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PAPER

Respiratory rate monitoring in ICU patients and healthy volunteers using electrical impedance tomography: a validation study

J J Wisse^{1,2} , M J W Flinsenberg^{1,3}, A H Jonkman¹ , T G Goos^{2,3} and D Gommers¹ ¹ Department of Adult Intensive Care, Erasmus Medical Center, Rotterdam, The Netherlands² Department of Neonatal and Pediatric Intensive Care, Erasmus Medical Center—Sophia Children's Hospital, Rotterdam, The Netherlands³ Department of Mechanical, Maritime and Materials Engineering, Delft University of Technology, Delft, The NetherlandsE-mail: j.j.smit@erasmusmc.nl**Keywords:** respiratory rate, electrical impedance tomography, intensive care unit, capnography, bioimpedance ECGSupplementary material for this article is available [online](#)**Abstract**

Objective. The respiratory rate (RR) is considered one of the most informative vital signals. A well-validated standard for RR measurement in mechanically ventilated patient is capnography; a noninvasive technique for expiratory CO₂ measurements. Reliable RR measurements in spontaneously breathing patients remains a challenge as continuous mainstream capnography measurements are not available. This study aimed to assess the accuracy of RR measurement using electrical impedance tomography (EIT) in healthy volunteers and intensive care unit (ICU) patients on mechanical ventilation and spontaneously breathing post-extubation. Comparator methods included RR derived from both capnography and bioimpedance electrocardiogram (ECG) measurements. **Approach.** Twenty healthy volunteers wore an EIT belt and ECG electrodes while breathing through a capnometer within a 10–40 breaths per minute (BPM) range. Nineteen ICU patients underwent similar measurements during pressure support ventilation and spontaneously breathing after extubation from mechanical ventilation. Stable periods with regular breathing and no artefacts were selected, and agreement between measurement methods was assessed using Bland–Altman analysis for repeated measurements. **Main result.** Bland–Altman analysis revealed a bias less than 0.2 BPM, with tight limits of agreement (LOA) ± 1.5 BPM in healthy volunteers and ventilated ICU patients when comparing EIT to capnography. Spontaneously breathing ICU patients had wider LOA (± 2.5 BPM) when comparing EIT to ECG bioimpedance, but gold standard comparison was unavailable. RR measurements were stable for 91% of the time for capnography, 68% for EIT, and 64% of the ECG bioimpedance signals. After extubation, the percentage of stable periods decreased to 48% for EIT signals and to 55% for ECG bioimpedance. **Significance.** In periods of stable breathing, EIT demonstrated excellent RR measurement accuracy in healthy volunteers and ICU patients. However, stability of both EIT and ECG bioimpedance RR measurements declined in spontaneously breathing patients to approximately 50% of the time.

List of abbreviations

ECG	Electrocardiography
EIT	Electrical impedance tomography
etCO ₂	End-tidal CO ₂
CO ₂	Carbon dioxide
CV _{capno_breath-duration}	Coefficient of variation of capnography breath duration
CV _{capno_etco2}	Coefficient of variation of capnography end-expiratory CO ₂ level

CV _{capno_tidal}	Coefficient of variation of capnography tidal difference in CO ₂ level
CV _{ecg_breath_duration}	Coefficient of variation of ECG breath duration
CV _{ecg_tidal}	Coefficient of variation of ECG tidal difference
CV _{EIT_tidal-volume}	Coefficient of variation of EIT tidal volume
CV _{EIT_breath-duration}	Coefficient of variation of EIT breath duration
CV _{EIT_EELI}	Coefficient of variation of EIT end-expiratory lung impedance
BPM	Breath per minute
ICU	Intensive care Unit
LOA	Limits of agreement
RR	Respiratory rate
RR _{DETECTED}	Respiratory rate detected with the STBP algorithm in the ECG bioimpedance waveform
RR _{ECG}	Respiratory rate measured with bioimpedance ECG after application of strict selection criteria based on the ECG waveform
RR _{EIT}	Respiratory rate measured with EIT
RR _{CAPNO}	Respiratory rate measured with capnography
RR _{ECGmonitor}	Respiratory rate measured with bioimpedance ECG
STBP	Stable tidal breathing period

