Peer-reviewed version available at *Diseases* **2018**, *6*, 81; doi:10.3390/diseases6030081

Article

Innovative Multi-Site Photoplethysmography Analysis for Quantifying Pulse Amplitude and Timing Variability Characteristics in Peripheral Arterial Disease

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Abstract: Photoplethysmography (PPG) is a simple-to-perform vascular optics measurement technique that can detect changes in blood volume in the microvascular tissue bed. Beat-to-beat analysis of the PPG waveform enables the study of the variability of pulse features such as amplitude and pulse arrival time (PAT), and when quantified in the time and frequency domains, has considerable potential to shed light on perfusion changes associated with peripheral arterial disease (PAD). In this pilot study innovative multi-site bilateral finger and toe PPG recordings from 43 healthy control subjects and 31 PAD subjects were compared (recordings each at least 5 minutes, collected in a warm temperature-controlled room). Beat-to-beat normalized amplitude and PAT variability was then quantified in the time-domain using SD and IQR measures and in the frequency-domain bilaterally using Magnitude Squared Coherence (MSC). Significantly reduced normalized amplitude variability (healthy control 0.0384 (IQR 0.0217-0.0744) vs PAD 0.0160 (0.0080-0.0338) ($p<0.001$) and significantly increased PAT variability (healthy control 0.0063 (0.0052-0.0086) vs PAD 0.0093 (0.0078-0.0144) ($p<0.001$) was demonstrated in PAD using the time-domain analysis. Frequency-domain analysis demonstrated significantly lower MSC values across a range of frequency bands for PAD patients. These changes suggest a loss of right-to-left body side coherence and cardiovascular control in PAD. This study has also demonstrated the feasibility of using these measurement and analysis methods in studies investigating multi-site PPG variability for a wide range of cardiac and vascular patient groups.

Keywords: Cardiovascular variability; heart-rate variability; peripheral arterial disease; photoplethysmography; pulse

1. Introduction

Photoplethysmography (PPG) is a simple-to-perform vascular optical measurement technique that is often used to detect blood volume changes in the microvascular bed [1]. PPG can use a low-cost light-emitting diode (LED) and photodiode configuration to measure the light absorption and transmission in tissue, with changes in light detected by the PPG photodiode providing a pulse waveform with features that can be extracted and utilized diagnostically [2]. Although the PPG signal is not fully understood it has been used by many researchers to provide useful information about the cardiovascular system [1]. PPG can be performed using a single site measurement or using multiple measurement sites across the body, for example the right and left ear lobes, index finger pads and great toe pads [1].

A PPG waveform has a pulsatile 'AC' component superimposed on a low-frequency 'DC' component [2]. Beat-to-beat analysis of the AC waveform produces a number of features that have been described in the literature, for example timing, amplitude and shape and the variability of each of these. Amplitude, typically the measure from the systolic peak to the foot of the pulse, is an indicator of arterial blood flow and tissue volume changes [3]. Studies have found that amplitude can be used as an estimate for blood pressure or stroke volume and also as an indicator of sympathetic tone [3, 4]. Another important characteristic of the PPG waveform is the pulse arrival time (PAT), which has been measured using the foot or the peak of the pulse. PAT requires an ECG as well as a PPG and is often measured as the time between an ECG R-wave peak and a 1st dominant peak in the PPG signal [5]. A short PAT has been shown to correlate well with increased blood pressure [6] and has also been shown to relate to arterial stiffness and compliance [7-9]. Allen *et al* [10] found PAT increased with disease severity and was linked to a reduced arterial compliance and BP reductions due to arterial stenosis. Pulse width and pulse area can also both be calculated from the PPG waveform. Studies have shown that both the pulse *width* and the pulse *area* increase when peripheral vascular resistance increases [11, 12]. The variability of PPG waveforms is body site specific [13] and studying the signals from a range of bilateral body sites simultaneously is of considerable interest in PPG research.

The more slowly varying constituents of the PPG signal, often group together and called the DC component, have also been studied in detail including in the frequency-domain, for example using wavelet and/or conventional Fourier Transform based analyses that have been reported for heart rate variability (HRV) and laser Doppler flowmetry (LDF) studies. Specific frequency band ranges for LDF were first described in 1999 [14] and have since been redesigned with the discovery of oscillations at very low frequencies (the different bands and their attributed physiological origin are summarized in Table 1). The frequency band ranges can differ between individuals and also be modified by exercise and some disease processes, therefore an ideal universal division of the frequency ranges is impossible [14]. However, this type of quantification using the frequency domain provides a promising way forward to identify physiological processes and particularly those at the lower frequencies of cardiovascular variability. Nitzan *et al* has published key research in the analysis of the low frequency and beat-to-beat changes in PPG signals from different peripheral body sites, and his work has included the assessment of such oscillations in health and in diabetic patients. Nitzan and co-workers studied baseline and amplitude fluctuations from PPG signals and demonstrated that these low frequency (0.02-0.05 Hz) oscillations were mediated by sympathetic activity [16, 17], and studies before and after sympathectomy supported this [18]. Nitzan also demonstrated that the cross-correlation of pulse signal changes between right-left body sides was high in healthy individuals but this reduced significantly in diabetic patients [19]. There is considerable further scope to utilize time and/or frequency domain pulse wave analysis techniques to study a wide range of cardiovascular related diseases.

