

# Biological variation of the cardiac index in patients with stable chronic heart failure: inert gas rebreathing compared with impedance cardiography†

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## Abstract

**Aims** In chronic heart failure (CHF), changes in cardiac function define the course of the disease. The cardiac index (CI) is the most adequate indicator of cardiac function. Interpretation of serial CI measurements, however, requires knowledge of the biological variation of CI. Because measurements of CI can be confounded by the clinical situation or the method applied, biological variation might be subject to the same confounders.

**Methods and results** We prospectively included 50 CHF patients who met rigid criteria for clinical stability. CI was measured by both inert gas rebreathing (IGR) and impedance cardiography (ICG) in weekly intervals over 3 weeks—each measurement performed at rest (IGR<sub>rest</sub>/ICG<sub>rest</sub>) and during low-exercise 10 Watt pedalling (IGR<sub>10W</sub>/ICG<sub>10W</sub>). Intra-class correlation coefficients (ICCs), reference change values, and minimal important differences of CI were determined for IGR<sub>rest</sub>, ICG<sub>rest</sub>, IGR<sub>10W</sub> and ICG<sub>10W</sub>. Impedance cardiography and IGR showed moderate agreement at rest (20% (6–36)) and good agreement at 10 Watt (−4% (−23–16)). Depending on time interval, measurement modality for CI, and mode, ICC ranged between 0.42 and 0.78, ICC values for IGR were lower than those for ICG. Reference change value ranged between 3 and 15%, and minimal important difference ranged between 0.2 and 0.5 L/min/m<sup>2</sup>. Values for IGR were lower at rest and higher at 10 Watt than those for ICG.

**Conclusion** Non-invasive measurements of CI are stable over time. Measurement modalities for CI, however, are not interchangeable. Biological variation is less pronounced when obtained by ICG. The influence of low-level exercise on stability of CI depends on the measurement modality.

**Keywords** Cardiac index; Inert gas rebreathing; Impedance cardiography; Biological variation; Chronic heart failure

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## Introduction

Hemodynamic variables such as the cardiac index (CI) provide valuable diagnostic and prognostic information in the management of patients with chronic heart failure (CHF).<sup>1</sup> The gold standard methods for measuring hemodynamic variables are thermodilution and the direct Fick's method. However, due to the invasive nature of these methods, they are only rarely used in the routine monitoring of CHF patients. The Fick method has been largely replaced by the inert gas

rebreathing (IGR) and impedance cardiography (ICG) methods, which allow non-invasive measurement of hemodynamic variables including the cardiac index.

Several studies have shown high precision and acceptable agreement between hemodynamic variables measured by IGR or ICG with those obtained by thermodilution,<sup>2–4</sup> while other studies have suggested they are not sufficiently accurate.<sup>5–8</sup> However, many of these studies were conducted in unstable patients in a pre-operative or critical care setting.<sup>4,6,7</sup> Very few studies have investigated the

precision and accuracy of non-invasive methods for the measurement of hemodynamic variables in patients with stable CHF.<sup>2,3,8</sup> Moreover, in some studies that used non-invasive methods, their clinical applicability was based on single point measurements rather than time-dependent trends in values for hemodynamic variables.<sup>7,9</sup> This may limit the applicability of the IGR and ICG methods in these studies, since they may be more accurate at measuring changes rather than absolute values in hemodynamic variables.<sup>8</sup>

The use of hemodynamic data for disease monitoring in CHF patients requires knowledge of the effect of biological variation on values for hemodynamic variables so that changes in these values during the course of the patient's treatment can be interpreted correctly as either a change due to worsening or improvement in the patient's disease or change due to inherent biological variation.

Biological variation can be regarded as the random variation that occurs around a homeostatic set point for a particular variable. Within-subject biological variation refers to all biological (non-disease-related) sources of variation that can alter any individual's test results, including, but not limited to the following: seasonal and geographic variation, gender, and pulsatile and circadian biorhythms.<sup>10</sup> It is inherent to any biological system and can only be measured in the strict absence of any change induced by disease instability or intervention.

Due to their close and immediate neurohormonal regulation, hemodynamic variables may be confounded by the clinical situation during measurement.<sup>11–13</sup> The biological variation of hemodynamic variables might therefore be subject to the same confounders. As only little is known about the biological variation of values for CI and its potential confounders in CHF patients,<sup>14,15</sup> we determined the repeatability (intra-class correlation coefficient (ICC)), reference change value (RCV), and minimal important difference (MID)—the most commonly accepted parameters for assessing biological variation—in a cohort of stable CHF patients whose CI was measured by IGR and ICG—both at rest and during low-level exercise.