Introduction

Monitoring of respiratory function is important in both clinical and research settings. The respiratory rate (RR) plays a crucial role in the diagnosis and management of various respiratory conditions and prediction of patient deterioration (Addison *et al* 2015). Therefore, it is important to have accurate and reliable RR measurements. In mechanically ventilated patients, RR can be read from the mechanical ventilator directly, or is computed from capnography waveforms with end-tidal CO₂ measurement (etCO₂) as a well-validated gold standard (Donnelly *et al* 2013, Subbe and Kinsella 2018). However, continuous mainstream capnography measurements in non-ventilated spontaneously breathing patients are not possible or require a dedicated oxygen mask (Gaucher *et al* 2012). It is of great importance to have accurate RR measurements in spontaneously breathing patients, especially for the timely identification of RR alterations following respiratory distress (Gandevia and McKenzie 2008). This enables health care providers to promptly recognize patient deterioration and to anticipate accordingly (Subbe and Kinsella 2018). In these patients, continuous RR is commonly available at the bedside. These RR measurements are derived from impedance changes induced by respiration (ECG bioimpedance) and measured by ECG electrodes. However, the accuracy of this method may be limited especially in dynamic situations, provided that the frequency spectrum of the heart rate and respiratory rate could overlap and is time-varying.

Another noninvasive technique to monitor respiratory physiology is electrical impedance tomography (EIT). This is a bedside, radiation free and non-invasive functional imaging modality to monitor both global and regional lung ventilation (Frerichs *et al* 2017). Before routine and clinical application, the reliability of the RR as measured from the global EIT signal should be evaluated.

The aim of this study is therefore to evaluate the accuracy of the RR measurement of the LuMon device in both healthy volunteers and intensive care (ICU) patients on mechanical ventilation and spontaneously breathing after extubation. Comparator methods were the RR as derived from capnography and/or the ECG bioimpedance measurement, according to their availability within these settings.

Methods

Study population

This study was performed in both healthy human subjects and intensive care patients. The study protocol was approved by the ethics committee of Erasmus MC (Rotterdam, the Netherlands) (MEC-2020-0521, MEC-2020-0767). All subjects or legal representatives provided written informed consent.

The used ICU patient data was a secondary analysis of an observational study in ICU patients around the weaning period from mechanical ventilation (Wisse *et al* 2024). Adults who were invasively ventilated for at least 24 h and considered ready for a spontaneous breathing trial within the next 48 h were considered for enrollment. Measurements were performed around extubation. All included patient were on pressure support ventilation

before extubation and spontaneously breathing with optional oxygen support via a nasal cannula after extubation. Admission diagnosis was not a predefined inclusion criterion. In both groups, the RR was measured using three methods: the RR as provided with EIT (RR_{EIT}), the capnography derived RR (RR_{capno}), and ECG bioimpedance derived RR ($RR_{ECGmonitor}$).

Devices and acquisition

- (1). Electrical impedance tomography: EIT measures impedance changes in the thorax using a belt embedded with 32 electrodes around the chest. Lung impedance changes are displayed in a global dynamic waveform (plethysmogram). Breaths are detected by evaluation of the course of the plethysmogram, an increase during the inspiratory phase followed by a decrease in the expiratory phase is a potential breath. EIT measurements were performed with the LuMonTM (Sentec AG, Therwil Switzerland) which has recently entered clinical practice (Khodadad *et al* 2018). The measurement range for RR_{EIT} is 5–60 breaths per minute (BPM) with a specified accuracy of ± 2 BPM (SenTec 2020). Small currents 0.7–3.8 mA_{rms} were injected with a frequency of 200 kHz $\pm 10\%$. The image rate of the EIT measurement was 50.2 Hz and the respiratory rate trend was reported with 1 Hz.
- (2). Capnography: RR_{capno} monitoring was performed with the Draeger Infinity mainstream CO₂ sensor (Draeger Infinity Acute Care System, Draegerwerk AG, Lübeck, Germany). It uses an infrared sensor to measure the etCO₂. The respiratory rate can be derived from the capnometry signal. The measurement range for mainstream measurements is 0–150 BPM. This device has a specified accuracy of ± 1 BPM in the range of 0–70 BPM, ± 2 BPM for 71–120 BPM and ± 3 BPM for 121–150 BPM. The sample frequency of the used trend report was 1 Hz.
- (3). ECG bioimpedance: the $RR_{ECGmonitor}$ is derived from impedance measurements. A high-frequency current is injected between two ECG-electrodes on the subjects' chest. The impedance between the two-ECG electrodes varies during inspiration and expiration by the expansion and relaxation of the chest (Charlton *et al* 2018). The respiratory rate can be derived from these impedance changes measured between ECG lead 1 or 2. $RR_{ECGmonitor}$ monitoring was performed with the Draeger Infinity (Draeger Infinity Acute Care System, Draegerwerk AG, Lübeck, Germany). The reported measurement range is 0–150 BPM with a specified accuracy of ± 1 BPM or 2% of the rate value, whichever is greater. The excitation frequency was 39.9 kHz with 50 μ A. The sample frequency of the used trend report was 1 Hz.

