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Using Time-Frequency Analysis of the Photoplethysmographic Waveform to Detect the Withdrawal of 900 mL of Blood

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BACKGROUND: We designed this study to determine if 900 mL of blood withdrawal during spontaneous breathing in healthy volunteers could be detected by examining the time-varying spectral amplitude of the photoplethysmographic (PPG) waveform in the heart rate frequency band and/or in the breathing rate frequency band before significant changes occurred in heart rate or arterial blood pressure. We also identified the best PPG probe site for early detection of blood volume loss by testing ear, finger, and forehead sites.

METHODS: Eight subjects had 900 mL of blood withdrawn followed by reinfusion of 900 mL of blood. Physiological monitoring included PPG waveforms from ear, finger, and forehead probe sites, standard electrocardiogram, and standard blood pressure cuff measurements. The time-varying amplitude sequences in the heart rate frequency band and breathing rate frequency band present in the PPG waveform were extracted from high-resolution time-frequency spectra. These amplitudes were used as a parameter for blood loss detection.

RESULTS: Heart rate and arterial blood pressure did not significantly change during the protocol. Using time-frequency analysis of the PPG waveform from ear, finger, and forehead probe sites, the amplitude signal extracted at the frequency corresponding to the heart rate significantly decreased when 900 mL of blood was withdrawn, relative to baseline (all $P < 0.05$); for the ear, the corresponding signal decreased when only 300 mL of blood was withdrawn.

The mean percent decrease in the amplitude of the heart rate component at 900 mL blood loss relative to baseline was 45.2% (38.2%), 42.0% (29.2%), and 42.3% (30.5%) for ear, finger, and forehead probe sites, respectively, with the lower 95% confidence limit shown in parentheses. After 900 mL blood reinfusion, the amplitude signal at the heart rate frequency showed a recovery towards baseline. There was a clear separation of amplitude values at the heart rate frequency between baseline and 900 mL blood withdrawal. Specificity and sensitivity were both found to be 87.5% with 95% confidence intervals (47.4%, 99.7%) for ear PPG signals for a chosen threshold value that was optimized to separate the 2 clusters of amplitude values (baseline and blood loss) at the heart rate frequency. Meanwhile, no significant changes in the spectral amplitude in the frequency band corresponding to respiration were found.

CONCLUSION: A time-frequency spectral method detected blood loss in spontaneously breathing subjects before the onset of significant changes in heart rate or blood pressure. Spectral amplitudes at the heart rate frequency band were found to significantly decrease during blood loss in spontaneously breathing subjects, whereas those at the breathing rate frequency band did not significantly change. This technique may serve as a valuable tool in intraoperative and trauma settings to detect and monitor hemorrhage. (Anesth Analg 2012;115:74–81)

Accurate detection of early blood volume loss is an important component of intraoperative and trauma care. A major difficulty for early detection of a Class I and II hemorrhage, which includes up to 15% and 30% blood volume loss,[1] respectively, is due to the presence of changes in vascular tone and contractility that obviate the need for significant changes in heart rate (HR) and arterial blood pressure (BP). Attempts have been made to diagnose Class I hemorrhage through analysis of information extracted from a pulse oximeter photoplethysmographic (PPG) waveform.[2,3]

The PPG waveform contains a pulsatile component at the HR frequency and a slower oscillatory component at

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the breathing rate (BR).[4] The influence of respiration on the PPG waveform has been shown to have a prominent effect during a hypovolemic state in mechanically ventilated patients where reductions in blood volume increased the respiratory-induced variation in the pulse amplitude of the PPG signal.[4–6] Although significant increases in the spectral power of the PPG at the respiratory frequency have been shown in spontaneously breathing subjects during blood withdrawal[2,3] and lower body negative pressure (LBNP),[7] other studies have shown the increased spectral power at the respiratory frequency to be inaccurate in detecting hypovolemia in spontaneously breathing subjects.[8] As an alternative to monitoring the respiratory PPG variability, McGrath et al.[9] used time-domain measures to show that PPG pulse amplitudes significantly decreased during hypovolemia in spontaneously breathing subjects.

