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Software for Analysis of Heart Rate and Blood Pressure Time-series Data from the Valsalva Maneuver

Teresa Jones[1], **Ana-Marija Hristovska**[2], **Mette S. Olufsen**[1]

[1] Department of Mathematics, North Carolina State University [2] Department of Anesthesiology & Intensive Care Medicine, Copenhagen University Hospital - Hvidovre

Corresponding Author

Mette S. Olufsen
msolufse@ncsu.edu

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Abstract

The Valsalva Maneuver (VM) is a low-risk highly accessible test that measures the baroreflex-induced heart rate (HR) and blood pressure (BP) response to forced breathing against 40 mmHg intrathoracic pressure for 15 s. This study demonstrates the ValsalvaAnalyzer software, which combines modeling and data analysis to extract biomarkers from time-series BP and electrocardiogram (ECG) data. The software, programmed in MATLAB, incorporates a graphic user interface, making data analysis and mathematical modeling predictions accessible to clinicians. The software calibrated for PCs and Macs reads ECG and BP data measured during the VM. It automatically identifies R and S peaks in the ECG signal, with integrated user verification to ensure the accuracy of the captured signals. The RR intervals are used to predict HR, and the QRS complex magnitude changes are used to predict respiration. Systolic and diastolic BP are captured, and the user identifies the onset and VM duration from the SBP. The software automatically detects the four VM phases and the intrathoracic pressure. The latter is obtained by merging the respiratory signal at rest with the 40 mmHg pressure the subject breathes against during the VM. The most commonly reported clinical VM markers computed from the HR and BP data are reported along with estimates of patient-specific parameters obtained using a differential equations model, which predicts sympathetic and parasympathetic dynamics. The final output, over 35 metrics, includes BP and HR, maximum and minimum HR, the Valsalva ratio, and measures of baroreflex sensitivity, all saved to a spreadsheet. The software is intended to analyze the VM data, but the methodology can be extended to other autonomic tests. Its strength lies in combining quantities extracted from raw data with model outputs, augmenting clinical data with a mathematical model providing insight into autonomic immeasurable quantities.

Introduction

The Valsalva Maneuver is a low-risk, non-invasive, inexpensive, and highly accessible test to measure the autonomic nervous system function through forced expiratory exercises[1,2]. The maneuver is performed by patients exhaling into a positive end-expiratory pressure (PEP) device connected to a manometer, holding an intrathoracic pressure of 40 mmHg for 15 s, typically in a supine or seated position[2,3]. This maneuver simultaneously challenges the autonomic and cardiovascular systems, mimicking the physiological response to stressors like straining while lifting heavy objects or equilibrating pressure when flying[4]. This test is used frequently in clinical settings[2,5], but more tools are needed to analyze data quantifying the underlying physiological mechanisms. Neurological modeling applied to the VM may facilitate the identification of improved diagnostic criteria and causal mechanisms for autonomic dysfunction[5].

The VM has four phases[6]. It is facilitated by a breath hold, which increases intrathoracic pressure, compressing the cardiac chambers and the thoracic aorta and emptying blood in the systematic circulation, thereby causing a transitory increase in BP. Phase I is characterized by a continuous increase in intrathoracic pressure, reducing the venous blood flow to the heart, the stroke volume, and the mean arterial BP. In response, the high-pressure arterial baroreceptors are activated. The decrease in BP during the early part of Phase II causes a parasympathetic withdrawal, increasing HR. During the late part of Phase II, sympathetic excess mediates vasoconstriction, increasing BP and HR. In healthy patients, BP recovery is the resting value before releasing the breath hold. Phase III is reciprocal to Phase I. This phase is initiated when the patient releases their breath hold, which causes sharp decreases in intrathoracic pressure and arterial BP, usually followed by an HR increase. In Phase IV, increased venous return to the heart and persisting vasoconstriction from late Phase II results in a marked BP increase called an overshoot. Stimulation of high-pressure arterial baroreceptors by the overshoot activates the vagal nerve to reduce HR. The patient is expected to recover to pretest values approximately 10-30 s after the VM onset[2,5].

The VM is used for bedside evaluation of autonomic and cardiovascular function[7]. The most common VM biomarker, the Valsalva ratio (VR)[8,9,10], measures parasympathetic function as the ratio between the longest RR interval after the breath-hold onset and the shortest RR interval. It has previously been established that postural orthostatic tachycardia (POTS) is associated with a high VR and a notable BP overshoot in Phase IV[11], also called N-pattern response[1]. Another metric is the vagal baroreflex sensitivity. This metric is estimated in the early part of Phases II and IV as the regression slope between RR intervals and characteristic BP changes[3,10,12,13,14]. Qualitative analysis of BP responses to the VM can identify different heart murmurs[9], and a distinctive square-wave response is a bedside indicator of impaired ventricular function and heart failure[15,16]. The absence of BP overshoot and the presence of bradycardia after breath-hold release is indicative of dysautonomia[13]. The V-pattern response with progressive BP decline in late Phase II and slow recovery in Phase IV is characteristic of neurogenic orthostatic hypotension, indicating alpha-adrenergic baroreflex failure[1]. Other studies have demonstrated the efficacy of using the VM to supplement head-up tilt testing in screening for orthostatic disorders[1,17,18].

Numerous studies have examined autonomic function by analyzing HR and BP responses during the VM[1], but no open-source automated systems are available to quantify the baroreflex function. Many studies have analyzed data from the VM[19,20,21,22], and a few software specialize in autonomic function, including VitalScan by Medeia[23] and Kubios[24]. VitalScan utilizes ECG and BP signals, but Kubios only analyzes ECG signals. The VitalScan website notes that this software assesses autonomic function but lacks a detailed description of calculated quantities. Kubios determines HR variability (HRV) and the VR[25]. To address these gaps, this study develops a new open-source software that calculates the most common VM indices[1,25].

The software analyzes ECG and BP data measured during a VM maneuver holding an intrathoracic pressure of 40 mmHg for 15 s. We recommend including at least 30 s stable data before and after the VM. After identifying HR and systolic BP, the VM onset and release, a set of clinical markers are extracted. In addition, this software uses the mathematical model by Randall et al.[20] to predict sympathetic and parasympathetic signaling along with parameters characterizing the baroreflex sensitivity. Two critical features are the ability to read data from noisy signals, to identify and remove artifacts, and the automatic detection with manual correction of VM markers. The latter is vital for patients with autonomic dysfunction, where purely automatic detection fails. This exposition includes all results for subject 1 and a protocol to extract results for subjects 2-8. Results for subject 1 is included in the code, text, and figures, and results for all subjects are shown in **Supplementary Figure 1**, **Supplementary Figure 2**, **Supplementary Figure 3**, **Supplementary Figure 4**, **Supplementary Figure 5**, **Supplementary Figure 6**, **Supplementary Figure 7**, and **Supplementary Figure 8**.

The Valsalva Analyzer software calculates adrenergic and vagal indices characterizing the response to the Valsalva maneuver (VM) from continuous ECG and BP measurements. To demonstrate the software, we provide a brief description of the experimental setup followed by a detailed description of the software. The software reads data extracted from patient records stored in LabChart. The analysis is demonstrated on a healthy control patient, but the software includes data from eight subjects with a range of autonomic responses. Below, we discuss patient examples and describe the protocol to install and run the software. The protocol includes references to figures generated within MATLAB. To distinguish these from figures included in the representative results, these are all referred to as MFigure #.

Protocol

Three example datasets were selected from adult male (age 30-45) blood donors eligible for blood donation according to the Danish legislation[26], three from a study examining the effects of preoperative opioids on adult (age 40-61) patients receiving knee and hip replacements[27], and one from a patient diagnosed with postural orthostatic tachycardia syndrome (POTS)[28]. The BD and opioid studies were approved by the local ethics committee in Denmark (H-19069845 and H-20071567, respectively), registered with the Danish data protection agency, and registered at ClinicalTrials.gov (NCT04499664 and NCT04902222, respectively). Frederiksberg and Bispebjerg Hospitals, Denmark ethics committee approved using data to diagnose POTS for research. All data were deidentified before being prepared as examples for this software, and all subjects could

speak and understand Danish and gave informed consent to participate in their respective studies.

NOTE: The software provides tools to extract markers from patient measurements of ECG and BP. Users can enter a subject ID number, age, sex, weight, and height. This information is optional. Users are encouraged to cite this manuscript. The software includes example data from measurements of ECG (channel 1), HR derived from ECG (channel 2), and BP (channel 3). **Table 1** includes a detailed patient description and patient notes. The eight example deidentified data sets provided with this software were extracted from previously published studies[26,27,28]. Data were selected to demonstrate characteristic features observed during the VM. The intention is to demonstrate the software's features, not to conduct a specific clinical study. Exclusion criteria for the blood donor and opioid studies include alcohol and drug abuse, and habitual use of opioids, arrhythmia or heart failure, history of orthostatic hypotension. Exclusion criteria not explicitly listed for each study are listed in[26,27].

Patient	Age (years)	Sex (m/f)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Notes
Subject 1	35	m	176	92	29.7 (ob)	**Normal response**
Subject 2	31	m	180	70	21.6 (nw)	**Normal response**
Subject 3	30	m	187	93	26.6 (ow)	**Large overshoot in phase IV. HR artefacts due to ECG signal noise**
Subject 4	42	m	175	76	24.5 (nw)	**V-response typical for autonomic dysfunction. Inadequate chronotropic effect in phase II. No BP recovery in late phase II. Missing overshoot in phase IV. Long PRT**
Subject 5	37	f	165	85	31.2 (ob)	**No overshoot in phase IV**
Subject 6	61	f	170	107	37.0 (ob)	**Inadequate BP recovery in late phase II. Maximal BP does not equal end BP in late phase II**
Subject 7	42	m	177	84	26.8 (ow)	**Missing overshoot in phase IV**
Subject 8	58	f	166	77	27.9 (ow)	**Negligible BP drop in early phase II. HR artefacts due to ECG signal noise. BP artefacts in phase IV**
nw: normal weight BMI (18.5-25), ow: over-weight (BMI (25-30), ob: obese (BMI > 30)[26]						

Table 1. Please click here to download this Table.

