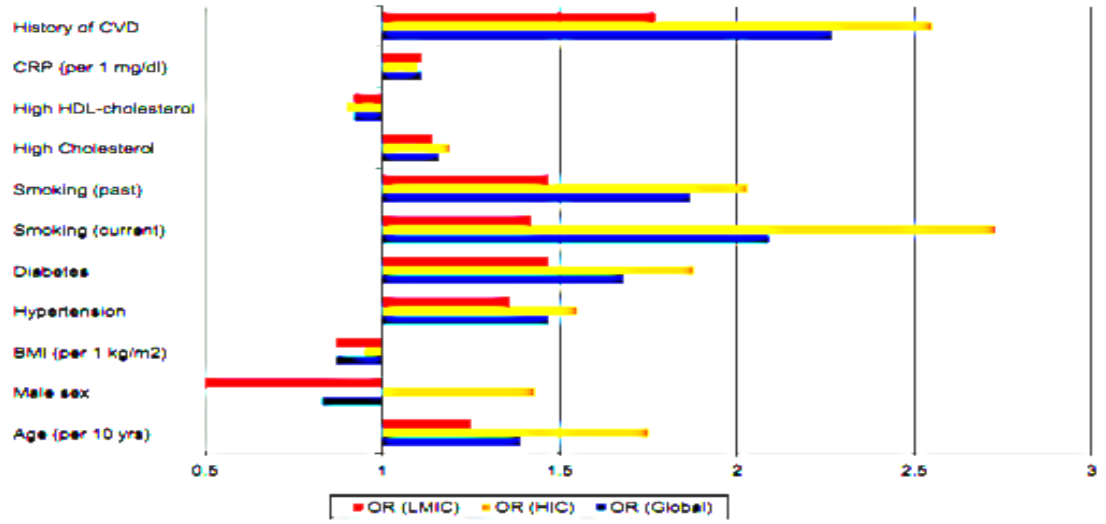


Figure 5: Odds ratios for peripheral artery disease in high income countries (HIC) and low to middle income countries (LMIC). BMI - body mass index, CVD - cardiovascular disease, CRP - C reactive protein, HDL: High-density lipoprotein (Reproduced with permission from Criqui and Aboyans 2015)

Diagnosis of PAD

Diagnostic Test Accuracy Studies

The ability of a test to distinguish patients with or without the presence of the target condition is its accuracy. Before selecting the best diagnostic tool for PAD, it is important to determine the role of the test to be used. There are three possible roles for a diagnostic test. The first being as a replacement to an existing test, in which case the index test will have to be compared against the existing test, using a reference standard. The second role is for triage or screening purposes. The role of this test is to identify true positives accurately and provide a low false negative rate, ie a test with high sensitivity. These tests may not be as accurate but are usually simple and inexpensive. The third role is as an add-on test, which would be used after an existing triage or screening pathway. This test would serve to identify false positives or false negatives. Challenges with Diagnostic test accuracy studies (DTA) have been the poor quality of reports, heterogeneity of methodology, different description of statistical terms, use of inappropriate statistical methods and misinterpretation of results (Leeflang et al., 2008; Bartlett & Frost, 2008; Bland & Altman, 1986). Therefore, DTA studies need to be interpreted carefully, as it impacts on patient management.

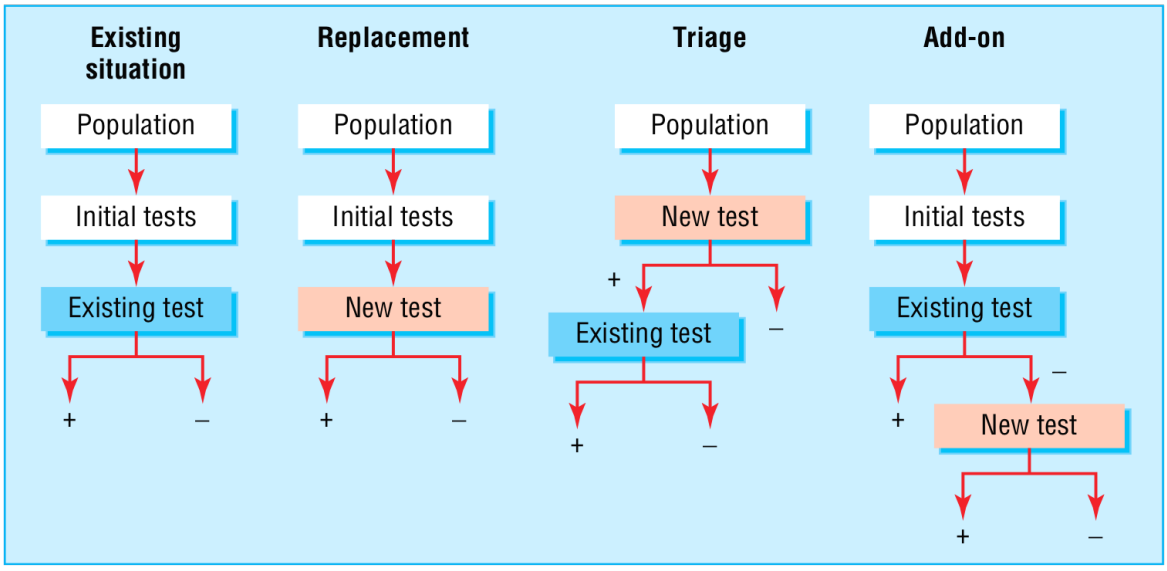


Figure 6: Three possible roles of diagnostic test (Reproduced with permission from Bossuyt P.M. 2006)

Features	Replacement test (Detecting herniated discs)		Triage test (Detecting pulmonary embolism)		Add-on test (Detecting distant metastasis)	
	New test (MRI)	Existing test (myelography)	New test (d-dimer)	Existing test (spiral CT)	New test (Positron Emission tomography)	Existing test (CT & US)
Accuracy	High	High	Low	High	High	High
Invasiveness	Non-invasive	Invasive	Non-invasive	Non-invasive	Non-invasive	Non-invasive
Waiting time	Yes	Yes	No	Yes	Yes	No
Knowledge and skills needed	Moderate	Moderate	Low	Moderate	Moderate	Moderate
Interpretable	Most tests	All tests	All tests	Most tests	Most tests	Most tests
Cost	High	High	Low	Higher	High	Medium

Table 2: Three possible roles of a diagnostic tests (Reproduced with permission from Bossuyt et al 2006).

A diagnostic test should be reliable (internal validity) and be applicable (external validity). Internal validity is affected by bias and precision. External validity of the study can be determined by using the PICO search strategy (population selection, index test, comparator and outcome) (Schmidt & Factor, 2013; Leeflang et al., 2008).

There are two main guidelines for assessing and reporting diagnostic accuracy studies. These are the STARD (Standard for Reporting Diagnostic accuracy studies) provided by the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network (Bossuyt et al., 2015) and for systematic reviews of DTA studies, the QUADAS 2 (Quality assessment tool for diagnostic accuracy studies) (Whiting, P. F. et al., 2011) recommended by the Cochrane collaboration.

The STARD checklist originally consisted of a 25-item checklist (Bossuyt et al., 2003) (Table 3), and has recently been updated to a 30-item checklist (Bossuyt et al., 2015) (Table 4). Early studies that compared diagnostic accuracy studies pre

and post STARD guidelines, showed no difference in the quality of reporting (Wilczynski, 2008). However, this has improved since the introduction of STARD more recently (Korevaar et al., 2015). The STARD checklist has now been adopted in more than 200 journals (Leeflang et al., 2008). Although the STARD checklist is very useful in guiding performance and interpretation of the methodology and how the overall study is performed, it does not provide advice on statistical analysis. The QUADAS quality assessment tool, developed in 2003 was a 14-item checklist useful for assessing reviews of diagnostic accuracy studies (Whiting, P. et al., 2003). Subsequent QUADAS-2 tool was developed which additionally assesses for bias and applicability (Whiting et al., 2011).

When a gold standard test is used, diagnostic accuracy studies usually provide the sensitivity, specificity, positive and negative predictive values, likelihood ratios and Area under the curve (AUC) of a Receiver Operating Characteristic (ROC) graph (Grimes DA, 2005; Bossuyt et al., 2015). Often a gold standard test may not be feasible to use either due to cost or invasiveness of the test (Naaktgeboren et al., 2016). When the reference test is not a gold standard, agreement statistics can be used. Agreement measures how close two measurements are (Bartlett & Frost, 2008). Bland-Altman method provides a graphical representation of the 95% limits of agreement, ie mean difference +/- 2SD, and is used for continuous variables. Kappa coefficient is used to report agreement of categorical variables (Kim, 2013). Perfect agreement is indicated by a kappa of 1, whereas a kappa of 0 indicates an agreement that is due to chance. Kappa coefficient can be 0 (poor), 0.01–0.20 (slight), 0.21–0.40 (fair), 0.41– 0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1 (almost perfect) (Landis JR, 1977).

Reliability is the measurement error due to inherent variability in 'true' test subjects. It is measured as Intraclass correlation (ICC). Repeatability is the variation in repeat measurements under constant conditions (Bland & Altman, 2003; Bartlett & Frost, 2008). If these conditions were to change, either in its method or the environment, then reproducibility is measured (Bartlett & Frost, 2008). Guidelines for reporting reliability and agreement studies (GRRAS) have been proposed for use by the EQUATOR network (Kottner et al., 2011). Although other guidelines have been suggested, including QAREL (Quality appraisal tool for studies of diagnostic reliability) (Lucas et al., 2013), for the purposes of assessing a diagnostic tool for a particular purpose, the STARD and QUADAS checklist suffice. An important and often misinterpreted, misreported statistic in DTA studies is the correlation coefficient. It measures if two methods measure the same quantity, but do not represent causality or agreement (Bland & Altman, 1986).

SECTION AND TOPIC	ITEM #		ON PAGE #
TITLE/ ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	
Test methods	7	The reference standard and its rationale.	
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	
	13	Methods for calculating test reproducibility, if done.	
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	
	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	
	22	How indeterminate results, missing data and outliers of the index tests were handled.	
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	

Table 3: STARD 25 - item checklist for DTA studies Bossuyt et al 2003.

SECTION & TOPIC	NO	ITEM
TITLE OF ABSTRACT		
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive value, or AUC
ABSTRACT		
	2	Structured summary of study design, methods, results and conclusions (for specific guidance, see STARD for Abstracts)
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test
	4	Study and hypotheses
METHODS		
Study design	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)
Participants	6	Eligibility criteria
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates)
	9	Whether participants formed a consecutive, random or convenience series
Test methods	10a	Index test, in sufficient detail to allow replication
	10b	Reference standard, in sufficient detail to allow replication
	11	Rationale for choosing the reference standard (if alternative exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory
	13a	Whether clinical information and reference standard results were available to the performers or readers of the index test
	13b	Whether clinical information and index test results were available to the assessors of the reference standard
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy
	15	How indeterminate index test or reference standard were handled
	16	How missing data on the index test and reference standard were handled.
	17	Any analysis of variability in diagnostic accuracy, distinguishing pre-specified from exploratory
	18	Intended sample size and how it was determined
RESULTS		
Participants	19	Flow of participants using a diagram
	20	Baseline demographic and clinical characteristics of participants
	21a	Distribution of severity of disease in those with the target condition
	21b	Distribution of alternative diagnoses in those without the target condition
	22	Time interval and any clinical interventions between index test and reference standard
Test results	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence interval)
	25	Any adverse events from performing the index test or the reference standard
DISCUSSION		
	26	Study limitations, including sources of potential bias, statistical uncertainty and generalizability
	27	Implications for practice, including the intended use and clinical role of the index test
OTHER INFORMATION		
	28	Registration number and name of registry
	29	Where the full study protocol can be accessed
	30	Sources of funding and other support; Role of funders

Table 4: STARD 2015 - An Updated list of essential items for reporting diagnostic accuracy studies Bossuyt et al 2015.

Current Recommendations

The diagnosis of PAD is based on a good history including risk factors, clinical symptoms and signs. Often the first examination performed is pulse palpation. In claudicants, pulse palpation has a positive predictive value (PPV) that ranges between 17.7% and 48.7% with a negative predictive value (NPV) between 90.1% and 97% (Criqui, M. H. et al., 1985). Subsequent studies have varied results. Aubert et al demonstrated a PPV ranging between 41.5% and 58.4% and a NPV between 73.9% and 89.8% in diabetics. The author suggested a combined pulse palpation and ABPI measurement strategy, which yielded a sensitivity of 92.3% and a NPV of 89.8% (Aubert et al., 2014). Williams et al reported a sensitivity of 93%, 87% and 81% and a specificity of 70%, 53% and 56% using pulse examination of controls, diabetics and diabetics with neuropathy respectively (Williams et al., 2005). The results of these studies reflect the subjective nature of pulse palpation, which is dependent on the examiner, as shown by Brearley et al where the sensitivity for the larger femoral pulse was 95% but ranging between 33 to 60% for vessels below the knee, between different medical staff (Brearley et al., 1992).

NICE has recommended the use of the Doppler based ankle brachial pressure index (ABPI) for the diagnosis of PAD. The recommendation is for ABPI to be done at rest, with the patient supine. The ratio of the highest ankle pressure divided by the highest arm pressure using an appropriately sized cuff and a Doppler probe to assess the pulse provides the ABPI. A ratio between 0.9 and 1.3 is considered to be normal. Ratios under 0.9 are indicative of PAD and those below 0.5 indicative

of CLI (NICE CG 147, 2012) . False negatives occur in the elderly (Wikstrom et al., 2008; Meijer et al., 1998; Carter et al., 2013), diabetics (Williams et al., 2005; Premalatha et al., 2002; Potier et al., 2011; Li et al., 2012) and in patients with chronic renal failure (O'Hare et al., 2004; Leskinen et al., 2002; Chen et al., 2012; Yezvlin & Gemelli, 2012). ABPI is often used in conjunction with the six-minute walk test (Fletcher et al., 2001). Exercise induced decrease in ABPI ratios are indicative of aortoiliac disease and have been suggested to be a secondary healthcare test (Crawford et al., 2016). A pressure drop of 15-20mmHg is indicative of stenosis (Wennberg, 2013). Significant decreases in ABPI of >0.15 does not exist until at least 60% stenosis has occurred (Baril & Marone, 2012). ABPI primarily remains the modality for diagnosis by GP (NICE CG 147, 2012; Norgren et al., 2007). In secondary care, NICE recommends the use of duplex ultrasonography as first line imaging (NICE CG 147, 2012). This is an easily available test that is quick and cheap, with a sensitivity of 88% and specificity of 96% for the detection of a 50% or more stenosis (Collins et al., 2007). However, it is operator dependent and requires a trained technician to perform. Additional imaging modalities suggested are computed tomography angiography (CTA) or contrast enhanced magnetic resonance angiography (MRA) (NICE CG 147, 2012). CTA has sensitivity of 91% and specificity of 91% (Collins et al., 2007). It carries the risks of radiation and renal impairment or injury. It is also time consuming and not easily available (Ouwendijk et al., 2008). Contrast enhanced MRA has a higher sensitivity of 95% and specificity of 97%. This review also found minimal difference in the sensitivity and specificity between contrast-enhanced MRA and duplex ultrasonography for the detection of above and below knee 50% stenosis. For contrast enhanced MRA, the sensitivity and specificity were 87% and 93% respectively (above knee) and 83%

and 92% respectively (below knee). For duplex ultrasonography, the sensitivity and specificity was 88% and 95% respectively (above knee) and 84% and 93% respectively (below knee) (Collins et al., 2007). MRA is more expensive than CTA and duplex ultrasonography. It is dependent on patient selection, is difficult to interpret due to artefacts and is not easily available (Ouwendijk et al., 2008). Therefore, duplex ultrasonography is a reasonable clinical diagnostic test commonly used in secondary care to triage patients before further investigations or management decisions are made.

Role of provocation tests in diagnostics

Exercise or stress tests has been used in combination with ABPI to diagnose PAD. Originally, stress tests were developed for cardiopulmonary assessment. In addition to challenging the cardiovascular system, the peripheral vasculature would also be challenged. In the case of PAD, stress testing is a functional capacity assessment, based on reproducing the claudication symptoms of patients. These patients have varying walking distances based on their comorbidities and conditions that could affect their normal walking (Coughlin et al., 2001). An optimum stress test should be reproducible, sensitive, safe, well tolerated and cost effective (Hiatt et al., 2014).

The most commonly used tests are the treadmill test and the six-minute walk test. The treadmill test has stringent criteria and requires the test to be standardized, to produce reliable results. The treadmill test is either a constant load test at a fixed walking rate and inclination (Degischer et al., 2002), or a graded treadmill test, wherein the inclination is increased every 2 minutes (Hiatt, W. R. et al., 1988). The

constant load test is regarded as the gold standard test for PAD stress testing (Coughlin et al., 2006; Coughlin et al., 2001). During the test, the patient should not hold on to the treadmill bars for support and should not stop until the patient reaches their maximum limit (Hiatt et al., 2014). The safety of this test has not been studied in the PAD population. Some studies have reported episodes of angina or shortness of breath with treadmill tests (Cameron et al., 1997; McPhail et al., 2001). A more practical test is the six-minute walk test. It is much easier to perform in comparison to the treadmill and more reflective of patients normal walking. In this test, patients walk at their normal pace. It has been suggested as a feasible alternative to the treadmill test for PAD (Hiatt et al., 2014). A variety of walking test exists, which is either time or distance based. Of these, the six-minute walk test has been the most researched and established (Solway et al., 2001). It is often difficult to perform as patients with shortness of breath, angina or joint problems and it has been demonstrated to be difficult to complete (Laing & Greenhalgh, 1980; Yamamoto et al., 2009). Concerns have been raised about its safety and recommendations exist to ensure patient safety. Unstable angina and myocardial infarction in the previous month, is an absolute contraindication for this test (American Thoracic Society, 2002).

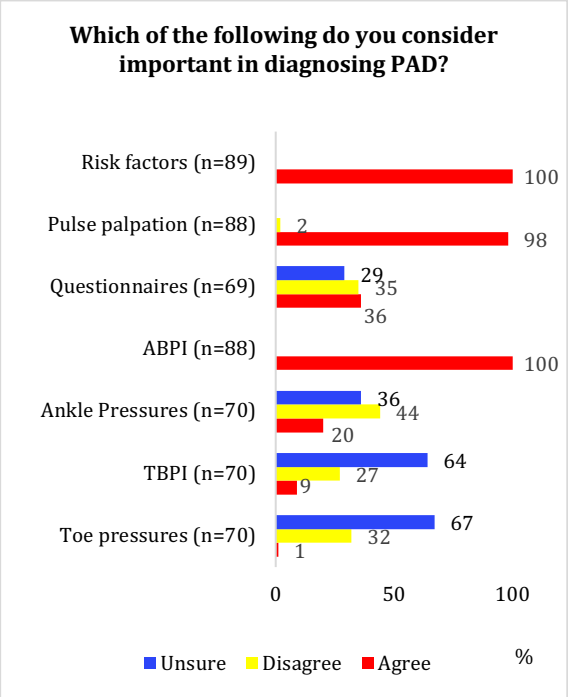
Alternative methods for reproducing a stress test specific for PAD has been assessed, including the use of ultrasound telemetry in the double physiological walking test (Coughlin et al., 2001; Coughlin et al., 2006), plantar flexion test (Yamamoto et al., 2009), the Stresst'er ergometer (Cameron et al., 1997; Cheetham et al., 2004), active pedal plantar flexion (McPhail et al., 2001), post occlusive reactive hyperemia test (Hoyer et al., 2013; de Graaff et al., 2000; Kragelj

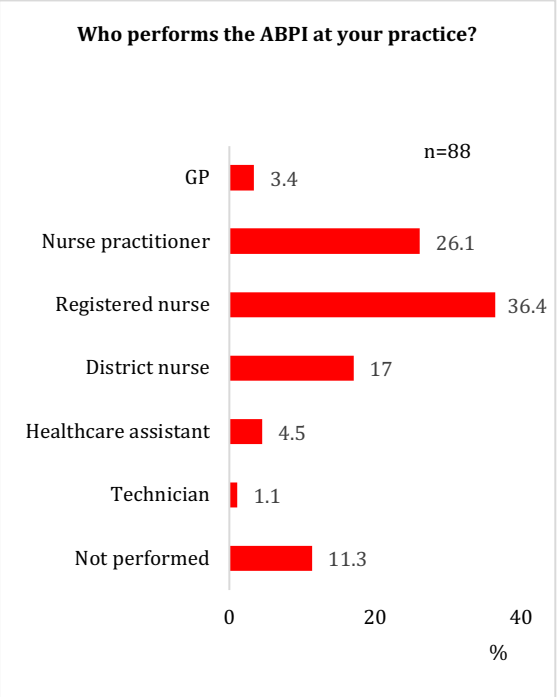
et al., 2001), isokinetic strength and endurance testing (Ritti-Dias et al., 2010). Studies have demonstrated a drop in systolic blood pressure in patients with PAD after stress testing (Wennberg, 2013; Baril & Marone, 2012). Existing studies demonstrate that the drop in ABPI pressure readings remains the same irrespective of the duration of the exercise (Laing & Greenhalgh, 1980; Cheetham et al., 2004), although the recovery time would be longer if the duration or the amount of 'stress' was higher (Laing & Greenhalgh, 1980). The best stress test for PAD has not been determined as yet. However, tests are increasingly less complicated, simpler to perform and specific to peripheral circulation. With increasing age, participants may have concomitant cardiopulmonary and joint disease which could limit their walking ability (Dziubek et al., 2015).

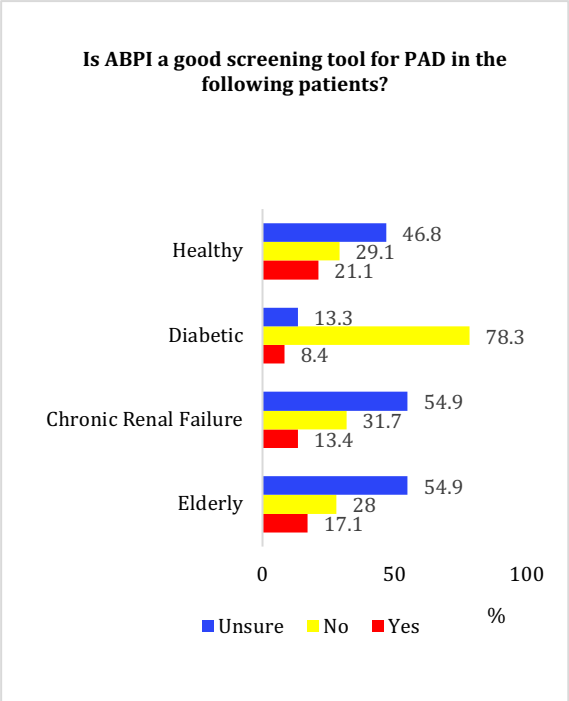
Challenges in primary care

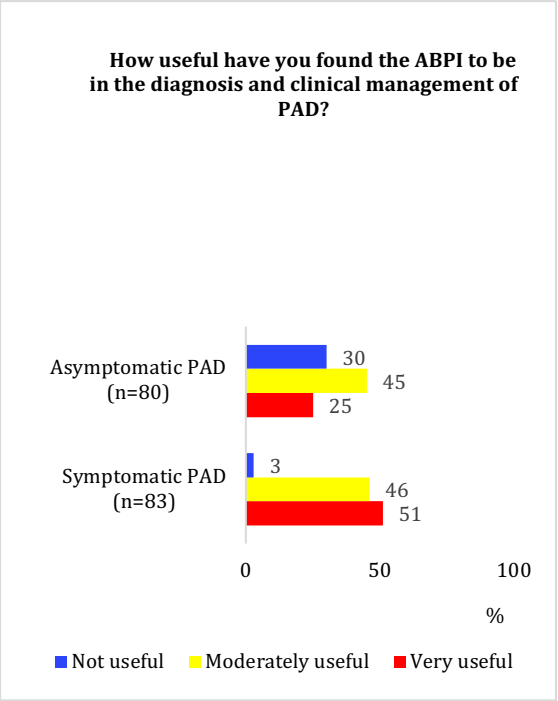
Recent and historical surveys on the use of ABPI have suggested that GPs should receive targeted training to perform and analyze ABPIs. Interestingly similar recommendations were made over a decade ago, following a large multicenter programme that assessed the practice and perceptions of primary care clinicians, in the PAD awareness, risk and treatment: New resources for survival (PARTNERS) in the USA (Hirsch et al., 2001). Following this, a PARTNERS preceptorship programme enrolled and trained primary care staff in the technique of performing and interpreting ABPIs. An ABPI usage survey conducted on participants of both the PARTNERS and the PARTNERS preceptorship programme summarized that primary care clinicians accepted ABPI as a simple diagnostic tool and their role to diagnose PAD despite existing barriers (Mohler III et al., 2004).

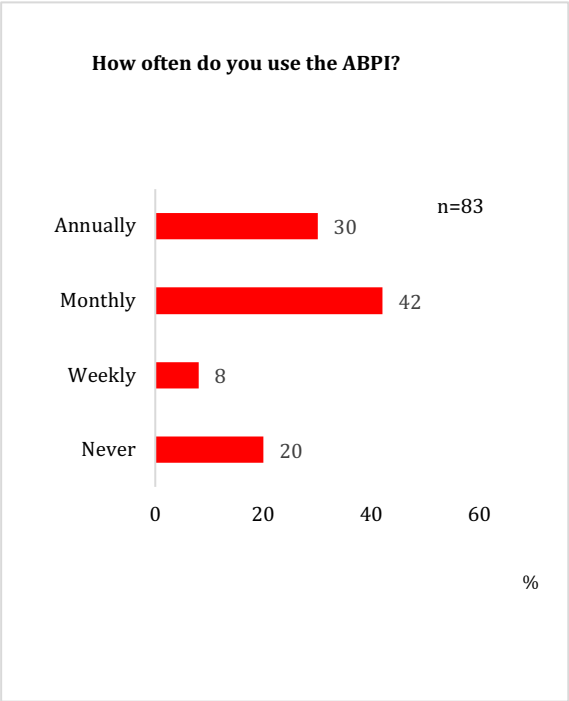
In Europe, surveys have shown that ABPI is rarely used to diagnose PAD in primary care (Davies et al., 2014; Blacher et al., 2006; Yap Kannan et al., 2016). In our survey, Leicestershire GPs opinions on ABPI and limitations to its implementation in primary care practice were studied. 70% and 97% of GPs found ABPI useful for the diagnosis of asymptomatic and symptomatic PAD respectively, although interestingly only 69% regarded it as a feasible test in primary care practice. Similar to previous surveys, limitations to the use of ABPI in primary care are time constraints and staff training. Additional limitations found were staff availability, financial constraints, availability of space and equipment, the presence of wounds, performance of a walk test and the interpretation of results (Figure 7) (Yap Kannan et al., 2016). ABPI has been reported to be cumbersome in nature, needs specially trained staff and is inaccurate (Moyer, 2013).

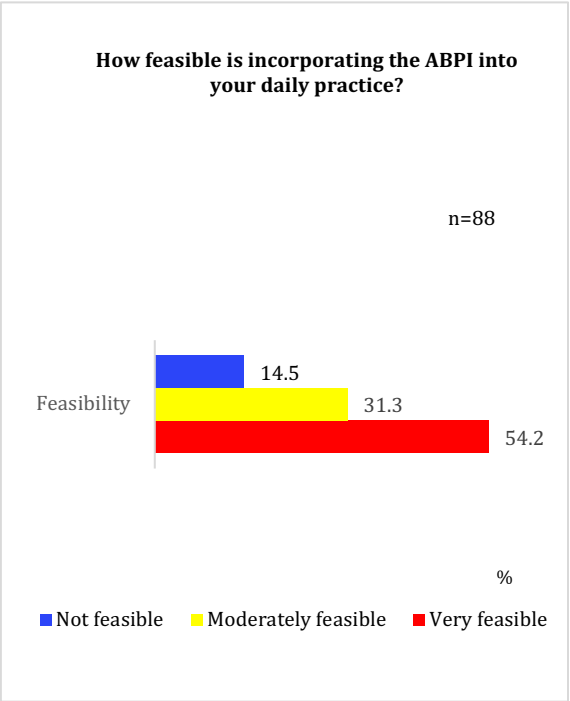





Is ABPI a good screening tool for PAD in the following patients?
Healthy
46.8
29.1
21.1
Diabetic
13.3
78.3
8.4
Chronic Renal Failure
54.9
31.7
13.4
Elderly
54.9
28
17.1
0
50
100
%
Unsure
No
Yes


How useful have you found the ABPI to be in the diagnosis and clinical management of PAD?
Asymptomatic PAD (n=80)
30
45
25
Symptomatic PAD (n=83)
3
46
51
0
50
100
%
Not useful
Moderately useful
Very useful


How often do you use the ABPI?
n=83
Annually
30
Monthly
42
Weekly
8
Never
20
0
20
40
60
%


How feasible is incorporating the ABPI into your daily practice?
n=88
Feasibility
14.5
31.3
54.2
%
Not feasible
Moderately feasible
Very feasible

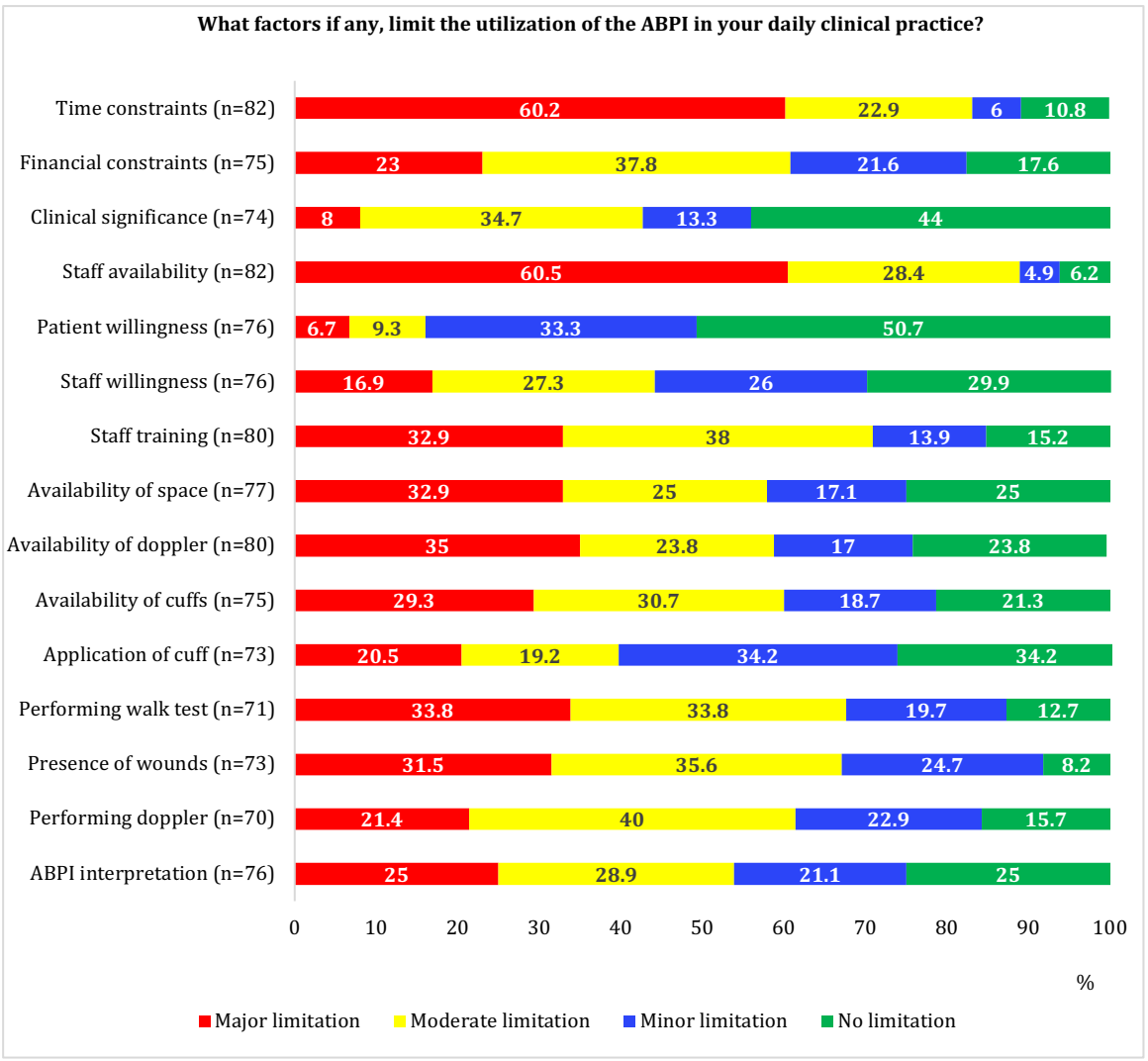


Figure 7: PAD survey responses. Yap Kannan et al 2016.

Recommendations for or against the use of ABPI have been divided (NICE CG 147, 2012; Moyer, 2013). Previous meta-analyses demonstrated a low sensitivity (15-79%) and high specificity (83-100%) (Dachun et al., 2010; Chung et al., 2010). There have been efforts to improve the sensitivity and specificity of ABPI, using Infrared based ABPI (Ro et al., 2013), automated oscillometric ABPI (Hamel et al., 2010; Clairotte et al., 2009; Premanath & Raghunath, 2010), pulse oximeter based ABPI (Papanas et al., 2010; Parameswaran et al., 2005), questionnaires combined with ABPI (He et al., 2006), modified calculation of ABPI (Schroder et al., 2006), refined pulse palpation with Doppler ABPI (Aubert et al., 2014), pulse wave velocity combined with ABPI (Khandanpour et al., 2009a) and photoplethysmography

based ABPI (Khandanpour et al., 2009b) with mixed results. ABPI measurements can be done with a handheld Doppler or in some countries using the strain gauge plethysmography technique (Joensen et al., 2008). A recent Cochrane review has suggested the insufficient evidence for the use of ABPI in diagnosing PAD (Crawford et al., 2016).

Management of PAD

Management of PAD often depends on the presence and severity of its symptoms or its sequelae. PAD patients can therefore be divided into symptomatic and asymptomatic groups. 7-15% of asymptomatic patients may develop intermittent claudication in 5 years (Fowkes et al., 1991). The aim in the management of PAD is to alleviate patient symptoms, increase ambulatory function, improve their quality of life and reduce cardiovascular risks (Hiatt et al., 2014). First line management of PAD is to manage modifiable factors, which includes lifestyle advice, smoking cessation, optimization of statins, antiplatelets, diabetic medication and control of hypertension (Bhasin & Scott, 2007). Intervention is considered when conservative measures fail. Detailed discussion of management is beyond the scope of this thesis.

Summary

Early identification of PAD would allow early management by conservative means. The use of ABPI to triage patients with claudication has been questioned due to the lack of evidence (Crawford et al., 2016). Current evidence suggests that more studies are required to assess the use of ABPI and newer diagnostic tests. In this study, I have set out to assess the reliability of novel diagnostics as triaging tool.

Chapter 2: Review of novel diagnostics and aim of study

This study is aimed at identifying alternative devices that can be used to diagnose PAD. It should therefore be easy to use, interpret, cost effective and be useful in both primary and secondary care. Diagnostic devices for PAD, either measure structure or function or both. The structural presence of PAD does not imply that treatment is necessary, especially in the absence of symptoms or signs. Structural assessment of PAD is commonly performed in secondary care, ie Magnetic resonance angiography (MRA), computed tomography angiography (CTA) or duplex. The most commonly used assessment used to date to diagnose PAD has been the ABPI. However, the role of ABPI however has been questioned recently (Crawford et al., 2016). To get the best results from novel diagnostic devices, it is important to understand how it works, the environment best suited for these devices, its strengths and weaknesses. To understand better these aspects of novel diagnostics, a topical review of alternative diagnostic devices for PAD was conducted.

Topical review of alternative devices

To understand the role of alternative diagnostic devices, a systematic review was conducted. Searches were conducted using a PICO search strategy (Table 5). Search terms used were combined using the BOOLEAN terms ‘or’ for each of the subsections, ie population (P), Investigation (I), Comparison (C) and Outcome (O). Each of these search categories were then combined using the BOOLEAN term ‘And’ (Table 5). Published literature on diagnostic test accuracies (DTA) of primary non-invasive diagnostic tests for PAD, available from Medline, Embase and Cochrane library database are reported in this topical review (Moher et al., 2009). The search

was limited to humans. Angiography or duplex imaging were accepted as the gold standard for PAD diagnosis, with ABPI considered an imperfect reference standard (Khan et al., 2008).

Initial search results yielded 11,723 articles. Of this, 127 eligible articles based on the title and abstracts were identified. Full texts of eligible articles were reviewed. Novel diagnostics not used for PAD (n=58), with inappropriate reporting outcomes (n=32), reporting prognostic accuracies (n=15), invasive (n=3) or that required specialist technical skills (n=5) were excluded (Figure 8). Fourteen articles were selected, with an additional 2 articles included from the reference list of full text articles shortlisted. Acceptable gold standards ranged from a minimum investigation of duplex to more sophisticated tests, which were computed tomography angiograms (CTA), magnetic resonance angiograms (MRA) or catheter angiography. Of the resulting 16 articles, 8 studies utilized an acceptable gold standard (duplex, computed tomography) for this review. PRISMA guidelines and flowchart are used to illustrate patient selection (Figure 8). In these studies, we report the sensitivity and specificity according to the standards for reporting of diagnostic accuracy (STARD) (Bossuyt et al., 2003) (Table 7). The remaining 8 articles were assessed according to the guidelines for reporting reliability and agreement studies (GRRAS) (Kottner et al., 2011), as ABPI was the reference standard (Table 8). In these studies, we report the limits of agreement and percent agreement (Kottner et al., 2011; Bland & Altman, 1986; Landis JR, 1977; Viera & Garrett, 2005; Meier, 2007), where available. When appropriate reporting outcomes were not provided, a 2 by 2 contingency table was reconstructed and re-analyzed with available data.

1. Population*	Peripheral arterial disease, peripheral arterial occlusive disease, PAD, PAOD, claudicants, critical limb ischemia, CLI, acute limb ischemia, ALI, Fontaine, Rutherford
2. AND investigation*	Ankle brachial pressure index, ankle brachial index, ABPI, ABI, magnetic resonance angiography, magnetic resonance angiogram, MRA, magnetic resonance imaging, computed tomography, computed tomography angiography, computed tomography angiogram, CT, CTA, angiography, digital subtraction angiography, DSA and duplex ultrasound
3. AND Comparison*	Toe brachial pressure index, toe brachial index, TBPI, TBI, laser doppler flowmetry, laser doppler fluxmetry, LDF, laser doppler imaging, LDI, laser speckle contrast imaging, LSCI, laser speckle contrast analysis, LASCA, laser doppler anemometer, LDA, near infrared spectroscopy, NIRS, capillaroscopy, dermatoscopy, spectroscopy, transcutaneous oximetry, $tcpO_2$, $pctO_2$, oximetry, pulse oximetry, thermometry, infrared thermometry, IR thermometry, thermography, infrared, IR, IRT, thermal imaging, hyperspectral imager, multispectral imager, thermal imager, hybrid, non-invasive, handheld, pulse volume recording, pulse wave velocity, photoplethysmography, PPG, skin perfusion pressure, SPP and segmental pressure
4. AND Outcome*	Diagnosis, detection, recognition, interpretation and identification

Table 5: PICO search strategy; *Boolean term OR for each search term

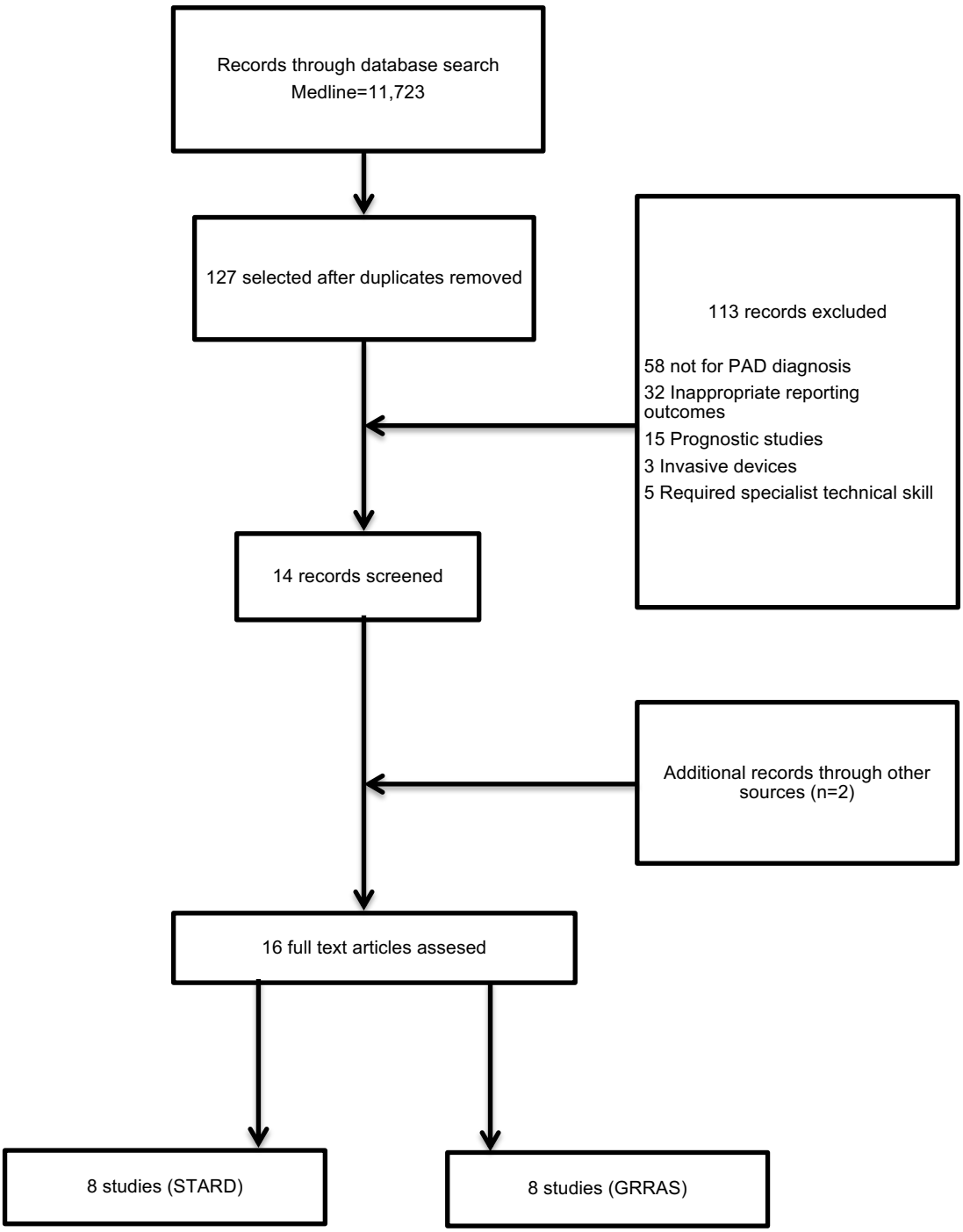


Figure 8: PRISMA flow diagram for selection of DTA studies.

Pressure measurement-based devices

TBPI

Digital arteries are not affected by atherosclerosis, making the Toe Brachial Pressure Index (TBPI) a suitable alternative to ABPI (Mukherjee & Eagle, 2010; Ubbink, 2004). However, the feasibility of toe pressures in detecting peripheral arterial disease (Bonham et al., 2010), is usually limited by the absence of digits or presence of wounds (Ezio et al., 2010). As a consequence, studies comparing TBPI to angiographic or duplex imaging has been restricted to certain group of patients (Høyer et al., 2013), with varied results. Selective testing with TBPI when ABPI is raised >1.3 has been suggested (Brooks et al., 2001). Prior to the agreed cut off of 0.7 (Norgren et al., 2007), the cut off for TBI was variable and lacked standardization, with studies using TBPI ranging from 0.54 to 0.75 (Norgren et al., 2007; Rooke et al., 2011). Current TBPI measurements are time consuming, require training and are usually performed in the vascular laboratory.

Plethysmography

Plethysmography is a device that measures a change in blood volume over time in a limb. There are divided into electrical, mechanical plethysmography and photoplethysmography. Electrical plethysmography measure voltage changes in relation to blood flow or blood volume. Measurements could be done using impedance (IPG), mercury or silastic (SGP), photoelectric (PPG) or phleborheography (PRG). Mechanical plethysmography is based on displacement of air or water in relation to volume changes. Of the three techniques, Photoplethysmography has shown great promise. A decade ago, functional photoplethysmography (FPPG) was reported to be a quick, cuffless technique that involves placing an optical probe on the second toe at

supine and 45 degree elevation. FPPG was only slightly more sensitive than ABPI (83% vs 80%) in detecting PAD in diabetics, using duplex angiography as the reference standard (n=26) (Alnaeb et al., 2007). The same group then included measurement of skin redness using red light in the assessment of non-diabetics (n=68). Despite this, the sensitivity of fPPG was 86% versus 80% for ABPI, with a PPV of 85% and a NPV of 86%. Unfortunately, the normal range for the 'perfusion index' was not provided, and the predictive values of ABPI are not analysed. Allen et al reported a significant agreement between PPG and ABPI, with a negative and positive predictive value of 99% and 98% respectively (Allen et al., 2008). ABPI was used as the reference standard. At the time, analysis was time consuming, sensitive to body position and temperature changes and underestimated perfusion (Jorfeldt et al., 2003).

However, a recent review by Castaneda et al, the newer wearable PPG devices are reported to be better, uncomplicated devices capable of measuring heart rate, pulse oximetry and provides more information when analysing the second derivative wave. These devices use green LED light which is less sensitive to motion artefact and penetrate deep into tissue. The newer PPG devices have a light source and photodetector and measure in 2 distinct patterns, the transmission and reflectance mode. In the former, there is tissue between the light source and sensor, suitable for earlobe or fingertip measurements. In the latter, the sensor and light source are on the same side to measure reflected light, suitable for the ankle, wrist, forearm and torso (Castaneda et al., 2018) . The use of these devices has not been tested as yet for PAD diagnosis.

Transfer Function Index of Pulse Volume Recordings

Pulse volume recordings (PVR) are independent of arterial calcification and can be obtained from segmental pressure recordings (Ro et al., 2013). The PVR from each cuff assessed is averaged using a 128-point Fast Fourier Transformation (FFT) producing a frequency response curve for each waveform. The ratio of the distal (outflow) frequency response curve to the proximal (inflow) frequency response curve (FTp) determines the transfer function. The area under the transfer function curve is called the transfer function index (TFI), which represents the patency of the segment of blood vessel interested (Doverspike et al., 2004). Nakashima et al utilised TFI of the ankle (TFI_{ankle}) and calf (TFI_{calf}), together with a venous occlusion test, to produce a measure of arterial inflow into the limb (Nakashima et al., 2009). A ratio of <1 was regarded as indicative of PAD. Since the selection of devices for this study, a further study conducted by Zainuer et al compared ABPI, TBPI and TFI in patients with PAD with >50% stenosis diagnosed by CT, MR or duplex as the reference standard (Zainuer et al., 2016) . In this latter study, a cut-off value for TFI of <1.025 is more accurate than an ABPI <0.9 or a TBPI <0.65 in diagnosing PAD.

It is interesting to note that in this study that TFI is only slightly more sensitive than ABPI in detecting non-calcified PAD. TFI apparently takes 5 – 10 minutes to perform, not painful for the patient and is easy to interpret (Ihlberg et al., 2001; Tan & Civil, 2003; Nakashima et al., 2009). The drawbacks however are that it may be unreliable in patients with arrhythmias and is operator dependent (Tan & Civil, 2003; Ihlberg et al., 2001).

Perfusion measurement-based diagnostics

Laser Technology

Laser technology is divided into Laser Doppler and Laser Speckle technologies. Laser Doppler measures microcirculatory flow and works on the principle of the 'doppler shift'. When the photon (from the laser source) hits the moving blood cells, some of these will scatter and some reflected back to the detector. These reflected photon beams are measured and provide a relative measure of total blood flow (flux) which is the concentration of RBCs multiplied by their average velocity at that point, expressed as arbritary perfusion units. Blood flow can be detected using a fibre optic probe (Laser Doppler Flowmetry, LDF) or imaging systems (Laser Doppler Imaging, LDI).

Studies assessing the diagnostic potential of LDF have been limited by heterogeneous parameters used in different studies. The absence of an agreed cut off threshold for PAD also lends difficulty in interpretation of results and its clinical significance. These parameters are either perfusion or time-based measurements (Hoffmann et al., 2008). Time rather than perfusion related parameters offer a better interday and intraday reproducibility for PAD diagnosis, in comparison to transcutaneous oximetry and capillaroscopy measurements. The latter have good interday and intraday reproducibility for all its parameters (Klomp et al., 2000). LDF waveforms were differentiated into 3 types by Ray et al. Type 1 curves had one rise in the LDF curve within 10 seconds and was proposed to be associated with normal flow or single proximal occlusion but good infrapopliteal run off. A type 2 curve had 2 initial rises in the curve and was proposed to be associated with multiple proximal

occlusions but good distal run off. The type 3 curve do not have any rise in the LDF and proposed to be associated with proximal occlusion and no distal run off. Using these criteria, the author concluded that the PPV for PAD using type 1 and 2 curves was 83%, and 86% for type 3 curves (Ray et al., 1999). Although all patients had ABPI and digital subtraction angiography (DSA), there is lack of substantial analysis to allow clinical utilisation of the results.

LDF is very sensitive to low pressure change (de Graaff et al., 2000; Ubbink, 2004). It has been used as an aide for ABPI and TBPI measurements in combination with a post occlusive reactive hyperaemia test. This role of LDF has been compared to Strain Gauge Plethysmography (SGP) based ABPI (Hoyer et al., 2013) and Photoplethysmography based ABPI. LDF measurements were possible on all toes, not dependent on the size of the toe and not as sensitive to movement as PPG (de Graaff et al., 2000).

LDF measures microcirculatory changes independent of skin colour and oxygen perfusion, with a penetration depth of 1mm irrespective of setup and different models of skin (Fredriksson et al., 2009). LDF provides single point measurements. Its main drawback is, that it is influenced by ambient light, patient movement, position and pressure of the skin probe (Hoffmann et al., 2008). However, measurements at a fixed marked point can produce good reproducibility (Roustit & Cracowski, 2012).

Other laser technologies available are the Laser Doppler Imaging (LDI) and the Laser Speckle Contrast Imaging (LSCI). LDI is a scanning method that is non-contact based and measures perfusion over a larger area of skin (up to 50x50cm^2) (O'Doherty et al., 2009) . LSCI is based on the random interference pattern produced when coherent light is scattered from a medium onto the camera detector. The red blood cell motion

scatters particles can be analysed to determine blood flow (Boas & Dunn, 2010). Specialised LSCI equipment is expensive, requires technical skill and is difficult to interpret. Attempts have been made to produce more cost-effective alternatives with good agreement with LSCI (Richards et al., 2013). Both LDI and LSCI have not been used to diagnose PAD in humans as yet. These techniques are non-contact and overcome the pressure artefacts. However, neither can distinguish RBC movement from movements caused by artefacts (Eriksson et al., 2014).

Laser Doppler Flowmetry

Laser Speckle Contrast Imager

Figure 9: LDF and LSCI (Courtesy Moor Instruments)

Capillaroscopy/Dermatoscopy

Capillaroscopy is a technology used to assess the morphology of capillaries, mainly in the nailfolds where capillaries are horizontal in position. On normal skin, capillary loops are perpendicular to the surface of the skin only allowing capillary density to be assessed. Capillary density does not correlate with the abnormal ABPIs of claudicants and patients with CLI (Lamah et al., 1999). Capillaroscopes are big, not portable, time consuming and require specially trained staff and have not been used for PAD diagnosis yet.

Videomicroscopic techniques are contact based and include Optical Polarization Spectral imaging (OPS) and Side stream Dark Field (SDF). Both allow point measurement and direct visualization of RBCs, which appear dark. Videomicroscopic techniques are sensitive to pressure and movement (Eriksson et al., 2014). Difference between perfusion devices are described in table 6.

	Video-microscopy *(OPS & SDF imaging)*	***Laser Doppler Perfusion Imaging***	**Laser Speckle Contrast Imaging**
Variables measured	Vessel diameter, RBC velocity, Heterogeneity of perfusion	RBC mean velocity RBC concentration	RBC mean velocity RBC concentration
Contact	Yes	No	No
Measuring depth	~500µm	~1 – 1.5mm	~300 µm
Pros	Direct visualization of microcirculation	Non-contact	Non-contact Fast
Cons	Motion artifacts Pressure artifacts Time consuming analysis	Motion artifacts Long measurement time Relative measurements	Motion artifacts Relative measurements

Abbreviations: OPS – Orthogonal polarization spectral, SDF – Side-stream darkfield, RBC – Red blood cells

Table 6: Differences between perfusion measurement devices (Eriksson et al. 2014).

Oxygen measurement-based diagnostics

Pulse oximetry

Pulse oximeters have become a vital part of clinical practice. The pulse oximeter consists of a light emitting diode and the light detector (photo-detector). The light emitting diode emits two different wavelengths of light (red and infrared). Most of it is absorbed by blood and tissue. This value remains constant. Over a short period of time, the only variable is the pulsatile oxygenated blood. This can then be isolated from the constants and measured (Philips Medical Systems SpO Monitoring, 2002). Pulse oximetry is commonly used on the fingers, earlobes and the toes. The pulse oximeter probes are either hinged, rubber or disposable plaster based. The pulse

oximeter is an attractive tool because it is easy to use, affordable, operator independent and does not require calibration (Couse et al., 1994).

One often quoted study showed a significant correlation between pulse oximetry and transcutaneous oximetry (Joyce et al., 1990), although this is not unexpected given both devices measure oxygen levels (Bland & Altman, 2003). The sensitivity, specificity, PPV and NPV of pocket pulse oximeter-based finger to toe oxygen saturations in patients with ABPI diagnosed PAD was reported to be 42.6%, 79.1%, 35.7% and 83.4 % respectively (Ena et al., 2013). ABPI was used as the reference standard in this study and therefore the results are not truly diagnostic. Similarly, Bianchi et al studied the accuracy of the Lanarkshire oximetry index in patients with Doppler ABPI diagnosed PAD. The Lanarkshire oximetry index (LOI) essentially substitutes the pulse oximeter for the handheld Doppler device. The index was developed as a means of identifying patients with PAD, prior to application of compression dressings for venous ulcers. The author reported a sensitivity and specificity of 93.3% and 89.1% respectively for a LOI <0.9 and a sensitivity and specificity of 40% and 99.3% for LOI <0.8. Once again Doppler ABPI has been used as the reference standard (Bianchi et al., 2008). Parameswaran et al studied the use of pulse oximetry in diagnosing PAD in patients with type 2 diabetes. In this study, a monophasic waveform on any vessel was considered diagnostic for PAD. He reported a sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of 77%, 97%, 30% and 24.8% respectively (Parameswaran et al., 2005). Once again, waveform analysis is not an appropriate reference standard. Therefore, results need to be interpreted with caution.

