



Myocardial performance assessment in neonates by strain and strain rate by tissue Doppler is improved by one-segment analysis – a quality improvement cohort study

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Title: Myocardial performance assessment in neonates by strain and strain rate by tissue Doppler is improved by one-segment analysis – a quality improvement cohort study

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ABSTRACT

Objectives To investigate one-segment strain and strain rate indices as measures of myocardial performance in asphyxiated term neonates.

Design Quality improvement cohort study.

Setting Newborns admitted to a neonatal intensive care unit at a Norwegian University

Hospital for perinatal asphyxia and non-asphyxiated newborn recruited from the Maternity

Ward at the same hospital.

Participants Twenty asphyxiated and 48 non-asphyxiated term neonates.

Primary outcome measure Strain and strain rate indices and repeatability measures. One-segment longitudinal strain and strain rate by tissue Doppler were assessed on day 1, 2 and 3 of life in 9 heart walls. Repeatability was compared against measurements from two-segment analyses previously performed in the same images.

Results The 95% limits of agreement were significantly better for the one-segment than two-segment repeatability analyses, the inter-rater peak systolic strain (PSS) was (-3.1, 3.3) vs. (-11.4, 18.3) %, the inter-rater peak systolic strain rate (PSSR) was (-0.38, 0.40) vs. (-0.79, 1.15) /s, the intra-rater PSS was (-2.5, 2.6) vs. (-8.0, 9.8) % and the intra-rater PSSR (-0.23, 0.25) vs. (-0.75, 0.80) /s ($p < 0.05$). The myocardial performance was lower in the asphyxiated neonates (indices closer to zero) than in the non-asphyxiated neonates, PSS was -17.8 (0.6) (mean (SEM)) vs. -21.2 (0.3) %, PSSR -1.43 (0.08) vs. -1.61 (0.03) /s, early diastolic strain rate 1.72 (0.11) vs. 2.00 (0.11) /s and strain rate during the atrial systole 1.92 (0.17) vs. 2.27 (0.10) /s ($p < 0.05$), despite no difference in fractional shortening (29.0 (0.5) vs. 29.1 (1.0)%) ($p > 0.05$).

Conclusions One-segment strain and strain rate assessed the reduced myocardial performance in asphyxiated neonates with significantly improved reproducibility as compared to two-segment analysis and was therefore more feasible than two-segment analyses for assessment of myocardial performance after perinatal asphyxia.

Article Summary

Article focus

Strain and strain rate by tissue Doppler are sensitive indices for assessment of myocardial performance in neonates.

Use of one-segment analyses might improve repeatability and make the analyses more suitable for clinical practice.

In this study, the feasibility and reliability of one-segment strain and strain rate analyses for assessment of the myocardial performance in asphyxiated and healthy term neonates are assessed.

Key messages

The variation between repeated measurements in neonates was reduced by use of one-segment strain and strain rate analyses as compared to two-segment analyses.

One-segment strain and strain rate indices of myocardial performance in systole and diastole is reduced in perinatal asphyxia.

One-segment analysis might be a promising modality for assessing myocardial performance in the daily care of asphyxiated term neonates.

Strengths and limitations of this study

The measurements and the variability can be directly compared between the one-segment and two-segment analyses, as the same tissue Doppler images were used in both analysis procedures.

As an unselected group of asphyxiated neonates was studied, this study was not design for quantifying the myocardial performance after perinatal asphyxia. A larger study should be conducted to study in more detail the impact from asphyxia on the myocardial performance.