## Methods

### Patients

We prospectively recruited 50 patients with stable CHF from the heart failure outpatient clinic of the University Hospital, Heidelberg, Germany. The diagnosis of heart failure was established according to published guidelines.<sup>16</sup> To be eligible for this study, patients had to meet all of the following 11 criteria: (i) CHF diagnosis and at least 1 year of follow-up at our clinic; (ii) stable clinical condition

with no hospitalization attributable to worsening heart failure within the previous 12 weeks; (iii) New York Heart Association (NYHA) functional class I–III; (iv) subjectively stable clinical condition since the last visit to the outpatient clinic, as judged both by the patient and the physician; (v) ischemic or idiopathic dilated cardiomyopathy as the aetiology of CHF; (vi) in case of ischemic origin, no revascularization planned during the next 8 weeks; (vii) age >18 years; (viii) complete adherence to guidelines of medical treatment regarding class of drugs; (ix) individually optimized doses of guideline-recommended drugs for at least 4 weeks before study inclusion; (x) no recent involuntary change of weight exceeding 2 kg within 4 weeks before inclusion; and (xi) stability of rhythm between visits.

The study conformed to the principles of the declaration of Helsinki and was approved by the local ethics committee.

### Study protocol

After the screening visit ( $V_0$ ), patients were seen in our clinic for three study visits ( $V_1$ ,  $V_2$ , and  $V_3$ ) at weekly intervals ( $7 \pm 1$  day). All visits included performance of the following: patient history; physical examination; collection of blood samples; 12-lead electrocardiogram (ECG); read-out of external ECG event recorder (at follow-up visits); and non-invasive testing for CI using both the IGR and ICG methods at rest and during low (10 Watt) exercise pedalling. Altogether, 12 measurements of CI were performed in each of the 50 patients (four measurements at each visit).

Patients with sinus rhythm or a pacemaker rhythm at  $V_1$  were provided with an external tele-ECG loop recorder (Vitaphone® Tele-ECG-Loop-Recorder 3100 BT and Vitaphone® Tele-ECG-Loop-Recorder 3300 BT, vitaphone GmbH, Mannheim, Germany) in order to exclude intermittent atrial fibrillation. Tele-ECG loop recorder monitoring was continued until  $V_3$ .

Hemodynamic measurements were performed in the mornings after overnight fasting using Innocor®, Innovision, Odense, Denmark for IGR and CardioScreen 2000, Medis GmbH, Ilmenau, Germany for ICG. After 10 min of rest in a semi-recumbent position on an ergometer, CI was measured by both IGR and ICG ( $IGR_{rest}$  and  $ICG_{rest}$ ). Subsequently, patients started low-exercise pedalling at 10 Watt with a pedalling rate of 50–60/min. After 3.5 min of pedalling, IGR and ICG measurements were repeated ( $IGR_{10W}$  and  $ICG_{10W}$ ).

### Inert gas rebreathing

Rebreathings were performed in a closed system, which consisted of a three-way respiratory valve connecting a facemask, an anti-static rubber bag, and an infrared photo-acoustic gas analyser. Patients rebreathed a gas mixture of nitrous oxide ( $N_2O$ ) (0.5%) and sulphur hexafluoride

(0.1%) in oxygen, diluted with atmospheric air, from an anaesthesia bag of size 3–6 L, depending on patient's sex, height, and age. The rebreathing manoeuvre was started after a normal expiration at a breathing rate of 20/min. A constant ventilation rate was ensured by having the patient breathe in synchrony with a graphical tachometer on the computer screen, and a constant ventilation volume was ensured by requesting the patient to empty the rebreathing bag completely with each breath. Rebreathing was typically performed over 5–8 breaths, of which the last 2–3 breaths were used for the calculation of pulmonary blood flow and CI. Between the measurements, an interval of 5 min was strictly adhered in order to guarantee the complete elimination of N<sub>2</sub>O. The details of CI calculations using Innocor® IGR (Innovision) have been described previously.<sup>9</sup>

### Impedance cardiography

According to the manufacturer's guidelines, four pairs of standard electrocardiographic electrodes were placed at both sides of the neck and both sides of the inferior aspect of the thorax at the level of the xiphoid process with an inter-electrode gap of 5 cm. Verification of the correct signal quality was accomplished by visualization of the ECG, the impedance waveform, and its first derivative. Detailed descriptions of ICG parameters and their calculations have been published elsewhere.<sup>17</sup>