Set up

A 6-lead ECG was connected to each study participant and the appropriate size EIT belt was placed around the thorax according to the manufacturer's instructions. Volunteers were asked to breathe through a mainstream capnometer connected to a mask. Capnography measurements in intubated ICU patients were performed with a mainstream capnometer connected to the endotracheal tube. Capnography measurements were not available in spontaneously breathing ICU patients.

Respiratory rate comparison

Volunteers were asked to breath in a regular pattern with 4 different rates, each lasting 1 min and with an adjustment time of 20 s in between. Rates varied between 10–20 BPM, 20–30 BPM, 30–40 BPM and 10–30 BPM. An animation was shown to the participant, facilitating the volunteer to follow the suggested breathing rate and to announce the start and stop of each measurement.

No breathing rate instructions were given to the ICU patients; measurements were purely observational within clinical care.

Data synchronization

The three respiratory waveforms (global EIT, capnography and ECG bioimpedance signal) were synchronized and corrected for any offset and drift. Synchronization was performed manually at multiple time points based on the respiratory waveforms. This is needed because of the unknown algorithms calculating the respiratory rates using different averaging times. Matching time periods for the stable tidal breathing periods (STBP) of the different measurements were included in the analysis.

Stable tidal breathing period detection

Data processing was performed in Matlab R2022a (Mathworks, Natick, USA). To find STBP in the EIT signal, a method proposed by Haris *et al* was used for identification and analysis of stable breathing period in EIT recordings (Haris *et al* 2021). Three criteria were used for identification of STBPs, namely the coefficient of

variation of tidal volume ($CV_{EIT_tidal-volume}$), breath duration ($CV_{EIT_breath-duration}$) and end-expiratory lung impedance (CV_{EIT_EELI}). A period was selected as an STBP when it had a duration of at least 30 s and when $CV_{EIT_tidal-volume} < 0.25$, $CV_{EIT_breath-duration} < 0.3$ and $CV_{EIT_EELI} < 0.2$.

We developed a similar algorithm for detection of STBP in the capnography and bioimpedance signals. The three criteria for identification of STBPs in the capnography signal were, the coefficient of variation of breath duration ($CV_{capno_breath-duration}$), the end-expiratory CO_2 level (CV_{capno_etco2}) and the tidal difference in CO_2 level (CV_{capno_tidal}). A period was selected as an STBP when it had a duration of at least 30 s and when $CV_{capno_breath-duration} < 0.1$, $CV_{capno_etco2} < 0.3$ and $CV_{capno_tidal} < 0.2$.

Two criteria were used to identify a STBP in the bioimpedance signal breath duration ($CV_{ecg_breath_duration}$) and the tidal impedance difference (CV_{ecg_tidal}). A period was selected as an STBP when it had a duration of at least 30 s and when $CV_{ecg_breath_duration} < 0.3$, $CV_{ecg_tidal} < 0.2$. Stricter STBP criteria for the ECG bioimpedance signal ($RR_{ECG_monitor}$) were necessary for reliable comparison and the periods where the respiratory rate was close the detected heart rate were discarded (see supplement 1 for a detailed description). This stricter selection of $RR_{ECG_monitor}$ based on the ECG waveform is called RR_{ECG} .

The percentage of time where the signals were stable was calculated based on STBP detection without limiting the period of the STBP to 30 s.

Manual waveform counting

An additional analysis was done to evaluate if the RR as provided by the LuMon EIT reflected the RR as based on manual counting of the EIT, capnography and bioimpedance waveforms. This manual counting was performed by three different experts; for further details, see supplement 2.