From a methodological standpoint, the use of standard pulse oximeter finger probes on adult toes is flawed. Current pulse oximeters are meant for fingers or earlobes. For readings on the toe to be accurate, the seal around the toe needs to be able to reduce penetration of external light and keep the light emitting diode and light detector in the same line. Currently, there are no adult sized toe pulse oximeters. An oximeter that is too tight around an inappropriately sized digit will compress the digit resulting in venous pulsations. The pulse oximeter identifies arterial flow by its pulsatile motion. Additional venous pulsations would also be measured in this case, resulting in false readings (Pulse Oximetry & Training Manual, 2011; Philips Medical Systems SpO Monitoring, 2002).

Couse et al performed a pilot study (n=27) to assess the time to return of pulsatile flow as detected using the pulse oximeter, using the post occlusive reactive hyperaemia test as the stress test. Although, the numbers were small, it was interesting to note that the time to return of flow in patients with angiographically proven PAD was longer than for control patients. No further studies have reported the accuracy of pulse oximetry by this method since.

Since its humble beginnings, pulse oximeters have advanced from wired devices to wireless Bluetooth devices, which are not sensitive to movement. In a small prospective study (n=49) in Korea, signal extraction pulse oximetry was used to identify patients with PAD. All patients subsequently underwent CTA as part of their management. The reported sensitivity, specificity, PPV and NPV was 87.06%, 87.8%, 84.3% and 90% respectively (Kwon & Lee, 2012). This new device has the added advantage of being insensitive to movement compared to conventional pulse oximeters. In the assessment of the presence of PAD in asymptomatic diabetics,

Kumar et al reported a sensitivity, specificity, PPV and NPV of 74.1%, 95.7%, 83.3% and 92.7% respectively compared to ABPI which was 70.3%, 87.1%, 61.3% and 91% for the same parameters. The author used duplex as a reference standard (Kumar et al., 2016).

Limited good quality data on the accuracy is available for the role of pulse oximetry in PAD. Technology has advanced and more recent studies have used appropriate reference standards. Adherence to STARD and QUADAS guidelines would help overcome poor methodological quality of diagnostic studies (Brownrigg et al., 2016).

Transcutaneous oximetry

Transcutaneous oximetry ($tcpO_2$) measures superficial skin oxygen levels. It is not similar to pulse oximetry, as local heating of the skin with the probe is required before measurements are taken. This allows for maximal vasodilation and provides levels of oxygenation at the temperature of the probe. Readings will need to be adjusted for body temperature during analysis. In healthy feet, tcpO2 levels should be more than 50 mmHg (Hauser & Shoemaker, 1983), with threshold levels for hypoxia varying between 30 to 40mmHg (Andrews et al., 2013; Hauser & Shoemaker, 1983; Ubbink et al., 1997; Bunt & Holloway, 1996). 98% of patients with tcpO2 levels less than 30mmHg undergoing angiographic examination have significant arterial disease (Bunt & Holloway, 1996). In a study of diabetic patients with critical limb ischemia with angiographic evidence of significant PAD, although the ankle pressure was more than 70mmHg, the foot oxygen tension was less than 50mmHg, prompting the authors to conclude that foot oxygen tension as an essential tool for diagnosis of CLI in diabetics irrespective of the ankle pressures (Ezio et al., 2010). Haemodialysis patients with abnormal tcpO2 of less than 40mmHg at the outset, are 7 times more likely to die at

1 year than those with normal tcpO2 and may warrant more aggressive treatment (Benhamou et al., 2012).

Buttock pain can be caused by PAD or lumbar spinal stenosis. Approximately one out of 7 patients with buttock claudication have proximal without distal arterial disease. Exercise based tcpO2 can help diagnose this condition even when ABPI is borderline or normal (Gernigon et al., 2013). Abraham et al assessed 3 parameters of tcpO2, i,e Rest (baseline), Min (lowest value during exercise) and the Drop (difference between baseline to the minimum value). All 3 parameters were assessed in 2 groups of patients with angiographically proven PAD (buttock and calf claudicants). Using ROC analysis, the Drop values provided the highest diagnostic performance for both buttock claudicants, with a reported sensitivity, specificity, PPV and NPV of 78.8%, 85.7%, 83.9% and 81.1% respectively (Abraham et al., 2003).

Another measure of transcutaneous oxygen level is the Transcutaneous Regional Perfusion Index (RPI). Raw tcpO2 values are sensitive to body position and more importantly to the oxygen levels of the whole body. It may be falsely depressed in the presence of cellulitis or oedema (Bunt & Holloway, 1996). As RPI is a ratio of tcpO2 levels in the area studied and the chest tcpO2, the changes that occur due to these is eliminated and is more reflective of local tissue perfusion oxygen pressures (Hauser & Shoemaker, 1983). It is a consistent and accurate tool for assessment of PAD in diabetics (Hauser et al., 1984).

In the past, the use of tcpO2 has been limited due to a large variability in cut off values and agreement of measurements to be used. Additionally, this is a time-consuming test and may not be feasible as a triage tool. Nevertheless, efforts to standardize

values and improve reporting outcomes have started (Fife et al., 2009), which will allow its translation into common practice. The application of an affordable transcutaneous oximeter as a suitable triage tool, needs to be assessed. Its role in prognostication device has been studied but is not the aim of this study (Bouyé et al., 2005; Abraham et al., 2003).

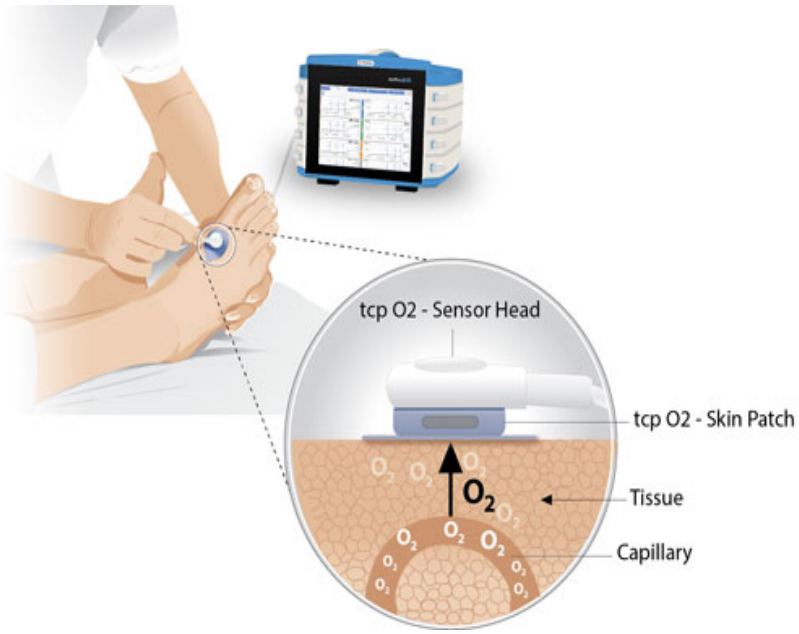


Figure 10: Transcutaneous oximetry. Reproduced from Perimed instruments Image courtesy Perimed instruments (https://www.perimed-instruments.com)

Near Infrared Spectroscopy (NIRS)

NIRS measures the oxyhaemoglobin levels, by measuring the differential absorption of Near-infrared light by haemoglobin (Bauer et al., 2004; Kragelj et al., 2001). Light penetration depth of NIRS depends on the tissue optical properties and the source-detector separation. Penetration depth is one half to one third of the source-detector separation (Yu, 2012). Adipose tissue thickness can influence NIRS measurements (Nakashima et al., 2009). Previous studies have focussed on the correlation of NIRS

with ABPI (Malagoni et al., 2010; Kooijman et al., 1997; Cheatle et al., 1991; Watanabe et al., 2004). Unlike transcutaneous oximetry, NIRS offers the advantage of measuring oxygenation beneath the surface of the skin (Bouyé et al., 2005; Abraham et al., 2003).

NIRS measurements are either stated as absolute values or time related values. The recovery time of StO2 (tissue oxygen saturation) is a good measure to detect abnormalities in blood flow (Sugano et al., 2003; Comerota et al., 2003). Another NIRS parameter, the resting muscle oxygen consumption ($RmVO_2$) is performed after venous occlusion (Malagoni et al., 2010), represents local muscle capillarization and is higher in severe PAD (Manfredini et al., 2012). The recovery time (T_{50} and T_{100}), following an exercise test in claudicants demonstrates a significantly faster recovery of StO_2 than the ABPI, and is proportional to the amount of tissue. This is because the oxygen is under-utilised during exercise and is therefore more easily available when tissue is recovering. The artery however, remains dilated to provide more oxygen to the hypoxic tissues to repay the 'oxygen debt' (Watanabe et al., 2004). Therefore, poor flow does not imply poor oxygenation (Bouyé et al., 2005).

Although the StO_2 levels may be low post exercise, claudicants may still have a SBP>50mmHg even once maximum walking distance has been achieved (Khurana et al., 2013). A study on a small series of non-diabetic patients, looked at StO2 changes in healthy and PAD patients following exercise, demonstrating no change with mild exercise. However, with increasing severity of exercise, there was an initial increase in StO2 levels in both groups followed by a decline in StO2 levels, which was prolonged in PAD patients (Bauer et al., 2004). This is suggested to be secondary to

the ‘metabolic inertia’ due to a proposed mitochondrial myopathy and a lack of ATP in PAD patients when faced with increasing demands (Greenhaf et al., 2004).

NIRS is a promising tool that provides deeper tissue oxygenation and needs further studies to determine its agreement with ABPI, sensitivity, specificity and diagnostic accuracy, before it can be utilised as a diagnostic tool for PAD screening. Real time wireless utilization of NIRS has already been investigated in sport medicine studies (Hesford et al., 2012). More affordable versions of the device are available for commercial use at the moment.

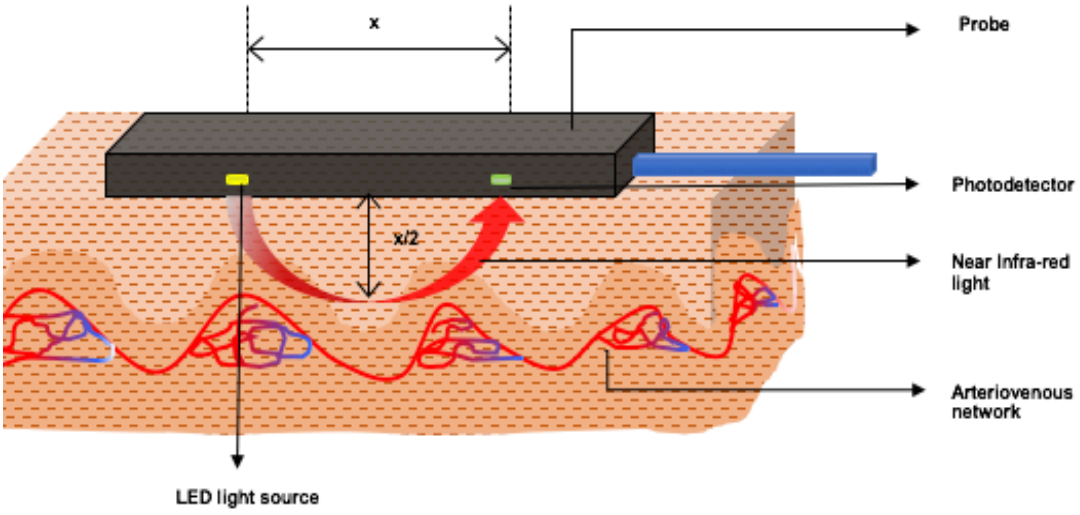


Figure 11: Near infrared spectroscopy. The penetration depth is half (x/2) the distance between the LED light source and the photodetector (x).

Hyperspectral cameras

Hyperspectral imaging is based on the analysis and interpretation of the electromagnetic spectrum of colours. Superimposition of spectral signatures (imprints) creates the hyperspectral image.

Chin et al set out to demonstrate its usefulness in PAD patient comparing hyperspectral imaging in 9 angiosomes of the leg against ABPI. The findings of this study demonstrated an inverse relationship of deoxygenated haemoglobin with ABPI. There was no significant relationship between ABPI and oxygenated haemoglobin as determined by hyperspectral imaging. This was particularly so, at the plantar aspect of the foot, which was attributed to the rich arteriovenous anastomoses present in this region of glabrous skin (Chin et al., 2011).

Hyperspectral cameras require a complex setup which is time consuming and require technical expertise to interpret data obtained. These factors and its current costs may limit its utilisation as a feasible triage diagnostic technology at present.

Temperature measurement-based diagnostics

Thermography

Thermoregulation is essential for normal physiological processes (Bouzida et al., 2009). The human body emits heat (Hardy, 1934) and electromagnetic radiation known as infrared or thermal radiation, which can be subdivided into the near, medium and far infrared radiation (Jones, 1998; Maldague, 2001). Medical thermography can be either contact or non-contact based. Skin flap perfusion at the site of anastomoses

has been studied in plastic surgery (Armstrong et al., 2006) and breast oncological surgery (Lawson, 1956).

It has been suggested that temperature of identical areas on either limb, should not differ by more than 1°C, with any difference of more than 2.2°C being abnormal (Armstrong et al., 2003). The use of this temperature assessment has been suggested to indicate early diabetic foot disease (Armstrong DG, 1996).

Absence of neurological reflexes leads to lack of thermoregulation, due to inability to vasodilate, causing peripheries to have increased temperatures and is a useful predictive sign of foot ulceration and subclinical inflammation of the feet (Roback, 2010). Armstrong and Lavery used temperature monitoring as a means of screening and self-monitoring for diabetic foot re-ulceration in three randomized trials (Lavery et al., 2004; Armstrong et al., 2007). Temperature is elevated in areas of inflammation at least 4.8 times in areas that are at risk of re-ulceration in diabetics (Armstrong et al., 2007) and this increase can alert diabetics to reduce activity and re-ulceration rates (Lavery et al., 2007).

Thermal imaging is influenced by the position, in which images are taken, hot water consumption, room temperature, periods of the day images are taken. It is not influenced by the amount of time taken for the body to acclimatise to room temperature (Nkengne et al., 2013). Contact based surface temperature measurements are sensitive to excessive pressure from the sensors (Bharara et al., 2006). Although infrared thermography is normal at rest in patients with and without PAD, post exercise induced changes are significant (Huang, C. L. et al., 2011).

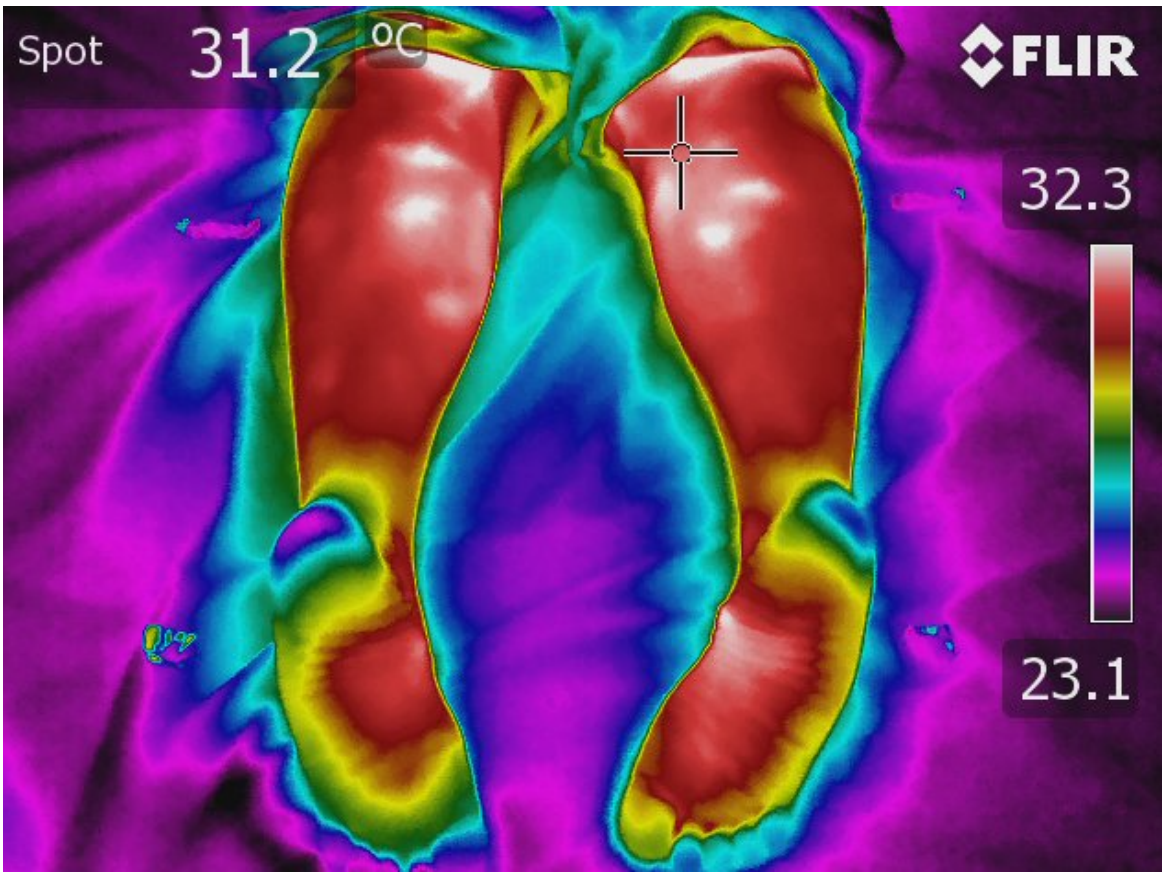


Figure 12: Thermal image of calf and heel in normal patient

Other alternatives

Hybrid Devices

Hybrid devices that combine different technologies are being developed. In the past, Laser Doppler Anemometer (LDA) combined the use of LDF with capillaroscopy to estimate the exact flow through a single capillary. It includes a source of laser beam that is split. The optical image forming system measures the backscattered signal. LDA was compared against LDF and tcpO2 in the peri-ulcerous skin of patients with venous and mixed arterio-venous ulcers, the latter all having an ABPI < 0.59 +/- 0.07. In contrast to tcpO2 and LDF devices, the LDA was able to detect an increase in blood flow and capillary density after the administration of Prostaglandin E1 (Stucker et al.,

2004). The advantage of the LDA is in its ability to assess capillary blood velocity and capillary density, despite the skin capillaries being perpendicular to skin surface, producing more reproducible results (Stucker et al., 1996).

Another hybrid device, combines blood flow using Diffuse Correlation Spectroscopy (DCS) and oxygenation using Diffuse Reflectance Spectroscopy (DRS) (Yu et al., 2005). Instead of the single scattering of light, multiple scattering occurs from a light source. This traverses through different paths in the tissue providing a better measurement of perfusion and oxygenation. This technology has shown much promise in the field of head and neck, breast and neurosurgery (Yu, 2012). DCS combined with NIRS (DCS-NIRS) has been studied to evaluate the Tissue Metabolic Rate of Oxygen consumption ($TMRO_2$), the metabolic index, derived from the oxygenation levels and flow results (Yu et al., 2005; Yu et al., 2011; Yu, 2012).

The role of DCS-NIRS and LDA as a triaging tool for PAD diagnosis needs further assessment. However, there is much promise, with newer more portable devices being tested in small studies in healthy participants, which need further validation. The advantage of such a technology that combines oxygenation and perfusion measurement, is that it is more affordable, smaller, easier to operate and measures both oxygenation and perfusion parameters through the same volume of tissue (Shang et al., 2009).

Smart phone technology

It is inevitable that smart phone technology will become part of our practice in the future (Yamada et al., 2011; Woods et al., 2011). Smart phone technology has been used in diabetes care (Charpentier et al., 2011), falls prevention (Boulos et al., 2011),

delivery of physical activity intervention (Kirwan et al., 2012; Worringham et al., 2011) and ECG recording (Oresko et al., 2010; Hii & Chung, 2011). A new exciting development is a portable handheld smart phone doppler ultrasound (Huang, C. C. et al., 2012) or smart phone enabled flow monitoring device (Wu et al., 2016). Although initial experiences with the pocket-siized echocardiograms are currently unable to replace the standard echocardiogram (The European Association of Echocardiography) (Sicari et al., 2011), further studies will be required to validate smart phone technology in PAD.

Table 7 shows studies that have compared novel diagnostics against an accepted standard for PAD diagnosis. Table 8 provides details of studies that have not used accepted standards. The analysis of these studies has been interpreted as agreement due to this.

Table 7: Diagnostic test accuracies of novel devices assessed using STARD criteria

Author	Study details and aims	Parameters (Cutoff)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Conclusion
Zainuer et al 2016	Study aimed to identify the area under the curve (AUC) for ABPI, TBPI and the transfer function index (TFI) to in significant PAD (>50% stenosis), confirmed by duplex, CTA or MRA. Analysis on calcified (C), non-calcified (NC) and all participants (C & NC) (n=102; 192 limbs)	ABPI <0.9 C & NC C (49 limbs) NC (63 limbs) TBPI <0.65 C & NC C (49 limbs) NC (63 limbs) TFI 1.025 C & NC C (49 limbs) NC (63 limbs)	83 60 80 87.9 87.9 77.5 93.1 90 94.4	70% 100% 95.7% 80% 82.5% 82.6% 86.3% 88.9% 80%	96.9% 71.4% 94.4% 84.4% 88.9% 87.9% 93.8% 92.9% 95.7%	A cut-off value for TFI of <1.025 is more accurate than an ABPI <0.9 and a TBPI <0.65 in diagnosing significant PAD.
(Ro et al. 2013)	Retrospective study to assess the sensitivity and specificity of doppler ABPI, continuous wave doppler waveform analysis (CWA) and photo-plethysmography waveform analysis (PPGA) against angiography for PAD diagnosis (n=97; 197 legs)	ABPI (<0.9) CWA PPGA	Na	69.3 (61.9-75.9) 90.8 (85.3-94.8) 81.6 (85.3-94.8)	96.8 (83.8-99.4) 64.5 (45.4-80.8) 77.4 (58.9-90.4)	In the presence of below knee stenosis, the sensitivity of ABPI is reduced (14.8%), compared to CWA (92%) and PPGA (67%).

Table 7: Diagnostic test accuracies of novel devices assessed using STARD criteria

Author	Study details and aims	Parameters (Cutoff)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Conclusion
(Williams et al. 2005)	Prospective study evaluating the efficacy of foot pulses, ABPI, TBPI and continuous wave doppler waveform analysis (CWA), against colour duplex imaging confirmed presence of PAD in diabetics (n=68; 130 legs)	Control Pulse palpation ABPI (<0.9 or >1.3) TBPI (<0.75) CWA	Not applicable	 93 83 100 86		CWA was the most effective test for diabetics
		Diabetic; no neuropathy Pulse palpation ABPI (<0.9 or >1.3) TBPI (<0.75) CWA	Not applicable	 87 100 91 100	 53 88 65 92	
		Diabetic neuropathy Pulse palpation ABPI (<0.9 or >1.3) TBPI (<0.75) CWA	Not applicable	 81 53 100 94	 56 95 61 66	
(Benhamou et al. 2012)	Prospective study of ABPI against transcutaneous oximetry ($tcpO_2$) in diagnosing duplex confirmed PAD in hemodialysis patients (n=48; 48 legs)	ABPI (<0.9) $tcpO_2$ (<40mmHg)	Not applicable	71.4 100	93.8 85.2	A tcpO2 <40 mmHg will help identify patients with end stage renal disease with PAD.

Table 7: Diagnostic test accuracies of novel devices assessed using STARD criteria

Author	Study details and aims	Parameters (Cutoff)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Conclusion
(Ezio et al. 2010)	Prospective study assessing the feasibility of transcutaneous oximetry ($tcpO_2$) and doppler based ankle pressures (AP) in diabetics with critical limb ischemia, with significant stenosis (>50%) confirmed by digital subtraction angiography (DSA); (n=261)	$tcpO_2$ (<30mmHg) $tcpO_2$ (<50mmHg) AP (<70mmHg)	Not applicable	81.6 (CLI) 100 (PAD) 32.9		Ankle and toe pressures were immeasurable in 41.8% and 71.6% with CLI. Although AP>70mmHg does not exclude the presence of PAD, a tcpO2 <50mmHg is capable of detecting all patients with PAD.
(Bouyé et al. 2005)	Prospective study comparing transcutaneous oximetry (DROP), near infrared spectroscopy (recovery time) and ABPI against angiography to detect PAD in the hypogastric circulation of buttock claudicants (n=30; 60 legs)	DROP (-15 mmHg) Recovery time (>240s) ABPI (<0.9&>1.3)	0.7188	94 (81.4-99.3) 33 (18.6-51) 64 (46.2-78.2)	62 (67.6-97.3) 87 (40.6-81.2) 50 (29.1-70.9)	NIRS showed a lower diagnostic accuracy compared to tcpO2 (33% vs 94%) in the detection of PAD in the hypogastric circulation, using the cutoff values.
(Manfredini et al. 2009)	Prospective study of the diagnostic accuracy of NIRS in claudicants, attempting to quantify muscle metabolic response during exercise and identify the best parameters suitable to diagnose and assess severity of PAD respectively. All patients underwent an echo colour doppler (n=95; 129 lower limbs with PAD, 61 normal legs)	dHb (≤197) O_2 Hb (≤ 76) HHb (>280)	0.932 (0.886-0.963)+ 0.91 (0.86-0.947)+ 0.861 (0.803-0.906)+	87.6 78.3 71.3	93.4 95 91.8	Dynamic NIRS evaluation of muscle metabolic response detects PAD. The parameter with the highest diagnostic accuracy was the differential haemoglobin levels.

Table 7: Diagnostic test accuracies of novel devices assessed using STARD criteria

Author	Study details and aims	Parameters (Cutoff)	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Conclusion
(Abraham et al. 2003)	Prospective study comparing transcutaneous oximetry against the angiographic presence of 75% or more stenosis in the hypogastric circulation of proximal (pc) and non-proximal claudicants (npc) (n=77)	PC group DROP (-16 mmHg) REST (81 mmHg) MIN (63 mmHg)	0.91±0.03 0.45±0.07 0.73±0.06	82.8 58.6 62.1	82.1 32.8 85.7	Transcutaneous oximetry is sensitive to the presence of PAD in the proximal hypogastric circulation. The DROP parameter (difference between chest and lower limb $tcpO_2$). A -16mmHg cutoff is more sensitive compared to the REST and MIN parameters in PAD.
		NPC group DROP (-15 mmHg) REST (89 mmHg) MIN (68 mmHg)	0.86±0.05 0.52±0.07 0.65±0.07	78.8 78.8 54.5	85.7 37.1 68.6	
(Doverspike et al. 2004)	Prospective diagnostic accuracy study of TFI in PAD detected by angiography, duplex, ABPI or TBPI (n=146 lower limbs)	TFI (0.94) TFI (0.90) TFI (0.91) TFI (0.92) TFI (0.95) TFI (0.96)		92 79 97 93 98 100	90 100 84 87 84 70	The ROC Curve showed the best cutoff for TFI to be 0.94 with a sensitivity of 92% and specificity of 94%.

Table 7: Diagnostic test accuracies of novel devices assessed using STARD criteria

Table 8: Agreement between novel devices and ABPI using GRRAS criteria.

Author	Study details and aims	Index test	Imperfect reference standard	PPA (95%CI)	NPA (95% CI)	Cohens' Kappa (95% CI)	Conclusion
(Hoyer, Sandermann & Petersen 2013)	Prospective, randomized study to determine accuracy of automated portable device (APD) against mercury-in-silastic, strain-gauge plethysmography (SGP) to measure TBPI for PAD diagnosis (n=204).	**APD**	**SGP**				Although the overall agreement is 90.2%, the SGP has a good positive percent agreement (PPA), with APD for PAD diagnosis
		TBPI <0.7 ABPI ≤ 0.9 or TBPI <0.7		98.8	58.1		
		Ankle pressure <50mmHg or		98.8	61		
		Toe pressure <30mmHg		83.3	97.8		
(Hoyer, Sandermann, Paludan et al. 2013)	Prospective study comparing accuracy of laser doppler flowmetry (LDF) against strain gauge plethysmography (SGP) based segmental pressure indices (n=200).	**LDF**	**SGP**				LDF has a moderate agreement with SGP for the diagnosis of PAD including CLI.
		ABPI ≤ 0.9 or TBPI <0.7		96.6	85.7	0.775 (0.631-0.919)	
		Ankle pressure <50mmHg or Toe pressure <30mmHg		78.2	96.6	0.78 (0.624 – 0.936)	
(Moosa et al. 2013)	Prospective study assessing agreement between doppler ABPI and photoplethysmographic (PPG) TBPI, in the diagnosis of PAD (n=182).	**PPG**	**Doppler**				The agreement between ABPI and TBPI is fair.
		TBPI <0.7	ABPI <0.9 or >1.3	35	75.4	0.09	
			ABPI <0.9	90	75.4	0.23	

Table 8: Agreement between novel devices and ABPI using GRRAS criteria.

Author	Study details and aims	Index test	Imperfect reference standard	PPA (95%CI)	NPA (95% CI)	Cohens' Kappa (95% CI)	Conclusion
(C. L. Huang et al. 2011)	Prospective study using infra-red thermography to investigate exercise induced temperature change (eTC) after a 6MWT for PAD diagnosis (n=51).	**eTC**	**ABPI**				Resting temperature of legs in PAD and non-PAD patients are equal. Post exercise, PAD patients have a decrease in temperature.
		-0.99[+]	<0.9	81.7	65	0.457	
(Papanas et al. 2010)	Prospective study evaluating the Lanarkshire Oximetry Index (LOI) against ABPI, for the diagnosis of PAD in type 2 diabetics (n=161; 322 lower limbs)	**LOI**	**ABPI**				Agreement between LOI and ABPI was moderate.
		<0.9	ABPI <0.9	93	89	0.569[+∞]	
		<0.8				0.611[+∞]	
		<0.9	ABPI <0.9 (excluding >1.2)	40	99.3	0.747[+∞]	
		<0.8				0.595[+∞]	
		<0.9	ABPI <0.9 (excluding >1.3)			0.584[+∞]	
		<0.8				0.609[+∞]	
(Comerota et al. 2003)	Prospective study comparing near infrared spectroscopy (NIRS) against ABPI for PAD diagnosis. Time to 50% and 100% recovery of baseline oxygen saturation (T_{50} and T_{100} respectively) were assessed. (n=49)	**Recovery time**	**Doppler**				Although resting oxygen saturation (StO_2) is similar between claudicants and patients without PAD, there is a significantly greater drop in StO_2 and longer recovery time in claudicants.
		T_{50} ≥70 sec [∞]	ABPI < 0.9	89	85	0.634*	
		T_{100} ≥165sec [∞]	ABPI < 0.9	88	81	0.588*	

Table 8: Agreement between novel devices and ABPI using GRRAS criteria.

Author	Study details and aims	Index test	Imperfect reference standard	PPA (95%CI)	NPA (95% CI)	Cohens' Kappa (95% CI)	Conclusion
(Brooks et al. 2001)	Prospective study comparing the agreement between TBPI and ABPI (n=227).	**Doppler or PPG**	**Doppler**				TBPI offers no advantage over ABPI except if there is overt calcification, where the TBPI is superior.
		TBPI ≤0.54 or ≥0.94	ABPI <0.9 or ≥1.3	70.7	79.1	0.443	
		TBPI ≤0.54	ABPI <0.9	84	79.1	0.477	
(Allen et al. 2008)	Prospective study comparing toe pulse wave analysis obtained by photoplethysmography (PPGA) against ABPI (n=111; 222 legs). Parameters were assessed in both toes. Analysis was performed for each toe and as a comparison between each toe. Parameters were: a) pttf ‡ : Transit time from an ECGs R wave to the trough of a pulse wave. b) pttp ∂ : Transit time from the ECGs R wave to the peak of the pulse wave. c) Rise time: Transit time from trough to peak on the pulse waveform. Shape index: Total area of the pulse waveform falling outside the normal range of normalized shape profiles.	**PPGA**	**ABPI**				PPGA has a moderate to substantial agreement with ABPI for detecting PAD and critical limb ischemia, when absolute values or a difference between values for both toes was measured. For the purpose of PAD diagnosis alone, shape index has the best agreement with ABPI.
		Individual toes					
		Shape Index	<0.9	88.9	90.6	0.75	
		Rise time		75.9	88.8	0.63	
		pttf ‡		64.8	91.3	0.58	
		Bilateral differences					
		pttf ‡	<0.9	71.9	93.3	0.68	
		pttp ∂		75	93.3	0.7	
		Rise time		56.3	94.7	0.56	

*Recalculated from available raw data; ⁺ Statistically significant; ∞ Receiver operator characteristic (ROC) curve analysis

∂From review; Kappa coefficient can be 0 (poor), 0.01–0.20 (slight), 0.21–0.40 (fair), 0.41– 0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1 (almost perfect) (Landis JR 1977).

Table 8: Agreement between novel devices and ABPI using GRRAS criteria.

Summary

Devices were selected based on their strength, weakness, feasibility and availability.

Summary of these are listed in table 9 below.

Pressure measurement-based novel diagnostics					
Device	**Advantage**	**Disadvantage**	**Feasible**	**Available**	**Selected**
Plethysmography	Tested method.	Sensitive to motion artefacts and temperature changes. Time consuming analysis.	No	No	No
TFI-PVR	Not sensitive to arterial wall calcification.	Operator dependent. Unreliable in arrhythmias. Expensive. Bulky.	No	No	No
Perfusion measurement-based diagnostics					
Device	**Advantage**	**Disadvantage**	**Feasible**	**Available**	**Selected**
LDF	Contact based. Measures average velocity of blood cells.	Sensitive to motion artefacts, ambient light, position and pressure applied by probe. Expensive.	Yes	Yes	Yes
LDI	Non-contact based. Measures perfusion over large area.	Sensitive to motion. Complex analysis. Expensive.	No	Yes	No
LSCI	Non-contact based. Measures perfusion over large area.	Complex analysis. Expensive.	No	Yes	No
Capillaroscopy	Analyses capillary density, anatomy and flow.	Time consuming. Requires specially trained staff. Not portable.	No	No	No
Oxygen measurement-based diagnostics					
Device	**Advantage**	**Disadvantage**	**Feasible**	**Available**	**Selected**
Pulse oximetry	Simple. Easy to use. Inexpensive.	Applicability for lower limb oxygenation uncertain.	Yes	Yes	Yes
Transcutaneous Oximetry	Simple.	Time consuming analysis. Heating element required on skin. Expensive.	Yes	Yes	Yes
NIRS	Simple to use.	Time consuming analysis.	Yes	Yes	Yes

	Wireless device.	Expensive.			
Hyperspectral camera	Mobile.	Complex setup. Technical expertise. Expensive.	No	Yes	No
Temperature measurement-based devices					
Device	**Advantage**	**Disadvantage**	**Feasible**	**Available**	**Selected**
Infrared thermometer	User friendly. Affordable. Small and mobile.	Accuracy in PAD unknown.	Yes	Yes	Yes
Infrared camera	User friendly.	Costly. Complicated setup.	Yes	Yes	Yes
Other alternatives					
Device	**Advantage**	**Disadvantage**	**Feasible**	**Available**	**Selected**
LDA	Assesses capillary blood velocity and density	Time consuming Complex setup	No	No	No
Smart phone technology	Easy to carry.	Not validated for medical use	No	Yes	No

Table 9: Summary of novel diagnostic and selection process.

Aim of study

The aim of this study is to identify novel diagnostic devices that measure perfusion, oxygenation and temperature changes for the diagnosis of PAD. Historical studies using such devices had methodological or analytical flaws. Recent addition of agreed reporting standards have led to an improvement in the understanding of reporting diagnostic test accuracy studies, its interpretation and the reporting of results.

In secondary care, PAD diagnostic investigations, usually begin with the use of duplex ultrasound. This modality acts as an effective tool to detect PAD and plan further investigations or management. However, its lack of availability out of routine working hours, in primary care and its dependence on the technician, makes it less applicable for use in a non-elective setting. In primary care, traditionally ABPI has been used as the referral tool by general practitioners to refer patients with PAD. Evidence suggests

that there has been poor uptake of its use (Yap Kannan et al., 2016). Its usefulness in detecting PAD in claudicants has been brought into question in a recent Cochrane review (Crawford et al., 2016).

The selection of a diagnostic device depends on its performance characteristics and operational characteristics (Banoo et al., 2010). Additionally, knowledge of the anatomy of the microcirculation can help determine potential sites for examination. For example, glabrous skin sites are more sensitive to temperature changes (Huang et al., 2011), oxygenated-deoxygenated hemoglobin levels (Chin et al., 2011) and are rich in arteriovenous anastomoses (Khaodhiar et al., 2007). These sites have the added advantage of not being prone to varicose veins and oedema, which affects some measurements (Huang et al., 2011).

A pragmatic study design was chosen to allow for a more translatable and applicable results (Hunink & Krestin, 2002), reflecting day-to-day clinical practice. Environmental factors were therefore not controlled.

Methods

Chapter 3: Methods

Recruitment Process

Participants were approached in person, in clinics, whilst awaiting other tests or procedures, or as inpatients in the hospital. A brief explanation of the tests and an information leaflet was provided. A total of 150 participants were recruited. The breakdown of the participants is shown in the table below (Table 10).

	Total Participants	Legs studied
***REC requirement pilot**	1	1
Pilot tests	12 controls 11 patients	12 11
Infrared thermometry versus Infrared thermography	10	20
Oxygen and Thermometry study	82 patients 43 controls	97 43

Table 10: Distribution of recruited participants in study. *REC : Regional Ethics Committee

Inclusion criteria were participants who were being investigated for symptoms of PAD, and diagnosed by either ABPI, duplex imaging, CT or MR angiograms to confirm diagnosis. Healthy controls were asymptomatic, and were recruited from the hospital and university staff, all of whom had an ABPI. Exclusion criteria were patients who were unable to consent for the study or who elected not to participate.

Provocation tests of limb circulatory reserve

A detailed explanation of individual study protocols is provided with the respective studies in the results section. All protocols involved a provocation test of limb circulatory reserve. Tests were either actively performed or passively measured. In

both active and passive tests, measurements were taken before, during and after the test.

Active tests involved exercise of the whole body or a single limb. The whole body exercise was the 6-minute walk test (6MWT), which involved walking up and down a corridor for 6 minutes. This a shortened form of the 12-minute walk test originally described for cardiopulmonary testing. The duration was shortened to accommodate patients with respiratory disease. Its main indication is for the assessment of response to medical intervention for severe heart or lung disease (American Thoracic Society, 2002). Over the years it has been adapted for use in PAD diagnosis as well (Montgomery & Gardner, 1998; Cahan et al., 1999). One main drawback of this test is that patients with musculoskeletal pain may not tolerate it well (American Thoracic Society, 2002).

Single limb exercise was the 1-minute flexion extension test (1MF). Participants were either supine or sat with the legs level with the hips and asked to dorsi and plantar-flex their ankle for a full minute. This latter test is similar to the heel-rise test (Monteiro et al., 2013) and the Stresst'er test (Cameron et al., 1997). This test targets the calf muscles. This test was developed during the pilot phase of this study.

The passive test was the post-occlusive reactive hyperemia (PORH) test, which involved inflating a thigh cuff up to 30mmHg above the highest brachial systolic blood pressure (Figure 14). Three minutes was deemed suitable for the PORH, based on previous studies (Kragelj et al., 2001; Roustit & Cracowski, 2013), which demonstrated it to be a valid and acceptable model for testing ischemia recovery.

Morales et al reported that of 54 participants, only 3 were unable to tolerate 3 minute cuff inflation (Morales et al., 2005). Figure 13 illustrates the provocation tests used.

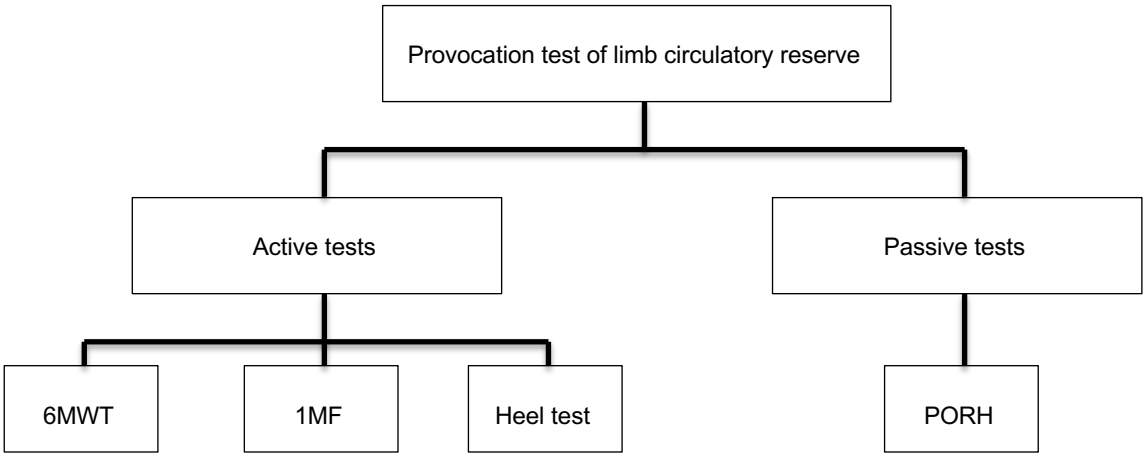


Figure 13: Classification of provocation tests of limb circulatory reserve used during period of study. 6MWT: 6-minute walk test, 1MF: 1-minute flexion extension test; PORH: Post occlusive reactive hyperemia test.

Post-occlusive reactive hyperemia device

The initial PORH device used during the pilot phase was the MoorVMS-PRES. The device has a maximum inflation of 250mmHg. It can inflate a leg cuff in less than 10 seconds. This device was acquired from Moor instruments for a period of 2 weeks. The device subsequently used was the Hokanson E20/AG101 rapid cuff inflator. The cuff (Hokanson SC12 thigh cuff) measures 13x85cm. This device is much larger than the MoorVMS-PRES but was already available in the department. The AG101 is a source of clean compressed air for the E20 rapid cuff inflator. Both devices are shown below (Figure 14a and b).

a

b

Figure 14: a) MoorVMS-PRES Pressure cuff controller (Image reproduced from us.moor.co.uk
b) Hokanson E20/AG101 Rapid cuff inflator (Image reproduced from www.hokansonvascular.com

Novel diagnostic devices

Devices deemed suitable in the review, were acquired from some companies for 2 to 2 and a half weeks. Some were already available. Attempts to acquire the Perimed transcutaneous oximetry for the period of the pilot was not successful (Table 11).

Company	Device name	Property	Parameter measured	Duration acquired
Artinis	Portamon	NIRS	Oxygen	2 weeks
Artinis	Portalite	NIRS	Oxygen	2 weeks
Moor Instruments	MoorVMS-PRES	PORH	Arterial Compression device	2.5 weeks
Moor Instruments	MoorVMS-OXY	StO_2	Oxygen	2.5 weeks
Moor Instruments	MoorVMS-LDF	LDF Temperature	Perfusion Temperature	2.5 weeks
Nonin Medical	Nonin Onyx Vantage 9590	Pulse oximeter	Oxygen	2 weeks
HubDIC	HubDIC FS-700	Infra-red thermometry	Temperature	Procured
FLIR	T650sc	Infra-red thermography	Temperature	In department
Hokanson	Hokanson E20/AG101	PORH	Arterial Compression device	In department
Huntleigh	Dopplex D900	Duplex	Blood flow	In department

Table 11: Characteristics of devices loaned from companies for pilot study.

Near Infra-red Spectroscopy

Two different companies were approached for oxygenation measurement devices. The first was the MoorVMS-OXY, which uses white light spectroscopy. It requires probes to be attached to the skin and connected to a monitor, which in turn is connected to the laptop. The wavelength used range from 500-650nm. The probe was placed on the skin overlying the dorsalis pedis artery at the ankle.

The second devices borrowed were from Artinis (Portamon and Portalite). The Portamon device is applied directly on to the skin, as the light emission is directly from

the device. The Portalite is smaller than Portamon and has a light emitting diodes connected to the main device via a lead. The Artinis devices measure oxy, de-oxy and total haemoglobin. Both devices are Bluetooth enabled and data is transferred in real-time onto a laptop via an Oxysoft software. The light emitting diodes have 3 distances between receiver and transmitter (30, 35 and 40mm respectively). The wavelengths used were 760 and 850nm. The penetration depth is 2.5cm. Images of these devices are in Figure 15.

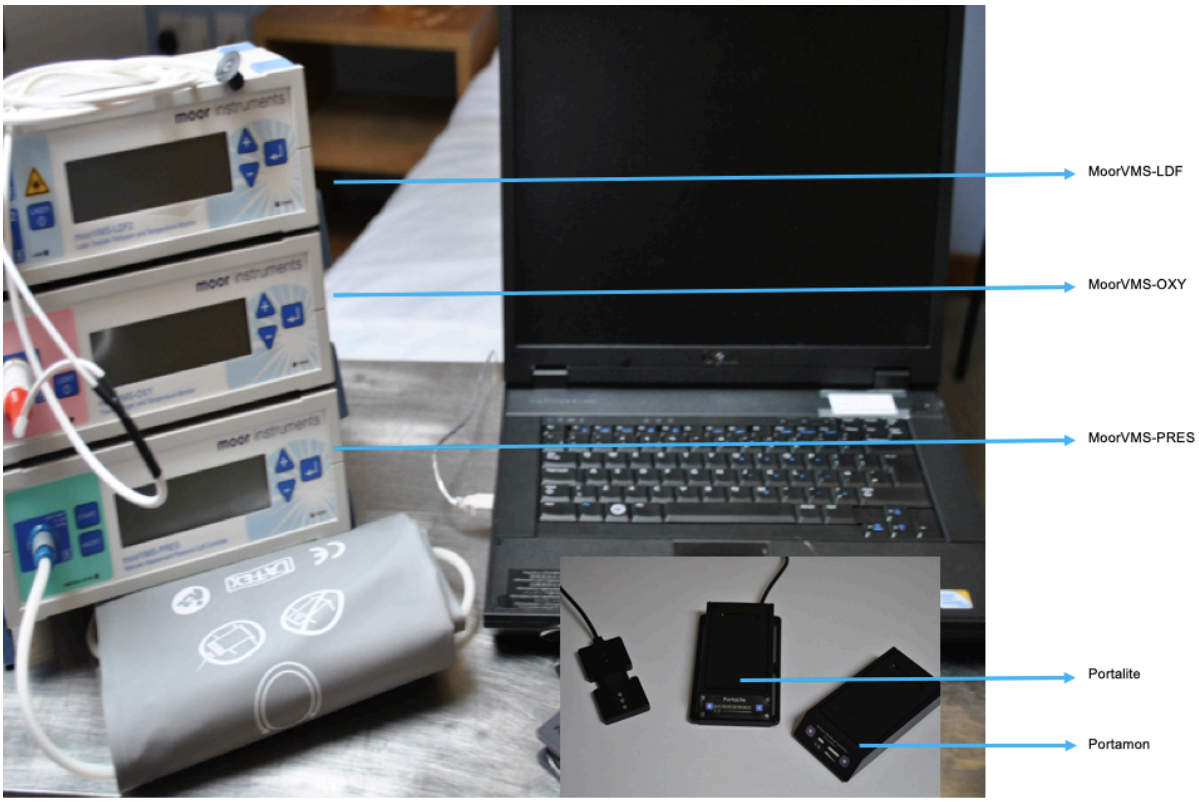


Figure 15: MoorVMS-LDF, MoorVMS-OXY, MoorVMS-PRES, Portalite and Portamon device on display

Laser Doppler flowmetry

The MoorVMS-LDF is a device that uses a wavelength of 785nm +/- 10nm with an angular spread of laser light from the probe tip of 26°. Measurements are taken as Perfusion Units (PU). It has a precision of ±3% and an accuracy of ±10%. It has a

measurement range of 0-1000 PU. The LDF probe was positioned on the dorsum of the forefoot over the dorsalis pedis pulse.

Infrared Thermometry

The non-contact infra-red thermometer, HubDiC FS-700 is capable of measuring temperature at body settings (22°C to 42.5°C) and object settings (10°C to 80°C). The device is commonly available and inexpensive (Figure 16). In the initial pilot tests, temperature was measured on the medial side of the calf (5cm below and medial to the tibial tuberosity), 5cm, 10cm and 15 cm above the medial malleolus (MM), 5cm above the lateral malleolus (LM), the skin overlying the calcaneal fat pad and over both temporal arteries in the forehead.

Figure 16: HubDIC FS-700 non-contact Infra-red thermometer.

Handheld vascular dopplex

This is an advanced vascular doppler with a 8MHz probe (D900). It provides bidirectional blood flow information and has the option to be linked to the Dopplex

reporter software package or Dopplex Printa II for waveform recordings. The device was mainly used for the assessment of ABPI in healthy participants and was available from the vascular ward.

Figure 17: D900 handheld doppler

Pulse oximetry

The Nonin Onyx Vantage 9590 pulse oximeter is meant for fingertip pulse oximetry and is a wireless device. Unlike NIRS, pulse oximeters can only provide static oxy-haemoglobin percentages usually at the fingertips. More recent versions can provide measurements at the wrist. However, to date there are no devices suitable for the toes or lower limbs. As explained in Chapter 2, the use of pulse oximeters in the toes can lead to false readings. Pulse oximeters use red and infra-red light as well.

Figure 18: Nonin Onyx Vantage 9590 pulse oximeter

Infrared thermography

All objects emit heat. Most of this is in the visible light spectrum. However, when an object is not warm enough to emit heat in this spectrum, it will emit in the infra-red spectrum. The infra-red camera is able to detect this heat signature, produce a thermal image and measure its temperatures. In this study, the camera was acquired periodically courtesy of the University of Leicester Space Research Centre. The device is costly and not feasible for day to day use. For the purpose of this research, it was used in comparison to the infrared thermometry both in the laboratory and in-vivo pilot test, as elaborated in chapter 6.

Figure 19: Thermal infra-red camera

Regional Ethical Committee requirements

Feasibility of duration of experiment

One of the requirements of the Regional Ethics Committee (REC) was for the experiment to be done within 60 minutes. Pilot studies were conducted to determine the best provocation test, novel diagnostic, site of measurement and protocol. To assess the feasibility of the duration of the experiment, all provocation tests were used. A healthy participant was given an initial 12 minutes to acclimatize to room temperature. He was requested to complete the EuroQOL 5D (EQ5D) and PAD questionnaire, whilst having the ABPI measurements taken, sat up on a trolley. These provided information on quality of life (EUROQOL) and symptoms of peripheral arterial disease (PAD questionnaire).

The Portamon device was placed on the medial gastrocnemius (MG) 10cm below and medial to the tibial tuberosity. The Portalite probe was placed on the heel, 10cm from the calcaneal tip on the sole. The MoorVMS-LDF and MoorVMS-Oxy probe were placed on the dorsum of the foot overlying the dorsalis pedis artery. The MoorVMS-PRES cuff was positioned on the upper thigh of the left leg.

With the exception of temperature measurements during the period of cuff inflation, all other parameters were measured for 3 minutes before inflation of the cuff, continuously during the 3-minute PORH and continued on for 3 minutes after deflation. Temperature measurements were measured 5cm,10cm and 15cm above the MM, 5cm above the LM and over both temporal arteries on the forehead. The pressure cuff was inflated to 30mmHg above the highest systolic arm blood pressure.

At the end of the PORH test, the participant was given 5 minutes to rest before performing the 6MWT. The experimental time was 30 minutes, with an additional 14 minutes in between for the participant to prepare, leading to a total of 44 minutes (Figure 20). The participant did not experience any pain during the experiment.

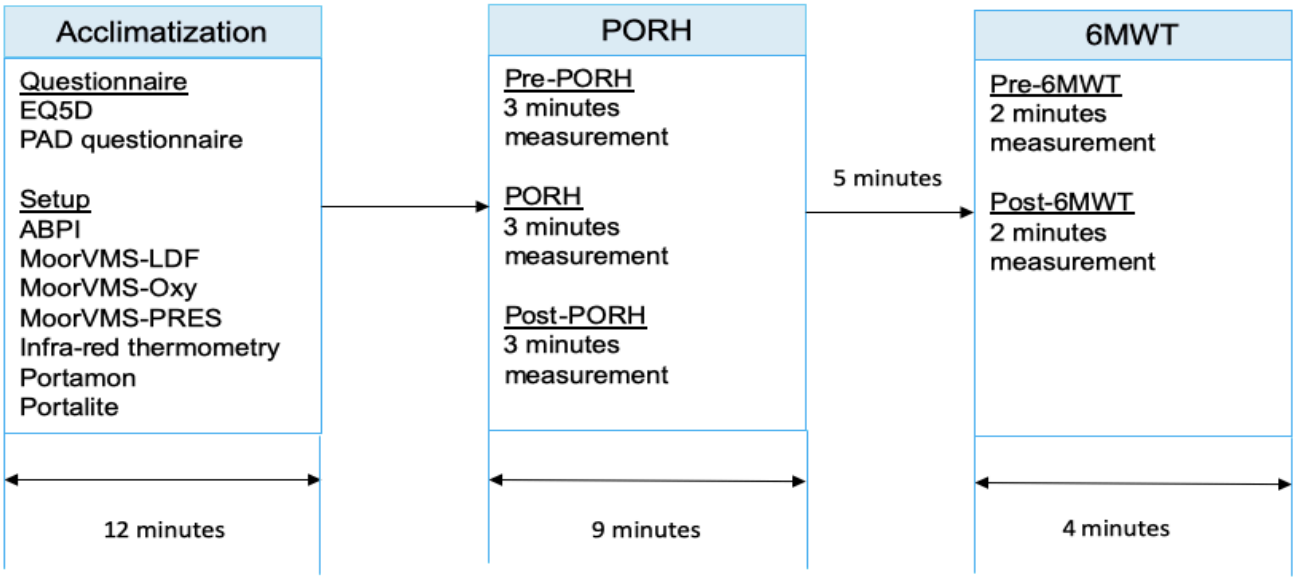


Figure 20: Study design for time trial pilot; EQ5D: EuroQOL 5D questionnaire, PAD: Peripheral Arterial Disease, LDF: Laser Doppler Flowmetry, PORH: Post occlusive reactive hyperemia test, NIRS: Near infra-red spectroscopy, 1MF: 1 minute flexion extension test, 6MWT: 6 minute walk test.

Feasibility of the environment of study

The study design was pragmatic and performed by one examiner (RK). The room temperature and humidity were not controlled in order to simulate a routine clinic environment setting. No air conditioning was allowed and air movement was kept to a minimum. This was primarily because of temperature measurements are affected by air movement. Light settings were documented, but not controlled.