### Statistics

#### *Agreement between impedance cardiography and inert gas rebreathing*

The agreement of CI measurements between IGR and ICG was analysed by calculating both Pearson's correlation coefficients and standardized mean differences (SMDs) of CI values obtained by IGR and ICG. The SMD is used for contrasting two groups on a continuous dependent variable. It is defined as SMD = (difference in mean outcome/variable of interest between groups)/(standard deviation of outcome/variable of interest among participants).<sup>18</sup>

The pooled standard deviation adjusts the differences between groups for both the scale and precision of measurement and the size of the population sample used.<sup>19</sup> Cohen offered the following guidelines for interpreting the magnitude of the SMD: small, SMD <0.2; medium, SMD ≈0.5; and large, SMD ≈0.8.<sup>20</sup>

#### *Coefficient of variation and reference change value*

The coefficient of variation (CV) was calculated as follows: CV = 100 × (standard deviation/mean). The total CV (CV<sub>t</sub>) and the analytical CV (CV<sub>a</sub>) provided the basis for calculating the individual biological CV (CV<sub>i</sub>), where

$$CV_i = (CV_t^2 - CV_a^2)^{1/2}$$

Values for CV<sub>a</sub> were taken from the technical manuals of the IGR and ICG devices. Values for CV considered indicative of low, moderate, and high imprecision were <6%, 6–10%, and >10%, respectively.<sup>15</sup>

Reference change values were calculated from median CV<sub>t</sub> values, according to the formula:

$$RCV = Z \times 2^{[1/2]} (CV_a^2/n_a + CV_i^2/n_s)^{[1/2]}$$

where Z = 1.96 (i.e. the Z-score for 95% confidence level with a 2-tailed P <0.05), n<sub>a</sub> is the number of replicate assays, and n<sub>s</sub> is the number of patient samples.

Within-subject variation was calculated and reported using an intra-class correlation. The ICC was determined using CI measurements at V<sub>1</sub> and V<sub>2</sub>, and it was calculated separately for IGR and ICG measurements. Following Rosner,<sup>21</sup> we suggest that ICC <0.4 indicates poor reliability, 0.4 ≤ ICC <0.75 as fair to good reliability and ICC ≥0.75 as excellent reliability.

#### *Minimal important difference*

The MID was determined using the one-standard error of measurement (one-SEM)-based approach developed by Wyrwich *et al.*<sup>22</sup> following the equation:

$$MID = SD \times \text{SQRT}(1 - r),$$

where SD is the population standard deviation, SQRT is the abbreviation for square-root, and r is the reliability coefficient (i.e. the degree of absolute agreement among measurements). We selected the ICC as the reliability coefficient used in the earlier equation because it accounts for the proportion of variance in test values due to between-subject variation; it is simple to calculate; and it provides an estimate of MID that is in good agreement with other methods.<sup>22,23</sup>

The relationship between relative changes in CI and the respective CI value at V<sub>1</sub> was assessed following the method proposed by Bland and Altman.<sup>24</sup> Analyses were carried out separately for measurements at rest and at low-exercise pedalling. Calculations were obtained using MedCalc software version 12.7 (Ostend, Belgium) and results were displayed using GraphPad Prism version 6.02 for Windows (GraphPad Software, La Jolla, CA, USA). A P-value of 0.05 was regarded as statistically significant.

#### *Subgroup analysis*

All analyses were repeated in the subgroup of patients with atrial fibrillation at baseline. In addition, heart rate (HR) measurements obtained by either IGR or ICG were analysed in this subgroup.

## Results

### Patient characteristics

The clinical characteristics for all patients are shown in *Table 1*. Although the mean left ventricular ejection fraction was significantly reduced, the majority of patients were in NYHA class I and II. Correspondingly, the mean 6 min walking distance was mildly reduced. The majority of patients were in sinus rhythm.

### Tele-electrocardiogram-loop-recorder

Tele-ECG-loop-recorder monitoring was provided in 42 patients with sinus rhythm or pacemaker activity at  $V_1$ . No episode of atrial fibrillation was detected in any of these patients during 2 weeks of monitoring.

