Pocket-size imaging device: effectiveness for ward-based transthoracic studies†

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Aims
Pocket-size imaging devices (PSID) are now available; their potential role in a hospital environment has been investigated but still remains undefined.

Methods
We evaluated the effectiveness of PSID in 92 patients referred for bedside transthoracic echocardiogram (TTE). Patients were included where there was a focused clinical question: quantification of left ventricular function (LVF); presence of regional wall motion abnormalities (RWMA); evidence of pericardial effusion, exclusion of significant valve pathology. Each patient underwent an echocardiography evaluation using PSID and TTE. In 83 patients \( k = 90\%, 95\% \text{ CI} (82.2–95.4) \), it was possible to answer the clinical question by PSID examination alone. There was agreement between the findings of PSID and TTE in 86 cases \( k = 47\%, 95\% \text{ CI} (12.8–82.0) \), in three cases, the clinical question was not answered by both modalities. When the clinical question was focused on LVF, the agreement was excellent \( k = 96\%, 95\% \text{ CI} (95.3–97.9) \), as was the agreement in the detection of RWMA \( k = 94.5\%, 95\% \text{ CI} (82.4–95.1) \). There was also good concordance in the detection of valve pathology and pericardial effusion. Using PSID, the reduction in the scanning and reporting time was 66%. The cost-effectiveness analysis produced very favourable results: with PSE, we obtained an overall cost saving per scan of 76%, compared with TTE.

Conclusion
This study demonstrates that PSID can provide a valuable alternative to TTE in the presence of focused clinical questions and can provide an efficient way of delivering a ward-based transthoracic echo service.

Keywords
Transthoracic echocardiogram • Cost-effectiveness • Pocket-size imaging devices

Introduction
Echocardiography, at present, is the first-line imaging tool in cardiology, due to its diagnostic accuracy, feasibility, and cost-effectiveness. Bedside echocardiography allows an immediate assessment of the heart in critically ill patients unable to be transferred to the echocardiography department; this avoids delay in diagnosis and, therefore, in appropriate treatment.

Improvements in technology, including miniaturization have led to the development of pocket-size imaging devices (PSID) with good image quality and extreme portability. These attributes make PSID potentially suitable for bedside Echo studies in a hospital environment. Recent studies have shown a good correlation between PSID and standard echo machines in various other clinical environments1–8 and a focused echocardiography study has been demonstrated useful for patient management in different clinical settings.9–12

We assessed the clinical and economical usefulness of PSID in the echocardiography department of a busy tertiary hospital, specifically, in the setting of bedside echo requests. Common clinical queries include the quantification of left ventricular systolic function (LVF), presence of regional wall motion abnormalities (RWMA), evidence of pericardial effusion, and exclusion of significant valve pathology.

We evaluated the usefulness of the PSID as a screening tool for those patients who would benefit from an immediate clinical decision based on focused echocardiography findings. This would

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† The study was performed at King’s College Hospital London.

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consequently reduce the number of full bedside echocardiograms thereby increasing efficiency and decreasing the overall cost.

Methods

The study included a group of 92 male and female inpatients at King’s College Hospital London (UK) with a clinical indication for echocardiography in whom a bedside (portable) study had been deemed necessary for diagnostic purposes. This study was based on an internal audit project created with the purpose of confirming the diagnostic efficacy and safety of PSID in a clinical environment. The cost-effectiveness analysis was also performed to further assess the economic impact of the device. Patient enrolment was performed over a 3-month period and three PSID sessions per week were performed. Patients were included where there was a focused clinical question for the quantification of LVF, presence of RWMA, evidence of pericardial effusion, and exclusion of significant valve pathology.

For RWMA assessment, the left ventricle (LV) was divided into seven segments: anterior wall, lateral wall, posterior wall, inferior wall, inferior-septum, anterior septum, and apex. In a subgroup of patients, RWMA assessment was performed using the American Society of Echo 16-segment model of the LV. Two experienced and accredited sonographers performed a focused scan (B-mode and colour flow imaging) on these patients with the PSID device (Vscan; GE Medical). No measurements were performed and LVF and structures were only visually assessed. On the same day, each patient also underwent a full bedside echocardiography evaluation (transthoracic echocardiogram (TTE)). Exclusion criteria included the suspicion of endocarditis, left ventricular thrombus, or increased right ventricular pressure, where it can be assumed that standard TTE (possibly with contrast) would be required anyway.

