TITLE PAGE

TITLE: RESPIRATORY VARIATION IN AORTIC BLOOD FLOW PEAK VELOCITY AND RESPONSE TO A FLUID CHALLENGE IN VENTILATED NEONATES

SHORT RUNNING TITLE: PRELOAD RESPONSIVENESS IN CRITICALLY ILL NEONATES

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KEYWORDS

Fluid responsiveness; point of care ultrasound, critically ill neonate, echocardiography, fluid challenge

ABSTRACT

OBJECTIVE: To assess whether respiratory variation in aortic blood flow peak velocity (ΔVpeak) can predict preload responsiveness in mechanically ventilated and hemodynamically unstable neonates.

DESIGN: Prospective observational diagnostic accuracy study

SETTING: Third level neonatal intensive care unit.

PATIENTS: Hemodynamically unstable neonates under mechanical ventilation

INTERVENTIONS: Fluid challenge with 10 ml/kg of normal saline over 20 minutes.

MEASUREMENTS AND MAIN RESULTS: ΔVpeak and superior vena cava flow were measured at baseline (T0), immediately upon completion of the fluid infusion (T1) and at one hour after fluid administration (T2). Our main outcome was preload responsiveness which was defined as an increase in superior vena cava flow of at least 10% from T0 to T1 ($\Delta SVCf_{T1\text{-}T0}$). Forty-six infants with a median (IQR) gestational age of 30.5 (28-36) weeks were included. Twenty-nine (63%) infants were fluid responders, and 17 (37%) were nonresponders Fluid responders had a higher baseline (T0) ΔVpeak than nonresponders [9% (8.2-10.8) vs 5.5% (3.7-6.6); p<0.001]. Baseline ΔVpeak was correlated with $\Delta SVCf_{T1\text{-}T0}$ (*rho*=0.841, p<0.001). The area under the ROC curve of ΔVpeak to predict preload responsiveness was 0.912 (95% CI: 0.82-1). An ΔVpeak cut-off point of 7.8% provided a 90% (95% CI: 71-97) sensitivity, 88% (95% CI: 62-98) specificity, 7.6 (95% CI: 2-28) positive likelihood ratio and 0.11 (95%CI: 0.03-0.34) negative likelihood ratio to predict preload responsiveness.

CONNCLUSIONS: ΔVpeak accurately predicted the response to a fluid challenge in hemodynamically unstable neonates under mechanical ventilation. This value could inform medical staff about fluid status and may aid in the individualization of fluid resuscitation contributing to reduce fluid overload.

RESPIRATORY VARIATION IN AORTIC BLOOD FLOW PEAK VELOCITY AND RESPONSE TO A FLUID CHALLENGE IN VENTILATED NEONATES

INTRODUCTION

In critically ill children with circulatory shock, fluid resuscitation may be lifesaving. However, inappropriate fluid administration or failure to initiate fluid deresuscitation after shock reversal may result in fluid overload and is associated to increased morbidity and mortality in critically ill children. (1) Although such data in neonates are limited, it is recognized that fluid overload is also a poor outcome factor. (2–4) Fluid boluses are recommended during initial shock resuscitation in neonates despite the lack of high-quality evidence to support this practice. (5, 6) Animal studies have shown that the neonatal myocardium has a limited preload reserve. (7–9) Therefore, neonates may be especially vulnerable to the adverse effects of excessive fluid administration.

The assessment of preload responsiveness (PR) has been recommended to guide fluid resuscitation (10, 11), with some data indicating reduced mortality and ICU stay in association with this practice. (11) The respiratory variation in peak aortic blood flow velocity (ΔVpeak) is one of the dynamic indexes of preload (DIPs) that has shown good accuracy in critically ill children. (12) Unfortunately, no information in neonates exists. The main limitation of ΔVpeak and other DIPs is that accuracy is reduced in the setting of low tidal volume (TV) ventilation, which is currently the main practice in intensive care medicine across all ages. (13, 14) There are important developmental differences in cardiac performance and cardiopulmonary interactions between neonates and adults, which may influence the performance of the DIPs and preclude the extrapolation of adult data to the neonatal population. (15)

In this pilot study, we assessed the utility of ΔVpeak as a predictor of PR in hemodynamically unstable ventilated neonates.

MATERIALS AND METHODS

Design and participants

This was a prospective observational diagnostic accuracy study in a third-level neonatal intensive care unit (NICU) over a 2-year period (from January 1, 2018, to December 31, 2019). Consecutive mechanically ventilated and hemodynamically unstable neonates who received a first fluid bolus for suspected hypovolemia were eligible. Infants with congenital heart defects (CHDs), pulmonary hypertension (PH) and/or right heart failure, active bleeding, who were on high-frequency oscillatory ventilation or those whose representative failed to provide informed consent were excluded. In cases of study protocol violations or missing data, infants were prospectively excluded.

Study protocol

Infants were ventilated in synchronized intermittent positive pressure ventilation (IPPV) mode with a guaranteed TV set at 4-5 ml/kg. As per local NICU guidelines, intubated term infants were sedated with continuous infusions of fentanyl (0.5-2 mcg/kg/h) with or without midazolam (0.05-0.2 mg/kg/h). Preterm infants received occasional boluses of fentanyl (1-2 mcg/kg) or midazolam (0.05-0.1 mg/kg) to ensure adaptation to the ventilator. All children remained passive with the ventilator. Neuromuscular blockade was not used. Respiratory and hemodynamic support was performed according to international recommendations and local NICU guidelines (supplemental online content; supplemental methods and supplemantal figure s1). (6, 16) Fluid boluses (10 ml/kg of normal saline over 20 minutes) were indicated only in cases of suspected hypovolemia, which included the following: hypotension despite escalating catecholamine doses, unexplained or persistent elevated lactate (>2.5 mmol/l), sustained tachycardia (heart rate above the 90th percentile for age) (17) with poor peripheral perfusion and/or echocardiographic findings suggestive of hypovolemia (defined in Table 1). When a fluid bolus was deemed necessary, functional echocardiography was performed by the main