In our work, to more accurately capture time-varying amplitudes of pulse oximeter signals that may reflect a signature of blood loss, we used a time-frequency spectral approach[10] to detect such variations in the pulse oximeter amplitudes at the frequency location corresponding to HR. The HR frequency is easy to discriminate because it is the dominant amplitude in the spectrum. Using this approach, we previously found significant decreases in the spectral amplitude at the frequency corresponding to HR starting as early as 20% of the LBNP tolerance (maximum negative pressure a volunteer can tolerate without syncope) in spontaneously breathing healthy subjects.[11]

We hypothesized that the amplitudes of the cardiac pulse and respiration-induced oscillations in the pulse oximeter signal change significantly during the withdrawal of 900 mL blood in spontaneously breathing subjects, and that they can be detected before significant changes occur in HR and BP. Additionally, we hypothesized that the ear lobe would be the optimal site for detecting blood volume loss by considering simultaneous PPG measurements of the ear, finger, and forehead because it has been shown to be least influenced by local peripheral vascular resistance changes.[12]

METHODS

Experimental Protocol

The protocol was approved by the Yale-New Haven Hospital IRB Committee and written informed consent was obtained from all subjects. Healthy volunteers (n = 8 male, age 28 ± 2.9 years (mean ± SD), height 174.6 ± 5.2 cm, and weight 80.1 ± 8.9 kg) with no known cardiovascular or systemic disease participated in this study. The subjects were instructed to abstain from caffeine and other known vasoconstrictive compounds for a minimum of 4 hours before participation in the study. The subjects were placed in a semirecumbent position on a stretcher at a room temperature of ~21°C. A 16-gauge IV catheter was inserted into an antecubital vein after application of subcutaneous lidocaine. The catheter was attached to a CPDA-1 bag for subsequent blood withdrawal. A hematocrit of at least 36 was confirmed before blood withdrawal was started. After a venous tourniquet was applied to the arm, 900 mL of blood was allowed to drain by gravity with a withdrawal time that varied by subject from 12 to 40 minutes. Volume of blood withdrawn was determined by continuously weighing the withdrawn blood. Reinfusion of the same 900 mL of blood was accomplished linearly over a period of 20 minutes. Patients were instructed to breathe normally during withdrawal as well as reinfusion. The study endpoints included changes in HR or BP that exceeded 15% of baseline and/or the development of any signs or symptoms of hypovolemia.

Data Acquisition

Three infrared PPG-probes (Modified Model 520A, Oxypleth®, Novametrix/Respironics, Wallingford, CT) were placed at the finger, forehead, and ear. The auto-gain function and other filtering algorithms were disabled during PPG recording. Standard electrocardiograms were simultaneously recorded alongside PPG measurements at 200 Hz using a microprocessor-based data acquisition system (PowerLab 16, ADInstruments, Colorado Springs, CO). The time events of the experimental protocol including baseline, amounts of blood withdrawn, and reinfusion were marked in the LabChart® 7 (ADInstruments) file during the data acquisition process. Changes in BP were monitored on a beat-by-beat basis with a noninvasive finger arterial pressure monitor (Ohmeda 2300 Finapres, Boulder, CO). Furthermore, intermittent cuff pressures were also monitored on the leg because intermittent cuff inflation on the arm may have disturbed other hemodynamic monitoring. HR was evaluated at each level of the blood withdrawal protocol using beat-to-beat R wave peak detection from the electrocardiogram recordings.

Estimation of HR and BR Amplitudes

We extracted 2-minute PPG data segments by the end of each level of the experimental protocol: baseline, blood withdrawal of 300, 600, and 900 mL, and after reinfusion of 900 mL. A summary of the analysis methods to estimate the amplitude from the PPG signal is shown in Figure 1. From each 2 minutes of PPG data, a 1-minute window was shifted in 10-second intervals producing 7 PPG segments at each protocol level. PPG segments were downsampled to 20 Hz followed by removal of the mean and linear trend. Segments were then normalized to unit variance, and the time-frequency spectrum of each was generated by variable frequency complex demodulation. The variable frequency complex demodulation algorithm has been reported in detail,[10,11,13] and an online supplemental appendix (Appendix 1, see Supplemental Digital Content 1, http://links.lww.com/AA/A392) is available containing the technical details. For PPG signals recorded during spontaneous breathing, the amplitude and frequency of the HR and BR would both be expected to change over time, and use of time-frequency analysis allows us to continuously track these amplitude signals.