Peripheral arterial disease (PAD) is a common condition that can severely affect the quality of life and life expectancy. PAD affects as many as 17.3 per 10 000 person years in the UK [20], although incidence is thought to be as high as 15-20% in people over 70 years of age with just a quarter of them displaying symptoms [21, 22]. PAD is associated with several atherosclerotic co-morbidities including coronary artery disease and cerebrovascular disease, with a prevalence of 40% to 60% in PAD patients [21]. The first diagnostic test performed for PAD is often the Ankle Brachial Pressure Index (ABPI). ABPI is the ratio of ankle BP to brachial BP, and a value of less than 0.9 is indicative of PAD [23]. ABPI is a non-invasive, inexpensive method that can be as high as 95% sensitivity and 99% specificity, when compared to computed tomography angiography (CTA), but is not without its limitations [23, 24]. ABPI is user-dependent, therefore prone to potential error, and is non-diagnostic in patients with heavily calcified vessels because the arteries are non-compressible [24]. Imaging is considered the gold-standard diagnostic test but is often only used in patients being considered for surgery [25]. Duplex ultrasound is a safe and effective test often used for surveillance but can be highly operator-dependent, therefore CTA or magnetic resonance angiography (MRA) are often

used to confirm and localize disease for surgical planning [26]. However, both CTA and MRA are expensive, time consuming and invasive [27]. CTA is particularly limited due to the use of iodinated contrast which is nephrotoxic so can cause acute kidney injury [27]. PPG technology is potentially offering new diagnostic tools for the low-cost, non-invasive and safe assessments of PAD with capability to be accessible and portable. One way that PPG is being used is as an automated, user-independent method of measuring BP for ABPI calculations in PAD, demonstrating that PPG is at least as effective as Doppler ultrasound and can be more effective than manual measurements [28-30]. More recently pulse wave analysis (PWA) has been used as a tool for diagnosing PAD with changes in pulse amplitude, PAT, shape and a number of other pulse features have been shown to relate to the presence of atherosclerosis meaning that PPG is a useful non-invasive diagnostic tool [31-33].

This exploratory study aims to quantify and contrast the cardiovascular variability of multi-site PPG waveform features in health and compared to patients with peripheral arterial disease. In this pilot a unique data set of multisite PPG recordings collected from previous multi-site PPG studies by the Dr John Allen's Newcastle vascular optics group will be used to investigate and quantify differences in variability in both the time and frequency domains between healthy controls and PAD patients.

2. Materials and Methods

2.1 Participants and datasets

This exploratory study used a unique data set comprising of data collected from various former multi-site PPG studies by the Newcastle group. The subject recruitment has previously been documented by Nath *et al* [34] and Allen *et al* [35]. In summary, patients were recruited from the Newcastle upon Tyne Hospitals NHS Foundation Trust and healthy participants recruited from hospital staff groups, the University of the Third Age (U3A, Wearside Branch), and the Institution of Engineering and Technology (IET) retired members group. Ethical approvals had been obtained for the earlier data collection studies and with each participant gave their written informed consent.

2.2 Multi-site PPG system and measurements

The innovative multi-site PPG measurement system set-up and measurements have also been previously described in the literature by Nath *et al* [34] and Allen *et al* [35], and as originally developed by Allen and Murray [13]. The multi-site PPG system employed was a six-channel measurement system, facilitating the recording of pulse waveforms from the tissue pads of bilateral ear lobes, index fingers and halluces. The PPG probes were reflection mode and with near infrared (950 nm) LED operating wavelengths. The amplifier bandwidth was 0.15 to 20 Hz with pulse channels matched electronically and optically for the 3 pairs of right / left side channels, so that differences between sides were likely to be physiological rather than due to systematic bias from the equipment.

The recordings were all made with the patient in the supine position, in a warm temperature (25 ± 1°C) and relative humidity (25± 1%) controlled room with a minimum 10-minute acclimatization period. The recordings were made for at least 5 minutes to a computer using 16-bit analogue-to-digital conversion at either 2000 or 2500 Hz sampling rate, depending on which former study was used to collect the data. A single lead ECG was measured simultaneously to provide a cardiac timing reference for the subsequent pulse wave analysis (PWA). The gain of the pulse amplifiers was also recorded and used in the subsequent analysis.