Feasibility of provocation test

Following the initial experiment to study the feasibility of duration of the experiment, a further study with the acquired devices was performed. The aim was to assess the feasibility of the provocation test, devices and site of measurement. For this pilot, the PORH was the baseline provocation test. Healthy participants and patients were recruited. One of 12 healthy and 6 of 11 patient participants, were unable to tolerate the PORH provocation test. Measurements were not possible in the patient participants as a result. The 1MF test was developed as a result, which is a simple bedside test, equivalent to the heel-rise test and does not cause any discomfort to any patient participants, who could not tolerate the PORH. These led to a revision of the study design.

Revised study design

For the reproducibility of the devices (as discussed in Chapter 4), the provocation test used was the PORH. For further studies, the provocation tests dictated patient participation. The sequence of testing was to perform the 1MF provocation test prior to the 6MWT with a 5-minute break at the very least between tests (as discussed in chapter 5 and 6). Patients not keen to perform the 6MWT but were happy to proceed with the 1MF test were studied for this provocation test alone. Temperature measurements were performed over the MG and the heel pad. Oxygenation measurements were performed on the MG alone. As it was not possible to place the Portalite probe on the sole whilst the patient performed the 6MWT, this site was not

used for oxygen levels. The final revised study design was still within the 60 minute duration requirement and is illustrated in Figure 21.

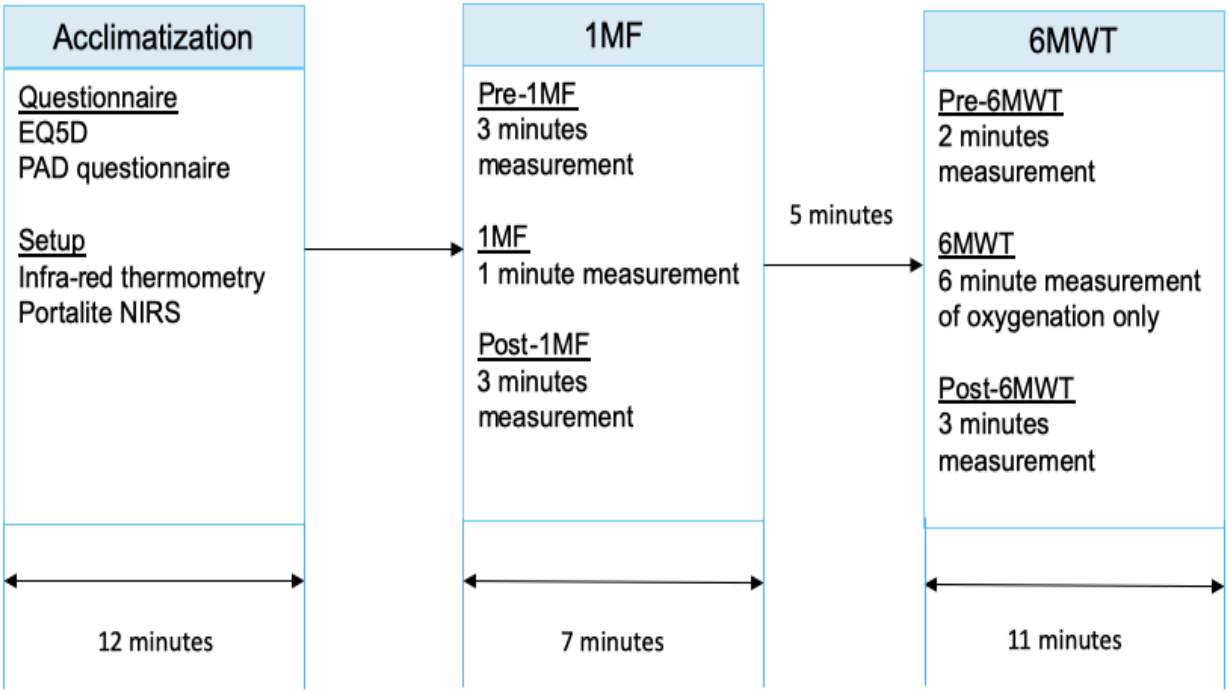


Figure 21: Study design used for further experiments; EQ5D: EuroQOL 5D questionnaire, PAD: Peripheral Arterial Disease, NIRS: Near infra-red spectroscopy, 1MF: 1 minute flexion extension test, 6MWT: 6 minute walk test.

Feasibility of diagnostic device

In the process of selecting the diagnostic device, the equipment characteristics and site of measurement were reviewed (Chapter 2). In the initial pilot, the sites of measurements, rationale and issues with equipment or site were identified, as shown in Table 12.

Device	Site	Rationale	Issues
NIRS	MG	Overlying site of incision for below knee popliteal artery	None
	Heel	Glabrous skin No edema	Not suitable for the provocation tests used (6MWT & 1MF)
Temperature	MG	Overlying site of incision for below knee popliteal artery	None
	5,10 &15cm above MM	Overlying site of perforators	Time consuming to locate with handheld doppler and perhaps unnecessary as heel site adequate
	5cm above LM	Overlying site of perforators	Time consuming to locate with handheld doppler and perhaps unnecessary as heel site adequate
	Heel	Glabrous skin No edema	None
LDF	Dorsalis pedis (DP)	To measure perfusion directly over artery	Too sensitive to slightest movement
Nonin	Hallux	Lower limb oxygen levels	Not appropriately sized for toes
MoorVMS-OXY	DP	Measure oxygenation directly over DP	Too expensive Not wireless compared to NIRS Sensitive to movement

Table 12: Site of measurement using novel diagnostic devices and potential issues identified; MG: Medial gastrocnemius, MM: Medial malleolus, LM: Lateral malleolus, DP: Dorsalis pedis.

Selection of devices for further studies

The devices selected for the final study design, along with the rationale and how these were procured are listed in Table 13.

Device	Selected	Reason	Site	Procurement
NIRS	Yes (Portalite)	Wireless Not sensitive to motion	MG	Small Research Grant NIHR Biomedical Research Unit Leicester.
Temperature	Yes	Non-contact Affordable, Light Object and skin settings available	MG Heel	Self funded
LDF	No	Sensitive to motion resulting in large fluctuations in measurements	Not applicable	Not applicable
Nonin	No	Not appropriate size for toes and sensitive to motion	Not applicable	Not applicable
MoorVMS-Oxy	No	Does not have added advantage of being wireless More expensive than NIRS	Not applicable	Not applicable

Table 13: Devices selected, rationale and how these were procured; MG- Medial gastrocnemius

Of the novel diagnostics tested in the pilot study, the HubDIC infra-red thermometer and the Artinis Portalite were deemed suitable. The Portalite had the added advantage of being wireless allowing for measurements to be done in real time during the provocation tests (1MF and 6MWT). The MoorVMS-LDF was too sensitive to slight movements, providing large fluctuations in measurements (Figure 22 a & b). The Nonin pulse oximeter was not suitable to be carried forward to future studies, as the size was not appropriate.

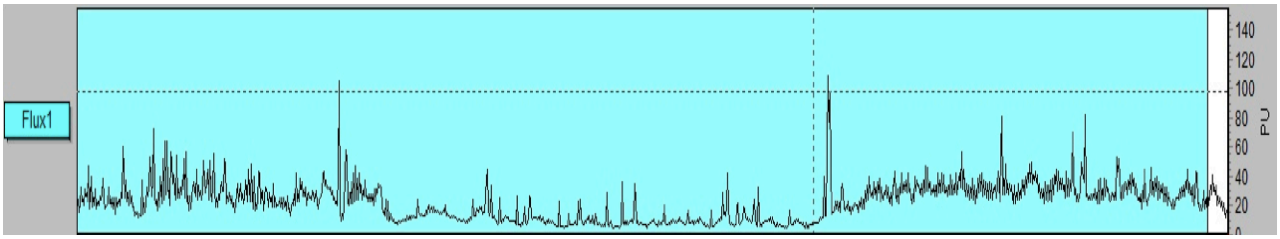
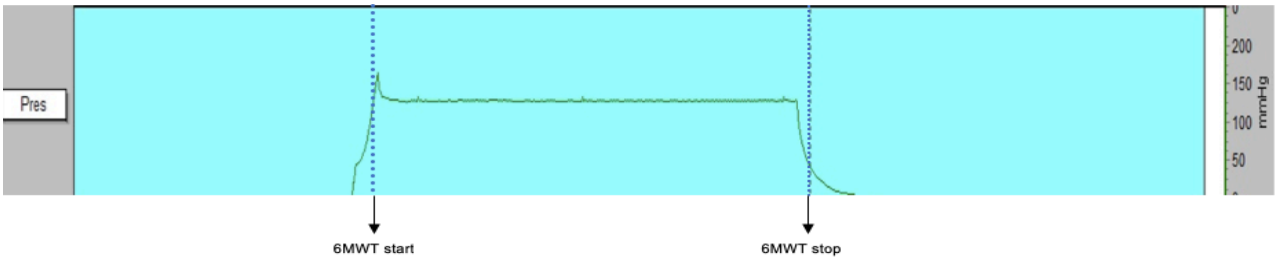


Figure 22 a) LDF Perfusion Units recorded by Moor-VMS LDF



PORH start PORH stop

Figure 22 b: LDF Perfusion Units changes during PORH test

Preconditioning

With the selected study design, there was concern if the initial provocation test (1MF) could affect the temperature and oxygenation measurements of the subsequent provocation test (6MWT). Ischemic preconditioning is a well-researched field in cardiovascular and sports medicine. This involves occluding the blood flow to an organ or limb momentarily for a few minutes with the intention to improve metabolic and vascular pathways, ultimately resulting in the protection of cellular processes in target organs or limbs. Most of the studies vary in terms of the occlusion method and the number of times this is done (cycles). M.Caru et al found the duration of occlusion to vary between 2 to 10 minutes with the number of cycles to be between 2 to 8 (Caru et al., 2019). Another review by Tapuria et al, suggests that repetitive brief ischemic episodes of the same duration renders the tissue tolerant to preconditioning (Tapuria et al., 2007).

In the case of this study, the time for the 1MF provocation test was 1 minute. No repetitions were done. Unlike other studies, where the duration of rest was between 1 to 2 minutes, participants in this study were given an adequate 5 minutes to recover between provocation tests. Therefore, the effect of any preconditioning was minimal on this study.

Statistical analysis

Graph Pad Prism 7 was used for statistical analysis of the results. A brief description of tests used and the rationale for their selection are provided in the sections below.

Limits of agreement

The purpose of the pilot study was to demonstrate the reproducibility of measurements taken at different times of the day or on different days. For this, a test of equivalence was performed in the form of Bland-Altman Limits of Agreement (LOA). This test was also used to compare the infrared thermometer and infrared camera to the black body radiator. The LOA in this case is the difference between measurements taken by individual devices at 2 different settings (inter-day and intra-day). The LOA estimates the range within which there is going to be a difference between 2 measurements. Most differences will lie between the d $\pm$1.96s range. The narrower this range, the closer the readings are to each other, the better the reproducibility. When the bias or mean difference (d) between two measurements is zero, the reproducibility is perfect (Figure 23).

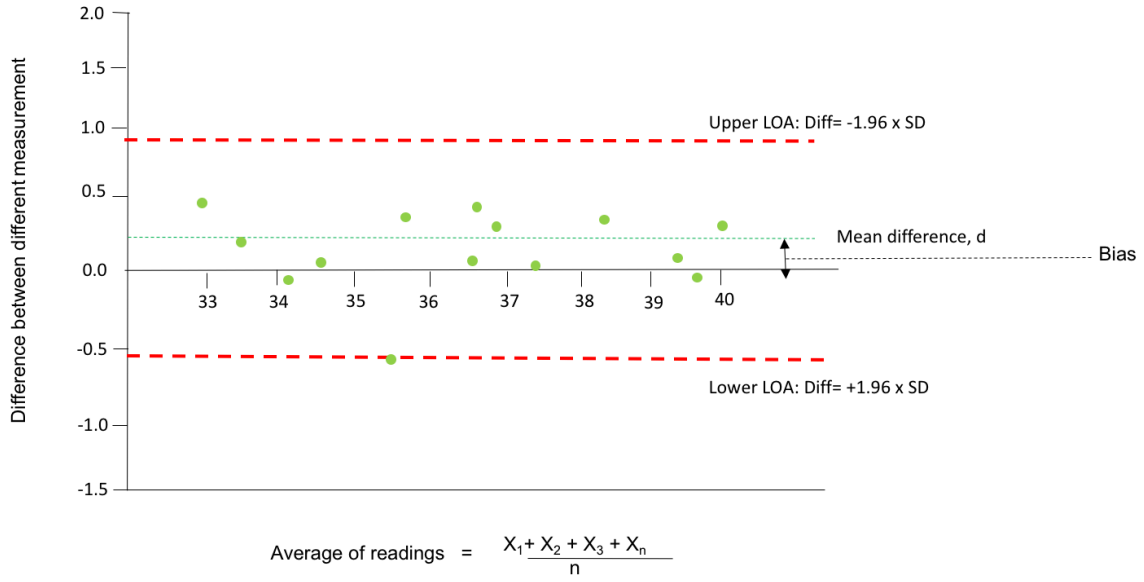


Figure 23: Example of Limits of Agreement (LOA); Mean difference (d), Standard deviation of mean difference (s).

Analysis of data from thermometry and oxygenation study

The aim of these studies was to assess if NIRS and infrared thermometry are able to identify patients with PAD. In both studies, the distribution of data was first assessed with histograms. Normality testing was done using D'Agostino & Pearson normality test. As data was not normally distributed, as described in chapter 5 and 6, the non-parametric Mann Whitney U test was used.

The Mann Whitney U test compares two groups, which are unpaired. Graph Pad Prism assigns a rank of 1 for the smallest number and n for the largest number, irrespective of the group. n represents the total number of recruits. The mean of the ranks in each group are compared and a p value <0.05 is considered significant, ie the null hypothesis can be rejected. The null hypothesis is that there is no difference between the two tested methods.

Youden Index

Scatter plots are used to compare the median values. If results were significantly different, a ROC curve was created. From this ROC, the area under the curve (AUC) and best cutoff value is reported. To determine the best cutoff value, the Youden index was calculated using the formula (Sensitivity + Specificity)-1 (Ruopp et al., 2008; Hajian-Tilaki, 2013). Optimum sensitivity and specificity was determined using this cut off value (Figure 24).

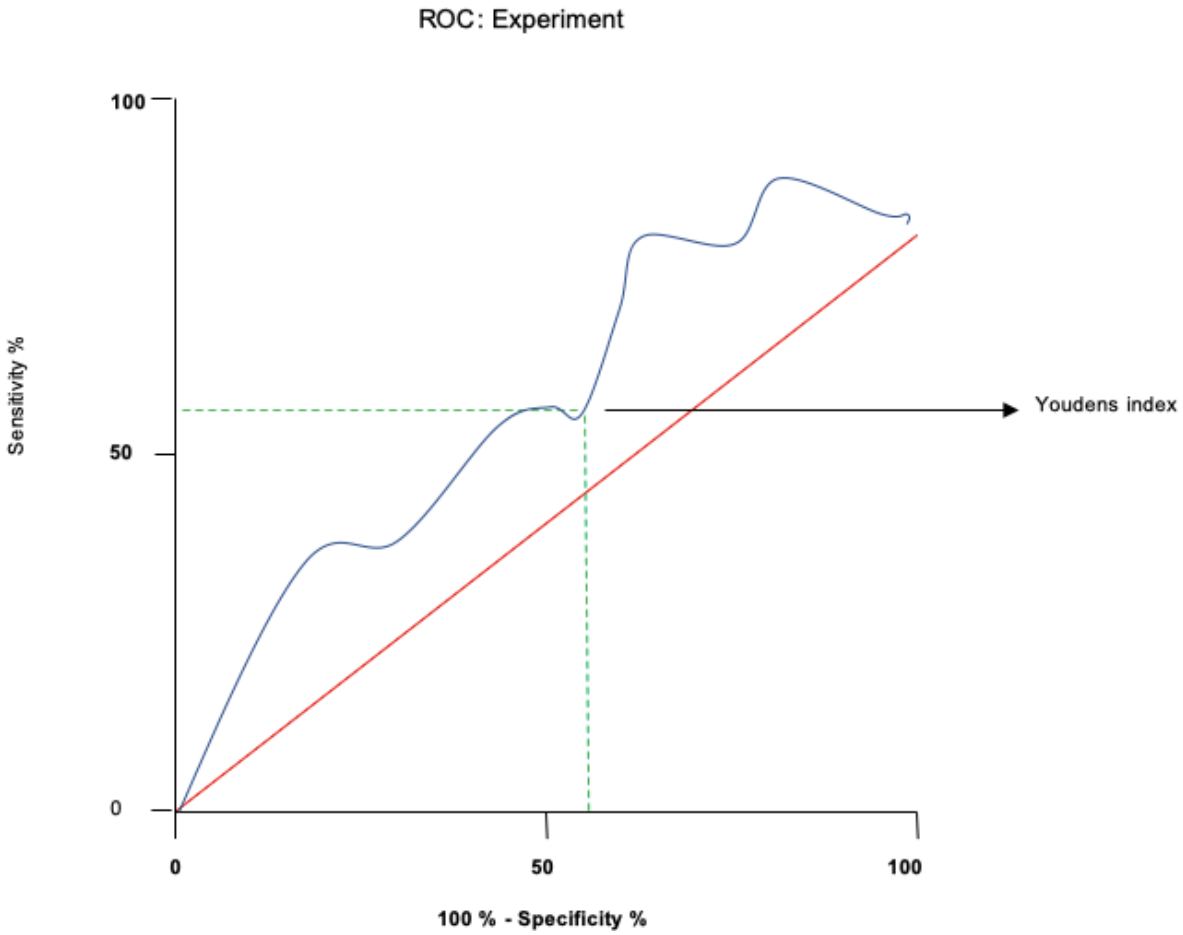


Figure 24: Youden index calculated from the ROC Curve

In order to compare more than 2 sets of data, the Kruskal-Wallis test for non-parametric data is used.

Summary

The PORH as a provocation test was not feasible to be carried forward in this study. Therefore, simpler provocation tests, such as the 6-minute walk test (6MWT) and the 1-minute Flexion-Extension test (1MF) were used. The devices used were the HubDIC Infrared thermometer and the Portalite NIRS device. The site of measurement was both the MG and heel for temperature and the MG for oxygenation.

RESULTS

Chapter 4: Precision studies

Aims

The purpose of this pilot test was to assess the precision (reproducibility) of the diagnostic devices selected.

Introduction

Diagnostic precision of a device is measured as its repeatability or reproducibility. Measurement variations performed in unchanged environmental, device and performer conditions elicit repeatability. Where there is any change, reproducibility is reported. There is a great deal of variability in the reporting of repeatability and reproducibility in the literature. Some studies advocate an Intra-class Correlation Coefficient (ICC), with an ICC >0.7 being significant. Another method is to use the Bland Altman Limits of Agreement (LOA) (Koo & Li, 2016; Bland & Altman, 1986) . The LOA provides a value of the bias. A bias of zero, implies absolute agreement and a perfectly reproducible or repeatable device (Bland & Altman, 1986; McAlinden et al., 2011). In this study, the conditions were changed in the process of measurement (variation in time and environment), therefore reproducibility is reported.

Reproducibility of infra-red thermometry, NIRS measured oxygenation (SaO_2) and LDF measured arbitrary perfusion unit, were assessed. Inter-day and intra-day reproducibility are reported for each diagnostic device. In the case of thermometry, two parameters were measured. These were the absolute temperature and the temperature ratio. The temperature ratio was taken as the temperature 5, 10 and 15mm above medial malleolus and 5mm above lateral malleolus, divided by the highest temporal temperature.

Protocol

For each participant, separate measurements were performed at 3 different times by a single observer (RK). Visit 1 was performed on a separate day from visit 2 and 3. The latter 2 were performed on the same day, at least 3 or more hours apart. Inter-day agreement was assessed between visit 1 versus visit 2 and visit 3 respectively. Throughout the duration of the procedure, participants were requested not to move their legs. The examination was performed with the participants' hips level with the feet. Participants' wearing long trousers were asked to either remove or roll these up to expose their calf and feet. The baseline PORH provocation test was used to recruit both healthy and patient participants.

The MoorVMS-PRES was used to perform the post occlusive reactive hyperemia test. Prior to the test, ABPI was measured using a manual BP cuff and a handheld vascular doppler D900. The thigh cuff was positioned over the upper thigh. Measurements were taken for an initial 3 minutes. The cuff was then inflated using the thigh cuff (21x84cm). The pressure was maintained at 30mmHg above the highest brachial systolic blood pressure reading for 3 minutes before rapid deflation. Deflation was sooner in those who could not tolerate the procedure. Measurements were then continued for another 3 minutes. The MoorVMS-PRES was replaced by the Hokanson E20/AG101 rapid cuff inflator and Hokanson SC12 thigh cuff (13x85cm), when it was returned after the period of loan for the MoorVMS-PRES completed. The latter device offered no additional advantage and was functionally similar to the Hokanson device, apart from being smaller.

Results

Results are displayed using the Bland Altman method of comparison. Inter-day reproducibility is represented by Visit 1 versus Visit 2 and Visit 1 versus Visit 3 (Figure 25). Intra-day reproducibility is represented by Visit 2 versus Visit 3 (Figure 26). For the purpose of comparison, the Y-axis is uniform between the devices. However, the averages (X-axis) cannot be uniform due to differences in the averages measured by the devices. The results are displayed based on the parameters on the same axes next to each other and have not been superimposed. This is to allow better appreciation of the LOA of each device.

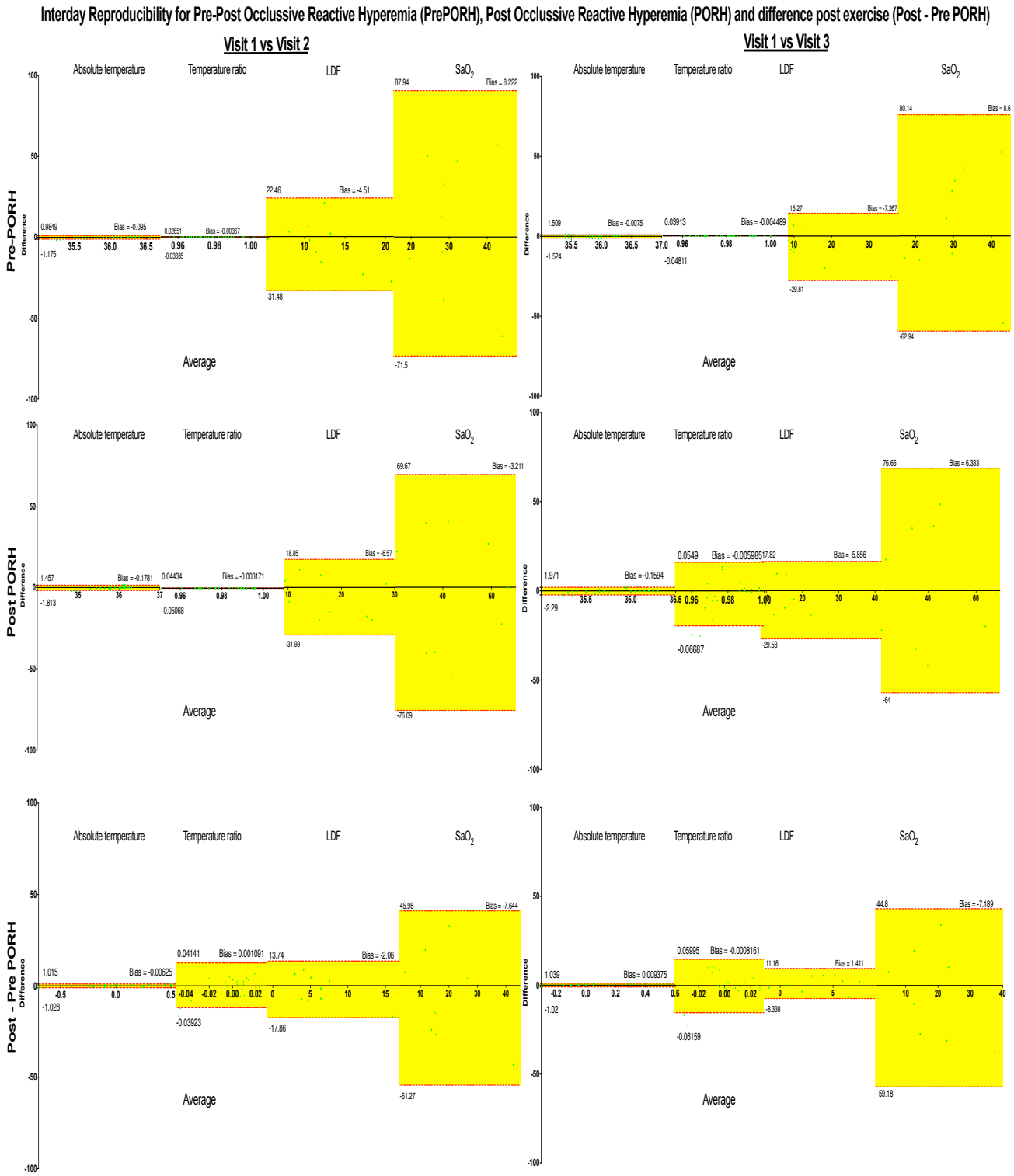


Figure 25: Inter-day reproducibility of devices pre-PORH, post-PORH and the difference between before and after a provocation test.

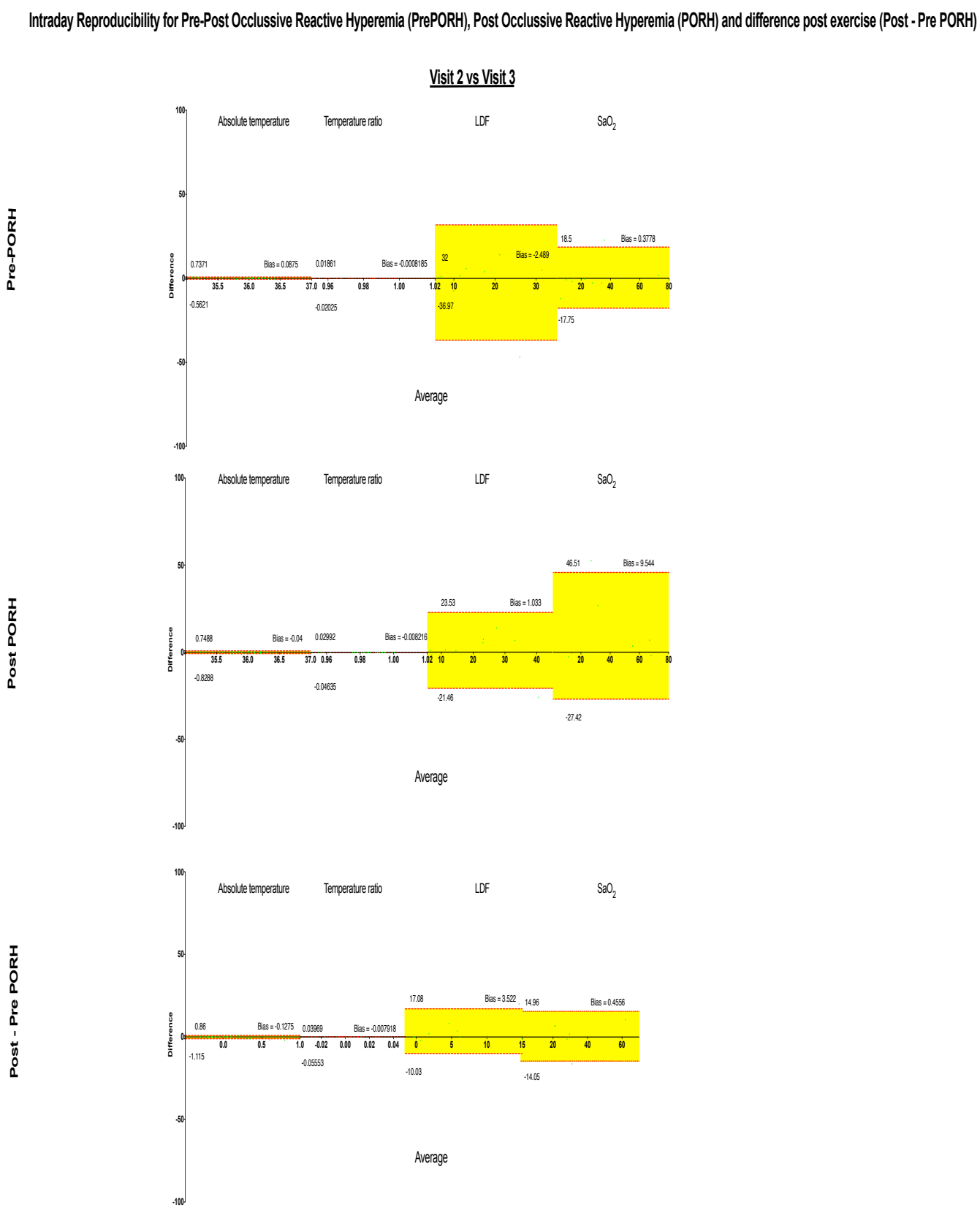


Figure 26: Intra-day reproducibility of devices pre-PORH, post-PORH and the difference between before and after a provocation test.

The bias of all temperature measurements (absolute, ratio and the difference after provocation) pre and post PORH test, is less than the manufacturer reported accuracy of 0.3°C, for the infra-red thermometer. The LOA is narrow. These reflect a very good inter-day and intra-day reproducibility. Temperature measured with the infra-red thermometers are reproducible, irrespective of when measurements were done. This is true whether absolute temperatures or temperature ratios are measured. As would be expected, temperature ratios can only be below 1, and therefore have the smallest bias and LOA, implying an almost perfect reproducibility.

The LDF based measurements of arbitrary perfusion units have a large bias between inter-day and intra-day measurements. The LOA for LDF is wide, suggesting a poor reproducibility. One possible explanation for this is that the number of participants was small in this study (Bland & Altman, 1986). Nevertheless, there was a great deal of variability in measurements with the slightest movement, making interpretation of results very difficult.

In the case of NIRS, the LOA is very wide for SaO_2 measurements, using the Portamon and Portalite device. The interday agreement is poor with a high degree of bias. However, in the case of intraday measurements, there appears to be a smaller, more acceptable bias for baseline readings and difference in oxygenation levels post provocation test. The device itself was well tolerated by participants and was easy to use.

Discussion

Feasibility of devices, provocation tests and reproducibility, were assessed in this pilot. The initial choice of a provocation test was the Post Occlusive Reactive Hyperemia (PORH) based on literature evidence as reported in Chapter 2. However, the PORH test was not tolerated by 1 of 12 healthy participants and 6 of 11 patient participants. The analysis of data for the latter group was not possible. Therefore, an assessment of the 6-minute walk test and 1-minute flexion extension test was performed on healthy participants and patient participants in this pilot study. Both these tests were well tolerated.

Infrared thermometers (IRTh), Laser Doppler Flowmetry (LDF) and the Near Infrared Spectroscopic (NIRS) devices (Portamon and Portalite) were well tolerated by all participants. In terms of cost, the infrared thermometer was an affordable device, followed by the NIRS devices with the most expensive being the LDF device. In the case of the LDF, there was a great range of measurement variability. The Nonin pulse oximeter was attempted for use on the toe, but it was too small for the big toe and too big for the smaller toes. Therefore, these results are not reliable, based on the review (Chapter 2).

Thermometry

A recent review by Houghton et al demonstrated that temperature measurement helps predict the development of ulcers in patients with diabetic neuropathy (Houghton et al., 2016). Recent articles assessing reproducibility of non-contact infrared thermometers have reported a good reproducibility with a low bias of less than 0.5°C, in different medical fields (Foto et al., 2007). In this pilot study, the inter-day and intra-day agreement for thermometry was good, with low values of bias, below the 0.5°C temperature range, suggestive of a good reproducibility for all parameters, pre and post provocation test. The lowest bias was reported

with temperature ratio measurements. This is not surprising, as maximum values for ratios can only be 1. Participants tolerated the procedure with the infrared thermometer without any difficulties.

Laser Doppler Flowmetry

Studies on the reproducibility of LDF have had varied results, with some quoting an acceptable ICC value above 0.7 (Svalestad et al., 2010) whilst others have accepted ICC values above 0.4 (Puissant et al., 2013). Nevertheless, in this pilot study, it was not possible to reliably analyze this parameter, as the LDF perfusion units varied with the slightest movement.

Oximetry

McLay et al recently studied the intra-day and inter-day repeatability of NIRS on the popliteal artery site of 9 healthy participants using a post occlusive reactive hyperemia test. The author reports a good repeatability for intra-day and inter-day measurements with an ICC of 0.92 and 0.94 respectively (McLay et al., 2016) . Another study of NIRS in 40 Dutch cyclists, demonstrated a high ICC, ranging from 0.69 to 0.99 for all parameters studied (van der Zwaard et al., 2016). In contrast, previous studies reported ICC ranging from -0.3 to 0.6 for different parameters (Southern et al., 2013) . The author has reported poor reproducibility for resting and exercise metabolism, but a good reproducibility for recovery kinetics. These papers report a good reproducibility for NIRS. In this pilot study, I have assessed the reproducibility of individual subjects at different time intervals using Bland-Altman Limits of agreement (Bland & Altman, 1986), which has the additional advantage of providing a bias value, compared to the ICC. The only parameters that appear to have differences close to

zero, between measurements are the pre-PORH and the difference of SaO_2 post exercise (Post-Pre PORH).

Conclusion

From this pilot study, temperature assessment and oxygenation were deemed the two parameters suitable for further evaluation. These are discussed in Chapter 5 and 6 respectively. Furthermore, the PORH as a provocation test was not feasible to be carried forward in this study. Therefore, simpler provocation tests, such as the 6-minute walk test (6MWT) and the 1-minute Flexion-Extension test (1MF) were used.

Chapter 5. Accuracy Study: Oxygenation

Aim

The aim of this study was to assess if NIRS can identify patients with PAD. The hypothesis being that there is a decrease in oxygenation (SaO_2) with a provocation test and that SaO_2 recovery time was longer post exercise.

Methods

Participants

The study subjects were divided into two groups (controls and patients). Controls had normal ABPIs and no significant drop in ABPI (>0.15) post exercise. Participants were recruited from the university or hospital staff. Patients had confirmed evidence of PAD, either in the form of duplex, CT or MR. They were inpatients or outpatients awaiting investigations, procedures or appointments. Patients were recruited from the vascular study lab, clinics, wards and angiography suite prior to angiograms.

Provocation test

The provocations tests used were the 1MF or the 6MWT. Participants who did not wish to perform a 6MWT were recruited for the 1MF provocation test group. The protocol used for both participant groups were the same.

Device

The Portalite is a Bluetooth operated NIRS devices that measures oxyhaemoglobin (oxyHb), deoxyhemoglobin (deoxyHb), total hemoglobin (tHb) and tissue saturation index (TSI), up to a 150 meter radius. It consists of 3 light emitting diodes that measure in 2 wavelengths (760nm and 850nm). It has one channel that measures absolute

oxygenated hemoglobin percentage (TSI%: Tissue Saturation Index %) and 3 channels to measure the other relative concentrations (oxyHb, deoxyHb and tHb) levels. The optode distance is 30, 35 and 40mm apart. Data is collected in real time using the Oxysoft software.

Study protocol

The study environment was not temperature or humidity controlled, to mimic a clinical setting. Air movement was kept to a minimum. Participants were allowed to acclimatize to the room temperature for the initial 15 minutes. During this time, patients were requested to remove their socks and shoes, to enable temperature measurements. Participants were requested to sit or lie on a trolley as long as the hips and ankles were at the same horizontal level. The NIRS device was then positioned on the MG, approximately 10cm below and medial to the tibial tuberosity (Figure 27). A crepe bandage was wrapped around the NIRS device to minimize external light exposure, during the study. The device was then synced to the Oxysoft software.

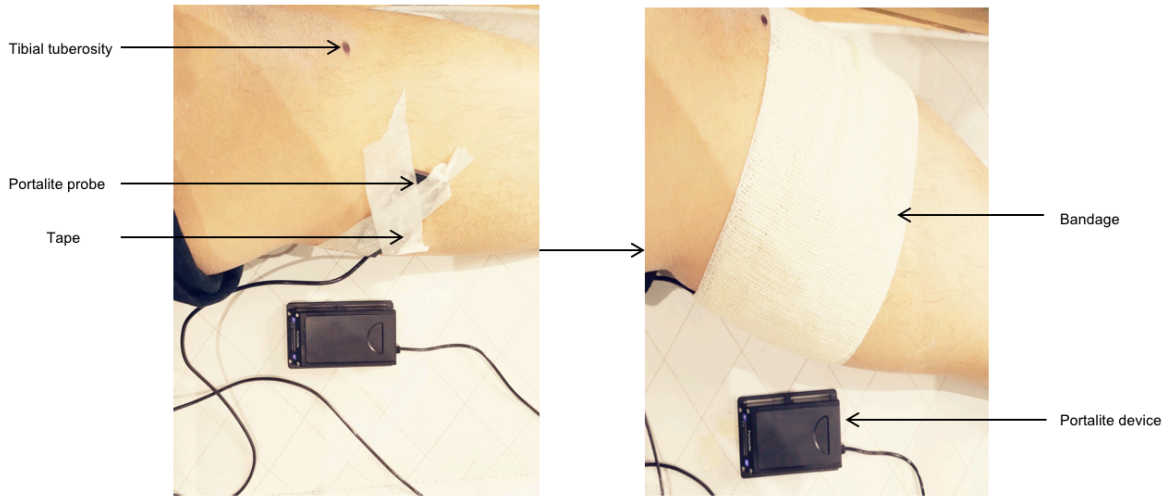


Figure 27: The Portalite probe is placed approximately 10cm medial and below the tibial tuberosity. The bandage is then applied over it.

Baseline measurements were then taken from the MG with the patient at rest for 3-5 minutes. Subjects were requested not to make any sudden movements of their toes, knees or hips during this time. After baseline measurements were taken, subjects were requested to fully dorsiflex and plantarflex their ankle on the trolley for one full minute. The duration of the exercise was recorded on the Oxysoft software. At the end of the exercise, participants were asked to rest their feet and keep still for 5 minutes. Measurements with the NIRS device were continued during this time. At the end of 5 minutes, the NIRS device was stopped. Participants were asked to rest their legs. During this time, participants demographics, comorbidities, symptoms were taken. Therefore, there was a 5-minute rest period from the end of the exercise to the start of the next provocation test. As discussed in Chapter 3, this eliminated the effect of any preconditioning. The individual study protocol for both the 1MF and 6MWT can be understood by the schematic illustration below (Figure 28).

Following this, the Portalite was synced again to the computer and baseline readings were measured again for a further 3-5 minutes. At the end of baseline measurements, participants were requested to walk on a corridor back and forth for 6 minutes. Duration of the walk was recorded on the Oxysoft software. Participants then returned to their initial position. Measurements continued for a further 5 minutes after exercise had ceased. Patients in whom the other leg was to be studied were given a further 15 minutes to rest, before undergoing the study protocol for the other leg again. Not all patients who underwent the 1MF provocation test underwent the 6MWT. This was dependent on patient choice or if they were unable or found it difficult to walk. The protocol for the control was the same, except that in the control group, only the left leg was studied.

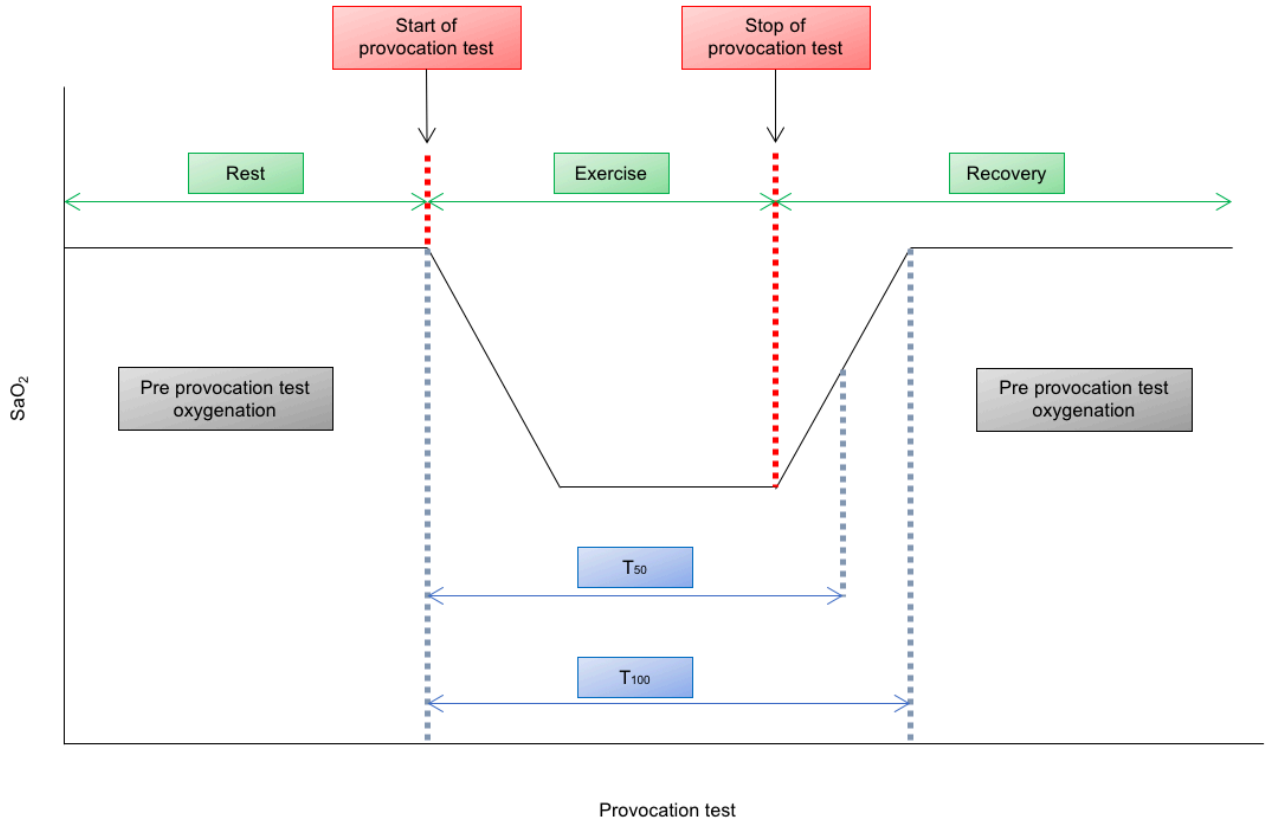


Figure 28: Schematic depiction of exercises, the 6-Minute Walk Test (6MWT) and the 1-Minute Flexion Extension Test (1MF). T_{50} : Time taken for recovery of half the baseline SaO_2; T_{100}: Time taken for complete recovery of the baseline SaO_2.

Parameter studied

The main parameters outlined to be studied were the tissue saturation index (TSI) which is the ratio of oxy-haemoglobin (OxyHb) to total haemoglobin (tHb). Baseline TSI (bTSI), difference in TSI pre and post exercise (dTSI) and the time it took to return to the baseline TSI (T_{100}) were studied. The results are outlined based on the type of test performed and the results from these parameters (Figure 29 a & b).

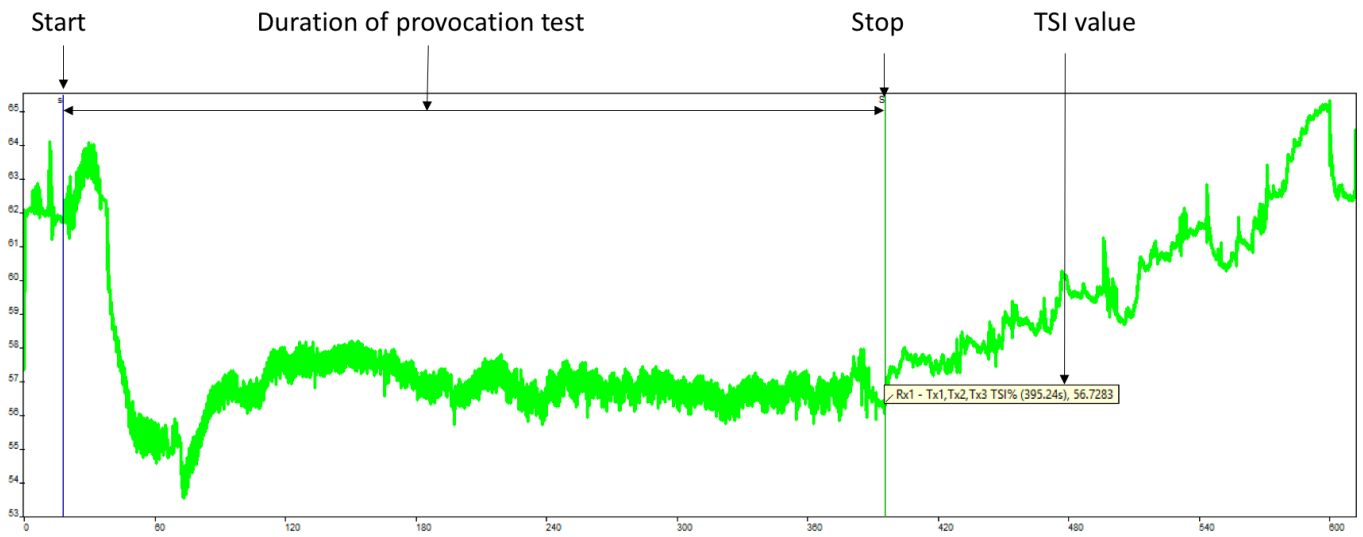


Figure 29a: Tissue Saturation Index of patient performing 6MWT

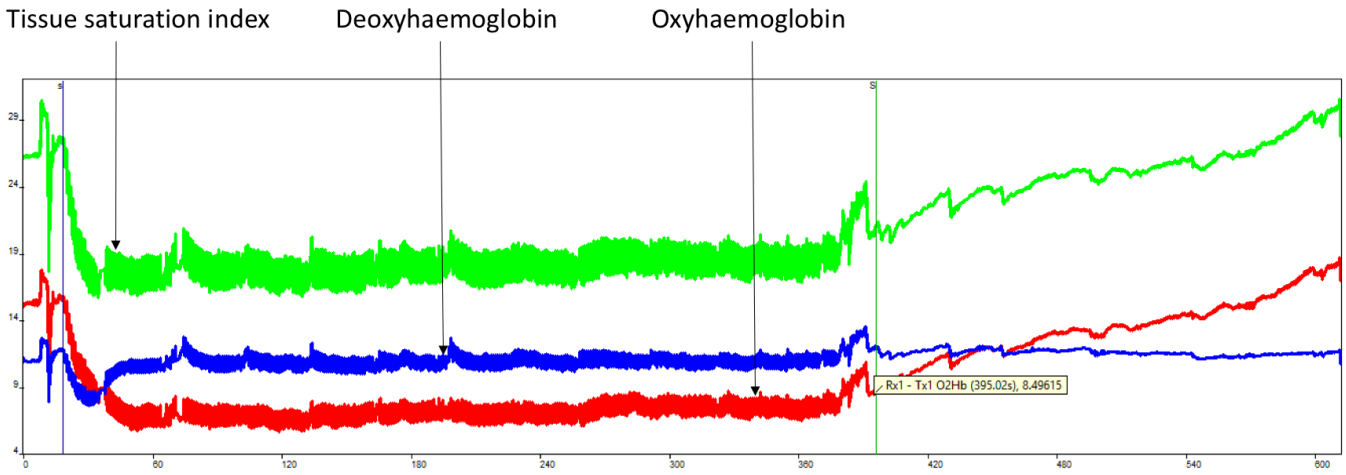


Figure 29b: Graph depicting changes in oxy, deoxy and total Hb in the same healthy patient in Fig 29a.

Unlike healthy participants, the expectation in patients with PAD, was to find a slower time to recovery and a much lower SaO_2 levels both at baseline and after the provocation test.

Statistical analysis

Statistical analysis was performed using the PRISM 7 software. Distribution of data was studied. Parametric or non-parametric tests were used accordingly. Histograms are used to depict the distribution of results. With non-normal distribution of data, a

Mann Whitney U test was used. Scatter plots are used to compare the median values. If results were significantly different, a ROC curve was created. To determine the best cutoff value, the Youden index was calculated using the formula (Sensitivity + Specificity)-1 (Hajian-Tilaki, 2013; Ruopp et al., 2008). Sensitivity and specificity was determined using this cut off value.

Results

Oxygenation measurement using 6-minute walk test in patients versus controls

All control subjects were able to complete the 6MWT. The table below depicts the demographic profile for patients and controls.

Characteristics	Patient group	Control group
Number of legs studied	56	43
Demographics		
Median age	68 (46 to 90)	47 (32 to 76)
Caucasians: Asians: Africans	51:1:0	36:6:1
Male: Female	46:6	9:34
Risk factors		
Diabetic	17 (32.7%)	2 (4.6%)
Hypertensive	25 (48.1%)	1 (2.3%)
Previous myocardial infarction/angina	15 (28.8%)	1 (2.3%)
Atrial fibrillation	2 (3.8%)	1 (2.3%)
History of Malignancy	5 (9.6%)	2 (4.6%)
Hyperlipidemia	31 (59.6%)	2 (4.6%)
History of TIA/CVA	3 (5.8%)	0 (0%)
COPD	7 (13.5%)	1 (2.3%)
Smoker	33 (63.5%)	11 (25.6%)
PAD		
Non-PAD: IC: CLI	0:41:11	44:0:0
Left: Right: Bilateral leg	24:22:5	44:0:0
Able to complete the 6MWT	37.5% (21 of 56)	100%

Table 14: Characteristics of participants undergoing the 6-minute walk test (6MWT) for measurement of oxygenation.

Baseline measurements

Analysis of Baseline Tissue Saturation Index (bTSI) in patients and controls undergoing the 6-minute walk test (6MWT)

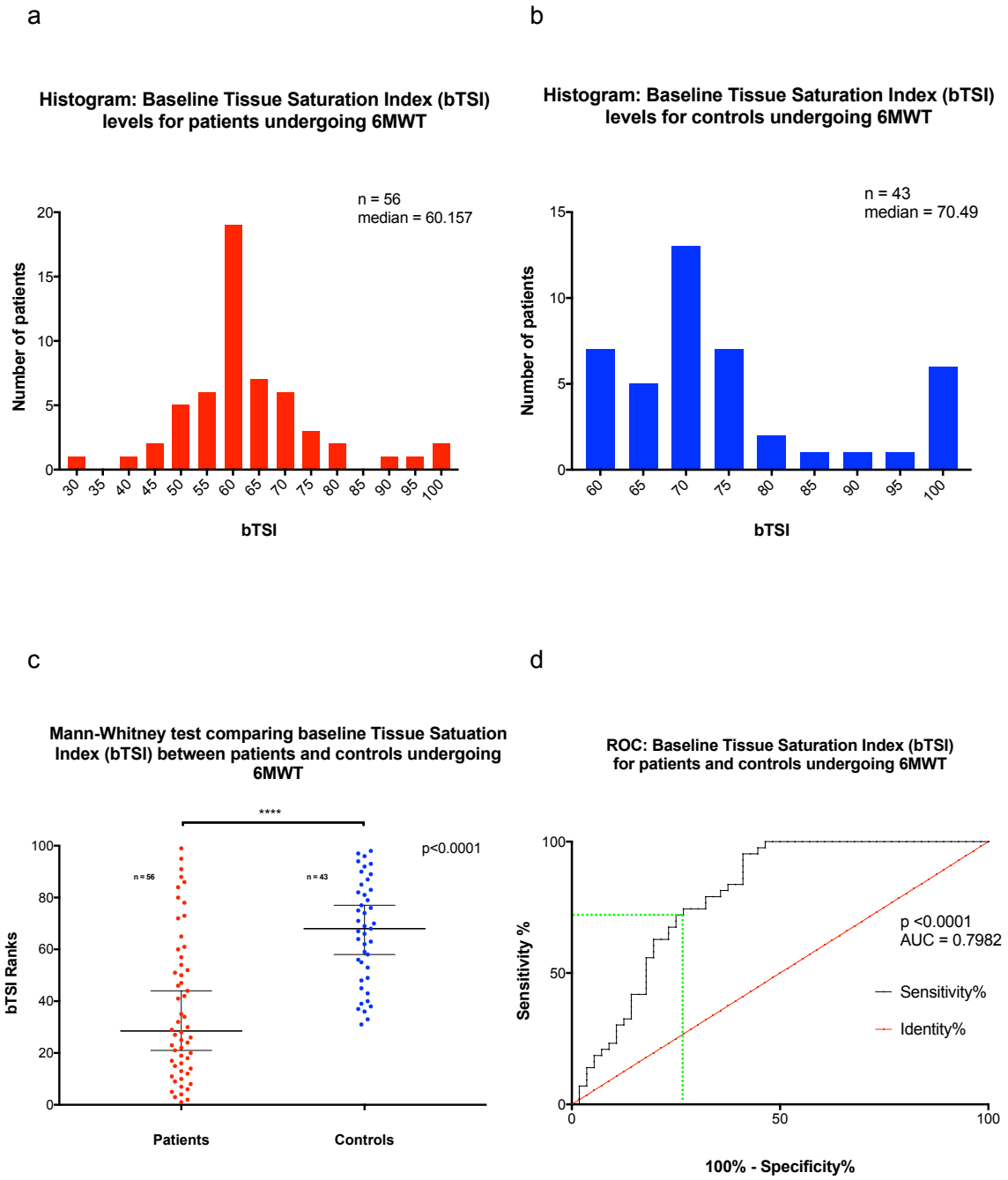


Figure 30: (a and b) The histograms show a non-normal distribution of data. (c) A Mann-Whitney U test was performed demonstrating a lower baseline TSI (bTSI) for patients that was significant (p<0.0001). Median baseline TSI levels were 60.16 (95% CI: 56.31 to 68.92) for patients and 70.49 (95%CI: 65.27 to 77.86) for controls. (d) ROC curve: Using a best Youden Index of 0.4763, gave a sensitivity and specificity of 73.21% and 74.42% respectively. The corresponding cutoff value was 66.58. AUC was 0.7982 (p<0.0001). The Likelihood ratio for PAD using this value was 2.862.