The time-frequency spectrum contains oscillatory PPG amplitudes at all frequency and time locations. The maximum amplitudes in the HR and BR frequency ranges were extracted at each time point from the time-frequency spectrum to constitute the PPG amplitude sequences.[11] The amplitude values in the HR frequency band contain the cardiac pulse components of the PPG waveform and are similar to the time-domain pulse amplitude described by McGrath et al.[9] The BR amplitude sequence corresponds to

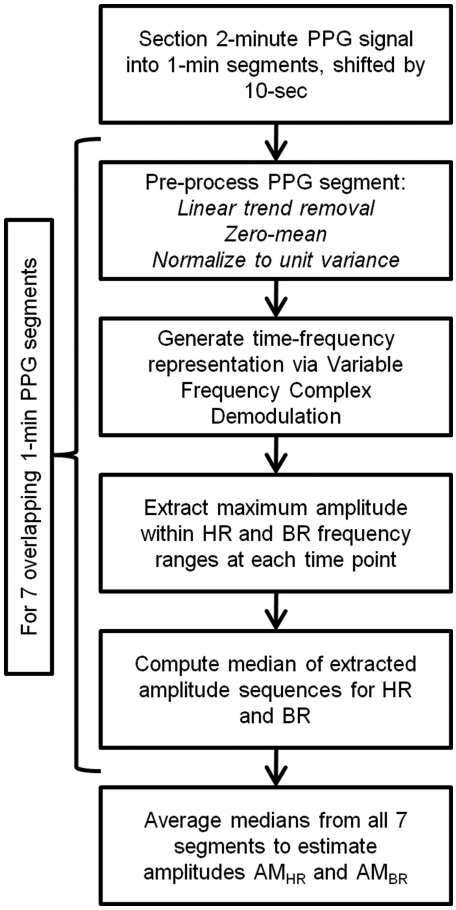

Figure 1. An overview of the method used to estimate the amplitudes from the heart rate (HR) and breathing rate (BR) components (AM_{HR} and AM_{BR}) at each level of the experimental protocol from the photoplethysmographic (PPG) waveform.

the respiration-induced oscillations in the PPG waveform.[2,8] The HR frequency range was defined as ± 0.2 Hz about the HR found as the maximum peak in the frequency spectrum of the PPG segment, and the BR frequency range was fixed at 0.05 to 0.35 Hz. The initial and final 5 seconds of the time-frequency representation were not considered for amplitude extraction because of an edge effect intrinsic to the variable frequency complex demodulation method that diminishes its accuracy at the beginning and end of a time series.[10,13] The median values of the amplitude sequences within the HR and BR frequency ranges were computed for each of the 7 PPG segments (1 minute data window shifted by 10 seconds). The mean of the 7 PPG segment amplitude estimates were then computed for both frequency ranges as estimates of the amplitudes at the HR and BR frequencies and are denoted as AM_{HR} and AM_{BR}, respectively. Percent changes in AM_{HR} and AM_{BR} values were calculated for each stage of analysis with respect to the individual's baseline for the three PPG signals. All analysis was performed offline using Matlab®.

Statistical Analysis

Data are reported as mean ± SD. The null hypothesis that percent change in AM_{HR} and AM_{BR}, HR, and BP measurements did not change at each protocol level was assessed. Normality of each measure, including percentage change, was assessed using D'Agostino and Pearson Omnibus normality test with $P > 0.05$ considered data from a normal distribution.[14] The AM_{HR} ear, all AM_{BR}, HR, and leg-cuff systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were determined to be normal, and AM_{HR} finger, AM_{HR} forehead, and Finapres SBP and DBP measurements were determined to be nonnormal. The null hypothesis was rejected for $P < 0.05$ from 1-way repeated measures ANOVA for normal data or nonparametric Friedman repeated-measures ANOVA on Ranks otherwise. Mauchly's Test of Sphericity was performed before parametric repeated-measures ANOVA analysis, and if the data were determined to be nonspherical the Greenhouse-Geisser correction was applied. If the null hypothesis was rejected for repeated measures assessment, pairwise comparisons between all stages of the protocol (10 total pairs) were made using either Holm-Sidak test, if the data were not rejected from being from a normal distribution, or nonparametric Tukey test with $P < 0.05$ considered significant. SigmaStat 3.5 (SPSS, Chicago, IL) was used for statistical comparisons. Threshold values for AM_{HR} were examined using receiver operating characteristic (ROC) curve analysis for their specificity and sensitivity when applied to the detection of blood loss between baseline and 900 mL withdrawal. The threshold value with the greatest specificity and sensitivity was selected. ROC confidence limits for specificity and sensitivity were determined using the method described by Kerekes.[15]