Pocket-size study

The ultrasound medical device used in this study was a Vscan (GE Medical Systems). This device is suitable for carrying in a coat pocket and measures 135 × 73 × 28 mm and weighs 390 g. The PSID has a 2D-grey scale and live colour Doppler imaging. It is not possible to perform MMode or to obtain spectral Doppler data. A specific algorithm permits automatic storage of the images without using an ECG signal. Each study was automatically assigned an examination number. All the images were saved on the device and transferred after each session to a workstation using commercial software (Gateway GE Vingmed Ultrasound).

A focused exam was performed at bedside with the patient in the left lateral decubitus position. The echocardiography assessment was obtained using a parasternal long-axis and short-axis view, an apical four-chamber, apical two-chamber, apical three-chamber, and a subcostal view. After each scan, the sonographer was asked to answer specific questions regarding the left and right ventricular function and dimensions, the presence of RWMA, left atrial enlargement, and to comment on the presence of pericardial effusion or major cardiac valve disease. Each question required a yes/no answer. The 16-segment wall motion score index was used for the assessment of RWMA (Figure 1).

As previously mentioned, no measurements were performed during and after PSID examination and the LVF and cardiac structures were only visually assessed by the two expert sonographers. As has been previously demonstrated, there is a good correlation between visual assessment and biplane measurement of LVF performed by an experienced operator.13–15

The overall quality of the study was graded as poor, average, or good. The images were defined as poor when there was suboptimal endocardial border delineation with suboptimal visualization of more than six myocardial segments and/or the cardiac valves were not well visualized in all the views. The images were defined as average quality when there was suboptimal endocardial border delineation with two to six myocardial segments not optimally visualized or the cardiac valves were not well visualized in more than one view. A good quality study was defined by a good endocardial border delineation (less than two myocardial segments not optimally visualized) and good valve visualization with optimal visualization of all the cardiac anatomy (Figure 1).

The scanning time was calculated as the time taken from the beginning to the end of the examination.

Portable TTE study

On the same day of the PSID, each patient underwent a full transthoracic study performed at bedside using Philips CX 50 or a GE Vivid I. These machines are capable of second harmonic B-Mode imaging, Mmode, continuous wave, pulsed wave, and colour Doppler. The images were stored on the hospital server and measurements were made using dedicated image viewing software (Xcelera R3.2L1 SP2 version). All the standard measurements were performed offline. The cardiac structures were analysed in accordance with the British Society of Echocardiography guidelines.16 Left ventricular ejection fraction (EF) was calculated by Simpson’s biplane from apical four- and two-chamber views.

Interpretation and comparison of both PSID and TTE results was performed by an experienced echocardiography fellow. The main purpose was to assess if a PSID evaluation was accurate enough to answer the clinical question and to assess if the PSID findings were concordant with the TTE findings. To determine the inter-observer variability, an experience sonographer blinded to the previous results, reassessed if the clinical question had been answered by PSID and if the findings were concordant in between the two modalities. To assess the intra-observer variability, the same sonographer blinded to his previous analysis reassessed the LVF and RWMA in a subgroup of patients.

Statistical analysis

All statistical analyses have been performed using STATA version 12.1 (StataCorp LP, College Station, TX). For descriptive purposes, patient characteristics, demographic, and medical history data are presented as means with standard deviations for continuous variables and frequency counts and percentages for categorical variables. Kappa statistics17 were used to estimate the level of inter-observer agreement and intra-observer reproducibility.

Kappa coefficient is a statistical measure of inter-rater agreement for qualitative items. It is generally robust measure that takes into account the agreement occurring by chance. Kappa is always ≥1 with a value close to 1 implying good agreement.

Kappa values were given with their 95% confidence intervals or with the standard error.

Cost-effectiveness analysis

The cost-effectiveness analysis was based on the cost-minimization analysis. The overall costs of a PSID study were compared with the overall costs of a TTE study. The overall cost for both modalities was calculated using the combination of staff cost, cost and maintenance of the equipment, hospital costs, time taken for a sonographer to travel between patients, plus mean scanning and reporting time.