Differences in oxygen value pre and post exercise

Analysis of difference in Tissue Saturation Index (dTSI) in patients and controls after the 6-minute walk test (6MWT)

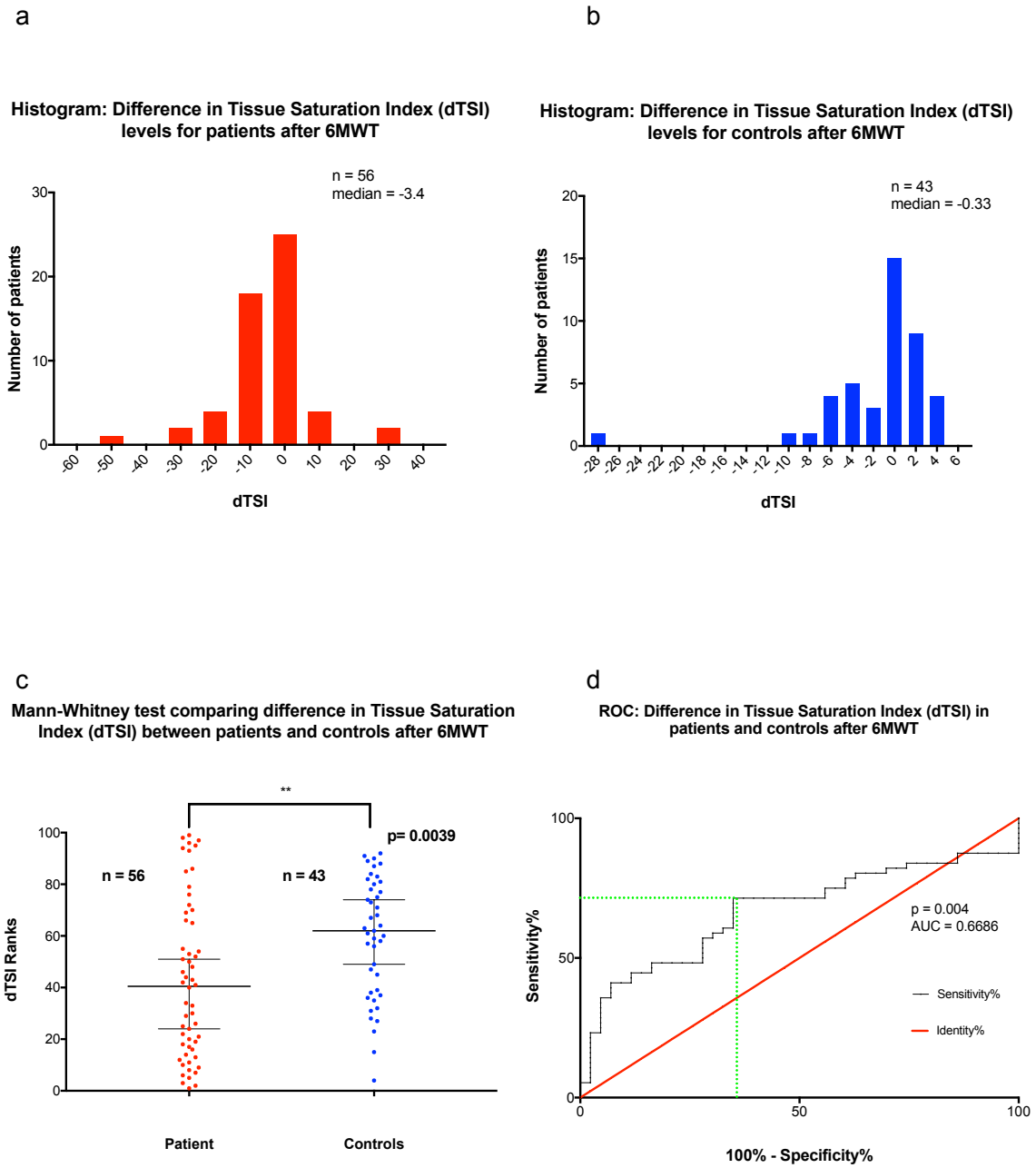


Figure 31: (a and b) The graphs above show that the difference in TSI pre and post exercise (dTSI) is not normally distributed in both patients and controls. (c) The median difference post 6MWT in TSI (dTSI) for patients is -3.401(95%CI: -8.394 to -1.791) in the patient group compared to -0.33 (95%CI: -3.132 to 0.1021) in the control group respectively. There is a significant (p=0.0039) decrease in oxygenation levels in patients compared to controls. (d) ROC curve: The highest Youden index was 0.3655. The corresponding cut off value was -1.068, with a sensitivity and specificity of 71.43% and 65.12% respectively. AUC was 0.6686 (p = 0.004). The likelihood ratio is 2.048.

Time to recovery to baseline oxygen values

Analysis of recovery time to baseline Tissue Saturation Index (T_{100}) in patients and controls after the 6-minute walk test (6MWT)

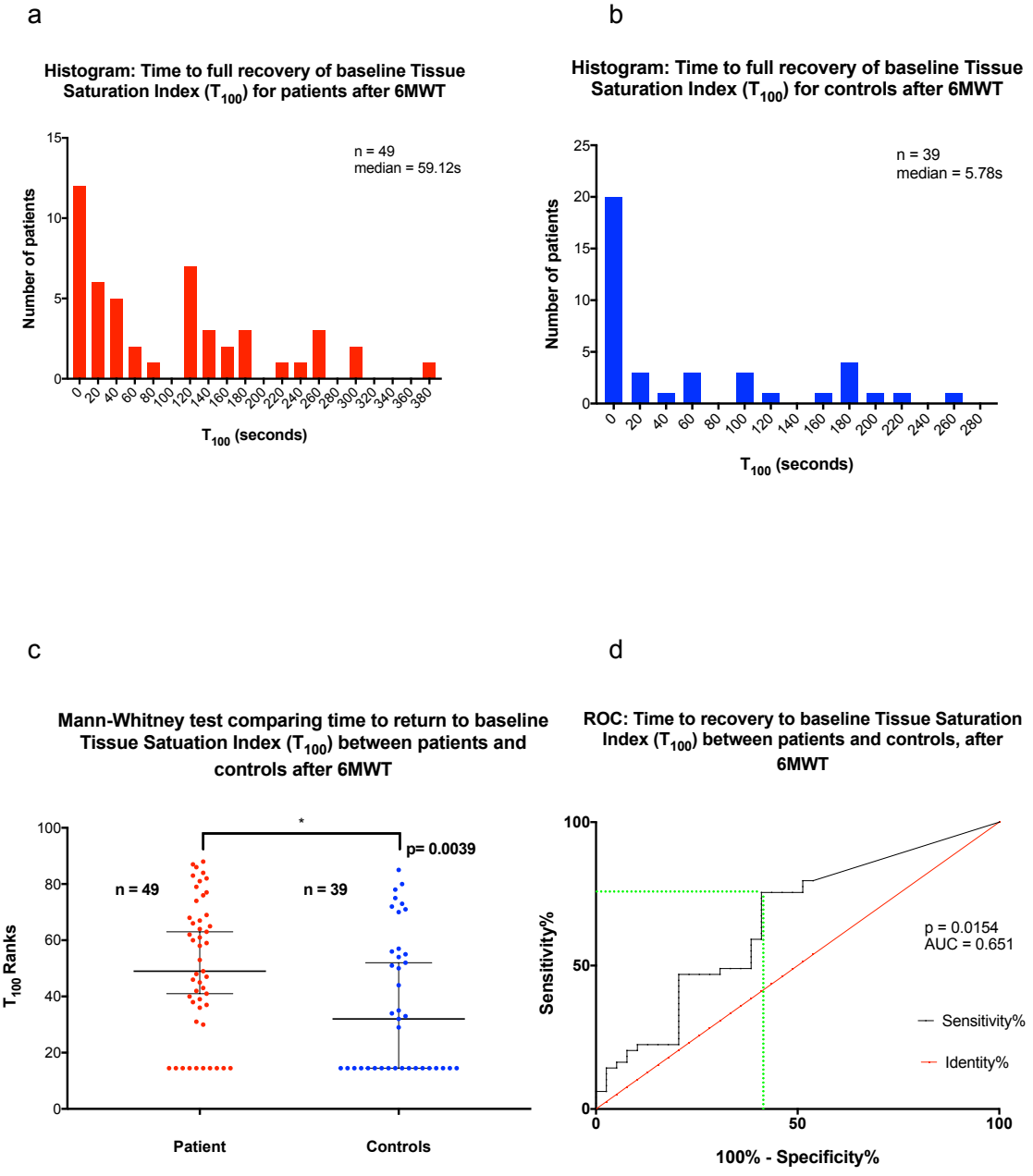


Figure 32: (a and b) The graphs below show the distribution of data for patients and controls. The data is not normally distributed. (c) A Mann-Whitney U test was therefore used. The median T_{100} value for patients is 59.12 seconds (CI: 72.31 to 129.9) compared to 5.78 seconds (CI 32.46 to 84.19) for controls (p=0.0134). (d) ROC curve: The highest Youden index was 0.3448. With a corresponding cut off value of 20.76 seconds, the sensitivity and specificity was 75.51% and 58.97% respectively. AUC was 0.651 (p=0.0154). The likelihood ratio is 1.841.

Summary of results

Tissue Saturation Index (TSI) after 6MWT			
Parameter	**Baseline TSI (bTSI)**	**TSI Difference (dTSI)**	**Recovery time (T_{100}; sec)**
Cutoff	66.58	-1.068	20.76
Sensitivity (%)	73.21	71.43%	75.51%
Specificity (%)	74.42	65.12%	58.97%
LR	2.862	2.048	1.841
AUC	0.7982	0.6686	0.651
p value	<0.0001	0.0042	0.0154

Table 15: Summary of results from Tissues Saturation Index measurement before and after the 6-minute walk test. 6MWT= 6-minute walk test; bTSI= Baseline tissue saturation index, dTSI= Difference in tissue saturation index after the 6MWT; T_{100}= time of recovery to baseline tissue saturation index post 6MWT; AUC = Area under the curve; LR = Likelihood ratio.

Of the participants, only 21% were CLI, and only 38.5% were able to complete a walk test. In this study, TSI values were measured at the medial gastrocnemius (MG). Using a TSI cutoff value of 66.58 for the baseline TSI (bTSI) parameter, the sensitivity and specificity were almost equal (sensitivity 73.2%, specificity 74.42%). Post 6MWT, a decrease in TSI (dTSI), by 1 unit yields an almost similar sensitivity (71.43%) to that of the bTSI parameter, at the cost of a reduced specificity (65.12&). Recovery time (T_{100}) of 20 seconds is a little more sensitive (75.51%), compared to bTSI and dTSI, suggesting that a return to baseline TSI values within 20 seconds is a more sensitive but less specific test (58.97%).

The easiest parameter to assess would be the bTSI. T_{100} and dTSI offers a slightly more sensitive test at the cost of being less specific. However, this is only marginally so. This would suggest that baseline TSI parameters taken at the MG, provides a reasonable test of oxygenation between patients and controls. Interestingly although less than half of patients were able to complete the 6MWT, their dTSI was still

available for calculation, and 87.5% of these patients had recovery time available for calculation. This suggests that the duration may actually be dependent on each individual's absolute claudication distance rather than a pre-determined time.

Oxygenation measurement using 1-minute flexion extension test in patients versus controls

The table below describes the demographic profile of the patients and controls.

Characteristics	Patient group	Control group
Number of legs studied	107	44
Demographics		
Median age	70 (46 to 87)	47 (32 to 76)
Caucasians: Asians: Africans	100:3	36:6:2
Male: Female	86:17	11:33
Risk factors		
Diabetic	35 (34%)	2 (4.5%)
Hypertensive	55 (53.4%)	1 (2.3%)
Previous myocardial infarction/angina	33 (32%)	1 (2.3%)
Atrial fibrillation	8 (7.8%)	1 (2.3%)
History of Malignancy	6 (5.8%)	2 (4.5%)
Hyperlipidemia	59 (57.3%)	2 (4.5%)
History of TIA/CVA	11 (10.7%)	0 (0%)
COPD	13 (12.6%)	1 (2.3%)
Smoker	69 (67%)	12 (27.3%)
PAD		
Non PAD: IC: CLI	0:72:31	44:0:0
Left: Right: Bilateral leg	49:34:18	44:0:0
Able to complete the 1MF	88.8% (95 of 107)	100%

Table 16: Characteristics of participants undergoing the 1-minute flexion extension test for measurement of oxygenation.

Baseline measurements

Analysis of baseline Tissue Saturation Index (bTSI) in patients and controls undergoing the 1-minute flexion extension test (1MF)

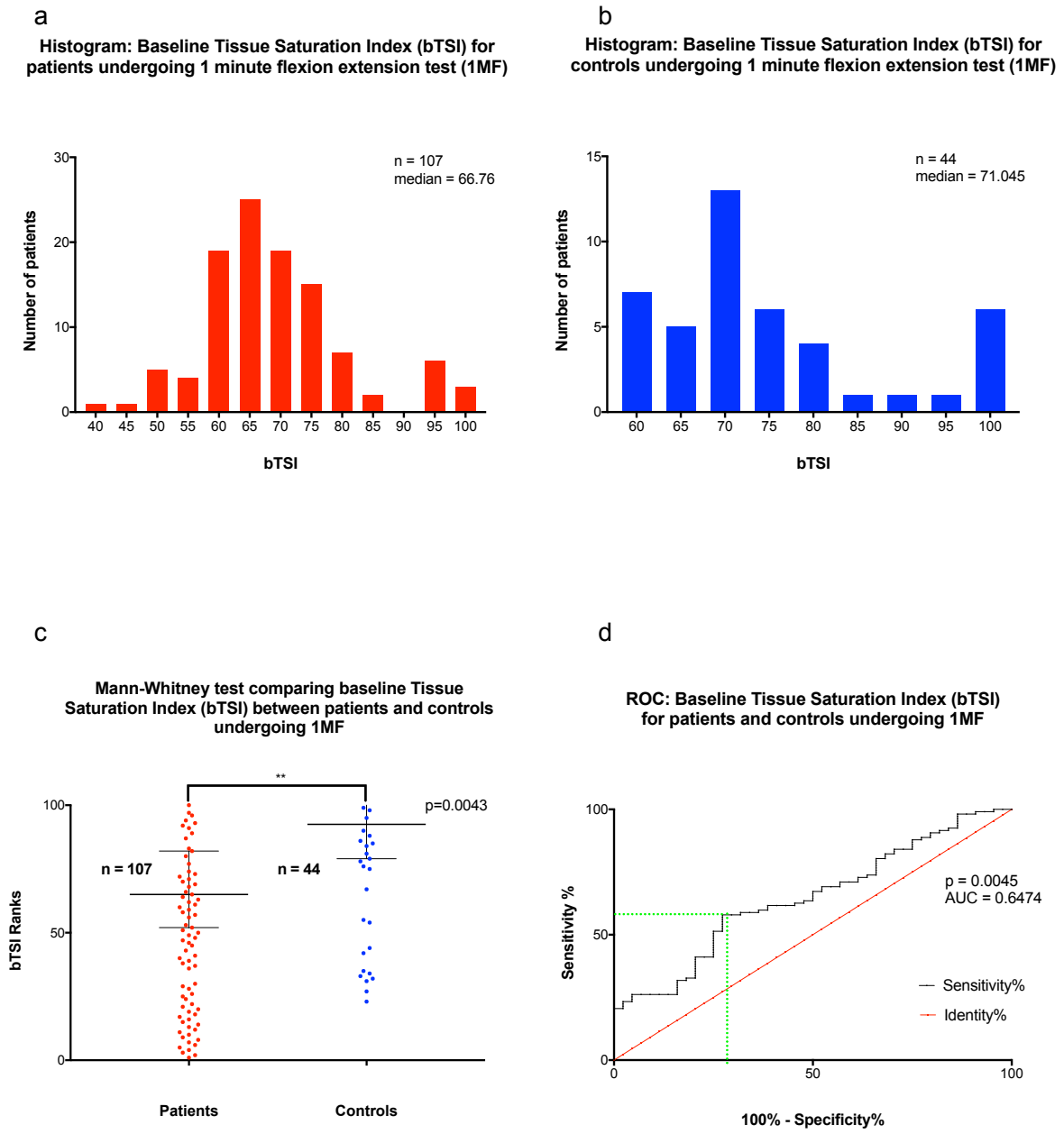


Fig 33: (a and b) Baseline oxygen values (bTSI) were not normally distributed for controls and patients in the 1-minute flexion extension group (1MF). (c) A Mann-Whitney U test demonstrates that the median bTSI for patients (68.94; 95% CI:62.04 to 75.44) was significantly lower (p=0.0043) compared to the median bTSI for controls (75.14; 95% CI: 65.71 to 79.72). (d) ROC curve: The best Youden index was 0.3067. With an AUC of 0.6474 (p=0.0045) and a cutoff value of 68.92, the corresponding sensitivity and specificity was 57.94% and 72.73% respectively. Likelihood ratio was 2.125.

Differences in oxygen value pre and post exercise

Analysis of difference in Tissue Saturation Index (dTSI) in patients and controls after the 1-minute Flexion Extension (1MF) test

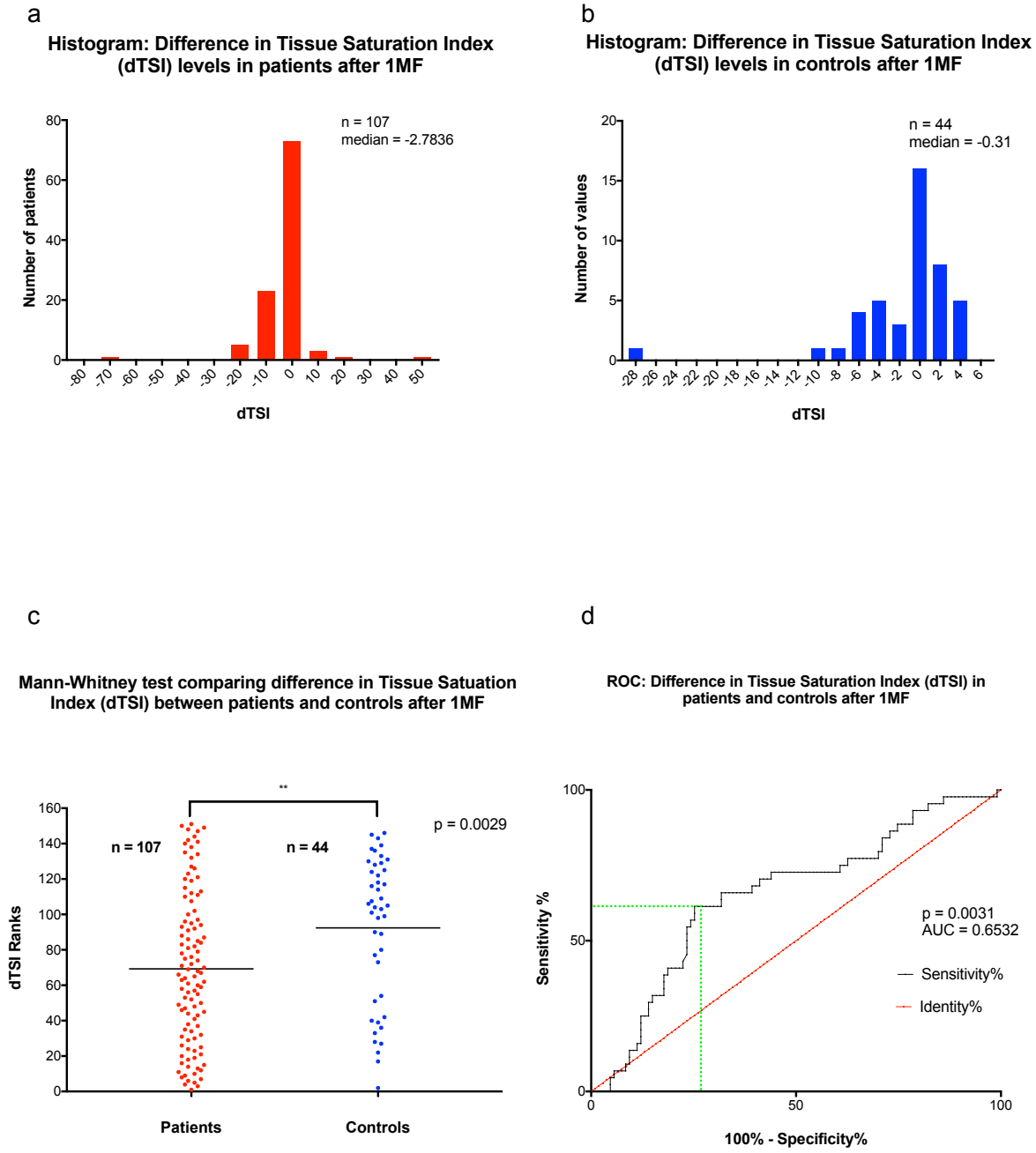


Fig 34: (a and b) The histograms below show the distribution of data for patients and controls. The data is not normally distributed. (c) A non-parametric Mann-Whitney U test was used. This demonstrates a significant decrease in TSI after exercise (dTSI) in controls (p=0.0029). The median dTSI for patients was -2.784 (CI: -5.537 to -1.694) compared to controls, who had a median of -0.31 (CI: -3.017 to 0.1639). (d) The best Youden index was calculated to be 0.3613. Cutoff value was -0.573, providing a sensitivity of 61.36% and specificity of 74.77%. The AUC is 0.6532 (p = 0.0031).

Time taken for recovery to baseline values (T_{100})

Analysis of recovery time to baseline Tissue Saturation Index (T_{100}) in patients and controls after the 1-minute flexion extension test (1MF)

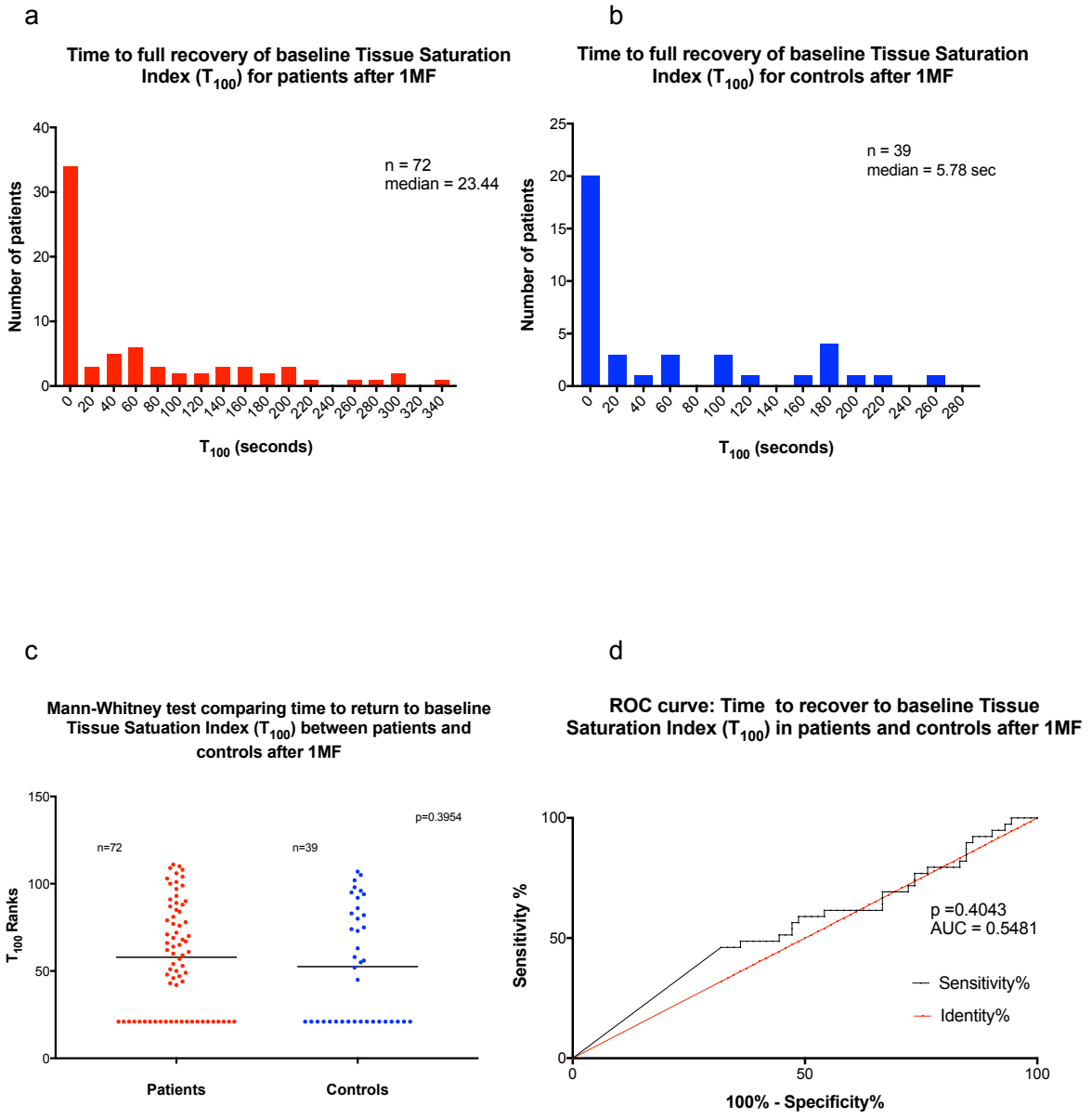


Fig 35: (a &b) The histograms depict the recovery time (T_{100}) for patients and controls. This data is not normally distributed. (c) Mann Whitney U test was used for analysis. The median time taken for recovery to baseline oxygenation levels in controls and patient were not statistically significant from each other (p=0.3954). The median T_{100} for patients was 23.44seconds (95% CI: 46.05 to 87.99) versus controls with a median T_{100} of 5.78 seconds (95% CI: 32.46 to 84.19). (d) ROC curve: The best Youden index was 0.1421. The corresponding cutoff value was 0.52 seconds. This yielded a sensitivity of 68.06% and specificity of 46.15%. The AUC was 0.5481 (p = 0.4043).

Summary of results

Tissue Saturation Index (TSI) after 1MF			
Parameter	**Baseline TSI (bTSI)**	**TSI Difference (dTSI)**	**Recovery time (T_{100}; sec)**
Cutoff	68.92	-0.5739	0.52
Sensitivity (%)	57.94	61.36%	46.15%
Specificity (%)	72.73	74.77%	68.06%
LR	2.125	2.432	1.445
AUC	0.6474	0.6532	0.5481
p value	0.0043	0.0031	0.4043

Table 17: Summary of results of the NIRS device for parameters of baseline TSI, Difference in TSI post exercise and time to full recovery of baseline StO_2 (T_{100}), using the 1MF test; 1MF = 1-minute flexion extension test; bTSI = baseline tissue saturation index; dTSI = Difference in tissue saturation index after the 1MF test; T_{100}= Time taken for recovery to baseline TSI values. LR = Likelihood ratio; AUC = Area under Curve.

30.1% of the participants in this group had CLI. The cutoff for bTSI in the 1MF group was 68.92, yielding a sensitivity and specificity of 57.94% and 72.73% respectively (p=0.0043). In the case of dTSI, the cutoff value of -0.5739 yields a sensitivity and specificity of 61.36% and 74.77% respectively (p=0.0031). The recovery time in the 1MF group (T_{100}) was not significantly different between patients and controls (p=0.4043). The sensitivity for bTSI levels compared to the 6MWT (57.94% vs 73.21%). This could be explained by more patients with CLI in the 1MF group (1MF vs 6MWT: 30.1% vs 21%).

Comparison of 6-Minute Walk Test (6MWT) and 1-minute flexion extension test (1MF)

Comparison of baseline Tissue Saturation Index (bTSI), difference in Tissue Saturation Index (dTSI) and time to recovery to baseline TSI (T_{100}) between the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF) group

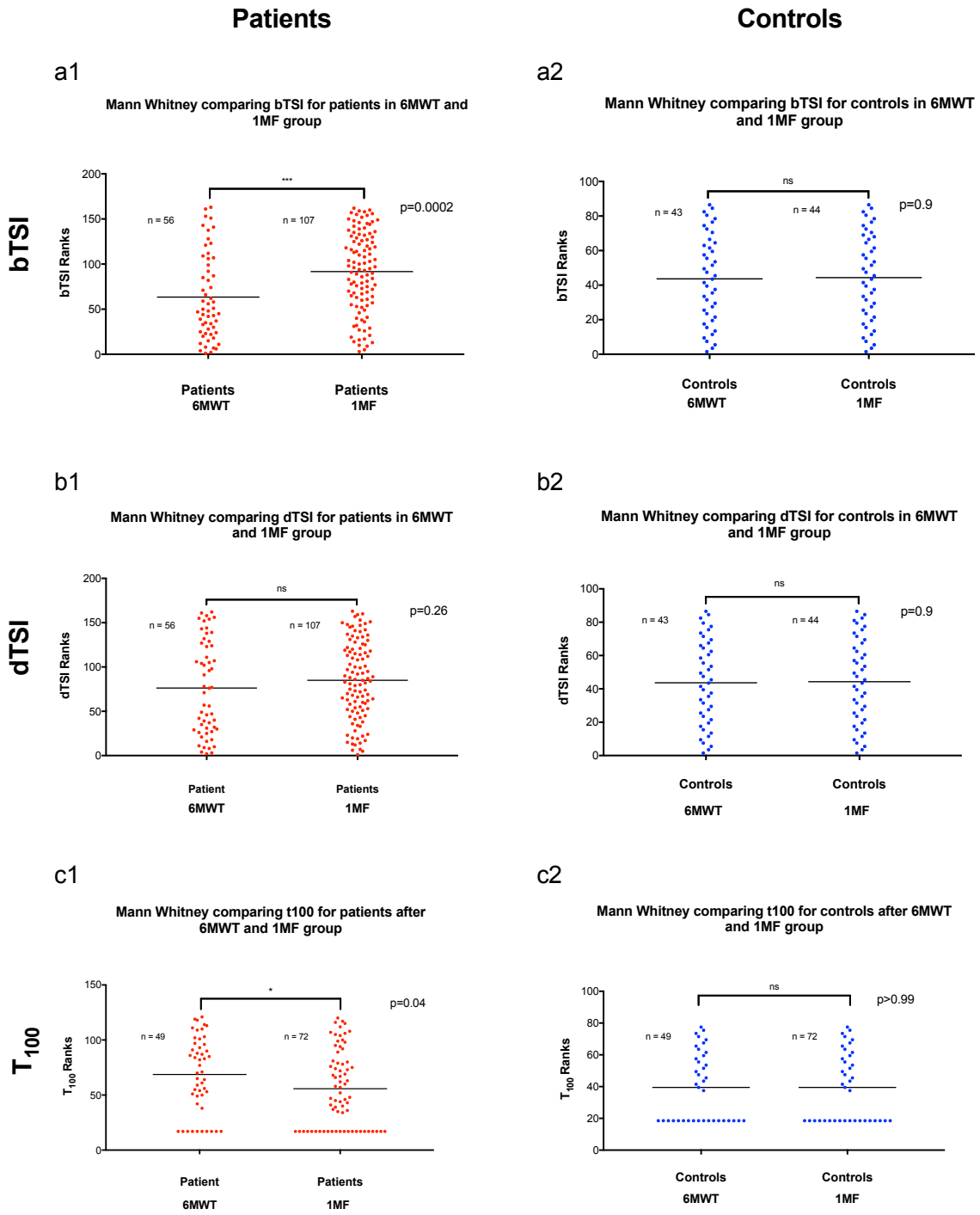


Fig 36: The graph above shows the difference between the 2 different provocation tests for each parameter (bTSI, dTSI, T_{100}) between patients and controls respectively. The Mann-Whitney U test was used for the non-parametric distribution of data.

Tissue Saturation Index (TSI) parameter			
Parameter	bTSI	dTSI	T100
Patients (6MWT vs 1MF)	Median 60.16 vs 66.76 p = 0.0002	Median -3.401 vs -2.784 p = 0.26	Median 59.12 vs 23.44 p = 0.04
Controls (6MWT vs 1MF)	Median 70.49 vs 71.05 p = 0.9008	Median -0.33 vs -0.31 p = 0.9	Median 5.78 vs 5.78 p >0.99

Table 18: TSI parameters pre and post provocation tests, along with their median values and statistical significance.

In graphs a1 and a2, the bTSI was significantly lower (p=0.0002) in patients undergoing the 6MWT (Median 60.16; 95%CI: 56.31 to 68.92) compared to 1MF (Median 66.76; 95%CI: 62.04 to 75.44). In comparison, there was no statistically significant difference (p=0.9) between controls undergoing 6MWT (Median 70.49; 95%CI: 65.27 to 77.86) and 1MF (Median 71.05; 95%CI: 65.71 to 79.72).

Graphs b1 and b2, demonstrate that the dTSI was not statistically different between patients (p=0.26) and controls (p=0.9) undergoing 6MWT and 1MF. The median dTSI for patients undergoing the 6MWT was -3.401 (95%CI: -9.43 to 0.1297) and for the 1MF was -2.784 (95%CI: -5.23 to -0.4614). The median dTSI for controls undergoing 6MWT was -0.33 (95%CI: -3.85 to 1.332) and for those undergoing 1MF was -0.31 (95%CI: -3.77 to 1.44).

The time to recovery to baseline TSI (T_{100}) depicted in graphs c1 and c2 demonstrate that T_{100} was significantly longer in those undergoing the 6MWT compared to patients undergoing 1MF (p=0.04). There is no statistically significant difference (p>0.99) between controls undergoing 6MWT and 1MF. The median T_{100} for patients undergoing 6MWT was 59.12 seconds (95%CI: 14.74 to 154.3 seconds) and median T_{100} for patients undergoing 1MF was 23.44 seconds (95%CI: 0 to 110.8 seconds). The median T_{100} for controls undergoing the 6MWT was 5.78 seconds (95%CI: 0

to109.2 seconds) and for those undergoing 1MF, the median T_{100} was 5.78 seconds (95%CI: 0 to 109.2 seconds).

The control participants did not exhibit any difference in the bTSI, dTSI and T_{100} levels for both 6MWT and 1MF groups. Patients however exhibited a difference between test groups (6MWT and 1MF) for the parameters of bTSI and T_{100}. Patients in the 6MWT group had a lower bTSI to start with, compared to the 1MF group. These patients (6MWT) also took longer to recover after the 6MWT. Although this is not likely to be due to preconditioning, as the duration of the test (1 minute) is not sufficient to cause this effect and with adequate rest between the 2 tests.

These results suggest that although all 3 parameters provide a similar sensitivity and specificity for both provocation test, the dTSI parameter is most reliable irrespective of the study population and the type of test used, i.e if using a different provocation test or using a different study population, the dTSI is more reliable.

Discussion

Baseline tissue saturation index (bTSI)

Baseline oxygen measurements in patients and controls undergoing the two different tests were significantly different from each other. This was consistent with previous systematic reviews (Verdi & Nini, 2008; Komiyama et al., 2002; Comerota et al., 2003; Kooijman et al., 1997). Interestingly, the baseline readings for patients undergoing the 6MWT and the 1MF, were also significantly different from each other (p=0.0002) compared to controls whose baseline reading was not statistically different (p=0.9).

Two possible explanations for these are either due to more patients in the 1MF group with CLI compared to the 6MWT group (30.1% vs 21%).

Difference in tissue saturation index (dTSI)

In a recent systematic review by Boezeman et al, 12 papers were studying the role of NIRS in the diagnosis of PAD were included (n=848) (Boezeman et al., 2016). Deoxygenation and rate of deoxygenation was significantly higher in claudicants (Kooijman et al., 1997; Bauer et al., 2004; Mesquita et al., 2013). Time to half and full recovery (T_{50} and T_{100}) of baseline oxygenation levels were significantly longer in claudicants (McCully et al., 1997; Comerota et al., 2003; Mesquita et al., 2013; Kooijman et al., 1997). The ratio of patient: controls for these papers was 14:35, 11:15, 26:31, 39:63 respectively, with the largest group of patients provided by McCully et al in 1997 (McCully et al., 1997).

The latter study bears some similarities with this study in terms of the provocation test used. This study employed a 1-minute repeated flexion, using a seated calf machine with spinal support. The author reports a sensitivity of 51-76% and specificity of 57-76% for T_{50}, with a best cutoff value of 40 seconds. Unfortunately, the gold standard for the diagnosis of claudication was made by history and clinical examination. Therefore, the reported statistics are not in a true sense, sensitivity or specificity. Comerota et al used ABPI as a gold standard, studying the ebb in oxygen levels during exercise. The author reported a lower ebb in StO_2, greater deoxygenation, T_{50} (89% and 85% sensitivity and specificity) and T_{100} (sensitivity 88%; specificity 81%) (Comerota et al., 2003). The only paper to employ an acceptable standard was by Manfredini et al using duplex ultrasound as the diagnostic standard in patients. However, the statistical analysis of the paper compared the area under curve (AUC) for oxyHb, DeoxyHb and Total Hb (Manfredini et al., 2009). The main criticism of the paper, was the translational potential of the analysis required to make this technique convenient in day to day practice, mainly in terms of the complex statistical analysis required (Boezeman et al., 2016).

Recovery time to baseline TSI (T_{100})

Although T_{50} may be significantly different between PAD and non-PAD patients, its analysis requires looking in to detail the graph and manually finding the T_{50}. This makes the process tedious, and not applicable in day to day practice. In my study, the focus has been on the translational potential of the diagnostic test used. I intended to study the T_{100} of NIRS, simply because of its similarity to the pulse oximeter, which is used on a day to day basis. However, the latter device is not suitable to be used in the lower limbs, due to factors explained in previous chapters. The pulse oximeter is

only capable of measuring oxyhaemoglobin levels. To date, no paper has looked at the oxygen levels before and after exercise. Additionally, current technology of the pulse oximeter does not allow for continuous measurement of the oxygenation post exercise, which can be used to help identify a cutoff value or time at which measurements can be taken. In my current study all patients were diagnosed by duplex as a minimum. In some cases, patients were diagnosed with CT or MRI. Duplex is the commonest, most feasible test used to diagnose PAD in regular clinics. The main limitation of my study is that control subjects did not have a duplex, but had a baseline and post exercise ABPI.

Bouyé et al reported buttock claudication reported a sensitivity of 33% and specificity of 87% using a recovery cutoff time of >240 seconds for buttock claudication (Bouyé et al., 2005). Gardner et al found a T_{100} time of 225+/-140 seconds in his study using a graded treadmill protocol (Gardner et al., 2008). In comparison, the median time to recovery to tissue saturation index, was longer in patients who performed the 6MWT (59.12s) compared to those who performed the 1MF test (23.44s). It appears that the longer the duration of the exercise, the higher the sensitivity of the test using the dTSI, irrespective of the study population.

Contrary to these results, Mesquita et al found no significant difference in the type of exercise for oxygen drop post exercise (treadmill vs pedal flexion) in PAD patients. This could be explained by the use of mean values for StO2 parameters, which could have caused a bias in the StO2 levels. This was acknowledged by the author, as the mild PAD patient parameters have a wider confidence interval, and to some extent behave like healthy patients (Mesquita et al., 2013).

Conclusion

NIRS offers the advantage as a one-off triage test to identify PAD patients. The sensitivity and specificity vary depending on the duration of exercise and the parameter studied. Using the 6MWT, the bTSI cutoff value of 66.58 provides a sensitivity of 73.2% and specificity of 74.42%, distinguishing PAD from non-PAD ($p<0.0001$). Using the dTSI cutoff of -1.068, produces a sensitivity of 71.43% and specificity of 65.12% ($p=0.0042$). A T_{100} of 20.76 seconds provides a sensitivity of 75.51% and specificity of 58.97% ($p=0.0154$). Using the 1MF test, the bTSI cutoff yields a sensitivity of 57.94% and specificity of 72.73% ($p=0.0043$), whereas a dTSI cutoff of -0.5739 yields a sensitivity of 61.36% and specificity of 74.77% (p=00031).

The ability of NIRS to monitor real time oxygen changes, allows for the determination of a cutoff point and the time to return to baseline oxygen values. What this study adds to current knowledge, is that oximetry can be used fairly reliably as a triage test before the use of duplex. Based on my study findings, baseline TSI (bTSI), differential TSI (dTSI) and time to return to baseline TSI (T_{100}) can all be used, depending on the duration of the test. However, the dTSI is the more reliable of the 3 parameters. Future studies are required for comparison and such studies may then justify the use of a potential pulse oximetry device suitable for the lower limbs.

Chapter 6. Accuracy study - Thermometry

Aim

The aim of this study was to assess if infra-red thermometry (IRTh) can identify patients with PAD. The hypothesis being that, there is a decrease in temperature following a provocation test.

Introduction

IRTh has not been used to diagnosed PAD in previous studies. Additionally, previous validation studies on infra-red thermometers, have not used appropriate gold standards. In order to validate the use of infra-red thermometers, a laboratory-based and clinical setting was necessary. The findings of these studies are described in 3 main sections. These are

Section 1: Validation of infra-red thermometers as a traceable fixed point for thermometry in clinical practice: Laboratory-based study.

Section 2: Pilot study comparing Infrared camera versus infrared thermometers in a clinical setting.

Section 3: Baseline and Exercise induced temperature changes in patients versus controls.

Section 1: Validation of infrared thermometers as a traceable fixed point for thermometry in clinical practice – laboratory based study.

Introduction

Temperature assessment or thermometry is influenced by the operational characteristics of the measurement device, the environment in which measurements are taken and the subject or object characteristics. Three important concepts in thermometry are the 'measurement traceability', 'fixed points' and the 'emissivity' of the surface measured. 'Measurement traceability' is the unbroken chain of calibration to a 'fixed point'. All devices that measure temperature need to be calibrated to a 'fixed point' as defined by the International Temperature Scale of 1990. These 'fixed points' have an established temperature range against which calibration can be performed (Preston, 1990). The National physical laboratory (NPL) is a national measurement standards body in the UK that ensures calibration of devices to a traceable 'fixed point'. A common laboratory based 'fixed point' is the 'near' blackbody radiator, which uses a calibrated sensor to confirm the blackbody cavity temperature (Rusby, 2012). The best calibrated sensor suited to measure cavity temperature in a blackbody radiator is the standard platinum resistance thermometer (Preston, 1990). The 'emissivity' of an object can be defined as the ratio of the amount of heat that is radiated from the surface of the object to the heat radiated by a perfect black body. A perfect blackbody radiator has an emissivity of 1. A 'near perfect' blackbody radiator has an emissivity greater than 0.995 (Rusby, 2012). Some temperature devices autocorrect the temperature based on the emissivity of the surface whereas some require the known or estimated emissivity to be keyed in, to be able to provide a

reliable measurement. It is not possible to directly measure body temperature with current reference 'fixed points' such as the blackbody radiator or platinum resistance thermometer. Furthermore with the discontinuation of the use of mercury in glass thermometers in clinical care, there is a gap in the 'measurement traceability' between laboratory and clinical thermometry (Robinson et al., 1998; Smith et al., 2010). A reference 'fixed point' for human body measurement should consider both the device used and the site of measurement. Temperature measurement with oesophageal, pulmonary artery, bladder, rectal, tympanic, axillary thermometers are some 'reference standards' used in thermometry studies, due to the lack of a true reference method for measuring skin temperature (Smith et al., 2010). None of these are validated as 'fixed points' for thermometry (Preston, 1990). The use of an imperfect reference standard provides results that are misleading (Bland & Altman, 1986).

Various devices were suggested as alternatives to the mercury in glass thermometer including compact electrical thermometers with maximum device, phase change (dot matrix) thermometers, infrared tympanic thermometers, continuous reading electrical thermometers and thermography (Rusby, 2012). Of these, radiation thermometry is increasingly popular due to its convenience, reduced infection transmission and better acceptance by patients.

This study focuses on two non-contact infrared based devices that are used in clinical practice, the thermal infrared camera and the non-contact infrared temporal thermometer. The emissivity of the thermal infrared camera can be adjusted to match the known emissivity of the object studied (Suleman et al., 2002). Non-contact infrared temporal thermometer has inbuilt algorithms that correct the temperature based on

the emissivity of the object (Rusby, 2012). The calibration of contact based infrared thermometers against blackbody radiators has been performed in the past (Smith et al., 2010).

Aim

No study to date has compared temperature measurements made by the non-contact infrared temporal thermometer and the thermal infrared camera against each other and a blackbody radiator or its calibrated sensor, the platinum resistance thermometer. We report the agreement and correlation between the non-contact infrared thermometer, thermal infrared camera and platinum resistance thermometer against each other and the blackbody radiator.

Methods

The study was performed in a temperature and humidity controlled room. The room temperature was maintained at 24°C and the humidity kept at 30%. A Hyperion R Model 982 Isotech blackbody radiator with an in-built digital temperature controller that allowed the cavity temperature to be set at any value between -10 to 80°C. The traceability of the blackbody cavity temperature was established using a separate temperature indicator and an included platinum resistance thermometer. This near perfect blackbody radiator was used as the reference 'fixed point'. Devices tested included a thermal infrared camera (FLIR T650sc) and a non-contact infrared thermometer (FS-700). Measurements were made 2 hours after activation of the camera. The FLIR T650sc thermal infrared camera with a manufacturer reported

<0.02°C sensitivity and ±1°C accuracy, capable of measuring temperatures between -40°C to 2000°C. Minimum focal distance for this device is 25cm. The FS-700 non-contact infrared temporal thermometer is capable of measuring body temperatures between 22°C and 42.5°C (±0.3°C accuracy) and object temperatures between 10°C and 80°C (±2°C accuracy), provided the relative humidity is below 95%. The infrared thermometer has an object and body setting. For the purpose of this study, the device was set at the object setting. The recommended distance for measuring temperature using this device is 3 to 5 cm.

Blackbody cavity temperature was escalated at 0.5°C increments between 20°C and 40°C. At each increment, the cavity temperature was established with the platinum resistance thermometer. The thermal infrared camera was set up on a fixed mount at a fixed distance of 20cm from the blackbody aperture during measurements. The infrared thermometer was set up on a fixed mount 5cm away from the blackbody aperture. Blackbody cavity temperature was initially measured with the thermal infrared camera followed by the infrared thermometer at object settings. In the case of the infrared thermometer, measurements were only compared for the temperature range of 30°C to 40°C, as any measurement below or above this temperature is provided as ‘low’ or ‘high’ respectively, on the digital display of this thermometer.

A systematic bias < 0.2°C, 95% limits of agreement < 0.3°C and a correlation > 0.9 were deemed acceptable from previous studies (Casa et al., 2007; Smith et al., 2010), using a blackbody cavity or standard platinum resistance thermometer as a reference standard (Rusby, 2012). When comparing the thermal camera to the infrared thermometer, a systematic bias <0.3°C and a 95% limits of agreement <0.9°C were

set as acceptable thresholds (Kelechi et al., 2006). The emissivity of the thermal infrared camera was set at 0.995 to match that of the black body radiator. The infrared thermometer autocorrected for surface emissivity and could not be manually adjusted.

Statistical analysis was performed using the GraphPad Prism 6 software. The mean difference (bias), 95% limits of agreement (95% LOA), and the Pearson correlation coefficient (r) were calculated for all comparisons. The degree to which the thermal camera and infrared thermometer vary from the reference 'fixed point' is given by the limits of agreement.

Results

For the range of temperatures measured (20°C to 40°C), measurements with the platinum resistance thermometer were all within ±0.2°C of the blackbody cavity temperature, compared to 80.5% (33 of 41) of measurements taken with the thermal camera. When the platinum resistance thermometer was considered as the reference standard, 75.6% (31 of 41) of measurements taken with the thermal infrared camera were within ±0.2°C of the platinum resistance thermometer temperature. In the case of the infrared thermometer, none of the readings (30°C to 40°C) were within the acceptable range of ±0.2°C, when either the blackbody cavity or the platinum resistance thermometer was regarded as the reference standard. Measurements with the infrared thermometer were all outside the ±0.3°C acceptable range, when the thermal infrared camera was regarded as the reference standard.

The thermal infrared camera and platinum resistance thermometer demonstrated perfect correlation with the blackbody cavity temperature ($r = 1$; $p<0.0001$), and a near perfect correlation with the infrared thermometer ($r =0.993$; $p<0.0001$). Similarly the platinum resistance thermometer has a perfect correlation with the thermal infrared camera ($r=1$; $p<0.0001$) and near perfect correlation with the infrared thermometer ($r=0.994$; $p<0.0001$). The correlation between the thermal infrared camera and the infrared thermometer was very strong ($r=0.994$; $p<0.0001$) (Figure 33).

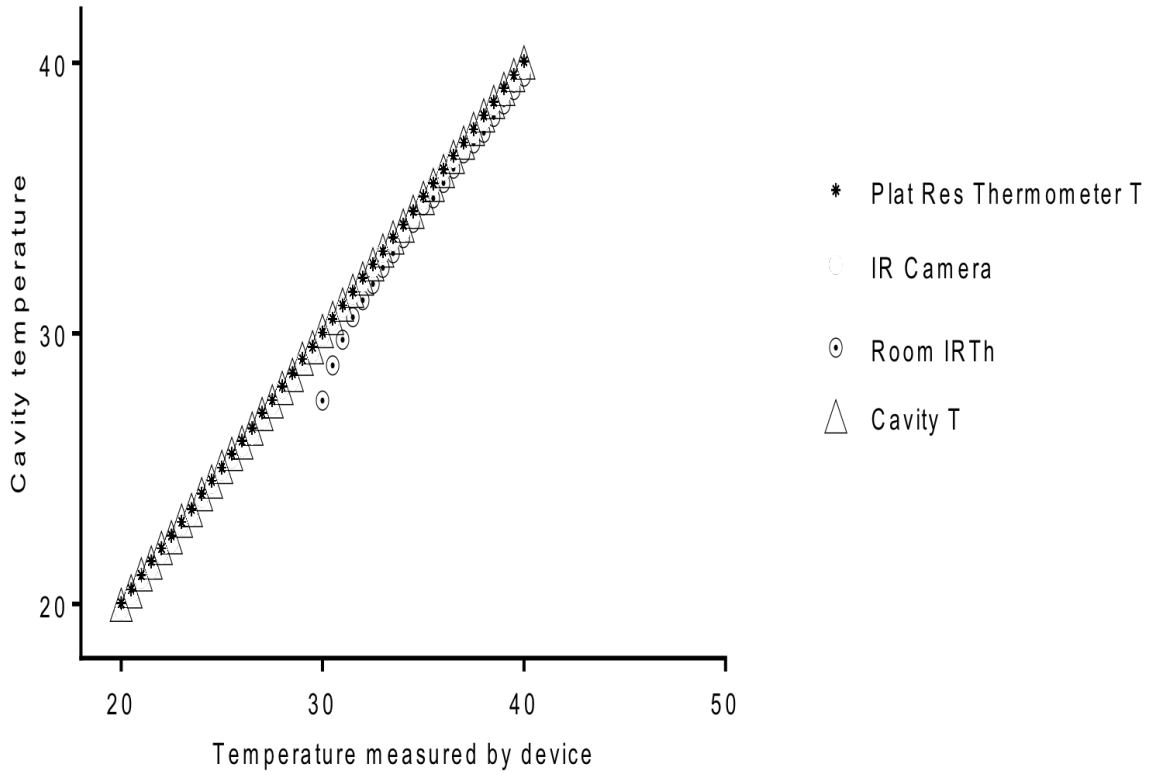


Figure 37: Correlation between blackbody cavity, platinum resistance thermometer, thermal infrared camera and infrared thermometer; All correlations were statistically significant (p<0.01)

Pearson Correlation Coefficient	Blackbody cavity temperature	Platinum resistance thermometer	Thermal infrared camera	Non-contact infrared temporal thermometer
Blackbody cavity temperature		1	1	0.952
Platinum resistance thermometer	1		1	0.95
Thermal infrared camera	1	1		0.948
Non-contact infrared temporal thermometer	0.952	0.95	0.948	

Table 19: Correlation between Blackbody cavity, platinum resistance thermometer, Thermal infrared camera and Non-contact infrared temporal thermometer

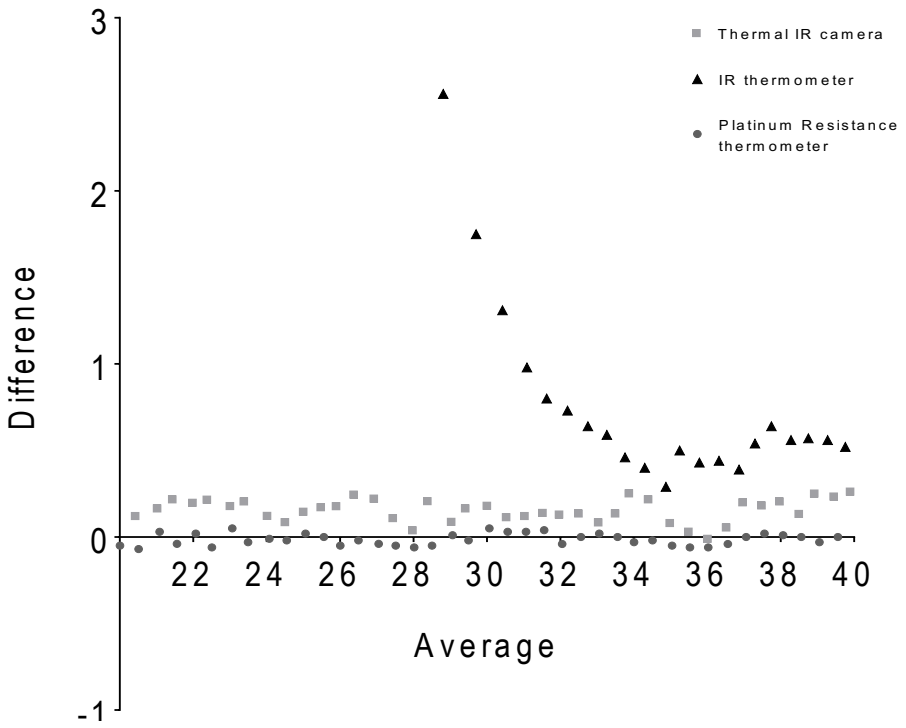


Figure 38: Agreement between the blackbody cavity and the platinum resistance thermometer, thermal infrared camera and infrared thermometer; Blackbody cavity temperature is underestimated by the platinum resistance thermometer (Bias: -0.015°C; SD of bias: 0.034°C; 95% LoA: -0.08°C to 0.05°C), but overestimated by both thermal infrared camera (Bias: 0.16°C; SD of bias: 0.065°C; 95% LoA: 0.03°C to 0.28°C) and the infrared thermometer (Bias: 0.75°C; SD of bias: 0.53°C; 95% LoA: -0.3°C to 1.79°C).

Device (°C)	Mean bias (°C)	SD of bias (°C)	95% limits of agreement
Acceptable limits	±0.2	0.15	0.3
Platinum resistance thermometer	-0.015	0.034	-0.08 to 0.05
Thermal infrared camera	0.16	0.065	0.03 to 0.28
Infrared thermometer	0.75	0.53	-0.3 to 1.79

Table 20: Agreement of the Platinum resistance thermometer, Thermal infrared camera and Non-contact infrared temporal thermometer to the Blackbody radiator.