1. Experimental setup

1. Collect continuous measurements of BP from a finger blood pressure cuff placed on the index and middle fingers on the non-dominant hand (**Figure 1A**). Position the hand at the heart's level to eliminate gravity's effects (**Figure 1B**).
2. Measure ECG using a standard 3-electrode ECG with electrodes placed at equidistance from the heart at the left and right atrium and on the left side lower edge of the rib cage. After instrumentation, perform the Valsalva maneuver by the subject exhaling into a positive end-expiratory pressure (PEP) device connected to a manometer (**Figure 1C,D**).
3. Ask the subject to breathe normally for 1-5 min until a stable signal is generated. To have sufficient data for analysis, record stable signals for at least 30 s before and after the VM. Perform VM by asking the subject to exhale for 15 s, holding a pressure of 40 mmHg (**Figure 1D**). During the recording, if possible, turn calibration off to avoid gaps in BP signal.
4. The software analyzes signals exported from LabChart to MATLAB at 1,000 Hz. In the Export as MATLAB menu, include channels with ECG, HR, and BP signals, and intrathoracic pressure if recorded. Note the channel numbers for each signal. In the menu, choose **32-bit floating pint, Upsample to same rate**, uncomment **Comments** and **Event markers**. Click **OK** to export .mat file and **Cancel** to stop the export.

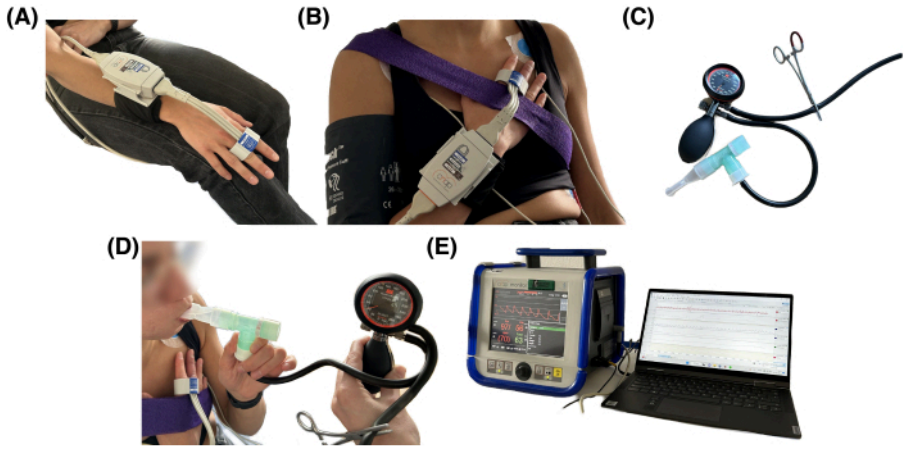

Figure 1: Instrumentation, BP cuff, ECG placement, monitor, VM equipment. (**A**) Mounting of the BP cuff on the index and middle finger on the non-dominant hand. (**B**) The finger BP cuffs are fastened at the level of the heart, using a cohesive CoFlex bandage to eliminate the effects of gravity. (**C**) PEP device attached to a manometer. (**D**) The seated subject exhales into a positive end-expiratory pressure (PEP) device connected to a manometer, holding an intrathoracic pressure of 40 mmHg for 15 seconds. (**E**) The CNAP module continuously measures BP and is connected to a computer that processes and saves data using LabChart. Please click here to view a larger version of this figure.

2. Software installation, data upload, and startup

NOTE: This protocol describes how to export signals from LabChart to MATLAB, but the protocol does not depend on recordings stored in this software. We refer to their manuals for signals recorded by other software and note that conversion may be needed to export recordings in the correct format. Data must include continuous time-series measurements of ECG and BP.

1. Download the ValsalvaAnalyzer Software. Clone the GitHub Repository at https://github.com/msolufse/ValsalvaAnalyzer. Click the green **Code** button. Click **Download** ZIP.
2. Navigate to the folder ValsalvaAnalyzer. The main script DriverBasic.m should be replaced by the ValsalvaAnalyzer folder, and all other scripts (.m files) should be in the Core folder. The software includes subfolders: Figures, Markers, Optimized, Sensitivities, and WS.

   NOTE: The folder Figures stores generated figures. This folder contains two subfolders (Data and Model_fits): Data stores for figures generated from the data analysis and figures generated by the differential equations model. The Labchart folder includes the exported .mat files but not the original LabChart files. The folder Markers hold the spreadsheets with clinical ratios (one file per subject). The Sensitivities and Optimized folders include .mat files with the sensitivities and estimated parameter values. The folder WS contains .mat files generated when cleaning data. The main folder ValsalvaAnalyzer includes DriverBasic.m, the core script needed to run the program. When the software is downloaded. The folder Labchart includes a .mat files for each of the eight example subjects, while the folders with results (Markers, Sensitivities, Optimized, and WS) only have results for Subject 1. As the example datasets in the folder are analyzed, output files will be stored in these folders. The file Patientinfo.xls (and Table 1) includes patient information (age (years), sex (m/f), height (cm), weight (kg), and BMI (kg/m^2)) for each of the eight example datasets. The weight is characterized as normal (nw), overweight (ow) or obese (ob)[29], and it is noted if the subject has a normal or pathological VM response.
3. To run the software, go to the folder ValsalvaAnalyzer and open the file **DriverBasic.m** in MATLAB. In the top panel, click **Editor**, and then click the **Green triangle labeled Run** to execute the program.

3. Software platform

NOTE: The software distributed by GitHub has been tested on Windows (Windows 11 Education) and Mac (MacOS Sonoma, version 14.3) and uses MATLAB (version R2023a). Defaults are set to the MacOS environment with suggestions for Windows.

1. From the pop-up menu **Select Figure Parameters**, select the software platform type, the figure font size, marker size, and linewidth.
2. Click **OK** to accept and proceed to Step 4 or click **Cancel** to end the program.

4. Patient selection

NOTE: This step involves the selection and analysis of data. The software will read .mat files from the Labchart folder.

1. Select any number of patients from the list using the mouse. The button **Select all** marks all patients. Patient labels are determined from the filenames. These are used in all exported files. Click **OK** to proceed to Step 5 or **Cancel** to exit the program.

5. Selection of operation

NOTE: The data analysis methods are listed in a menu providing available operations. These include methods for cleaning ECG and BP data, identifying VM phases, and computing VM features. The former is carried out on the raw data using the sampling rate embedded in the measurements (the attached examples are sampled at 1,000 Hz). The identification VM features use cleaned HR and systolic SBP signals subsampled at 10 Hz. The subsampled data are also used to determine sympathetic and parasympathetic signaling obtained from solving the differential equations model by Randall et al.[20] Generated figures are saved as .png files and stored in the Figures folder and generated numbers are stored in a spreadsheet (.xlsx) in the Markers folder. The layout of the functions within this software is illustrated in **Figure 2**.

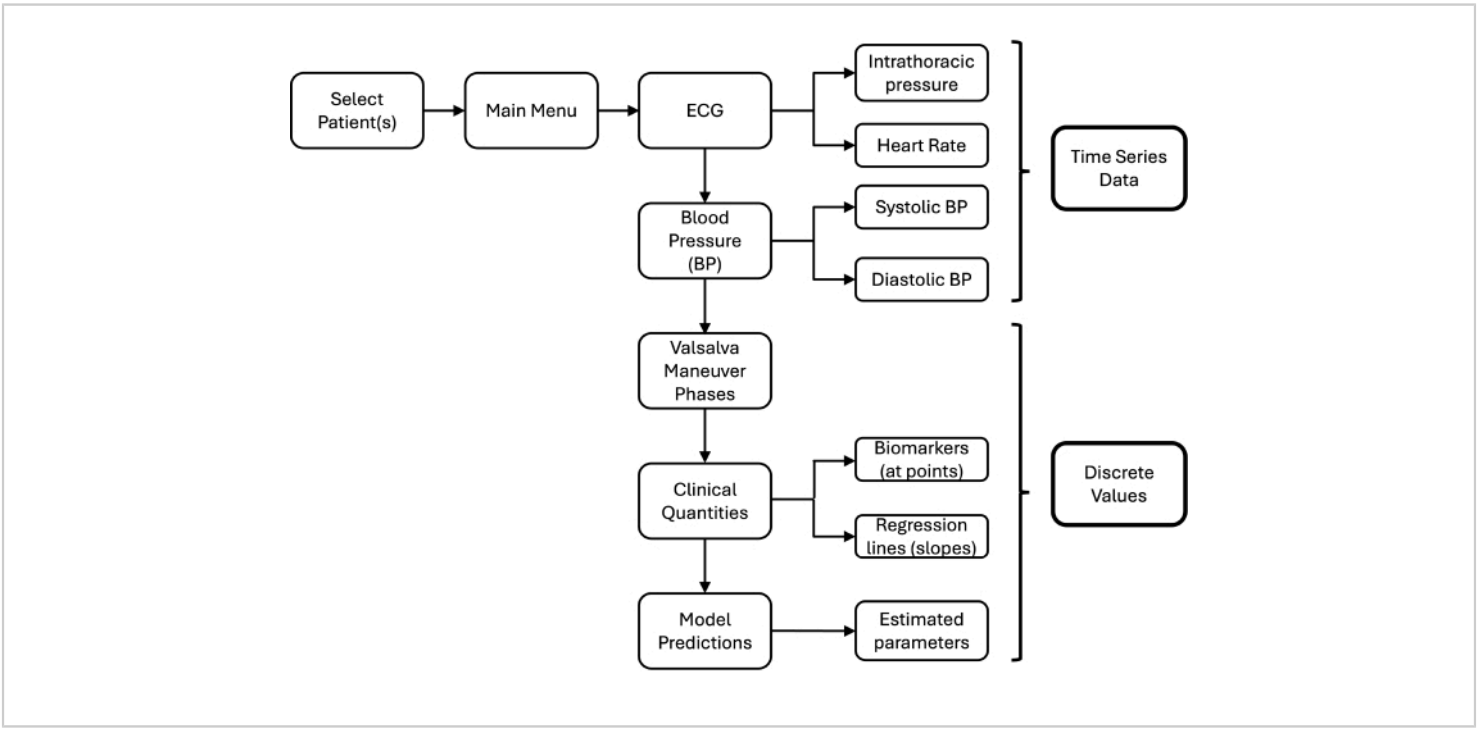

Figure 2: Software operations. After selecting patients, the software provides the option to correct measured signals, including (1) the ECG, from which heart rate and respiration are extracted, (2) the beat-to-beat blood pressure (BP) signal from which systolic and diastolic BP are extracted. After these procedures, the software identifies the Valsalva maneuver

phases and extracts clinical biomarkers. Finally, we provide the option of running a computational model predicting baroreflex function. Please click here to view a larger version of this figure.