### Measurements of cardiac index

Overall, 575 CI measurements were performed in 50 patients. There was one missing CI measurement in 25 patients. Results of serial CI measurements with respect to the method applied ( $IGR_{rest}/ICG_{rest}/IGR_{10W}/ICG_{10W}$ ) are shown in *Table 2*. CI measured at rest was lower as compared with measurements at 10 Watt pedalling irrespective of the method of determination applied ( $P < 0.001$  for  $IGR_{rest}$  vs.  $IGR_{10W}$  and  $P < 0.001$  for  $ICG_{rest}$  vs.  $ICG_{10W}$ ).

### Agreement of inert gas rebreathing and impedance cardiography

The Pearson's correlation coefficients were  $r = 0.10$  for  $ICG_{rest}$  vs.  $IGR_{rest}$  ( $P = 0.23$ ) and  $r = 0.24$  for  $ICG_{10W}$  vs.  $IGR_{10W}$  ( $P < 0.01$ ). At rest, mean CI derived by ICG was significantly higher as compared with IGR ( $2.86 \pm 0.43$  vs.  $2.30 \pm 0.71$  L/min/m<sup>2</sup>;  $P < 0.001$ ). The SMD of  $ICG_{rest}$  vs.  $IGR_{rest}$  was 20.27%. In contrast to rest measurements, ICG measurements at 10 Watt pedalling resulted in significantly lower CI values as compared with IGR ( $3.00 \pm 0.54$  vs.  $3.13 \pm 0.74$  L/min/m<sup>2</sup>;  $P = 0.02$ ). The SMD of  $ICG_{10W}$  vs.  $IGR_{10W}$  was  $-7.45\%$ .

### Stability of measurements, reference change values and minimal important differences

The CV was  $<6\%$  with all measurement strategies, indicating low biological variability. The respective ICCs ranged between 0.416 and 0.780, showing acceptable reproducibility of measurements. The Bland–Altman plots of the individual absolute differences of CI measurements between  $V_1$  and  $V_3$  vs. the respective mean values are depicted in *Figure 1*. CVs, RCVs, ICCs,

**Table 1** Baseline characteristics

Characteristic <sup>a</sup>	Value (n (%))
Men	42 (84)
Age (years)	63 ± 11
Aetiology	
Ischemic	27 (54)
Dilated cardiomyopathy	23 (46)
Co-morbidity	
Hypertension	49 (98)
Diabetes	17 (34)
Dyslipidaemia	34 (68)
COPD	2 (4)
Systolic BP (mmHg)	125 ± 18
Diastolic BP (mmHg)	76 ± 10
Heart rate (1/min)	66 ± 8
Heart rhythm	
Sinus rhythm	41 (82)
Atrial fibrillation	8 (16)
Pacemaker	1 (2)
BMI (kg/m <sup>2</sup> )	29 ± 5
NYHA	
I	17 (35)
II	28 (57)
III	4 (8)
LVEF	35 ± 7
6 MWT (m)	491 ± 91
Sodium (mmol/L (135–145))	140 ± 3
Potassium (mmol/L (3.5–4.8))	4.4 ± 0.5
eGFR (mL/min/1.73m <sup>2</sup> (90–150))	79 ± 22
Urea (mmol/L (<7.515))	6.85 ± 2.17
Hemoglobin (mmol/L (♂: 8.073–10.557; ♀: 7.452–9.315))	8.82 ± 0.75
Treatment	
Beta blocker	49 (98)
ACE inhibitor	32 (64)
ARB	21 (42)
Aldosterone Antagonist	25 (50)
Ivabradine	3 (6)
Aspirine	16 (32)
ICD	14 (28)
Pacemaker	1 (2)
CRT	3 (6)

<sup>a</sup>Values in parentheses represent normal range values in the local laboratory.

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate using the Modification of Diet in Renal Disease equation; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional class; 6 MWT, 6 minute walk test.

and MIDs of CI with respect to the measurement strategy applied are shown in *Tables 3* ( $V_1$  vs.  $V_2$ ) and *4* ( $V_1$  vs.  $V_3$ ). Overall, the biological variation of CI was lowest when measured by ICG at rest, while it was highest for IGR measurements at rest.

