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# Echocardiography does not prolong peri-shock pause in cardiopulmonary resuscitation using the COACH-RED protocol with non-expert sonographers in simulated cardiac arrest



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## Abstract

**Objective:** Focused echocardiography during peri-shock pause (PSP) can prognosticate and detect reversible causes in cardiac arrest but minimising interruptions to chest compressions improves outcome. The COACH-RED protocol was adapted from the COACHED protocol to systematically incorporate echocardiography into rhythm check without prolonging PSP beyond the recommended 10s. The primary objective of this study was to test the feasibility of emergency nurses learning to perform all roles in the COACH-RED protocol. PSP duration and change in participant confidence were secondary outcomes.

**Methods:** After an initial two-hour workshop, five ALS-trained nurses were assessed for the correct use of COACH-RED protocol, without critical error, in three simulated cardiac arrest scenarios of four cycles each. Assessments were repeated on days 7 and 35. On day 35, three COACHED scenarios were also assessed for comparison. Participant roles per scenario and cardiac rhythm per cycle were randomised. Participants completed questionnaires on their confidence levels. Sessions were videotaped for accurate measurement of PSP duration and results tabulated for simple comparison. Statistical analysis was not performed due to small sample size.

**Results:** There were no critical errors, two minor team-leading errors and two minor echosonography errors. Minor errors occurred in separate scenarios resulting in a 100% pass rate overall by predetermined criteria. Echocardiographic recordings were 100% adequate. Overall median PSP was 9.35s for COACH-RED and 6.94s for COACHED. Sub-group analysis of COACH-RED revealed median PSP 10.80s in shockable rhythms and 8.74s (~2s less) in non-shockable rhythms. Mean participant confidence in performing COACH-RED improved from 1.6 to 4.6, on a 5-point scale.

**Conclusion:** The COACH-RED protocol can be effectively performed by ALS-trained nurses, in all roles of this protocol, including echocardiography, in a simulated environment, after a single training session. Using this protocol, focused echocardiography does not prolong PSP beyond 10s.

Keywords: Advanced cardiac life support, Cardiac arrest, Cardiopulmonary resuscitation, Echocardiography, Education, Nursing, Simulation training

Abbreviations: ALS, Advanced Life Support; ARC, Australian Resuscitation Council; CPR, Cardiopulmonary resuscitation; ED, Emergency Department; IQR, Interquartile Range; PEA, Pulseless Electrical Activity; PSP, Peri-Shock Pause; SAH, Sydney Adventist Hospital; VF, Ventricular Fibrillation; VT, Ventricular Tachycardia.

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### Introduction

Bedside ultrasound performed by clinicians has become increasingly widespread over the past 35 years and is now considered a core skill requirement of specialist training by most emergency medicine colleges and societies worldwide.<sup>1–7</sup> Echocardiography during a cardiac arrest has been used to identify reversible causes such as cardiac tamponade, hypovolaemia and pulmonary embolus and is increasingly being used as a decision aid for termination of resuscitation since the absence of spontaneous organised cardiac activity on echocardiography has been reported to predict a poor prognosis.<sup>8–12</sup> Conversely, the identification of fine ventricular fibrillation or pseudo-pulseless electrical activity has been associated with improved prognosis.<sup>8–12</sup> However, the evidence for echocardiography leading to a positive change of outcome in cardiac arrest is lacking, and in recent years some studies have suggested a negative effect through prolongation of pauses in chest compressions.<sup>13–16</sup>

Studies in animals and humans have shown vastly improved chances of successful defibrillation and survival when pauses in chest compressions are minimised.<sup>17–28</sup> To minimise peri-shock pauses (PSP) in compressions, most resuscitation councils now recommend continuing compressions whilst charging the defibrillator. However, there is some variation as to whether this occurs before or after a pause for rhythm assessment.<sup>29–35</sup> The Australian Resuscitation Council (ARC) recommends anticipatory charging of the defibrillator before pausing for rhythm assessment, with or without subsequent defibrillation, aiming to keep the PSP less than 10s.<sup>34</sup>

In New South Wales, Australia, COACHED (Fig. 1) is a commonly used mnemonic algorithm guiding a pause for rhythm check with anticipatory charging.<sup>36</sup> Mnemonics and algorithms as cognitive aids have been shown to reduce cognitive load, reduce systematic errors,

