A computerized integrated system for the assessment of central and peripheral chemoreflex sensitivity

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

The assessment of chemoreflex sensitivity (CRS) is of major importance in studies investigating the adaptation of ventilation to the needs of the human body. Increased sensitivity of chemoreceptors to both hypoxia and hypercapnia has recently been shown to be a powerful and independent prognosticator in heart failure (HF) patients, thus highlighting the importance of the assessment of CRS also in the clinical setting. In spite of this, the measurement of CRS is currently limited to the research setting. One possible reason might be the lack of suitable commercial equipments.

On the basis of these considerations, we designed a system to carry out a comprehensive assessment of CRS, including both central and peripheral chemoreceptors. The system is based on the integration of different commercial devices and is entirely managed by a custom software written in Matlab language. The main features of our system are: (1) the implementation of standard methods (the Read’s rebreathing test, the CO\textsubscript{2} single breath test and the transient hypoxia test) suitable for both pathological and healthy subjects, (2) data quality assurance and reduction of subjective judgment in the analysis through advanced analysis procedures and statistical outliers rejection, and (3) full interactive control of every step of the recording and analysis procedures.

The system is currently used in our Institution in the assessment of CRS in HF patients, chronic obstructive pulmonary disease patients and healthy subjects. It has proven to be very effective and easy to use even by clinical personnel without a specific background in respiratory function assessment.

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1. \textbf{Introduction}

The peripheral and central chemoreflex are major reflex arches which adapt ventilation to the needs of the body and contribute to its homeostasis. The importance of assessing chemoreflex sensitivity (CRS) has been, and still is, the subject of extensive research in basic physiological studies, including investigations on the effects of high altitude on respiratory control [1,2], in studies on the ventilatory response to exercise [3,4], and in studies on the mechanisms underlying sleep-related breathing disorders [5,6]. Recently, increased sensitivity of chemoreceptors to both hypoxia and hypercapnia has been shown to be a powerful and independent predictor of mortality in heart failure (HF) patients [7,8], thus highlighting the importance of CRS also in the clinical setting. Notwithstanding this, the measurement of CRS (also...
commonly referred to as ventilatory response) is currently limited to the research setting.

We argued that one of the possible reasons might be the lack of commercial equipments to carry out a complete assessment of CRS, including the response of both central and peripheral chemoreceptors.

On the basis of these considerations, we developed a system which integrates in a friendly and efficient environment, a set of well established ventilatory response tests suitable for healthy subjects and for patients with HF, namely the Read’s rebreathing test [6,8–10], the CO₂ single breath test [6,10,11] and the transient hypoxia test [12–14].

We also included the measurement of circulatory delay, as estimated by the lung-to-ear circulation time (LECT), since the interplay between augmented CRS and increased circulatory delay plays a major role in the genesis of ventilatory instability in HF patients [15].

The system is based on the integration of different commercially available devices and is entirely managed by a custom software written in Matlab language.

2. System description

The system is composed of 3 main functional blocks: (1) a workstation (signal acquisition and monitoring, management of the entire system, analysis), (2) a set of devices providing relevant signals (O₂, CO₂, respiratory flow, O₂ saturation and ECG), and (3) pneumatic circuitry (valves, controllers and gas bags). Fig. 1 provides a picture of the overall system.

In Fig. 2, a flow diagram describing the different operational phases to perform an experimental session is reported. All these phases are managed through a friendly graphical user interface.

The first step is the calibration of the gas analyzers (using a calibrating cylinder) and the calibration of the pneumotach (using a 3-L calibrating syringe). In the second step, bags and tubing are filled with the proper gas mixtures, using the guided semi-automatic procedures implemented. The third step is the insertion of patient’s data in the dedicated data-base. A link to the Hospital information system is also provided. Finally, the scheduled tests can be performed.