### Subgroup analysis

A total of eight patients (16%) had atrial fibrillation in the baseline ECG. Mean resting HR at  $V_1$  was  $73 \pm 7$ /min as compared with  $64 \pm 7$ /min in patients with sinus rhythm

**Table 2** Measurements of cardiac index at the respective visit, performed at rest and at 10 Watt pedalling, separate for method of determination (inert gas rebreathing and impedance cardiography)

	CI (V <sub>1</sub> ) (L/min/m <sup>2</sup> )	CI (V <sub>2</sub> ) (L/min/m <sup>2</sup> )	CI (V <sub>3</sub> ) (L/min/m <sup>2</sup> )	CI <sub>mean</sub> (L/min/m <sup>2</sup> )	P-value
IGR <sub>rest</sub>	2.19 ± 0.55	2.33 ± 0.67	2.40 ± 0.89	2.30 ± 0.71	0.36
ICG <sub>rest</sub>	2.89 ± 0.41	2.86 ± 0.48	2.82 ± 0.41	2.86 ± 0.43	0.76
IGR <sub>10W</sub>	3.18 ± 0.73	3.07 ± 0.72	3.14 ± 0.77	3.13 ± 0.74	0.73
ICG <sub>10W</sub>	2.98 ± 0.61	3.01 ± 0.51	3.01 ± 0.49	3.00 ± 0.54	0.96

CI, cardiac index; V<sub>1</sub>, visit 1; V<sub>2</sub>, visit 2 (1 week after visit 1); V<sub>3</sub>, visit 3 (2 weeks after visit 1); IGR<sub>rest</sub>, inert gas rebreathing measured at rest; IGR<sub>10W</sub>, inert gas rebreathing measured at 10 Watt pedalling; ICG<sub>rest</sub>, impedance cardiography measured at rest; ICG<sub>10W</sub>, impedance cardiography measured at 10 Watt pedalling.

Values are presented as mean ± standard deviation. The P-value corresponds to the analysis of covariance of CI at V<sub>1</sub>, V<sub>2</sub>, and V<sub>3</sub>.

( $P < 0.01$ ). HR measured by IGR<sub>rest</sub> did not differ from HR measurement obtained by ICG<sub>rest</sub> ( $P = 0.56$ ). In contrast, HR at low exercise pedalling obtained by IGR<sub>10W</sub> was significantly higher when compared with measurements using ICG<sub>10W</sub> ( $P = 0.03$ ). HR measurements were stable for each method over time (Supporting information, Table S1).

Analyses of CI measurements in patients with atrial fibrillation mainly confirmed the results from the general cohort. Again, CI measurements were stable over time for each method (Table S2). The agreement of ICG and IGR measurements,

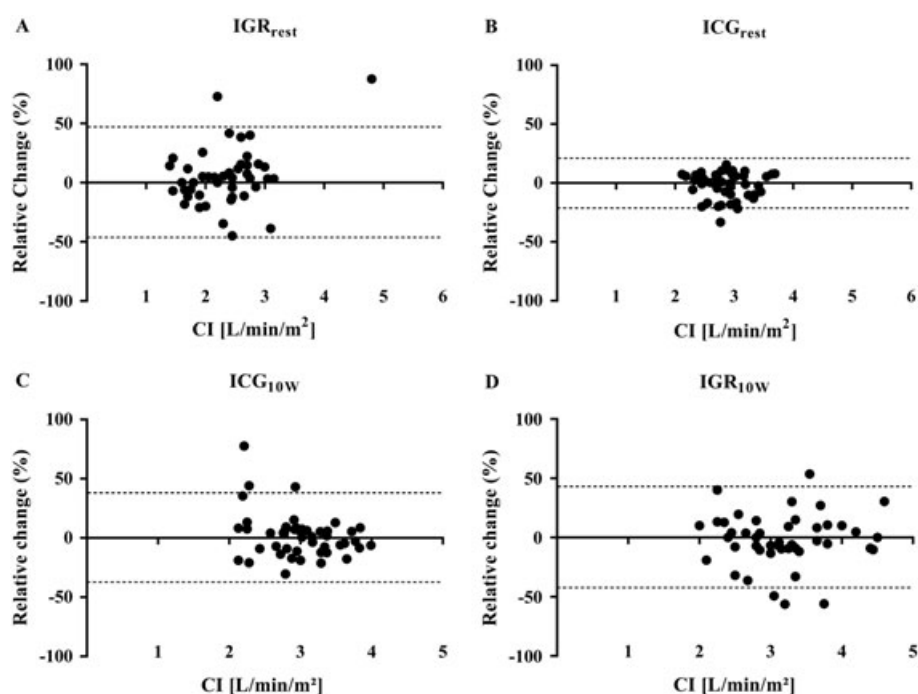
however, was poor, while CI measured by IGR<sub>rest</sub> was lower as compared with IGR<sub>10W</sub> ( $P < 0.0001$ ), this was not true for ICG measurements ( $P = 0.62$  for ICG<sub>rest</sub> vs. ICG<sub>10W</sub>). ICG measurements both at rest and at low-exercise pedalling resulted in higher CI values as compared with IGR measurements ( $P < 0.001$  for ICG<sub>rest</sub> vs. IGR<sub>rest</sub> and  $P = 0.02$  for ICG<sub>10W</sub> vs. IGR<sub>10W</sub> respectively). The Pearson's correlation coefficients were  $r = 0.01$  for ICG<sub>rest</sub> vs. IGR<sub>rest</sub> ( $P = 0.96$ ) and  $r = 0.11$  for ICG<sub>10W</sub> vs. IGR<sub>10W</sub> ( $P = 0.65$ ). The SMD of ICG<sub>rest</sub> vs. IGR<sub>rest</sub> was 37.43%, while it was 8.65% for ICG<sub>10W</sub> vs. IGR<sub>10W</sub>.