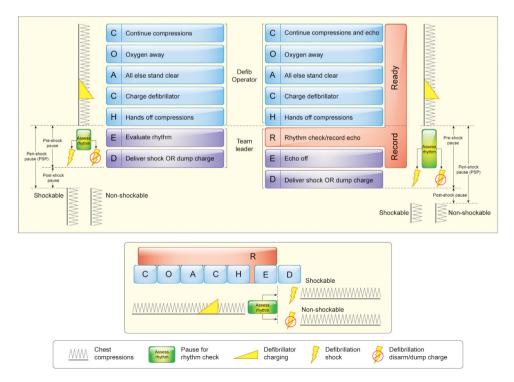
expedite tasks, establish a common language and allow for teamsharing of a mental model in stressful situations.<sup>37–44</sup> To date, only one study has evaluated the COACHED protocol, and its use was found to reduce the pause in compressions from 8s to 6s in a simulated environment.<sup>36</sup>

The COACH-RED protocol was created to systematically incorporate focused echocardiography into the rhythm check without prolonging the pause in compressions beyond the 10s recommended by the Australian Resuscitation Council (Fig. 1).<sup>34,45</sup> The authors video-recorded themselves using the algorithm in a simulated environment and reported the PSP to be well below 10s-6.45s for non-shockable and 8.47s for shockable rhythms.45-47 In Australia. the COACHED protocol is often primarily used by senior nurses in charge of the defibrillator, allowing the team leader (usually a medical doctor) to release some cognitive load during this process whilst maintaining situational awareness.<sup>36</sup> The primary aim of this study was to examine the feasibility of teaching nurses to perform each role in the COACH-RED protocol safely and to retain the ability after one then five weeks. Uniquely, whilst every participant was trained in Advanced Life Support (ALS) and COACHED, none had previous formal training in echocardiography or COACH-RED. We hypothesised that using COACH-RED would require a slightly longer PSP compared to COACHED, but not beyond 10s.

## **Methods**

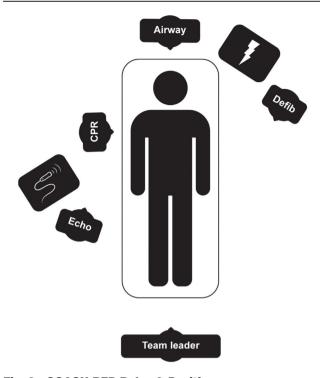
#### Study design

This was a pilot, prospective simulation study aimed at training a convenience sample of five participants so they could safely perform each role in the COACH-RED protocol (see Figs. 1 and 2).





The original author of the COACHED protocol is unknown. The COACH-RED protocol is reproduced here (with some alterations) with permission from Finn et al., 2018.<sup>6</sup>



# Fig. 2 - COACH-RED Roles & Positions.

Reproduced with permission and minor alterations from from Finn et al., 2018.<sup>6</sup> Airway: participant with bagvalve mask at the head of the bed. Team Leader: participant at the foot of the bed with a clear overview. CPR: participant responsible for chest compressions to the right of the bed. Echo: participant operating the ultrasound machine, strategically placed to not interfere with compressions or view of team leader. Defib: participant operating the defibrillator as illustrated by box with lightning bolt, must have a clear view of all other participants before delivering or disarming the charge.

Competency was assessed immediately, one week after initial training and again one month later, to check retention of knowledge, skill and confidence. Fig. 3 illustrates the components of the three sessions. The initial session comprised a 45-minute PowerPoint presentation followed by a practical session. The material covered in the presentation included a literature review of the role of focused echocardiography in cardiac arrest, normal subcostal echocardiographic findings and examples of abnormal subcostal echocardiographic findings. Abnormal findings included enlarged right ventricle, cardiac tamponade and lack of spontaneous cardiac activity. The COACH-RED protocol, as depicted in Fig. 1, was then taught and the two videos from the original publication by Finn et al. (2019) were shown to participants.<sup>46,47</sup>

Each assessment consisted of three scenarios of four cycles each. Participants kept the same role for each scenario, but rhythm varied from one cycle to the next. Participants were given 30min to practise before each assessment and time was allowed for further training after assessments if required. In addition to COACH-RED, three scenarios of COACHED were also assessed during the final assessment, to compare the two protocols and the difference in PSP duration.