INTRODUCTION

Perinatal asphyxia can lead to impaired oxygen supply to the myocardium. Affection of the heart after asphyxia is usually part of a multi-organ involvement.¹⁻⁷ Myocardial injury can occur in both ventricles.⁸ Autopsies have shown that the necrotic areas frequently are scattered, with the papillary muscles and the subendocardial regions most prone to injury.^{9;10}

The reduced myocardial performance following perinatal asphyxia^{11;12} can contribute to increase end-organ damage and mortality.¹³ Standard methods for cardiovascular monitoring in neonates such as blood pressure, capillary refill time, urinary output, biochemical markers and electrocardiograms are of limited value.¹⁴⁻¹⁶

Reduced myocardial performance after perinatal asphyxia can be assessed by cardiac ultrasound,^{14;17-20} traditionally based on left ventricle cavity measures such as fractional shortening and ejection fraction.²¹ Some studies have shown a reduction in these indices after perinatal asphyxia^{14;17;19;22} while others have not.^{13;23-25}

Newer tissue Doppler derived indices have been introduced in neonates such as atrioventricular (AV) plane velocities²⁶⁻²⁹ and strain and strain rate in myocardial segments.²⁹⁻³³ Especially the AV-plane velocities^{25;34} and two-segment systolic longitudinal strain and strain rate³³ have proven more sensitive than fractional shortening as markers for reduced myocardial performance after perinatal asphyxia. Strain and strain rate have the additional advantage that they are normalized for heart size, compared to velocities and AV-plane motion. Strain rate in ventricle diastole has yet to be assessed in asphyxiated neonates. Newer modalities using two-dimensional analyses of grey-scale images have been used for deformation analysis also in neonates,^{35;36} but in neonates its use might be limited by a low temporal resolution. At a low temporal resolution the true peak values might be missed and hence false low peak values might be assessed.

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3 Because strain and strain rate indices have the advantages over tissue velocities that they are
4 normalized for heart size, they may be more suitable for comparing the performance of hearts
5 of different size and during growth. However, the signal-to-noise ratio and the reproducibility
6 are poor in small segments³⁷ and the analysis procedure is complex.³²
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11 We have earlier shown a low reproducibility in two-segment strain and strain rate analyses in
12 neonates.^{32;33} In one-segment analyses the segments can be larger, improving both signal-to-
13 noise ratio and reproducibility.^{37;38} The analysis procedure can be simplified by omitting the
14 tracking procedure^{32;33} and by use of one sample area per wall instead of two^{32;33} or three.^{30;31}
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21 The main obstacles against use of strain and strain rate analyses in routine neonatal care might
22 therefore be overcome by use of one large segment from each wall set stationary in the image
23 sector. Accordingly, the aim of this study was to investigate one-segment strain and strain rate
24 analyses in asphyxiated and non-asphyxiated term neonates, to compare one-segment
25 repeatability indices against repeatability indices previously assessed for two-segment
26 analyses in the same images,³³ and assess if differences in myocardial performances between
27 asphyxiated and non-asphyxiated neonates could be detected by one-segment analyses.
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37 MATERIALS AND METHODS

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39 Materials: The one-segment analyses were performed in all images used earlier for two-
40 segment analyses in twenty asphyxiated and forty-eight non-asphyxiated neonates^{32;33} (table
41 1) obtained in March 2005 - March 2007. The asphyxiated neonates were recruited among
42 neonates born at gestational age ≥ 37 weeks and admitted to the Neonatal Intensive Care Unit
43 at Oslo University Hospital, Ullevål, diagnosed with asphyxia. The decision for admittance was
44 based on the clinical condition after birth and the department's guidelines for transferral.
45 Hence, an unselected group of asphyxiated neonates were included. Eight neonates received
46 isotonic saline volume expansion, three were treated with conventional mechanical
47 ventilation, one with high-frequency ventilation and nitric oxide and one was treated with
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dopamine. Nineteen of them survived. The non-asphyxiated neonates were recruited from the Maternity Ward at Oslo University Hospital, Ullevål.

Table 1 Demographic data and Fractional Shortening (long axis m-mode) in the asphyxiated and non-asphyxiated neonates.