researcher (an intensivist expert in point of care ultrasound) immediately before the fluid challenge (T0), immediately after fluid challenge (T1) and at 1 hour after fluid administration (T2). Superior vena cava flow (SVCf) was chosen as the reference index to estimate cardiac output (CO) according to current ACCM guidelines.(6) The axial area of the SVC was traced in a short-axis view at the level of the right pulmonary artery as described by Ficial B et al. (18) The maximal and minimal areas of the SVC within a cardiac cycle were obtained, and values from five consecutive cardiac cycles were averaged. The pulsed Doppler wave of the SVC was obtained from the subcostal long-axis view, with the Doppler gate placed at the same level where the SVC area was traced. The velocity-time integral (VTI) of the Doppler signal was traced, and the values from 10 consecutive heartbeats were averaged to calculate the SVCf (supplemental figure s2). ΔVpeak was measured in the left ventricle (LV) outflow tract, and values from 5 respiratory cycles were averaged. ΔVpeak was calculated as ΔVpeak(%)= 100 × (Vpeak max-Vpeak min)/([Vpeak max + Vpeak min]/2) (supplemental figure s3). (19) During ΔVpeak measurement, the respiratory rate (RR) and TV were transiently adjusted to 25 breaths per minute and 5 ml/kg, respectively, to ensure a heart rate/respiratory rate ratio $\geq$4 in all patients. (20) For our main analysis, PR was defined as a prespecified >10% increase in SVCf at T1 compared to baseline ($\Delta SVCf_{T1-T0}$). Accordingly, the study cohort was subdivided into two groups: fluid responders (F-Rs) and fluid nonresponders (F-NRs). PR at T2 ($\geq$10% $\Delta SVCf_{T2-T0}$) and using an alternative threshold of $\geq$15% in ΔSVCf were also analyzed.

Statistical analysis

Categorical data are summarized as numbers and percentages. Continuous data are summarized as medians and interquartile ranges (IQRs). Unadjusted comparisons between study groups were performed using nonparametric tests. Comparisons were adjusted by gestational age and postnatal days of life to account for age-related physiological variability in the parameters measured during the fluid challenge. Rho-Spearman correlation coefficients were used to assess

the relationship between hemodynamic parameters and $\Delta SVCf_{T1\text{-}To}$ and $\Delta SVCf_{T2\text{-}To}$. The diagnostic accuracy of ΔVpeak and other hemodynamic parameters to predict PR was assessed by receiver operating characteristic (ROC) analysis. Optimal cut-off points were selected by the Youden method, and the cut-off of 12% for ΔVpeak was also explored.

Intra- and interobserver agreements in ΔVpeak and SVCf measurement were evaluated by repeating 50 individual and 30 averaged measurements of ΔVpeak and SVCf, respectively. The second observer was a neonatologist trained in targeted echocardiography who was blinded to patient data. Intra- and interobserver variabilities were evaluated by calculating the standard error of measurement (SEM) and the minimal detectable difference (MDD). Intra- and interobserver reliabilities were assessed by intraclass correlation coefficients (ICCs) and Bland Altman plots. (21)

Based on a recent metanalysis of 11 studies assessing ΔVpeak performance in children, we expected a PR rate of 52% (median value). (12) Fifteen non-responders and 16 responders would achieve a power of 0.8 with a type I error of 0.05 using a conservative target area under the ROC curve (AUROC) of 0.75 (lower range of previously reported). (22) The SPSS (IBM Corp., Armonk, N.Y., USA) statistical package version 22 was used for analysis.

Ethical aspects

The study protocol was approved by the local institutional review board (IRB), and parents provided informed consent for participation.

RESULTS

During the study period, 161 infants were assessed for eligibility. Ninety-two patients did not meet the inclusion criteria. Of the remaining 79 infants, 33 were excluded for several reasons,

and forty-six infants completed the analysis. (Figure 1). The clinical characteristics of the cohort are shown in Table 1. The cohort's median (IQR) percent $\Delta SVCf_{T1-T0}$ and $\Delta SVCf_{T2-T0}$ were 12.7% (7.6-14.7) and 3.6% (-1.87-10.91), respectively (p<0.001). According to our definition of PR for the primary analysis, 29 infants (63%) were classified as F-Rs, and 17 (37%) were classified as F-NRs. Only 12/29 (44.1%) infants remained F-Rs at T2 (26.1% of the whole cohort). The cohort's median (IQR) ΔVpeak at baseline (T0) was 8.3% (6-9.7). F-Rs had a higher baseline ΔVpeak than F-NRs [9% (8.2-10.8) vs. 5.5% (3.7-6.6); p<0.001]. Those who remained F-Rs at T2 had a higher baseline ΔVpeak [10.2% (8.9-14.2) vs 7.3% (5-8.9); p<0.001] and a greater $\Delta SVCf_{T1-T0}$ [17.1% (14.9-20.1) vs 9.7% (5.4-13.4); p<0.001] compared to the rest of the cohort and compared to the subgroup who were initially F-Rs at T1 but did not sustain PR at T2 [ΔVpeak 10.2% (8.9-14.5) vs 8.5% (7.9-9.1); p=0.003 and $\Delta SVCf_{T1-T0}$ 17.1% (14.9-20.1) vs 13.3% (11.56-13.9); p<0.001]. Preterm infants younger than 32 weeks of GA were less likely to be F-Rs than older infants [13/26 (50%) vs. 16/20 (80%); p=0.037]. However, there were no statistically significant differences in baseline ΔVpeak [8.1% (5.3-9.4) vs 8.9% (7-9.9); p=0.292], $\Delta SVCf_{T1-T0}$ [9.7% (5.4-14.4) vs 13.7% (11.1-15.2); p=0.126] or $\Delta SVCf_{T2-T0}$ [2.8% (-1.8-11.6) vs 3.8% (-1.9-10); p=0.666] compared to older children.