To avoid selection bias, participants were randomly allocated roles for each scenario. An online randomiser programme was used for this purpose.<sup>48</sup> Similarly, shockable vs non-shockable rhythms were randomly allocated to individual cycles in advance.

# Setting

We used identical equipment in all three sessions, which were carried out in the Sim Lab of University of Sydney's Clinical School located at Sydney Adventist Hospital (SAH), New South Wales, Australia. The study took five weeks to complete from the initial session on 13th November 2019 to the third session on 18th December 2019. The CPR manikin was a Blue Phantom<sup>™</sup> 1800-FAST ultrasound phantom (© CAE Healthcare, Florida, USA) allowing for subcostal echocardiographic views. The ultrasound machine was a Philips Sparg (© Philips, Bothell, USA), with standard Cardiac Preset using phased array probe and cine-loop recordings set to 10s. An adult-sized bagvalve-mask (© Mayo Healthcare, Sydney, Australia) and the ALSi system (© ISimulate, Sydney, Australia) allowed for high-fidelity simulation. Canon EOS 60D (© Canon, Tokyo, Japan) and Nikon D750 (© Nikon, Tokyo, Japan) cameras on tripods were used to videorecord sessions from different angles, capturing all participants whilst a GoPro Hero3 (© GoPro Inc, San Mateo, USA) was placed nearer the manikin to record the PSP. None of the participants had used the manikin or ultrasound machine prior to this study.

#### Participants

Inclusion criteria were predefined as healthcare staff of the Emergency Department (ED) at SAH, with at least three years of

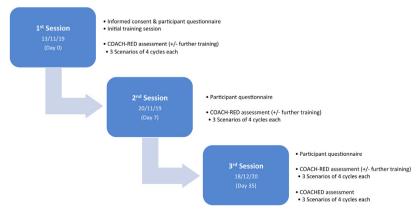


Fig. 3 - Study Protocol Flowchart.

experience in the department and up to date with ALS training. Written informed consent was obtained from participants using an opt-in signed consent form explaining the study design, rationale and that sessions would be filmed with recordings potentially published for data transparency. Participants were excluded if they were unable to attend all three sessions.

#### Data collection and analysis

Questionnaires were used to gather baseline participant characteristics as well as experience and confidence with CPR and focused echocardiography at the start of every session and the end of the final session. Researchers marked participants performance in real-time during assessments using a mark sheet with predefined criteria. Adobe Premiere Pro v14.0 (© Adobe Inc, San Jose, USA) was used to slow down the 60 frames per second GoPro Hero3 footage and measure PSP to an accuracy of 0.016s per frame. Data were tabulated in Excel 365 (© Microsoft Corp, Washington, USA) and simple descriptive statistics used for comparison. As this was a pilot study with small sample size, no statistical analysis was performed.

#### Ethics

The study was approved by the Adventist Healthcare Limited Human Research Ethics Committee (HREC 2019-036) and received no funding.

#### Study outcomes

The primary outcome was defined as the correct performance of the COACH-RED protocol by the team. To pass a scenario, participants had to correctly perform COACH-RED in three out of four cycles with at least one satisfactory echocardiographic recording per scenario, and participants were required to pass two out of three scenarios per each assessment. A critical error was defined as anything which could potentially be dangerous for participants, such as defibrillating whilst echosonographer or other touching the manikin or misinterpretation of the rhythm. The correct performance was marked using predefined assessment criteria for each role, in Table 1.

Secondary outcomes were the length of peri-shock pause (PSP) for both COACH-RED and COACHED protocols as well as participant self-reported confidence and feedback.

#### **Results**

#### Participants

Five participants met inclusion criteria and were all ALS-trained nurses. All participants voluntarily consented to participate and attended all three sessions. The participant mean age was 40 (range 32–53) with a 3:2 male to female ratio. Mean experience working in ED was 12 years (range 8–20) and mean time since ALS certification was 7.4 months (range 3–15). All participants were familiar with the COACHED protocol prior to this study, having used it regularly in previous cardiac arrest resuscitations. None had any previous formal training in focused echocardiography or the COACH-RED protocol.

