Non-Invasive Rodent Cardiac Output: Comparison of a Compact Clinical Monitor with Echocardiography

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Abstract

Background: There are limited options for non-invasive cardiac output (CO) measurement in rodents. This study aimed to compare a commercially available human CO monitor, USCOM® (USCOM Ltd, Sydney, Australia), with specialised rodent echocardiography for rat CO measurement.

Methods: With institutional ethics committee approval, twenty-one anaesthetised, mechanically-ventilated male Sprague-Dawley rats (573±96g) were studied during refinement and study of an endotoxic shock model. Pulsed-wave Doppler echocardiography (15MHz rodent probe) was used to measure left ventricular (LV) outflow velocity and calculate stroke volume and CO. USCOM (v1.7; 2.2MHz) CO measurements followed each echocardiographic examination. USCOM CO was measured by combining continuous wave Doppler with predicted outflow tract diameter (OTD-U).

Results: 21 paired measurements were analysed. Mean echocardiographic CO was 113mL/min (range 46–236). Mean USCOM CO was 245mL/min (range 75-553). Paired echocardiographic and USCOM measurements demonstrated significant correlations for heart rate (r=0.92, P≤0.0001) and CO (r=0.68, P=0.001). Bland Altman analysis of CO demonstrated mean bias of -131mL/min and precision of 52mL/min. Linear regression analysis yielded a simple correction factor for USCOM OTD estimation. Following application of this correction factor (0.68*OTD-U), mean bias improved to -0.1mL/min with precision of 38mL/min.

Conclusions: USCOM (v1.7) is not interchangeable with pulsed-wave Doppler echocardiography for measuring rat CO. We propose a simple correction factor that should improve performance of this device in the rodent laboratory. Incorporation into a rat-specific algorithm should be evaluated prospectively across a range of potential applications.

Introduction

Rodent models are often studied in critical care research. Cardiac output (CO) measurement in such models is desirable but often impractical. Non-invasive CO measurement is appealing because it facilitates serial measurement of haemodynamics in vivo. Rodent echocardiography incorporating pulsed-wave Doppler (PWD) has recently been compared with thermodilution [1], but it requires relatively expensive equipment and specialised technicians.

USCOM® (USCOM Ltd, Sydney, Australia) is a clinical monitoring device that uses continuous-wave Doppler (CWD) combined with an algorithm to estimate left ventricular outflow tract diameter (OTD) to accurately measure stroke volume (SV) and CO in humans [2, 3]. In comparison to echocardiography, USCOM is compact, more portable, less expensive and requires less training. It has shown excellent agreement with aortic flow probes in anaesthetised dogs [4]. However, it has not previously been studied in rats. This study aimed to compare USCOM with rodent echocardiography for CO measurement in rats.

Methods

Twenty-one male Sprague-Dawley rats were studied. The rats were fed autoclaved rat chow and allowed water ad libitum. They were housed in a room with constant temperature (21°C) and 12 hour light-dark cycle. Animals were allowed to acclimatise for at least one week prior to experimentation. Approval for the study was granted by the University of Queensland Animal Ethics Committee (protocol 675/05).

Experimental model. This study was a planned sub-study of an endotoxic model of rodent sepsis. During model refinement it was necessary to trial different anaesthetic techniques and variable doses of endotoxin (E.coli O55:B5, Sigma, MO, USA). Anaesthesia was induced by either intra-peritoneal injection of buprenorphine / xylazine (150mcg/kg and 10mg/kg respectively), ketamine / xylazine (60mg/kg and 10mg/kg respectively) or subcutaneous alphaxalone (15mg/kg). Mechanical ventilation (Harvard Small Animal Ventilator Model 683, Harvard Apparatus MA, USA) was performed via tracheostomy (14G insyte cannula, Becton Dickinson Infusion Therapy Systems Inc. UT, USA). Anaesthesia was

maintained with isoflurane (0.5-1%) in the buprenorphine / xylazine and alphaxalone groups. The ketamine / xylazine group received intra-peritoneal supplementation as required. After baseline assessment (echocardiography and USCOM), rats continued into refinement or study of an endotoxic shock model.