2.3 Pulse Wave Analysis and signal quality control

Pulse wave analysis was carried out exclusively using bespoke research software (MATLAB, 2012 version, MathWorks Inc.). Prior to the beat-to-beat feature extraction of pulse timing and amplitude data a semi-automatic process of quality control was performed in order to minimize noise related artifact, and to identify poor data sets to be excluded from the final analysis [10]. This QA checking process was performed on all recordings prior to time domain and also frequency domain analyses and quantification where occasional noisy beats were interpolated out. Beat-to-beat amplitude and also timing measurement quality can be affected by factors such as excessive movement during recording, cardiac arrhythmias (such as atrial fibrillation or ventricular ectopic beats), and occasionally for severe vascular disease when a pulse cannot be detected easily ('a flat liner').

Our group focused specifically on finger and toe recordings rather than ear recordings because PAD typically presents in the lower limbs and occasionally upper limbs of patients [36]. In addition, artifact / noise appeared to affect the ear site significantly more than other sites, the morphologies at the ears appeared more complicated than more distal sites, the ear site links only to the external carotid artery rather than the internal carotid artery, and it was considered that they would have added little clarity to this pilot study evaluation. Furthermore, previous literature also assessing the use of PPG for PAD diagnosis have more commonly used finger and toe sites but not the ear sites [31-33].

Quality control was an extensive and meticulous process that involved manually checking each of the ~300+ heart beats individually in hundreds of site PPG recordings, using a bespoke MATLAB GUI-based processing program previously written by Allen *et al* [10] and as used in numerous other published studies [including 13, 35, 37]. Toe site recordings were first quality checked and were excluded if they did not meet acceptable quality suitable for further consideration in the study. Subjects whose toe signals passed the quality control then had finger signals quality checked and further exclusions were made where necessary.

2.3.1 Time-domain analysis

Beat-to-beat PAT over the full recording was calculated from the ECG R wave to the foot of each pulse. Beat-to-beat amplitude was calculated from the difference in height between the 1st dominant peak and the foot of each pulse; this amplitude was then normalized using the respective site PPG amplifier gain settings noted at the time of the reading, this normalization allows individuals to be fairly summarized within a group and compared between groups [35]. From each site the median values for the beat-to-beat PAT and normalized amplitude measures were calculated. To quantify the variability of each measure both the standard deviation (SD) and the interquartile range (IQR) of PAT and amplitude over the length of a pulse recording were calculated. Both SD and IQR were compared between subject groups, the former more parametric and the latter non-parametric for data that might not have a normal distribution.

2.3.2 Frequency-domain analysis

The Magnitude Squared Coherence (MSC) was calculated and quantified the coherence of bilateral toe and finger recordings at different frequency ranges for the PPG waveforms for the frequency bands defined in Table 1 (Signal processing script using bespoke MATLAB signal processing scripts). MSC gives a value between 0 and 1 [38] for which an MSC value closer to 1 indicates more coherence between two signals for a specified frequency band. The frequency ranges were determined using previous work from Stefanovska et al [14, 39, 40]: very low frequency (VLF 0.0095-0.021 Hz); low frequency (LF 0.021-0.052 Hz); medium frequency (MF 0.052-0.145 Hz); high frequency (HF 0.145-0.6 Hz); alternating current (AC 0.6-2 Hz). The frequency-domain analysis was

performed on resampled beat-to-beat amplitude and PAT data (4 Hz) [41]. Right and left great toes and right and left index fingers were compared using MSC. Overall i.e. median MSC estimates were then calculated for each frequency band and each subject group compared.

Table 1: Frequency intervals and their attributed physiological origin [14]. NO nitric oxide.

Frequency Band	Frequency (Hz)	Physiological origin
Alternating Current (AC)	0.6 - 2.0	Heartbeat
High frequency (HF)	0.145 - 0.6	Respiratory activity
Medium frequency (MF)	0.052 - 0.145	Intrinsic myogenic activity
Low frequency (LF)	0.021 - 0.052	Neurogenic (sympathetic) activity
Very low frequency (VLF)	0.0095 - 0.021	NO-dependent endothelial activity

2.4 Statistical analysis

Statistical analysis was carried out using the Minitab statistical package software (version 17). Data was tested for normality using the Anderson-Darling test and all variables were found to be non-parametric, therefore only non-parametric statistical analysis was carried out. Continuous variables are reported as median with the interquartile range (Q1-Q3) values. The Mann-Whitney U test was carried out to test for statistical significance between groups. A p value <0.05 was considered to be statistically significant.