SVCf increased at T1 in both F-Rs and F-NRs and remained higher at T2 compared to baseline, but F-Rs attained significantly higher SVCf values at T1 and T2 than F-NRs. As mentioned previously, baseline (T0) ΔVpeak was higher in F-Rs than in F-NRs and decreased significantly at T1 and T2 in only F-Rs (Figure 2). The mitral Doppler E/A ratio increased with fluid administration in both groups but remained lower in F-Rs than in N-FRs from T0 to T2. Mean arterial pressure (MAP) increased during fluid challenge in both groups, without significant differences between them, while heart rate (HR) decreased significantly only in F-Rs, although the difference between F-Rs and F-NRs was not statistically significant after adjustment for GA and postnatal age (e-Table 1).

There were strong correlations between ΔVpeak and $\Delta SVCf_{T1-T0}$ (*rho* coefficient=0.841; p<0.001) and $\Delta SVCf_{T2-T0}$ (*rho*= 0.653; p<0.001). Conversely, the mitral E/A ratio was inversely correlated with $\Delta SVCf_{T1-T0}$ (*rho* -0.662, p<0.001) and $\Delta SVCf_{T2-T0}$ (*rho* -0.479; p=0.001). MAP, HR, LV shortening fraction (LVSF) and lactate were not correlated with $\Delta SVCf_{T1-T0}$ or $\Delta SVCf_{T2-T0}$ (Figure 3 and supplemental figures s4 and s5). The AUROC curve of ΔVpeak to predict FR was 0.912 (95% CI: 0.82-1). An optimal cut-off of 7.8% provided a sensitivity and specificity of 90% (95% CI: 71-97) and 88% (95% CI: 62-98), respectively (Figure 4). The performance of ΔVpeak in predicting PR at T2 and with an alternative threshold of 15% in $\Delta SVCf_{T1-T0}$ and $\Delta SVCf_{T2-T0}$ was also explored (Table 2). Additionally, the mitral E/A ratio had AUROC curves to predict preload unresponsiveness of 0.89 (95% CI: 0.81-0.98) at T1 and 0.820 (95% CI: 0.64-0.90) at T2. A cut-off >1.15 provided 100% (95% CI: 85-100) specificity to diagnose fluid unresponsiveness at both the T1 and T2 timepoints, with 47% (95% CI: 23-71) and 41% (95% CI: 19-66) sensitivities, respectively. MAP, HR, lactate and LVSF were of limited diagnostic value to predict PR (AUROC curves ranging 0.640 to 0.730, data not shown).

The intraobserver calculated SEM for ΔVpeak was 0.37% (95% CI: 0.29-0.44) with an MDD of 1.02%. The intraobserver SEM for SVCf was 2.2 (95% CI: 1.4-2.9) ml/kg/min with an MDD of 6.6 ml/kg/min. The interobserver SEM for ΔVpeak was 0.42% (95% CI: 0.34-0.5) with an MDD of 1.15%. The interobserver SEM for SVCf was 3.9 (95% CI: 2.5-5.4) ml/kg/min with an MDD of 10.9 ml/kg/min. The ICCs of intraobserver measurements of ΔVpeak and SCVf were 0.995 (95% CI: 0.992-0.997) and 0.92 (95% CI: 0.838-0.98), respectively. The ICCs of interobserver measurements of ΔVpeak and SCVf were 0.97 (95% CI: 0.93-0.98) and 0.910 (95% CI: 0.83-0.95), respectively. A complete description of observer agreement analysis can be found in supplemental Table s2 and figures s6 and s7.

DISCUSSION

In this pilot study, ΔVpeak accurately predicted PR in ventilated critically ill neonates who were hemodynamically unstable and received a fluid challenge. ΔVpeak was strongly correlated with changes in SVCf. However, the effects of crystalloids on SVCf dissipated rapidly, and a minority of infants sustained PR one hour after fluid administration. (23) We also observed that the rates of F-Rs when PR was defined as a >15% increase in SVCf were only 26.1% and 15.2% immediately and 1 hour after fluid challenge, respectively. Preterm infants were less likely to be F-Rs than term infants, suggesting reduced cardiac performance in more immature infants. Taken together, our findings support that neonates have a limited preload reserve and call into question the pertinence of repeated crystalloid boluses as a strategy to improve CO and tissue perfusion in hemodynamically unstable neonates. (5) The use of ΔVpeak could be informative to titrate and individualize fluid resuscitation and decide when repeated fluid boluses might be indicated.

Our study is the first to date to assess PR using a DIP in neonates; thus, we cannot compare our results with those of other similar cohorts. We found a median ΔVpeak at baseline and a discriminative cut-off of approximately 8%, which is lower than that previously reported in older children (approximately 12%). (12) However, ΔVpeak was reasonably discriminative of PR. We hypothesize that lower ΔVpeak values are indicative of a limited preload reserve in the neonatal myocardium. In this line, animal and human data have shown that while the Frank-Starling relationship is preserved in neonates, the curve plateaus at a lower diastolic ventricular pressure than those in older children and adults. (7, 24, 25)

The results of systematic reviews and meta-analyses have shown that ΔVpeak is the only DIP that consistently predicts PR in children. (26–28) ΔVpeak is more accurate than DIP based on blood pressure tracing, plethysmography and the passive leg raising (PLR) test, all of which have shown excellent performance in adults. (29–31) The inaccuracies of blood pressure and plethysmography-based indexes in children are related to the highly compliant arteries that

partially absorb pressure changes caused by variations in SV; thus reducing the ability of these techniques to reflect changes in loading conditions. (32–34) The PLR test is an appealing method to predict FR because it is not affected by most of the limitations of respiratory indexes. (30) Unfortunately, the results in children have been disappointing. (35)

Our data challenge the concept that DIP are not reliable under protective low TV ventilation during the neonatal period. (14) Previous studies have established that a minimum TV of 7-8 ml/kg is needed for respiratory DIP to accurately predict PR. (13) It has been argued that otherwise, the generated driving pressure (Paw) may be insufficient to induce phasic changes in preload, resulting in false-negative results. (36) However, it is worth noting that the pleural pressure (Ppl), which is determined by the elastance of the chest wall and respiratory system, is the main determinant of ventilator-induced variations in preload. (37) To fully understand the differences in cardiopulmonary interactions between neonates and adults, the physiology of venous return should be considered. Cardiac filling depends on transmural filling pressure, which is the difference between intravascular pressure and extravascular pressure (pleural pressure). (38) Intravascular pressures are much lower in neonates compared to adults. Thus, a minimal increase in intrapericardial (pleural) pressure during inspiration results in significant decreases in transmural filling pressure and cardiac filling. On this basis, neonates are known to be more vulnerable to the adverse hemodynamic effects of positive pressure ventilation than adults. (39, 40) Our cohort was composed of hemodynamically unstable infants with suspected hypovolemia, and presumably, the effects of positive pressure ventilation on venous return were accentuated. It is probable that in these conditions, phasic changes in intrapericardial pressure are significant for ΔVpeak to perform well in the prediction of PR, despite the use of "low" TVs.