1. Select operation(s) to be performed in the **Subset selection menu**. Complete operations in descending order, e.g., patient information should be inputted before the ECG is analyzed. If more than one subject and task is selected from the Operation Selection menu in step 4, the software will complete the first task for all subjects before moving to the next task.
    1. The software includes the following operations: Patient Information (Operation 1, Step 6); Electrocardiogram (ECG; Operation 2, Step 7); Heart Rate (HR; Operation 3, Step 8); Respiration (Operation 4, Step 9); Blood Pressure (BP; Operation 5, Step 10); VM Phases (Operation 6, Step 11); Clinical Ratios (Operation 7, Step 12); Model Prediction (Nominal; Operation 8, Step 13); Sensitivity Analysis (Operation 9, Step 14); Optimization (Operation 10, Step 15); Plot Model Predictions (Operation 11, Step 16); Summary (Operation 12, Step 17).
2. After selecting operations, Click **OK** to proceed to the operation or click **Cancel** to return to Step 4.

6. Patient information (Operation 1)

NOTE: The first operation involves inputting patient characteristics (ID, Age, Sex, Height, and Weight) the channel numbers from the exported Labchart file (ECG, HR, BP, intrathoracic pressure - if available), and identifying the time range for the data analysis.

1. Enter patient ID (integer), Age (integer, years), Sex (m/f, male/female), Height (real number, cm), and Weight (real number, kg). Click **OK** to proceed or **Cancel** to return to Step 5. The software will run without selections. Characteristic values for the eight subjects are listed in **Table 1**. These values are not used in the data analysis but may be helpful for summary statistics.
2. Identify in what channel each signal is stored, default values are: Channel 1 (ECG), channel 2 (HR), channel 3 (BP), channel 4 (intrathoracic pressure - Pth) is set to 0. The example datasets 1-8 does not include this signal.
3. MFigure 1 (**Figure 3**) displays the ECG (mV) top, HR (bpm) center, and BP (mmHg) bottom as functions of time (seconds). Select data to analyze. Include approximately 20 s before and after the VM.
4. Positioning the crosshair, click once with the mouse at the start (~20 s before the VM) and a second time at the end (~20 s after the VM). The selected data will appear in red in MFigure 1. Click **Save and Exit**. MFigure 1, with the selected data, will be saved in the folder Figures/Data under the name [patient name] + _dataAnalyzed.png.

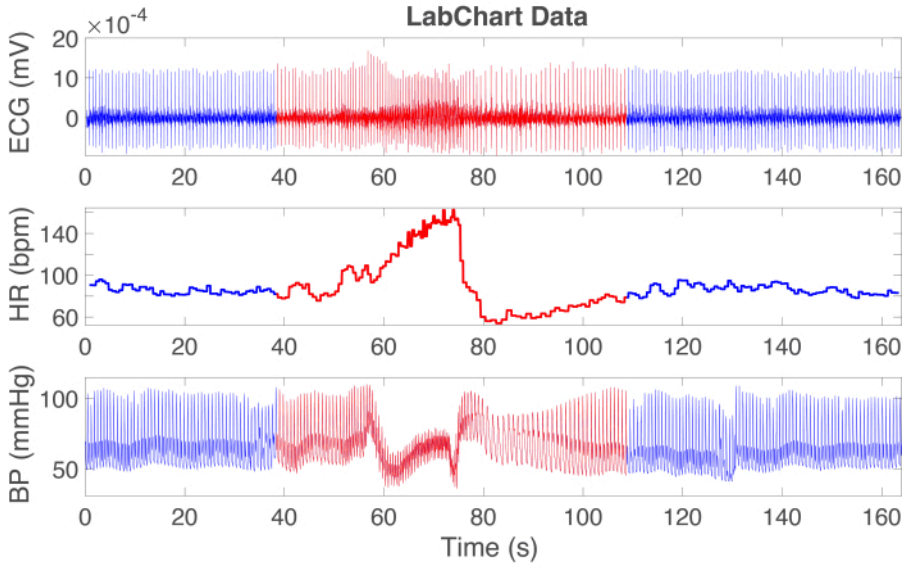

Figure 3: Graphs depict the ECG (mV, top), heart rate (HR bpm, center), and blood pressure (mmHg, bottom) data. The blue trace shows all data extracted from LabChart, and the red trace shows data selected for analysis in this study. The red region containing ECG, HR, and BP starting and ending ~20 sec before and after the VM maneuver. Please click here to view a larger version of this figure.

7. Electrocardiogram (Operation 2)

NOTE: Operation 2 involves identifying R and Q or S peaks in the ECG signal (removing extra and/or adding missing peaks). This operation is performed on the raw data sampled at 1,000 Hz. The magnitude of the QRS complex (the absolute distance between the R and Q or S peaks) is used to create the respiration signal before and after the breath hold.

1. MFigure 1 displays the ECG signal (black line). Automatically detected R peaks are marked with red and Q or S peaks with blue circles. The objective is to correct misplaced peaks. The total number of R and Q or S peaks is printed to the right of the graph. This task can only be completed if the number of R and S peaks are the same. To correct the misplaced peaks, scroll with the hand to the right through the signal and stop when the peaks to be corrected are in the window. An example of an extra wrongly placed S peak and the signal after the peak has been removed is shown in **Figure 4**.

NOTE: The R and S peaks are identified using the methodology described by Randall et al.[20]. This algorithm uses findpeaks.m to find peaks between 25% to 200% of the mean signal. The sampling rate is 1,000 Hz (encoded in the data), and the MinPeakDistance is set to 1.5. R peaks are found from the raw signal, and S (or Q) peaks are found by analyzing the negative of the signal. It should be noted that for some datasets, the algorithm will identify S peaks and for some Q peaks.

Examples are shown in **Figure 4A**. The QRS magnitude is used to determine respiration as described by Randell et al.[20].

2. Repeat correction until the number of S and R peaks are the same using the steps described below.
    1. To correct the misplaced peaks, scroll to the right and stop when the peak(s) to be corrected are in the window.
    2. If a peak is missing, placed wrongly, or an extra peak is marked, scroll to the location of the peak. Press **Enter** on the keyboard, and a crosshair appears. Click on the point to correct. The next menu query is: Add or remove point? Select either **Add** (step 7.2.3), **Remove** (step 7.2.4), or **Cancel**, returning to Step 7.2.1.
    3. Click **Add**, and the point marked will be added and appear in red (R peak) or blue (Speak). The program will use the exact location of the click and automatically classify the point as R or S.
    4. Click **Remove**, and the point marked will be removed. Repeat this step if the point does not disappear, returning to task 7.2.2.
3. Repeat step 7.2 until all R and S peaks are identified correctly and the number of R and S (or Q) peaks are the same. Then press **Enter** on the keyboard. On the prompt asking to correct points, click **No**. Continue with operation 3 (HR). If the time series has no errors, but the number of R and S (or Q) peaks is not identical. To correct this error, return to Step 7.2. Note that for consistency within a signal, choose either S or Q peaks.
4. If the user clicks **No** when the number of R and S peaks is not identical, a new menu pops up, noting that the **Number of R and S peaks must be equal**. Inspect data. Click **OK** and the code returns to Step 7.2.

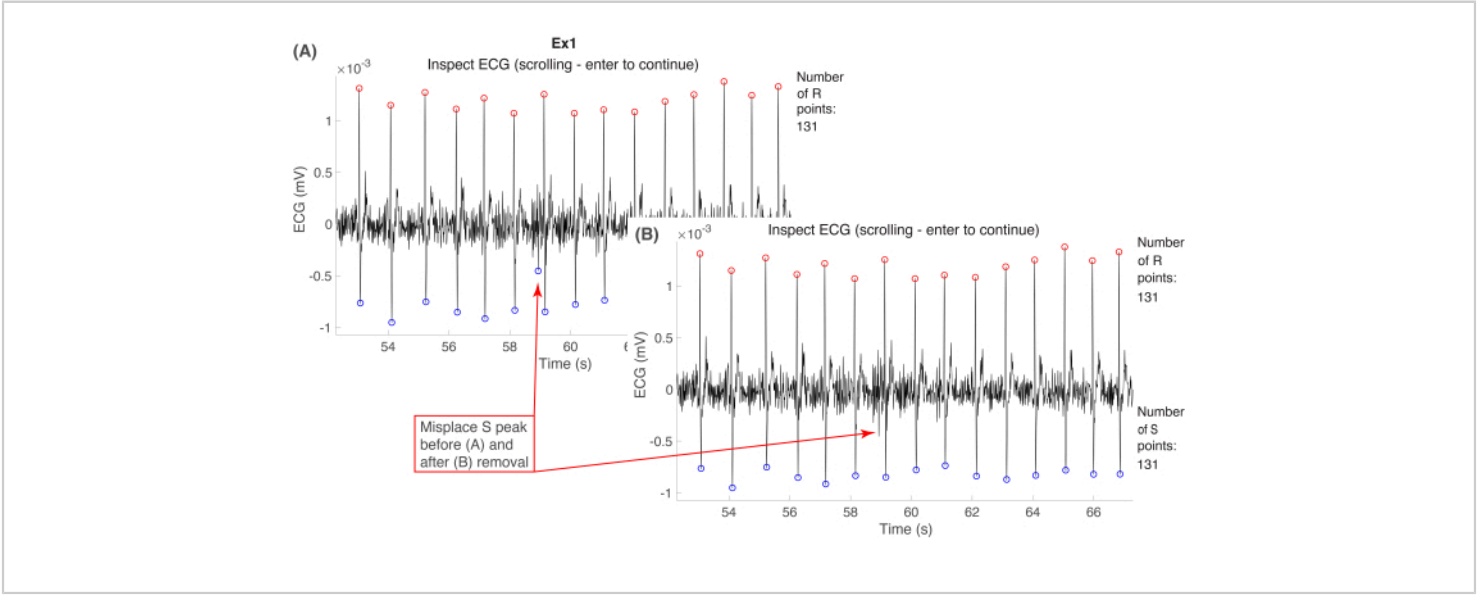

Figure 4: Graphs used to guide ECG (mV) correction. The figure shows the ECG trace (black), R waves (red circles), and S waves (blue circles). (**A**) The graph has a misplaced S wave. In (**B**), this S-wave has been removed. A clean ECG

signal will have the same number of R and S peaks, as noted on the right side of the graph. Please click here to view a larger version of this figure.