Results

Patients’ baseline characteristics are shown in Table 1. The mean age of the patient was 64 ± 17 years, 59% were male. The mean EF was 50 ± 13%.
The patient population includes mainly patients from the cardiac, the liver, the haematology, the vascular ward, and from the stroke unit. Eighteen patients presented with acute myocardial infarction, 5 with acute coronary syndrome, 6 with arrhythmias, 2 with cardiac arrest, 7 with valve disease, 7 with heart failure, 1 with pericarditis, and 1 with dilated cardiomyopathy. Among the non-cardiac patients, 3 of them presented with vascular disease, 4 with haematology disorders, 14 with liver failure, 7 with stroke, 2 with respiratory failure, and 15 with miscellaneous diagnosis.

In all the patients included in the study, the echocardiographic diagnosis was important to confirm or exclude a clinical suspicion or to optimize the management.

The assessment of LVF and RWMA was the most common clinical question (47%), followed by the assessment of LV function alone (42%), presence of pericardial effusion (9%), and exclusion of severe valve disease (2%). In 16% of PSID studies, the quality of the pictures was considered good based upon the previously described methodology, in 66% of cases, it was considered average and, in 17% of the cases, it was considered poor. The picture quality in the TTE group was found to be good in 29% of the studies, average in 63%, poor in 8%. In 19% of cases, the image quality was deemed poorer on PSID compared with conventional TTE. We assessed whether there was an association between BMI and image quality. However, using Fisher’s exact test, there was no significant statistical correlation between BMI and PSID image quality ($P = 0.067$). Moreover, no significant statistical difference has been found in the quality of the pictures obtained by TTE ($P = 0.782$) and PSID ($P = 0.999$) when categorizing for the BMI > 25. This is probably because suboptimal image quality is not only related to a
high BMI; it is also influenced by the chest conformation, the position of the heart in the chest, by the presence of lung disease, and by patient mobility or cooperation.

In the 90% of cases, 83 patients \( k = 90\%, \text{ 95\% CI (82.2–95.4)} \), it was possible to successfully answer the clinical question by PSID examination alone. There was agreement in between the two modalities in 86 cases \( k = 47\%, \text{ 95\% CI (12.8–82.0)} \). In five of the six cases of no agreement between the two modalities, the clinical question was mainly on LV function assessment. This was not possible with PSID due to poor image quality. The last case of disagreement between TTE and PSID concerned the exclusion of significant valve disease. The overall picture quality was graded as average. The sonographer reported the mitral valve findings as ‘possible previous mitral valve repair’. However, the patient had moderate mitral valve stenosis, recognized on TTE. Interestingly enough a second sonographer, during inter-observer variability analysis, recognized the presence of mitral valve stenosis. So this may have been an issue related to operator experience rather than a limitation of PSID.

Using the \( k \)-statistic, when the clinical question was focused on LVF, the agreement was \( k = 96\%, \text{ 95\% CI (95.3–97.9)} \). In three cases, as discussed above, it was not possible to assess the LV function by PSID due to the suboptimal quality of the pictures. In one case, the LV dysfunction was overestimated by PSID.

The agreement in the detection of RWMA between the PSID and TTE is shown in Table 2.

In a subgroup of patients, a second sonographer performed the RWMA analysis using a 16-segment model of the LV both in PSID and TTE studies. The agreement between the two modalities for each LV segment was excellent and the results are shown in Table 3.

In three patients, the clinical question was to exclude significant valve pathology and this was possible by PSID in two of them (Figure 3). In the third case, as already discussed, the PSID findings were misleading. When the question was focused on the presence of pericardial effusion, the inter-observer agreement was excellent (100%; \( k = 100\% \)).