3. Results

3.1 Subject demographics

Multisite PPG pulse recordings from 84 participants were originally included for this study. Ten subjects were excluded because of poor quality toe signal recordings, the most common cause being linked to cardiac arrhythmia (n=5), as well as excessive movement artifact type noise (n=3) and signal flat lining (n=2; likely severe disease cases). Of the remaining 74 subjects, a further 4 recordings were excluded because of poor quality based on finger site PPG measurements, however the good quality toe signals from these subjects were still used for summary analysis at that site. Therefore, a total number of 148 toe and 140 finger QA checked body sites were available for the main analysis.

Of the 74 participants used in the final analysis 43 were healthy controls (ABPI ≥ 0.9) and 31 participants had significant PAD in at least one leg (ABPI < 0.9). In the healthy control group there were 20 males, 10 diabetic patients with median age of 68 (55-77) years of age. In the study PAD group there were 15 males, 9 diabetic patients with median age of 69 (64-75) years of age. There was no significant difference in the overall ages for the two groups ($p=0.48$).

Table 2: Study participant demographics.

Variable	Healthy Control (n=43)	PAD (n=31)
Age (years)	68 (55-77)	69 (64-75)
Sex (male)	20	15
Height (cm)	169 (162-174)	167 (159-172)
Heart-rate (bpm)	69 (64-77)	70 (61-79)
Systolic BP (mmHg)	142 (124-158)	158 (144-177)

3.2. Toe site PPG variability in the time-domain comparing PAD to healthy controls

Variability was measured both in term of the SD and the IQR in the recording period to compare the measurements. Overall in both SD and IQR variability in the toes, there was significantly lower amplitude variability and significantly higher PAT variability in PAD compared to the healthy control group (see Table 3).

Table 3: Toe time-domain variability results comparing PAD and control. Median (IQR) values are shown. With a.u. arbitrary units.

Measure	Healthy Control legs (n=86)	PAD legs (n=62)	p-value
SD amplitude (a.u.)	0.0295 (0.0172-0.0536)	0.0124 (0.0056-0.0270)	**<0.0001**
IQR amplitude (a.u.)	0.0384 (0.0217-0.0744)	0.0160 (0.0080-0.0338)	**<0.0001**
SD PAT (s)	0.00535 (0.0041-0.0074)	0.00845 (0.0061-0.0114)	**<0.0001**
IQR PAT (s)	0.00626 (0.0052-0.0086)	0.00933 (0.0078-0.0144)	**<0.0001**

In this study the normalized amplitude variability was significantly lower in PAD for both SD and IQR with a median SD of 0.0124 (0.0056 - 0.0270) a.u. and a median IQR of 0.0160 (0.0080 - 0.0338) a.u., compared to the control group which had median SD of 0.0295 (0.0172-0.0536) a.u. and a median IQR of 0.0384 (0.0217-0.0744) a.u. Statistical testing showed highly significant differences in both SD and IQR giving p-values of <0.0001. In contrast, PAT variability was significantly higher in PAD for both SD and IQR with median SD PAT of 0.00845 (0.0061-0.0114) s and median IQR is 0.0093 (0.0078-0.0144) s compared to the significantly lower values in the control group of 0.0054 (0.0041-0.0074) s and 0.0063 (0.0052-0.0086) s for PAT SD and IQR, respectively. Again, both PAT SD and IQR yielded highly significant p-values of <0.0001.