#### Primary outcomes

Table 2 summarises the results of the COACH-RED and COACHED assessments, grouped into primary and secondary outcomes. Fig. 4 depicts still images from the cine-loop recordings obtained in each cycle. There were only four cycles of incorrect performance; no critical errors and all echocardiographic recordings were satisfactory, resulting in a 100% pass rate for the primary outcome. Two role errors occurred due to the echosonographer not removing the probe promptly when instructed, and two were due to the team leader failing to instruct a shock and resumption of compressions in a shockable rhythm. These errors were in separate COACH-RED scenarios, allowing for the 100% pass rate. There were no errors in the COACHED scenarios.

#### Secondary outcomes

#### Peri-Shock pause

Peri-shock pause (PSP) per individual cycle and their averages are shown in Table 2. Of all cycles using COACH-RED, 69% had a PSP of

Table 1 – COACH-RED Assessment Criteria/Checklist.									
Role	Responsibilities	Pass/Fail							
Airway	Removes oxygen at appropriate time								
	Safe Overall								
CPR	Hands off chest at appropriate time								
	Hands back on chest promptly								
	Safe overall								
Defib	Performs COACH-RED								
	Charges defibrillator								
	Checks all clear including US probe								
	Disarms/Delivers appropriately								
	Safe overall								
Echo	Prepares machine with correct preset & positioning								
	Records adequate subcostal image ( $\geq$ 1 from 4 cycles)								
	Removes probe at appropriate time								
	Safe Overall								
Team Leader	Performs COACH-RED								
	Interprets rhythm								
	Instructs disarm/deliver shock appropriately								
	Instructs to continue compressions promptly								

Assessments				Primary Outcome - Pass?					Secondary Outcome Peri-Shock Pause (PSP) (second					
Da	iy Sce	nario	Cycle	Туре	Airway	CPR	Defib	Echo	Team Leader	Overall Pass?	PSP	Median	PSP	
0		1	1	Non-Shockable	Y	Y	Y	Y	Y	Y	9.82	10.48	9.70	9.35
			2	Shockable	Y	Y	Y	Y	Y		11.14			(IQR 2.3
			3	Non-Shockable	Y	Y	Y	Y	Y		9.67			
			4	Shockable	Y	Y	Y	Y	Ν		15.36			
		2	1	Non-Shockable	Y	Y	Y	Y	Y	Y	7.92	9.89		
			2	Non-Shockable	Y	Y	Y	Y	Y		9.15			
			3	Shockable	Y	Y	Y	Y	Y		12.15			
			4	Shockable	Y	Y	Y	Y	Y		10.62			
		3	1	Shockable	Y	Y	Y	Y	Y	Y	9.72	9.25		
			2	Non-Shockable	Y	Y	Y	Y	Y		9.15			
			3	Shockable	Y	Y	Y	Y	Y		9.35			
			4	Non-Shockable	Y	Y	Y	Y	Y		8.87			
7		1	1	Non-Shockable	Ŷ	Ŷ	Ŷ	Ŷ	Ŷ	Y	9.34	10.75	9.79	
÷.,		•	2	Non-Shockable	Ŷ	Ŷ	Ŷ	Ŷ	Ŷ	·	10.98		0.1.0	
			3	Shockable	Ŷ	Ŷ	Ŷ	Ŷ	Ŷ		15.64			
			4	Non-Shockable	Ý	Ý	Ý	Ý	Ŷ		10.52			
2 3	2	1	Non-Shockable	Y	Ý	Ý	Ý	Ý	Y	8.58	10.84			
	2	2	Shockable	Y	Ý	Ý	N	Ý		11.89	10.04			
			3	Shockable	Y	Y	Y	Y	Y		12			
			3 4	Non-Shockable	Y Y	Y	Y Y	Y	Y Y		12 9.79			
		~			r Y	Y	Y Y		Y Y	V		0.00		
		3	1	Non-Shockable	Y Y	Y Y	Y Y	Y		Y	8.15	8.82		
			2	Shockable	-	-		Y	Y		9.49			
			3	Shockable	Y	Y	Y	N	Y		9.79			
	_		4	Non-Shockable	Y	Y	Y	Y	Y		8.11			
35	)	1	1	Shockable	Y	Y	Y	Y	Y	Y	10.95	9.66	8.36	
			2	Non-Shockable	Y	Y	Y	Y	Y		8.36			
			3	Shockable	Y	Y	Y	Y	N		11.03			
			4	Non-Shockable	Y	Y	Y	Y	Y		7.75			
		2	1	Shockable	Y	Y	Y	Y	Y	Y	8.79	7.59		
			2	Shockable	Y	Y	Y	Y	Y		7.81			
			3	Shockable	Y	Y	Y	Y	Y		7.37			
			4	Non-Shockable	Y	Y	Y	Y	Y		6.83			
		3	1	Shockable	Y	Y	Y	Y	Y	Y	9.13	8.60		
			2	Non-Shockable	Y	Y	Y	Y	Y		7.87			
			3	Shockable	Y	Y	Y	Y	Y		8.84			
			4	Shockable	Y	Y	Y	Y	Y		8.35			
35	5	1	1	Shockable	Y	Y	Y	Y	Y	Y	7.07	7.20	6.94	
)			2	Non-Shockable	Y	Y	Y	Y	Y		7.25		(IQR 0.6	61)
			3	Non-Shockable	Y	Y	Y	Y	Y		8.97			
			4	Shockable	Y	Y	Y	Y	Y		7.14			
+ = )		2	1	Shockable	Y	Y	Y	Y	Y	Y	6.79	6.74		
			2	Non-Shockable	Y	Y	Y	Y	Y		6.46			
			3	Non-Shockable	Y	Y	Y	Y	Y		7.35			
			4	Shockable	Y	Y	Y	Y	Y		6.68			
		3	1	Shockable	Y	Y	Y	Y	Y	Y	6.8	6.70		
			2	Shockable	Y	Y	Y	Y	Y		6.6			
			3	Non-Shockable	Ŷ	Ŷ	Ŷ	Ŷ	Ŷ		8.86			
			4	Non-Shockable	Ŷ	Ŷ	Ŷ	Ŷ	Ŷ		6.56			