In patients with atrial fibrillation, the CV ranged between 1 and 8%, indicating low to moderate biological variability. The respective ICCs were  $>0.70$  in the majority of cases, showing good reproducibility of measurements. The 2 week reproducibility of ICG measurements at low-exercise pedalling, however, was poor. CVs, RCVs, ICCs, and MIDs of CI in patients with atrial fibrillation with respect to the measurement strategy applied are shown in Tables S3 (V<sub>1</sub> vs. V<sub>2</sub>) and S4 (V<sub>1</sub> vs. V<sub>3</sub>). As in the general cohort, the biological variation of CI was lowest when measured by ICG at rest.

## Discussion

Non-invasive measurement of hemodynamic variables, especially CI, is a promising tool in the monitoring of CHF patients.

**Figure 1** Bland–Altman plot for CI values between V<sub>1</sub> and V<sub>3</sub> for IGR<sub>rest</sub> (A), ICG<sub>rest</sub> (B), IGR<sub>10W</sub> (C), or ICG<sub>10W</sub> (D). CI, cardiac index; V<sub>1</sub>, first study visit; V<sub>3</sub>, third study visit; IGR<sub>rest</sub>, inert gas rebreathing measured at rest; ICG<sub>rest</sub>, impedance cardiography measured at rest; IGR<sub>10W</sub>, inert gas rebreathing measured at low-exercise pedalling; ICG<sub>10W</sub>, impedance cardiography measured at low-exercise pedalling.



**Table 3** Coefficients of variation, reference change values and minimal important differences of cardiac index measurements performed at rest and at 10 Watt pedalling using inert gas rebreathing and impedance cardiography (visit 1 vs. visit 2)

	IGR <sub>rest</sub> (%)	ICG <sub>rest</sub> (%)	IGR <sub>10W</sub> (%)	ICG <sub>10W</sub> (%)
CV <sub>a</sub> (%)	2	2	2	2
CV <sub>i</sub> (%)	5.44 (2.82–9.78)	1.12 (0.65–4.26)	3.80 (0.86–8.48)	3.34 (1.76–6.56)
CV <sub>t</sub> (%)	5.80 (2.99–9.98)	2.29 (1.19–4.71)	4.29 (1.32–8.71)	3.89 (2.02–6.86)
RCV (L/min/m <sup>2</sup> )	0.33	0.10	0.34	0.28
RCV (% of CI)	15.14	3.32	10.59	9.33
ICC	0.532	0.689	0.780	0.665
MID (L/min/m <sup>2</sup> )	0.37	0.22	0.34	0.35
MID (% of CI)	15.87	7.76	11.02	11.78

CI, cardiac index; CV, coefficient of variation; CV<sub>a</sub>, analytical CV; CV<sub>i</sub>, individual CV; CV<sub>t</sub>, total CV; ICC, intraclass correlation coefficient; ICG<sub>rest</sub>, impedance cardiography measured at rest; ICG<sub>10W</sub>, impedance cardiography measured at 10 Watt pedalling; IGR<sub>rest</sub>, inert gas rebreathing measured at rest; IGR<sub>10W</sub>, inert gas rebreathing measured at 10 Watt pedalling; RCV, reference change value; MID, minimal important difference. Values are represented as median (interquartile range).