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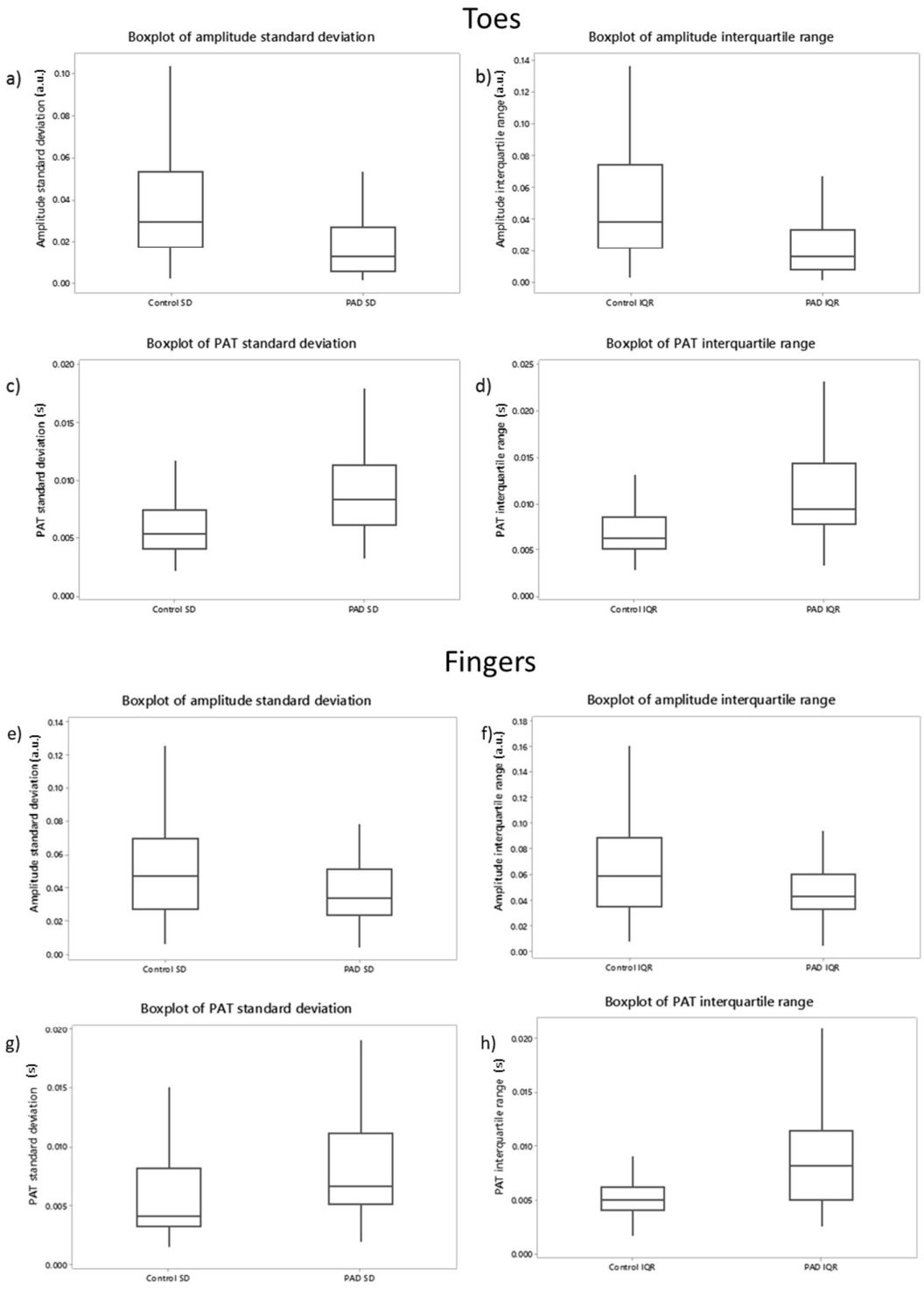

Figure 1: Boxplots of variability for the PPG waveforms: a) Toe normalized amplitude variability (SD) was significantly lower in the PAD group compared to the healthy controls ($p<0.0001$), b) Toe normalized amplitude variability (IQR) was also significantly lower in PAD ($p<0.0001$), c) Toe PAT variability (SD) was significantly higher in the PAD group ($p<0.0001$), d) Toe PAT variability (IQR) was also significantly higher in PAD ($p<0.0001$). e) Finger amplitude (SD) was significantly lower in PAD ($p=0.013$). f) Finger amplitude (IQR) was significantly lower in PAD ($p=0.013$). g) Finger PAT (SD) was significantly higher in PAD group ($p=0.0015$), and h) Finger PAT (IQR) was also significantly higher in PAD ($p<0.0001$).

3.3 Changes in the frequency-domain with PAD for the toe site

The MSC was calculated using the resampled PAT and showing significantly lower overall MSC values in PAD across the range of frequencies. For normalized amplitude these were found to be statistically significant for the lower 2 frequency bands (see Table 4).

Table 4: MSC analysis using resampled normalized amplitude and PAT frequency data for the toe site. Median (IQR) values are shown.

Frequency Band	**Healthy Control legs (n=86)**	**PAD legs (n=62)**	**p-value**
Amplitude VLF	0.685 (0.514-0.835)	0.466 (0.394-0.685)	**<0.005**
Amplitude LF	0.704 (0.563-0.799)	0.484 (0.342-0.704)	**<0.005**
Amplitude MF	0.611 (0.430-0.707)	0.536 (0.394-0.656)	0.161
Amplitude HF	0.684 (0.545-0.839)	0.551 (0.444-0.735)	**0.015**
Amplitude AC	0.526 (0.446-0.653)	0.497 (0.415-0.567)	0.117
PAT VLF	0.870 (0.727-0.937)	0.701 (0.543-0.899)	**<0.05**
PAT LF	0.704 (0.546-0.882)	0.445 (0.269-0.646)	**<0.001**
PAT MF	0.631 (0.479- 0.865)	0.435 (0.377-0.557)	**<0.001**
PAT HF	0.755 (0.575-0.904)	0.517 (0.468-0.699)	**<0.005**
PAT AC	0.685 (0.467-0.838)	0.426 (0.373-0.767)	**<0.01**

3.4 Finger site PPG variability in the time-domain comparing PAD to healthy controls

Variability in normalized amplitude and PAT in the finger sites correlates with results from the toe site measurements as discussed above. As for the toe site there was also less variability in the normalized amplitude but more variability in PAT in the PAD patients when compared to the healthy control group (see Table 5).