A third experienced sonographer, blinded to the previous results, reinterpreted the images previously obtained in order to assess inter-observer variability (using the seven-segment model). This was very satisfactory with an agreement of 79%. The agreement between the

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**Table 1 Patients’ baseline characteristics**

<table>
<thead>
<tr>
<th>Continuous variables</th>
<th>Mean ± SD (range)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 17</td>
<td>17–98</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>124 ± 25</td>
<td>80–201</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72 ± 15</td>
<td>46–126</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>79 ± 18</td>
<td>48–140</td>
</tr>
<tr>
<td>EF</td>
<td>50 ± 13</td>
<td>15–65</td>
</tr>
<tr>
<td>BMI</td>
<td>27.7 ± 6.2</td>
<td>16–41</td>
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</table>

<table>
<thead>
<tr>
<th>Discrete variables</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>59</td>
</tr>
<tr>
<td>Atrial fibrillation during scan</td>
<td>9</td>
</tr>
<tr>
<td>Prior atrial fibrillation</td>
<td>22</td>
</tr>
<tr>
<td>Hypertension</td>
<td>42</td>
</tr>
<tr>
<td>Diabetes</td>
<td>22</td>
</tr>
<tr>
<td>Current smoke</td>
<td>28</td>
</tr>
<tr>
<td>Ex smoke</td>
<td>20</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>39</td>
</tr>
<tr>
<td>Prior MI</td>
<td>34</td>
</tr>
<tr>
<td>Prior angina</td>
<td>11</td>
</tr>
<tr>
<td>Heart failure</td>
<td>13</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>4</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>16</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

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**Figure 2** Comparison between parasternal long-axis views obtained using TTE (A) and PSID (B) in a patient with a normal LV.
The prevalence of abnormal echo findings in the study population was assessed using both PSID and TTE with related modalities in visualizing and interpreting pathological findings. In fact, there were no statistically significant differences between the two modalities in visualizing and interpreting pathological findings. The agreement in between the two modalities was excellent when the clinical question was focused on the LVF. The scanning time was 16.4 ± 1.8 min for TTE and 5.9 ± 0.5 min for PSID. The mean reporting time was estimated as 5 min for a PSID and 15 min for a TTE.

### Cost-effective analysis results

As mentioned previously, the overall cost for both modalities was calculated using the combination of staff cost, cost and maintenance of the equipment, hospital costs, time taken for a sonographer to travel between patients, plus mean scanning and reporting time. The purchase cost of the VSCAN was £5,000 (USD 7,827, €5,815), the maintenance was calculated as £500 (USD 783, €582) per year. The cost for a portable TTE machine as used in this study is £50,000 (USD 78,272, €58,148) with the maintenance cost of £5,000 (USD 7,827, €5,815) per year.

The cost has been calculated per single scan. The cost of a trans-thoracic bedside echocardiogram has been calculated at £88 (USD 137.76, €102.34). This included: the cost of a band seven echocardiographer, estimated at £47.70 (USD 74.36, €55.24) allowing for the scanning time and time spent by the echocardiographer in reaching the patient and setting up the machine; the cost of the instruments (purchase + maintenance) was £17 (USD 26.61, €19.77) and all the associated hospital costs have been estimated £22.90 (USD 35.85, €26.63).

The cost of a PSID has been calculated at £21 (USD 32.87, €24.42). The cost of a band seven echocardiographer per exam was calculated £15.20 (USD 23.79, €17.68), the cost of the instrument (purchase + maintenance) was £0.38 (USD 0.59, €0.44) and all the associated hospital costs have been estimated £5.45 (USD 8.53, €6.34).

This led to an overall cost saving per scan of 76%, using the PSID, moreover, PSID led to a reduction in the scanning and reporting time of 66% compared with TTE.

### Discussion

In our busy tertiary referral hospital, many bedside echocardiograms (TTE) are requested on a daily basis (a mean of 35 and max of 50). This can affect the waiting time for inpatient and for outpatient echocardiograms due to limited resources. However, the most common queries are simple and focused, including the assessment of the LVF and of RWMA, the exclusion of pericardial effusion and significant valve pathology. Performing a complete bedside echo is time-consuming and results in considerable strain on departmental resources. Moreover, a considerable amount of time is required to transport the standard echo machines throughout the hospital between the wards and the departments. The results of this study show that a pocket-size imaging device used by experienced sonographers improves the efficiency of ward-base echocardiography services and reduces the overall cost without significantly compromising diagnostic accuracy. In experienced operators’ hands, the lack of spectral Doppler information overall did not appear to be a major disadvantage in answering the focused questions mentioned above. In 90% of cases, it was possible to successfully answer the clinical question by PSID examination. When it was not possible, this was mainly due to poor image quality (both with TTE and PSID), and the PSID was not considered diagnostic. The agreement in between the two modalities was excellent when the clinical question was focused on the LVF [k = 96%, 95% CI (95.3–97.9)]. In the assessment of the two observers on the quantification of the LVF was 84% with a k = 75%. The agreement on the visualization of RWMA was excellent 92% with a k = 84%. The agreement in the detection of each specific segment abnormality by the different operators is shown in Table 4.