**Table 4** Coefficients of variation, reference change values and minimal important differences of cardiac index measurements performed at rest and at 10 Watt pedalling using inert gas rebreathing and impedance cardiography (visit 1 vs. visit 3)

	IGR <sub>rest</sub> (%)	ICG <sub>rest</sub> (%)	IGR <sub>10W</sub> (%)	ICG <sub>10W</sub> (%)
CV <sub>a</sub> (%)	2	2	2	2
CV <sub>i</sub> (%)	5.53 (2.32–9.89)	2.99 (1.70–4.87)	4.63 (3.02–9.38)	3.71 (2.58–6.96)
CV <sub>t</sub> (%)	5.88 (2.53–10.09)	3.60 (1.97–5.26)	5.04 (3.18–9.59)	4.21 (2.77–7.24)
RCV (L/min/m <sup>2</sup> )	0.37	0.24	0.41	0.31
RCV (% of CI)	15.37	8.39	12.88	10.34
ICC	0.416	0.713	0.544	0.622
MID (L/min/m <sup>2</sup> )	0.41	0.22	0.49	0.37
MID (% of CI)	17.73	7.46	15.86	12.51

CI, cardiac index; CV, coefficient of variation; CV<sub>a</sub>, analytical CV; CV<sub>i</sub>, individual CV; CV<sub>t</sub>, total CV; ICC, intraclass correlation coefficient; ICG<sub>rest</sub>, impedance cardiography measured at rest; ICG<sub>10W</sub>, impedance cardiography measured at 10 Watt pedalling; IGR<sub>rest</sub>, inert gas rebreathing measured at rest; IGR<sub>10W</sub>, inert gas rebreathing measured at 10 Watt pedalling; RCV, reference change value; MID, minimal important difference. Values are represented as median (interquartile range).

Interpretation of change in values for these variables, however, requires knowledge of the biological variation of the respective hemodynamic variables in the population of interest. We determined RCV and MID for CI in CHF patients while accounting both for the method of determination and the possibly confounding effect of the measurement setting. Our main results are as follows:

- In stable CHF patients, intermediate-term reproducibility of non-invasive CI measurements is acceptable for all measurement strategies investigated.
- The agreement of IGR and ICG measurements at rest is moderate. Although it improves when measurements are performed during low-exercise pedalling, CI values obtained by IGR and ICG were still significantly different and may therefore not be used interchangeably.
- The biological variation of CI is lower when obtained by ICG than by IGR.
- Low-level exercise (10 Watt) lowers biological variation for IGR but increases it for ICG, resulting in similar biological variation for both methods during 10 Watt.

- These results were essentially unchanged in the subgroup of patients with atrial fibrillation at baseline.

We chose IGR and ICG to determine CI because of their non-invasive nature, ease of use, reproducibility, and accuracy, making them ideally suited for routine clinical use.<sup>2–4</sup> In addition, several studies have reported that these methods are also useful in the non-invasive measurement of cardiac output (CO) levels in cardiac patients.<sup>4,9,17,25</sup> In addition, studies have shown that IGR provides reliable measurements of CO during cardiopulmonary exercise in healthy subjects.<sup>26</sup>

Our study is the first to describe the biological variation of CI expressed as either RCV or MID in stable CHF patients. We prefer to use CI over CO measurement as it facilitates between-subject comparison of cardiac function. In addition, CI, but not CO, is accepted as a major criterion for high-urgency heart transplantation in the Eurotransplant.<sup>27</sup>

There are little data on the biological variability of CO measurements.<sup>14,15,26,28,29</sup> Moreover, studies addressing this issue differ significantly in design, patient selection, and methods

for measuring CO.<sup>26,28,29</sup> We are aware of only two studies on the variation of values for CO in CHF patients: Moser *et al.* performed thermodilution over 2 hours in 39 patients with advanced CHF,<sup>14</sup> while Jakovljevic *et al.* reported the reproducibility of CO over a 1 week interval in 19 CHF patients who underwent IGR at rest and during near-maximal exercise.<sup>15</sup> The CVs ranged from 3.4 to 7.2% at rest and 5.4% during exercise. These values correspond well to those obtained in our study.

Our study contributes significantly to both the understanding of measurement principles and the concept of biological variation in the measurement of CI for the following reasons: we assessed the variation of values for CI over longer intervals than previous studies; our patient cohort consisted of carefully selected CHF patients who met rigid criteria for heart failure stability; we accounted for the influence of both measurement technique and physical activity on values for CI; and we determined values for biological variation using two different approaches—RCV and MID. In addition, our study is the first to provide a separate subgroup analysis of stable CHF patients with atrial fibrillation.