8. HR (Operation 3)

NOTE: This step involves converting the RR intervals to HR. After the ECG signal has been corrected (as described above), for most datasets, the HR signal is smooth. However, if the HR signal has artifacts (example shown in **Figure 5A**). Operation 3 provides the opportunity to correct the signal (example shown in **Figure 5B**).

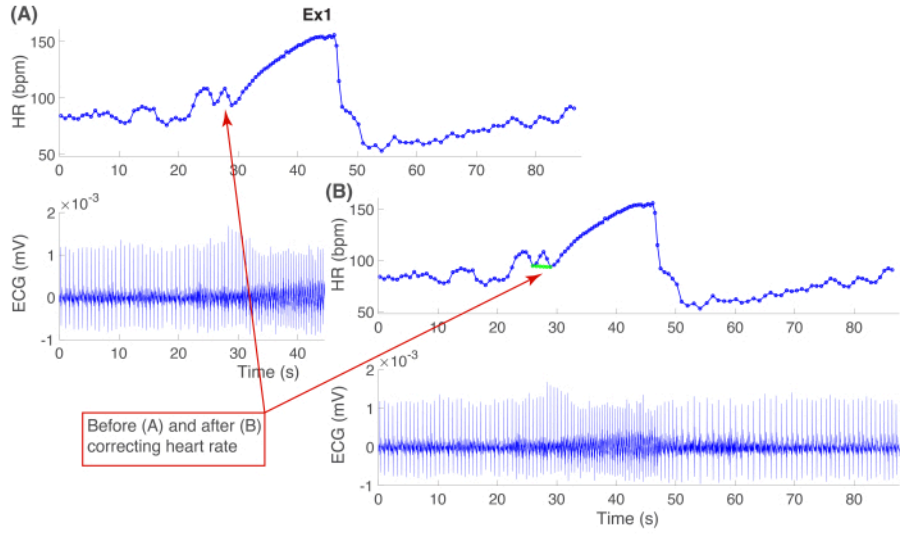

Figure 5: Graphs used to guide heart rate (HR, bpm) correction. (**A**) Heart rate (blue line) generated from the corrected ECG. The small blue circles mark the times at which the heart beats. (**B**) Example of a spline connecting two points (green line), removing an artifact from the heart rate signal. Please click here to view a larger version of this figure.

1. MFigure 1 (**Figure 5A**) displays HR (bpm) in the top panel and ECG (mV) in the bottom panel. The HR (bpm) is computed from the corrected ECG RR peaks. If the HR signal does not have artifacts, click **Save and Exit**, and continue to Operation 4 (Respiration). If there are errors in the data (compare the two panels in **Figure 5**), click **Correct Heart Rate** and proceed to Step 8.2.
2. Scroll along the HR signal and locate artifacts. Press **Enter** on the keyboard when viewing a region to correct. Proceed to Step 8.3.
3. Click **OK** on the menu querying **Click at points to connect**. Align the crosshair over the first point before the artifact and click once with the mouse. Then, align the crosshair over the first point after the artifact and click a second time with the mouse. A linear spline (plotted in

green) connects the two points. A menu asks, **Accept change?** Answers: **Yes** (proceed to Step 8.3.1), **Undo** (go to Step 8.3.2), and **Add** change (go to Step 8.3.3).

1. Select **Yes** to accept the linear spline, exit this operation, and return to Step 4. Select **Undo** to remove the linear spline and return to Step 8.2. Select **Add** to keep the linear spline and return to Step 8.2 to allow additional corrections.

4. MFigure 1 displays the HR (bpm) and ECG (mV) in the top and bottom panels. The figure is saved in the folder Figures/Data under the name [patient name] + _HeartRateECG.png. To continue, press **Save and exit**. The signals will be stored at the sample rate (1,000 Hz for the example datasets) embedded in the measurements.

9. Respiration (Operation 4)

NOTE: A respiration signal is extracted from the corrected ECG signal by computing the QRS complex magnitude, fitting a piecewise cubic Hermite interpolating polynomial spline (using interp1.m with the pchip method) through this difference as described in the study by Randall et al.[20].

1. MFigure 1 (**Figure 6**) depicts the respiratory signal extracted from the difference between the corrected R and S peaks. Inspect the graph, click **Save and exit**, and continue to operation 5 (blood pressure). MFigure 1 will be saved in the folder Figures/Data under the name [patient name] + _RespiratorySignal.png.

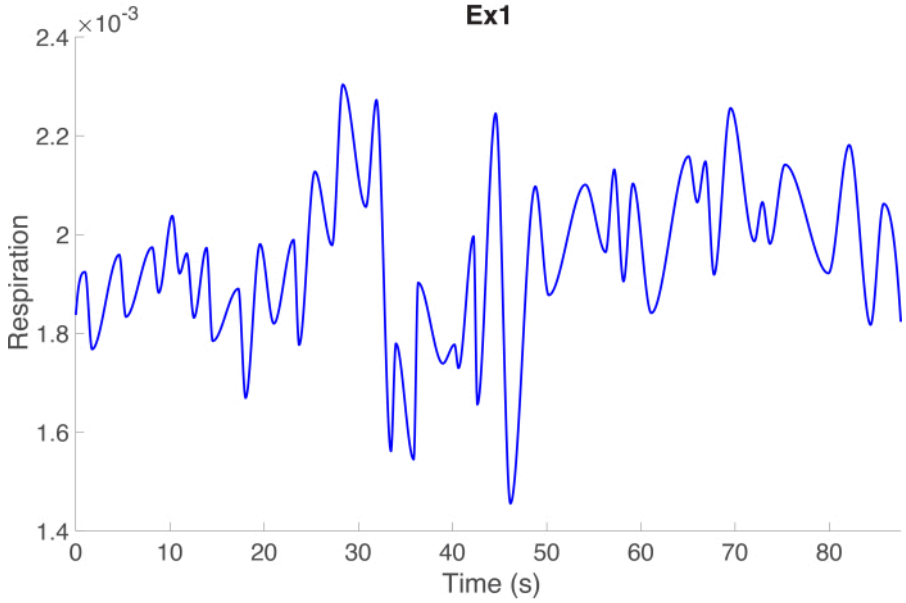

Figure 6: Respiratory signal. Respiratory signal (blue line) generated from the QRS interval amplitude changes, as described by Randall et al.[20]. Please click here to view a larger version of this figure.

10. Blood Pressure (Operation 5)

NOTE: This step involves extracting systolic and diastolic BP. Two curves are formed by generating a spline through selected data points. For this operation, the user can correct the automatically detected curves. Given the significant change in BP, correction is likely needed immediately following the breath hold release.

1. MFigure 1 (shown in **Figure 7A**) displays a zoomed window with the BP data. Align the crosshair with a BP peak and click once with the mouse. Then, align the crosshair on the next peak to the right and click again. The distance between the two peaks estimates the cardiac cycle length. This distance is needed to identify systolic and diastolic peaks. MFigure 2 (**Figure 7B,C**) appears, displaying automatically detected systolic and diastolic signals.

   NOTE: The systolic and diastolic peaks are found using peaks2.m, which inputs the length of the cardiac cycle at rest to set the minimum peak distance. Similar to R. The systolic peaks are found directly from the signal, and diastolic peaks are found by analyzing the negative signal.

2. Systolic BP correction: A menu appears, prompting: **Do you want to correct systolic points?** Press **Yes** (Step 10.4) to initiate a protocol for fixing the systolic BP and **No** (Step 10.3) to proceed to correct the diastolic BP.

3. Diastolic BP correction starts with a menu asking: **Do you want to correct diastolic points?** Press **Yes** (Step 10.4) to initiate a protocol for fixing the diastolic BP and **No** to proceed. Press **Save and exit** and continue with operation 6 (Valsalva maneuver phases).

   NOTE: The protocol for correcting diastolic BP is identical to the one correcting systolic BP; both are described in Step 10.4.

4. MFigure 2 shows a zoom of the first 40 s data. Inspect the region and keep scrolling until a wrong point appears (corrected trace shown in **Figure 7B,C**). Press **Enter** on the keyboard and place the cross-hair on the last correct peak before the misplaced point(s), click on this point, and continue moving the cross-hair, clicking on all points to be corrected, ending with a correct point. Press **Enter** when finished. A dashed line appears connecting the corrected points (examples shown in **Figure 7B,C**).

   NOTE: Correction can be repeated until no more segments need to be modified. For each section, the corrected points are connected with a red (SBP) and green (DBP) dashed line attached to existing points at each end. The continuous BP signal, plotted in blue, is used as a guide. The system will record peaks clicked even if they do not align with the BP signal. This operation corrects the systolic and diastolic signals. Within each signal, only correct points associated with the signal, i.e., do not try to correct diastolic points when correcting the systolic BP or vice versa.