3. Hardware and software specifications

The system comprises:

- a commercial device for pulmonary function testing (model Quark PFT, Cosmed, Pavona di Albano, Rome, Italy), slightly modified by the manufacturer to provide O₂, CO₂ and respiratory flow signals as analog output. The device is equipped with a multi-use pneumotach (Lilly based technology), paramagnetic O₂ analyzer (rise time <130 ms) and digital infrared CO₂ analyzer (rise time <100 ms).
- an inflatable balloon-type automated directional breathing valve (Series 2550C Automated Directional Control Valve, Four-way Gatlin-Shape, Hans-Rudolph, Kansas City, MO) with quick inflation and deflation time (80 ms), designed for minimal resistance to breathing
- a balloon valve controller (Series 2530AF Inflatable three Balloon-Valve Controller, Hans-Rudolph, Kansas City, MO), with double piston assembly actuated from TTL external control, supplied with compressed air from an external tank
- an Y shape 2-way non-rebreathing valve (Series 2630 Medium Y shape 2-Way NRBV, Hans-Rudolph, Kansas City, MO)
- a set of non diffusing gas bags, 15 L capacity (Hans-Rudolph, Kansas City, MO)
- 35 mm diameter clean-bor tubing (Vacumed, Ventura, CA)
- a fast response (3 s) pulse oxymeter with ear probe providing analog output (Model Biox 3740, Datex-Ohmeda)
- a data acquisition board, with 16 differential BNC analog inputs (16-bit, 250 kS/s), 4 BNC analog outputs (16-bit, 833 kS/s), 48 digital I/O (model NI USB-6229, National Instruments, Austin, TX)
- a personal computer (Pentium 4 class under Windows XP)
4. Computational methods and theory

4.1. Tests procedures

For all tests, the subject is requested to seat, relax listening to light music through headphones, and breath quietly through a mouthpiece connected to the inflatable balloon automated directional breathing valve. The controller can switch the valve to let the subject inhale either room air or different mixtures of gas from non-diffusing gas bags. The valve is initially set to let the subject breath room air.

All signals are digitized (100 Hz sampling frequency), and processed in real time. Lung volume is computed integrating the flow signal and reported to BTPS conditions applying proper correction for inspiratory and expiratory phases. From the lung volume, both tidal volume and breath duration are computed on a breath-by-breath basis, and the ratio between these two numbers, multiplied by 60, provides the breath-by-breath minute ventilation. End-tidal CO2 (PetCO2) is computed as the maximum value of CO2 measured around the end of the each expiratory phase. Finally, breath-by-breath inspiratory drive was computed as the slope (least square regression) of the lung volume signal in the linear portion of inspiration [16].

O2, CO2 and lung volume signals are smoothly displayed on the screen (polygraph style) and breath-by-breath values of minute ventilation, tidal volume and PetCO2 are continuously displayed, together with heart rate (HR, computed beat-by-beat from the ECG) and arterial oxygen saturation.

4.2. CO2 single breath test

This test is implemented as originally proposed by McClean [11], with modifications for HF patients [6]. Once stable ventilation is achieved, the test starts and, after 6 breaths, during the expiratory phase, the valve is automatically switched so as to let the subject take a single breath of a 13% CO2, 21% O2, 66% N2 gas mixture. In normal subjects, a transient increase in ventilation is typically seen within a 20 s response interval, while in HF patients this interval is extended to 33 s due to the enlarged circulatory delay.

The change in PetCO2 (i.e. the stimulus for chemoreceptors) is automatically computed as the difference between the PetCO2 value for the CO2 enriched breath and the average value of PetCO2 over the 5 breaths preceding CO2 inhalation. The difference between the maximum value of minute ventilation in the response interval and its average value over the 5 breaths preceding CO2 inhalation, is also automatically computed (Fig. 3). The ratio between the change in ventilation and the change in PetCO2 is taken as the estimated ventilatory response for that test.