Statistics

For respiratory rate analysis the RR_{capno} was used as the gold standard. Both the $RR_{ECG_monitor}$, RR_{ECG} and RR_{EIT} were compared against this gold standard. The accuracy and limits of agreement (LOA) for the different breathing frequencies were compared for both measuring techniques. In ICU patients after extubation, the only remaining comparison was RR_{ECG} against RR_{EIT} , because capnography measurements were unavailable in patients off the ventilator.

Comparisons were evaluated using a Bland Altman analysis to measure LOA. The Bland Altman analysis was corrected for repeated measures within each subject to account for the variable amount of breaths per setting and subject, using bootstrap resampling ($N = 1000$) for the confidence intervals of limits of agreements for the volunteers (Martin Bland and Altman 1986, Parker *et al* 2016; Pinheiro *et al* 2020). The 95% confidence intervals were computed. Statistical tests were performed with R version 2022.07.2 (RStudio, Posit Software, Boston MA, USA).

Results

Study population

A diverse group of 20 healthy volunteers (male 9, female 11, age: 26 (24–30.25)), BMI: 21.93 (21.13–24.86), and 19 ICU patients were enrolled (male 11, female 8, age: 63 (51.5–69)), BMI: 25 (24–32.5). The studied ICU patients exhibited a broad range of admission diagnosis, such as sepsis, trauma, respiratory, cardiovascular and neurological conditions, resulting in a varying degree of lung (in)homogeneity. The total measurement duration was 79 min in healthy volunteers and 679 h in ICU patients.

Stable tidal breathing periods

Respiratory waveforms from the volunteer population were included in the analysis if they were continuously stable for a minimum of 30 s. 69/79 of the one-minute capnography waveforms were included, 72/79 of the EIT waveforms and 61/79 of the ECG waveforms.

In the ICU population 91% of the capnography signal was classified stable, 68% of the EIT signal and 64% of the ECG bioimpedance signal were classified as stable. The percentage of stability decreased after extubation from 67% to 55% for the ECG bioimpedance and 73% to 48% for the EIT signal. The RR displayed on the patient's bedside monitor often corresponded to the heart rate. Figure 1 provides representative examples of stable and unstable waveform of the capnometer, EIT and ECG bioimpedance signals.

Analysis volunteers

Table 1 summarizes the Bland–Altman results for the volunteers with the corresponding Bland–Altman plots visualized in figure 2. The bias of comparison 1 (RR_{EIT} versus RR_{capno}) is 0.16 BPM with LOA within ± 1 BPM. The bias of comparison 2 ($RR_{ECG_monitor}$ versus RR_{capno}) was -0.08 with LOA within ± 2 BPM.