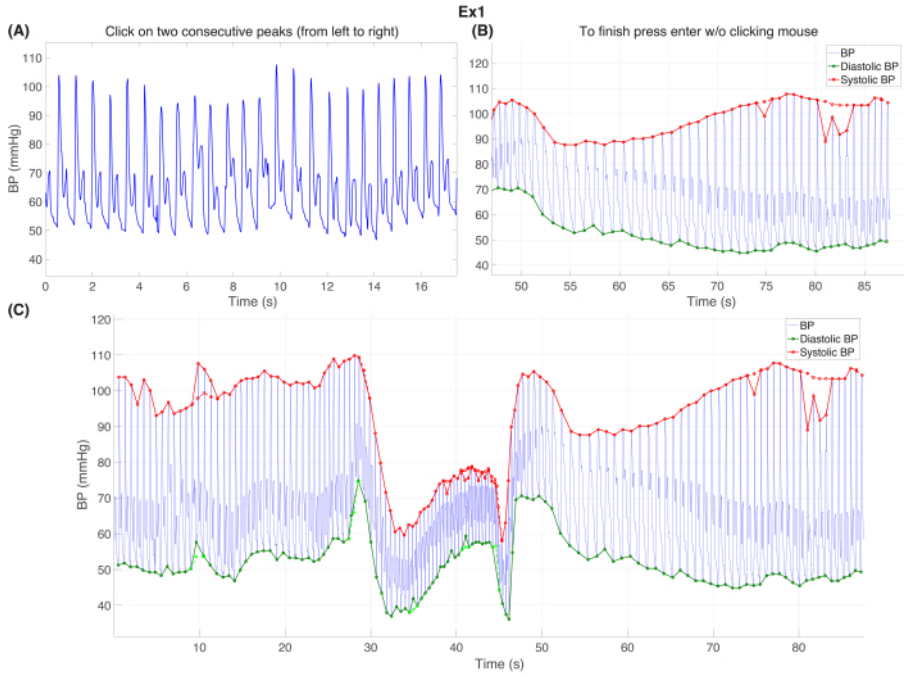

Figure 7: Blood pressure correction. (**A**) Zoom of the BP signal at rest. The user is asked to click on two consecutive peaks to determine the average length of the cardiac cycle. (**B**) A zoom of the original and corrected systolic (red) and diastolic (green) pressure. In this panel, the continuous blood pressure measurements (mmHg) are shown in blue. (**C**) The original and corrected systolic (red) and diastolic (green) blood pressure signals over the time range analyzed. In all panels, the continuous beat-to-beat BP signal is shown with a blue line, the SBP with a red line, and the DBP with a green line. For the SBP and DBP signals, each cardiac cycle is marked by small stars. Please click here to view a larger version of this figure.

11. Valsalva Maneuver (VM) Phases (Operation 6)

NOTE: Operation 6 involves the detection of the VM. This operation uses HR, SBP, DBP, and the intrathoracic pressure (if available) data subsampled to 10 Hz. The user is asked to identify the onset and release of the breath hold. The breath hold starts at the lowest BP value before the first peak and is released at the BP value before the second BP drop. After identifying these points, the software determines the four VM phases from the characteristics of the signals. These can be corrected manually, which is especially important when analyzing data for abnormal hemodynamic responses.

1. MFigure 1 depicts the continuous (thin line) and systolic (SBP, bold line) BP in the top panel (mmHg), HR (bpm) in the 2nd panel, respiration (Resp, mV) in the 3rd panel, and ECG (mV) in the bottom panel If intrathoracic pressure is available, this signal will be shown in the

3rd panel (Pth, mmHg), and the respiratory signal (Resp, mV) in the 4th panel. The Valsalva phases are automatically detected, and the software continues to step 1.3 for datasets without Pth measurements. To mark the VM start, align the crosshair with the breath-hold onset (the SBP minimum immediately before the significant SBP rise and HR decrease) and click once with the mouse.

2. To mark the VM end, align the crosshair with the breath-hold end (the BP value immediately before the 2nd SBP drop) and click once with the mouse. These points are used to determine the four VM phases in MFigure 2, displaying BP (mmHg) in the top panel, HR (bpm) in the center panel, and respiration (Resp, mV) in the bottom panel. If the intrathoracic pressure (mmHg) is measured it is shown between the heart rate and respiration panels.

3. A menu queries**: Accept indices?** Select **Yes** to complete the operation and continue to Operation 7 (clinical ratios). Select **No** to inspect the automated detection of phases delineated by vertical lines.

4. A menu queries: **Index Correction**. Select one, multiple, or all phases, then click **OK** to proceed to Step 10.4 for correction or **Cancel** to return to Step 11.1.

5. A crosshair appears in MFigure 2. The second line of the title describes the phase being corrected. For the selected phase, click the time, marking the onset of the phase. Repeat this operation until all selected phases have been corrected. The corrected times are shown with red vertical lines. Once all selected phases have been corrected, the menu reappears, querying: **Accept indices?** Clicking **Yes** continues to Step 11.5, and **No** reverts to Step 11.1. Note that the phases must be corrected in sequential order.

6. MFigure 3 (**Figure 8**) displays the final VM phases. The figure shows BP (mmHg) top panel, HR (bpm) center panel, and thoracic pressure (Pth, mmHg) bottom panel. This signal is obtained by merging the extracted respiratory signal with the measured or computed intrathoracic pressure enforced during the breath-hold. The four phases are shaded in gray. Click **Save and exit** and continue to operation 7 (Clinical ratios). This figure will be saved in the folder Figures/Data under the name [patient name] + _VMphases.png.

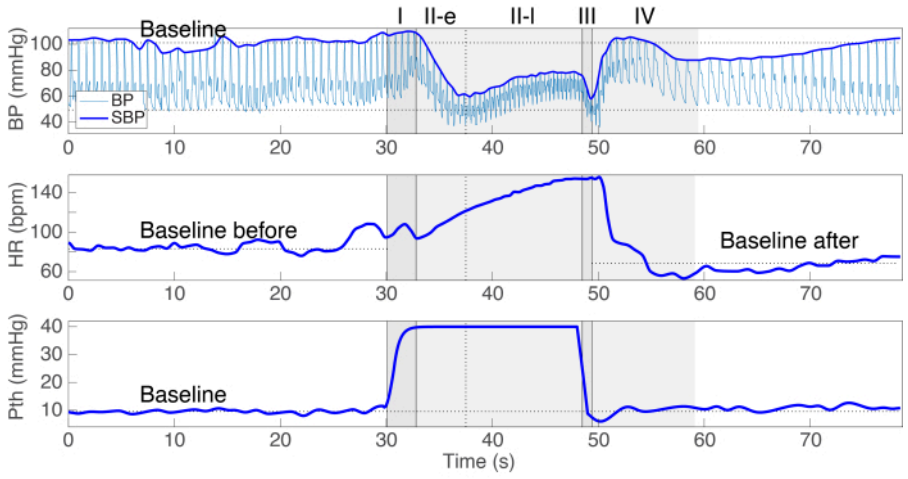

Figure 8: Valsalva Phases I-IV. The top graph shows continuous (light blue) and systolic (thick blue line) blood pressure; the second panel shows heart rate, and the bottom panel shows thoracic pressure. The latter is obtained by merging the respiratory signal with pressure during breath-hold (set to 40 mmHg). The Valsalva Phases I and III are marked with gray panels. Baseline values (mean SBP, HR before and after the VM) are denoted by horizontal dashed lines. Please click here to view a larger version of this figure.

12. Clinical ratios (Operation 7)

NOTE: This step computes clinical ratios characterizing the VM using HR, RR, and SBP, data subsampled to 10 Hz. All factors are listed in Table 2. These include patient characteristics (patient ID, age, sex, height, and weight), duration of the VM phases, minimum and maximum BP, HR, RR intervals within each VM phase[30,31], and pressure recovery time[32,33]. The slope and goodness of fit (R^2 value) of the HR and RR regression lines in early Phase II (cyan line) and IV (brown line), characterizing vagal stimulation and pressure increase in early (cyan line) and late (blue line) Phase II and early Phase IV (brown line). The latter determines sympathetic stimulation. In addition, the software characterizes the change in SBP, vagal[1,32,34,35], and adrenergic (BRS)[1,32,33,36] markers. Again, the automatically detected phases and points can be corrected as needed. For example, the maximum BP and minimum HR in early Phase IV are often misaligned. **Figure 9** depicts the clinical ratios before (panel **Figure 9A**) and after (panel **Figure 9B**) correction. **Figure 9C** depicts the ratios adapted from Palamarchuk et al.[1] and Sandroni et al.[31]. Note quantities displayed in this figure are derived from the values extracted from the data described in Steps 12.1-12.4.