Another factor that may influence the performance of DIP is the ratio of RR to HR. (20) If the respiratory cycle is too short in relation to the cardiac cycle, the pulmonary transit time becomes

too long at high RR, and left ventricular SV barely reflects the respiratory variation in preload. (36) We took the precaution to measure ΔVpeak at an RR of 25 bpm, which resulted in a median HR/RR ratio of 6.2. This may have increased the sensitivity in detecting PR and should be taken into account in the design of future studies.

We found that a high E/A ratio (which indicates reduced LV distensibility) identified F-NRs with high specificity. Our data suggest that a high baseline E/A or its increase during a fluid challenge indicates exhausted preload reserve and may be useful to identify the safe limits of fluid resuscitation. (41)

Our research has some limitations. This was a single-center study with a relatively small sample size, and it should be considered as a proof of concept. Our threshold to define PR for the primary analysis was 10% compared to 15% in other studies, which might have increased the sensitivity of ΔVpeak. However, ΔVpeak was also reasonably accurate when using the 15% threshold. We used SVCf as the reference standard to define PR. SVCf is a partial measure of the CO directed to the upper body and does not reflect whole-body systemic flow. This is in contrast to all previous studies utilizing echocardiography that measured SV at the left ventricular outflow track. However, measurement at the ventricular outflow is not suitable to estimate effective CO in neonates because it is affected by physiological intracardiac shunts. (42) In contrast, SVCf is unaffected by shunts and reflects flow to the central nervous system. Of note, low SVCf ($\leq$40 ml/kg/min) is associated with an increased risk of ischemic brain injury, mortality and poor long-term neurological outcomes, supporting the concept that it is a clinically meaningful measurement. (43) The main limitation of SVCf is that, as with all Doppler-echocardiography-based measurements of blood flow, it has important intra- and interobserver variabilities. The main source of error is measurement of vessel radius, which has to be squared to calculate flow. (44) This can lead to an exponential amplification of any minimal error. We tried to minimize this bias by tracing the SVC axial area instead of measuring its diameter. This

has the additional advantage of measuring the actual area, without making the geometric assumption that the SVC is a perfect circle, which is not. This method has been shown to reduce variability in SCVf measurement compared to the traditional method. (18) We have shown excellent and good-to-excellent intra- and interobserver agreements with this method; therefore, we think that the repeatability of our index and standard reference methods is not influencing our results. Finally, being pragmatic, echocardiography is the most useful method that can estimate CO in preterm infants and neonates for whom invasive monitoring, such as pulmonary artery catheterization or transpulmonary thermodilution, are either unreliable, impractical or even dangerous. (42, 45, 46)

The main strength of our study is the consistency of the study protocol. We considered only the initial fluid bolus administered to the child, avoiding the confounding effect of previous fluid resuscitation. We excluded infants with PH and right heart failure, which are known to cause false positive variations in DIP. (47) Ventilator settings and hemodynamic management were standardized. In addition, during ΔVpeak measurement, the ventilator RR and TV were equalized for all patients. All these factors contribute to reducing the influence of confounders and increasing the internal validity of the results.

CONCLUSIONS

Our study indicates that ΔVpeak may be a useful method to assess PR in ventilated neonates. According to our results, repeated fluid boluses in hemodynamically unstable neonates are questionable and potentially unsafe. Other strategies, such as low-dose vasopressors, may cause sustained increases in preload and CO and may be worthy of exploration in future studies. ΔVpeak could be informative of the effects of different interventions and provide valuable data for individualized hemodynamic management.

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Ignacio Oulego Erroz takes full responsibility of the content of the manuscript, including data and analysis.

Author's contributions:

IOE; conceived and designed the study, analyzed data and drafted the manuscript.

PAQ: conceived the study, acquired data and critically reviewed the manuscript.

SST: acquired and analyzed data and critically reviewed the manuscript.

ARN conceived and designed the study and critically reviewed the manuscript.

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FIGURE LEGENDS

Figure 1: Flow diagram of the study

Footnote: CHD: congenital heart defects; HFOV: high frequency oscillatory ventilation; PPHN: persistent pulmonary hypertension of the newborn

Figure 2: Change in ΔVpeak and superior vena cava flow during fluid challenge in fluid responders (F-R) and nonresponders (F-NR)

Footnote: Intra-group comparisons: ΔVpeak in F-R: T1 vs T0 p<0.001; T2 vs T0 p=0.018: ΔVpeak in F-NR: T1 vs T0, p=0.484; T2 vs T0 p=0.212. SVCf in F-R: T1 vs T0 p<0.001; T2 vs T0 p<0.001. F-NR: T1 vs T0, p<0.001; T2 vs T0 p=0.238. Inter-group comparisons: ΔVpeak FR vs F-NR T0 (p=0<0.001) T1 (p=0<0.001), T2 (p=0<0.001). SVCf F-R vs F-NR T0 (p=NS), T1 (p<0.05), T2 (p<0.05)

Figure 3: Correlation between ΔVpeak and superior vena cava flow immediately after the fluid challenge (T1) and one hour after the fluid challenge (T2)

Footnote: p values for the *rho* Spearmen correlation coefficient.