Demographic data	Asphyxiated	Non-asphyxiated	p-value
No neonates (no of exams)	20 (53)	48 (138)	
Gestational age (weeks), median (range)	40 (37, 42)	41 (37, 42)	0.100 *
Birth weight (kg) median (range)	3.4 (3.0, 3.7)	3.7 (3.5, 3.8)	0.022 **
Apgar score median (25 and 75 percentiles)			
1 min	2 (1, 3)	9 (9, 9)	<0.001 *
5 min	5 (4, 6)	9 (9, 10)	0.001 *
10 min	7 (6, 8)		
Fractional shortening % (95% CI)	29.1 (26.9, 31.2)	29.0 (27.9, 30.0)	0.922 **

95% CI: 95% confidence interval for mean, *: Mann-Whitney U test, **: T-test

Measurements

Echocardiography was performed on day one, two and three of life.^{32;33} Images were recorded with a 2.4 MHz probe (5S probe, Vivid 7 Dimension, v 4.0.1 built 1644, GE Vingmed, Horten, Norway). The sector angle was 30 degrees or less and the tissue Doppler frame rate was 170-220/second in most images. Congenital heart defects were ruled out on day one. A grey-scale parasternal image for assessment of the fractional shortening was obtained at each examination.

Protocol

The nine walls were recorded from five apical views. The left lateral wall, the septum and the right lateral wall was recorded from the apical four-chamber view; the left inferior and left anterior wall from the apical left two-chamber view, the left inferiolateral wall and the anterior

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3 septum from the apical long-axis view; the right inferior wall from the right inferior two-
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5 chamber view and the right superior wall from the right superior two-chamber view.³²
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8 In 19 of the asphyxiated neonates the injury of the myocardium was assessed biochemically by
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10 the cardiac troponin T (Roche Diagnostics, GmbH, Mannheim, Germany). These neonates
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12 were grouped according to the peak values. As 97 nanograms per litre has been suggested as
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14 the upper reference limit in neonates,³⁹ values less than 100 nanograms per litre were
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16 regarded normal.
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20 21 22 **Data analysis**

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24 One segment from each wall was analysed (figure 1). The end-systolic lengths of the
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26 myocardial walls parallel to the ultrasound beam were 25 millimetres or higher and the widths
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28 of the myocardial walls were 4 millimetres or higher. The sample area was set stationary in the
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30 ultrasound sector. Due to the motion of the heart during the heart cycles, a segment length of
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32 21 millimetres and a width of 3 millimetres were therefore used, to ensure that the whole
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34 segment was within the myocardial wall throughout the cardiac cycle. The sample area was
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36 defined by a strain length of 20 millimetres and a length of 1 millimetre and width of 3
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38 millimetres for the region-of-interest. The peak systolic strain, peak systolic strain rate, early
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40 diastolic strain rate and strain rate during the atrial systole were obtained from curves
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42 averaged over three consecutive cycles.
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47 For the two-segment peak systolic strain and strain rate³² analyses (figure 1) used in the
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49 repeatability analyses, a strain length of 10 millimetres and a length of 1 millimetre and a
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51 width of 3 millimetres for the region-of-interest had been used, leading to length of 11
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53 millimetres and a width of 3 millimetres for the sample area. A semi-automatic tracking
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55 algorithm had been used to keep the two-segment region-of-interest centred in the segment
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57 within the ultrasound sector.
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3 For all analyses, 40 milliseconds Gaussian smoothing and linear drift compensation for the
4 Lagrangian strain curves were used. The start of the strain calculation was at the start of the
5 ventricle systole, defined by the first peak of the QRS complex. The end of the ventricle systole
6 was defined by the notch in the tissue Doppler displacement curve caused by the closure of
7 the aortic valve.⁴⁰ There was little or no post-systolic strain in the measurements.
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14 The fractional shortening was assessed from the parasternal long-axis view.^{21;41}
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17 The one-segment indices for each examination were assessed by averaging measurements
18 from all walls eligible for analysis. Indices for the left, septum and right groups of walls were
19 assessed by averaging measurements within the respective group. The peak systolic indices
20 from the septum and left walls were averaged into left/septum values. Indices for each
21 neonate were assessed by averaging the indices from the examination on day one, two and
22 three.
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31 All changes are described in absolute magnitudes; a peak systolic strain of -20% is regarded as
32 a higher value than -15%, and a change to the latter is described as a decrease.
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36 **Statistics and repeatability analyses**