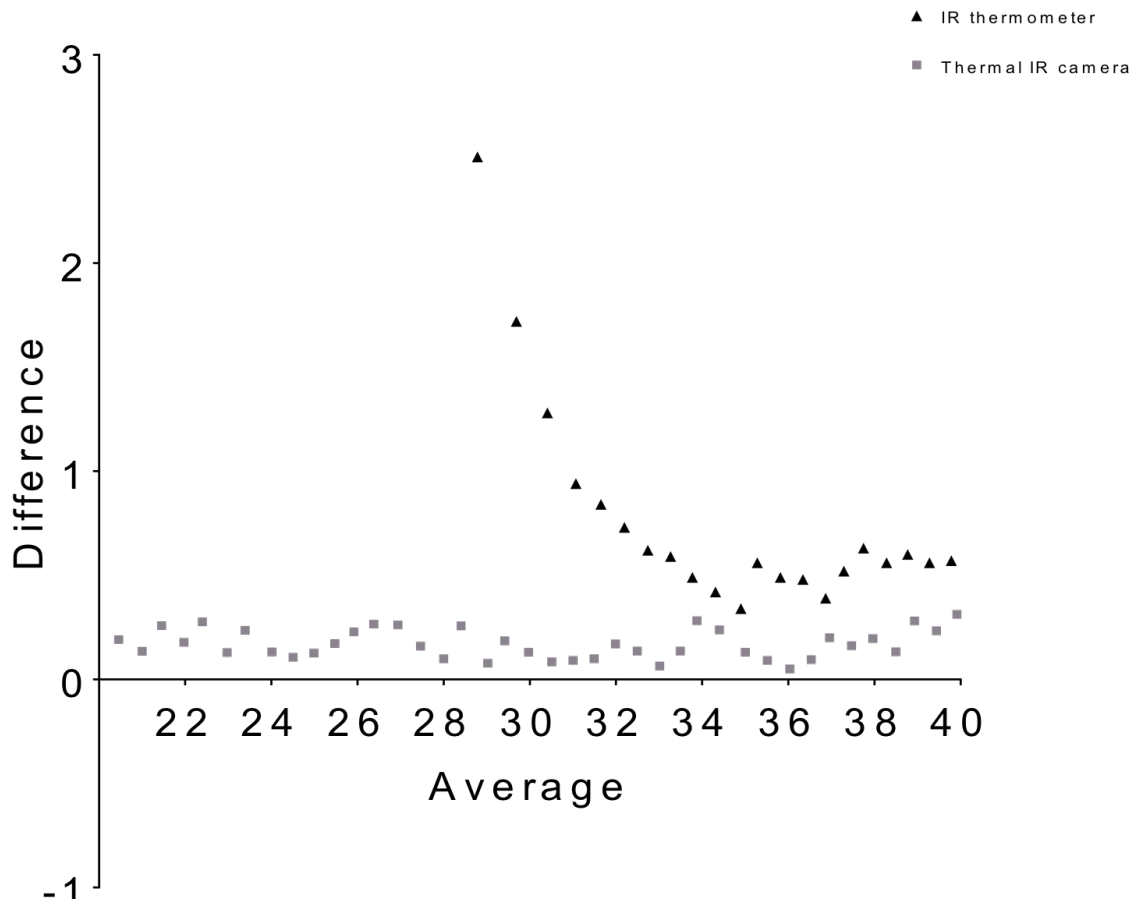


Figure 39: Agreement between the platinum resistance thermometer and the thermal infrared camera and infrared thermometer; Platinum resistance thermometer based temperature is overestimated by the infrared camera (Bias: 0.17°C; SD of bias: 0.07°C; 95% LoA: 0.03°C to 0.31°C) and the infrared thermometer (Bias: 0.75°C; SD of bias: 0.51°C; 95% LoA: -0.25°C to 1.76°C).

Device	Mean bias (°C)	SD of bias (°C)	95% limits of agreement (°C)
Acceptable limits	± 0.2	0.15	0.3
Thermal infrared camera	0.17	0.07	0.03 to 0.31
Infrared thermometer	0.75	0.51	-0.25 to 1.76

Table 21: Agreement of the Thermal infrared camera and Non-contact infrared temporal thermometer to the Platinum resistance thermometer.

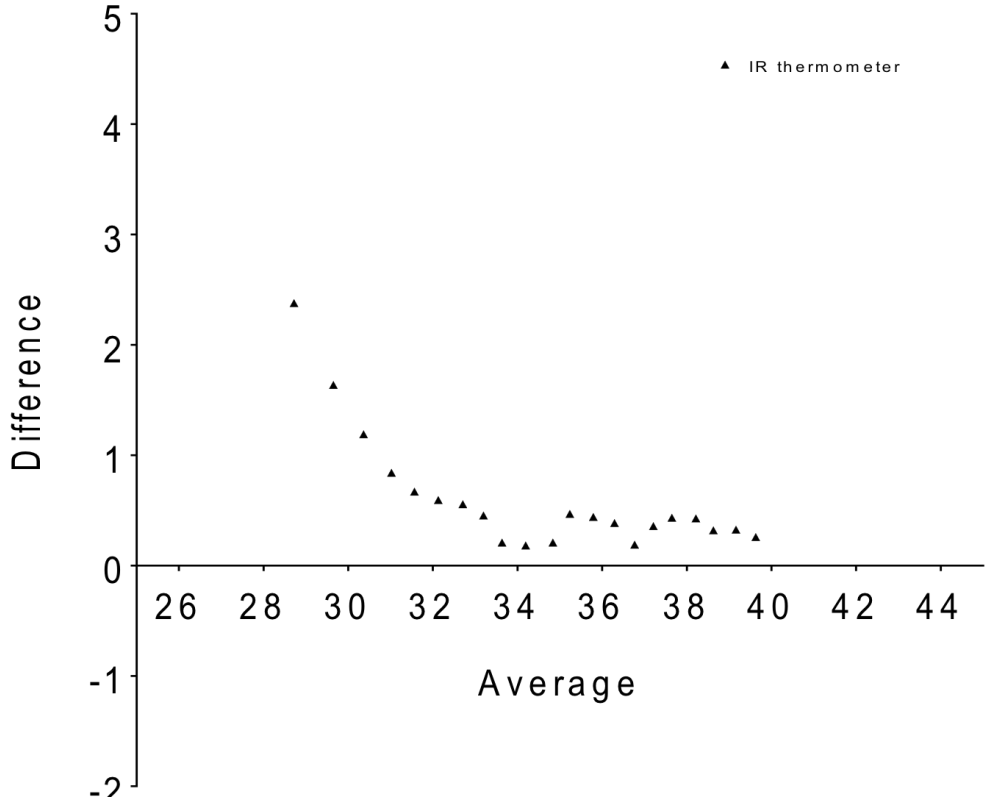


Figure 40: Agreement between the platinum resistance thermometer and the thermal infrared camera and infrared thermometer; The infrared thermometer overestimates the cavity temperature measured by the infrared camera (Bias: 0.596°C; SD of bias: 0.538°C; 95% LoA: -0.46°C to 1.65°C).

Device	Mean bias (°C)	SD of bias (°C)	95% limits of agreement (°C)
Acceptable limits	±0.2	0.15	0.3
Infrared thermometer	0.596	0.538	-0.46 to 1.65

Table 22: Agreement of the Non-contact infrared temporal thermometer to the Thermal infrared camera.

Discussion

Thermometry as a screening tool for pyrexia has become increasingly important, especially since the recent pandemic outbreaks (Bitar et al., 2009; Wong & Wong, 2006), where an easy to use, reliable and accurate assessment of fever was required. Our study assesses the potential role of the thermal infrared camera and the non-contact temporal thermometer as a reference 'fixed point' to ensure 'measurement traceability'. The thermal infrared camera and non-contact infrared temporal thermometer are unique because both these devices can be used to measure black body cavity and human body skin temperature.

Most studies on thermometry have used imperfect reference standards, which will provide percent agreement (Meier, 2007), rather than measures of accuracy (Bland & Altman, 1986). The majority of 'reference standards' have lacked measurement traceability to a known reference 'fixed point' (Rusby, 2012). Apart from oral, tympanic and axillary thermometry, the other 'reference standards' are invasive, inconvenient, risks traumatic injury and infection transmission. Even though calibrated mercury thermometers were traceable, these are no longer recommended in the EU and studies that did use these would have been affected by the site from which temperature is measured. For example rectal temperature is influenced by the anatomy and contents of the rectum, oral temperature is affected by intubation, ingestion, smoking, tachypnea and gum chewing, axillary temperature is affected by diaphoresis and airflow (Robinson et al., 1998; Hayward et al., 1984). Pulmonary artery and oesophageal temperatures measurement with Swan-Ganz catheters and thermocouples respectively are invasive, lack measurement traceability and may differ from temperature on the surface of the skin (Steketee, 1973). Infrared tympanic

thermometers have to be inserted at a certain angle into the ear canal and is affected by earwax (Daanen, 2006; McCarthy & Heusch, 2006).

The human skin emits infrared radiation. Its emissivity is approximately 0.98±0.01 for the wavelength range of 2-14 μm (Steketee, 1973). Infrared thermometers have been subjected to clinical research for over a decade (Bitar et al., 2009; Chan et al., 2004; Dodd et al., 2006). Non-contact infrared thermometer received mixed reviews with some studies showing it to be more specific than sensitive (Bitar et al., 2009). A recent study reported a high sensitivity and specificity of 97% respectively, for fever detection in children with non-contact infrared thermometers, although rectal thermometry was the gold standard (Teran et al., 2011). With axillary thermometers as the reference standard, the sensitivity and specificity is reduced to a range of 88.7% to 94.3% and 89.9 to 90.5% respectively (Apa et al., 2013; Chiappini et al., 2011). Thermal infrared camera has been used for fever screening with a sensitivity and specificity that varies depending on the distance between camera and the skin, site of study, the cut-off temperature for fever and the reference standard used. According to Chan et al, the sensitivity of infrared camera was 67% if 38°C was used as the cut-off for fever, and 83% if this cut-off was reduced to 37.5°C, with tympanic and oral thermometry as the 'reference standard' (Chan et al., 2004). Recent reviews suggest a variable positive predictive value ranging from 0.9% to 76%, negative predictive value of 86.1 to 99.7% with non-contact infrared thermometers (Bitar et al., 2009).

Smith et al compared a wireless contact-based thermometer, the ibutton® against a thermocouple for the measurement of a water bath followed by body temperature measurement at 3 different ambient temperatures (10°C, 20°C and 30°C). Both the ibutton® and thermocouple demonstrated a mean bias of <0.2°C and correlations

>0.999 against the water bath (Smith et al., 2010). In our study, the platinum resistance thermometer and the thermal camera both demonstrated a mean bias <0.2°C, but the infrared thermometer had a mean bias that exceeded 0.2°C. When using the platinum resistance thermometer as the reference standard, the mean bias for the thermal infrared camera was still <0.2°C, with the mean bias of the infrared thermometer >0.2°C. The correlation coefficient for the platinum resistance thermometer, thermal infrared camera and infrared thermometer were all >0.9. Ng et al compared three non-contact infrared thermometers against a 'reference standard' electronic thermometer to measure surface temperature of a water bath. The average difference between the measured temperatures was 0.56°C, with a 95% limits of agreement of -1.00°C to 2.12°C (Ng et al., 2005), compared to our findings with the blackbody radiator (mean bias:0.75; 95% limits of agreement of -0.25 to 1.76) where the limits of agreement were narrower by 1°C.

When using non-contact infrared thermometers, the distance between the thermometer and the source (Bitar et al., 2009), time to acclimatization (Erenbeck et al., 2013), selection of devices and interpretation of results (Wong & Wong, 2006) need to be considered. In our study, we chose 20cm from the black body aperture, as the cavity plate to aperture distance is 9cm. Therefore, the total distance from the camera to the cavity plate is 29cm. This is above the expected minimum distance required for the thermal infrared camera, which is 25cm. In the case of the infrared thermometer, it is not possible to position the thermometer within the 3-5cm of the cavity plate due to the depth of the cavity (9cm). In this case, the total distance from cavity plate to the infrared thermometer is 14cm. This may have affected the temperature measurements taken with the infrared thermometer. Despite this, in our study the infrared thermometer overestimated the temperature by 0.75°C compared

to the manufacturer ±2°C accuracy at object settings. Similarly, we found that the thermal infrared camera overestimated the black body cavity temperature by 0.16°C, compared to the manufacturer reported accuracy of ±1°C. Results of our study suggests that the thermal infrared cameras is a suitable alternative as a mobile, traceable reference fixed point for thermometry studies of the future. Further studies are required to assess this device in a clinical setting. In the case of non-contact infrared temporal thermometer, further studies comparing it to a calibrated 'fixed point' within the recommended distance may be useful to assess its potential as a traceable 'fixed point'.

Conclusion

The infrared camera is a potential traceable temperature reference fixed point that could serve as a reference standard for future diagnostic accuracy studies. The infrared thermometer may need to be compared against another 'fixed point' before deciding on its potential as a 'fixed point' for measurement traceability. However, at object settings, the temperature variation is within 1°C.

Section 2: Pilot study comparing Infrared camera versus infrared thermometers in a clinical setting

Introduction

Results of my initial study have shown that infrared thermography can be used as a reference fixed point for temperature assessment (Yap Kannan *et al.*, 2015). To apply this to a clinical setting, I planned a study to compare the infrared thermography (IRTg) and infrared thermometry (IRTh) on human subjects. I set out to determine the limits of agreement between the two measurement devices using two sites, namely the heel and medial gastrocnemius. There is no data on the agreement between these 2 devices on human subjects.

Aim

The purpose of this study was to determine if the temperature difference pre and post exercise detected using the infrared thermography (IRTg) and infrared thermometry (IRTh) were in agreement.

Methods

Participants

Ten healthy subjects were recruited for these tests. All patients had their ABPIs checked. Temperature measurements were taken before and after a 6-minute walk test (6MWT), respectively.

Device

Devices used were similar to those used in the laboratory-based study, except for the blackbody radiator. These included the thermal infrared camera (FLIR T650sc) and the non-contact infrared thermometer (FS-700). The FLIR T650sc thermal infrared camera has a manufacturer reported <0.02°C sensitivity and ±1°C accuracy and is capable of measuring temperatures between -40°C to 2000°C. The infrared thermometer has an object and body setting. In this study, the device was set at the body settings. The FS-700 non-contact infrared temporal thermometer is capable of measuring body temperatures between 22°C and 42.5°C (±0.3°C accuracy) and object temperatures between 10°C and 80°C (±2°C accuracy), provided the relative humidity is below 95%. The recommended distance for measuring temperature is 3 to 5 cm. The infrared thermometer autocorrects for surface emissivity and cannot be manually adjusted.

Study protocol

The temperature of the room was not controlled, to make it similar to a regular clinical setting. All participants were allowed 15 minutes to acclimatize to the room temperature. Room temperature was measured before each reading. Humidity was also monitored prior to measurements. The distance between the participants' leg and the infrared thermometer was set to be within the recommended distance of 3 to 5cm. The FLIR T650sc thermal infrared camera was positioned on a stand one meter from the bed. The emissivity setting was kept 0.98.

Participants were requested to remove their footwear including socks and rest their feet for 15 minutes in the study location. They were then asked to lie prone with their calves exposed. Baseline temperature measurements were taken bilaterally, over the temporal and radial arteries with the infrared thermometer. Participants then lay prone on the bed. A marker pen was used to mark the reference spot on the calf for the infrared thermometer. This mark was placed 10cm inferior to the medial femoral condyle on both legs. An adhesive reflective foil tape was placed on to the bed at this level, so that the participant can lie prone in the same position after the exercise test. Similarly, the heel was marked 10cm from the tip of the heel towards the toes on each leg. A tape measure was used to confirm the position on the bed and this was marked with an emissivity tape for the level of the medial gastrocnemius and the level of the heel. The emissivity tape is cooler and appears different to surrounding temperature making it more visible in the infrared camera (Figure 37).

A baseline thermal infrared image of the leg in this position was taken with the infrared camera. Temperature measurements were taken with the infrared thermometer at the locations previously marked. Participants were then provided with theatre shoes to walk up and down a straight corridor for 6 minutes. On completion of this, participants' baseline temperature at bilateral temporal region and bilateral radials were retaken before resuming the prone position on the bed. Participants were carefully repositioned so that the markers on the skin corresponded to a straight line between the previously marked emissivity tapes at both the level of the medial gastrocnemius and the heel, using a tape measure. Temperature measurements were taken using the infrared thermometer and images were taken using the infrared camera at 1, 2 and 3 minutes post exercise, for each leg.

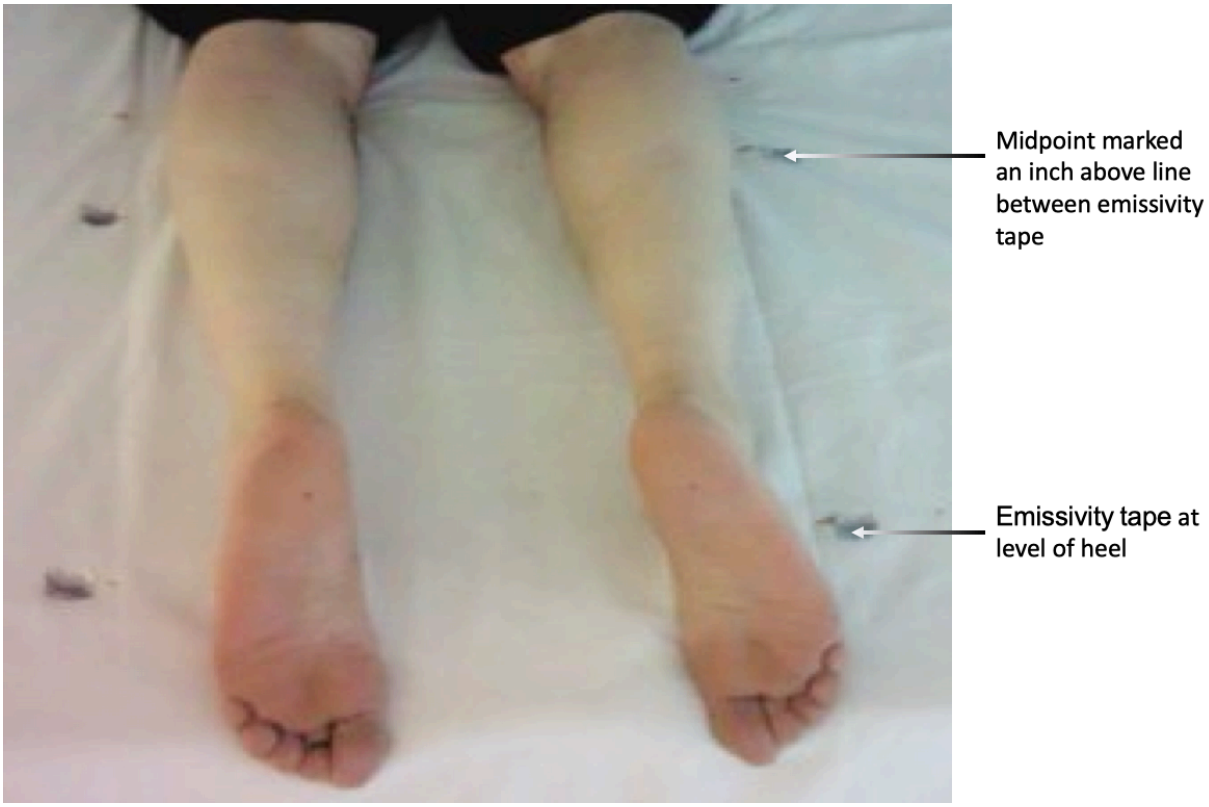


Figure 41: Image taken with infrared camera (baseline image).

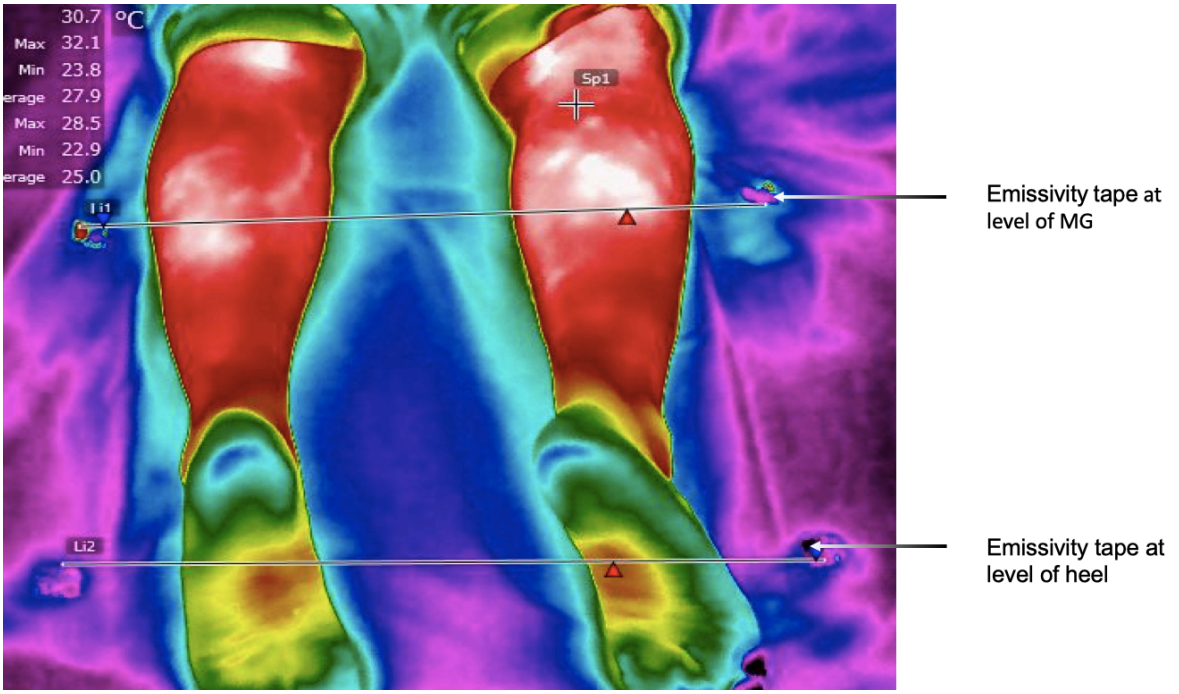


Figure 42: Infrared image of the baseline image.

A line is drawn between the 2 emissivity tapes at the level of the medial gastrocnemius and heel respectively. The temperature along this line is depicted in the graph below

(Figure 38). The midpoint temperature of the site measured was taken as the temperature for IRTg. A mark on the skin was made an inch above this midpoint, to allow temperature measurement at the same site as the camera by the infrared thermometer. This was not placed directly on the site to be measured to avoid temperature changes that may be caused by the skin marker itself.

Parameters

The difference between the post-exercise and pre-exercise temperature was calculated as the exercise induced temperature change (eTC). Exercise induced temperature change (eTC) was measured as the difference between the pre and post exercise temperature at 1 minute (eTC1), 2 minutes (eTC2) and 3 minutes (eTC3). These were calculated for both devices at the 2 respective sites (heel and medial gastrocnemius).

Statistical analysis

The agreement of the temperature difference between the infrared thermometer and infrared thermography was calculated using Bland Altman limits of agreement. A 95% limits of agreement <0.9°C were set as acceptable thresholds (Kelechi et al., 2006). Devices are in agreement if the mean differences were within +/-0.5°C and LOA within +/- 1°C (Kelechi et al., 2006; Bach et al., 2015). Analysis was done using the GraphPad Prism 7 software. Mean difference (bias), 95% limits of agreement (95% LOA) and the Pearson correlation coefficient was calculated for this comparison.

Results

Eight men and two women participated in the study. The median age was 34 years. Eight participants were Caucasian and two were of Indian origin. There were no significant comorbidities in this cohort of participants. All patients had normal ABPIs. The average room temperature was 22.6°C (Range: 21 to 23°C). Average humidity was 24.3 (Range: 22 to 32).

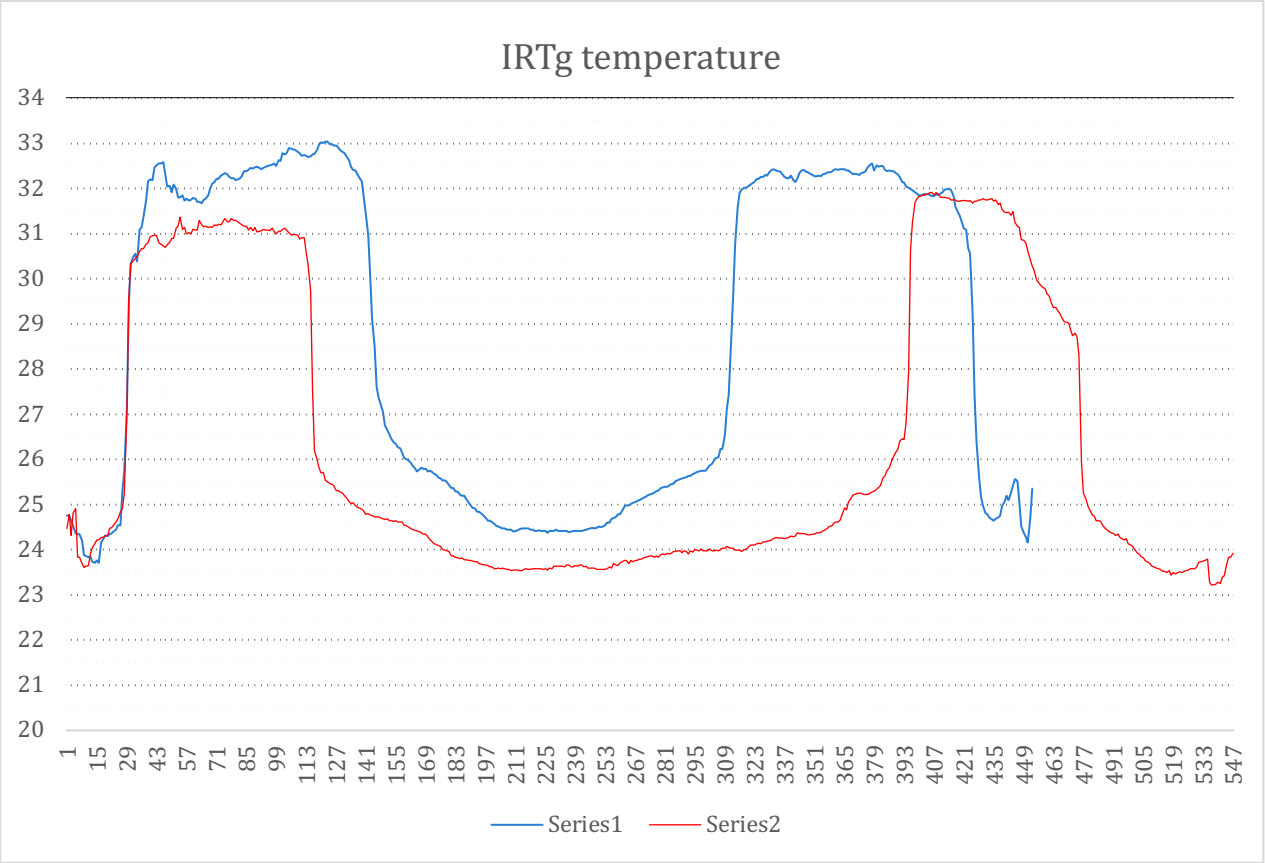


Figure 43: Temperature along the line drawn between the two emissivity tapes for the medial gastrocnemius (Series 1) and the heel (Series 2)

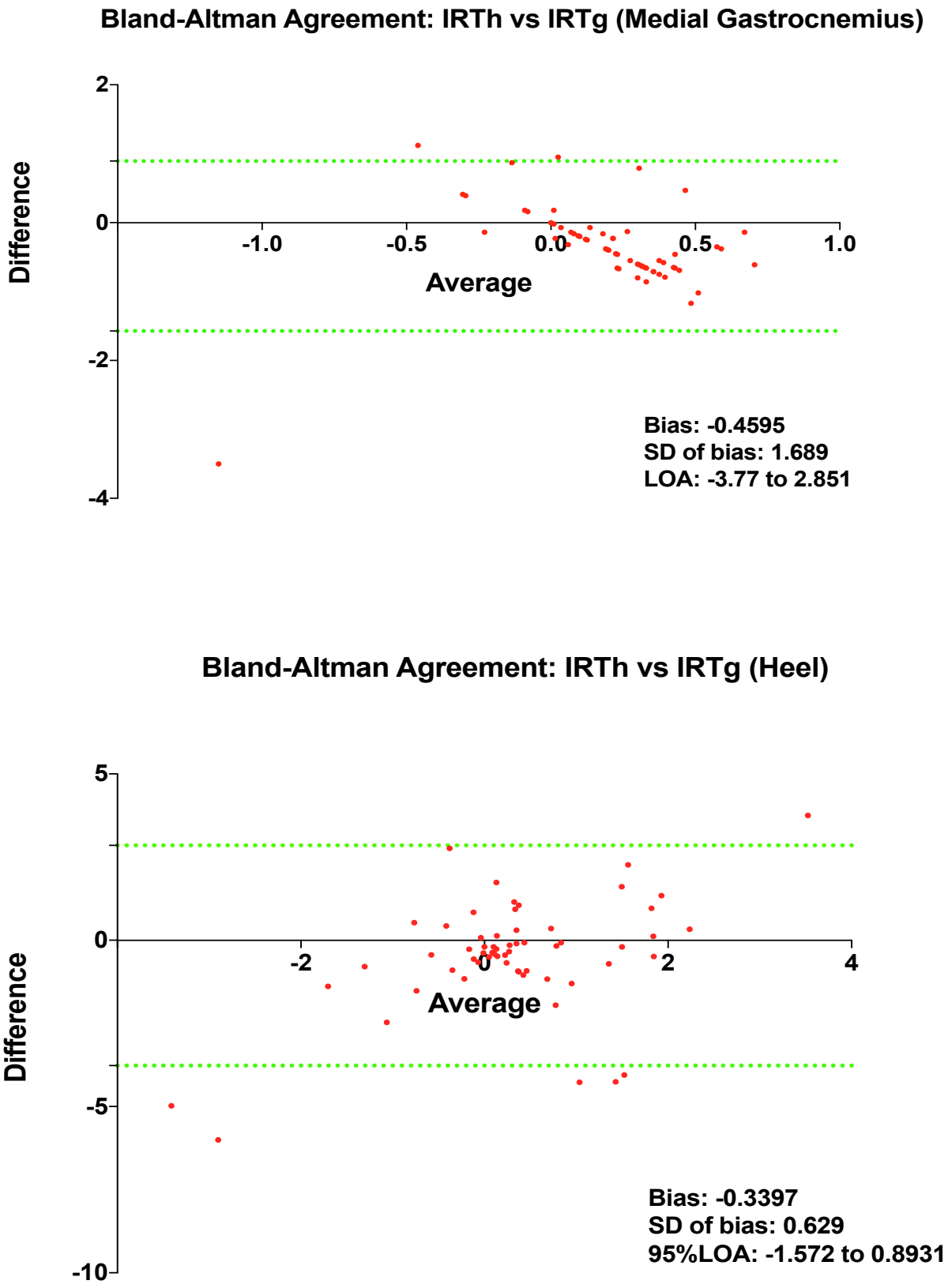


Figure 44: Results show a good agreement between both Infrared thermography (IRTg) and Infrared thermometry (IRTh), at 1, 2 and 3-minutes post exercise over the Medial Gastrocnemius and heel respectively, as depicted in the Bland-Altman plot shown below. The bias is well within the set +/-0.5°C.

Discussion

At present, there are no gold standard for temperature measurement of the skin in clinical practice. In the laboratory-based setting, I demonstrated that the infra-red camera has near perfect agreement with the black body radiator and hence is a traceable fixed point for temperature assessment (Yap Kannan et al., 2015), and therefore is in effect, a gold standard for measuring skin temperature in any setting. This study was performed to assess if infra-red thermometers are as good as infra-red cameras in a clinical setting, for measuring temperature changes post exercise. The infra-red thermometer was set at its body temperature setting for this study, which is different to that used in the laboratory setting. Unlike the laboratory setting, where the temperature measurements taken with the infra-red thermometer were beyond the optimal measurement distance due to the shape of the black body radiator, in this study the measurements were obtained at its optimal distance.

Previous studies have looked at the effect of exercise on temperature by infra-red thermography. Huang et al studied temperature changes post exercise in high risk PAD patients, with infra-red thermography. The author found a decrease in temperature, post exercise in these patients (Huang et al., 2011; Buono et al., 2007). In this clinical study, the bias for both the medial gastrocnemius and heel was within the acceptable cutoff, which was set at ±0.5°C (Kelechi et al., 2006). Likewise, Buono et al, demonstrated that there is a good correlation between contact and non-contact IR thermometers in measuring skin temperature at rest or during exercise, irrespective of environmental temperature (Buono et al., 2007) . However, in neither study, is agreement between these two devices nor its accuracy assessed. This study

is the first to describe an agreement between the infra-red thermometer and a traceable fixed point (infra-red camera) (Yap Kannan et al., 2015). As the study population is small, no comments can be made on the accuracy of the infrared thermometer. Nevertheless, this study proves that the infra-red thermometer has a good agreement with the infrared camera which is a traceable fixed point.

Conclusion

The infra-red thermometer has a very good agreement with the infra-red camera at its body setting. It has potential as traceable temperature reference fixed point, and hence a potential gold standard for temperature measurement. At body setting, it has a temperature variation within ±0.5°C. It is therefore a valid device to assess temperature changes post exercise.

Section 3: Baseline and Exercise induced temperature changes in patients versus controls

Aim

The aim of this study was to assess if IRTh can identify patients with PAD. The questions asked in this aspect of the study, was whether exercise induced temperature change (eTC) was different with the type of exercise, site of measurement and time of measurement.

Methods

Participants

The study subjects were the same group from the oxygenation study. The first group consisted of healthy controls with normal ABPIs and no significant drop in ABPI (>0.15) post exercise. Controls recruited were either university or hospital staff. The second group consisted of patients with confirmed evidence of PAD by duplex. All patients were elective admissions or had outpatient procedures or appointments. Patients were recruited from the vascular study lab, clinics, wards and angiography suite prior to angiograms. Both groups were subject to two forms of exercise testing. The first being the 1-minute flexion extension test for a duration of one minute (1MF) and the second being the 6-minute walk test (6MWT). The protocol for patients and controls were the same.

Device

The HubDic FS-700 Infrared thermometer is a non-contact based infrared thermometer, capable of measuring body temperatures between 22°C and 42.5°C (±0.3°C accuracy) and object temperatures between 10°C and 80°C (±2°C accuracy), provided the relative humidity is kept below 95%. The infrared thermometer has an object and body setting. For the purpose of this study, the settings were kept for body temperature measurements. The recommended distance for measuring temperature using this device is 3 to 5cm.

Study protocol

The study environment was neither temperature nor humidity controlled, to mimic a regular clinical setting. Air movement was kept to a minimum. Participants were allowed to acclimatize to the room temperature for the initial few minutes. During this time, patients were requested to remove their socks and shoes, for temperature measurements. Patients were requested to keep their legs flat either on a trolley or a chair. Baseline temperature measurements were then taken after an initial rest period of 5 minutes. Measurements were taken from the medial gastrocnemius (MG), 5cm below and medial to the tibial tuberosity. Participants were then requested to fully dorsiflex and plantarflex their ankle for one full minute. A timer was used to determine the duration the test was performed. Participants unable to complete the task, were asked to inform the researcher. Temperature measurements were taken at 1, 2 and 3 minutes post exercise from the medial gastrocnemius and the heel. Participants were given a total of 10 minutes rest period between the two exercise tests (6MWT and 1MF). During this time demographic details, comorbidities and relevant history was obtained.

At the end of baseline measurements, subjects were requested to walk on a corridor back and forth for 6 minutes. Subjects who could not walk the full duration of 6 minutes were allowed to stop and return to the examination trolley or chair with the legs in pre-exercise position. Measurements were taken with the leg kept in the pre-exercise position at 1,2 and 3 minutes from the medial gastrocnemius and the heel. If the other leg of the patient was to be studied, patients were allowed to rest for a further 15 minutes before re-examining the other leg in the same manner. In the control group, only the left leg was studied.

Parameter studied

The exercise induced temperature change (eTC) was studied. Changes in temperature at 1, 2 and 3 minutes (eTC1, eTC2 and eTC3 respectively) were studied. Each parameter was measured at the heel and medial gastrocnemius respectively.

Statistical analysis

Analysis was performed using the PRISM 7 software. Distribution of data was assessed using the D'Agostino and Pearson normality test. With non-parametric distribution of data, a Mann Whitney U test was used. Histograms are used to depict the distribution of results. The ranks are proportional to the temperature, ie the higher the rank, bigger the exercise induced temperature difference or higher the baseline temperature.

Results

Characteristics	Patient group		Control group	
	6MWT	1MF	6MWT	1MF
Number of limbs at eTC1	48	97	41	42
Number of limbs at eTC2	48	95	39	42
Number of limbs at eTC3	40	39	39	39
Demographics				
Median age	68	69.5	47	47
Caucasians: Asians	47:1:0	95:2:0	34:7:0	34:7:0
Male: Female	43:5	87:10	11:30	11:30
Risk factors				
Diabetic	18 (37.5%)	37 (38.1%)	2 (4.9%)	2 (4.9%)
Hypertensive	28 (58.3%)	56 (57.7%)	2 (4.9%)	2 (4.9%)
Previous myocardial infarction/angina	16 (33.3%)	36 (37.1%)	1 (2.4%)	1 (2.4%)
Atrial fibrillation	5 (10.4%)	7 (7.2%)	1 (2.4%)	1 (2.4%)
History of Malignancy	5 (10.4%)	9 (9.3%)	2 (4.9%)	2 (4.9%)
Hyperlipidemia	33 (68.8%)	58 (59.8%)	2 (4.9%)	2 (4.9%)
History of TIA or CVA	3 (6.3%)	14 (14.4%)	0 (0%)	0 (0%)
COPD	10 (20.8%)	16 (16.5%)	1 (2.4%)	1 (2.4%)
Smoker	37 (77%)	65 (67%)	12 (29.3%)	12 (29.3%)
PAD				
Non-PAD: IC: CLI	0:39:9	0:78:25	41:0:0	41:0:0
Left: Right: Bilateral leg	27:26:10	63:45:22	41:0:0	41:0:0
Able to complete the 6MWT	32 (66.7%)	54 (55.7%)	41 (100%)	41 (100%)
Median duration of 6MWT	6 minutes	6 minutes	6 minutes	6 minutes

Table 23: Study subject characteristics for exercise induced temperature change (eTC) undergoing 6-minute walk test (6MWT).

Section 3 of the study is presented in 4 subdivisions. Each section compares different characteristics of the study. The subsections and questions asked are:

A) Comparison of type of participants (patients versus controls)

i) Is there a difference between baseline temperatures, eTC1, eTC2 and eTC3 post provocation test, measured at the heel or the MG in the 6MWT and 1MF group, between patients and controls?

B) Comparison of provocation test (6MWT versus 1MF)

i) Is there an eTC between the type of exercise used for controls and patients at the level of the MG and or the heel?

C) Comparison of site of measurement (MG versus heel)

i) Is there a baseline or eTC between controls and patients undergoing the 6MWT or 1MF, at the MG and or the heel?

D) Comparison of timing of measurement (eTC1 versus eTC2 and eTC3)?

i) Is there an eTC difference at 1, 2 and 3-minutes post provocation, between patients and controls at the MG or the heel?

Subsection A: Comparison between patients and controls

i) Is there a difference between baseline temperatures, eTC1, eTC2 and eTC3 post provocation test, measured at the heel or the MG in the 6MWT and 1MF group, between patients and controls?

1) Baseline temperature analysis

Histogram of baseline temperature over the medial gastrocnemius (MG) in controls and patients undergoing the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF)

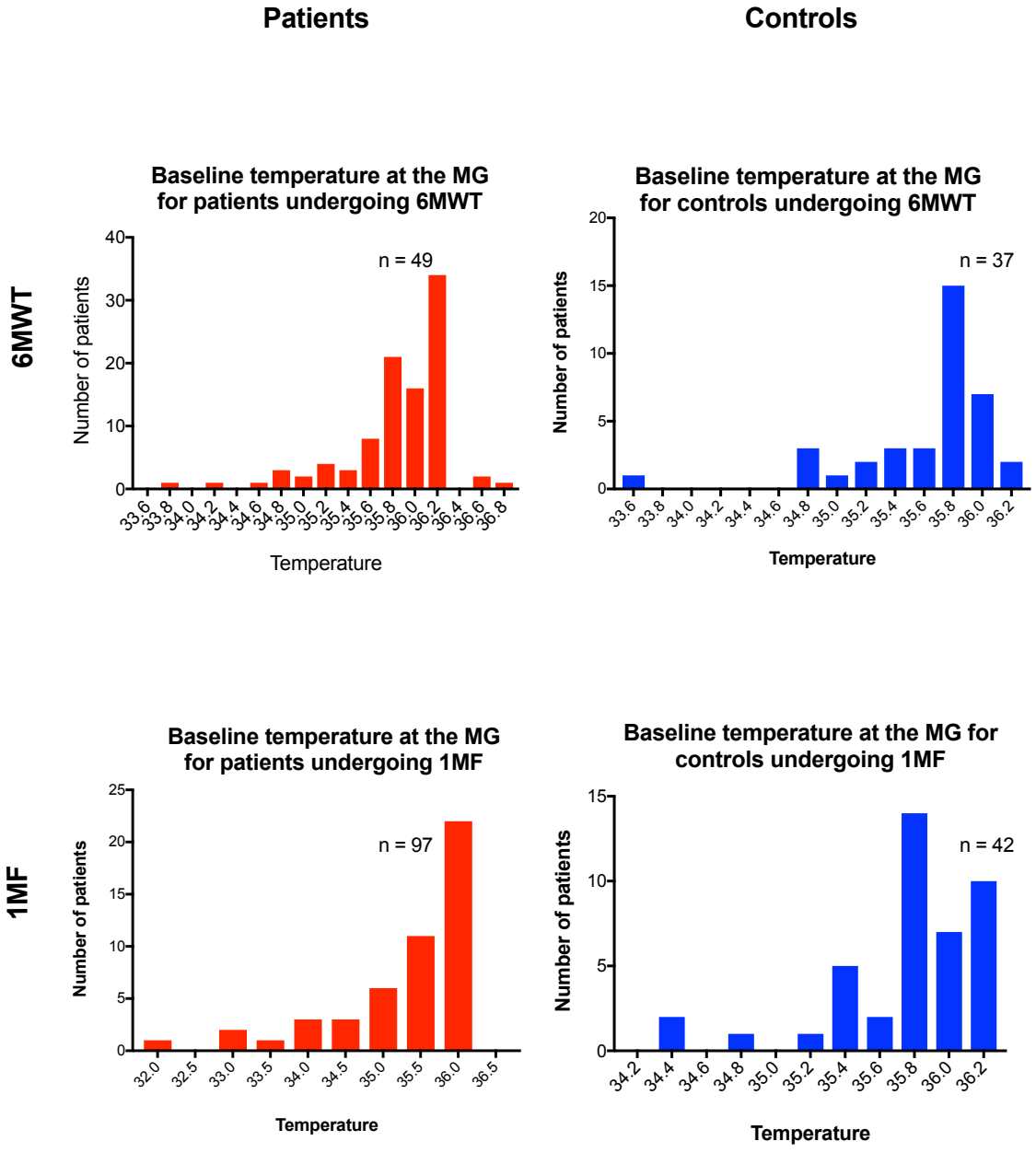


Figure 45: The baseline temperature measurements over the medial gastrocnemius (MG), for patients and controls undergoing the 6-minute walk test (6MWT) and the 1-minute flexion extension test (1MF) demonstrate data that is not normally distributed, as per the D'Agostino & Pearson normality test.

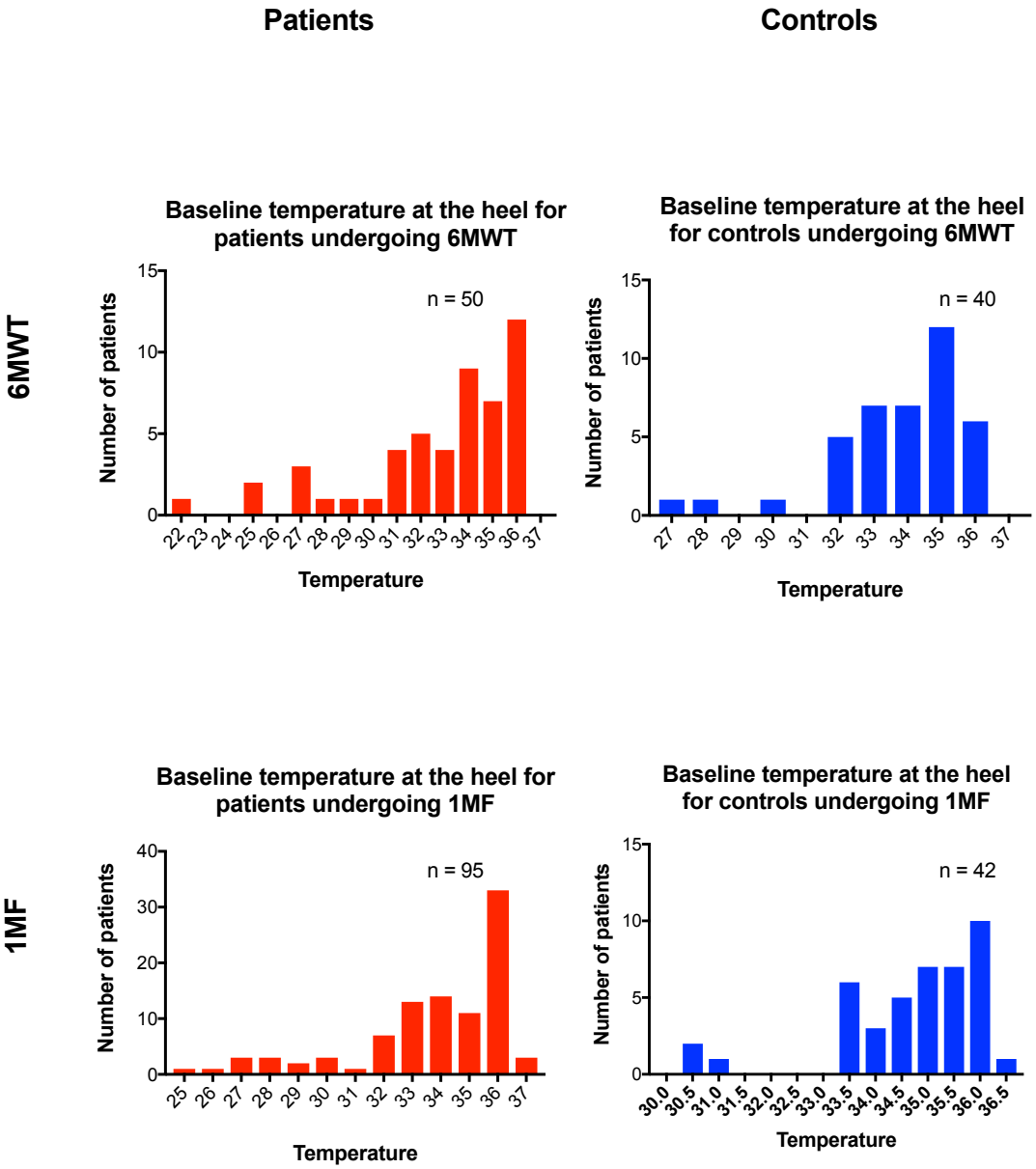


Figure 46: The baseline temperature measurements over the heel, for patients and controls undergoing the 6-minute walk test (6MWT) and the 1-minute flexion extension test (1MF) demonstrate data that is not normally distributed, , as per the D'Agostino & Pearson normality test.

As both histograms for the heel and MG site are not normally distributed, the Mann-Whitney U test was the non-parametric choice for statistical analysis to compare the data.

Mann Whitney U test: Comparison of baseline temperature at the medial gastrocnemius (MG) and the heel for controls and patients undergoing the 6-minute walk test (6MWT) and the 1-minute flexion extension test (1MF)

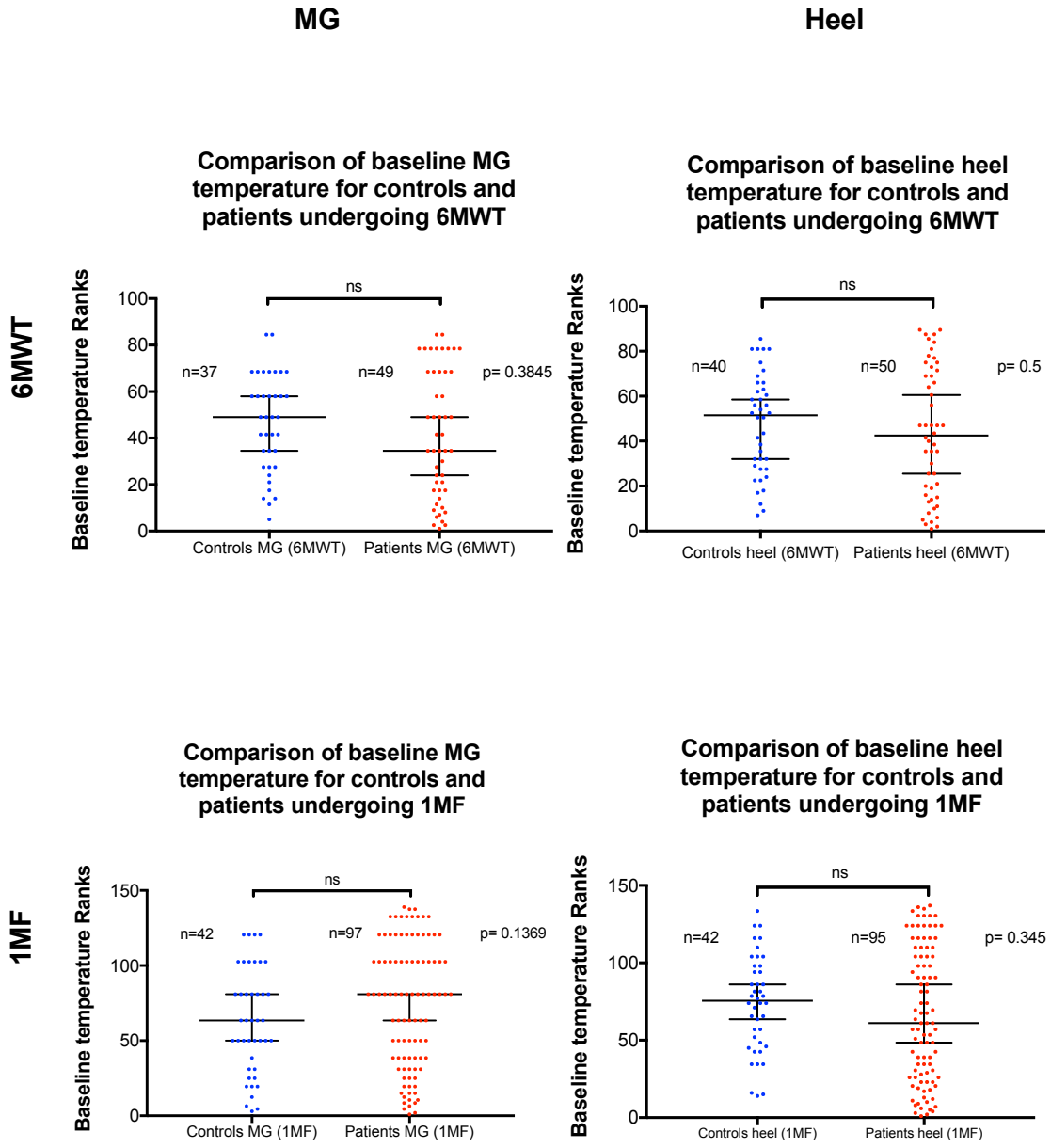


Figure 47: The Mann Whitney U test suggests no significant difference of baseline temperature measurements between patients and controls who were either in the 6-minute walk test (6MWT) or the 1-minute flexion extension test group (1MF).

Summary of results

Baseline temperature				
6MWT				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	37	49	40	50
Median	35.8	35.6	34.3	33.9
IQR	35.4 - 35.95	35 – 36	32.85 - 35.15	30.98 - 31.82
Min- Max	33.6 - 36.2	32.1 - 36.2	27.3 - 36.2	22.1 - 36.4
P value	0.3845		0.5059	
1MF				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	42	97	42	95
Median	35.9	36	35.05	34.4
IQR	35.6 - 36.03	35.7 - 36.1	33.88 - 35.8	32.6 – 36
Min- Max	34.4 - 36.2	33.7 - 36.7	30.3 - 36.4	25.2 - 36.8
P value	0.1369		0.345	

Table 24: Baseline temperatures at the Medial Gastrocnemius (MG) and heel for patients and controls undergoing the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

The results show no significant difference in baseline temperature at the MG or heel of controls and patients. Therefore, at rest there is no difference in temperature between patients and controls.