Normalized amplitude variability, measured in both SD and IQR, was significantly lower in the PAD patient group. Median amplitude SD was 0.0336 (0.0239-0.0511) a.u. in the PAD group compared to 0.0473 (0.0275-0.0695) a.u. in the control group, and median amplitude IQR was 0.0435 (0.0331-0.0603) a.u. in the PAD group compared to 0.0591 (0.0353-0.0890) a.u. in the control group. Both SD and IQR differences between the two groups are statistically significant with p-values of less than 0.05. There were also statistically significant differences in PAT variability between the control and PAD groups similar to those found in the toe sites. PAT SD was higher in PAD patients with a median of 0.00664 (0.0051-0.0111) s compared to a lower PAT SD of 0.0041 (0.0033-0.0082) s in the controls. PAT IQR was also increased in the PAD group with a median value of 0.0082 (0.0050-0.0114) s compared to 0.0050 (0.0041-0.0062) s. The differences between the two groups in PAT SD and also IQR measures were highly statistically significant (p-values <0.005).

3.5 Changes in the frequency-domain with PAD for the fingers

The bilateral frequency coherence of the resampled amplitude and PAT data will be compared here. Differences in MSC values in amplitude coherence were most significant at the LF and MF bands, similar to results in the smoothed raw data above. PAT MSC differences between the two subject groups were significant for MF and HF bands (see Table 6).

Table 5: Time-domain variability results in the fingers comparing PAD and control groups. Median (IQR) values are shown.

Measure	Healthy Control arms (n=78)	PAD patient arms (n=62)	p-value
Amplitude SD (a.u.)	0.0473 (0.0275-0.0695)	0.0336 (0.0239-0.0511)	**<0.05**
Amplitude IQR (a.u.)	0.0591 (0.0353-0.0890)	0.0435 (0.0331-0.0603)	**<0.05**
PAT SD (s)	0.00411 (0.0033-0.0082)	0.0066 (0.00513-0.0111)	**<0.005**
PAT IQR (s)	0.0050 (0.0041-0.0062)	0.0082 (0.0050-0.0114)	**<0.0001**

Table 6: Resampled normalized amplitude and PAT frequency analysis in the fingers results. Median (IQR) values are shown.

Measure	Healthy Control arms (n=78)	PAD arms (n=62)	p-value
Amplitude VLF	0.804 (0.573-0.931)	0.696 (0.500-0.922)	0.159
Amplitude LF	0.834 (0.664-0.924)	0.680 (0.487-0.787)	**<0.005**
Amplitude MF	0.673 (0.548-0.768)	0.532 (0.404-0.694)	**<0.01**
Amplitude HF	0.631 (0.515-0.782)	0.573 (0.458-0.699)	0.375
Amplitude AC	0.492 (0.440-0.622)	0.486 (0.402-0.580)	0.471
PAT VLF	0.750 (0.468-0.939)	0.633 (0.464-0.858)	0.523
PAT LF	0.518 (0.325-0.746)	0.514 (0.380-0.637)	0.611
PAT MF	0.581 (0.378-0.808)	0.439 (0.349-0.557)	**<0.05**
PAT HF	0.644 (0.499-0.833)	0.492 (0.398-0.733)	**<0.05**
PAT AC	0.540 (0.409-0.822)	0.434 (0.363-0.661)	0.065

MSC values are lower across the range of frequencies in the resampled amplitude data in PAD patients, however, there were only statistically significant differences for the LF and MF bands. In the LF range, median amplitude MSC is 0.834 (0.664-0.924) in the control group compared to 0.680 (0.487-0.787) in the PAD group. Amplitude MSC was also higher in the control group in the MF bands with a median MSC of 0.673 (0.548-0.768) compared to 0.532 (0.404-0.694) in the PAD patients. Statistically significant p-values in the LF band (p=0.002) and MF band (p=0.008) were found. There were no other statistically significant differences for the other frequency bands however.