less than 10s, with a median of 9.35 (IQR 2.34, Range 6.83–15.36) seconds. There were two outlying COACH-RED cycles with PSP greater than 15s, resulting in a mean PSP greater than 10s for day 0 and day 7. Excluding these, with median values, the averages for all three COACH-RED sessions were below 10s. COACH-RED performance improved during the study period – 83% of cycles had a PSP of lesser than 10s by the third session. The average PSP also improved from the first to the third scenario on each assessment.

With COACHED protocol, 100% of cycles had a PSP less of than 10s with a median of 6.94 (IQR 0.615, range of 6.46–8.97) seconds. The difference in median between COACH-RED and COACHED was 2.41 s.

Fig. 5 illustrates the discrepancy in PSP for shockable and nonshockable rhythms. Only two of the non-shockable COACH-RED cycles resulted in a PSP greater than 10s compared to nine for shockable rhythms. Shockable rhythms were thus much more likely to

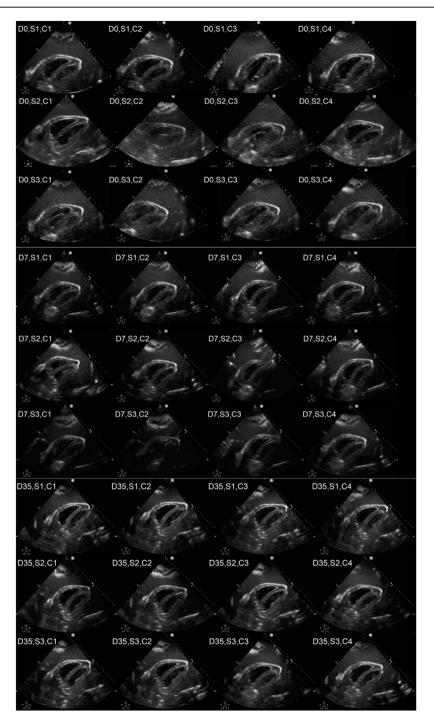


Fig. 4 - Stills from COACH-RED Recordings.

result in a PSP greater than 10s and all the primary outcome errors and delays beyond 15s occurred in shockable rhythm cycles.

# Confidence

Fig. 6 depicts the trend in confidence recorded by the participant questionnaire before each session and after the final session. The questionnaire after the final session also asked participants whether they thought COACH-RED should be implemented in their ED and which roles they would be happy to perform. All participants answered

yes to the first question and were happy to perform all roles except for one participant who stated they would not feel comfortable in the role of echosonographer yet.