2) Exercise induced temperature change analysis (6MWT)

Histograms of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) in controls and patients after the 6-minute walk test (6MWT)

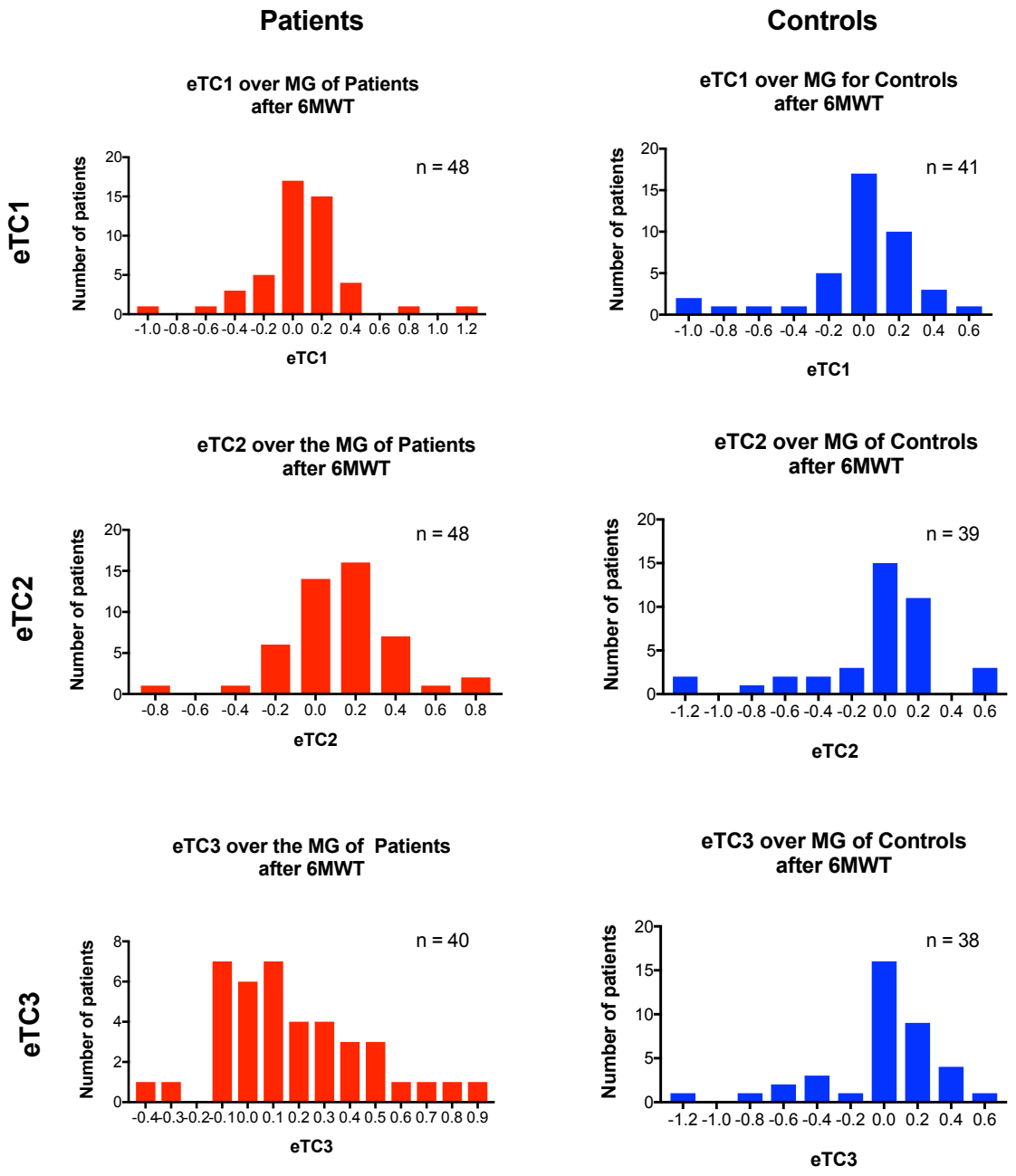


Figure 48: The histograms above compare the data distribution for patients and controls undergoing the 6-minute walk test (6MWT) group. These measurements were taken at the medial gastrocnemius only. The data is not normally distributed, as per the D'Agostino-Pearson omnibus normality test. Therefore a non-parametric Mann-Whitney U test was used to compare the data between the 2 groups of patients, as shown in Figure 44.

Mann Whitney test of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) in controls and patients after the 6-minute walk test (6MWT)

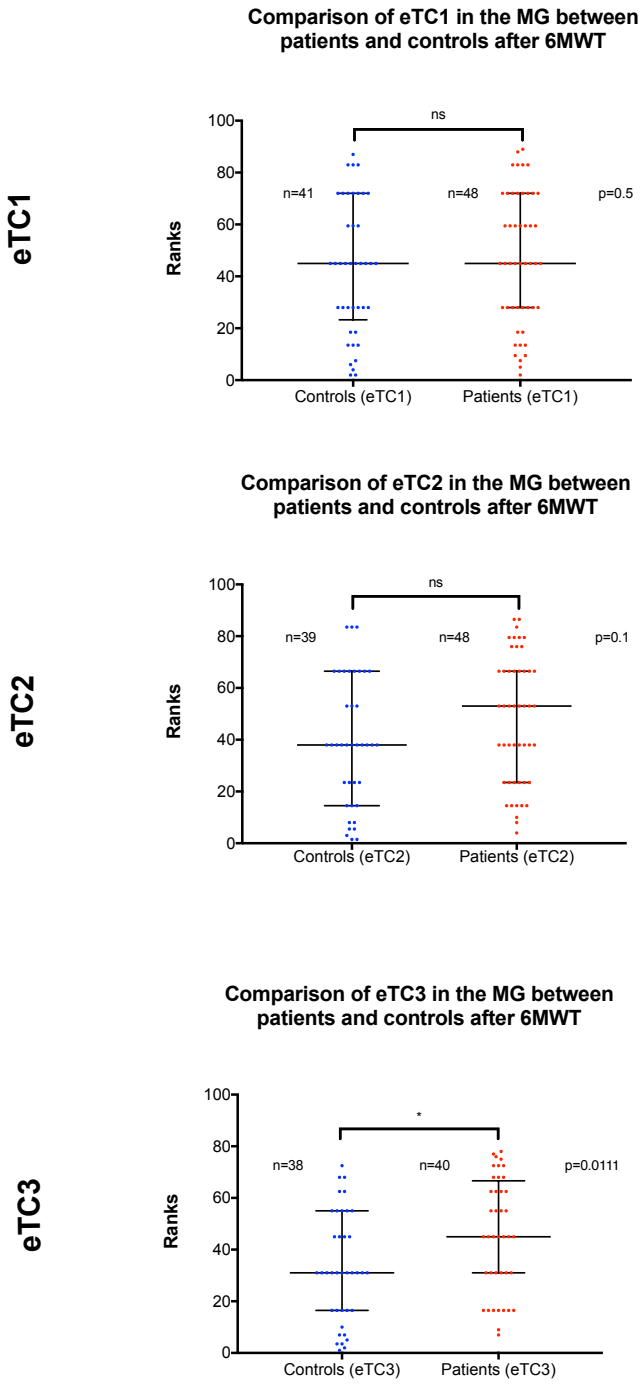


Figure 49: The results of the comparison between controls and patients suggest that the temperature difference gradually improves in controls at 3 minutes compared to patients whose temperature difference remain persistently high. At 3 minutes this difference between patients and controls is significantly different. This could be explained by the blood being diverted by the arteriovenous shunts away from the skin and preferentially to the muscles.

Histogram of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients after the 6-minute walk test (6MWT)

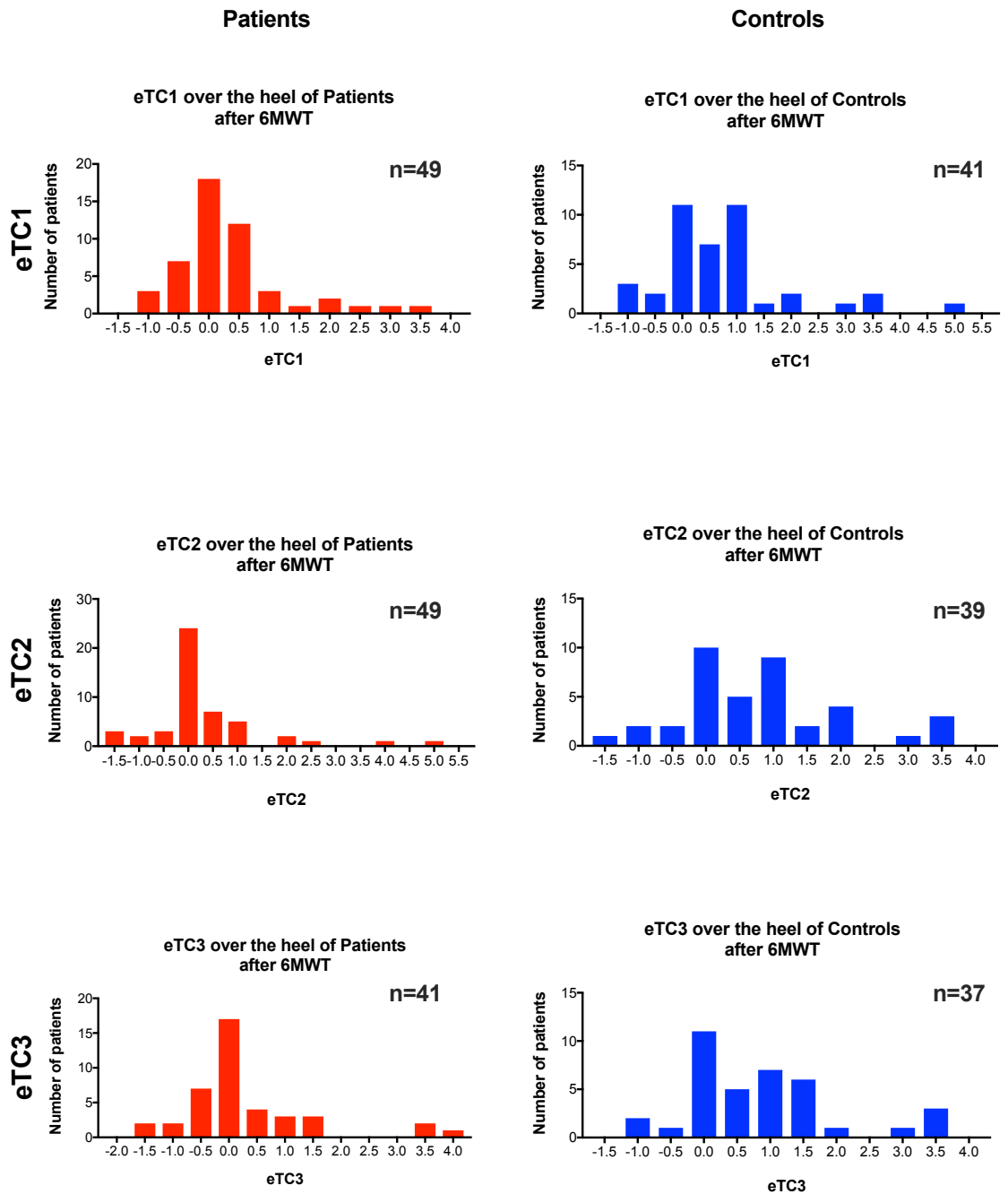


Figure 50: The exercise induced temperature change at the level of the heel is not normally distributed in both patients and controls undergoing the 6-minute walk test, as depicted in the histograms above, as per the d'Agostino-Pearson omnibus normality test.

Mann Whitney test of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients after the 6-minute walk test (6MWT)

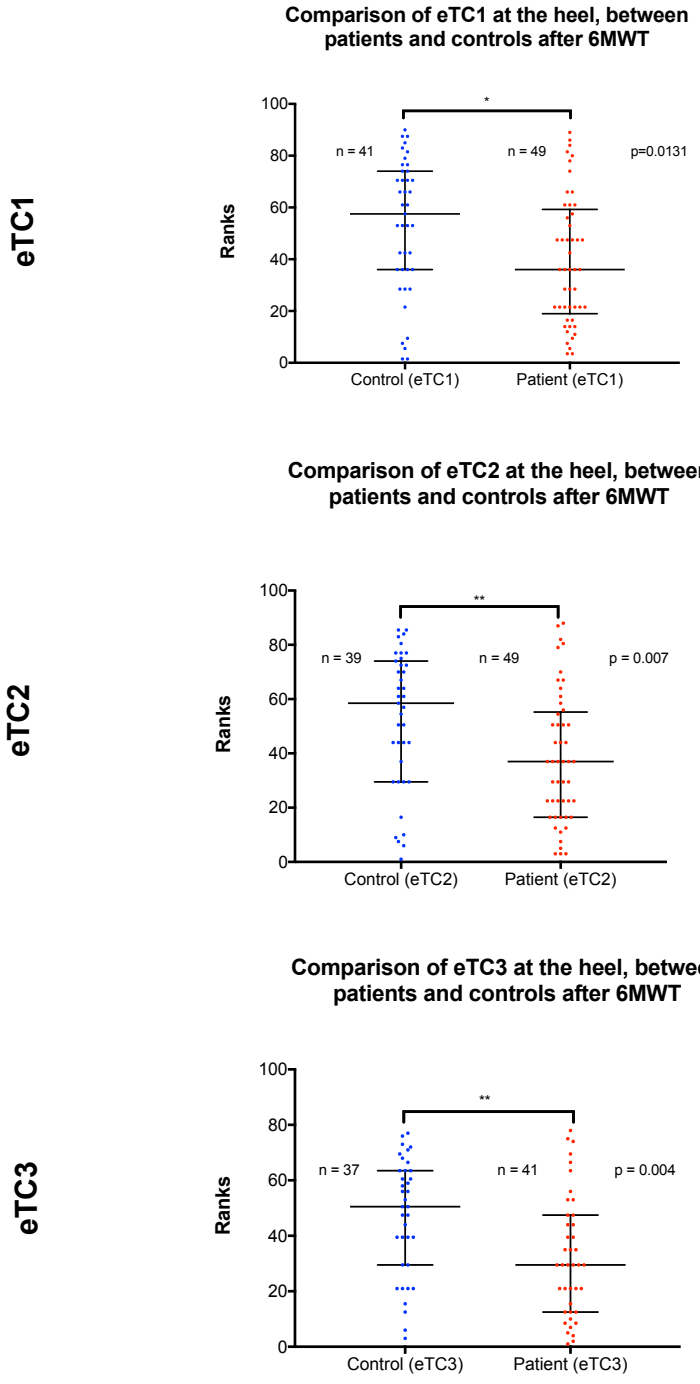


Figure 51: The results from the exercise induced temperature change at the heel after the 6-minute walk test (6MWT) suggests that there is a significant difference between patients and controls, with patients exhibiting a lower median temperature drop after 6MWT compared to controls.

2) Exercise induced temperature change analysis (1MF group)

Histogram of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) in controls and patients after 1 minute flexion extension test (1MF)

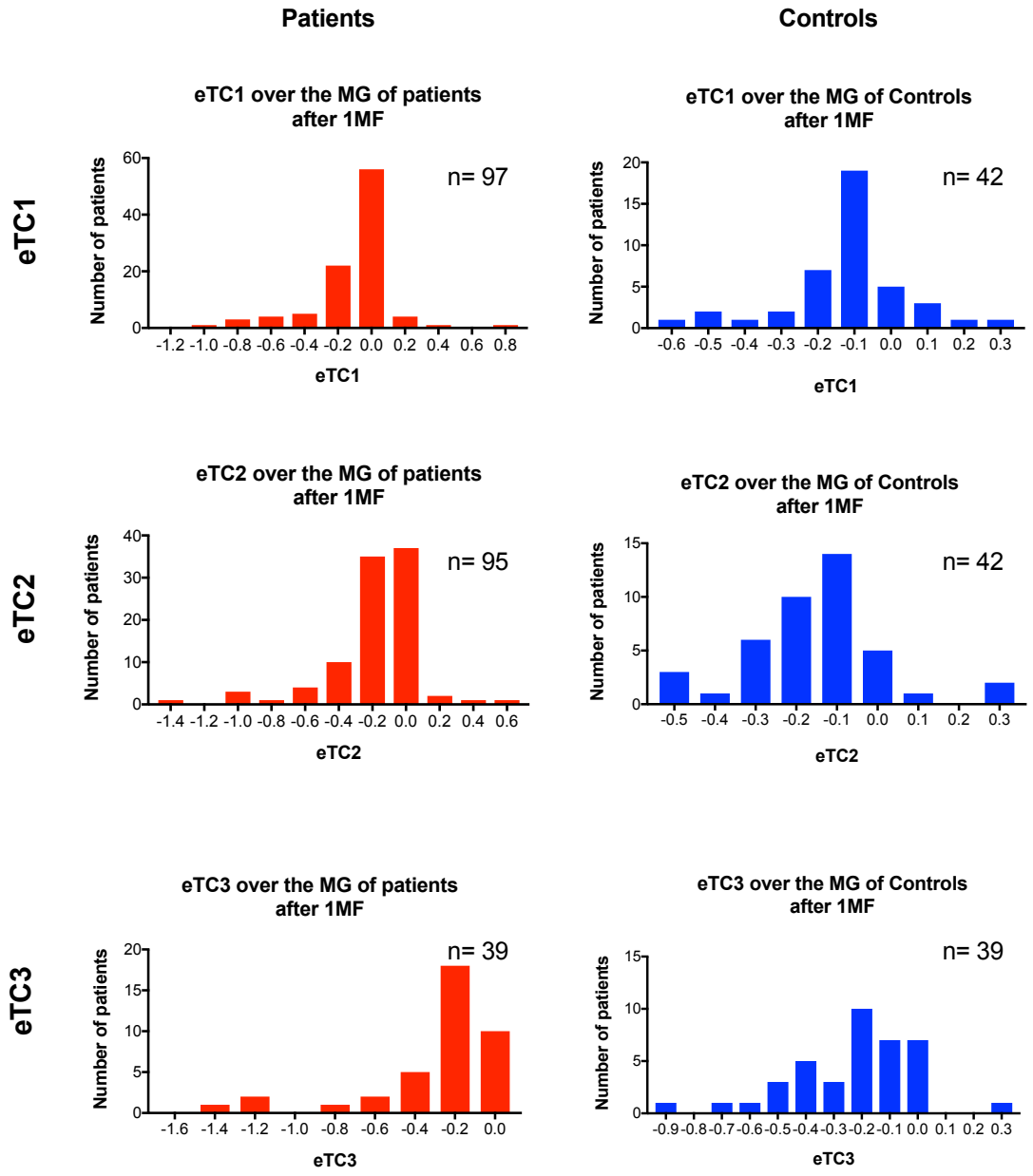
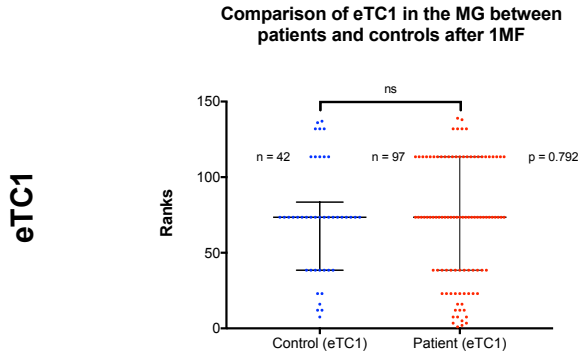
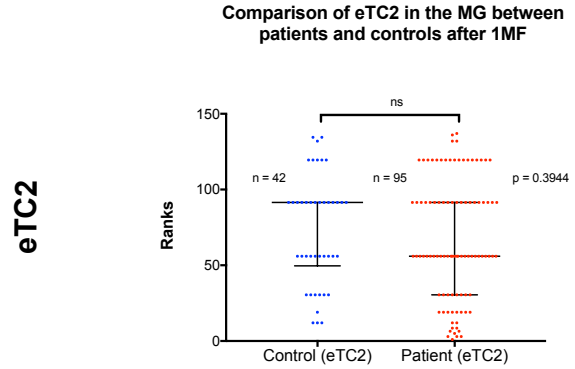


Figure 52: The data for patients and controls undergoing the 1-minute flexion extension test (1MF) show that the measurements taken at the medial gastrocnemius (MG) are not normally distributed, as per the d'Agostino-Pearson omnibus normality test.

Mann Whitney test of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) in controls and patients after the 1 minute flexion-extension test (1MF)





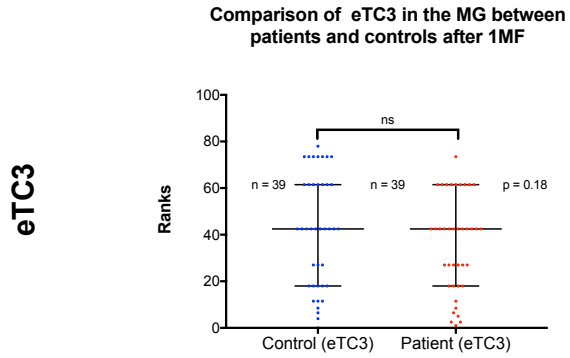


Figure 53: Unlike the 6-minute walk test (6MWT) group, where patients exhibited a persistently increased temperature difference post exercise at 3 minutes, the 1-minute flexion extension (1MF) test does not produce similar results. There is no statistically significant difference at the level of the medial gastrocnemius (MG), between patients and controls.

Histogram of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients after the 1 minute flexion extension test (1MF)

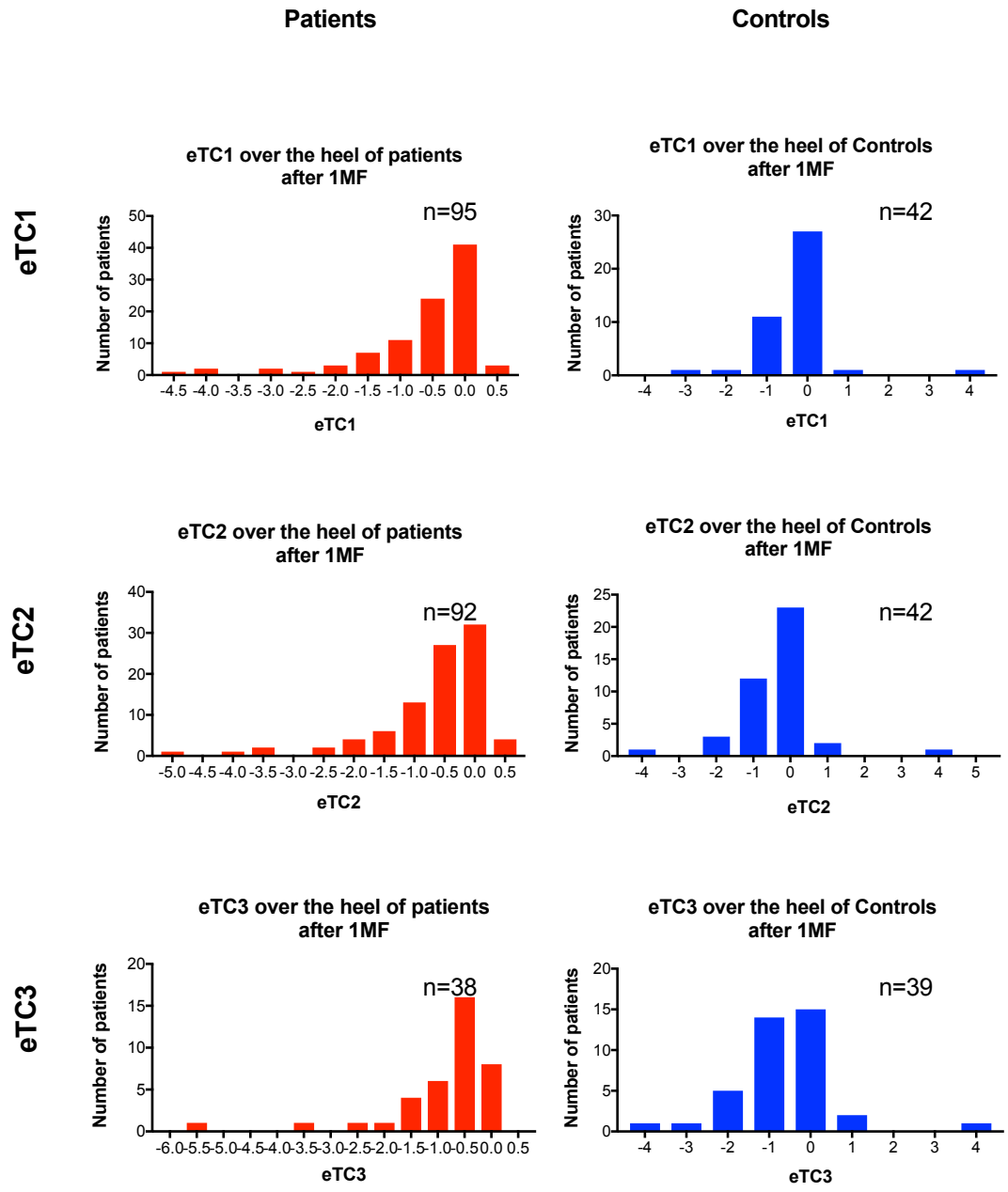
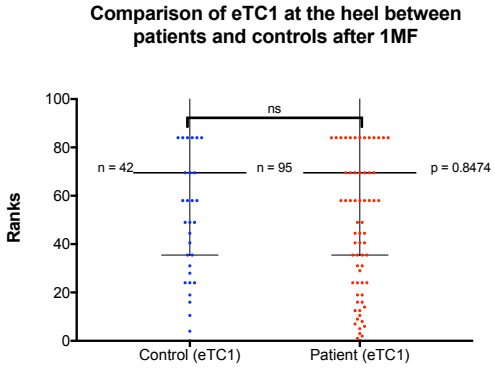


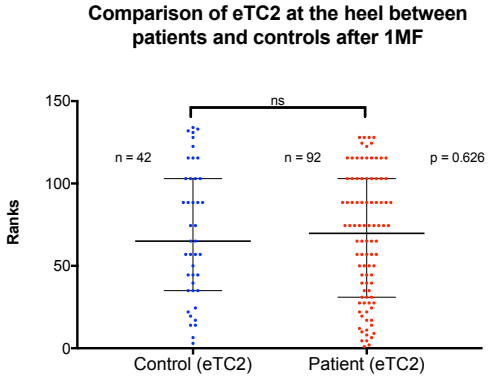
Figure 54: Data for exercise induced temperature change (eTC) at the heel for patients and controls undergoing the 1-minute flexion extension test (1MF) is not normally distributed, as per the d'Agostino-Pearson normality test.

Mann Whitney test of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients after the 1 minute flexion extension (1MF) test

eTC1



eTC2



eTC3

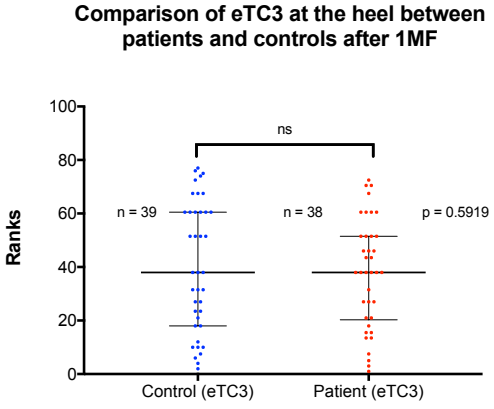


Figure 55: The analysis shows no significant difference between controls and patients undergoing the 1-minute flexion extension test (1MF) test, at the level of the heel at 1, 2 and 3 minutes post exercise.

Summary of results

Exercise induced temperature change at 1 minute				
6MWT				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	41	48	41	49
Median	0	0	0.6	0.1
IQR	-0.15 - 0.2	-0.1 - 0.2	0.1 – 1	-0.15 - 0.65
Min- Max	-1 - 0.6	-1 – 1.2	-1.2 – 1	-1.1 - 3.7
P value	0.5009		0.0131	
1MF				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	42	97	42	95
Median	-0.1	-0.2	-0.3	-0.3
IQR	-0.075 - -0.2	-0.2 – 0	-0.8 - -0.1	-0.8 - -0.1
Min- Max	-0.6 - 0.3	-1.1 - 0.8	-3.5 - -0.1	-4.4 - -0.1
P value	0.7928		0.8474	

Table 25: Exercise induced temperature change at 1 minute (eTC1) at the Medial Gastrocnemius (MG) and heel for patients and controls undergoing the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

eTC1 post 6MWT, there is a significant difference between patients and controls at the heel, with a smaller temperature increase in patients compared to controls. However, at the level of the MG, there is no significant temperature difference between patients and controls.

Exercise induced temperature change at 2 minutes (eTC2)				
6MWT				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	39	48	39	49
Median	0	0.1	0.7	0.1
IQR	-0.2 - 0.2	-0.1 - 0.2	0-1.3	-0.2-0.45
Min - Max	-1.2 - 0.5	-0.8 - 0.8	-1.5-3.7	-1.3-5.1
P value	0.1094		0.0073	
1MF				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	42	95	42	92
Median	-0.1	-0.2	-0.4	-0.35
IQR	-0.225 - -0.1	-0.3 - -0.1	-0.9 - -0.1	-1 - -0.1
Min - Max	-0.5 - 0.3	-1.4 - 0.6	-3.9 - 4.1	-4.8 - 0.3
P value	0.3944		0.626	

Table 26: Exercise induced temperature change at 2 minute (eTC2) at the Medial Gastrocnemius (MG) and heel for patients and controls undergoing the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

Similar to the results in Table 25, there is a significant temperature difference between controls and patients after a 6MWT at the heel. Controls exhibited a significant temperature increase that persists at 2 minutes, which patients did not ($p<0.007$).

Exercise induced temperature change at 3 minutes (eTC3)				
6MWT				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	38	40	37	41
Median	0	0.1	0.6	0
IQR	-0.1 - 0.2	0 - 0.375	0 - 1.3	-0.3 - 0.4
Min- Max	-1.2 - 0.5	-0.4 - 0.9	-1.2 - 3.7	-1.7 – 4
P value	0.0111		0.0042	
1MF				
Site	**MG**		**Heel**	
Participant	**Control**	**Patient**	**Control**	**Patient**
N	39	39	39	38
Median	-0.2	-0.2	-0.6	-0.6
IQR	-0.4 - -0.1	-0.4 - -0.1	-1.2 - -0.2	-1.05 - -0.3
Min- Max	-0.9- -0.1	-1.5 - -0.1	-3.9 - 3.7	-5.7 - 0.1
P value	0.1811		0.5919	

Table 27: Exercise induced temperature change at 3 minute (eTC3) at the Medial Gastrocnemius (MG) and heel for patients and controls undergoing the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

eTC3 post 6MWT, at the heel, controls had the persistent temperature elevation that is significantly different to patients (p=0.004). Additionally, eTC3 at the MG, is significantly different between patients and controls (p=0.01).

Subsection B: Comparison of 6-minute walk test (6MWT) against the 1-minute flexion extension test (1MF)

i) Is there an eTC between the type of exercise used for controls and patients at the level of the MG and or the heel?

1) Exercise induced temperature change (eTC) at the Medial Gastrocnemius(MG)

Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the Medial Gastrocnemius (MG) in controls and patients after the 6-minute walk test and the 1-minute flexion extension test (1MF)

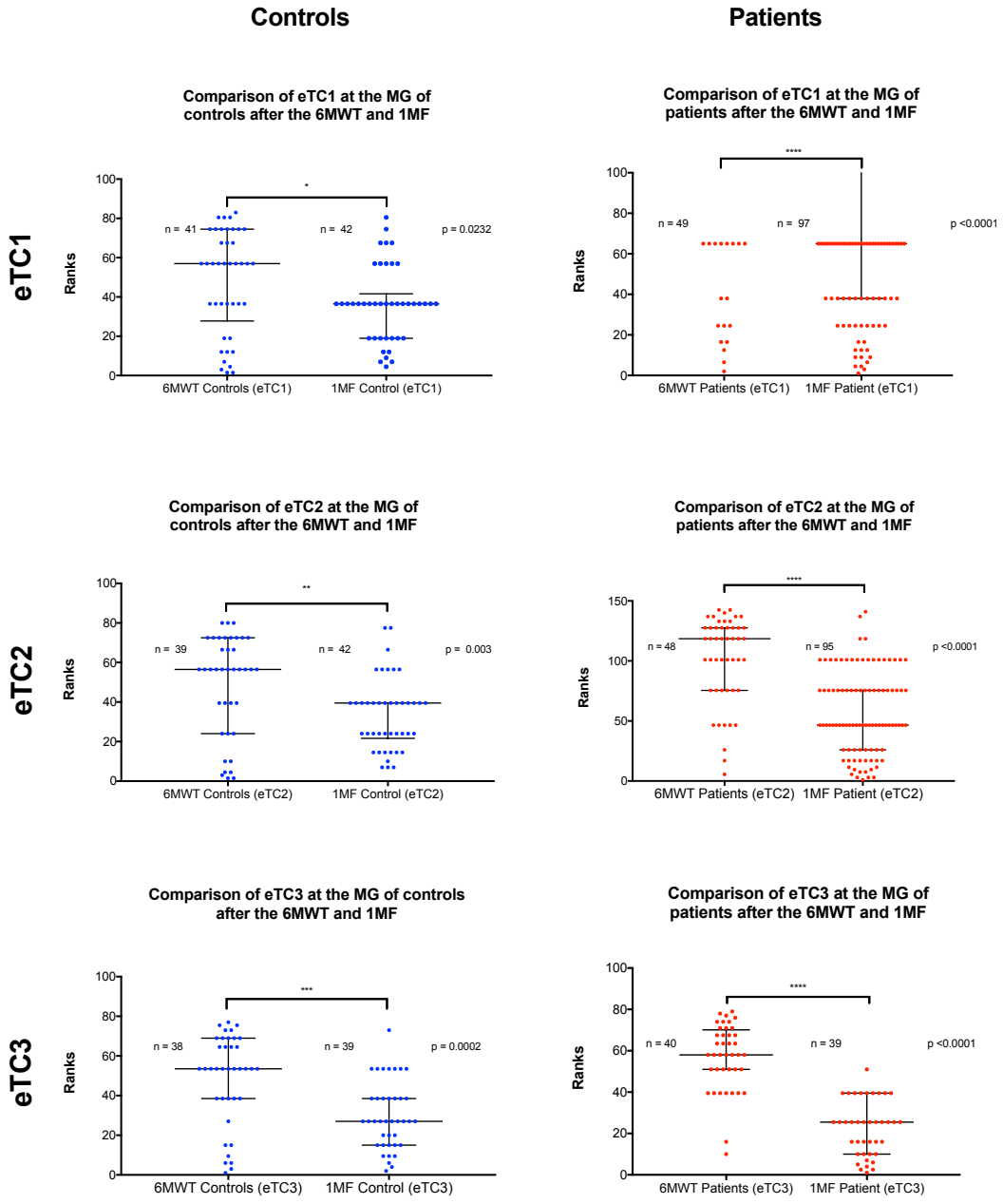


Figure 56: The results demonstrate that at the level of the medial gastrocnemius, there is a significant difference between the 6-minute walk test (6MWT) and the 1-minute flexion extension test (1MF). The exercise induced temperature change was higher for those undergoing the 6-minute walk test at 1,2 and 3 minutes post exercise in both patients and in controls.

1b) Exercise induced temperature changes over the heel

Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients after the 6 minute walk test (6MWT) and the 1 minute flexion extension test (1MF)

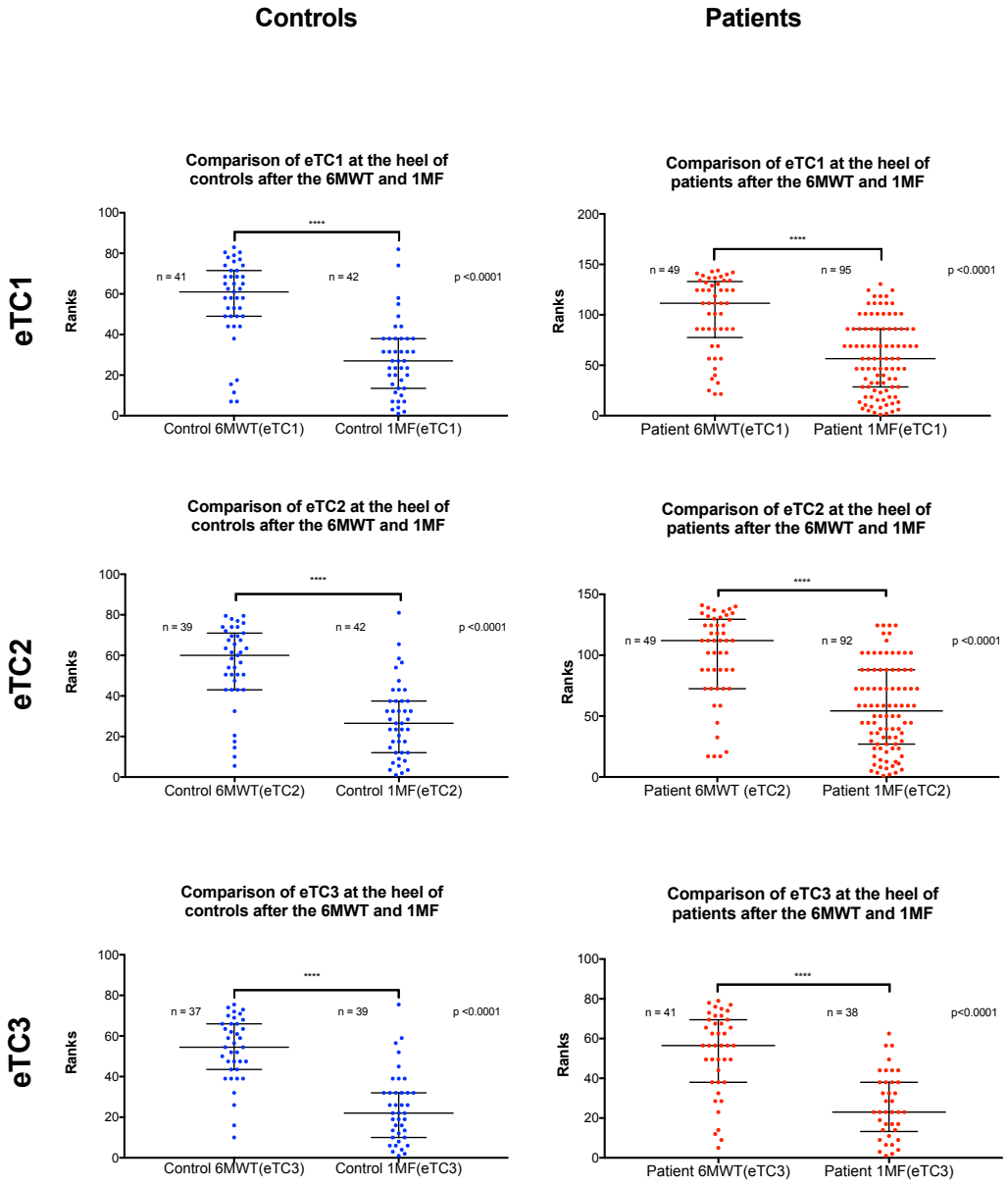


Figure 57: Similar to results from the medial gastrocnemius, there is a significant difference between the 6-minute walk test (6MWT) and the 1-minute flexion extension (1MF) test in both patients and controls. This suggests that the longer the duration of the test, the more significant the temperature change.

Summary of results

Comparison of 6MWT against 1MF				
Exercise induced temperature change at 1 minute (eTC1)				
Site	**MG**		**MG**	
Participant	**Controls**		**Patients**	
Provocation	**6MWT**	**1MF**	**6MWT**	**1MF**
N	41	42	48	97
Median	0	-0.1	0	-0.1
IQR	-0.15 - 0.2	-0.075 - -0.2	-0.1 - 0.2	-0.2 – 0
Min - Max	-1 - 0.6	-0.6 - 0.3	-1 - 1.2	-1.1 - 0.8
P value	0.0232		<0.0001	
Exercise induced temperature change at 2 minutes (eTC2)				
Site	**MG**		**MG**	
	Controls		**Patients**	
Participant	**6MWT**	**1MF**	**6MWT**	**1MF**
N	39	42	48	95
Median	0	-0.1	0.1	-0.2
IQR	-0.2 - 0.2	-0.1 - -0.225	-0.1 - 0.2	-0.3 - -0.1
Min - Max	-1.2 - 0.2	-0.5 - 0.3	-0.8 - 0.8	-1.4 - 0.6
P value	0.003		<0.0001	
Exercise induced temperature change at 3 minutes (eTC3)				
Site	**MG**		**MG**	
	Controls		**Patients**	
Participant	**6MWT**	**1MF**	**6MWT**	**1MF**
N	38	39	40	39
Median	0	-0.2	0.1	-0.2
IQR	-0.1 - 0.2	-0.4 - -0.1	0 - 0.375	-0.4 - -0.1
Min – Max	-1.2 - 0.5	-0.9 - 0.3	-0.4 - 0.9	-1.5 – 0
P value	0.0002		<0.0001	

Table 28: Comparison of the 6-minute walk test (6MWT) against the 1-minute flexion extension test (1MF) at the Medial Gastrocnemius (MG) assessing the Exercise induced temperature change at 1 minute (eTC1), 2 minutes (eTC2), 3 minutes (eTC3).

The result of this section demonstrates that there is a significant difference between the 6MWT and 1MF, for patients and even for controls, at both the heel and MG. Combining results from Section A and this section, the non-significant difference between controls and patients after the 1MF provocation test, is not due to differences between study subjects, but to the duration of the type of provocation test itself.

Subsection C: Comparison of measurement site: Heel against Medial Gastrocnemius (MG)

i) Is there a baseline or eTC between controls and patients undergoing the 6MWT or 1MF, at the MG and or the heel?

1) Exercise induced temperature change (6MWT group)

Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) and heel in controls and patients after the 6-minute walk test (6MWT)

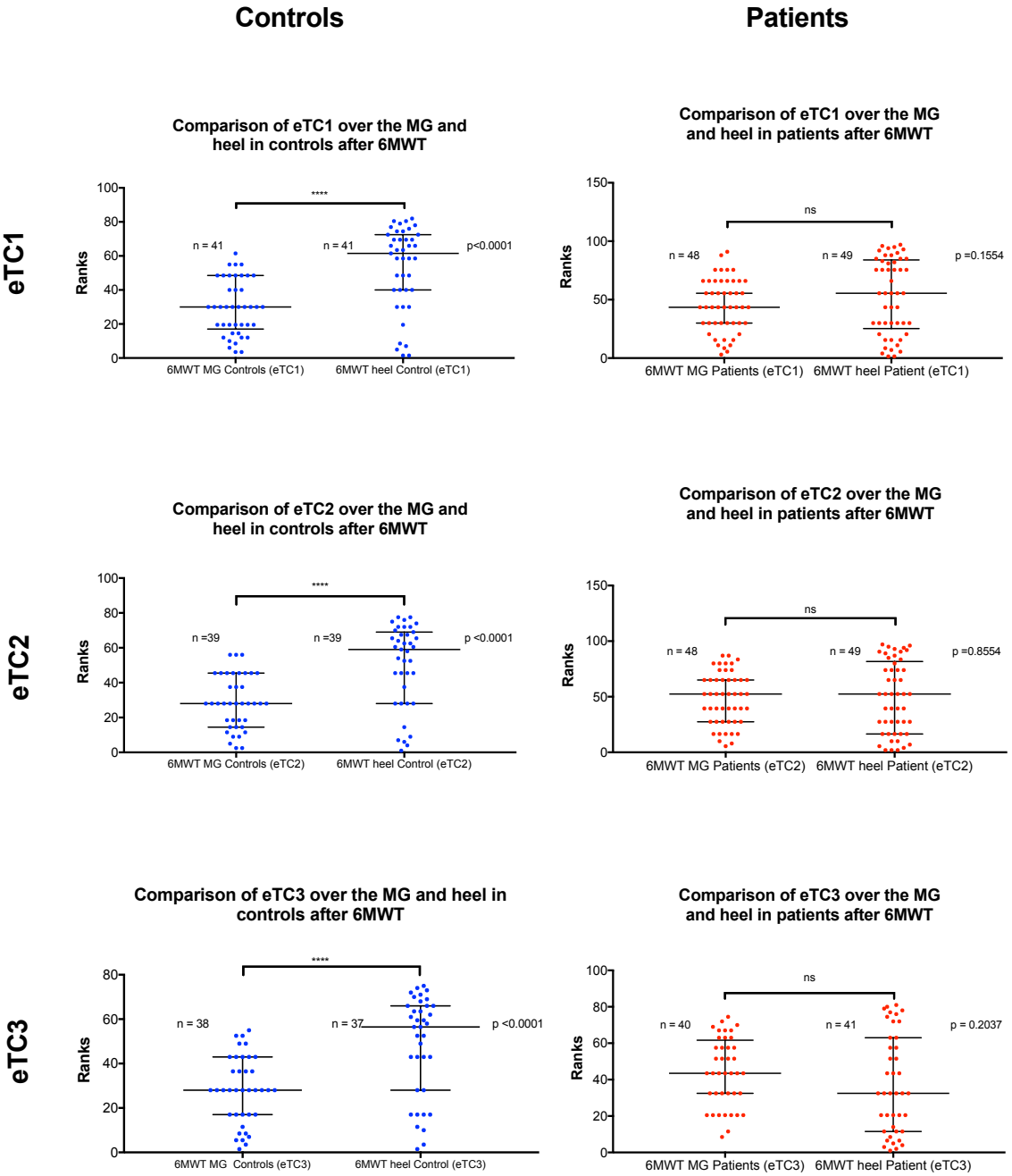


Figure 58: There is a significant difference amongst controls who underwent the 6-minute walk test (6MWT). Controls showed a higher exercise induced temperature difference at the heel compared to the medial gastrocnemius. This was different to patients who did not exhibit any significant exercise induced temperature change for either site.

Summary of results

Comparison of MG against Heel				
Exercise induced temperature change at 1 minute (eTC1)				
Site	**6MWT**		**6MWT**	
Participant	**Controls**		**Patients**	
Provocation	**MG**	**Heel**	**MG**	**Heel**
N	41	41	48	49
Median	0	0.6	0	0.1
IQR	-0.15 - 0.2	0.1 – 1	-0.1 - 0.2	-0.15 - 0.65
Min – Max	-1 - 0.6	-1.2 - 5.2	-1 - 1.2	-1.1 - 3.7
P value	<0.0001		0.1554	
Exercise induced temperature change at 2 minutes (eTC2)				
Site	**6MWT**		**6MWT**	
	Controls		**Patients**	
Participant	**MG**	**Heel**	**MG**	**Heel**
N	39	39	48	49
Median	0	0.7	0.1	0.1
IQR	-0.2 - 0.2	0 - 1.3	-0.1 - 0.2	-0.2 - 0.45
Min – Max	-1.2 - 0.5	-1.5 - 3.7	-0.8 - 0.8	-1.3 - 5.1
P value	<0.0001		0.8554	
Exercise induced temperature change at 3 minutes (eTC3)				
Site	**6MWT**		**6MWT**	
	Controls		**Patients**	
Participant	**MG**	**Heel**	**MG**	**Heel**
N	38	37	40	41
Median	0	0.6	0.1	0
IQR	-0.1 - 0.2	0 - 1.3	0 - 0.375	-0.3 - 0.4
Min – Max	-1.2 - 0.5	-1.2 - 3.7	-0.4 - 0.9	-1.7 – 4
P value	<0.0001		0.2037	

Table 29: Comparison of the Medial Gastrocnemius against the heel for Exercise induced temperature change at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) measurement after the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

From the previous sections, the 6MWT was shown to be a better test. The analysis of this section is presented separately based on the provocation test used (6MWT and 1MF). The results suggest that in contrast to controls, patients did not demonstrate a significant eTC between the MG and heel, post 6MWT. Based on the result with the control group, there should be a significant difference in eTC post 6MWT.

1) Exercise induced temperature change (1MF group)

Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) and heel in controls and patients after the 1-minute flexion extension test (1MF)

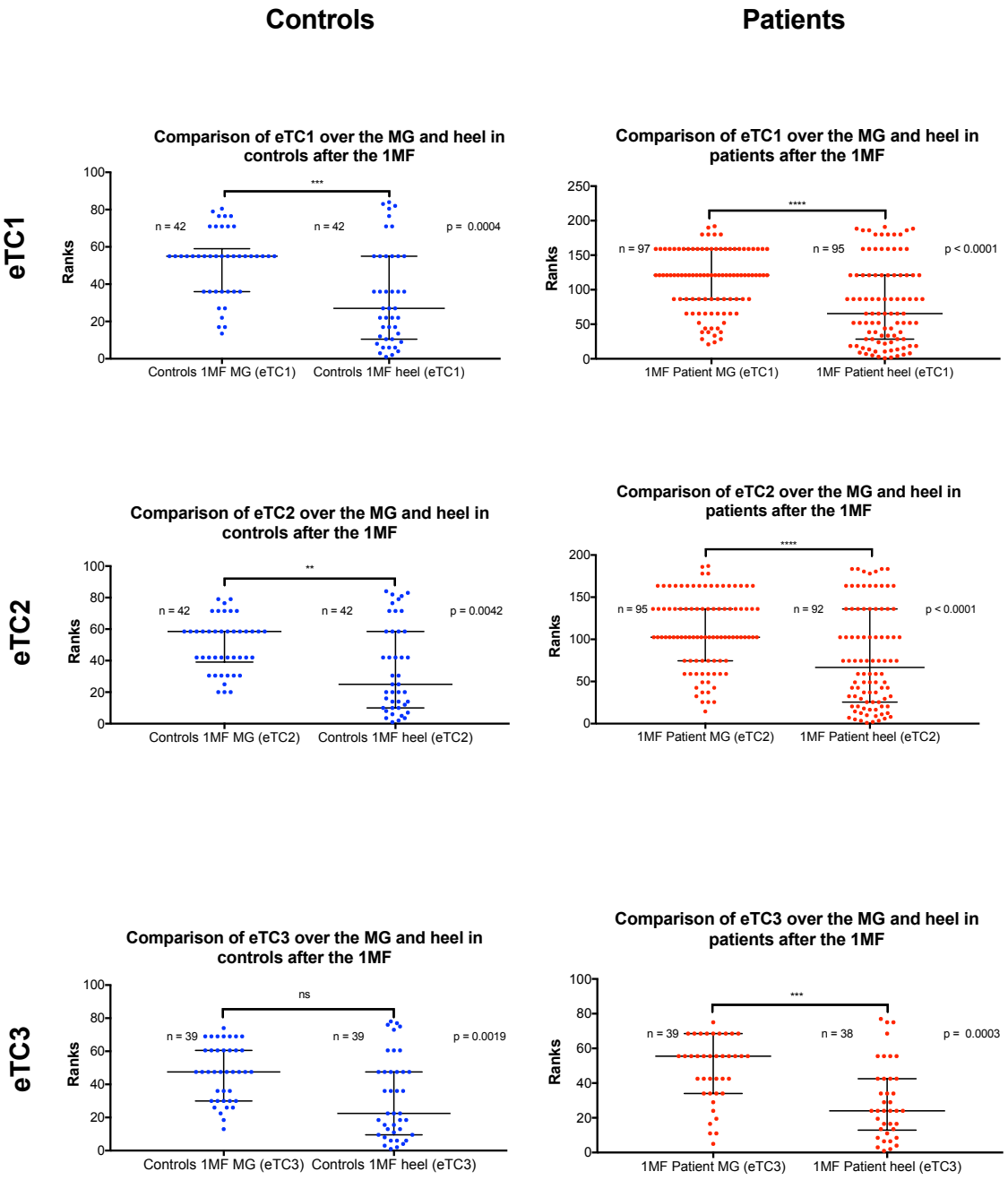


Figure 59: Results suggest that with the 1-minute flexion extension test, both patients and controls exhibit a significant difference in the exercise induced temperature change with the heel measurements having a lower temperature difference compared to the medial gastrocnemius.

Summary of results

Comparison of Medial Gastrocnemius (MG) against Heel				
Exercise induced temperature change at 1 minute (eTC1)				
Site	**1MF**		**1MF**	
Participant	**Controls**		**Patients**	
Provocation	**MG**	**Heel**	**MG**	**Heel**
N	42	42	97	95
Median	-0.1	-0.3	-0.1	-0.3
IQR	-0.075 - -0.2	-0.1 - -0.8	-0.2 – 0	-0.8 - -0.1
Min - Max	-0.6 - 0.3	-3.5 - 3.9	-1.1 - 0.8	-4.4 - 0.5
P value	0.0004		<0.0001	
Exercise induced temperature change at 2 minutes (eTC2)				
Site	**1MF**		**1MF**	
	Controls		**Patients**	
Participant	**MG**	**Heel**	**MG**	**Heel**
N	42	42	95	92
Median	-0.1	-0.4	-0.2	-0.35
IQR	-0.1 - -0.225	-0.9- -0.1	-0.3 - -0.1	-1 - -0.1
Min – Max	-0.5 - 0.3	-3.9 - 4.1	-1.4 - 0.6	-4.8 - 0.3
P value	0.0042		<0.0001	
Exercise induced temperature change at 3 minutes (eTC3)				
Site	**1MF**		**1MF**	
	Controls		**Patients**	
Participant	**MG**	**Heel**	**MG**	**Heel**
N	39	39	39	38
Median	-0.2	-0.6	-0.2	-0.6
IQR	-0.4 - -0.1	-1.2 - -0.2	-0.4 - -0.1	-1.05 - 0.1
Min – Max	-0.9 - 0.3	-3.9 - 3.7	-1.5 – 0	-5.7 - 0.1
P value	0.0019		0.0003	

Table 30: Comparison of the Medial Gastrocnemius against the heel for Exercise induced temperature change at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) measurement after the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

The results post 1MF demonstrate a significant post 1MF eTC between MG and heel. With the 1MF, gravity is eliminated. This significant decrease in temperature for both patients' and controls' MG and heel post 1MF, remains even at 3 minutes for both groups. Unlike the post 6MWT results at the MG, where controls had an increase in temperature post 6MWT, the heel site demonstrates a decrease in temperature.

Subsection D: Comparison of timing of measurement (eTC1 versus eTC2 and eTC3)?

i) Is there an eTC difference at 1, 2 and 3-minutes post provocation test, between patients and controls, at the MG or the heel?

1a) Exercise induced temperature changes over the medial gastrocnemius

One-way ANOVA: Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the medial gastrocnemius (MG) in controls and patients undergoing the 6 minute walk test or the 1 minute flexion extension test (1MF)

Controls | **Patients**

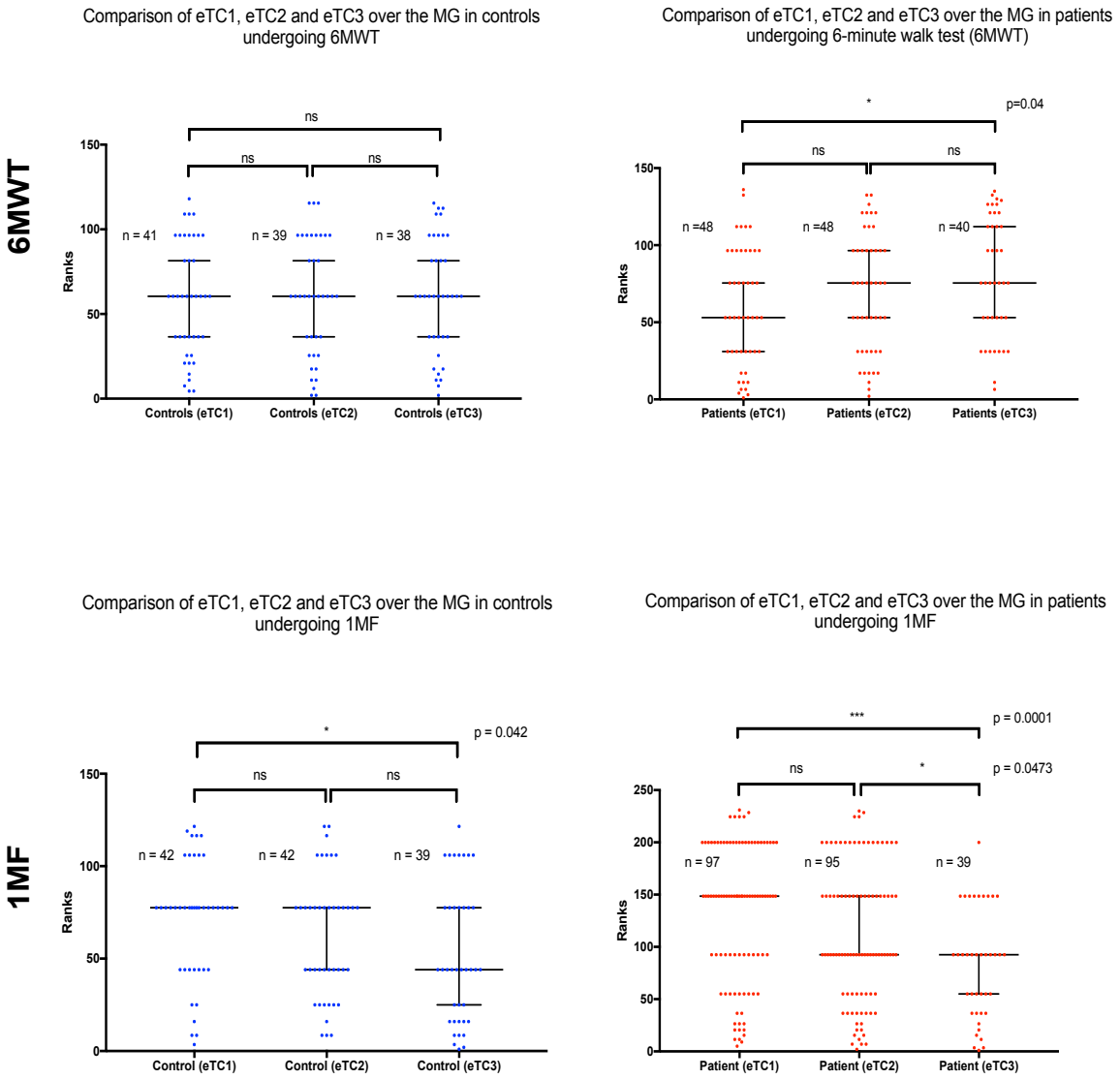


Figure 60: The results indicate that with the 1MF test, measuring at the MG, there is a significant decrease in the temperature decrease after 3 minutes. This is true for patients and controls, between eTC1 and eTC3. With the 6MWT, there is a significant difference between eTC1 and eTC3 for patients. However, there is no apparent difference for controls, between eTC1, eTC2 and eTC3.