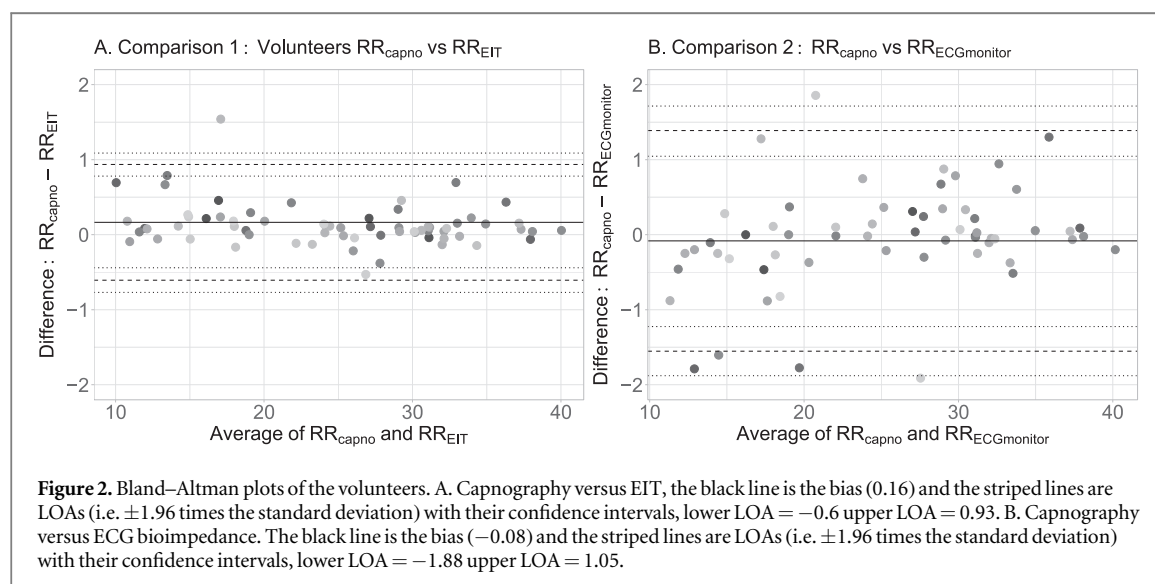
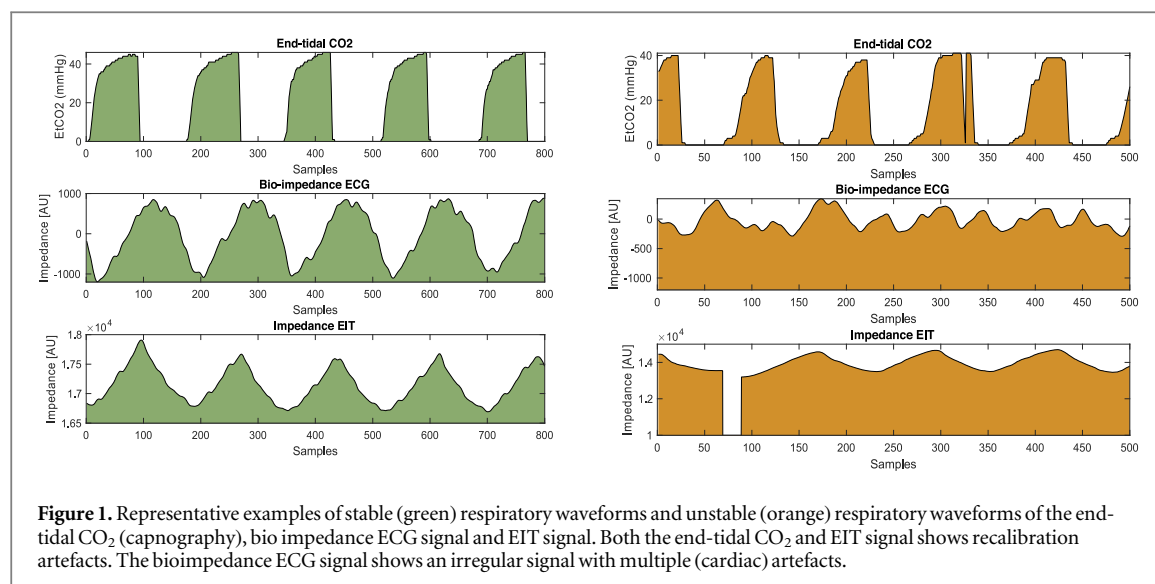


Table 1. Bland–Altman comparison of the STBP periods in healthy volunteers.

	Bias (BPM)	SD (BPM)	Lower LoA	Upper LoA
Comparison 1: RR_{EIT} versus RR_{capno}	0.16	0.39	-0.6	0.93
95% confidence interval (Bootstrapped $N = 1000$)	0.07	0.33	-0.77	0.78
	0.26	0.46	-0.44	1.10
Comparison 2: $RR_{ECGmonitor}$ versus RR_{capno}	-0.08	0.75	-1.55	1.39
95% confidence interval (Bootstrapped $N = 1000$)	-0.28	0.62	-1.88	1.05
	0.11	0.88	-1.21	1.71

Analysis ICU patients

Table 2 summarizes the Bland–Altman results for the adult ICU patients, the corresponding Bland–Altman plots are visualized in figure 3 and supplemental figure A-3. The bias of RR_{EIT} versus RR_{capno} was 0.16 and the lower and upper LOA were within ± 1.2 BPM. The bias of the $RR_{ECGmonitor}$ as displayed on the patient's bedside monitor versus RR_{capno} was larger with wide limits of agreement of ± 8 BPM; evaluation of the actual ECG signal confirmed that this displayed rate often reflected the heart rate instead of respiratory rate (supplemental figure A-3). After removing data periods where $RR_{ECGmonitor}$ closely reflected the heart rate instead of respiratory rate (further referred to as RR_{ECG} , see Methods section and supplement 1), bioimpedance performance improved: bias of -0.05 with limits of agreement of ± 1.3 BPM. The performance in spontaneous breathing ICU patients