Echocardiography (Reference Standard). Prior to trans-thoracic echocardiography, the left anterior chest wall was shaved and ultrasound gel applied. Echocardiographic examinations were performed by a single, experienced echocardiographer. In the supine position, CO was measured by a combination of two-dimensional and PWD echocardiography according to ASE guidelines [5]. A commercially available echocardiographic system (Vivid 5, GE Healthcare) with a dedicated 15MHz rodent probe was used.

Aortic annulus diameter (OTDE) was determined as the maximum of three measurements from zoomed parasternal long axis view. PWD interrogation of LV outflow tract velocity was guided by apical 5 chamber view.

PWD images were recorded at a sweep speed of 200mm/s. Recordings were digitally transferred to an online PowerMac computer and stored on magnetic optical disks for subsequent analysis in the EchoPac software without loss of image frames. Echocardiographic recordings were analysed at least 1 month after the experiments by a single observer blinded to USCOM results. PWD measurements were taken from five consecutive cardiac cycles beginning in expiration.

Heart rate (HR), velocity time integral (VTI) and peak velocity (Vpeak) were recorded. SV was calculated as the product of the VTI and cross-sectional area of the aortic annulus $[\pi.(OTDE/2)2]$. CO was calculated as the product of mean SV and mean HR.

USCOM measurements. The device for this study was identical to that sold for human clinical use. A 2.2 MHz probe was used in combination with version 1.7 of the operating software. USCOM measurements were performed by a single investigator blinded to echocardiography. The USCOM transducer was placed over the cardiac apex and directed toward the right scapula. The 'best' signal, as identified visually by maximal amplitude and fullest contour, was recorded. Measurements were made off-line using the touch point calliper method for five consecutive cardiac cycles beginning in expiration. HR, VTI and Vpeak were recorded. Outflow tract diameter (OTDU) was estimated by the device's weight-based neonatal (human) algorithm. The specific details of this algorithm were not available to the investigators. SV

was calculated as the product of VTI and outflow tract circular cross-sectional area $[\pi.(OTDU/2)2]$. CO was calculated as the product of mean HR and mean SV.

Statistics. Due to variation in experimental protocol during model refinement, the first paired echocardiographic and USCOM measurement on each rat was studied. Analysis was performed by SPSS, version 14.0 for Windows (SPSS Inc, Chicago, IL, USA) and Stata/SE 9.2 for Windows (StataCorp LP, College Station, TX USA). Bias and precision were calculated as described by Bland and Altman [6, 7]. Correlation coefficients (Pearson) and linear regression analysis was performed on paired echocardiographic and USCOM data. A P-value ≤0.05 was regarded as significant. Unless otherwise stated, results are presented as mean±SD.

Results

Twenty-one male Sprague-Dawley rats were studied. The rats were fed autoclaved rat chow and allowed water ad libitum. They were housed in a room with constant temperature (21°C) and 12 hour light-dark cycle. Animals were allowed to acclimatise for at least one week prior to experimentation. Approval for the study was granted by the University of Queensland Animal Ethics Committee (protocol 675/05).

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Correlation coefficients (Pearson) and linear regression analysis was performed on paired echocardiographic and USCOM data. A P-value ≤0.05 was regarded as significant. Unless otherwise stated, results are presented as mean±SD.

Discussion

The main finding of this study is that USCOM (v1.7) is not interchangeable with PWD echocardiography for measuring CO in rats. There are a number of potential reasons for this. First, the weight-based algorithm used by USCOM was developed to estimate human neonatal OTD, rather than rat OTD. Second, USCOM uses CWD, while echocardiographic measurement was performed using PWD. Finally, it was not possible to simultaneously perform USCOM and echocardiographic measurements because both techniques require application of an ultrasound probe to the same portion of rat chest wall.