Figure 4: Receiver operating characteristic (ROC) curve for baseline ΔVpeak in the prediction of preload responsiveness

Foot note: Preload responsiveness is defined as an increase of at least 10% in superior vena cava flow from baseline to completion of the fluid challenge ($\Delta SVCf_{T1-T0}$ >10%)

LEGENDS FOR SUPPLEMENTAL DIGITAL CONTENT

Supplemental methods: Description of respiratory and hemodynamic general management during the study period

Supplemental Table s1: Haemodynamic variables during fluid challenge. The results are presented as medians (IQRs). Comparison between fluid responders (F-Rs) and non-responders (F-NRs).

Supplemental table s2: table ΔVpeak and SVCf intra and interobserver variability

Supplemental figure s1: Protocol for screening and treatment of low systemic blood flow (LSBF) in preterm infants with a gestational age of less than 30 weeks.

Supplemental figure s2: Echocardiographic measurement of superior vena cava flow

Supplemental figure s3: Representative aortic pulse Doppler tracing of a fluid responder and a fluid non responder with the correspondent values in ΔVpeak(%)

Supplemental figure s4: Correlation between percentual change in SVCf from baseline to T1 and hemodynamic parameters ($\Delta SVCf_{T1-T0}$)

Supplemental figure s5: Correlation between percentual change in SVCf from baseline to T2 and hemodynamic parameters ($\Delta SVCf_{T2-T0}$)

Supplemental figure s6: Intra and interobserver Bland Altman plot (ΔVpeak)

Supplemental figure s7: Intra and interobserver Bland Altman plot (superior vena cava flow)