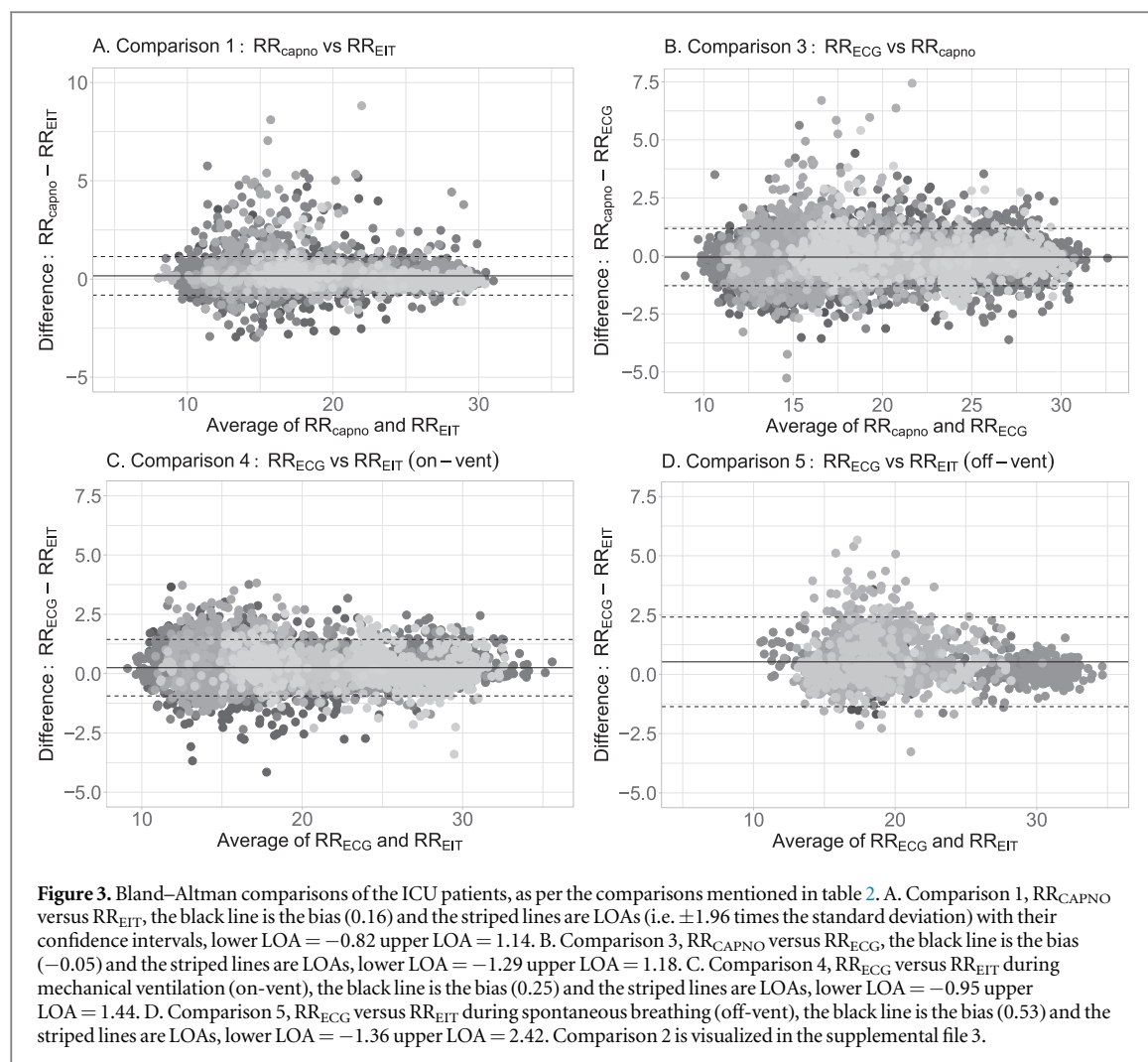


Table 2. Bland–Altman comparison of the STBP periods in ICU patients.