RESULTS

Average initial blood volumes were estimated to be 5.1 ± 0.4 L based on each subject's height and weight using Nadler's formula,[16] and percent blood loss was estimated at 5.9 ± 0.5%, 11.7 ± 1.0%, and 17.6 ± 1.5% for the 300, 600, and 900 mL withdrawal points, respectively, determined by continuously weighing the volume in the bag and then applying Nadler's formula. HR and BP values obtained at each blood withdrawal level are presented in Table 1. Finapres BP measurements were not obtainable in three subjects for all the stages of the protocol because of difficulties with cuff alignment. No significant changes were found for any of the vital sign measures throughout the experimental protocol.

A sample ear-PPG signal and its time-frequency spectrum showing the dominant amplitudes at each time and frequency location are shown in Figure 2a and 2b, respectively. Two dominant amplitudes occur across time that can be observed to have varying frequencies within bounded ranges in the time-frequency representation, outlined in Figure 2b. These constitute the main dynamics in the PPG signal and consist of an HR ridge, representing the high frequency component in the PPG waveform related to the cardiac pulse (green box in Fig. 2b), and a BR ridge, corresponding to the amplitude of the respiration-induced oscillations (red box in Fig. 2b). The collection of the largest instantaneous amplitude at each time sample within the HR and BR frequency bands constitute the amplitude sequences of each (Fig. 2, c and d).

Percent changes of the AM_{HR} and AM_{BR} parameters obtained from the 8 healthy subjects at each level of the

Table 1. Heart Rate and Arterial Blood Pressure (BP) Measurements Obtained During the Blood Withdrawal Protocol

		Leg cuff (n = 8)		Finapres (n = 5)	
	Heart rate (n = 8) (beats/min)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Baseline	72.7 ± 7.6	141.1 ± 8.5	64.4 ± 4.4	126.4 ± 7.4	65.5 ± 8.4
300 ml	71.6 ± 8.2	148.0 ± 9.4	67.8 ± 6.0	135.2 ± 14.1	68.3 ± 17.8
600 ml	74.8 ± 9.7	142.6 ± 7.1	67.5 ± 5.5	128.4 ± 15.7	67.1 ± 16.0
900 ml	76.7 ± 8.6	142.5 ± 9.0	66.5 ± 7.2	124.4 ± 14.1	68.5 ± 12.9
Post-reinfusion	74.5 ± 6.4	148.0 ± 9.9	66.0 ± 2.8	144.9 ± 20.3	79.4 ± 15.3

No significant changes occurred in heart rate or BP throughout the protocol ($P < 0.05$ considered significant).

blood withdrawal protocol are provided in Figure 3 for ear, finger, and forehead probe sites. AM_{HR} from the ear PPG showed a significant decrease ($P < 0.05$) at 300 mL and 600 mL of blood loss compared to baseline. Furthermore, AM_{HR} decreased with respect to baseline by the 900 mL withdrawal point in all PPG sites (Fig. 4, a, c, and e) (all $P < 0.05$). Exact P values for multiple comparison testing are listed in Table 2. At 900 mL, the mean percent decrease in AM_{HR} reached 45.2% (38.2%), 42.0% (29.2%), and 42.3% (30.5%) for ear, finger, and forehead PPG signals, respectively, with the lower 95% confidence limit for the sample mean shown in parentheses. After 900 mL of reinfusion, AM_{HR} showed a recovery towards the baseline value and was significantly higher than the 900 mL blood withdrawal time points for the ear location; after reinfusion, no site had AM_{HR} that differed significantly from baseline.