#### Experience

Participants were asked if they had participated in cardiac arrests between sessions. Two participants were involved in a cardiac arrest in the week between the first two sessions and used the COACHED but not the COACH-RED protocol.

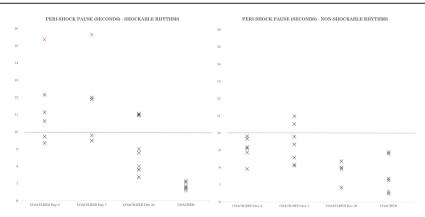


Fig. 5 - Peri-Shock Pauses for Shockable vs Non-shockable Rhythms.

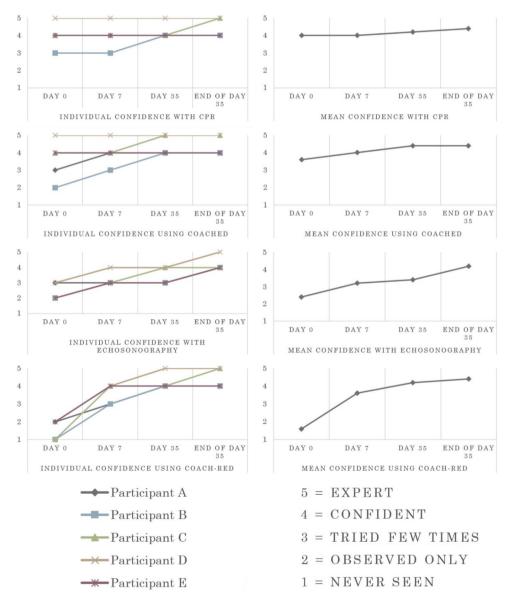


Fig. 6 - Individual Participant & Mean Confidence.

#### **Discussion**

Emergency physicians have been using focused echocardiography during cardiac arrest to identify reversible causes such as hypovolaemia, pulmonary embolus and pericardial effusion since 1985.<sup>8,49,50</sup> At least 13 different protocols have been created for this purpose – FATE, Rapid Cardiac Ultrasound, FEER, CAUSE, FEEL, modified FEER, RUSH, PEA, GDE, SESAME, SHoC, CASA and most recently COACH-RED.<sup>45,51–61</sup> Most of these protocols have focused on the echocardiographic views and the pathology to identify whereas COACH-RED was created in response to publications in 2017 and 2018 suggesting that ultrasound during a cardiac arrest was associated with prolongation of pauses in chest compressions.<sup>14,15,45</sup> The detrimental effect of a pause in chest compressions was theorised over twenty years ago, proven in animal models in 2002 and later supported by computer-based physiological analysis.<sup>62–65</sup>

A pause in chest compressions is currently necessary for evaluation of the cardiac rhythm during cardiac arrest and subsequent defibrillation where appropriate. The total pause (peri-shock pause) can be divided into pre-shock and post-shock pauses. In 2010, a seminal paper showed that defibrillator charging during chest compressions was safe and could reduce the pre-shock pause from 13.3 to 2.6s.<sup>66</sup> The same study compared charging before rhythm check (anticipatory charging) with charging after rhythm check and found that both pre-shock and post-shock pauses were one or two seconds longer for anticipatory charging but the overall pause in compressions in the 30s leading up to defibrillation was significantly shorter with anticipatory charging, by 7-10 seconds.<sup>66</sup> It remains unknown whether it is beneficial to have a much shorter overall pause in the 30s leading up to defibrillation or an extremely short pause directly before defibrillation, and thus resuscitation guidelines differ between Europe, USA and Australasia.31,32,34

The European Resuscitation Council (ERC) recommend two pauses—one for rhythm assessment and a second for defibrillation, with chest compressions in between whilst charging the defibrillator.<sup>31</sup> The American Heart Association (AHA) have the same advice as to the ERC for the first rhythm assessment, but if the first assessment finds a shockable rhythm, they recommend anticipatory charging before subsequent rhythm assessments.<sup>32</sup> The Australian & New Zealand Resuscitation Guidelines recommend anticipatory charging, with a peri-shock pause duration less than 10s, in keeping with the ILCOR guidelines of 2015, which led to the creation of the COACHED and COACH-RED protocols.<sup>13,34,36,45</sup>