In our study, CI values obtained by ICG<sub>rest</sub> were significantly higher than values obtained by IGR<sub>rest</sub> irrespective of heart rhythm, and agreement between values for ICG<sub>rest</sub> and IGR<sub>rest</sub> was moderate. This latter finding corresponds to the findings in the study by Trinkmann *et al.* who also reported acceptable agreement between ICG and IGR methods in the determination of CO.<sup>25</sup> Here, in the subset of patients with low CO (<4.1 L/min), ICG also resulted in significantly higher CO values as compared with IGR. Thus, in patients with systolic CHF, ICG<sub>rest</sub> seems to overestimate cardiac function as compared with IGR<sub>rest</sub>. This is of clinical importance, as values obtained by either method at rest may not be interchangeable.

Lastly, we found that agreement between CI values obtained by IGR and ICG improved when performed in CHF patients undergoing low-exercise pedalling. This may be due to the improved standardization of test conditions (e.g. ambient conditions, body posture, and muscle tension) when performing IGR or ICG in these patients.<sup>11–13</sup> Nevertheless, mean CI values determined by ICG<sub>10W</sub> were significantly lower as compared with IGR<sub>10W</sub>. Therefore, for measurements of CI at rest as for measurements during low-exercise pedalling, values obtained by either method should not be used interchangeably in the monitoring of in patients with CHF.

## Limitations

As we did not perform invasive verification of our values for CI, we cannot comment on the accuracy of our IGR and ICG methods. However, both methods have previously shown both acceptable agreement with CI values obtained by thermodilution<sup>2–4</sup> and high precision<sup>3,4,9,17,25</sup>—a prerequisite for the interpretation of serial measurements of hemodynamic variables.

We cannot completely exclude a pre-selection bias in the selection of our patients, as presentation to our outpatient CHF clinic depends on referring physicians. Also, since measures of biological variation are context dependent, our results may not be applicable to other settings. In addition, because our patient cohort was comprised mostly of Caucasians, applying the results obtained from our study to populations from different ethnic/racial backgrounds may not be reliable.

Patients included in this study mostly suffered from mild CHF symptoms (NYHA I/II). Serial CI measurements in patients with severe heart failure symptoms (NYHA III/IV) may show a higher variation of CI. Such an analysis, however, would also include patients with relevant minor clinical changes and/or instabilities and therefore determine the clinical variation rather than the biological variation of CI in CHF, as clinical stability is the prerequisite for the determination of biological variation. We calculated RCVs and MIDs using well-established formulas; however, values for RCVs and MIDs may not only depend on the population of individuals studied and the clinical procedures used but also on the time interval between successive measurements of hemodynamic variables. RCVs and MIDs provided in our study were higher when calculated from data obtained in CHF patients over a two week interval as compared with a one week interval. Therefore, our RCV and MID values for CI by IGR or ICG methods may not be transferable to serial CI measurements over longer time intervals. However, differences between 1 week and 2 week RCVs and MIDs were small, and most studies on biological variation have considered the bias of different sampling intervals to be negligible.<sup>30</sup>

Subgroup analyses of CI measurements in patients with atrial fibrillation mainly confirmed the results of the general cohort. Due to the limited number of patients included into the subgroup analysis, however, the results should be interpreted with caution.

## Conclusion

In our prospectively recruited cohort of 50 patients with stable systolic CHF, values for CI by IGR or ICG methods are stable over time. In addition, values for CI obtained by either IGR or ICG show moderate agreement that improves when these values are determined during low-level exercise. Therefore, values for CI from IGR and ICG are method dependent and not interchangeable. Lastly, biological variation is lower for CI values obtained by ICG than by IGR.

## Conflict of Interest

None declared.

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## Supporting information

Supporting information may be found in the online version of this article.

**Table S1.** Heart rate of patients with atrial fibrillation during non-invasive measurements of cardiac index at the respective

visit, separate for method of determination (inert gas rebreathing and impedance cardiography).

**Table S2.** Measurements of cardiac index in patients with atrial fibrillation at the respective visit, performed at rest and at 10 Watt pedalling, separate for method of determination (inert gas rebreathing and impedance cardiography).

**Table S3.** Coefficients of variation, reference change values and minimal important differences of cardiac index measurements performed in patients with atrial fibrillation at rest and at 10 Watt pedalling using inert gas rebreathing and impedance cardiography (visit 1 vs. visit 2).

**Table S4.** Coefficients of variation, reference change values and minimal important differences of cardiac index measurements performed in patients with atrial fibrillation at rest and at 10 Watt pedalling using inert gas rebreathing and impedance cardiography (visit 1 vs. visit 3).

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