Comparison of timing of measurements						
Medial Gastrocnemius (MG)						
Provocation	**6MWT**			**6MWT**		
Participant	**Controls**			**Patients**		
Time	**eTC1**	**eTC2**	**eTC3**	**eTC1**	**eTC2**	**eTC3**
N	41	39	38	48	48	40
Median	0	0	0	0	0.1	0.1
IQR	-0.15 - 0.2	-0.2 - 0.2	-0.1 - 0.2	-0.1 - 0.2	-0.2 - 0.2	0 - 0.375
Min – Max	-1 - 0.6	-1.2 - 0.5	-1.2 - 0.5	-1 - 1.2	-0.8 - 0.8	-0.4 - 0.9
P value	eTC1 vs eTC2 >0.999			eTC1 vs eTC2: 0.6528		
	eTC1 vs eTC3 >0.999			eTC1 vs eTC3: 0.5889		
	eTC2 vs eTC3 >0.999			eTC2 vs eTC3: 0.0408		
Medial Gastrocnemius (MG)						
Site	**1MF**			**1MF**		
	Controls			**Patients**		
Time	**eTC1**	**eTC2**	**eTC3**	**eTC1**	**eTC2**	**eTC3**
N	42	42	39	97	95	39
Median	-0.1	-0.1	-0.2	-0.1	-0.2	-0.2
IQR	-0.2 - -0.075	-0.225 - -0.1	-0.4 - -0.1	-0.2 - 0	-0.3 - -0.1	-0.4 - -0.1
Min – Max	-0.6 - 0.3	-0.5 - 0.3	-0.9 – 0.3	-1.1 - 0.8	-1.4 - 0.6	-1.5 – 0
P value	eTC1 vs eTC2: 0.6956			eTC1 vs eTC2: 0.0803		
	eTC1 vs eTC3: 0.0421			eTC1 vs eTC3: 0.0001		
	eTC2 vs eTC3: 0.5986			eTC2 vs eTC3: 0.0473		

Table 31: Comparison of the Exercise induced temperature change at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) at the Medial Gastrocnemius for measurement after the 6-minute walk test (6MWT) and 1-minute flexion extension test (1MF).

After the 6MWT, there is a significant difference for eTC2 versus eTC3 at the MG, between patients but not between controls. Like the 6MWT, the 1MF as the provocation test, produces a significant difference for eTC2 versus eTC3 between patients, that is not seen in controls. With this latter test, the difference between eTC1 and eTC3 is significantly different for both patients and controls at the MG. Referring

back to results from section A, using the 6MWT there is a significant difference between patients and for controls for eTC3.

1b) Exercise induced temperature changes over the heel

One-way ANOVA: Comparison of Exercise induced temperature changes at 1,2 and 3 minutes (eTC1, eTC2 and eTC3) over the heel in controls and patients undergoing the 6 minute walk test or the 1 minute flexion extension test (1MF)

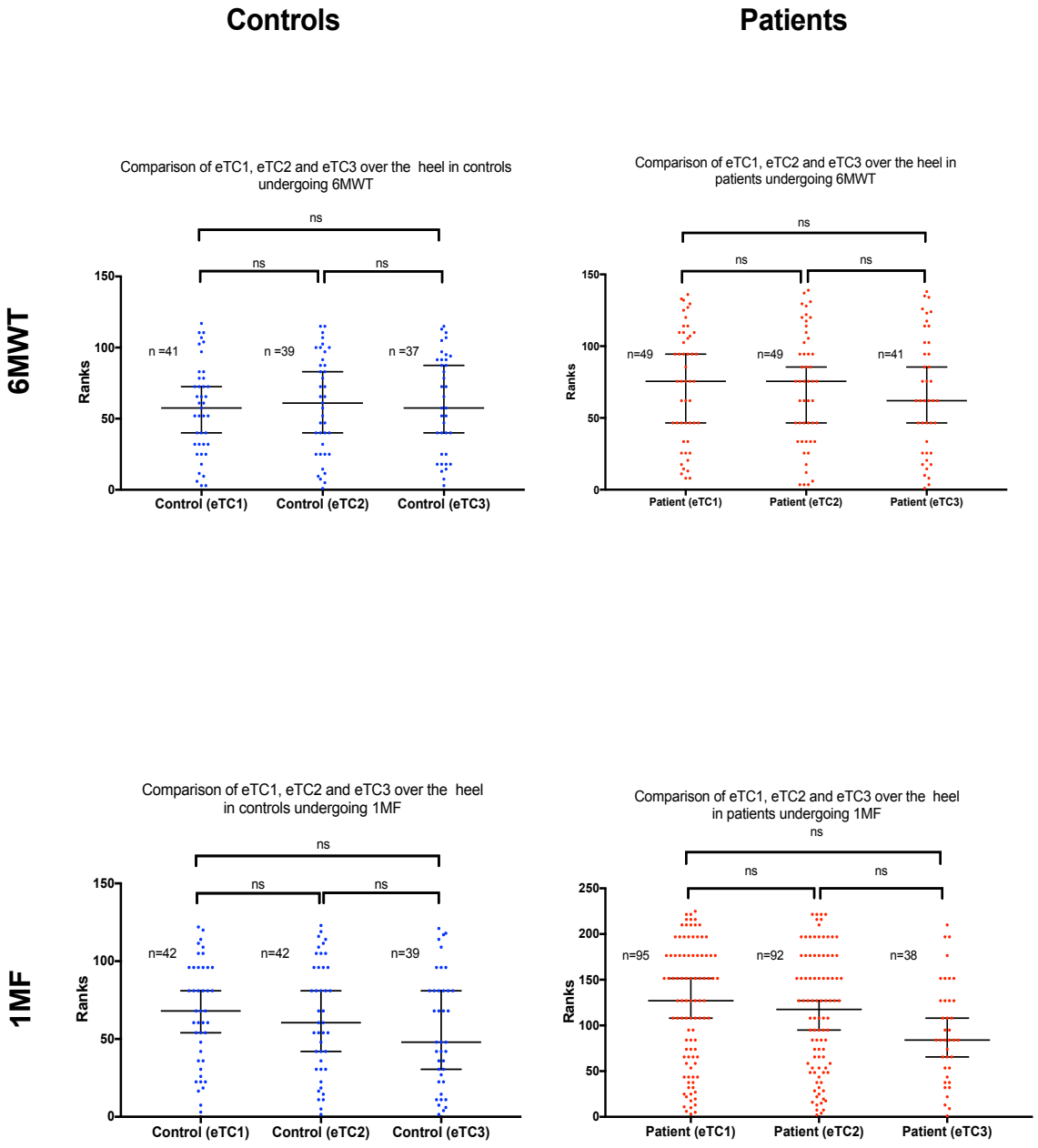


Figure 61: The results suggest that at the level of the heel, there is no statistically significant difference between patients and controls between eTC1, eTC2 and eTC3.

Summary of results

Comparison of timing of measurements						
Heel						
Provocation	**6MWT**			**6MWT**		
Participant	**Controls**			**Patients**		
Time	**eTC1**	**eTC2**	**eTC3**	**eTC1**	**eTC2**	**eTC3**
N	41	39	37	49	49	41
Median	0.6	0.7	0.6	0.1	0.1	0
IQR	0.1 - 1	0 - 1.3	0 - 1.3	-0.15 -0.65	-0.2 - 0.45	-0.3 - 0.4
Min – Max	-1.2 - 5.2	-1.5 - 3.7	-1.2 - 3.7	-1.1 - 3.7	-1.3 - 5.1	-1.7 – 4
P value	eTC1 vs eTC2 >0.999			eTC1 vs eTC2 >0.999		
	eTC1 vs eTC3 >0.999			eTC1 vs eTC3 >0.999		
	eTC2 vs eTC3 >0.999			eTC2 vs eTC3 >0.999		
Heel						
Site	**1MF**			**1MF**		
	Controls			**Patients**		
Time	**eTC1**	**eTC2**	**eTC3**	**eTC1**	**eTC2**	**eTC3**
N	42	42	39	95	92	38
Median	-0.3	-0.4	-0.6	-0.3	-0.35	-0.6
IQR	-0.8 - -0.1	-0.9 - -0.1	-1.2 - -0.2	-0.8 - -0.1	-1 - -0.1	-1.05 - -0.3
Min - Max	-3.5 - 3.9	-3.9 - 4.1	-3.9 - 3.7	-4.4 - 0.5	-4.8 - 0.3	-5.7 - 0.1
P value	eTC1 vs eTC2 >0.999			eTC1 vs eTC2 >0.999		
	eTC1 vs eTC3: 0.5125			eTC1 vs eTC3: 0.0692		
	eTC2 vs eTC3: 0.9249			eTC2 vs eTC3: 0.2932		

Table 32: Comparison of the exercise induced temperature change (eTC) at the heel between the 1st, 2nd and 3rd minutes.

At the level of the heel, there is no significant difference between eTC1, eTC2 and eTC3 between the controls and likewise between patients, using either 6MWT or 1MF. Looking back at results from section A, there is a significant difference between eTC1, eTC2 and eTC3 of patients and controls using the 6MWT at the level of the heel. This is not true for the 1MF test.

Receiver Operating Characteristics (ROC) Curve: Exercise Induced temperature change at 1,2, and 3 minutes (eTC1,eTC2 and eTC3) after the 6-minute walk test (6MWT) over the Medial Gastrocnemius (MG) and Heel

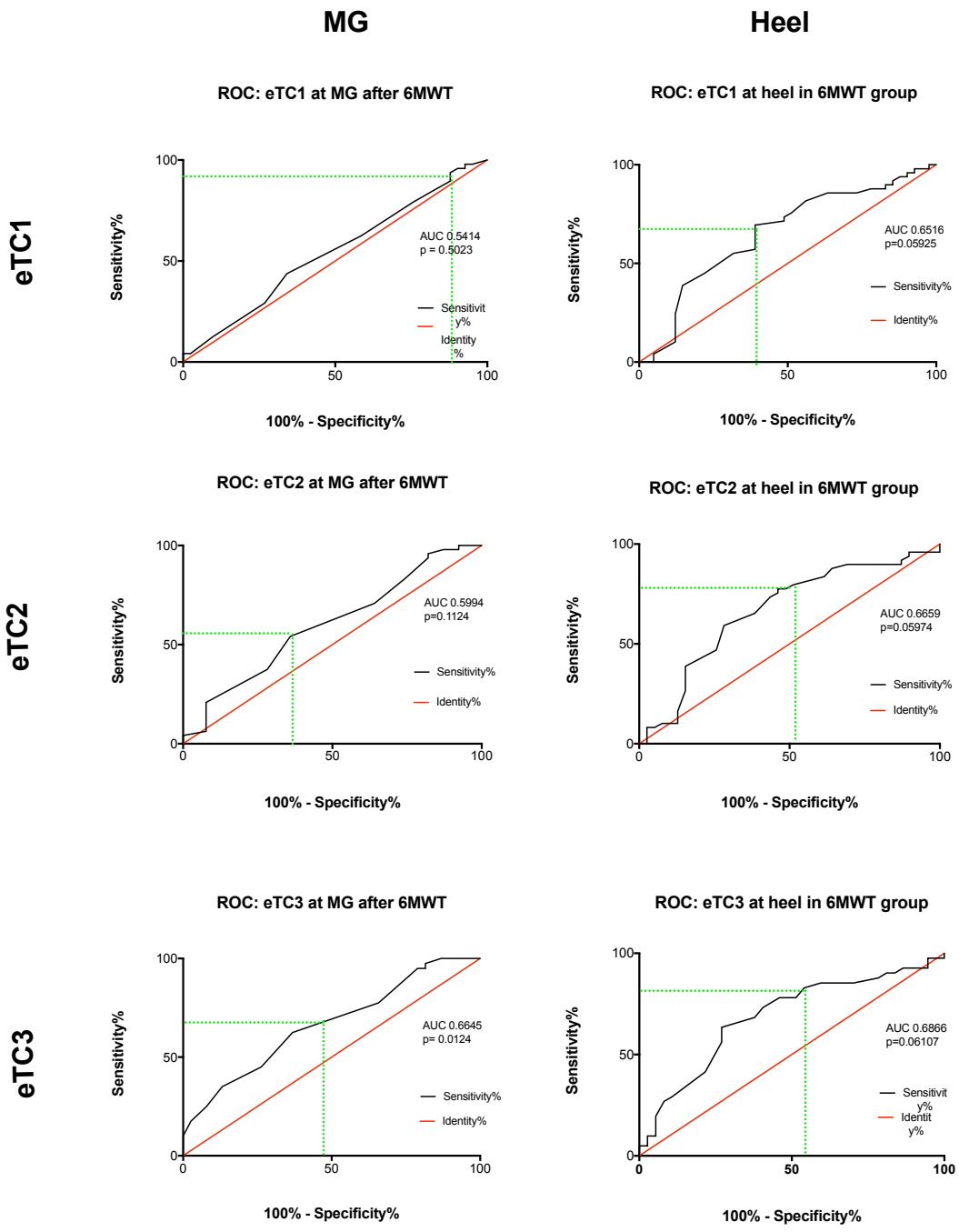


Figure 62: ROC curve determining the sensitivity of the exercise induced temperature changes (eTC) at the medial gastrocnemius and heel using the 6-minute walk test (6MWT). The cutoff for this exercise induced temperature change at 3 minutes (eTC3) was 0.05°C. The best Youden index was 0.2566 yielding a sensitivity and specificity of 62.5% and 63.16% respectively.

Receiver Operating Characteristics (ROC) Curve: Exercise Induced temperature change at 1,2, and 3 minutes (eTC1,eTC2 and eTC3) after the 1-minute flexion extension test (1MF) over the Medial Gastrocnemius (MG) and Heel

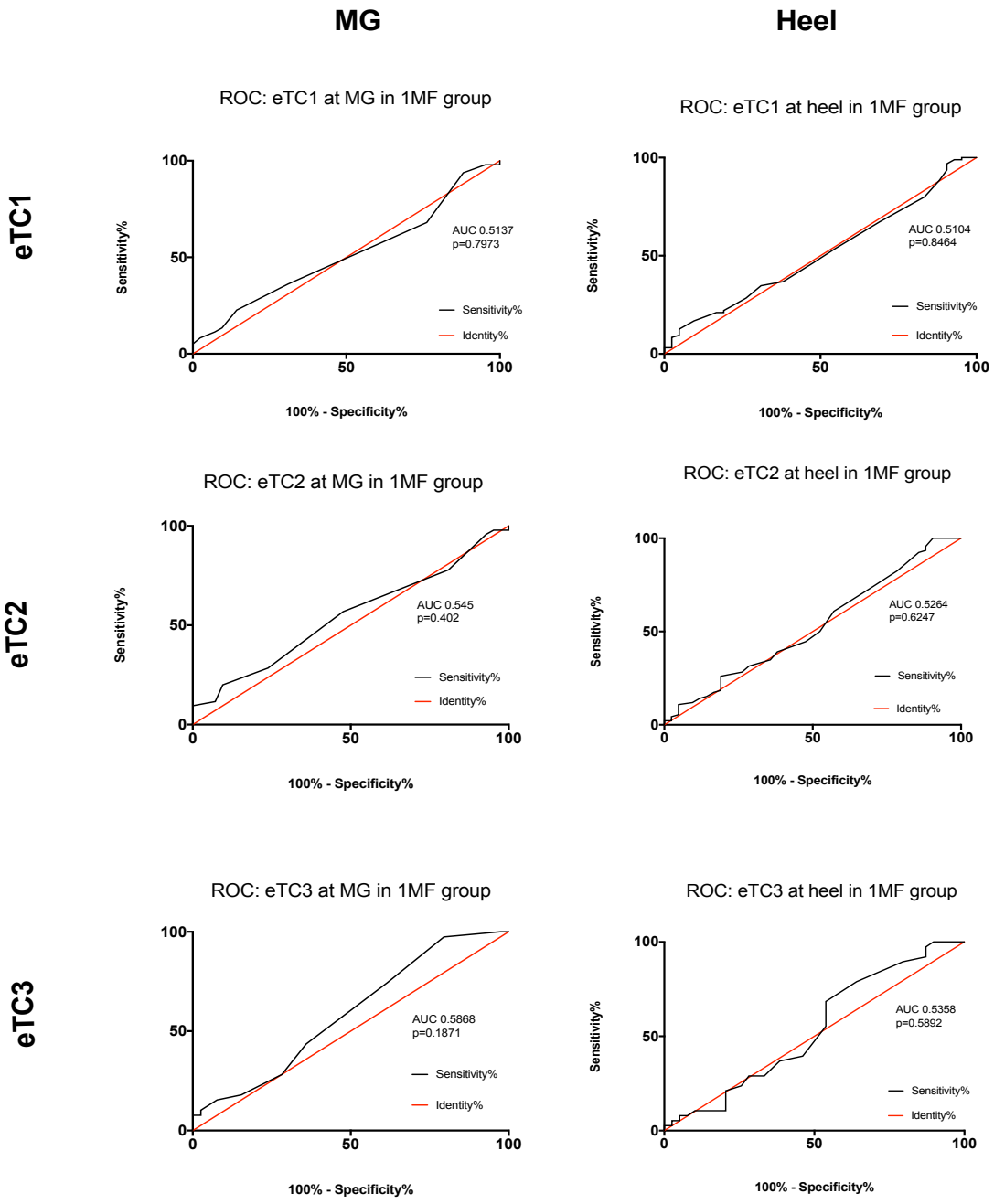


Figure 63: ROC curve determining the sensitivity of the exercise induced temperature changes (eTC) at the medial gastrocnemius and heel using the 1-minute flexion extension test (1MF). None of the Area under the curve (AUC) was significant enough to warrant a cutoff value and therefore accuracy.

Discussion

There aren't many studies that have compared temperature in the legs post exercise. The main study that is worth comparing against is the study by Huang et al who measured temperature changes in the shin and sole post 6MWT using infrared thermography at 1-minute post exercise (eTC1) in 27 controls and 28 PAD patients, diagnosed with ABPI<1. In my study, baseline temperature is no different between patients and controls, both at the MG and heel, which is similar to Huang et al (Huang et al., 2011).

In my study, there is a significant difference for eTC1 and eTC2 at the level of the heel between controls and patients, which is not seen at the MG, post 6MWT. However, 3-minutes post 6MWT (eTC3), there is a significant difference between patients and controls at this site and the heel. Further discussion is divided into the provocation test used.

6MWT

In healthy controls, on walking the median eTC over 3-minutes post 6MWT at the MG does not show significant change (Median 0°C). In patients however, on walking the median eTC in the MG is slightly elevated, but the median eTC shows no significant change over the 3-minutes post 6MWT (Median 0°C - 0.1°C). Huang et al found that the skin temperature at the shin (1-minute post 6MWT), was elevated by 0.3°C in controls, but reduced by -0.01°C in PAD patients (p=0.16). At the shin, he did not report any significant difference in temperature between patients and controls. This is not very different to the findings from my study, where there was no significant

difference until the 3rd minute post 6MWT (Patient vs control median eTC: 0.1°C vs 0°C, p=0.01). This means that the MG is a good site for temperature assessment after 3 minutes, between patients and controls.

At the heel, healthy controls and patients both exhibit a median temperature increase after the 6MWT, unlike Huangs' study, where patients versus controls eTC1 were significantly different (eTC1: -1.25°C vs 0.15°C, p<0.001). Patients in my study had a lower temperature increase at the heel after the 6MWT (Median 0°C - 0.1°C) compared to controls (Median 0.6°C - 0.7°C). eTC1, eTC2 and eTC3 was not significantly different up to 3-minutes (p>0.99) for these individual groups. However, after the 6MWT at the heel, the median eTC of patients versus controls was significantly different (eTC1: 0.1°C vs 0.6°C, p=0.01), (eTC2: 0.1°C vs 0.7°C, p=0.007) and (eTC3: 0°C vs 0.6°C, p=0.004).

The cutoff eTC3 at the MG, for distinguishing between patients and controls was 0.05°C, yielding a sensitivity of 62.5% and specificity of 63.16%. If measurements are taken at the heel, the cutoff temperature at 1 minute is 0.35°C (sensitivity 69.39%, specificity 60.98%), 0.55°C at 2-minutes (sensitivity 77.55%, specificity 53.85%) and 0.15°C at 3-minutes (sensitivity 63.41%, specificity 72.97%). Huangs' study suggested an eTC1 cutoff point for the sole was -0.99°C, with a sensitivity of 81.7% and specificity of 65%.

1MF

With gravity eliminated, in the control group the median eTC in the MG keeps decreasing over 3 minutes. This difference is significant between the 1st and 3rd minute post 1MF (Median -0.1°C - -0.2°C). In patients, the median eTC in the MG remains the same. Although, further analysis suggests that the eTC1 and eTC2 are significantly different from eTC3 in patients, there is no statistically significant difference between patients and controls overall. Therefore, assessment of temperature change at the MG using the 1MF test irrespective of the time of measurement is not useful. This could be as the duration of the 1MF is not adequate to elicit a temperature change.

At the heel of controls, the temperature decreases with time at 3-minutes. This decrease is not significant (Median -0.3°C - -0.6°C). In the case of patients, the median decrease is quite similar (-0.3°C - -0.6°C). In patients, the difference between eTC1 and eTC3, is significant. However, once again there is no significant difference between patients and controls, irrespective of timing of measurement.

Both these findings from the 1MF study are similar to the findings of a recent systematic review (Neves 2015). This systematic review excluded patient with diseases, and only included healthy adults and athletes. Temperature changes were measured at the muscle, before, during and after either a high intensity or low intensity exercise. With the short intensity exercises, the skin temperature initially decreases and gradually returns to normal values, whereas with high intensity exercises, the temperature increases and then gradually decreases slowly. This is similar to the

temperature decrease with short duration exercise (1MF) but increased or remained the same after the high intensity exercise (6MWT).

Therefore, the duration of the test seems to play an important role in eliciting a temperature change. It is clear that 6 minutes is an adequate duration and 1 minute is not sufficient. Further studies to determine the sufficient duration of exercise to elicit a temperature of significance. A further interesting finding is the significant temperature difference at the heel at 1,2 and 3-minutes post 6MWT amongst patients, which is only seen in the MG at 3 minutes. This could be explained by the possibility that the blood supply at the MG is better than the heel in PAD patients. The difference in temperature at the heel between patients and controls after the 6MWT could be explained by the shunting of blood to the active muscles via the arteriovenous shunts during exercise.

Conclusion

To the best of my knowledge, this is the first study that has used infrared thermometry for the diagnosis of PAD. The findings of this study suggest that a longer duration of exercise is better in eliciting temperature change. A temperature difference of 0.05°C, at 3 minutes after the 6MWT in the medial gastrocnemius yields a sensitivity of 62.5% and specificity of 63.16% for PAD.

Temperature measurement should be performed, based on the site of measurement. When measuring temperature change at the MG, temperature should be taken at 3

minutes post 6MWT. When measuring temperature change at the heel, measurements can be taken at 1,2 or 3-minutes post 6MWT.

Chapter 7: Conclusion

STARTREC (Simplifying Technologies for peripheral ARTerial disease RECognition) is aimed at identifying novel non-invasive technologies to study physiological parameters that have potential as a triage tool for the diagnosis of peripheral arterial disease (PAD). ABPI is and has been recommended but used sparsely particularly in primary care. This study looks at the best conditions, site and timing of measurement on the leg to measure these parameters. A topical review was performed to identify non-invasive novel diagnostics. Devices were selected based on previous research in this field, and a precision study was conducted to assess the reproducibility, ease of use and applicability in a pragmatic setting. The final devices selected were the infrared thermometer and the Near infrared spectroscopy. Participants parameters were measured at baseline and following 2 provocation tests. These provocation tests were the 1-minute flexion extension test and a 6-minute walk test. Patients had their diagnosis confirmed by imaging (duplex, CT or MR). Healthy subjects had PAD ruled out by ABPI. From previous meta-analysis, the ABPI was reported to have a sensitivity of 15-79% and a specificity of 83-100% for PAD diagnosis (Dachun et al., 2010). Additionally, the use of ABPI has been poor in primary care (Yap Kannan et al., 2016) with its use in triaging PAD amongst claudicants brought in to question (Crawford et al., 2016). This study assessed novel diagnostics based on 3 parameters. From the pilot studies, it became clear that the first parameter of perfusion is too sensitive to movement to assess and produce a meaningful result, and therefore not reliable at present for PAD diagnosis.

In the case of the oxygenation measurements, this study has demonstrated that baseline tissue saturation index (bTSI) and difference in tissue saturation index (dTSI) post provocation test (6MWT or 1MF) at the medial gastrocnemius (MG), can be used to aid PAD diagnosis. The sensitivity using the bTSI ranges from 57.94% to 73.2%, with a specificity ranging from 72.73% to 74.42%. The sensitivity of the dTSI ranges from 61.36% to 71.43%, with a specificity ranging from 65.12% to 74.77%. When using the T_{100} parameter, the more intense provocation test (6MWT) produces a sensitivity of 75.51% and specificity of 58.97%. bTSI is proportional to the severity of PAD, in contrast to dTSI which is not affected by this. The 1MF test duration is also sufficient to produce a significant dTSI. When using the T_{100} parameter, the longer duration of the provocation of the test, the better it is. However, analysis of this parameter requires technical expertise and is not efficient. The study found that at baseline, oxygen levels are not significantly different between healthy subjects and patients. However, there is a significant drop post exercise in oxygen levels (dTSI) and the time for recovery to baseline (T100), after a provocation test in patients, for both provocation tests. Future technologies capable of measuring the oxygen tissue saturation index at the MG immediately after the provocation test, could be equally useful.

With the thermometry study, baseline temperature and exercise induced temperature change at 1,2 and 3 minutes after both provocation tests were measured at the calf and heel. The results show that baseline temperature is similar between patients and healthy subjects. The 1MF test does not produce any significant results. With the 6MWT, at the calf, there is a rise in temperature in patients and healthy subjects, but by 3 minutes, the temperature starts to return to baseline only in healthy subjects. For

the same provocation test, at the heel, patients have a higher temperature compared to healthy subjects at 1, 2 and 3 minutes post provocation. Thermometry offers the advantage of being simple and convenient. The main limiting factor is the duration of the provocation test required to elicit a significant temperature change. A temperature difference of 0.05°C, at 3 minutes after the 6MWT in the medial gastrocnemius yields a sensitivity of 62.5% and specificity of 63.16% for PAD. At the heel, measurements can be taken at 1,2 or 3-minutes post 6MWT. 1-minute post 6MWT, a cutoff of 0.35°C yields a sensitivity of 69.39% and specificity of 60.98%. 2-minutes post 6MWT, a cutoff of 0.55°C yields a sensitivity of 77.55% and specificity of 53.85%. At 3-minutes post 6MWT, the sensitivity and specificity is 63.41% and 72.97% respectively using a cutoff of 0.15°C. Despite the duration, the measurements are easier to perform compared to the ABPI. In this study, the maximum duration of provocation was 6 minutes and the minimum at 1 minute. The time between 1 and 6 minutes was not assessed for this parameter. Future studies in thermometry can focus on the timing of measurement to determine the appropriate duration of exercise to elicit a significant temperature cutoff to distinguish PAD from non-PAD.

This study demonstrates that although provocation tests of longer duration appear to be better for temperature and oxygen measurement, shorter provocation tests can also be used to produce significant TSI changes. With regards to the site of measurement, distal sites are better for temperature assessment, but remain unstudied for oxygenation levels. The limitations of this study are that healthy subjects were diagnosed by ABPI, compared to duplex in patients. This was due to the ethical question of detecting asymptomatic PAD and its further management. Future studies taking into account findings from this study, will produce more useful information on

simplifying technologies for peripheral arterial disease recognition. In the precision study, criteria were not set out to determine applicability and ease of use, at the offset. Nevertheless, the risk of bias in both precision and accuracy studies are low as, there is no evidence on what results to expect.

Currently, oxygenation can be studied at the calf using NIRS, using a short provocation test such as the 1MF. Whether there is a difference that persists or is higher at the feet would be of interest. Newer developments in photoplethysmography and pulse oximetry at the foot are potential avenues for further research in this field.

Appendix

1. **Indemnity Insurance letter**
2. **Patient Information Sheet**
3. **Consent form**
4. **EuroQOL-5D questionnaire**
5. **Peripheral arterial disease questionnaire**
6. **GP Survey questionnaire**

1.Indemnity Insurance letter

Our Ref: sdb 2013-2014 – 204

20th December 2013

ESTATES AND FACILITIES MANAGEMENT DIVISION
Fielding Johnson Building
University Road
Leicester
LE1 7RH
Tel: +44 (0)116 229 7631
Fax: +44(0)116 229 7633

To whom it may concern,

UNIVERSITY OF LEICESTER CLINICAL TRIAL/PROFESSIONAL INDEMNITY INSURANCE

Title of Study: STARTREC Simplifying Technologies for peripheral ARTerial disease RECognition

Chief Investigator: Professor Rob Sayers
Student Investigator – Mr Ramesh Yap Kannan

I confirm that the University of Leicester will provide Clinical Trials and Professional Indemnity insurance cover in respect of its legal liability in relation to the above trial within the UK only.

Any significant departure from the programme of research as outlined in the application (such as changes in methodological approach, large delays in commencement of research, additional forms of data collection or major expansions in sample size) must be communicated to us.

The cover is provided subject to normal policy terms and conditions.

Sue Banbury

Sue Banbury
Insurance & Risk Manager
University of Leicester

Sdb16@le.ac.uk

2.Patient Information Sheet

Patient Information Sheet

Study Code

Participants Initials

STARTREC

Simplifying **T**echnologies for peripheral **ART**erial disease **REC**ognition

Study Description:

You are invited to participate in a study comparing non-invasive diagnostic methods to aid early diagnosis and predict progression of peripheral arterial disease (PAD).

This information sheet consists of two parts that would take 10 minutes to read.

- **Part 1** will tell you the purpose of the study and what it will mean to you.
- **Part 2** provides more detailed information about the conduct of the study (to be read if you are considering participation)

Please ask us if anything is unclear or if you would like more information.

Thank you for taking the time to read this information sheet.

Part 1

WHAT IS PERIPHERAL ARTERIAL DISEASE (PAD)?

PAD reduces blood flow to your limbs leading to reduced oxygen and nutrition reaching your limbs, which leads to pain, ulcers or dead tissue (symptoms). Any or a combination of these symptoms makes you symptomatic.

HOW IS IT DIAGNOSED?

PAD can be diagnosed by your doctor using simple tests in their practice, measuring blood pressure in your legs (non-invasive bedside test)

WHY CONDUCT THIS STUDY?

Current test to diagnose PAD are cumbersome and may not identify PAD in certain patients accurately. This study will assess available technologies and study if they provide a reasonable and reliable alternative to improve diagnosis of PAD.

HOW WILL THIS STUDY HELP?

This study will answer the question whether technological advancement can replace or improve current non-invasive bedside diagnosis of PAD.

WHY HAVE I BEEN INVITED?

You are being invited because, you are either:

- A healthy volunteer or a participant with diabetes without PAD
- Symptomatic with PAD undergoing outpatient visits and/or intervention
- Symptomatic with PAD undergoing emergency surgery or intervention

AM I ELIGIBLE?

You are eligible, unless:

- You have grafts in both legs.
- You have previous blood clots in your leg.

If you have a bypass graft in only one leg, you are still eligible to participate.

IS THERE ANY TREATMENT INVOLVED?

No. These tests are purely diagnostic. There is no treatment involved.

WILL THIS STUDY CHANGE ANY TREATMENT I AM MEANT TO HAVE?

No. This study will not change any aspect of treatment that you are meant to have.

WHAT DO I HAVE TO DO?

No special preparation is necessary. Please take own medications as you would.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

There is no treatment benefit for you if you participate. However, we hope results from this study will help improve diagnosis and management of patients in the future.

ARE THERE SIDE EFFECTS, RISKS AND DISCOMFORTS OF TAKING PART?

There is no risk of radiation with any of these tests. Potential complications include:

- Pain during inflation of the blood pressure cuff on your leg.
- Ischemia (lack of oxygen) to the leg.

You can inform the doctor if it is too painful, and tests will be stopped. To date there is no reported complications from the blood pressure cuff being applied on legs.

WILL I BE PAID FOR PARTICIPATING IN THE RESEARCH?

Unfortunately, we do not have sufficient funds to pay you for participating.

WILL MY GP BE INVOLVED?

Your GP would not be involved in the study.

WHAT IF I DECIDE TO PARTICIPATE OR NOT PARTICIPATE?

If you decide to participate in this study, any further questions you may have will be clarified. You will then undergo tests. If you decide not to participate, any ongoing or future treatment will not be affected. You can withdraw from the study without having to give any reason by contacting the study doctor for a 'Withdrawal of Consent' form.

HOW WILL MY DETAILS BE USED AND WILL IT BE CONFIDENTIAL?

By signing the consent form you consent to the study doctor and his staff collecting personal and medical information from you or your notes ('Study Data'). The study doctor, will use your study data for research and analysis. The data will be analyzed in accordance with UK Data Protection Act 1998. You have the right to request or correct information about you from the study data held by the study doctor. If you wish to make a request, please contact the study doctor (*page 3*). Your consent does not have a specific expiration date, but you may withdraw your consent at any time.

DO I HAVE TO COME BACK?

If you are:

- Healthy or if you have diabetes without PAD, no follow up tests will be needed.
- Symptomatic with PAD undergoing outpatient assessment or intervention, tests will be repeated within 6 months at your scheduled clinic appointment.
- Symptomatic with PAD and admitted for emergency surgery or intervention, tests will be repeated after your treatment and within 6 months, at your scheduled clinic appointment. You will not undergo a physiological test in this group.

WHO HAS REVIEWED THIS STUDY?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests.

WHOM SHOULD I CONTACT IF I NEED MORE INFORMATION OR HELP?

Whenever you have questions or concerns about the study, please contact:

Name: Mr.Ramesh Yap Kannan
Contact details: ryk1@le.ac.uk (email)
Address: Vascular Surgery Research Group,
RKCSB, Leicester Royal Infirmary, LE1 5WW

PART 2

WHAT ARE THE TESTS INVOLVED?

The first step is a questionnaire which can take up to 15 minutes. The tests would be performed on the palm and finger, thigh, calf, forefoot, sole and toe of your legs. These tests would be performed concurrently, before, during and after a 'physiological test'. The 'physiological test' involves inflating a blood pressure cuff around your leg for up to 5 minutes, or walking along the corridor for 5 minutes. Device tests will involve scanning test, photo tests or sticky probe tests. Scanning test are similar to supermarket scanners that will be used to scan your legs for up to 15 minutes. Photo test of your leg can take up to 10 minutes. Sticky probe tests will be stuck on to your skin and blood flow will be checked for up to 20 minutes. **Total time for these tests is one hour.**

WHAT WILL HAPPEN TO THE RESULTS OF THE STUDY?

Study data will be analyzed and findings may be published in medical literature. You will not be identified in any report or publication. If you have any questions about the study results, you should contact your study doctor.

WHAT IF THERE IS A PROBLEM?

If you have any concerns, you should speak with the study doctor. If you suffer any side effects or injuries after the tests, notify the study doctor immediately in order to receive medical treatment.

WHO SHOULD I SPEAK TO IF I WISH TO COMPLAIN?

If you wish to complain formally, you can do this through the NHS Complaints Procedure provided below:

Patient Information and Liaison Service
The Firs C/O Glenfield Hospital
Groby Road, Leicester, LE3 9QP
Phone: 080801788337
Email: pils@uhl-tr.nhs.uk

Thank you for taking time to read this information sheet.

3. Consent Form

Study Code _____

CONSENT FORM

Study: STARTREC

Simplifying ***T***echnologies for peripheral ***ART***erial disease ***REC***ognition

Please initial the box

1. I confirm that I have received verbal information and read and understand the information sheet dated (UK version 1, dated 1st of November 2013) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from Leicester University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree that my contact details may be kept, by the research team, following completion of the study in order that I may be contacted regarding any future studies. ☐
5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Participant (BLOCK CAPITALS)	Date	Signature
_____	_____	_____
Investigator (BLOCK CAPITALS)	Date	Signature

1 copy for participants, 1 copy for researcher, 1 copy to be kept with participants' notes

4. EuroQOL-5D questionnaire

Health Questionnaire

(English version for the UK)
(validated for use in Eire)

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

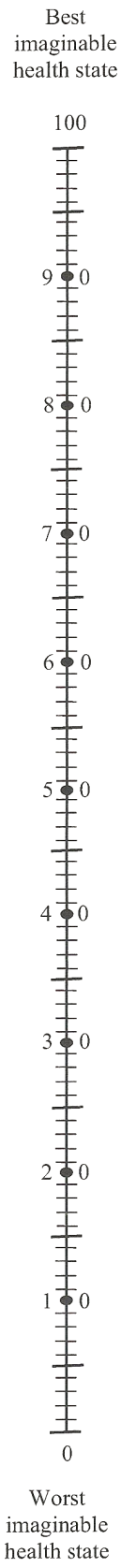
I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



5.The Peripheral Arterial Questionnaire

The Peripheral Arterial Questionnaire

The following questions refer to blockages in your arteries, particularly in your legs, and how they might affect your life. Please read and answer the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

1. Blockages in the arteries, often referred to as **peripheral vascular disease**, affect different people in different ways. Some feel cramping or aching while others feel fatigue. Which leg (or buttock) causes you the most severe **discomfort, fatigue, pain, aching or cramps**?

The **right** leg (buttock)	The **left** leg (buttock)	**Both** are the same	Neither
☐	☐	☐	☐

2. Please review the list below and indicate how much limitation you have had due to **your peripheral vascular disease** (discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)) over the past 4 weeks.

Please place an **X** in one box on each line

Activity	Extremely limited	Quite a bit limited	Moderately limited	Slightly limited	Not at all limited	Limited for other reasons or did not do the activity
Walking around your home	☐	☐	☐	☐	☐	☐
Walking 100-200 metres (100-200 yards) on **level ground**	☐	☐	☐	☐	☐	☐
Walking 100-200 metres (100-200 yards) **uphill**	☐	☐	☐	☐	☐	☐
Walking 300-400 metres (300-400 yards) on level ground	☐	☐	☐	☐	☐	☐
Hurrying or jogging (as if to catch a bus)	☐	☐	☐	☐	☐	☐
Vigorous work or exercise	☐	☐	☐	☐	☐	☐

3. Compared with 4 weeks ago, have your symptoms of **peripheral vascular disease** (discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)) changed?

My symptoms have become…

Much worse	Slightly worse	Not changed	Slightly better	Much better	I have had no symptoms over the past 4 weeks
☐	☐	☐	☐	☐	☐

4. Over the past 4 weeks, how many times did you have **discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)**?

All of the time	Several times a day	At least once a day	3 or more times a week but not every day	Once or twice a week	Less than once a week	Not at all over the past 4 weeks
☐	☐	☐	☐	☐	☐	☐

5. Over the past 4 weeks, how much have **discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)** bothered you?

They have been...

Extremely bothersome	**Moderately** bothersome	**Somewhat** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no leg discomfort**
☐	☐	☐	☐	☐	☐

6. Over the past 4 weeks, how often have you been woken up by **pain, aching or cramps in your legs or feet**?

Every night	3 or more times a week but not every night	Once or twice a week	Less than once a week	Not at all over the past 4 weeks
☐	☐	☐	☐	☐

7. How satisfied are you that everything possible is being done to treat your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

8. How satisfied are you with the explanations your doctor has given you about your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

9. Overall, how satisfied are you with the current treatment of your **peripheral vascular disease**?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

10. Over the <u>past 4 weeks</u>, how much has your **peripheral vascular disease** limited your enjoyment of life?

It has **extremely** limited my enjoyment of life	It has limited my enjoyment of life **quite a bit**	It has **moderately** limited my enjoyment of life	It has **slightly** limited my enjoyment of life	It has **not limited** my enjoyment of life at all
☐	☐	☐	☐	☐

11. If you had to spend the rest of your life with your **peripheral vascular disease** the way it is <u>right now</u>, how would you feel about that?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

12. Over the <u>past 4 weeks</u>, how often have you felt discouraged or 'down in the dumps' because of your **peripheral vascular disease**?

I felt that way **all of the time**	I felt that way **most of the time**	I **occasionally** felt that way	I **rarely** felt that way	I **did not** feel that way at all
☐	☐	☐	☐	☐

13. How much does your **peripheral vascular disease** affect your lifestyle? Please indicate how much your **discomfort, fatigue, pain, aching or cramps in your calves (or buttocks)** have limited your participation in the following activities over the past 4 weeks.

Please place an **X** in one box on each line

Activity	**Severely** limited	Limited **quite a bit**	**Moderately** limited	**Slightly** limited	**Did not** limit at all	Does not apply or did not do for other reasons
Hobbies, recreational activities	☐	☐	☐	☐	☐	☐
Going out to visit family or friends	☐	☐	☐	☐	☐	☐
Working or doing household chores	☐	☐	☐	☐	☐	☐

6.GP Survey Questionnaire

Peripheral Arterial Disease Screening Survey

We, at the Vascular Surgery Research Group, University of Leicester kindly request for a few minutes of your time to complete this survey to assess current practice in the diagnosis of Peripheral Arterial Disease (PAD) at primary care facilities.

1. Please select your profession

- ☐ General Practitioners
- ☐ Registered Nurse
- ☐ Nurse Practitioner
- ☐ Health Care Assistant
- ☐ Technician
- ☐ Other

2. Which of the following do you consider important in diagnosing PAD?

	Yes	No	Unsure
Risk Factors	☐	☐	☐
Pulse Examination	☐	☐	☐
Questionnaires	☐	☐	☐
Ankle Brachial Pressure Index (ABPI)	☐	☐	☐
Ankle Pressures	☐	☐	☐
Toe Brachial Pressure Index (TBPI)	☐	☐	☐
Toe Pressures	☐	☐	☐

3. Who performs the ABPI at your practice?

- ☐ General Practitioner
- ☐ Nurse Practitioner
- ☐ Registered Nurse
- ☐ Health Care Assistant
- ☐ Technician
- ☐ Other

4. How often do you use the ABPI?

- ☐ Weekly
- ☐ Monthly
- ☐ Annually
- ☐ Never

5. How useful have you found the ABPI to be in the diagnosis and clinical management of

	Very Useful	Moderately Useful	Not Useful
Asymptomatic PAD	☐	☐	☐
Symptomatic PAD	☐	☐	☐

6. How feasible is incorporating the ABPI into your daily practice?

- ☐ Very Feasible
- ☐ Moderately Feasible
- ☐ Not Feasible

7. Is ABPI a good screening tool for PAD in the following patients?

	Yes	No	Unsure
Healthy patients	☐	☐	☐
Diabetics	☐	☐	☐
Chronic Renal Failure	☐	☐	☐
Elderly (>65 years)	☐	☐	☐

8. What factors if any, limit the utilization of the ABPI in your clinical practice?

	No limitation	Major limitation	Moderate limitation	Minor limitation
Time Constraints	☐	☐	☐	☐
Financial Constraints	☐	☐	☐	☐
Clinical Significance	☐	☐	☐	☐
Staff Availability	☐	☐	☐	☐
Patient Willingness	☐	☐	☐	☐
Staff Willingness	☐	☐	☐	☐
Staff Training	☐	☐	☐	☐
Availability of Space	☐	☐	☐	☐
Availability of Doppler	☐	☐	☐	☐
Availability of Cuffs	☐	☐	☐	☐
Application of Cuff	☐	☐	☐	☐
Performing Walk test	☐	☐	☐	☐
Presence of Wounds	☐	☐	☐	☐
Performing Handheld Doppler examination	☐	☐	☐	☐
ABPI interpretation	☐	☐	☐	☐

Bibliography

Abraham, P., Picquet, J., Vielle, B., Sigaudo-Roussel, D., Paisant-Thouveny, F., Enon, B., Saumet, J.L. (2003). Transcutaneous oxygen pressure measurements on the buttocks during exercise to detect proximal arterial ischemia: Comparison with arteriography. *Circulation.* 107, 1896-1900.

Abularrage, C.J., Sidawy, A.N., Aidinian, G., Singh, N., Weiswasser, J.M., Arora, S. (2005). Evaluation of the microcirculation in vascular disease. *Journal of Vascular Surgery.* 42, 574-581.

Adler, A.I., Stevens, R.J., Neil, A., Stratton, I.M., Boulton, A.J., Holman, R.R. (2002). UKPDS 59: hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. *Diabetes Care.* 25, 894-899.

Allen, J., Overbook, K., Nath, A., Murray, A., Stansby, G. (2008). A prospective comparison of bilateral photoplethysmography versus the ankle-brachial pressure index for detecting and quantifying lower limb peripheral arterial disease. *Journal of Vascular Surgery.* 47, 794-802.

Allison, M.A., Criqui, M.H., McClelland, R.L., Scott, J.M., McDermott, M.M., Liu, K., Ham, R.O., Bertoni, A.G., Sharrett, A.R., Homma, S., Kori, S. (2006). The effect of novel cardiovascular risk factors on the ethnic-specific role for peripheral arterial disease in the Multi-Ethnic Study of Atherosclerosis (MESA). *Journal of the American College of Cardiology.* 48, 1190-1197.

Alnaeb, M.E., Crabtree, V.P., Boutin, A., Mikhailidis, D.P., Seifalian, A.M., Hamilton, G. (2007). Prospective assessment of lower-extremity peripheral arterial disease in diabetic patients using a novel automated optical device. *Angiology.* 58, 579-585.

Althouse, A.D., Abbott, J.D., Forker, A.D., Bertolet, M., Barinas-Mitchell, E., Thurston, R.C., Mulukutla, S., Aboyans, V., Brooks, M.M. (2014). BARI 2D Study Group. Risk factors for incident peripheral arterial disease in type 2 diabetes: results from the bypass angioplasty revascularisation investigation in type 2 diabetes (BARI 2D) trial. *Diabetes Care.* 37, 1346-1352.

American Thoracic Society (2002). ATS statement: guidelines for the six-minute walk test. *American Journal of Respiratory and Critical Care Medicine.* 166, 111-117.

Andrews, K.L., Dib, M.Y., Shives, T.C., Hoskin, T.L., Liedl, D.A., Boon, A.J. (2013). Non-invasive arterial studies including transcutaneous oxygen pressure measurements with the limbs elevated or dependent to predict healing after partial foot amputation. *American Journal of Physical Medicine and Rehabilitation.* 92, 385-392.

Apa, H., Gozmen, S., Bayram, N., Catıkoglu, A., Devri, F.I., Karaarskan, U., Gunay, I., Unal, N., Devrim, I. (2013). Clinical Accuracy of Tympanic Thermometer and

Noncontact Infrared Skin Thermometer in Paediatric Practice: An Alternative for Axillary Digital Thermometer. *Paediatric Emergency Care.* 29, 992-997.

Armstrong DG, L.L. (1996). Monitoring neuropathic ulcer healing with infrared dermal thermometry. *Journal of Foot and Ankle Surgery.* 35, 335-338.

Armstrong, D.G., Holtz-Neiderer, K., Wendel, C., Mohler, M.J., Kimbriel, H.R., Lavery, L.A. (2007). Skin temperature monitoring reduces the risk for diabetic foot ulceration in high-risk patients. *American Journal of Medicine.* 120, 1042-1046.

Armstrong, D.G., Lavery, L.A., Wunderlich, R.P., Bouton, A.J. (2003). Skin temperatures as a one-time screening tool do not predict future diabetic foot complications. *Journal of American Podiatric Medical Association.* 93, 443-447.

Armstrong, D.G., Lipsky, B.A., Polis, A.B., Abrasion, M.A. (2006). Does dermal thermometry predict clinical outcome in diabetic foot infection? Analysis of data from the SIDESTEP* trial. *International Wound Journal.* 3, 302-307.

Aubert, C.E., Cluzel, P., Kemel, S., Michel, P.L., Laat-Kiss, F., Dadon, M., Hartemann, A., Bourron, O. (2014). Influence of peripheral vascular calcification on efficiency of screening tests for peripheral arterial occlusive disease in diabetes - a cross sectional study. *Diabetic Medicine.* 31, 192-199.

Bach, A.J.E., Stewart, I.B., DIsher, A.E., Costello, J.T. (2015). A Comparison between conductive and infrared devices for measuring mean skin temperature at rest, during exercise in heat and recovery. *PLoS One.* 10, 1-13.

Banoo, S., Bell, D., Bossuyt, P., Herring, A., Mabey, D., Poole, F., Smith, P.G., Sriram, N., Wongsrichanalai, C., Linke, R., O'Brien, R., Perkins, M., Cunningham, J., Matsoso, P., Nathanson, C.M., Olliaro, P., Peeling, R.W., Ramsay, A. : TDR Diagnostics Evaluation Expert Panel (WHO/TDR) (2006, 2010). Evaluation of diagnostic tests for infectious diseases: general principles. *Nature Reviews Microbiology.* S28.

Baril, D.T. & Marone, L.K. (2012). Duplex evaluation following femoropopliteal angioplasty and stenting: Criteria and utility of surveillance. *Vascular and Endovascular Surgery.* 46, 353-357.

Bartlett, J.W. & Frost, C. (2008). Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound Obstetric Gynecology.* 31, 466-475.

Bauer, T.A., Brass, E.P., Hiatt, W.R. (2004). Impaired muscle oxygen use at onset of exercise in peripheral arterial disease. *Journal of Vascular Surgery.* 40, 488-493.

Benhamou, Y., Edet, S., Begarin, L., Cailleux, N., Hanoy, M., Bessin, C. (2012). Transcutaneous oximetry as predictive test of peripheral vascular revascularization in haemodialysis population. *Nephrology Dialysis Transplantation.* 27, 2066-2069.

Bharara, M., Cobb, J.E., Claremont, D.J. (2006). Thermography and thermometry in the assessment of diabetic neuropathic foot: a case for furthering the role of thermal techniques. *International Journal of Lower Extremity Wounds.* 5, 250-260.

Bhasin, N. & Scott, D.J.A. (2007). Ankle brachial index: Identifying cardiovascular risk and improving diagnostic accuracy. *Journal of the Royal Society of Medicine.* 100, 4-5.

Bianchi, J., Zamiri, M., Loney, M., McIntosh, H., Dawe, R.S., Douglas, W.S. (2008). Pulse oximetry index: A simple arterial assessment for patients with venous disease. *Journal of Wound Care.* 17, 253-260.

Bitar, D., Goubar, A., Desenclos, J.C. (2009). International travels and fever screening during epidemics: A literature review on the effectiveness and potential use of non-contact infrared thermometers. *Euro Surveillance.* 14, 1-5.

Blacher, J., Cacoub, P., Luizy, F., Mourad, J.J., Levesque, H., Benelbaz, J., Michon, P., Premalatha, G., Priollet, P. (2006). Peripheral arterial disease versus other localizations of vascular disease: the ATTEST study. *Journal of Vascular Surgery.* 44, 314-318.

Bland, J.M. & Altman, D.G. (2003). Applying the right statistics: Analyses of measurement studies. *Ultrasound Obstetric Gynecology.* 22, 85-93.

Bland, J.M. & Altman, D.G. (1986). Statistical Methods for assessing agreement between two methods of clinical measurement. *The Lancet.* 327, 307-310.

Boas, D.A. & Dunn, A.K. (2010). Laser speckle contrast imaging in biomedical optics. *Journal of Biomedical Optics.* 15, 011109.

Boezeman, R.P.E., Moll, F.L., Ünlü, Ç, de Vries, J.P.M. (2016). Systematic review of clinical applications of monitoring muscle tissue oxygenation with near-infrared spectroscopy in vascular disease. *Microvascular Research.* 104, 11-22.

Bonham, P.A., Kelechi, T., Mueller, M., Robison, J. (2010). Are toe pressures measured by a portable photoplethysmograph equivalent to standard laboratory tests? *Journal of Wound Ostomy and Continence Nursing.* 37, 475-486.

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L., Moher, D., Rennie, D., de Vet, H. C. W., Lijmer, J.G. (2003). The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Annals of Internal Medicine.* 138, 1-12.

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L., Lijmer, J.G., Moher, D., Rennie, D., de Vet, H. C. W, Kressel, H.Y., Rifai, N., Golub, R.M., Altman, D.G., Hooft, L., Korevaar, D.A., Cohen, J.F. (2015). STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies. *Radiology.* 277, 826-832.

Boulos, M.N., Wheeler, S., Tavares, C., Jones, R. (2011). How smartphones are changing the face of mobile and participatory healthcare: an overview, with example from eCAALYX. *Biomedical Engineering Online.* 10, 1-14.

Bouyé, P., Jacquinandi, V., Picquet, J., Thouveny, F., Liagre, J., Leftheriotis, G., Saumet, J.L., Abraham, P. (2005). Near-infrared spectroscopy and transcutaneous oxygen pressure during exercise to detect arterial ischemia at the buttock level: Comparison with arteriography. *Journal of Vascular Surgery.* 41, 994-999.

Bouzida, N., Bendada, A., Maldague, X.P. (2009). Visualization of body thermoregulation by infrared imaging. *Journal of Thermal Biology.* 34, 120-126.