Outflow tract diameter. In keeping with data from Bjornerheim and colleagues, we did not demonstrate a correlation between echocardiographically measured aortic annulus diameter and weight [8]. This contrasts with work by Slama and colleagues that demonstrated positive correlation between rat body weight and aortic annulus diameter (r= 0.80, PThe mean weight (and standard deviation) of the twenty-one Sprague-Dawley rats in our study was greater (573±96g; range 380-740) and seemed disproportionate to the range of nose-rump lengths observed (mean±SD = 26.2±1.1cm; range 24-28). This suggests that in our study, variation in weight might be largely attributed to differences in adiposity rather than lean body mass. Also, Slama and colleagues measured aortic annulus diameter using M-mode techniques. This might have offered better spatial resolution than our use of two-dimensional clips. The current data does not exclude the possibility of correlation between rat lean weight or length with OTD. For practicality, the use of rat length in an algorithm to estimate rat OTD warrants consideration.

Proposed correction factor. USCOM is a clinical monitoring device developed for use in humans. We applied the weight-based neonatal OTD algorithm in an attempt to measure rat CO. Bias and precision statistics were improved substantially by application of a simple correction factor.

We chose to correct calculations based upon a circular cross sectional area because this is consistent with the existing USCOM algorithm. An alternative explanation is that instead of circular cross sectional area, OTDU could be used to generate a triangular

cross sectional area in keeping with the effective aortic valve area [9]. The maximum area of an inscribed equilateral triangle is approximately half of the area of the circle that contains it (diameter = OTDU). In the present study, this alternative explanation would also account for correction of USCOM CO by a factor of approximately 0.5.

Cardiac output measurement. Our comparison of rat CO measurement with USCOM and echocardiography was performed using methods described by Bland and Altman [6, 7]. This approach is useful in determining whether a new method is suitable to replace an existing method. The current data suggests that USCOM is not interchangeable with PWD echocardiography for measuring CO in rats.

USCOM uses CWD, while echocardiographic measurement was performed using PWD. Except in the presence of sub- or supra-valvular pathology, maximal velocities, as measured by CWD, will represent flow through the aortic valve which is the smallest area of cross sectional flow. On the other hand, PWD aims to sample the velocity profile at the same position as the diameter measurement [5]. Also, PWD is spatially guided and is therefore less likely to be affected by angle of insonation than CWD measurements [10]. If the angle of insonation varied up to 20º, this would account for 6% underestimation (cos20º). These factors potentially explain the lack of correlation between VTI, Vpeak and SV of the two techniques.

The measurement of rodent cardiac out put lacks a 'gold standard.' We accepted PWD echocardiography as a reference standard because of its appeal as a non-invasive technique and the availability of data comparing it with rodent thermodilution techniques [1]. This comparison with thermodilution yielded limits of agreement of 32mL/min. This must be viewed in the context of the mean echocardiographic CO that was 73±4mL/min. The limitations of PWD as a reference standard might be addressed by comparison of USCOM with another technique, such as thermodilution, radiolabelled microspheres or flow probe. This would also allow prospective evaluation of a rat specific OTD algorithm and the ability of USCOM to track changes in CO. The later was not undertaken in the current study due to variations in anaesthesia and endotoxin dose during refinement of our endotoxin model.

Study limitation. In this study of non-invasive cardiac output measurement it was not possible to simultaneously perform USCOM and echocardiographic measurements. Both techniques require application of an ultrasound probe to the same portion of chest wall. USCOM measurements were necessarily delayed until completion of echocardiographic examination. As attested by the differences in paired HR, this is likely to have contributed to some of the observed difference between paired haemodynamic measurements.

Conclusion(s)

USCOM (v1.7) is not interchangeable with PWD echocardiography for measuring CO in rats. Based on linear regression analysis, we propose a simple correction factor that may improve performance of this device in the rodent laboratory. Incorporation into a rat-specific algorithm should be evaluated prospectively against an alternative, more robust reference standard and across a range of potential applications.

References

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Illustrations

Illustration 1

Figure 1 USCOM compared to echocardiographically measured heart rate.