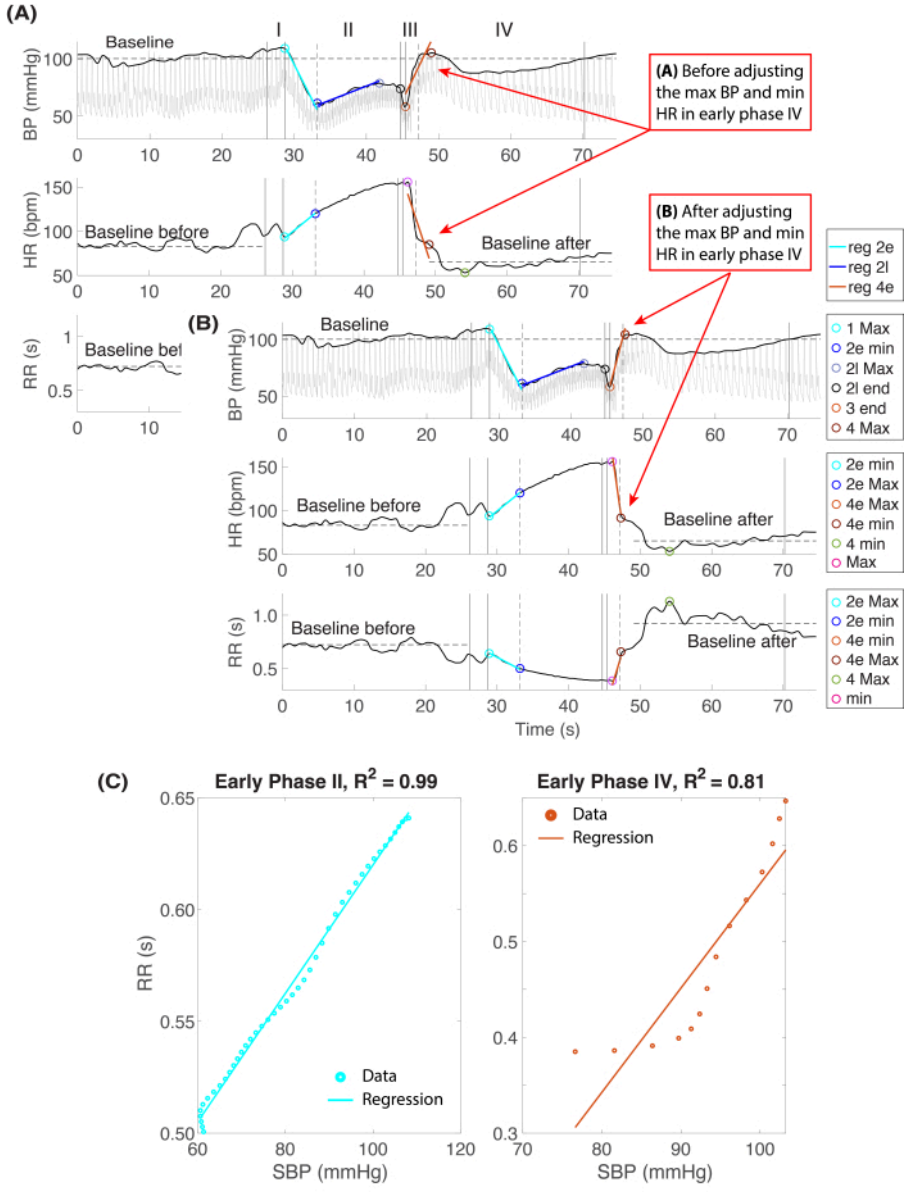

Figure 9: Clinical ratios. (A, B) Ratios and regression lines for blood pressure (BP, mmHg) in the top panel, heart rate (HR, bpm) in the center panel, and RR intervals (s) in the bottom panel. (A) shows the automatically detected ratios and panel (B) the corrected maximum BP and minimum HR in early Phase IV. (**C**) Regression line through corrected ratios. Please click here to view a larger version of this figure.

1. MFigure 1 (shown in **Figure 9**) depicts systolic BP (SBP, mmHg) in the top panel, HR (bpm) in the center panel, and the RR interval (s) in the bottom panel. Characteristic SBP, HR, and RR values are annotated with circular markers. A menu appears, querying: **Do you want to accept markers?** Inspect the markers. Click **Yes** if all

points are correct; this operation is completed, reverting to Step 4. Click **No** if a point needs to be adjusted.

2. A menu queries: **Select points to move**. The menu enables the selection of one, multiple, or all points. If indices have been selected, click **OK** to continue (Step 12.3) or **Cancel** to proceed without changing any indices, continuing to Step 12.4.

3. For each selected quantity, a pop-up menu lists the points to be corrected. Click **OK** to continue. Align the crosshair at the desired point and click once with the mouse. When all selected points have been corrected, MFigure 1 displays BP (mmHg) in the top panel, HR (bpm) in the center panel, and RR intervals (s) in the bottom panel. It shows the corrected points and regression lines during early and late Phase II and early Phase IV. Press **Save and continue** to operation 8 (Run model).

4. MFigure 2 (shown in **Figure 9C**) displays the regression lines relating the RR interval to the SBP and the goodness of fit (the R^2 value). **Press Save and exit**, returning to Step 4. MFigures 1 and 2 will be saved in the folder Figures/Data under the names [patient name] + ratios.png and [patient name] + _ratios_regression.png.

13. Run model (Operation 8)

NOTE: Operation 8 involves solving the baroreflex differential equations model from Randall et al.[20], which predicts sympathetic and parasympathetic signaling. This step runs the model with parameter values set using patient information and clinical ratios identified in Operation 7. This operation is needed to test nominal predictions; if nominal fits have significant errors, results from the optimization operation (Step 15) may not be successful for the specific dataset.

1. Solves the differential equations model by Randell et al.[20] using nominal patient-specific parameter values extracted from the data and the patient information entered in Step 5. MFigure 1 depicts BP (mmHg) top left panel, HR (bpm) data (blue) and model (magenta) top right panel, thoracic pressure (Pth, mmHg) bottom left panel, and parasympathetic (magenta) and sympathetic (dark purple) predictions bottom right panel. The results are depicted with a time resolution of 10 Hz, which corresponds to the resolution in the subsampled HR and SBP data. Click **Save and exit** and continue to operation 8 (Run model). This figure will be saved in the folder Figures/Model_fits as [patient name] + _nominal.png.

14. Sensitivity analysis (Operation 9)

NOTE: Sensitivity analysis is not required for data analysis. This analysis generates a graph depicting model parameters' sensitivity (or importance) for accurate HR prediction. Sensitivities are evaluated at a frequency of 10 Hz, corresponding to the subsampled HR and SBP data. The operation uses local sensitivity analysis described in detail by Randall et al.[20]

1. This operation computes the sensitivity of model parameters to HR. Results (on a log scale) depicting ranked sensitivities are shown in MFigure 1 (**Figure 10**). Click **Save and exit,** and continue to operation 10 (Optimization). Note that this computation takes a few minutes. The result shown in MFigure 1 is saved

in the folder Figures/Model_fits as [patient name] + _sensitivities.png.

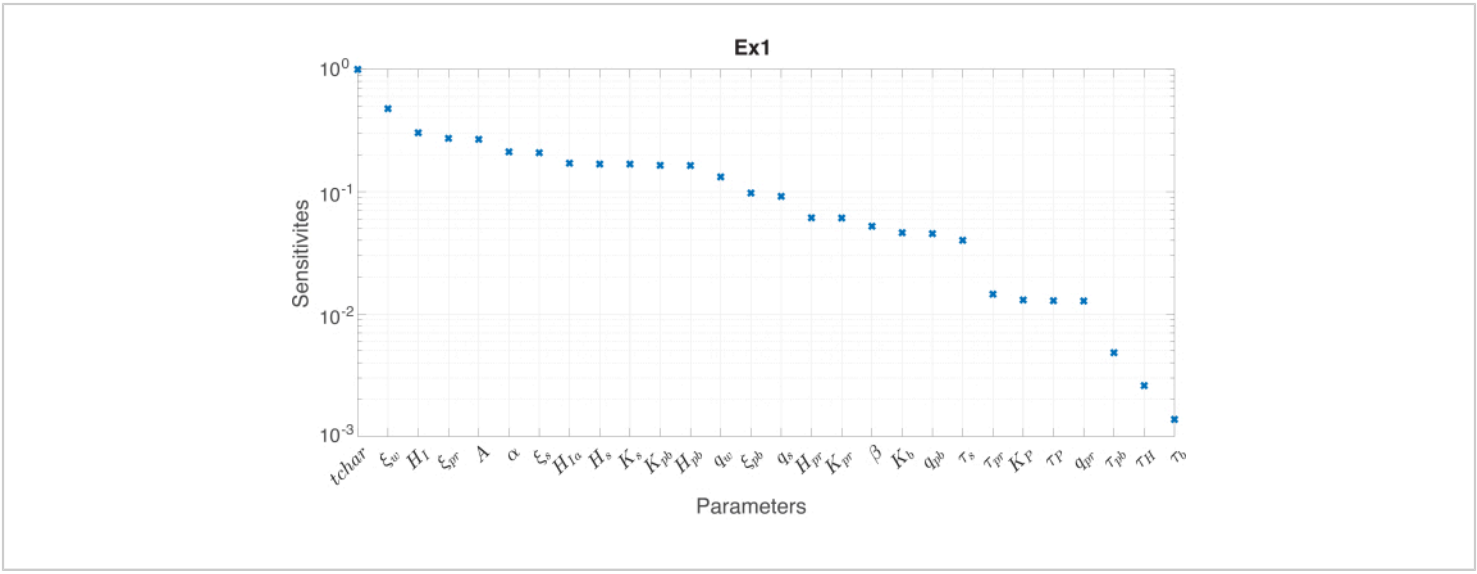

Figure 10: Sensitivity of model parameters to prediction of heart rate. The model and parameters are discussed in detail in the study by Randall et al.[20], and the estimated parameters are explained in **Table 2**. Please click here to view a larger version of this figure.

15. Optimization (Operation 10)

NOTE: This operation estimates a subset of identifiable parameters given the mathematical differential equations model and data availability (HR). The outcome is an HR model calibrated to data subsampled at 10 Hz. In addition to a set of estimated parameters, the optimized model predicts sympathetic and parasympathetic signals. If the simulation does not fit the data well, the predicted sympathetic and parasympathetic signals cannot be interpreted. Optimization is conducted using the Levenberg Marquardt method as described by Randall et al.[20].

1. The parameter estimation can take 5-10 min to complete. During computation, the MATLAB command window prints up to 30 lines of five numbers denoting (from left to right) the gradient norm, the least squares cost, the iteration number, and the Jacobian matrix condition number. When the optimization is complete, and continue to Operation 11 (Plot model predictions). The estimated parameter and a vector INDMAP are saved in the folder Optimized.

16. Plot model predictions (Operation 11)

NOTE: The results of model predictions with nominal (Step 13, Operation 8) and estimated (Step 15, Operation 10) parameter values are plotted at a resolution of 10 Hz corresponding to the subsampled data. If the HR prediction shown in the top right panel of MFigure 1 is reasonable,

the code predicts sympathetic and parasympathetic signaling (Bottom right panel of MFigure 1).

1. On the menu, **Select model predictions to view**, click **Nominal** to plot the model predictions from Step 13 and **Optimized** to view the optimized model predictions from Step 15. MFigure 1 (**Figure 11A** nominal parameters, and **Figure 11B** optimized parameters) depicts BP (mmHg) in the top left corner, HR (bpm) in the top right corner data (blue), and the model (magenta), the thoracic pressure (Pth, mmHg) is at the bottom left corner. The prediction of parasympathetic (magenta) and sympathetic (dark purple) signals are in the bottom right corner. Click **Save and exit**, and continue to Operation 12 (Summary). This figure will be saved in the folder Figures/Model_fits under the name [patient name] + _[action].pn, where [action=nominal] or [action=optimal] depending on the chosen action.