To assess the intra-observer variability, the first sonographer blinded to his previous results, reassessed the LVF and the RWMA in a subgroup of patients. The intra-observer variability was also very good as shown in Table 5. The query of left ventricular thrombus was one of our exclusion criteria because sometimes this echo diagnosis can be very challenging without the use of contrast; however, in one of the patients, it was possible to visualize an apical thrombus both with the TTE and PSID (Figure 4). We also assessed the prevalence of other abnormal findings diagnosed by PSID and TTE. In fact, there were no statistically significant differences between the two modalities in visualizing and interpreting pathological findings. The prevalence of abnormal echo findings in the study population by PSID and TTE with related P-values is reported in Table 6.
To obtain the best agreement between the two modalities when the RWMA was localized in the apex $k = 95\%$, 95% CI (87.3–100), in the inferior-septum $k = 83\%$, 95% CI (81.8–85.6), and in the inferior wall $k = 88\%$, 95% CI (86.9–90.6). There was also overall good concordance in the exclusion of major valve pathology.

In three cases, the clinical question was focused on the exclusion of severe valvular disease. In two cases, there was agreement between PSID and TTE. In one case, PSID was not able to achieve an accurate diagnosis: the transthoracic window was not optimal, the patient previously underwent a percutaneous mitral balloon valvuloplasty (not mentioned in the echo request form) and there was residual moderate mitral valve stenosis. In this case, a high-end TTE with spectral Doppler would have added further important information. Starting from these results, we observed that PSID is mainly useful to exclude a significant pathology rather than to assess the severity of the valve lesion. The use of PSID is not recommended to accurately grade valvular stenosis or regurgitation, especially, in the presence of mixed or multiple valvular disease. In these cases, a standard TTE examination is mandatory and the spectral Doppler is essential for a proper evaluation.

There was a significant difference in the scanning time between TTE and PSID: 16.4 ± 1.8 vs. 5.9 ± 0.5 min as well as in the mean reporting time. PSID examination reporting was quicker due to the lack of spectral Doppler and measurements; moreover, the LV systolic function was only visually assessed. Previous studies on the visual assessment of systolic function showed good agreement with the Simpson’s biplane-derived EF measurement, when it is performed by expert operators.\textsuperscript{13–15}

Our results regarding the clinical implications of the use of PSID are confirmed by a recently published study from Andersen \textit{et al.}\textsuperscript{19} However, this study did not include an assessment of the economic impact; the use of PSID may have on a ward-based echo service.

Previous studies\textsuperscript{20–23} analysed the cost-effectiveness of portable echo devices, but not in this setting. Vourvouri \textit{et al.}\textsuperscript{24} studied the PSID in a similar setting (requests for the cardiac evaluation of a patients with suspected cardiac disease from physicians in a non-

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**Table 4** Inter-observer variability in the detection of RWMA between PSID and TTE using a seven-segment model of the LV

<table>
<thead>
<tr>
<th>RWMA inter-observer variability</th>
<th>Agreement with 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apex</td>
<td>85% (60.0–76.7)</td>
</tr>
<tr>
<td>Anterior septum</td>
<td>79% (46.6–91.0)</td>
</tr>
<tr>
<td>Infero septum</td>
<td>84% (62.1–72.3)</td>
</tr>
<tr>
<td>Inferior wall</td>
<td>82% (64.8–80.3)</td>
</tr>
<tr>
<td>Anterior wall</td>
<td>83% (44.9–72.6)</td>
</tr>
<tr>
<td>Lateral wall</td>
<td>90% (60.4–72.6)</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>86% (61.5–76.0)</td>
</tr>
</tbody>
</table>