Heart Rate

r=0.92, P==0.0001

USCOM (beats/min)

Echocardiography (beats/min)
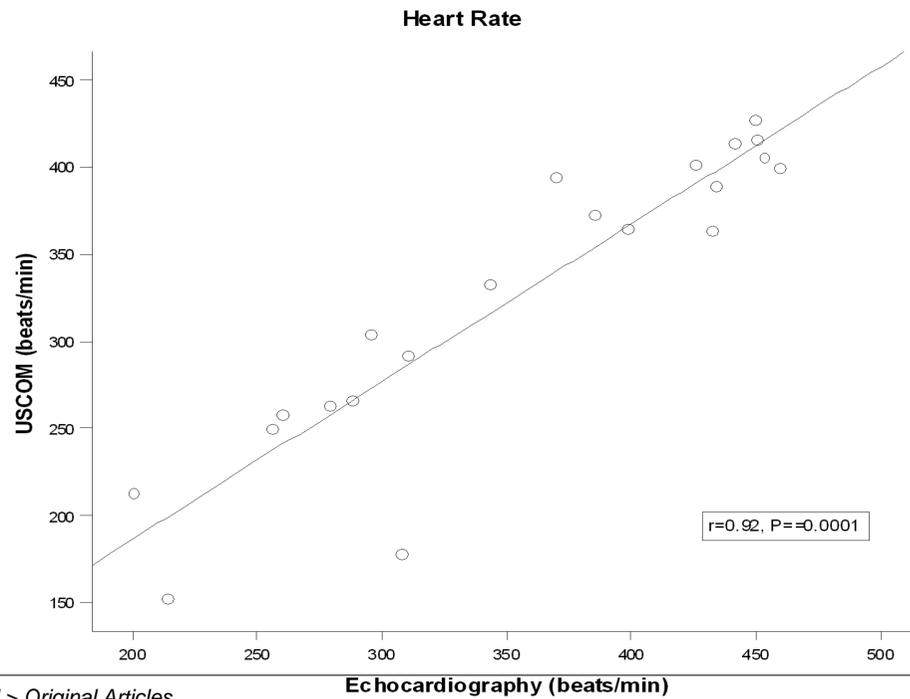
Illustration 2

Figure 2 USCOM compared to echocardiographically measured cardiac output.

Cardiac Output

r=0.68, P=0.001
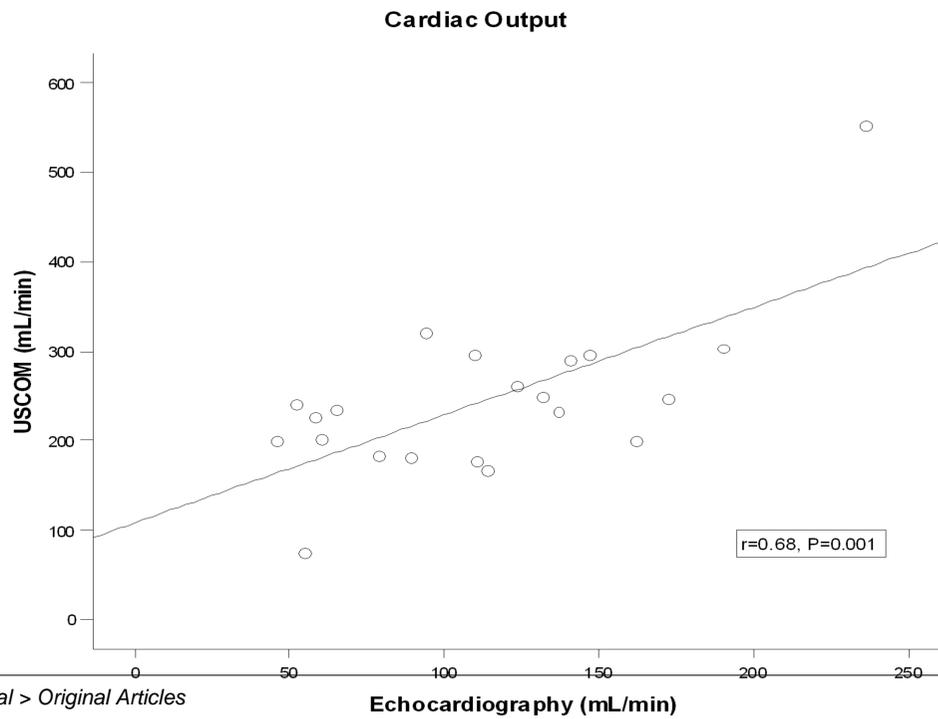
Illustration 3

Figure 3 Bland Altman plot of cardiac output (mL/min) prior to application of correction factor. Bias (line of best fit) is accompanied by lines representing limits of agreement (mean2SD).