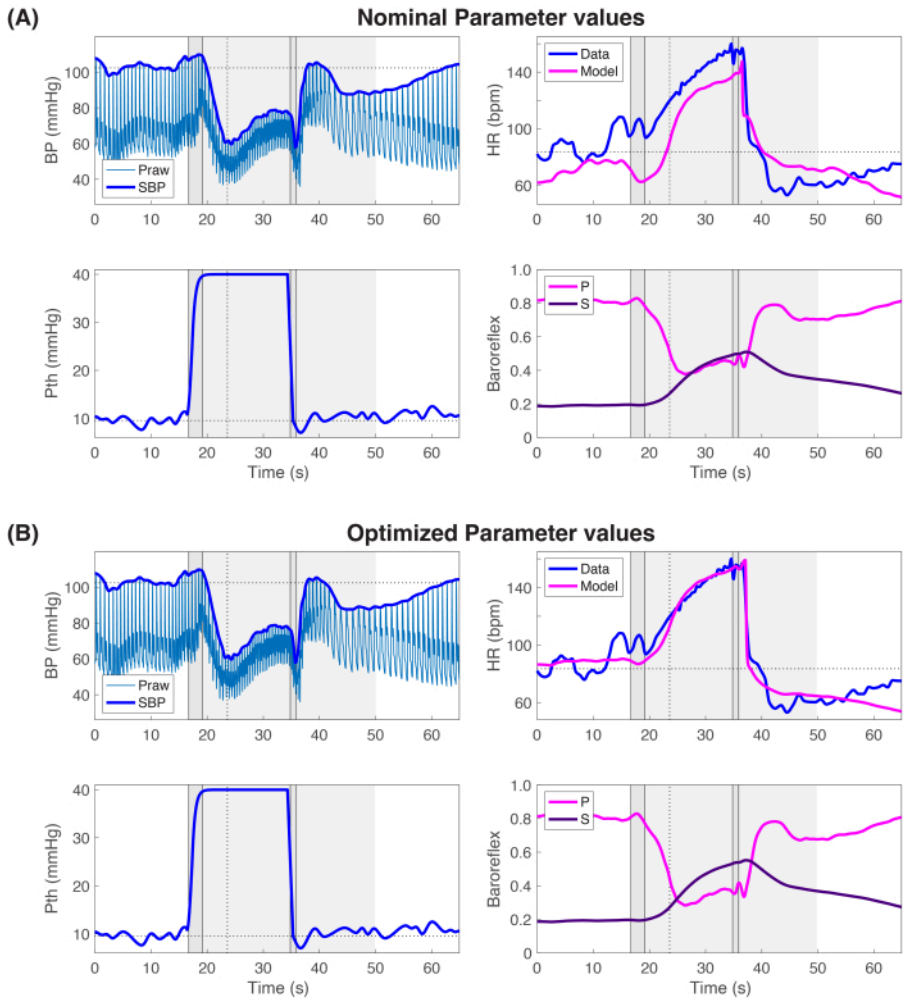

Figure 11: Model prediction. The model was predicted with (**A**) nominal and (**B**) optimized parameter values. The figure shows top left: blood pressure (SBP thick blue line and continuous BP light blue line, mmHg); top right: heart rate (HR, bpm),

model prediction (pink line) and data (blue line); bottom left: thoracic pressure (Pth, mmHg) dark blue line; and bottom right: predictions of parasympathetic (pink) and sympathetic (purple) activity. Both are non-dimensional. Please click here to view a larger version of this figure.

17. Summary (Operation 12)

1. A summary of findings is stored as an Excel spreadsheet (.xlsx) and as a comma-separated file (.csv). The menu **Save Data** queries: **Enter data summary file name (ex: FileName)**, enter the preferred name into the textbox.
2. If the files exist, a menu queries the user: **Add or overwrite the existing file**. This operation also prints outputs to the MATLAB command line. Click **OK** to generate the file and **Cancel** to output only to the command line. The saved file (.xlxs and .cvs) contains patient information (Operation 1, Step 6), Clinical Markers and Regression Lines (Operation 7, Step 12), and Nominal (or Optimized) parameter values (Operation 11, Step 16). For each regression line, the R^2 value indicates the goodness of fit.

   NOTE: This operation (Step 17) can be completed without running the modeling, sensitivity, and optimization steps (Operations 8-11).

Representative Results

The eight example datasets are selected to represent a diverse range of responses. **Figure 3**, **Figure 4**, **Figure 5**, **Figure 6**, **Figure 7**, **Figure 8**, **Figure 9**, **Figure 10**, and **Figure 11** present the results from a representative healthy control subject for each step within the algorithm. **Supplementary Figure 1**, **Supplementary Figure 2**, **Supplementary Figure 3**, **Supplementary Figure 4**, **Supplementary Figure 5**, **Supplementary Figure 6**, **Supplementary Figure 7**, and **Supplementary Figure 8** show results for all eight subjects. Results from each step are highlighted, emphasizing features that the user should consider as part of the analysis.

ECG and HR correction

For each dataset, the part of the signal chosen for analysis is highlighted in red in **Supplementary Figure 1**. For all but one dataset, ECG signal corrections eliminate the need to correct HR. **Supplementary Figure 2** shows HR for all subjects. The exception is subject 8, which had a very irregular ECG signal (lower right panel in **Supplementary Figures 1**). HR correction, illustrated in **Figure 5**, shows two HR signals with and without correction. After correcting the ECG (illustrated in **Figure 4**) and adjusting the HR (**Figure 5B**) for subject 8, all subjects have smooth HRs. The ECG signal is also used to determine respiration (example signal shown in **Figure 6**, and results from all eight subjects are shown in **Supplementary Figure 3**). As Randall et al.[20] reported, respiration is predicted by filtering the signal obtained by computing the magnitude of the QRS complex for each cardiac cycle. At rest, before and after the VM, intrathoracic pressure is assumed to relate linearly to respiration. As shown in **Figure 12B**, it should be noted that the software sometimes identifies the S wave and sometimes the Q waves. Both are adequate for calculating the change in intrathoracic pressure at rest, i.e., the software's outcomes are unaffected. During the breath hold, intrathoracic pressure is held at 40 mmHg as instructed by the examiner.

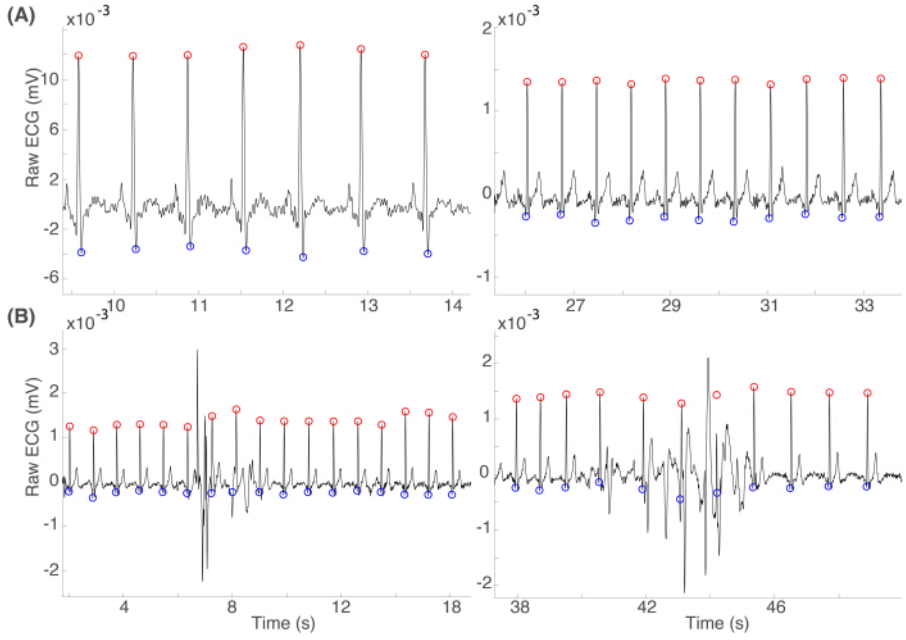

Figure 12: ECG signal (mV) black line. R waves are marked by red circles, and S or Q waves by blue circles. (**A**) An ECG signal where the algorithm identifies the Q waves (left) and S waves (right). (**B**) Two examples of arrhythmia. Please click here to view a larger version of this figure.

BP correction

Operation 5 involves BP correction. It is common for the recording sensors to miss a beat, or the sensor may detect a smaller or larger-than-normal peak. This study calibrates the peak detection algorithm with the cardiac cycle length. The user is asked to identify the cycle length by clicking on a zoomed section of the BP waveforms shown in **Figure 7A**. During the VM, HR changes significantly. As a result, the software often misses peaks.Systolic and diastolic signalsare corrected by connecting existing and new points with piecewise linear splines. The results of this process are illustrated in Figures 7B and C, showing the correction of a peak missed by the sensor (**Figure 7C**), extra-large (**Figure 7C**), and small (**Figure 7B**) peaks. Clinical biomarkers are detected from the corrected systolic BP. The differential equations model uses the corrected systolic and diastolic BP to predict sympathetic and parasympathetic signaling. Corrected systolic pressures for the eight example subjects are shown in **Supplementary Figure 4**.

Valsalva maneuver phases and clinical ratios

The software determines clinical markers after identifying the VM phases (shown in **Figure 8** and **Supplementary Figure 4**) in operation 6. All markers and slopes of regression lines are listed in **Table 2** and saved in the data summary. All regression lines are followed by an R^2 value included so the user can interpret the quality of the fit. While the step rarely fails, the software cannot identify all markers automatically. Examples of correct and misplaced markers are shown in **Figure 9**. **Figure 9A** shows a misplacement of the maximum

pressure and minimum heart in early Phase IV, and **Figure 9B** shows the correct placement after correction. **Figure 9C** shows regression lines during early Phases II and IV. The VM phases for the eight datasets are shown in **Supplementary Figure 4**, the clinical ratios in **Supplementary Figure 5**, and the regression lines are in **Supplementary Figure 6**. Subjects 1-3 are controls. Biomarkers for these subjects all fall within the normal range reported in other studies[37]. Subject 4 has a typical V-response and does not have BP recovery during late Phase II. The angle *α* (placement shown in **Figure 13**) is negative for this subject. To obtain the negative angle, the marker 2e_min is placed either at the end of early Phase II from a previously recorded normal response or at the average length of early Phase II in a healthy cohort. The marker 2l_Max, denoting the maximum pressure in late Phase II, should be placed at the end of late Phase II even though this is not a maximum. Lastly, this subject's PRT (return to baseline) is vastly prolonged; exporting sufficient data after the VM is crucial. Subjects 5 and 7 have missing overshoots, correctly identified by the software. Subject 6 has a minimal blood pressure recovery, followed by a BP drop towards the end of Phase II. This feature is captured by placing the marker 2l_Max in the center of late Phase II, and the slope of the BP recovery is computed over this interval, which is shorter than T_2l (the length of late Phase II). For this subject type, care must be taken when interpreting and -BRSa, as these values are functions of T_2l, the entire length of late Phase II. Also, HR drops after the BP in early Phase IV for some subjects. As a result, a regression line cannot be computed; an example is shown in Supplementary **Figure 6** for subject 6; the panel depicting RR as a function of SBP during early Phase IV is blank. Subject 8 has a negligible BP drop in early Phase II, also correctly identified by the software.