**Table 5** Intra-observer variability between the PSID and TTE in the detection of RWMA using a seven-segment model of the LV

<table>
<thead>
<tr>
<th>LV function</th>
<th>Kappa</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apex</td>
<td>0.9141</td>
<td>0.1128</td>
</tr>
<tr>
<td>Antero septum</td>
<td>0.6053</td>
<td>0.1193</td>
</tr>
<tr>
<td>Infero septum</td>
<td>0.6657</td>
<td>0.1098</td>
</tr>
<tr>
<td>Inferior wall</td>
<td>0.7340</td>
<td>0.1054</td>
</tr>
<tr>
<td>Anterior wall</td>
<td>0.9313</td>
<td>0.1151</td>
</tr>
<tr>
<td>Lateral wall</td>
<td>0.7805</td>
<td>0.1246</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>0.7273</td>
<td>0.1109</td>
</tr>
</tbody>
</table>

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Figure 3 (A) Four-chamber view obtained by TTE in a patient with ischaemic cardiomiopathy and moderate MR. (B) Two-chamber view obtained in the same patient by PSID.
cardiac department). His study showed a good concordance of clinical findings between portable and standard echo machines; there was an excellent agreement for the detection of abnormalities between the two devices (96%). The total cost of the examination was estimated €132 per patient with the TTE and €75 per patient with the PSID. This led to a reduction in the total cost of 33.4%. In our study, the cost of a TTE was calculated on the base of an accredited echocardiographer salary, the cost of the equipment, the maintenance of the device, and the scanning and reporting time, and it was UK £88. The estimated cost of a PSE was £21. The cost saving between the two modalities in our study was calculated per scan and corresponded to a reduction of 76% in cost. The slight difference in between our data and that of Vourvouri et al. is probably due to the large amount of studies performed per year by our busy echo department.

The results of this study show that the use of PSID would increase the number of bedside studies that could be performed per sonographer every day. This would significantly reduce the inpatient echocardiogram waiting list and would be less time and resource consuming for the echocardiography department. PSID is not an alternative to high-end echocardiography as it does not provide important information on diastolic function, valvular disease evaluation, exclusion of endocarditis, etc. However, in specific settings, it can quickly answer urgent clinical questions allowing prompt diagnosis and immediate treatment. This may also result in a reduced length of hospital stay.

**Limitations**

This is a single-centre study, involving a relatively small number of patients: focused on a very specific population (i.e. inpatients with a clinical indication for urgent echocardiography for diagnostic purposes). The major disadvantage of a PSID device is that there is no spectral Doppler, therefore, an appropriate quantification of valves disease could be challenging. On the PSID acquired images, no measurements were performed and the LVF was only visually assessed.

In accordance with the recently published recommendations for the use of pocket-sized echocardiography by the European Society of Echocardiography, the present study involves only experienced operators. The guidelines recommend a specific and dedicated training for cardiologists not fully conversant with echocardiography and deemed as mandatory training for non-cardiology personnel. Despite many research publications in this field, we did not test the use of this device in minimally trained hands.

**Conclusion**

The results of this study show how PSID used in a specific setting would be beneficial from a clinical and from a cost-effectiveness point of view. It can represent a valuable alternative to the standard way of delivering a ward-based transthoracic echo service.
Authors’ roles
S.G.: conception and design of the project, analysis, and interpretation of data. Drafting of the manuscript. Critical review of the manuscript. The final approval of the manuscript submitted. N.C.: performed the transthoracic and PSE studies. Analysis and interpretation of the data. Collection of data. Critical review of the manuscript. A.R.W.: performed the transthoracic and PSE studies; analysis and interpretation of the data; collection of data; critical review of the manuscript. A.G.I.: collection and interpretation of the data; critical review of the manuscript. F.V.: collection and interpretation of the data; critical review of the manuscript. P.T.W.: collection and interpretation of the data; critical review of the manuscript. A.D.: analysis of the data; critical review of the manuscript. J.R.: conception and design of the project; critical review of the manuscript. M.J.M.: conception and design of the project, critical review of the manuscript; the final approval of the manuscript submitted; he is responsible for the overall content as guarantor.

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