	Number of STBPs	On/Off vent	Bias (BPM)	SD (BPM)	Lower LoA	Upper LoA
Comparison 1: RR_{EIT} versus RR_{capno}	13009	On vent	0.16	0.5	-0.82	1.14
Comparison 2: $RR_{\text{ECGmonitor}}$ versus RR_{capno}	20562	On vent	-1.09	3.63	-8.19	6.01
Comparison 3: RR_{ECG} versus RR_{capno}	17908	On vent	-0.05	0.63	-1.29	1.18
Comparison 4: RR_{ECG} versus RR_{EIT}	10031	On vent	0.25	0.61	-0.95	1.44
Comparison 5: RR_{ECG} versus RR_{EIT}	2840	Off Vent	0.53	0.96	-1.36	2.42

could not be evaluated against capnography, and was therefore evaluated with RR_{ECG} against RR_{EIT} . The LOA were between -1.36 and 2.42 with a bias of 0.53 .

Manual waveforms counting

Manual counting of RR from capnography, bioimpedance and EIT waveforms confirmed that the LuMon-derived RR was reliable during stable breathing periods (average bias of -0.05 bpm; see supplemental file 2).

Discussion

In the present study, we evaluated the accuracy of respiratory rate measurements with EIT against ECG and capnography. We evaluated the performance in a heterogeneous group of volunteers and adult ICU patients.

Our findings reveal several important insights regarding the accuracy of the measurement techniques. Firstly, EIT provides a reliable respiratory rate agreement with $LOA \pm 1.5$ BPM from the reference capnography in both volunteers and ICU patients during stable tidal breathing periods. The performance of ECG bioimpedance RR as derived from the bedside monitor was accurate for the volunteer measurements with LOA of ± 2 BPM. The percentage of time with stable tidal breathing periods in ICU patients was 91% for capnography measurements, 68% for EIT measurements and 64% for ECG measurement. After extubation, when patients were more mobile, the percentage of stability decreased to 48% for EIT signals and to 55% for ECG bioimpedance.

All measured respiratory rates were within a clinically acceptable range of ± 2 BPM.

The volunteer study was performed in a controlled set-up with volunteers breathing at various suggested rates. All respiratory waveforms were visually inspected for stability before the start of the measurement and the breathing rates were stable. In contrast, the ICU patient data was part of an observational study during weaning from mechanical ventilation, with measurements up to 72 h and including periods with unstable EIT and ECG signals. During the observational measurement no extra attention was spent to the reliability of the ECG impedance waveform. We used the ECG impedance data as how it was continuously measured and shown on the patient's monitor. Our findings showed that the accuracy of the RR_{ECG} was low ($LOA \pm 8$ BPM) and the measured RR often corresponded to the heart rate (visual example in supplement 4). Clinically, with our specific patient bedside monitor it is possible to activate a warning on the monitor when the detected RR is too close to the actual heart rate and to set a certain breath detection threshold. These settings were not activated in our ICU. Therefore, to optimized comparisons between EIT and ECG signals, we applied our own selection criteria offline to eliminate STBPs from the ECG signal that demonstrated artefacts or when the RR was close to the actual heart rate. This strict selection was not performed in the healthy volunteer data due to the controlled and prospective study setup, where stability of the signals was confirmed prior to the measurements. In contrast, when applying the strict selection criteria in adult ICU patients we found that $RR_{ECG\text{monitor}}$ values displayed on the patient's bedside monitor were incorrect for over 50% of the time. Awareness of these artefacts and hence, more careful positioning of the ECG electrodes, and better bedside or offline algorithms to compute RR from ECG may improve reliability of RR monitoring.

Both EIT and ECG respiratory rate measurements are susceptible to changes in patient position, movement artefacts and electrode quality, making accurate measurement of respiratory rate especially challenging in spontaneously breathing patients. The percentage of time with stable tidal breathing decreased to $\pm 50\%$ of the time for both RR_{EIT} and RR_{ECG} in these patients after extubation. Future advancement in electrode positioning and quality could potentially mitigate some of these limitations.

We developed stable period detection algorithms for EIT, ECG and capnography measurements. We suggest manufacturers to incorporate stable period detection in algorithms in their software and alarm for unstable signals to ease reliable clinical interpretation of the vital signs for patient care.