Brearley, S., Shearman, C.P., Simms, M.H. (1992). Peripheral pulse palpation: an unreliable physical sign. *Annals of the Royal College of Surgeons of England.* 74, 169-171.

Brooks, B., Dean, R., Patel, S., Wu, B., Molyneaux, L., Yue, D.K. (2001). TBI or not TBI: that is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients? *Diabetic Medicine.* 18, 528-532.

Brownrigg, J.R.W., Hinchliffe, R.J., Apelqvist, J., Boyko, E.J., Fitridge, R., Mills, J.L., Reekers, J., Shearman, C.P., Zierler, R.E., Schaper, N.C. (2016). Effectiveness of bedside investigations to diagnose peripheral artery disease among people with diabetes mellitus: a systematic review. *Diabetes/Metabolism Research and Reviews.* 32, 119-127.

Bunt, T.J. & Holloway, G.A. (1996). $TcPO_2$ as an accurate predictor of therapy in limb salvage. *Annals of Vascular Surgery.* 10, 224-227.

Buono, M.J., Jechort, A., Marques, R., Smith, C., Welch, J. (2007). Comparison of infrared versus contact thermometry for measuring skin temperature during exercise in the heat. *Physiological Measurement.* 28, 855-859.

Cahan, M.A., Montgomery, P., Otis, R.B., Clancy, R., Flinn, W., Gardner, A. (1999). The effect of cigarette smoking status on six-minute walk distance in patients with intermittent claudication. *Angiology.* 50, 537-546.

Cameron, A.E.P., Porter, A., Rosser, S., Da Silva, A. E. C. F., De Cossards, L.M. (1997). The stresst'er ergometer as an alternative to treadmill testing in patients with claudication. *European Journal of Vascular and Endovascular Surgery.* 14, 433-438.

Carter, C., Martinez, M., Purnell, G., Chesbro, S.B. (2013). Screening for peripheral arterial disease in a long term care setting: Considerations for clinical practice. *Geriatric Rehabilitation.* 29, 203-209.

Caru, M., Levesque, A., Lalonde, F., Curnier, D. (2019). An overview of ischemic preconditioning in exercise performance: A systematic review. *Journal of Sport and Health Science.* 01, 1-15.

Casa, D.J., Becker, S.M., Ganio, M.S., Brown, C.M., Yeargin, S.W., Roti, M.W. (2007). Validity of devices that assess body temperature during outdoor exercise in the heat. *Journal of Athletic Training.* 42, 333-342.

Castaneda, D., Esparza, A., Ghamari, M., Soltanpur, C., Nazeran, H. (2018). A review on wearable photoplethysmography sensors and their potential future applications in health care. *International Journal of Biosensors & Bioelectronic.* 4, 195-202.

Cea-Soriano, L., Fowkes, F.G.R., Johansson, S., Allum, A.M., García Rodriguez, L.A. (2018). Time trends in peripheral artery disease incidence, prevalence and secondary preventive therapy: A cohort study in the health improvement network in the UK. *BMJ Open.* 8, e018184.

Chan, L.S., Cheung, G.T.Y., Lauder, I.J., Kumana, C.R. (2004). Screening for fever by remote-sensing infrared thermographic camera. *Journal of Travel Medicine.* 11, 273-279.

Charpentier, G., Benhamou, P.Y., Dardari, D., Clergeot, A., Franc, S., Schaepelynck-Belicar, P., Catargi, B., Melki, V., Chaillous, L., Farret, A., Bosson, J.L., Pennornas, A. (2011). The Diabeo software enabling individualized insulin dose adjustments combined with telemedicine support improves HbA_{1c} in poorly controlled type 1 diabetic patients: A 6-month, randomized, open-label, parallel-group, multicenter trial (TeleDiab 1 Study). *Diabetes Care.* 34, 533-539.

Cheatle, T.R., .Potter, A., Cope, M., Delpy, D.T., Smith, P.D.C., Scurr, J.H. (1991). Near infrared spectroscopy in peripheral vascular disease. *British Journal of Surgery.* 78, 405-408.

Cheetham, D.R., Ellis, M., Davies, A.H., Greenhalgh, R.M. (2004). Is the Stresst'er a reliable stress test to detect mild to moderate peripheral arterial disease? *European Journal of Vascular and Endovascular Surgery.* 27, 545-548.

Chen, S.C., Su, H.M., Chang, J.M., Liu, W.C., TEsai, Y.C., Lin, M.Y., Hwang, S.J., Chen, H.C. (2012). Increasing prevalence of peripheral arterial occlusive disease in hemodialysis patients: A 2 year follow up. *American Journal of the Medical Sciences.* 343, 440-445.

Chiappini, E., Sollai, S., Longhi, R., Morandini, L., Laghi, A., Osio, C.E. (2011). Performance of non-contact infrared thermometer for detecting febrile children in hospital and ambulatory settings. *Journal of Clinical Nursing.* 20, 1311-1318.

Chin, J.A., Wang, E.C., Kibbe, M.R. (2011). Evaluation of hyperspectral technology for assessing the presence and severity of peripheral artery disease. *Journal of Vascular Surgery.* 54, 1679-1688.

Chung, N.S., Han, S.H., Lim, S.H., Hong, Y.S., Won, J.H., Bae, J.I., Jo, J. (2010). Factors affecting the validity of ankle brachial index in the diagnosis of peripheral arterial obstructive disease. *Angiology.* 61, 392-396.

Clairotte, C., Retort, S., Potier, L., Roussel, R., Escoubet, B. (2009). Automated ankle brachial pressure index measurement by clinical staff for peripheral arterial disease diagnosis in non-diabetic and diabetic patients. *Diabetes Care.* 32, 1231-1236.

Collins, R., Burch, J., Cranny, G., Aguiar-Ibáñez, R., Craig, D., Wright, K., Berry, E., Gough, M., Kleijnen, J., Westwood, M. (2007). Duplex ultrasonography, magnetic resonance angiography, and computed tomography angiography for diagnosis and assessment of symptomatic, lower limb peripheral arterial disease: systematic review. *British Medical Journal.* 334, 1257-1261.

Comerota, A.J., Throm, R.C., Kelly, P., Jaff, M. (2003). Tissue (muscle) oxygen saturation (Sto2): A new measure of symptomatic lower-extremity arterial disease. *Journal of Vascular Surgery.* 38, 724-729.

Coughlin, P.A., Kent, P.J., Turton, E.P., Byrne, P., Berridge, D.C., Scott, D.J., Kester, R.C. (2001). A new device for the measurement of disease severity in patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery.* 22, 516-522.

Coughlin, P.A., Kent, P.J., Berridge, D.C., Scott, D.J.A., Kester, R.C. (2006). The PADHOC device is a better guide to the actual incapacity suffered by claudicants than the gold standard constant load treadmill test. *European Journal of Vascular & Endovascular Surgery.* 32, 651-656.

Couse, N.F. *et al* (1994). Pulse oximetry in the diagnosis of non-critical peripheral vascular insufficiency. *Journal of Royal Society of Medicine*. 87(9), 511-512.

Crandall, C.G., Musick, J., Hatch, J.P., Kellogg, D.L.J., Johnson, J.M. (1995). Cutaneous vascular and sudomotor responses to isometric exercise in humans. *J.Appl Physiol.* 79, 1946-1950.

Crawford, F., Welch, K., Andras, A., Chappell, F.M. (2016). Ankle brachial index for the diagnosis of lower limb peripheral arterial disease. *The Cochrane Database of Systematic Reviews.* 9, CD010680.

Criqui, M.H., Fronek, A., Klauber, M.R., Barrett-Connor, E., Gabriel, S. (1985). The sensitivity, specificity, and predictive value of traditional clinical evaluation of peripheral arterial disease: results from noninvasive testing in a defined population. *Circulation.* 71, 516.

Criqui, M. & Aboyans, V. (2015). Epidemiology of peripheral artery disease. *Circulation Research.* 116, 1509-1526.

Daanen, H.A.M. (2006). Infrared tympanic temperature and ear canal morphology. *Journal of Medical Engineering and Technology.* 30, 224-234.

Dachun, X., Jue, L., Liling, Z., Yawei, X., Dayi, H., Pagoto, S.L., Ma, Y. (2010). Sensitivity and specificity of the ankle-brachial index to diagnose peripheral artery disease: A structured review. *Vascular Medicine.* 15, 361-369.

Davies, J.H., Kenkre, J., Williams, E.M. (2014). Current utility of the ankle-brachial index (ABI) in general practice: Implications for its use in cardiovascular disease screening. *BMC Family Practice.* 15, 1-11.

De Graaff, J.C., Ubbink, D.T., Legemate, D.A., de Haan, R.J., Jacobs, M.J. (2000). The usefulness of a laser Doppler in the measurement of toe blood pressures. *Journal of Vascular Surgery.* 32, 1172-1179.

Degischer, S., Labs, K.H., Aschwanden, M., Tschoepl, M., Jaeger, K.A. (2002). Reproducibility of constant-load treadmill testing with various treadmill protocols and predictability of treadmill test results in patients with intermittent claudication. *Journal of Vascular Surgery.* 36, 83-88.

Dodd, S.R., Lancaster, G.A., Craig, J.V., Smyth, R.L., Williamson, P.R. (2006). In a systematic review, Infrared ear thermometry for fever diagnosis in children finds Poor Sensitivity. *Journal of Clinical Epidemiology.* 59, 354-357.

Doverspike, G.A., Bendick, P.J., Brown, D., Hubbard, J., Shelpman, D., Shanley, C.J. (2004). Evaluating the lower extremities for peripheral arterial obstructive disease using the Transfer Function Index. *Journal for Vascular Ultrasound.* 28, 9-12.

Dziubek, W., Bulinska, K., Stefanska, M., Kropielnicka, K., Jasinski, T., Jasinski, R., Pilch, U., Dabrowska, G., Skokowska-Telichowska, K., Wojcieszczyk-Latos, J., Kalka, D., Janus, A., Zywar, K., Paszkowski, R., Szuba, A. (2015). Peripheral arterial disease decreases muscle torque and functional walking capacity in elderly. *Maturitas.* 81, 480-486.

Ena, J., Argente, C.R., González-Sánchez, V., Algado, N., Verdú, G., Lozano, T., (2013). Use of pocket pulse oximeters for detecting peripheral arterial disease in patients with diabetes mellitus. *Journal of Diabetes Mellitus.* 3, 79-85.

Erenbeck, U., Torun, E., Ozkaya, E., Uzuner, S., Demir, A.D., Dundaroz, R. (2013). Skin temperature measurement using an Infrared thermometer on patients who have been exposed to cold. *Paediatrics International.* 55, 767-770.

Eriksson, S., Nilsson, J., Sturesson, C. (2014). Non-invasive imaging of microcirculation: a technology review. *Medical Devices (Auckland).* 7, 445-452.

Ezio, F., Giacomo, C., Maurizio, C., Antonella, Q., Vincenzo, C., Francesco, S. (2010). Evaluation of feasibility of ankle pressure and foot oximetry values for the detection of critical limb ischemia in diabetic patients. *Vascular and Endovascular Surgery.* 44, 184-189.

Faglia, E., Clerici, G.:.C., J., Gabrielli, L., Losa, S., Mantero, M., Caminiti, M., Curci, V., Lupattelli, T., Morabito, A. (2006). Early and five year amputation and survival rate of diabetic patients with critical limb ischemia: data of a cohort study of 564 patients. *European Journal of Vascular and Endovascular Surgery.* 32, 484-490.

Felix-Redondo, F.J., Fernandez-Berges, D., Grau, M., Baena-Diez, J.M., Mostaza, J.M., Vila, J. (2012). Prevalence and clinical characteristics of peripheral arterial disease in the study population Hermex. *Revista Espanola de Cardiologia.* 65, 726-733.

Fife, C.E., Smart, D.R., Sheffield, P.J., Hopf, H.W., Hawkins, G., Clarke, D. (2009). Transcutaneous oximetry in clinical practice: consensus statements from an expert panel based on evidence. *Undersea Hyperbaric Medicine.* 36, 43-53.

Fletcher, G.F., Balady, G.J., Amsterdam, E.A., Chaitman, B., Eckel, R., Fleg, J., Froelicher, V.F., Leon, A.S., Pina, I.L., Rodney, R., Simons-Morton, D.A., Williams, M.A., Bazzarre, T. (2001). Exercise standards for testing and training: A statement for healthcare professionals from the American Heart Association. *Circulation.* 104, 1694-1740.

Fontaine, R., Kim, M., Kieny, R. (1954). Surgical treatment of peripheral circulation disorders. *Hell Chir Acta.* 21, 499-533.

Forrest, K.Y., Becker, D.J., Kuller, I.H., Wolfsbon, S.K., Orchard, T.J. (2000). Are predictors of coronary heart disease and lower extremity arterial disease in type 1 diabetes the same? A prospective study. *Atherosclerosis.* 148, 894-899.

Foto, J.G., Brasseaux, D., Birke, J.A. (2007). Essential features of a hand-held infrared thermometer used to guide the treatment of neuropathic feet. *Journal of American Podiatric Medical Association.* 97 (5), 360-365.

Fowkes, F.G.R., Housley, E., Cawood, E.H.H., MacIntyre, C.C.A., Ruckley, C.V., Prescott, R.J. (1991). Edinburgh Artery Study: Prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *International Journal of Epidemiology.* 20, 384-392.

Fowkes, F.G.R., Rudan, D., Rudan, I., Aboyans, V., Denenberg, J.O., McDermott, M.M., Norman, P., Sampson, U.K.A., Williams, L.J., Mensah, G., Criqui, M.H. (2013). Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet, The.* 382, 1329-1340.

Fredriksson, I., Larsson, M., Strömberg, T. (2009). Measurement depth and volume in laser doppler flowmetry. *Microvascular Research.* 78, 4-13.

Gardner, A.W., Parker, D.E., Webb, N., Montgomery, P.S., Scott, K.J., Blevins, S.M. (2008). Calf muscle hemoglobin oxygen saturation characteristics and exercise performance in patients with intermittent claudication. *Journal of Vascular Surgery.* 48, 644-649.

Gernigon, M., Marchand, J., Ouedraogo, N., Leftheriotis, G., Picquet, J.M., Abraham, P. (2013). Proximal ischemia is a frequent cause of exercise-induced pain in patients with a normal ankle to brachial index at rest. *Pain Physician.* 16, 57-64.

Greenhaf, P.L., Campbell-O'Sullivan, S.P., Constantin-Teodosiu, D., Poucher, S.M., Roberts, P.A., Timmons, J.A. (2004). Metabolic inertia in contracting skeletal muscle: a novel approach for pharmacological intervention in peripheral vascular disease. *Br Journal of Clinical Pharmacology.* 57, 237-243.

Grimes DA, S.K. (2005). Refining clinical diagnosis with likelihood ratios. *Lancet.* 365, 1500-1505.

Hajian-Tilaki, K. (2013). Receiver Operating Characteristic (ROC) curve analysis for Medical Diagnostic Test Evaluation. *Caspian J Intern Med.* 4, 627-635.

Hamel, J.F., Foucaud, D., Fanello, S. (2010). Comparison of the automated oscillometric method with the gold standard doppler ultrasound method to assess the ankle brachial pressure index. *Angiology.* 61, 487-491.

Hardman, R.L., Jazaeri, O., Yi, J., Smith, M., Gupta, R. (2014). Overview of classification systems in peripheral arterial disease. *Seminars in Interventional Radiology.* 31, 378-387.

Hardy, J. (1934). The radiation of heat from the human body: III. The human skin as a black-body radiator. *The Journal of Clinical Investigation.* **13,** 615.

Hauser, C.J., Klein, S.R., Mehringer, C.M., Appel, P., Shoemaker, W.C. (1984). Assessment of perfusion in the diabetic foot by regional transcutaneous oximetry. *Diabetes.* 33, 527-531.

Hauser, C.J. & Shoemaker, W.C. (1983). Use of transcutaneous PO2 regional perfusion index to quantify tissue perfusion in peripheral vascular disease. *Annals of Surgery.* 197, 337-343.

Hayward, J.S., Eckerson, J.D., Kemna, D. (1984). Thermal and cardiovascular changes during three methods of resuscitation from mild hypothermia. *Resuscitation.* 11, 21-33.

He, Y., Jiang, Y., Wang, J., Fan, L., Li, X., Hu, F.B. (2006). Prevalence of peripheral arterial disease and its association with smoking in a population based study in Beijing, China. *Journal of Vascular Surgery.* 44, 333-338.

Hesford, C.M., Laing, S.J., Cardinale, M., Cooper, C.E. (2012). Asymmetry of quadriceps muscle oxygenation during elite short-track speed skating. *Medicine and Science in Sports and Exercise.* 44, 501-508.

Hiatt, W.R., Nawaz, D., Regensteiner, J.G., Hossack, K.F. (1988). The evaluation of exercise performance in patients with peripheral vascular disease. *Journal of Cardiopulmonary Rehabilitation.* 12, 525-532.

Hiatt, W.R., Rogers, R.K., Brass, E.P. (2014). The treadmill is a better functional test than the 6-minute walk test in therapeutic trials of patients with peripheral artery disease. *Circulation.* 130, 69-78.

Hii, P.C. & Chung, W.Y. (2011). A comprehensive ubiquitous healthcare solution on an Android™ mobile device. *Sensors.* 11, 6799-6815.

Hills, A.J., Shalhoub, J., Shepherd, A.C., (2009). Peripheral arterial disease. *British Journal of Hospital Medicine.* 344, 565.

Hirsch, A.T., Criqui, M.H., Treat-Jacobson, D., Regensteiner, J.G., Creager, M.A., Olin, J.W., Krook, S.H., Hiatt, W.R., Comerota, A.J., Walsh, M.E., McDermott, M.M., Hiatt, W.R. (2001). Peripheral arterial disease detection, awareness and treatment in primary care. *Jama.* 286, 1317-1324.

Hoffmann, M.J., Knudson, P.E., Silver-Thorn, M.B. (2008). A device for noninvasive assessment of vascular impairment risk in the lower extremity. *IEEE Transactions on Biomedical Engineering.* 55, 2786-2791.

Hooi, J.D., Kester, A.D., Stoffers, H.E., Overdijk, M.M., Van Ree, J.W., Knottnerus, J.A. (2001). Incidence of and risk factors for asymptomatic peripheral arterial occlusive disease: a longitudinal study. *American Journal of Epidemiology.* 153, 666-672.

Houghton, V.J., Bower, V.M., Chant, D.C. (2013). Is an increase in skin temperature predictive of neuropathic foot ulceration in people with diabetes? A systematic review and meta-analysis. *Journal of Foot and Ankle Research.* 6(31),1-14.

Hoyer, C., Sandermann, J., Paludan, J.P.D., Pavar, S., Petersen, L.J. (2013). Diagnostic accuracy of laser Doppler flowmetry versus strain gauge plethysmography for segmental pressure measurement. *Journal of Vascular Surgery.* 58, 1563-1570.

Høyer, C., Sandermann, J., Petersen, L.J. (2013). The toe-brachial index in the diagnosis of peripheral arterial disease. *Journal of Vascular Surgery.* 58, 231-238.

Huang, C.C., Lee, P.Y., Chen, P.Y., Liu, T.Y. (2012). Design and implementation of a smartphone-based portable ultrasound pulsed-wave Doppler device for blood flow measurement. *IEEE Trans Ultrasonic Ferroelectrics and Frequency Control.* 59, 182-188.

Huang, C.L., Wu, Y.W., Hwang, C.L., Jong, Y.S., Chao, C.L., Chen, W.J., Wu, Y.T., Yang, W.S. (2011). The application of infrared thermography in evaluation of patients at high risk for lower extremity peripheral arterial disease. *Journal of Vascular Surgery.* 54, 1074-1080.

Hunink, M.G. & Krestin, G.P. (2002). Study design for concurrent development, assessment, and implementation of new diagnostic imaging technology. *Radiology.* 222, 604-614.

Ihlberg, L.H.M., Mätzke, S., Albäck, N.A., Roth, W.D., Sovijärvi, A.R.A., Lepäntalo, M. (2001). Transfer Function Index of pulse volume recordings: A new method for vein graft surveillance. *Journal of Vascular Surgery.* 33, 546-553.

Jager, A., Kostense, P.J., Ruhé, H.G., Heine, R.J., Nijpels, G., Dekker, J.M. (1999). Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: Five-year follow-up of the Hoorn Study. *Arteriosclerosis Thrombosis and Vascular Biology.* 19, 617-624.

Joensen, J.B., Juul, S., Abrahamsen, J., Henneberg, E.W., Lindholt, J.S. (2008). Doppler ultrasound with strain gauge for measurement of systolic ankle blood pressure. *Angiology.* 59, 296-300.

Johnson, J.M., Pergola, P.M., Liao, F.K., Kellogg, D.L.J., Crandall, C.G. (1995). Skin of the dorsal aspect of human hands and fingers posseses an active vasodilator system. *Journal of Applied Physiology.* 78, 948-954.

Jones, B.F. (1998). A reappraisal of the use of infrared thermal image analysis in medicine. *IEEE Transactions on Medical Imaging.* 17, 1019-1027.

Joosten, M.M., Pai, J.K., Bertoia, M.L., Rimm, E.B., Spiegelman, D., Mittleman, M.A., Mukamal, K.J. (2012). Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. *Journal of the American Medical Association.* 308, 1660-1667.

Jorfeldt, L., Vedung, T., Forsstrom, E., Henriksson, J. (2003). Influence of leg position and environmental temperature on segmental volume expansion during venous occlusion plethysmography. *Clinical Science (London).* 104, 599-605.

Joyce, W.P., Walsh, K., Gough, D.B., Gorey, T.F., Fitzpatrick, J.M. (1990). Pulse oximetry: a new non-invasive assessment of peripheral arterial occlusive disease. *British Journal of Surgery.* 77, 1115-1117.

Jude, E.B., Oyibo, S.O., C., N., Boulton, A.J. (2001). Peripheral arterial disease in diabetic and non-diabetic patients: A comparison of severity and outcome. *Diabetes Care.* 24, 1433-1437.

Kallio, M., Forsblom, C., Groop, P.H., Groop, L., Lepäntalo, M. (2003). Development of new peripheral arterial occlusive disease in patients with Type 2 diabetes during a mean follow up of 11 years. *Diabetes Care.* 26, 1241-1245.

Katsilambros, N.L., Tsapogas, P.C., Arvanitis, M.P., T., N.A., Alexiou, Z.P., Rigas, K.L. (1996). Risk factors for lower extremity arterial disease in non-insulin dependent diabetic persons. *Diabetic Medicine.* 13, 2432-246.

Kelechi, T.J., Michel, Y., Wiseman, J. (2006). Are Infrared and Thermistor Thermometers interchangeable for measuring localized skin temperature? *Journal of Nursing Measurement.* 14, 19-30.

Khan, T., Farooqui, F., Niazi, K. (2008). Critical review of the ankle brachial index. *Current Cardiology Reviews.* 4, 101-106.

Khandanpour, N., Armon, M.P., Jennings, B., Clark, A., Meyer, F.J. (2009a). The association between ankle brachial pressure index and pulse wave velocity: Clinical implication of pulse wave velocity. *Angiology.* 60, 732-738.

Khandanpour, N., Armon, M.P., Jennings, B., Clark, A., Meyer, F.J. (2009b). Photoplethysmography, an easy and accurate method for measuring ankle brachial pressure index. Can photoplethysmography replace doppler? *Vascular and Endovascular Surgery.* 43, 578-582.

Khaodhiar, L., Dinh, T., Schomacker, K.T., Panasyuk, S.V., Freeman, J.E., Lew, R., Vo, T., Panasyuk, A.A., Lima, C., Giurini, J.M., Lyons, T.E., Veves, A. (2007). The Use of medical hyperspectral technology to evaluate microcirculatory changes in diabetic foot ulcers and to predict clinical outcomes. *Diabetes Care.* 30, 903-910.

Khurana, A., Stoner, J.A., Whitsett, T.L., Rathbun, S., Montgomery, P.S., Gardner, A.W. (2013). Clinical significance of ankle systolic blood pressure following exercise in assessing calf muscle in tissue ischemia in Peripheral Arterial Disease. *Angiology.* 64, 364-370.

Kim, H.Y. (2013). Statistical notes for clinical researchers: Evaluation of measurement error 2: Dahlberg's error, Bland-Altman method and Kappa coefficient. *Restorative Dentistry and Endodontics.* 182-185.

Kirwan, M., Duncan, M.J., Vandelanotte, C., Mummery, W.K. (2012). Using Smartphone technology to monitor physical activity in the 10,000 steps program: A matched case–control trial. *Journal of Medical Internet Research.* 14, e55.

Klomp, H.M., Wittens, C.H.A., van Urk, H. (2000). Transcutaneous oximetry, laser doppler fluxmetry, and capillary microscopy: Variability in patients with advanced atherosclerotic disease of the lower extremity. *Vascular and Endovascular Surgery.* 34, 234-243.

Komiyama, T., Onozuka, A., Miyata, T., Shigematsu, H. (2002). Oxygen Saturation Measurement of Calf Muscle During Exercise in Intermittent Claudication. *European Journal of Vascular and Endovascular Surgery.* 23, 388-392.

Koo, T.K. & Li, M.Y. (2016). A guideline of selecting and reporting Intraclass correlation coefficients for reliability research. *Journal of Chiropractic Medicine.* 15, 155-163.

Kooijman, H.M., Hopman, M.T.E., Colier, W. N. J. M., van der Vliet, J. A., Oeseburg, B. (1997a). Near Infrared Spectroscopy for non-invasive assessment of claudication. *Journal of Surgical Research.* 72, 1-7.

Korevaar, D.A., Wang, J., van Enst, W.A., Leeflang, M.M., Hooft, L., Smidt, N., Bossuyt, P.M. (2015). Reporting diagnostic accuracy studies: some improvements after 10 years of STARD. *Radiology.* 274, 781-789.

Kottner, J., Audigé, L., Brorson, S., Donner, A., Gajewski, B.J., Hróbjartsson, A., Roberts, C., Choukri, M., Streiner, D.L. (2011). Guidelines for reporting reliability

and agreement studies (GRRAS) were proposed. *Journal of Clinical Epidemiology.* 64, 96-106.

Kragelj, R., Jarm, T., Erjavec, T., Prestern-Strukelj, M., Miklavcic, D. (2001). Parameters of postocclusive reactive hyperemia measured by Near infrared spectroscopy in patients with peripheral vascular disease and in healthy volunteers. *Annals of Biomedical Engineering.* 29, 311-320.

Krause, D., Burghaus, I., Thiem, U., Trampisch, U.S., Trampisch, M., Klaassen-Mielke, R., Trampisch, H., Diehm, C., Rudolf, H. (2016). The risk of peripheral artery disease in older adults - seven-year results of the getABI study. *VASA. Zeitschrift Fur Gefasskrankheiten.* 45, 403-410.

Kumar, M.S., Lohiya, A., Ramesh, V., Behera, P., Palepu, S., Rizwan, S.A. (2016). Sensitivity and specificity of pulse oximetry and ankle-brachial index for screening asymptomatic peripheral vascular diseases in Type 2 Diabetes mellitus. *The Journal of the Association of Physicians of India.* 64, 38.

Kwon, J.N. & Lee, W.B. (2012). Utility of digital pulse oximetry in the screening of lower extremity arterial disease. 82, 94-100.

Laing, S.P. & Greenhalgh, R.M. (1980). Standard exercise test to assess peripheral arterial disease. *British Medical Journal.* 13-16.

Lamah, M., Mortimer, P.S., Dormandy, J.A. (1999). Quantitative study of capillary density in the skin of the foot in peripheral vascular disease. *British Journal of Surgery.* 86, 342-348.

Landis JR, K.G. (1977). The measurement of observer agreement for categorical data. *Biometrics.* 33, 159-174.

Lavery, L.A., Higgins, K.R., Lanctot, D.R., Constantinides, G.P., Zamorano, R.G., Armstrong, D.G., Athanasiou, K.A., Agrawal, C.M. (2004). Home monitoring of foot skin temperatures to prevent ulceration. *Diabetes Care.* 27, 2642-2647.

Lavery, L.A., Higgins, K.R., Lanctot, D.R., Constantinides, G.P., Zamorano, R.G., Athanasiou, K.A., Armstrong, D.G., Agrawal, C.M. (2007). Preventing diabetic foot ulcer recurrence in high-risk patients: use of temperature monitoring as a self-assessment tool. *Diabetes Care.* 30, 14-20.

Lawson, R.N. (1956). Implications of surface temperatures in the diagnosis of breast cancers. *Canadian Medical Association Journal.* 75, 309-310.

Leeflang, M.M.G., Deeks, J.J., Gastonia, C., Bossuyt, P.M.M. (2008). Systematic reviews of diagnostic test accuracy. *Annals of Internal Medicine.* 149, 889-897.

Leskinen, Y., Salenius, J.P., Lehtimaki, T., Huhtala, H., Saha, H. (2002). The prevalence of peripheral arterial disease and medial arterial calcification in patients with chronic renal failure: requirements for diagnostics. *American Journal of Kidney Diseases.* 40, 472-479.

Li, X., Wang, Y.Z., Yang, X.P., Xu, Z.R. (2012). Prevalence of and risk factors for abnormal ankle brachial index in patients with type 2 diabetes. *Journal of Diabetes.* 4, 140-146.

Lucas, N., Macaskill, P., Irwin, L., Moran, R., Rickards, L., Turner, R., Bogduk, N. (2013). The reliability of a quality appraisal tool for studies of diagnostic reliability (QAREL). *BMC Medical Research Methodology.* 13, 111.

Malagoni, A.M., Felisatti, M., Mandini, S., Mascoli, F., Manfredini, R., Basaglia, N., Zamboni, P., Manfredini, F. (2010). Resting muscle oxygen consumption by near-infrared spectroscopy in peripheral arterial disease: A parameter to be considered in a clinical setting? *Angiology.* 1, 530-536.

Maldague, X.P. (2001). *Theory and Practice of Infrared Technology for Nondestructive Testing.* First ed. New York: John Wiley and Sons.

Manfredini, F., Malagoni, A.M., Felisatti, M., Mandini, S., Mascoli, F., Manfredini, R., Basaglia, N., Zamboni, P. (2009). A dynamic objective evaluation of peripheral arterial disease by near infrared spectroscopy. *European Journal of Vascular and Endovascular Surgery.* 38, 441-448.

Manfredini, F., Malagoni, A.M., Mandini, S., Felisatti, M., Mascoli, F., Basaglia, N., Manfredini, R., Mikhailidis, D.P., Zamboni, P. (2012). Near-infrared spectroscopy assessment following exercise training in patients with intermittent claudication and in untrained healthy participants. *Vascular and Endovascular Surgery.* 46, 315-324.

Manfredini, F., Malagoni, A.M., Manfredini, R. (2013). Ankle brachial pressure index: faulty or overused. *Angiology.* 64, 89.

McAlinden, C., Khadka, J., Pesudovs, K. (2011). Statistical methods for conducting agreement (comparison of clinical tests) and precision (repeatability or reproducibility) studies in optometry and opthalmology. *Ophthalmic and Physiological Optics.* 31, 330-338.

McCarthy, P.W. & Heusch, A.I. (2006). The vagaries of ear temperature assessment. *Journal of Medical Engineering and Technology.* 30, 242-251.

McCully, K.K., Landsberg, L., Suarez, M., Hofmann, M., Posner, J.D. (1997). Identification of peripheral vascular disease in elderly subjects using optical spectroscopy. *Journal of Gerontology.* 52a, B165.

McLay, K.M., Nederveen, J.P., Pogliahi, S., Paterson, D.H., Murias, J.M. (2016). Repeatability of vascular responsiveness measures derived from near-infrared spectroscopy. *Physical Reports.* 4(9), pii:e12772.

McPhail, I.R., Spittle, P.C., Weston, S.A., Bailey, K.R. (2001). Intermittent claudication: An objective office-based assessment. *Journal of American College of Cardiology.* 37, 1381-1385.

Meier, K. (2007). Statistical guidance on reporting results from studies evaluating diagnostic tests: guidance for industry and FDA Staff. *Food and Drug Administration.* 1-39.

Meijer, W.T., Grobbee, D.E., Hunink, M.G., Hofman, A., Hoes, A.W. (2000). Determinants of peripheral arterial disease in the elderly: The Rotterdam study. *Archives of Internal Medicine.* 160, 2934-2938.

Meijer, W.T., Hoes, A.W., Rutgers, D., Bots, M.L., Hofman, A., Grebbe, D.E. (1998). Peripheral arterial disease in the elderly: The Rotterdam study. *Arteriosclerosis Thrombosis and Vascular Biology.* 18, 185-192.

Mesquita, R.C., Putt. M., Chandra, M., Yu, G., Xing, X., Han, S.W., Lech, G., Shang, Y., Durduran, T., Zhou, Mohler III, E.R. (2013). Diffuse optical characterization of an exercising patient group with peripheral arterial disease. *Journal of Biomedical Optics.* 18, 1-11.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. (2009). Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Journal of Clinical Epidemiology.* 62, 1006-1012.

Mohler III, E.R., Treat-Jacobson, D., Reilly, M.P., Cunningham, K.E., Miani, M., Criqui, M.H., Hiatt, W.R., Hirsch, A.T. (2004). Utility and barriers to performance of the ankle-brachial index in primary care practice. *Vascular Medicine.* 9, 253-260.

Mohler, E.R., Bundens, W., Denenberg, J., Medelilla, E., Hiatt, W.R., Chriqui, M.H., (2012). Progression of asymptomatic peripheral artery disease over 1 year. *Vascular Medicine.* 17, 10-16.

Monteiro, D.P., Britto, R.R., Lages, A.C.R., Basillo, M.L., Pires, M.C.O., Carvalho, M.L.V., Procópio, R.J., Pereira, D.A.G. (2013). Heel-rise test in the assessment of individuals with peripheral arterial occlusive disease. *Vascular Health and Risk Management.* 9, 29-35.

Montgomery, P.S. & Gardner, A.W. (1998). The clinical utility of a six-minute walk test in peripheral arterial occlusive disease patients. *Journal of American Geriatric Society.* 46, 706-711.

Morales, F., Graaff, R., Smit, A.J., Bertuglia, S., Petoukhova, A.L., Steenbergen, W., Leger, P., Rakhorst, G. (2005). How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: application of a standardised protocol to patients with peripheral arterial obstructive disease. *Microvascular Research.* 69, 17-23.

Morris, S., Tang, M., Geddes, C.R. (2006). Vascular anatomical basis of perforator skin flaps. *Cirugía Plástica Ibero-Latinoamerican.* 32, 1-5.

Moyer, V.A. (2013). Screening for peripheral artery disease and cardiovascular disease risk assessment with the ankle-brachial index in adults: U.S. Preventive

Service Task Force Recommendation Statement. *Annals of Internal Medicine.* 159, 342-348.

Miukherjee, D. & Eagle, K. (2010). The importance of early diagnosis and treatment in peripheral arterial disease: insights from the PARTNERS and REACH registries. *Current Vascular Pharmacology.* 8, 293-300.

Murabito, J.M., D'Agotino, R.B., Silbershatz, H., Wilson, W.F. (1997). Intermittent claudication. A risk profile from The Framingham Heart Study. *Circulation.* 96, 44-49.

Murabito, J.M., Evans, J.C., Nieto, K., Larson, M.G., Levy, D., Wilson, P.W. (2002). Prevalence and clinical correlates of peripheral arterial disease in the Framingham Offspring Study. *American Heart Journal.* 143, 961-965.

Naaktgeboren, C.A., de Groot, J. A. H., Rutjes, A.W.S., Bossuyt, P.M.M., Reitsma, J.B., Moons, K.G.M. (2016). Anticipating missing reference standard data when planning diagnostic accuracy studies. *British Medical Journal.* 352, i402.

Nakashima, R., Inoue, Y., Sugano, N., Jibiki, M., Kudo, T., Iwa, T. (2009). Efficacy of the transfer function index using the pulse volume recording as a parameter to assess intermittent claudication. *Surgery Today.* 39, 658-662.

Natarajan, S., Glick, H., Criqui, M., Horowitz, D., Lipsitz, S.R., Kinsman, B. (2003). Cholesterol measures to identify and treat individuals at risk for coronary heart disease. *American Journal of Preventative Medicine.* 25, 50-57.

Nehler, M.R., Duval, S., Diao, L., Annex, B.H., Hiatt, W.R., Rogers, K., Zakharyan, A., Hirsch, A.T. (2014). Epidemiology of peripheral arterial disease and critical limb ischemia in an insured national population. *Journal of Vascular Surgery.* 60, 686-695.

Newman, A.B., Siscovick, D.S., Manolo, T.A., Polak, J., Fried, L.P., Bohrani, N.O., Wolfsbon, S.K. (1993). Ankle-arm index as a marker of atherosclerosis in the Cardiovascular Health Study: Cardiovascular Heart Study (CHS) Collaborative Research Group. *Circulation.* 88, 837-845.

Ng, D.K., Chan, C.H., Chan, E.Y.T., Kwok, K.L., Chow, P.Y., Lau, W.P. (2005). A brief report on the normal range of forehead temperature as determined by noncontact, handheld, infrared thermometer. *American Journal of Infection Control.* 33, 227-229.

NICE CG 147, (2012). Lower limb peripheral arterial disease: Primary care (clinical audit tool: Implementing NICE Guidance). CG 147. National Institute of Health and Clinical Excellence.

Nkengne, A., Papillon, A., Bertin, C. (2013). Evaluation of the cellulite using a thermal infra-red camera. *Skin Research and Technology.* 19, e231–e237.

Norgren, L., Hiatt, W.R., Dormandy, J.A., Nehler, M.R., Harris, K.A., Fowkes, F.G.R. (2007). Inter-society consensus for the management of peripheral arterial disease (TASC II). *Journal of Vascular Surgery.* 45, s67.

O'Doherty, J., McNamara, P., Clancy, N.T., Enfield, J.G., Leahy, M.J. (2009). Comparison of instruments for investigation of microcirculatory blood flow and red blood cell concentration. *Journal of Biomedical Optics.* 14, 034025.

O'Hare, O.M., Vittinghoff, E., Hsia, J., Shilpak, M.G. (2004). Renal insufficiency and the risk of lower extremity peripheral arterial disease: results from the Heart and Estrogen/Progestin replacement study (HERS). *Journal of the American Society of Nephrology.* 15, 1046-1051.

Oresko, J.J., Duschl, H., Cheng, A.C. (2010). A wearable smartphone-based platform for real-time cardiovascular disease detection via electrocardiogram processing. *IEEE Transactions on Information Technology in Biomedicine.* 14, 734-740.

Ouwendijk, R., de Vries, M., Stijnen, T., Pattynama, P.M.T., van Sambeek, M. R. H. M., Buth, J., Tielbeek, A.V., van der Vliet, D. A., Hunink, M.G., Kitslaar, P. J. E. H., de Haan, M.W., van Engelshoven, J. M. A., Hunink, M.G.M. (2008). Multicenter randomized controlled trial of the costs and effects of noninvasive diagnostic imaging in patients with peripheral arterial disease: The DIPAD trial. *American Journal of Radiology.* 190, 1349-1357.

Papanas, N., Kakagia, D., Papatheodorou, K., Papazoglou, D., Alexandridou, M., Pagkalos, A., Karadimas, E.J., Maltezos, E. (2010). Lanarkshire oximetry index as a diagnostic tool for peripheral arterial disease in Type 2 Diabetes: A pilot study. *Angiology.* 61, 388-391.

Parameswaran, G.I., Brand, K., Dolan, J. (2005). Pulse oximetry as a potential screening tool for lower extremity arterial disease in asymptomatic patients with diabetes mellitus. *Archives of Internal Medicine.* 165, 442-446.

Philips Medical Systems SpO_2 Monitoring (2002). SpO2Booklet.book Page 1.

Potier, L., Abi Khalil, C., Mohammedi, K., Roussel, R. (2011). Use and utility of ankle brachial index in patients with diabetes. *European Journal of Vascular and Endovascular Surgery.* 41, 110-116.

Premalatha, G., Ravikumar, R., Sanjay, R., Deepa, R., Mohan, V. (2002). Comparison of colour duplex ultrasound and ankle brachial pressure index measurement in peripheral vascular disease in type 2 diabetic patients with foot infections. *Journal of the Association of Physicians of India.* 50, 1240-1244.

Premanath, M. & Raghunath, M. (2010). Ankle brachial index by oscillometry: A very useful method to assess peripheral arterial disease in diabetes. *International Journal of Diabetes in Developing Countries.* 30, 97-101.

Preston, T.H. (1990). The International Temperature Scale of 1990 (ITS-90). *Metrologia.* 27, 3-10.

Puissant, C., Abraham, P., Durand, S., Humeau-Heurtier, A., Faure, S., Leftheriotis, G., Rousseau, P., Mahe, G. (2013). Reproducibility of Non-Invasive assessment of skin endothelial function using Laser Doppler Flowmetry and Laser Speckle Contrast Imaging. *PLoS One.* 8, e61320.

Pulse Oximetry and Training Manual, (2011). World Health Organisation. 1-14.

Ray, S.A., Buckenham, T.M., Belli, A.M., Taylor, R.S., Dormandy, J.A. (1999). The association between laser doppler reactive hyperaemia curves and the distribution of peripheral arterial disease. *European Journal of Vascular and Endovascular Surgery.* 17, 245-248.

Richards, L.M., Shams Kazmi, S.M., Davis, J.L., Olin, K.E., Dunn, A.K. (2013). Low-cost laser speckle contrast imaging of blood flow using a webcam. *Biomedical Optics Express.* 4, 2269-2283.

Ritti-Dias, R.M., Basyches, M., Câmara, L., Puech-Leao, P., Battistella, L., Wolosker, N. (2010). Test-retest reliability of isokinetic strength and endurance tests in patients with intermittent claudication. *Vascular Medicine.* 15, 275-278.

Ro, H.D., Moon, H.J., Kim, J.H., Lee, K.M., Kim, S.J., Lee, D.Y. (2013). Photoplethysmography and continuous wave doppler ultrasound as a complimentary test of ankle brachial index in the detection of stenotic peripheral arterial disease. *Angiology.* 64, 314-320.

Roback, K. (2010). An overview of temperature monitoring devices for early detection of diabetic foot disorders. *Expert Review of Medical Devices.* 7, 711.

Robinson, J.L., Seal, R.F., Spady, D.W., Joffres, M.R. (1998). Comparison of esophageal, rectal, axillary, bladder, tympanic, and pulmonary artery temperatures in children. *Journal of Pediatrics.* 133, 553–556.

Rooke, T.W., Hirsch, A.T., Misra, S., Beckman, J.A., Findeiss, L.K., Golzarian, J., Gornik, H.L., Halperin, J.L., Jaff, M.R., Moneta, G.L., Olin, J.W., Stanley, J.C., White, C.J., White, J.V., Zierler, R.E. (2011). 2011 ACCF/AHA Focused update of the guideline for the management of patients with peripheral artery disease (Updating the 2005 guideline): A report of the American College of Cardiology Foundation/American Heart Association Task Force on practical guidelines. *Journal of the American College of Cardiology.* 58, 2020-2045.

Rose, G.A. (1962). The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. *Bulletin World Health Organization.* 27**,** 645-658.

Roustit, M. & Cracowski, J. (2013). Assessment of endothelial and neurovascular function in human skin microcirculation. *Trends in Pharmacological Sciences.* 34, 373-384.

Roustit, M. & Cracowski, J.L. (2012). Non-invasive assessment of skin microvascular function in humans: An insight into methods. *Microcirculation.* 19, 47-64.

Ruopp, M.D., Perkins, N.J., Whitcomb, B.W., Schisterman, E.F. (2008). Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. *Biomedical Journal.* 50, 419-430.

Rusby, R. (2012). Good Practice Guide no. 125: The beginner's guide to temperature measurement. *National Physical Laboratory.*

Rutherford, D.B., Baker, J.D., Ernst, C., Johnston, K.W., Porter, J.M., Ahn, S., Jones, D.N. (1997). Recommended standards for reports deadline with lower extremity ischemia: revised version. *Journal of Vascular Surgery.* 26, 517.

Saad, A.R., Stephens, D.P., Bennett, L.T., Charkoudian, N., Kosiba, W.A., Johnson, J.M. (2001). Influence of isometric exercise on blood flow and sweating in glabrous and nonglabrous human skin. *Journal of Applied Physiology.* 91, 2487-2493.

Schirmang, T.C., Ahn, S.H., Murphy, T.P. (2009). Peripheral arterial disease: update of overview and treatment. *Medicine & Health.* 92, 398-402.

Schmidt, R.L. & Factor, R.E. (2013). Understanding sources of bias in diagnostic accuracy studies. *Archives of Pathology and Laboratory Medicine.* 137, 558-565.

Schroder, F., Diehm, N., Kareem, S., Ames, M., Pira, A., Zwettler, U., Lawall, H., Diehm, C. (2006). A modified calculation of ankle-brachial pressure index is far more sensitive in the detection of peripheral arterial disease. *Journal of Vascular Surgery.* 44, 531-536.

Shang, Y., Zhao, Y., Cheng, R., Dong, L., Irwin, D., Yu, G. (2009). Portable optical tissue flow oximeter based on diffuse correlation spectroscopy. *Journal of Biomedical Optics.* 34, 3556-3558.

Sicari, R., Galderisi, M., Voigt, J.U., Habib, G., Zamorano, J.L., Lancellotti, P., Badano, L.P. (2011). The use of pocket-size imaging devices: a position statement of the European Association of Echocardiography. *European Journal of Echocardiography.* 12, 85-87.

Smith, A.D., Crabtree, D.R., Bizon, J.L.J., Walsh, N.P. (2010). The validity of wireless iButtons (R) and thermistors for human skin temperature measurement. *Physiological Measurement.* 31, 95-114.

Solway, S., Brooks, D., Lacasse, Y., Thomas, S. (2001). A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory Domain. *Chest.* 119, 256-270.

Southern, W.M., Ryan, T.E., Reynolds, M.A., McCully, K. (2013). Reproducibility of near-infrared spectroscopy measurements of oxidative function and post- exercise

recovery kinetics in the medial gastrocnemius. *Applied Physiology Nutrition and Metabolism*. 39(5), 521-529.

Steketee, J. (1973). Spectral emissivity of the skin and pericardium. *Physics in Medicine & Biology.* 18, 686-694.

Stoffers, H. E. J. H., Rinkens, P. E. L. M, Kester, A.D.M., Kaiser, V., Knottnerus, J.A. (1996). The prevalence of asymptomatic and unrecognized peripheral arterial occlusive disease. *International Journal of Epidemiology.* 25, 282-290.

Stucker, M., Baier, V., Reuther, T., Hoffmann, K., Kellam, K., Altmeyer, P. (1996). Capillary blood cell velocity in human skin capillaries located perpendicularly to the skin surface: measured by a new laser doppler anemometer. *Microvascular Research.* 52, 188-192.

Stucker, M., Huntermann, C., Bechara, F.G., Hoffmann, K., Altmeyer, P. (2004). Capillary blood cell velocity in periulcerous regions of the lower leg measured by laser Doppler anemometry. *Skin Research and Technology.* 10, 174-177.

Sugano, N., Inoue, Y., Iwai, T. (2003). Evaluation of buttock claudication with hypogastric artery stump pressure measurement and near infrared spectroscopy after abdominal aortic aneurysm repair. *European Journal of Vascular and Endovascular Surgery.* 26, 45-51.

Suleman, M.I., Doufas, A.G., Akca, O., Ducharme, M., Sessier, D.I. (2002). Insufficiency in a new temporal-artery thermometer for adult and paediatric patients. *Anaesthesia and Analgesia.* 95, 67-71.

Svalestad, J., Hellem, S., Vaagbo, G., Irgens, A., Thorsen, E. (2010). Reproducibility of transcutaneous oximetry and Laser Doppler Flowmetry in facial skin and gingival tissue. *Microvascular Research.* 79, 29-33.

Tan, C.P. & Civil, I. (2003). Transfer Function Index: Is it a reliable method for vein graft surveillance? *ANZ Journal of Surgery.* 73, 1032-1035.

Tapuria, N., Kumar, Y., Habib, M.M., Amara, M.A., Seifalian, A.M., Davidson, B.R. (2007). Remote ischemic preconditioning: A novel protective methods from ischemia reperfusion injury - A Review. *Journal of Surgical Research.* 150, 304-330.

Teran, C.G., Torrez-Llanos, J., Teran-Miranda, T.E., Balderrama, C., Shah, N.S., Villarroel, P. (2011). Clinical accuracy of non-contact infrared skin thermometer in paediatric practice. *Child Care, Health and Development.* 38, 471-476.

Tseng, C.H. (2003). Prevalence and risk factors of peripheral arterial obstructive disease in Taiwanese type 2 diabetic patients. *Angiology.* 54, 331-338.

Ubbink, D.T. (2004). Toe blood pressure measurements in patients suspected in leg ischaemia: a new laser doppler device compared with photo plethysmography. *European Journal of Vascular and Endovascular Surgery.* 27, 629-634.

Ubbink, D.T., Tulevski, I.I., den Hartog, D., Koelemay, M.J.W., Legemate, D.A., Jacobs, M. J. H. M. (1997). The value of non-invasive techniques for the assessment of critical limb ischaemia. *European Journal of Vascular and Endovascular Surgery.* 13, 296-300.

van der Zwaard, S., Jaspers, R.T., Blokland, I.J., Achterberg, C., Visser, J.M., den Uil, A.R., Hofmijster, M.J., Levels, K., Noordhof, D.A., de Haan, A., deKoning, J.J., ven der Laarse, W. J., de Ruiter, C.J. (2016). Oxygenation threshold derived from Near-Infrared Spectroscopy: Reliability and its relationship with the first ventilatory threshold. 11, e0162914.

Venus, M. Waterman, J., McNab, I. (2011). Basic physiology of the skin. *Surgery.* 29, 471-474.

Verdi, M. & Nini, A. (2008). Near Infrared spectroscopy for evaluation of peripheral vascular disease. A systematic review of literature. *European Journal of Vascular and Endovascular Surgery.* 35, 65-74.

Viera, A.J. & Garrett, J.M. (2005). Understanding interobserver agreement: The Kappa Statistic. *Family Medicine.* 37, 360-363.

Walløe, L. (2015). Arterio-venous anastomoses in the human skin and their role in temperature control. *Temperature.* 3, 92-103.

Watanabe, T., Matsushita, M., Nishikimi, N., Sakurai, T., Komori, K., Nimura, Y. (2004). Near infrared spectroscopy with treadmill exercise to access lower limb ischemia in patients with atherosclerotic occlusive disease. *Surgery Today.* 34, 849-854.

Wennberg, P.W. (2013). Approach to the patient with peripheral arterial disease. *Circulation.* 128, 2241-2250.

Whiting, P.F., Rutjes, A.W.S., Westwood, M.E., Mallett, S., Deeks, J.J., Reitsma, J.B., Leeflang, M.M.G., Sterne, J.A.C., Bossuyt, P.M.M. (2011). QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. *Annals of Internal Medicine.* 155, 529-536.

Whiting, P., Rutjes, A.W.S., Reitsma, J.B., Bossuyt, P.M.M., Kleijnen, J. (2003). The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Medical Research Methodology.* 3, 1-13.

Wikstrom, J., Hansen, T., Johansson, L., Lind, L.A., H. (2008). Ankle brachial index *Acta Radiologica.* 49, 143-149.

Wilczynski, N.L. (2008). Quality of reporting diagnostic accuracy studies: No change since STARD statement publication - Before and after study. *Radiology.* 248, 817-823.

Williams, D.T., Harding, K.G., Price, P. (2005). An evaluation of the efficacy of methods used in screening for lower limb arterial disease in diabetes. *Diabetes Care.* 28, 2206-2210.

Wong, J.J. & Wong, C.Y. (2006). Non-contact infrared thermal imagers for mass fever screening: State of the art or myth? *Hong Kong Medical Journal.* 12, 242– 244.

Woods, C.A., Dumbleton, K., Jones, L., Fonn, D. (2011). Patient use of smartphones to communicate subjective data in clinical trials. *Optometry and Vision Science.* 88, 290-294.

Worringham, C., Rojek, A., Stewart, I. (2011). Development and feasibility of a smartphone, ECG and GPS based system for remotely monitoring exercise in cardiac rehabilitation. *PLoS One.* 6, e14669.

Wu, K.J., Gregory, T.S., Reader, C., Leitmann, B., Huffinis, A., Donovan, S., Mosteller, L., Murrow, J., Tse, Z.T.H. (2016). Smartphone enabled flow monitoring device for peripheral artery disease. *Journal of Medical Devices.* 10, 020958.

Yamada, M., Aoyama, T., Okamoto, K., Nagai, K., Tanaka, B., Takemura, T. (2011). Using a smartphone while walking: a measure of dual-tasking ability as a falls risk assessment tool. *Age Ageing.* 40, 516-519.

Yamamoto, K., Miyata, T., Onozuka, A., Toyama, H., Ohtsu, H., Nagawa, H. (2009). Plantar flexion as an alternative to treadmill exercise for evaluating patients with intermittent claudication. *European Journal of Vascular and Endovascular Surgery.* 33, 325-329.

Yap Kannan, R., Dattani, N., Sayers, R.D., Bown, M.J. (2016). Survey of ankle-brachial pressure index use and its perceived barriers by general practitioners in the UK. *Postgraduate Medical Journal.* 92, 322-327.

Yap Kannan, R., Kerestzes, K., Hussain, S., Coats, T.J., Bown, M.J. (2015). Infrared cameras are potential traceable 'fixed points' for future thermometry studies. *Journal of Medical Engineering & Technology.* 39, 485-489.

Yezvlin, A.S. & Gemelli, G. (2012). Diagnosis and treatment of peripheral arterial disease in CKD patients. *Seminars in Dialysis.* 26, 240-251.

Yu, G. (2012). Diffuse Correlation Spectroscopy (DCS): A diagnostic tool for assessing tissue blood flow in vascular-related diseases and therapies. *Current Medical Imaging Reviews.* 8, 194-210.

Yu, G., Durduran, T., Lech, G., Zhou, C., Chance, B., Mohler, E.R. (2005). Time-dependent blood flow and oxygenation in human skeletal muscles measured with non-invasive near-infrared diffuse optical spectroscopies. *Journal of Biomedical Optics.* 10, 024027.

Yu, G., Shang, Y., Zhao, Y., Cheng, R., Dong, L., Saha, S.P. (2011). Intraoperative evaluation of revascularization effect on ischemic haemodynamics using near-infrared diffuse optical spectroscopies. *Journal of Biomedical Optics.* 16, 027004.

Zainuer, A., Inoue, Y., Kudo, T., Toyofuku, T., Igari, K., Kawano, T. (2016). Usefulness of the transfer function index for diagnosing peripheral arterial disease in patients with arterial calcification. *Journal of Medical and Dental Sciences.* 63, 29-35.