In this study, five ALS-trained emergency nurses successfully learned, retained and performed the COACH-RED protocol in a simulated CPR scenario after a single two-hour training session. This is not the first study to use non-expert sonographers, but we believe it to be the first study with nurses performing all roles of ALS, including echocardiography.<sup>16,31</sup>

It is not customary for nurses to team-lead or performs echosonography during resuscitation in Australia, but both roles were successfully performed with 100% adequacy of echocardiographic views recorded. This has implications for small or remote centres with limited emergency staff. Algorithms and mnemonics such as the 4H's and 4T's and COACHED already serve as useful memory aids allowing cognitive offloading during stressful scenarios, but in a centre with a single emergency doctor, the ability to step back during a rhythm check run entirely by nursing staff would allow even greater cognitive offloading; however, this was not evaluated by this study. The COACH-RED protocol resulted in a median peri-shock pause of 9.35s overall across 36 recorded cycles. To our knowledge, this was much faster than any other cardiac arrest echocardiography protocol in the literature.<sup>14,15,49–59,67</sup> Outside of the training and assessment sessions, one cardiac arrest resuscitation was attended by two of the participants where COACHED (in the role of Defib & CPR) but not COACH-RED protocol was used. One participant practised an echocardiographic view in a non-arrest scenario, once throughout the five-week period. Centres with a higher burden of cardiac arrests might expect more consistency of results as participants would be more likely to use the protocol regularly.

This study has further reinforced the use of the anticipatory charging COACHED protocol to achieve peri-shock pauses well under 10s.<sup>36</sup> The COACH-RED protocol was developed from COACHED, so as to be already familiar to emergency staff. We correctly hypothesised that using COACH-RED would require a slightly longer peri-shock pause compared to COACHED, but not beyond 10s. As expected, the addition of echocardiography increased the PSP on average by two seconds. Notably, most cycles with peri-shock pauses longer than 10s in this study were in shockable rhythms. Good quality CPR and defibrillation are the most important interventions for shockable rhythms with little role for echocardiography. Most utility for echocardiography in cardiac arrest has been proposed for the nonshockable rhythms.<sup>51-60</sup> Prognostication studies are most likely to find value in the first and last sonographic images obtained in a cardiac arrest.<sup>16</sup> Given this dichotomy, resuscitation team could use COACH-RED protocol in the first rhythm check and then either COACHED or COACH-RED for subsequent pauses depending on if the rhythm is likely to be shockable or non-shockable, respectively. Results from this study suggested that learning COACH-RED did not lead to any difficulties switching back to COACHED. Future studies could investigate this more specifically.

# Limitations

This was a small study of only five participants, limiting any statistical analysis. The positive results may not be extrapolatable to use in real patient resuscitations with higher stress levels, distracting factors and nuances of obtaining echocardiographic views in various body types within a limited time window. Although participants were blinded to the PSP time being recorded, they were aware of assessment taking place, potentially affecting their performance (Hawthorne effect). All participants were familiar with COACHED prior to the study, and these results may not apply to other settings. The current study focused solely on obtaining sub-costal echocardiographic views and did not investigate for non-cardiac pathologies such as tension pneumothorax or ruptured abdominal aortic aneurysm. It is hoped that future research will help investigate some of these questions.

# Conclusions

The COACH-RED protocol, which allows safe and effective incorporation of echocardiography into cardiopulmonary resuscitation, can be learned, performed and retained by ALS-trained nurses after a single training session. The COACH-RED protocol can be used to keep pauses in chest compressions (peri-shock pauses) under the 10s, as recommended by the Australian Resuscitation Council. This provides a foundation for future studies using COACH-RED in ALS training and real-time resuscitations.

# **Authors contributions**

All authors made a substantial contribution to the conception and design of the study, acquisition and analysis of data. Authors BT, BJ and VM drafted the manuscript. Authors BT and VM revised the manuscript. Author BT prepared all figures and tables. Author VM was the project supervisor responsible for the key ideas and the development of the study. All authors approved of the final version submitted for publication.

# **Conflicts of interest**

This study received no funding or grants, and the study authors have no competing interests to declare.

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