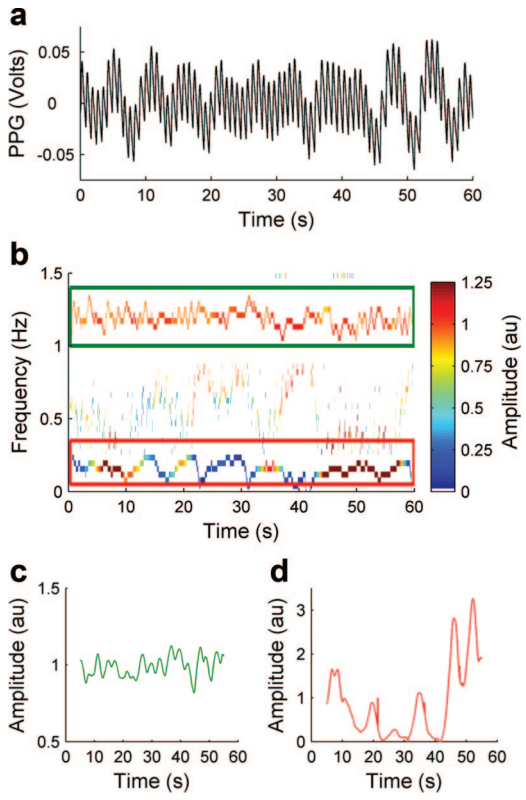

Figure 2. a: Representative measured ear photoplethysmographic (PPG) signal and its (b) time-frequency representation identifying the dominant amplitudes in the PPG signal at the appropriate frequency (y-axis) and time (x-axis) locations. The prominent amplitudes are seen near the heart rate (~1.2 Hz) and breathing rate (~0.2 Hz) ranges highlighted as boxes (green for heart rate and red for breathing rate), and it can be observed that these frequencies vary over time. The extracted amplitude sequences (shown in arbitrary units (au) after normalizing the PPG signal to unit variance) at the heart rate and breathing rate are shown in (c) and (d), respectively.

Absolute values of AM_{HR} were compared between baseline and 900 mL blood withdrawal conditions for ear, finger, and forehead PPG (Fig. 4). Areas under the ROC curve for the 3 measurement locations and their 95% confidence limits were found to be 0.89 (0.72, 1), 0.94 (0.92, 1), and 0.89 (0.72, 1) for ear, finger, and forehead, respectively. Threshold values with the highest specificity and sensitivity for absolute AM_{HR} were 0.55 for ear and finger PPG signals, and 0.68 for forehead PPG signals. Table 3 shows the specificity (percentage of baseline measurements correctly identified) and sensitivity (percentage of 900 mL withdrawal measurements correctly identified) of AM_{HR} given the above thresholds.

In contrast to AM_{HR}, percent changes in AM_{BR} from the ear, finger, and forehead probe sites were not significant during 900 mL blood loss (Fig. 3, b, d, and f).

DISCUSSION

The assessment of a high resolution based time-varying spectral method to detect progressive hypovolemia during spontaneous breathing using the PPG waveform was the focus of this study. Although the respiratory-induced PPG variability has been shown to be a promising predictor of hypovolemia in mechanically ventilated patients, it has proven inadequate for hypovolemic detection in spontaneously breathing subjects.[8] Our hypothesis was that one way to overcome this current limitation is to look for dynamic changes of the PPG spectral amplitudes at the HR and BR frequencies, as each vary over time. A technical challenge then required us to use a high-resolution time-frequency spectral technique to extract instantaneous amplitudes, which can be subsequently quantified to discern possible changes during blood loss.

Using the strategy outlined above, our present results show that the PPG spectral amplitude at the HR frequency significantly decreased at the stages of volume loss (Fig. 3, a, c, and e) whereas the amplitude at the BR frequency did not significantly change (Fig. 3, b, d, and f). Analysis of the HR amplitude sequences extracted from high-resolution time-frequency representations provided detection of 900 mL blood loss in spontaneously breathing subjects (Table 3) before any significant changes occurred in HR or BP (Table