In addition to markers shown in **Figure 9**, this software computes all ratios and BP differences (A-E) proposed by Palamarchuk[1] and others[31,38]. The latter are illustrated in **Figure 13**. These markers are saved in the spreadsheet. **Table 2** explains markers, including a description with units of all saved markers.

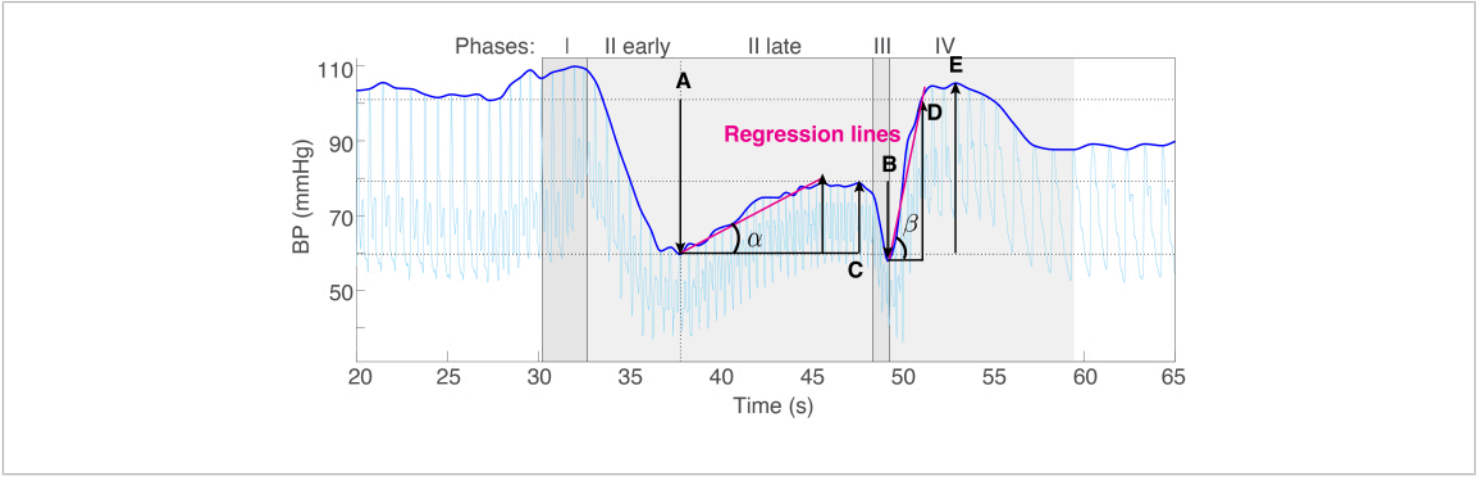

Figure 13: Continuous blood pressure (light blue line) and systolic blood pressure (thick blue line) during the VM. Clinical ratios A-E, defined by Palamarchuk et al.[1], are marked in black. Angles *α* and *β* are determined from regression lines, shown with pink lines. Please click here to view a larger version of this figure.

Prediction of sympathetic and parasympathetic activity

The software also includes the mathematical model developed by Randall et al.[20]. This model uses systolic BP and respiration signals to predict sympathetic and parasympathetic signaling. The latter is done by estimating model parameters that minimize the least squares error between model predictions and data. To ensure that only identifiable parameters are estimated, e.g., model parameters that can be determined uniquely given the data and the model, sensitivity analysis to assess the importance of model parameters; an example is shown in **Figure 10**. **Figure 11** depicts the model input (systolic BP and respiration) and output (HR) before (**Figure 11A**) and after (**Figure 11B**) estimating model parameters. The estimated and patient-specific model parameters are listed in the spreadsheet, and an explanation of the parameters is given in **Table 2**. The Table includes formulas and explanations for each index calculated. Characteristics typically used for the analysis of the Valsalva maneuver are inspired by definitions by Palamarchuk et al.[1], Moštak et al.[38], Garcia et al. [39], Vogel et al.[40], and Schrezenmaier et al.[36] are included. Sensitivity and model prediction results for all eight subjects are shown in **Supplementary Figures 7** and **8**.

Table 2. Please click here to download this Table.

Supplementary Figure 1: Selection of VM region (red) from the region exported from LabChart. Chanel 1 ECG (mV, top panel), Chanel 2 HR (bpm, center panel), and Chanel 3 BP (mmHg, bottom panel) for all eight example subjects. The HR signal generated from LabChart is not used by the ValsalvaAnalyzer software. Please click here to download this File.

Supplementary Figure 2: Corrected HR (bpm) and raw ECG (mV) signals for the eight example subjects. Please click here to download this File.

Supplementary Figure 3: Respiratory signals were obtained from the ECG (mV) signal by filtering beat-by-beat magnitude between the R and Q or S waves for all eight subjects. Please click here to download this File.

Supplementary Figure 4: The four phases of the VM for each of the eight subjects. Please click here to download this File.

Supplementary Figure 5: Clinical ratios for each of the eight subjects. Please click here to download this File.

Supplementary Figure 6: Regression lines fitting SBP (mmHg) to the RR intervals (s) in early Phases II and IV. Note only points included in both BP and RR signals are used. Please click here to download this File.

Supplementary Figure 7: Sensitivity ranking. Sensitivities of model parameters with respect to HR for the eight subjects. Please click here to download this File.

Supplementary Figure 8: Model predictions with optimized parameter values. Model prediction with optimized parameter values. The figure shows top left: blood pressure (SBP thick blue line and continuous BP light blue line, mmHg); top right: heart rate (HR, bpm), model prediction (pink line) and data (blue line); bottom left: thoracic pressure (Pth, mmHg) dark blue line; and bottom right: predictions of parasympathetic (pink) and sympathetic (purple) activity. Both are non-dimensional. Please click here to download this File.

Discussion

The ValsalvaAnalyzer software demonstrated in this study provides functionality to analyze clinical ECG and BP data recorded during the Valsalva maneuver. The software offers the ability to clean noisy signals, making it feasible to get consistent measures across a population. Using the corrected data, the software computes more than 35 markers that the user can interpret. The software aims to include all known quantities reported during the Valsalva maneuver reported in several studies[1,8,9,31,32,34,36,38,40]. One exception is HRV; this quantity is not included as data is not typically recorded over sufficiently long intervals to get reliable insight. However, it is a feature that will be considered in future versions. Additionally, several new clinical ratios (e.g., triangle areas *α*-Area and *β*-Area and the possibility to calculate negative angles) have been introduced. The software is demonstrated on data from eight subjects with both normal and compromised autonomic functions. Including a range of data reflects the versatility of the software. For example, as shown in the data from subject 4, BP drops in late Phase II. As a result, the angle is negative. This can be observed in the summary spreadsheet file (part of the git repository), which correctly determines a negative value.

The software is written in MATLAB, which requires a license. Future versions will be translated to an open-source environment, e.g., Python. One challenge is the discrepancy in formatting between PCs and MACs. A menu is generated initially, where the user selects the platform to overcome this challenge. While figures have been adapted, the font size cannot be changed in menus communicating with the user. Quantities listed in **Table 2** are reported in units of milliseconds, seconds, mmHg, and bpm (for HR). The summary table is saved in an Excel spreadsheet and comma-separated file. The reported values are easily converted to other units if required for a particular study. The software is written for clinical assessment, of VM data with and without measurements of intrathoracic pressure. The software assumes the VM is conducted using a standard 15 s breath hold at 40 mmHg. Note, the intrathoracic pressure was not measured in the 8 example datasets included with the software.

The software has a few limitations. If the code is disrupted before a task is completed, the program may stop within a folder other than the main folder. In this case, returning to the main directory is essential before completing the analysis. For example, the code may crash in the Core folder. To return to the main folder, type cd .. on the command line or use the mouse to return to the correct folder. Moreover, the software assumes that the respiration signal can be extracted directly from the QRS complex magnitude. This assumption relies on a linear relationship between tidal volume and change in QRS complex amplitude. The model used here is adapted from the study by Randall et al.[20] that translated the respiratory signal to intrathoracic pressure. This assumption was based on findings by Kobayashi[41], but results here were generated with a small sample size (n = 8). Therefore, in future studies, we propose to explore this topic further. Another limitation is the respiration curve extracted from the magnitude of the QRS, and no module allows for the correction of this signal. The respiration signal can be measured directly; more work is needed to incorporate this functionality. Finally, the optimization operation estimates a fixed set of parameters. This set can be modulated by changing indices in model_opt.m located in the Core directory. However, it should be noted that some of the model parameters, which are not estimated, are patient-specific. A

detailed discussion of model parameters and their impact can be found in the study by Randall et al.[20].

The mathematical model predicting sympathetic and parasympathetic signaling is fairly sensitive. It works well for most of the example subjects, but results are less accurate for the highly compromised subjects (e.g., subjects 4 and 8, see **Supplementary Figure 8**). Despite the weak signals, the model captures most of the HR. A good fit between model predictions of HR and data is necessary to trust sympathetic and parasympathetic signaling predictions. The model implemented here is adapted from the work by Randall et al.[20]. One discrepancy is that Randall's model includes a time delay in the sympathetic response. MATLAB cannot solve stiff delay differential equations. Therefore, we omitted this feature. It likely affects predictions, but they are still within physiological bounds. Most users will only use some computed quantities or may wish to calculate more of the combined indices proposed by Palamarchuk et al.[1] and others[8,36,40]. All the components required to calculate these are still included.

Disclosures

The authors have nothing to disclose.

Acknowledgments

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