Again, PAT MSC was lower for all frequency ranges in PAD. MSC was significantly lower ($p<0.05$) in the MF range in PAD patients with a median of 0.439 (0.349-0.557) compared to 0.581 (0.378-0.808) in the healthy control group. MSC was also significantly lower (p=0.035) in the HF range for PAD patients who had a median of 0.492 (0.398-0.733) compared to the healthy control

group of 0.644 (0.499-0.833). There were no other statistically significant differences for the other frequency ranges.

4. Discussion

This pilot study has quantified the variability in multi-site PPG waveforms in both the time-domain and the frequency-domain for healthy controls and an overall age-matched group of patients with peripheral arterial disease. Clear differences between the subject groups have been shown.

4.1 Variability in time-domain measures in health and PAD

Quantification of variability in amplitude, timing and shape measurements in PAD has not been extensively studied or discussed in the literature. Both SD and IQR measures of variability produced similar results in terms of demonstrating differences in variability in health and vascular disease. The pulse feature variability is considered to be derived from autonomic function [16, 17] - it was therefore expected that PAD patients would have less variability in all time domain measurements. Amplitude variability was expected to decrease in PAD and this study demonstrated lower amplitude variability for vascular patients using both SD and IQR measures. The difference in amplitude variability was more prominent in the lower limbs compared to the fingers. Variability is likely to be reduced in PAD patients because of a number of pathophysiological mechanisms, particularly autonomic dysfunction as well as reduced intrinsic myogenic activity and NO-dependent endothelial activity [14]. Further study is warranted using the methodologies described in this paper for diabetic patients who have peripheral autonomic neuropathy.

Interestingly, PAT variability was demonstrated to be higher overall for the PAD patient group compared to the healthy subjects. In both the toe and finger recordings, PAT variability was significantly higher in PAD patients in the toes and at the finger sites. Timing variability is more difficult to explain as it was initially expected to decrease with disease. This suggests that the PAT variability in the time-domain may not be related to sympathetic function and could instead be more affected by BP changes over arterial stenosis and occlusion found in PAD. These observations will be explored further in wider studies of the techniques employed.

Comparing amplitude variability with PAT variability demonstrates distinction between the healthy control and PAD groups (see Figure 2). The scatterplot shows the different cluster ranges although with a degree of overlap, with lower PAT variability and higher amplitude variability. In contrast, the PAD group has higher PAT variability and lower amplitude variability. The reasons for these differences are not well understood and warrant further study.

4.2 Variability in the frequency-domain in health and in PAD

Frequency analysis compared the MSC values of different signals from bilateral PPG measurement sites. The analysis computed the signal coherence at different frequency ranges that have been attributed to different physiological mechanisms. Loss of coherence between bilateral sites (MSC tending towards 0) in a particular frequency band means that the right-left body side variability synchronicity has been diminished, potentially meaning disease in the particular physiological origin of that frequency band range.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 August 2018 doi:10.20944/preprints201808.0206.v1

Peer-reviewed version available at Diseases 2018, 6, 81; doi:10.3390/diseases6030081