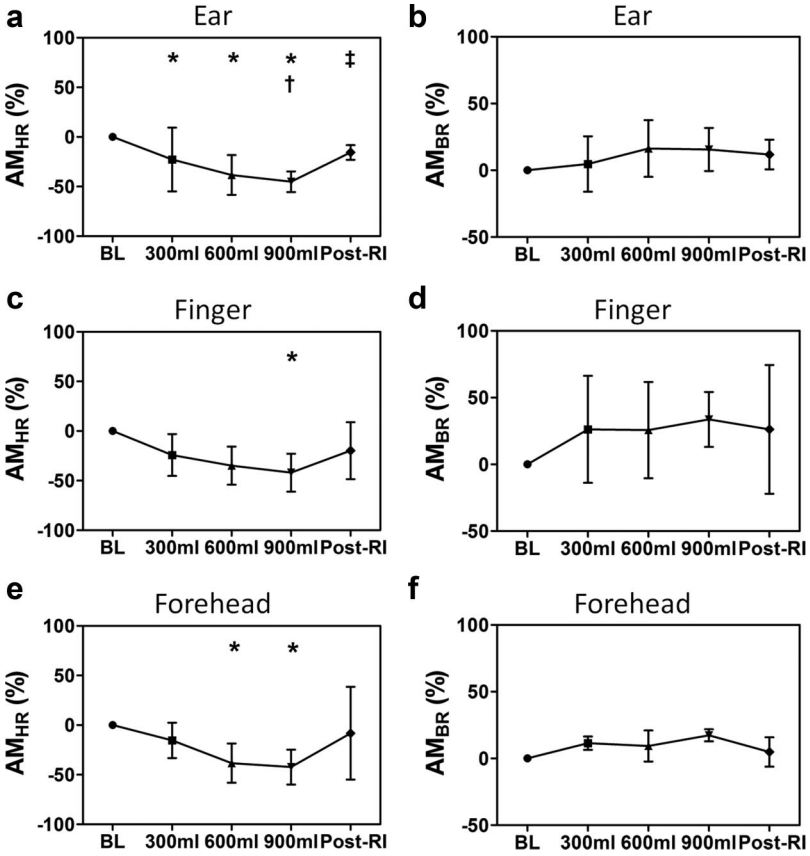

Figure 3. The percent changes in amplitudes at the heart rate (AM_{HR} for [a] ear, [c] finger, and [e] forehead PPG signals) and breathing rate (AM_{BR} for [b] ear, [d] finger, and [f] forehead PPG signals) during baseline (BL), blood withdrawal of 300, 600, and 900 mL, and post-reinfusion (Post-RI) of 900 mL blood are given in mean ± SD. Symbols denote post hoc statistical significance, $P < 0.05$, between the protocol level and either baseline (*), 300 mL blood withdrawal (†), or 900 mL blood withdrawal (‡). Table 2 contains P values for AM_{HR} multiple comparison testing. AM_{BR} measurements were not found to significantly change throughout the protocol ($P < 0.05$ considered significant).

1).[17,18] The AM_{HR} parameter measured from the ear significantly decreased at the early stage of 300 mL blood withdrawal through 900 mL withdrawal (Fig. 3a). The decrease in AM_{HR} represents a diminished pulse amplitude reflective of a combination of factors that may occur during blood loss, most notably a decrease in the end-diastolic pressure and stroke volume.[9]

For the AM_{HR} measure of ear PPG, we were able to find a threshold to separate baseline and 900 mL withdrawal time points with 87.5% sensitivity and specificity, although given our limited sample size ($n = 8$) the lower confidence limit for specificity and sensitivity was 47% (Table 3). This finding suggests that the present technique may have the potential to detect blood loss even if monitoring is initiated after blood loss has begun. This capability could be especially valuable in emergency and trauma situations where baseline values are not obtainable and the current blood volume status may be unknown. These changes occurred in the absence of changes in BP or HR. Of note, the higher SBP measurements from the leg cuff compared to Finapres are in agreement with the report by Moore et al.[19] demonstrating that leg cuff measurements are higher than measurements in the arm. We did not find significant changes in BP during blood withdrawal for either measurement.

Clinical pulse oximeters attached to the commonly used measurement sites may provide different signal dynamics; therefore, it is essential to determine the optimal probe site to monitor blood volume status.[20] From the present results, AM_{HR} measurements from all 3 probe sites significantly decreased throughout blood withdrawal compared to baseline. The ear PPG showed a significant decrease in AM_{HR} throughout 300, 600, and 900 mL withdrawal, but then returned to baseline levels during reinfusion; finger and forehead measurements did not match this performance (Table 2). Shelley et al.[20] showed the ear signal to have the strongest respiratory amplitude variation compared to the finger and forehead, and highlighted the shorter distance from measurement site to chest and the diminished sympathetic influence in the head vasculature as two contributing factors. From a practical point of view, the ear location may be optimal because it has the least interference with a subject's daily movements, which results in less motion and noise contamination in the PPG signal. All these factors suggest the ear as the best PPG probe site for detecting blood volume loss.