Illustration 4

Figure 4 Bland Altman plot of cardiac output (mL/min) following application of correction factor (0.68*OTD). Bias (line of best fit) is accompanied by lines representing limits of agreement (mean2SD). Note that axes are not scaled identically to Figure 3.
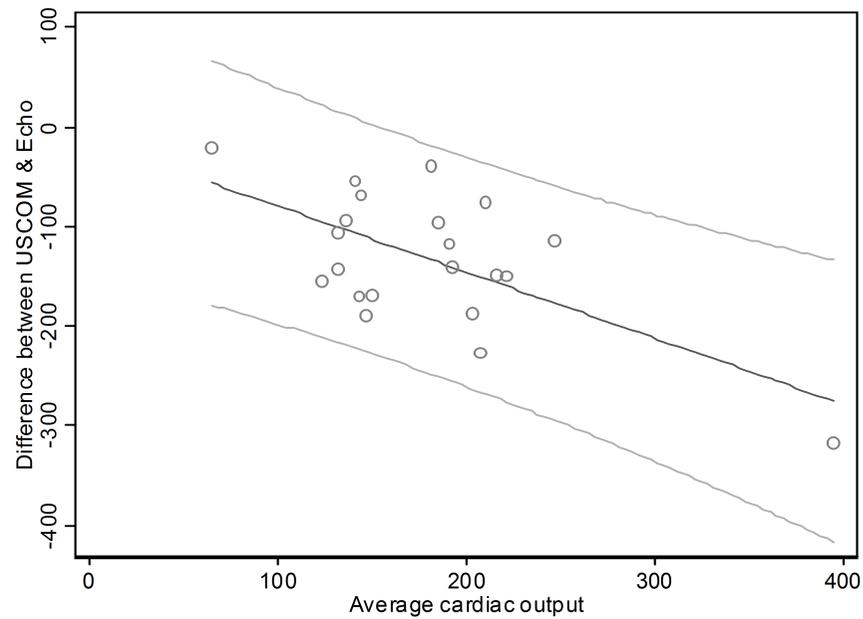
Illustration 5

Table 1 Comparison of USCOM with rodent echocardiography.

	Echocardiograh y (Mean±SD)	USCOM (Mean±SD)	r	P	Bias	Mean Bias	Pre
OTD (mm)	3.13±0.4	3.96±0.1	-0.46	0.04	0.88 – 2.2*OTD	-0.83	
HR(beats/min)	355±86	327±84	0.92	≤0.0001		28	
VTI (cm)	4.02±1.02	6.17±1.87	0.3	0.2	2.2 – 0.86*VTI	-2.1	
Vpeak (m/s)	0.81±0.18	1.18±0.3	0.3	0.2	0.39 – 0.76*Vpeak	-0.37	(
SV (mL)	0.31±0.1	0.76±0.24	0.1	0.7	0.26 – 1.34*SV	-0.45	(
CO (mL/min)	113±51	245±91	0.68	0.001	-13 – 0.66*CO	-131	

In the presence of a trend, bias is reported as an equation (line of best fit). "Mean Bias" refers to the mean difference between methods for each variable.

Illustration 6

Table 2 Results of comparison of USCOM with rodent echocardiography following application of correction factor (0.68) to OTD algorithm.

	$USCOM_C$ (Mean±SD)	Bias	Mean Bias	Precision
OTD_C (mm)	2.7±0.07	0.61 – 2.2*OTD	0.44	0.4
SV_C (mL)	0.35±0.1		-0.04	0.13
CO_C (mL/min)	113±42		-0.1	38

Only variables from table 1 affected by correction of OTD_U are displayed.
Variables are labelled with the subscript $_C$ to indicate application of the correction
factor. In the presence of a trend, bias is reported as an equation (line of best fit).
"Mean Bias" refers to the mean difference between methods for each variable.

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