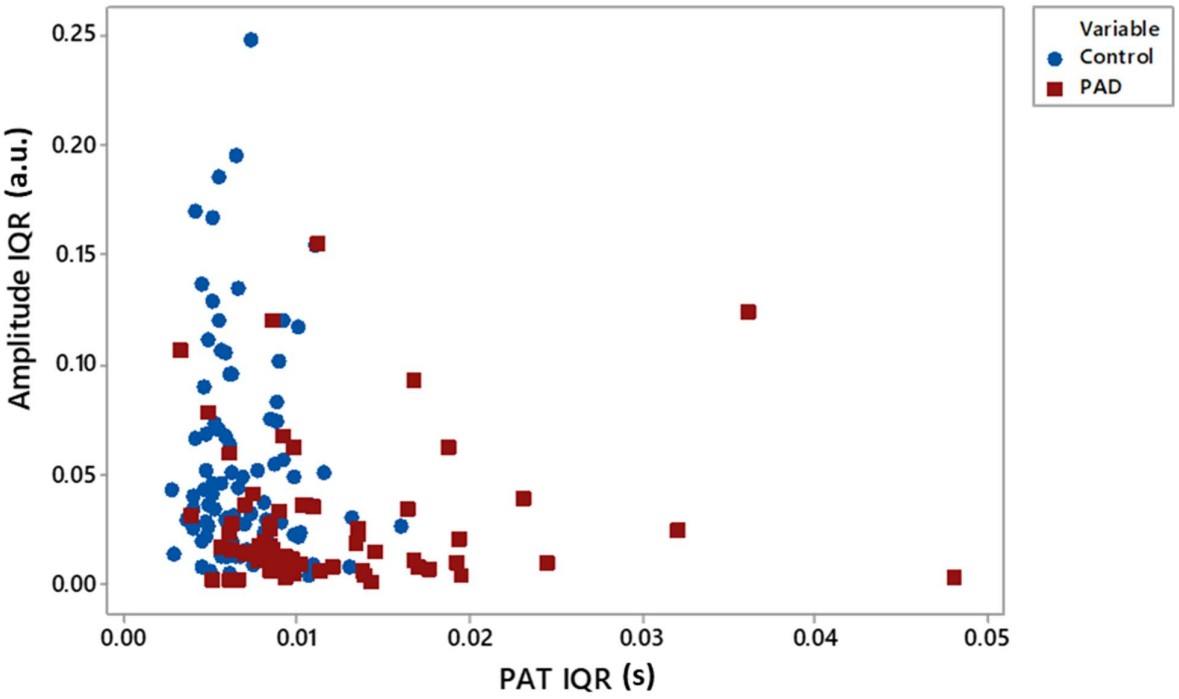

Figure 2: Scatterplot comparing amplitude IQR with PAD IQR. Graph demonstrates differences in PAT and amplitude variability in the PAD and control groups.

Frequency analysis was used to quantify variability in amplitude and PAT in healthy subjects and in PAD patients. In the toes, amplitude MSC values were significantly lower at VLF and LF bands, while PAT MSC values were significantly lower across all frequency band ranges in PAD patients. The fingers gave slightly different results again with amplitude MSCs being lower in LF and MF bands, while PAT MSCs are lower in MF-AC frequency bands in PAD patients. Lower normalized amplitude MSC values found in PAD patients suggest loss of variability synchronicity in the left and right fingers and toes; this is particularly prominent at lower frequency band ranges. In the toes the main difference between control and PAD groups are at the VLF and LF bands, which means amplitude variability could be attributed to NO-endothelial and sympathetic activity. Both NO-endothelial function and sympathetic endothelial function are diminished in PAD. Finger MSC differences are at slightly higher frequency bands which could suggest that the loss of NO-function could be less prominent in the upper limbs.

PAT variability coherence was lower across the full range of frequencies for the toe measurement site but was mostly lower for the higher frequency bands in the finger measurement site. The loss of coherence for PAT variability across all frequency ranges for the toes suggests that PAT variability could be affected by a range of pathophysiological mechanisms found in PAD. However, the most significant differences are found at LF and MF band ranges, meaning PAT variability could be most affected by loss of sympathetic function and myogenic activity. However, in the fingers these differences are slightly less prominent, again suggesting that the severity of PAD is likely to be lower in the upper limb arteries of PAD patients compared to the lower limb. These derived data sets are very interesting and warrant further investigation as ultimately the toe pulse variability measures could add value to PPG PAD diagnostics, have utility for assessing response to therapy, and to enable a better understanding of the pathophysiological mechanisms with disease.

5. Summary

In this pilot study the expected normal ranges for variability in multi-site PPG pulse amplitude and PAT timing measures have been quantified, along with their expected changes in peripheral arterial disease. The observed de-synchronization in PAD of the pulse amplitude and timing measures between right and left sides assessed using MSC coherence frequency-domain methods has also been shown. These data suggest a complex picture of changes in vascular autoregulation an endothelial and autonomic function in PAD and highlights the compensatory mechanisms involved in trying to maintain adequate distal perfusion. The time-domain and frequency-domain data determined in this pilot study will have value by informing the design of future multi-site PPG studies in peripheral arterial disease using this core and exciting measurement and analysis technology.

6. Patents etc.

Author Contributions: Conceptualization, J.A. and G.S.; Methodology, J.A.; Data Curation, J.A.; Software, J.A.; Resources, J.A.; Validation, J.A. and M.B; Formal Analysis, M.B.; Investigation, M.B.; Writing-Original Draft Preparation, M.B.; Writing-Review & Editing, J.A and G.S.; Supervision, J.A and G.S.

Funding: This specific research study received no external funding.

Acknowledgments: We acknowledge the use of multi-site pulse data collected from the earlier multi-disciplinary studies with Mr Alex Nath and Mr Klaus Overbeck. We are also very thankful to the Northern Medical Physics and Clinical Engineering Department for producing the prototype multi-site PPG measurement system as under the specification and joint design work with Dr John Allen.

Conflicts of Interest: Between 2014 and 2018 Dr John Allen was the Chief Investigator on an NIHR i4i funded grant (II-C1-0412-20003) to develop a miniaturized version of the multi-site PPG technology - specifically for peripheral arterial disease (PAD) detection in a primary care setting. He is a co-author on 2 published patents in relation to the GP device i.e. the pulse analysis algorithm for PAD detection and also a novel pulse sensor housing and attachment clip, respectively. There are no other potential conflicts of interest to report.

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