Our AM_{BR} measure, calculated from the time-frequency spectrum, provides the same information as the time-domain counterpart of the respiratory PPG amplitude (PPGr) described by Nilsson et al.,[8] and the frequency-domain–based respiratory-associated power described by Gesquiere et al.[2] The present results showed no significant changes in AM_{BR} values due to 900 mL blood loss, supporting the notion of Nilsson et al.[8] that the respiratory variations of the PPG waveform may not be a strong indicator of hypovolemia in spontaneously breathing subjects. Furthermore, both the plethysmogram variability index and Δ pulse oximeter plethysmographic measures, which also look at respiratory variability, were recently identified as inaccurate in predicting hemodynamic changes induced by passive leg elevation in spontaneously breathing volunteers.[21,22] Conflicting results are evident with respect to

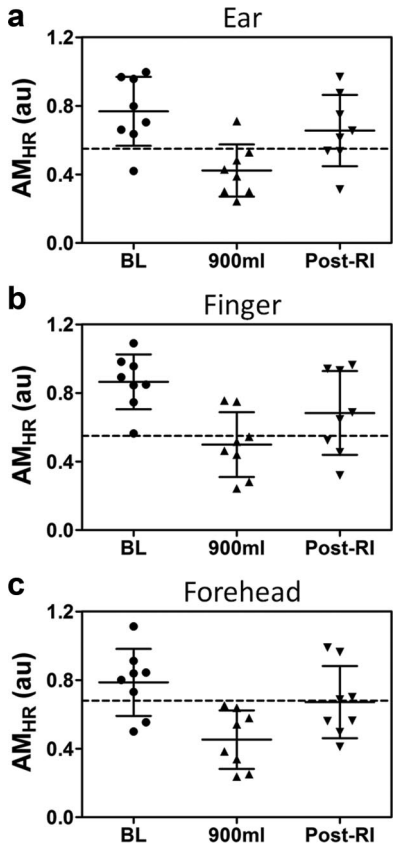

Figure 4. The comparison of absolute amplitudes at the heart rate frequency, AM_{HR}, in arbitrary units (au) between baseline (BL), 900 mL blood withdrawal and after reinfusion (Post-RI) conditions are given for (a) ear, (b) finger, and (c) forehead probe sites. Bars refer to mean ± SD. The optimal thresholds of the absolute AM_{HR} measures were found to be 0.55 for ear and finger photoplethysmographic (PPG), and 0.68 for forehead PPG (dotted lines).

Table 2. Adjusted *P* Values from Multiple Comparison Testing for Ear, Finger, and Forehead Amplitudes from the Heart Rate (AM_{HR}) Measurements from Each Protocol Level Assessed by the Holm-Sidak Test (Ear, After Parametric Repeated-Measures ANOVA with Greenhouse-Geisser Correction Applied) and Nonparametric Tukey Test (Finger and Forehead, After Nonparametric Repeated-Measures ANOVA)

	300 mL	600 mL	900 mL	Post-reinfusion
Baseline				
Ear	0.047*	<0.001*	<0.001*	0.17
Finger	0.24	0.056	0.014*	0.51
Forehead	0.80	0.005*	0.005*	0.61
300 mL				
Ear	—	0.21	0.042*	0.62
Finger	—	0.97	0.80	0.99
Forehead	—	0.12	0.12	0.99
600 mL				
Ear	—	—	0.40	0.053
Finger	—	—	0.99	0.80
Forehead	—	—	0.99	0.24
900 mL				
Ear	—	—	—	0.007*
Finger	—	—	—	0.51
Forehead	—	—	—	0.24

*Represents significant difference between groups ($P < 0.05$).

Table 3. Sensitivity and Specificity Between Baseline and 900 mL Withdrawal of Blood After Applying a Threshold to the Amplitudes from the Heart Rate (AM_{HR}) Absolute Values, 95% Confidence Intervals Shown in Parentheses

	Specificity	Sensitivity
Ear	87.5% (47.4%, 99.7%)	87.5% (47.4%, 99.7%)
Finger	100% (63.1%, 100%)	75% (34.9%, 96.8%)
Forehead	75% (34.9%, 96.8%)	100% (63.1%, 100%)

using respiratory variations as a marker of blood loss in spontaneously breathing subjects.

McGrath et al.[9] used time-domain techniques to compute the PPG pulse amplitude during LBNP and showed strong correlations with stroke volume from 0% to 100% LBNP tolerance. The present results of the AM_{HR} measure agree with this study as well as our previous LBNP study[11] in which the detection of simulated blood loss was shown to be possible at the early stage of 20% LBNP tolerance, and there was a linear decrease in AM_{HR} values corresponding to the progressive increase in LBNP. The present analysis allowed us to observe the respiration-induced oscillations and PPG pulse amplitude simultaneously. Based on previous results as well as our own, the pulse amplitude appears to be a better indicator of low volumes of blood loss during spontaneous breathing than the respiratory amplitude.