The test is repeated at 2 min intervals from each other. Each single test is analyzed after its completion and, if not properly carried out (e.g. due to an abnormal inspiration during the stimulus), it is excluded from further analysis. After 8 successful repetitions of the test, all estimated ventilatory responses are displayed in a scatterplot together with two outliers regions defined according to the Tukey [17] or the MAD (median absolute deviation) [18] criterion. A value is deemed to be an outlier if either the Tukey or the MAD criterion is exceeded. The ventilatory response for the examined patient is finally computed as the average of collected ventilatory responses not classified as outliers.

4.3. Transient hypoxia test

This test is implemented as originally proposed by Edelman [12]. Once stable ventilation is achieved, the test starts and, during the expiratory phase, the valve is automatically switched so as to let the subject take 2 breaths of 100% N2. Then the test is repeated, each time increasing the number of N2 breaths, until the minimum value of O2 saturation following the hypoxic stimulus is close to 75% or the maximum desaturation tolerated by the patient is reached. After that, the test is repeated reducing the number of N2 breaths, so as to provide a wide range of arterial oxygen saturations, typically from 75% to 100%. A total of 8 test repetitions are usually performed. Each repetition is preceded by a period of air breathing during which SaO2 and PetCO2 return to baseline values (about 2 min).

For each test, the average of the two consecutive breaths with the highest ventilation after inspiration of N2, is used to calculate the response minute ventilation, whereas the lowest arterial oxygen saturation is used as measurement of the corresponding hypoxic stimulus. The hypoxic chemoreflex sensitivity is finally computed as the slope of the least-squares regression line between all collected pairs of response minute ventilation and oxygen desaturations (Fig. 4a and b). In order to be protected against outliers, a robust linear regression algorithm was used, which iteratively reweights least squares with a bisquare weighting function [19].

4.4. Rebreathing test

The rebreathing test is implemented according to the Read’s method [9,20]. Once ventilation is stable, the test starts switching the valve during expiration from room air to a rebreathing bag containing a mixture of 7% CO2 and 93% O2. Initially, the subject is asked to take three deep breaths to ensure that the partial pressures of carbon dioxide in the bag, lungs and arterial blood quickly equilibrate with the mixed venous partial pressure.

Rebreathing is terminated after 4 min or when ventilation exceeds 100 L/min or PetCO2 exceeded 76 mmHg (≈10%) or if the subject feels the level of hyperpnea unbearable. The gain of the central controller (central chemoreflex sensitivity) is obtained by regressing breath-by-breath minute ventilation on PetCO2 in the linear portion of the response after the PetCO2 plateau has been attained (Fig. 5a and b). Interactive graphical facilities are provided to make the selection of the region of interest easy.
4.5. Lung-to-ear circulation time (LECT)

Before starting the test, the subject is given instructions on the five steps of the procedure: (1) breath quietly, (2) when asked, perform a full expiration without forcing, (3) hold his/her breath as long as possible, (4) take a forceful breath in as deep as possible, and (5) breath normally. Lung to ear circulation time is taken as the interval from the onset of the deep breath terminating the apnea to the nadir of the oxygen saturation measured at the ear (Fig. 6). The latter point is automatically identified as the minimum value of the parabolic fit to the SaO₂ signal (corrected for the fixed time delay introduced by the pulse oximeter) around its nadir. Three measurements are typically carried out in each subject and averaged to obtain the estimate of the LECT.

5. Samples of typical runs

5.1. CO₂ single breath

Fig. 3a shows the signals recorded during a typical CO₂ single breath test. The panels show (from top to bottom): the O₂ signal, CO₂ signal, lung volume, breath-by-breath minute ventilation and inspiratory drive.
The vertical gray strip shows the time during which the valve was switched from room air to the bag containing the gas mixture. This time encompasses a single inspiratory cycle, with commutation during the expiratory phases. During inspiration, the \( CO_2 \) signal reaches the value of 99 mmHg (13\%), the value of the gas mixture in the bag, and corresponding Pet\( CO_2 \) is increased with respect to baseline value, obtained as the average of Pet\( CO_2 \) on the 5 breaths preceding \( CO_2 \) inspiration. The difference between the value of Pet\( CO_2 \) after inspiration of \( CO_2 \) and the basal value constitutes the stimulus for chemoreceptors.