Figure 1

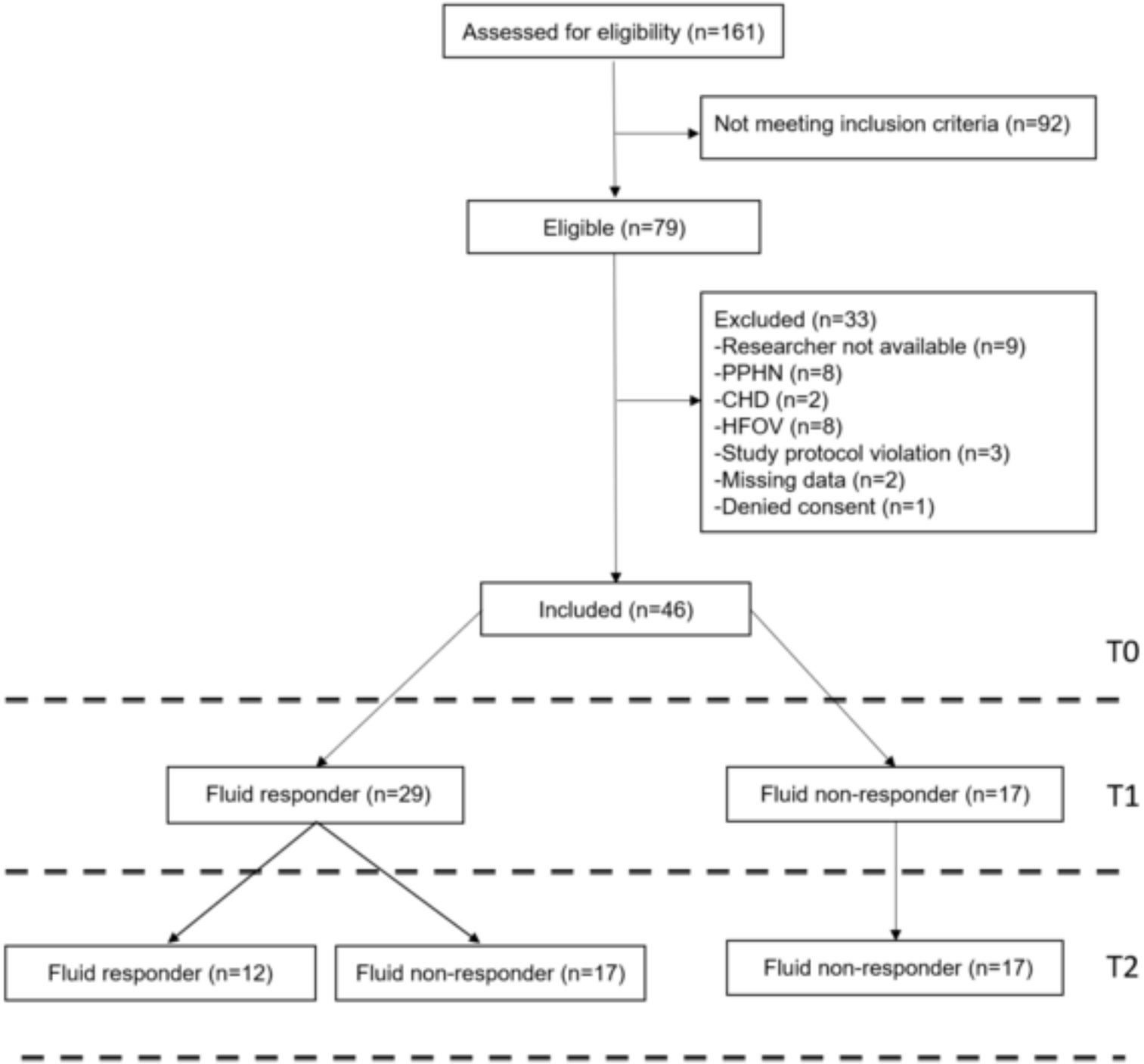

Figure 2

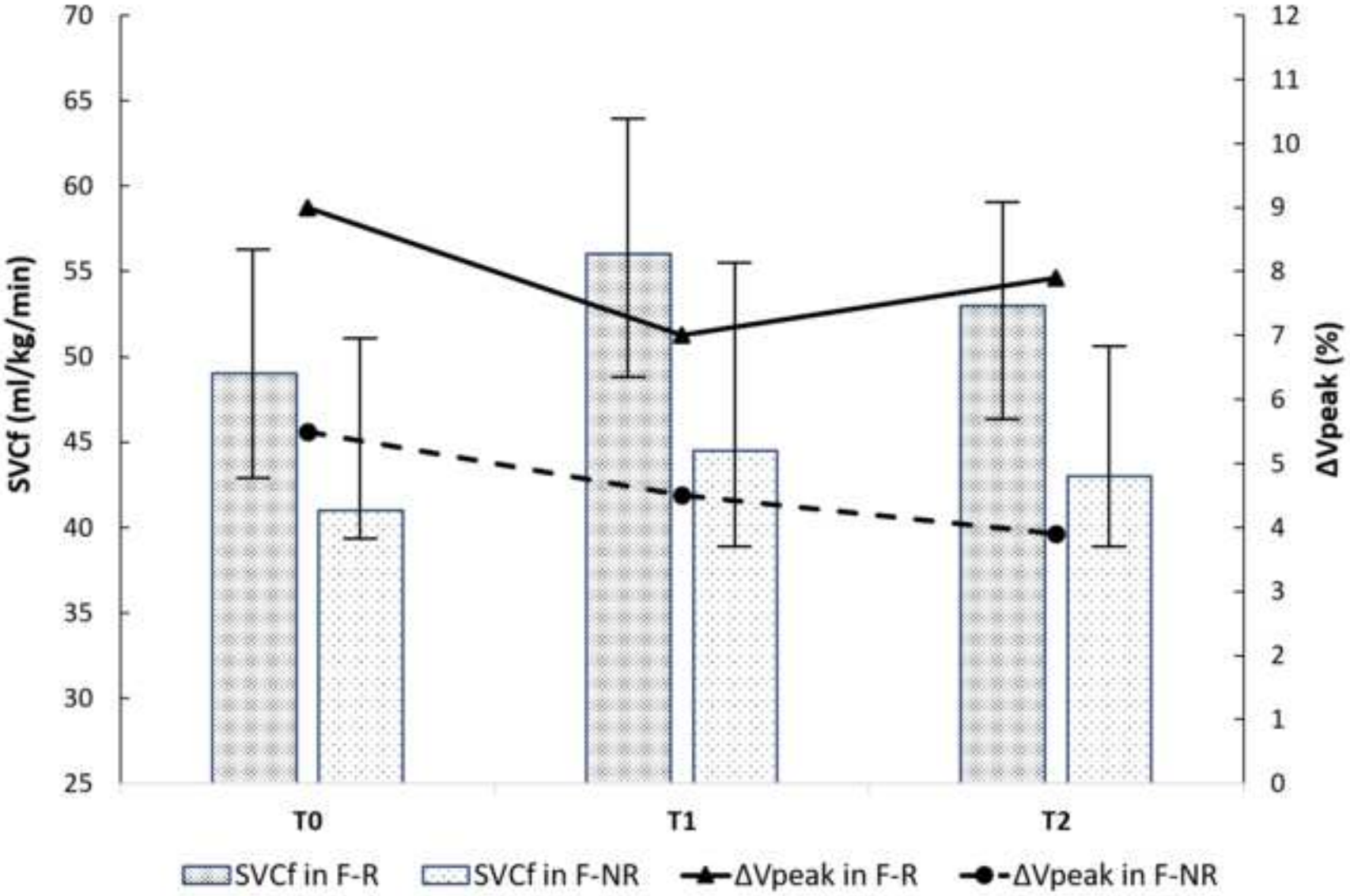

Figure 3

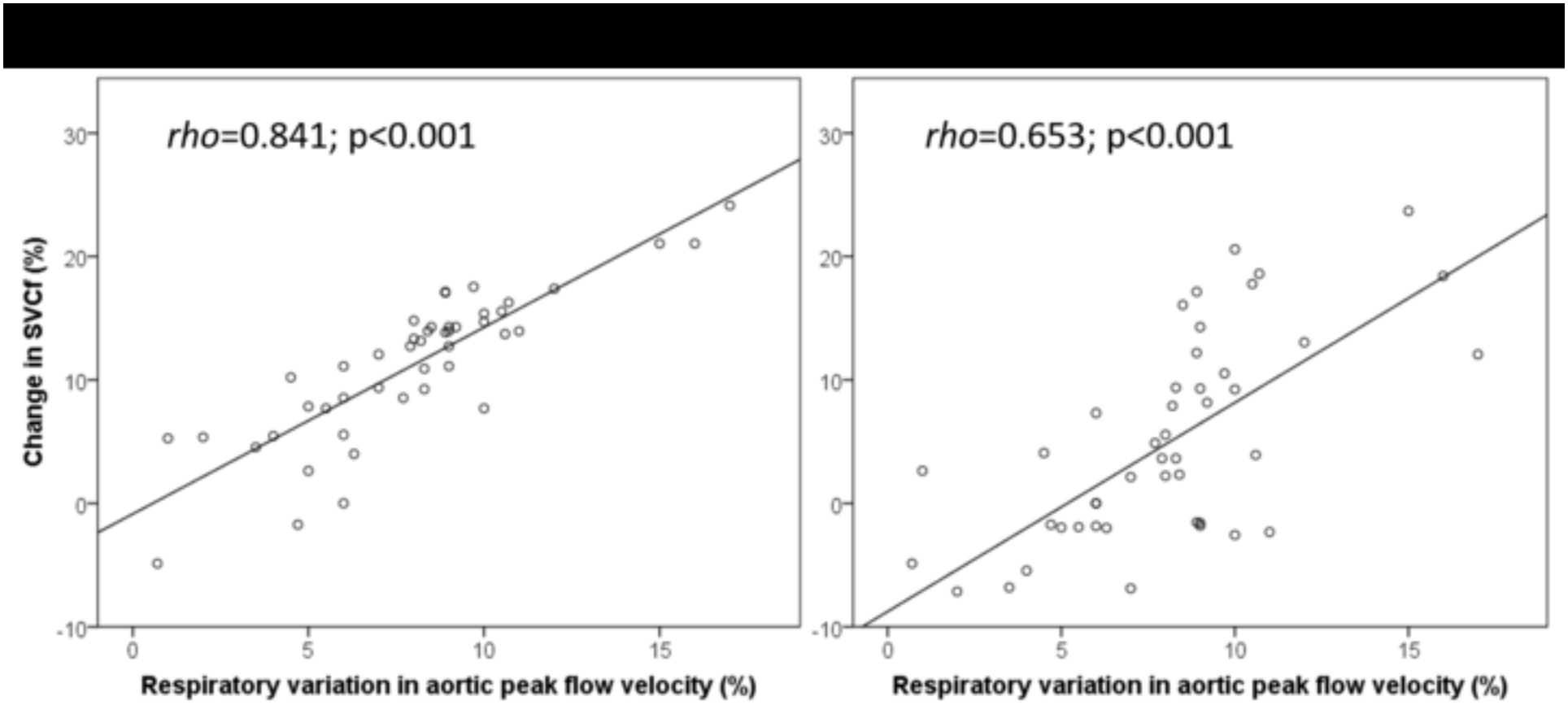

Figure 4

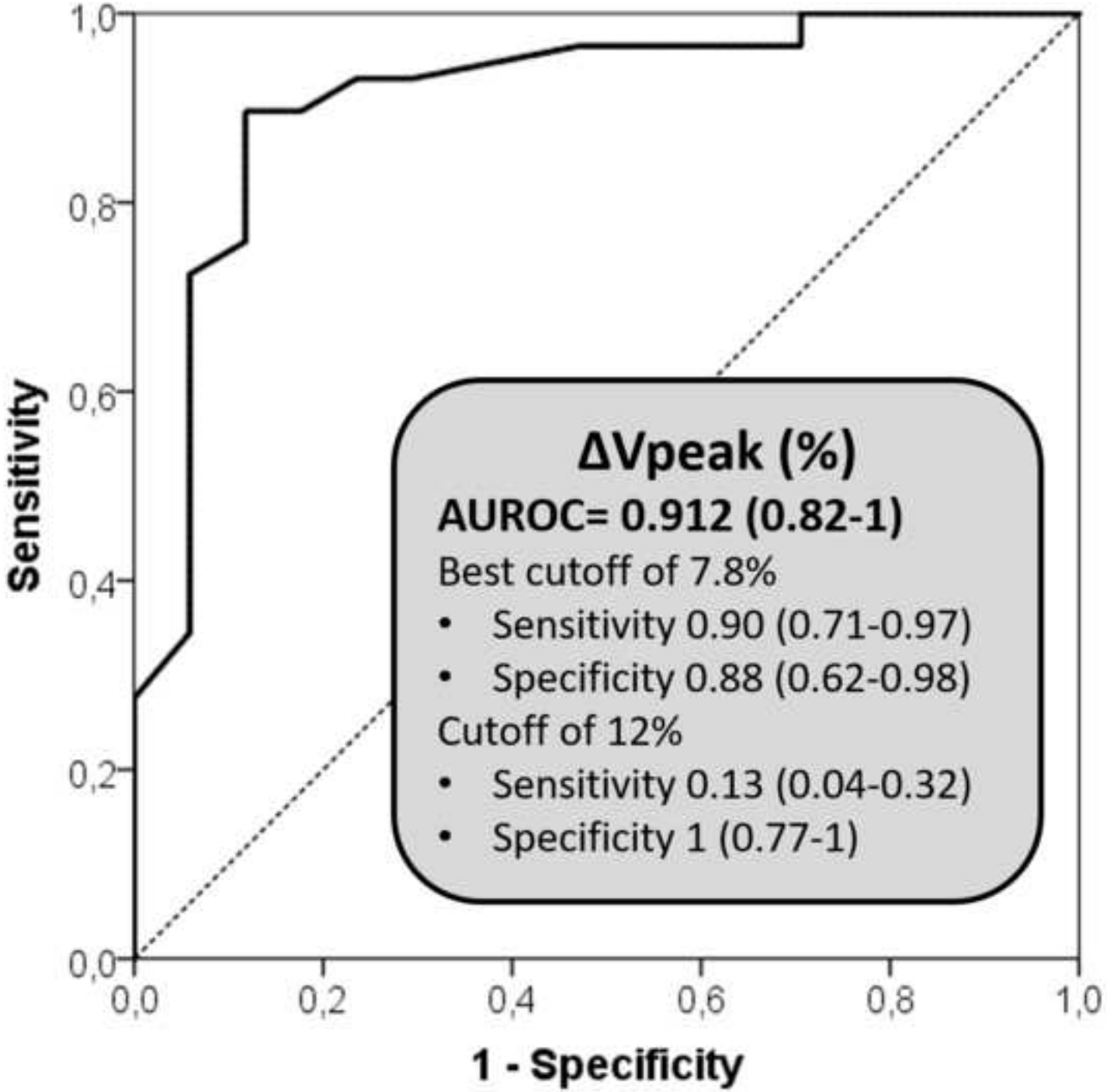

Table 1

Table 1: Patient characteristics at enrolment. Comparison between fluid responders (F-Rs) and non-responders (F-NRs). The results are presented as N (percentages) and medians (IQRs).