Strengths and limitations

This study has some strengths and limitations. We included respiratory data from both healthy volunteers and ICU patients with varying underlying causes of respiratory distress. The accuracy of respiratory rate measurement was evaluated over the entire physiological range of the respiratory rate. In addition, manual waveform counting was performed and the results confirm the reliable performance of the LuMon in measuring RR during stable breathing.

Some limitation should be acknowledged. This was a retrospective study and no sample size was calculated for this secondary analysis; however, we included a large number of breaths (i.e. from $> 20\,000$ STBPs). No subgroup analysis on age and gender was performed due to the small sample size and considering that patients were their own controls.

The respiratory rate measurement performance for spontaneous breathing ICU patients should be interpreted carefully for multiple reasons. First, the respiratory rate of the spontaneous breathing ICU patients could not be evaluated against the gold standard. Therefore, we performed the comparison of RR_{ECG} against RR_{EIT} . The use of sidestream capnography with sub-nasal sampling could help to increase the reliability of RR evaluation in spontaneous breathing ICU patients. However, in our ICU this method is not available and for this retrospective study we used clinically measurements for comparisons.

Second, the included STBP periods were based on our own algorithm, which could only be validated against RR_{capno} during invasive ventilation. Nevertheless, the results indicate that the RR_{EIT} performance in spontaneous breathing ICU patients is slightly less accurate compared to the RR_{EIT} performance in spontaneous breathing volunteers and mechanically ventilated ICU patients, but the performance is still within an acceptable range.

Clinical relevance

In patients on invasive mechanical ventilation, ventilator monitors provide reliable real time measurements of respiratory parameters, including respiratory rate, tidal volume and minute ventilation. Moreover, capnography offers comprehensive assessment of ventilatory status through the respiratory rate and measurement of exhaled CO₂. The role of respiratory rate monitoring by as EIT or ECG impedance is limited in this context, but of great importance in non-ventilated spontaneously breathing patients on the ward or in the ICU. In these settings accurate measurement of respiratory rate remains a challenge, and the limitations of conventional methods becomes evident. Intermittent manual assessment of respiratory rate is often still the standard of care, introducing subjectivity and limiting continuous monitoring (Van Leuvan and Mitchell 2008, Addison *et al* 2015). Having accurate RR measurements in spontaneously breathing patients is essential for early identification for respiratory distress and timely recognition can facilitate early intervention (Krieger *et al* 1986). Manual assessment also has variable accuracy, especially for the respiratory rate (Kellett and Sebat 2017).

Wireless sensors are increasingly used for continuous vital signal monitoring but variable accuracy of such remote systems were reported (Breteler *et al* 2020). Therefore, further investigation is essential to refine techniques and develop stable (wireless) respiratory rate measurement techniques suitable for spontaneously breathing patients.

Conclusion

Our study confirms the accuracy of the LuMon EIT respiratory rate detection algorithm, which exhibits a ± 2 BPM accuracy in both volunteers and mechanically ventilated ICU patients during stable periods (regular breathing, no artefacts). Nevertheless, the stability of RR measurements obtained with EIT and ECG bioimpedance declined notably when applied to spontaneously breathing ICU patients, with stability diminishing to approximately 50% of the time. The acquisition of reliable RR measurement in spontaneously breathing patients remain a significant challenge.

Data availability statement

The data cannot be made publicly available upon publication because they contain sensitive personal information. The data that support the findings of this study are available upon reasonable request from the authors.

Declarations

Ethics approval and consent to participate

The ethics board approved the study (MEC-2020-0521 and MEC-2020-0767) and informed consent was obtained. The study has been carried out in accordance with the Helsinki declaration for medical research involving humans.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest

All authors declared that they have no competing interests directly related to this study. This study was supported in part by Sentec AG, Therwil, Switzerland. Funders played no role in the design and conduct of the study; interpretation of the data; preparation, review, or approval of the manuscript; nor in the decision to submit the manuscript for publication. The opinions, results and conclusions reported in this paper are those of the authors.

Authors' contributions

Concept and design: JW, TG, DG. Data acquisition: JW Data analysis: JW, AJ, TG, MF Data interpretation: all authors. Manuscript drafting: JW, MF Manuscript revising for intellectual contact and final approval: all authors.

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