The reflex change in ventilation is given by the difference between the maximum value of minute ventilation (observed around 36s in this example) in the response interval (20s) and the average value of ventilation over the 5 breaths preceding \( CO_2 \) inhalation. The ratio between the change in ventilation and the change in Pet\( CO_2 \) gives the estimate of the ventilatory response. The ventilatory responses estimated in repeated tests and the average value of non-outlier data (solid line) are plotted in Fig. 3b. This value (0.27 L/min/mmHg), is taken as the ventilatory response of the patient. In this example, test #3 was considered an outlier, and not included in the averaging process. The Tukey and MAD thresholds for outliers detection are shown as horizontal broken lines.

5.2. **Transient hypoxia**

The signals recorded during a representative transient hypoxia test are shown in Fig. 4a. From top to bottom, the \( O_2 \) signal, \( CO_2 \) signal, lung volume, oxygen saturation measured at the ear, breath-by-breath minute ventilation and the signal switching the valve from room air to \( N_2 \) are shown.
5.3. **Rebreathing**

Fig. 5a shows the relevant signals recorded during a rebreathing test. From top to bottom, the CO₂ signal, lung volume and minute ventilation are displayed. After the three deep breaths taken by the subject at the beginning of the test, a linear increase in both CO₂ and minute ventilation (resulting from an increase of both breath-by-breath lung volume and breathing frequency) can be seen. The regression of minute ventilation on PetCO₂ in the linear portion of the response after the PetCO₂ plateau has been attained (identified by the two vertical broken lines in the minute ventilation panel) is displayed in Fig. 5b. The slope of this regression (6.18 L/min/mmHg in this example) gives the gain of the central controller (central chemoreflex sensitivity).

5.4. **Lung-to-ear circulation time**

The lung volume and oxygen saturation at the ear recorded during a test to measure LECT are shown in Fig. 6. The two
vertical markers indicate the onset of the deep breath terminating the apnea and the nadir of the oxygen saturation. The parabolic fit to the SaO2 signal around its nadir is plotted superimposed to the SaO2 signal. The distance (in seconds) between these markers, after subtracting the fixed time delay introduced by the pulse oxymeter, gives the lung-to-ear circulation time.

The system is currently used in our Institution for several research protocols involving HF patients, chronic obstructive pulmonary disease (COPD) patients and healthy subjects and has proven to be easy to use even by clinical personnel without a specific background in respiratory function assessment.

6. Concluding remarks

In this paper we have described a system designed to carry out a comprehensive assessment of chemoreflex sensitivity, including both central and peripheral chemoreceptors and to measure the lung-to-ear circulation time.

We have integrated different commercially available devices and developed a custom software in Matlab language. The choice of using Matlab and the Data Acquisition Toolbox allows flexibility and easy expandability of our system and provides direct support for most popular A/D boards, including National Instruments boards.

Every module in the package can be easily modified according to different protocols’ needs, and new modules performing different tasks can be added with small programming effort. Most parameters can be changed interactively, allowing easy adaptation of the software to different requirements, for example to manage healthy subjects or HF patients.

The main features of the system are: (1) the implementation of standard methods suitable for both pathological and healthy subjects, (2) data quality assurance and reduction of subjective judgment through advanced analysis procedures and statistical outliers rejection, and (3) full interactive control of every step of the recording and analysis process.

Availability

We will make our software and all information required to set up a replication of our system available to researchers interested in sharing research protocols with our group.

Conflict of interest

The authors have no conflicts of interest in regard to this research or its funding.

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