PATIENT CHARACTERISTICS	RESPONDERS (N=29)	NON-RESPONDERS (n=17)	p (R vs NR)	OVERALL (n=46)
Age (postnatal days)	2 (0.5-3.5) Range: 0-27	5 (2-10) Range: 0-24	0.011	2 (1-5) Range:0-27
Sex (male)	15 (51.7)	8 (47.1)	0.760	23 (50)
GA (weeks) <32 weeks	28 (25-31) 13 (44.8)	33 (29-37) 13 (76.5)	0.003 0.037	30.5 (28-36) 26 (56.5)
BW (grams)	1420 (994-3110)	1235 (847-2890)	0.001	1095 (690-1265)
Caesarean delivery	13 (44.8)	9 (52.9)	0.595	24 (52.2)
Apgar 5’	8 (6-9)	8 (6.5-9)	0.641	8 (6-9)
CRIB score 12 h	4 (3-6)	7 (3-9)	0.115	5 (3-7)
Previous surfactant	15 (51.7)	7 (41.1)	0.489	22 (47.8)
HsPDA	4 (13.7)	4 (23.5)	0.400	8 (17.4)
Main cause of hemodynamic compromise				
-Septic shock/NEC	8 (27.6)	9 (52.9)		17 (37)
-Low SBF*	2 (6.9)	2 (11.7)		4 (8.7)
-RDS	12 (41.4)	4 (23.5)	0.100	16 (34.8)
-HIE	6 (20.7)	0 (0)		6 (13)
-PDA-PLS	1 (3.5)	2 (11.7)		3 (6.5)
Clinical indications of fluid bolus				
-Low BP	17 (58.6)	13 (76.4)	0.004	30 (65.2)
-Tachycardia/poor perfusion	25 (86.2)	5 (29.4)	0.032	30 (65.2)
-Elevated lactate	18 (62.1)	5 (29.4)	0.013	23 (50)
-Hypovolemia on ECHO†	15 (51.7)	3 (17.6)	0.022	18 (39)
Ventilator settings				
-Tidal volume (ml/kg)	4.5 (4-5)	4 (3.9-4.2)	0.004	4.1 (4-4.8)
-PIP (cmH2O)	19 (17.5-22.5)	18 (16-21)	0.126	19 (17-22)
-Maw (cmH_2O)	14 (11-16)	12 (9.5-14)	0.203	13 (12-15)
-PEEP (cmH_2O)	6 (5-7)	5 (5-6)	0.139	6 (5-7)
-Paw (cmH_2O)	14 (12-16)	12 (11-14)	0.133	13 (11-15)
-FiO2 (%)	35 (22-50)	33 (24-35)	0.599	34 (24-46)
-RI	4.8 (2.6-7.1)	3.5 (2.8-4.7)	0.246	4.2 (2.7-6)
-Cest ml/cmH_20 (kg^{-1})	0.32 (0.30-0.36)	0.34 (0.29-0.36)	0.444	0.33 (0.29-0.36)
-HR/RR	6.2(5.6-6.7)	6 (5.7-6.2)	0.116	6.2 (5.7-6.6)
Lactate (mmol/l)	3 (2-5.5)	2.1 (1.5-2.9)	0.045	2.5 (1.7-4)
LV dysfunction	3 (10.3)	4 (23.5)	0.229	7 (15.2)
Catecholamine infusion				
Dopamine (N; mcg/kg/min)	28; 10 (8-13.5)	15; 11 (9-13)	0.235	43;10 (8-13)
Epinephrine (N; mcg/kg/min)	13; 0.1 (0.1-0.2)	12; 0.1 (0.1-0.15)	0.650	25;0.1 (0.1-0.15)
Dobutamine (N; mcg/kg/min)	9; 5 (5-6.5)	4; 7 (5-9.5)	0.330	13;5 (5-7.2)
VIS ‡	14 (8-25)	24 (11-25)	0.240	19.5 (10-25)

BW: birth weight; Cest: estatic compliance of the respiratory system (tidal volume/driving pressure (kg^{-1}); CRIB: clinical risk index for babies; ECHO: echocardiography; GA: gestational age; HR: heart rate; HIE: hypoxic-ischaemic encephalopathy; LV: left ventricle; Maw: mean airway pressure; NEC: necrotizing enterocolitis. PDA-PLS: patent ductus arteriosus post-ligation

syndrome. PIP: peak inspiratory pressure, Paw: driving pressure; PEEP: positive end-expiratory pressure; RR: respiratory rate; RDS: respiratory distress syndrome; RI: respiratory index; SBF: systemic blood flow. VIS: vasoactive-inotropic score. *Low SBF is defined as a SVCf $\leq$40 ml/kg/h in preterm infants and $\leq$50 ml/kg/min in term infants. † Hypovolemia on ECHO was defined as at least two of the following findings: subjective appearance of reduced chamber size, increased contractility visually ("*eyeballing*"), contact of anterior mitral valve with the intraventricular septum or *kissing* papillary muscles in the short axis view. ‡VIS is calculated as dopamine dose + dobutamine dose + (epinephrine dose x 100) + (norepinephrine dose x 100) + (milrinone dose x 10). LV dysfunction is defined as an LVSF <26% in preterm infants and <28% in term infants. RI is calculated as Paw x FiO2 and is used as an indicator of the level of ventilatory demand.[47]

Table 2: Prediction of preload responsiveness by ΔVpeak (%) according to the threshold in SCVf used to define fluid responsiveness (FR) and time of fluid challenge.

VARIABLES	**$\Delta SVCf_{T1-T0}$ (immediately after fluid challenge)**		**$\Delta SVCf_{T2-T0}$ (One hour after fluid challenge)**	
PR definition	**>10%**	**>15%**	**>10%**	**>15%**
FR, n (%)	**29 (63%)**	**10 (21.7%)**	**12 (26.1%)**	**7 (15.2)**
AUROC	0.912 (0.82-1)	0.920 (0.84-1)	0.890 (0.807-0.98)	0.850 (0.724-0.975)
Optimal Cut-off	**7.8%**	**9.4%**	**8.7%**	**9.8%**
Se	0.90 (0.71-0.97)	0.80 (0.44-0.96)	0.91 (0.59-0.99)	0.72 (0.3-0.94)
Sp	0.88 (0.62-0.98)	0.89 (0.72-0.96)	0.76 (0.58-0.88)	0.85 (0.68-0.93)
LR+	7.6 (2-28)	7.2 (2.7-19)	3.9 (2-7.3)	4.6 (1.9-11.1)
LR-	0.11 (0.03-0.34)	0.22 (0.06-0.78)	0.11 (0.01-0.72)	0.33 (0.1-1.1)
PPV	0.92 (0.75-0.98)	0.66 (0.35-0.88)	0.57 (0.34-0.79)	0.45 (0.24-0.81)
NPV	0.83 (0.57-0.95)	0.94 (0.78-0.98)	0.96 (0.79-0.99)	0.94 (0.79-0.99)

AUROC, area under the receiver operating characteristic curve; SVCf: superior vena cava flow; FR: fluid responder rate; PR: preload responsiveness; Se: sensitivity; Sp: specificity; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value. Values are provided with the 95% confidence interval.