By using a time-varying spectral method we can accurately track the amplitude at the HR frequency as both the amplitude and frequency change over time, which is expected during spontaneous breathing. Thus, our approach differs from others because we compute a time-frequency spectrum and then focus on our frequency ranges of interest, minimizing the effect of high- and low-frequency noise sources and motion artifacts that may appear outside of the HR frequency range. If artifacts are present in the extracted amplitude sequences, they are often not persistent for all time points. Another advantage of our computational approach is that the algorithm can be implemented for real-time detection of blood volume loss.[11]

Limitations

Zollei et al.[23] showed that there is an increase in sympathetic activity when 350 to 400 mL of blood is withdrawn over 5 minutes. The AM_{BR} measure may also reflect the sympathetic activity because of the overlap in the frequency range between sympathetic activity (0.04 to 0.15 Hz) and possible respiration rates (0.05 to 0.35 Hz) used for the computation of AM_{BR} series. Sympathetic activity could be assessed using PPG-variability measurements, which may allow us to distinguish changes in sympathetic activity from respiratory influences on the PPG waveform.[3] No significant changes in AM_{BR} suggest that the sympathetic activation did not directly impact PPG modulation at the breathing rate during 900 mL blood withdrawal, where the time of withdrawal varied from 12 to 40 minutes among the subjects. Rapid versus slow blood withdrawal may affect the sympathetic influence and timing of any influence. Sympathetic influences also vary based on measurement site location, and it was previously shown that the ear

is relatively immune to sympathetic induced vasoconstriction during the cold pressor test.[12] Therefore, we do not expect the sympathetic influence to significantly alter our results for the ear measurement location, which we have identified to be the best candidate for blood volume detection. Given these possible complications, our approach to detect signatures of blood loss in the HR frequency band is another advantage of our method.

Middleton et al.[3] outlined other limitations with the blood donation model including the inability to study uncontrolled hemorrhaging that may be expected in the clinical setting. In the present study, we focused on an early case of Class I blood loss, and an uncontrolled decrease in blood loss may quickly move past Class I leading to other hemodynamic effects that could be identified by HR or BP. We previously showed the successful application of this method to an independent LBNP study, which has a different set of limitations than the current blood donation model, and the present method was successful in detecting blood loss in both settings.[11]

This study was performed in a well-controlled environment with a limited number of volunteers. Further investigation with a larger number of participants and under various conditions is required to determine the full utility and potential of this method in clinical applications.

CONCLUSION

We have shown that blood loss in awake, spontaneously breathing subjects induces detectable changes in the PPG waveform. These changes, which have previously been elusive to clinicians and investigators, may be captured by the analysis of time-frequency spectra, in which we have shown that the spectral amplitudes at the heart rate frequency significantly decrease during blood loss. Our computational technique, which can be performed in real time, is a step towards providing effective monitoring of blood volume in patients experiencing internal or external hemorrhage.

DISCLOSURES

Name: Christopher G. Scully, MS.
Contribution: This author helped analyze the data and write the manuscript.
Attestation: Christopher G. Scully has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
Conflicts of Interest: The author has no conflicts of interest to declare.
Name: Nandakumar Selvaraj, PhD.
Contribution: This author helped analyze the data and write the manuscript.
Attestation: Nandakumar Selvaraj has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Name: Frederick W. Romberg, MS.
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Conflicts of Interest: The author has no conflicts of interest to declare.
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Conflicts of Interest: The author has no conflicts of interest to declare.
Name: John P. Florian, PhD.
Contribution: This author helped write the manuscript.
Attestation: John Florian approved the final manuscript.
Conflicts of Interest: The author has no conflicts of interest to declare.
Name: David G. Silverman, MD.
Contribution: This author helped design the study, conduct the study, and write the manuscript.
Attestation: David G. Silverman approved the final manuscript.
Conflicts of Interest: David G. Silverman has a patent pending for an alternative means of volume assessment, assigned to Yale University School of Medicine.
Name: Kirk H. Shelley, MD, PhD.
Contribution: This author helped design the study, conduct the study, and write the manuscript.
Attestation: Kirk H. Shelley approved the final manuscript.
Conflicts of Interest: Kirk H. Shelley has a patent pending for an alternative means of volume assessment, assigned to Yale University School of Medicine.
Name: Ki H. Chon, PhD.
Contribution: This author helped analyze the data and write the manuscript.
Attestation: Ki H. Chon has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
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