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39 Two-sided p-values and 95% confidence intervals were used. Variables with a normal
40 distribution were compared by one-way ANOVA and t-tests. LSD corrections were used for
41 post-hoc pair-wise comparisons. Variables with a marked non-normal distribution were
42 compared by Mann-Whitney U-tests. Bland-Altman plots, intra-class correlation coefficients,
43 mean errors and coefficients-of-variation were used in the repeatability analyses of the peak
44 systolic strain and strain rate measurements. Seven randomly selected walls from the
45 asphyxiated neonates and seven randomly selected walls from the non-asphyxiated neonates
46 were analysed twice by the same researcher (EN) several months apart and once by another
47 researcher (AS). These repeatability indices were compared against similar indices from the
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3 two-segment repeatability analyses performed earlier in the same groups.^{32;33} The limits of
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5 agreement between repeated measurements were compared by t-tests of the absolute
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7 differences between the paired observations. The mean errors were assessed as mean of the
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9 residuals within each paired measurements divided by the average of the paired
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11 measurements. The coefficients of variance were calculated as SD divided by the mean of the
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13 paired measurements, where SD was the standard deviation of the residuals between the
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15 paired measurements.
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17 18 19 **Ethics**

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21 Written parental consent was obtained, and the project was approved by the Norwegian
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23 South-East Regional Committee for Medical Research Ethics and by the Scientific Committee at
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25 Oslo University Hospital, Ullevål.
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32 **RESULTS**

33 34 35 **Reproducibility measurements**

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38 Bland-Altman plots are shown in figure 2 and repeatability indices are shown in table 2. The
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40 limits of agreement in the Bland-Altman plots were significantly different between one-
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42 segment and two-segment analyses ($p < 0.05$). The confidence intervals for the intra-class
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44 correlations coefficients overlapped only for the inter-rater analysis of peak systolic strain rate.
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Table 2. Repeatability measurements.

	One-segment intra-rater	One-segment inter-rater	Two-segment intra-rater ^{32;33}	Two-segment inter-rater ^{32;33}
PSS ICC	0.99 (0.95, 1)	0.98 (0.93, 0.99)	0.89 (0.80, 0.94)	0.76 (0.56, 0.87)
PSSR ICC	0.97 (0.92, 0.99)	0.93 (0.77, 0.98)	0.86 (0.74, 0.92)	0.76 (0.57, 0.87)
PSS Mean error %	5 (3, 7)	6 (3, 9)	15 (10, 21)	34 (23, 44)
PSSR Mean error %	6 (3, 9)	8 (4, 12)	19 (15, 23)	27 (18, 36)
PSS COV %	5.9	7.5	21.9	38.0
PSSR COV %	7.2	11.7	24.1	31.8

95% CI: 95% confidence interval for mean

PSS: Peak systolic strain

PSSR: Peak systolic strain rate

ICC: Intra-class correlation coefficients (mean (95% CI))

Mean error: (mean (95% CI))

COV: Coefficients of variance (mean)

Longitudinal strain and strain rate

The peak systolic strain, peak systolic strain rate, early diastolic strain rate and strain rate during the atrial systole were **all** decreased in the asphyxiated neonates (table 3). The left/septum peak systolic strain and strain rate were lower in the asphyxiated than in the non-asphyxiated neonates, the peak systolic strain was -15.5 (-14.6, -16.4) (mean (95% confidence intervals)) versus -21.2 (-20.7, -21.7) % and the peak systolic strain rate was -1.43 (-1.34, -1.66) versus 1.61 (-1.56, -1.66) /s ($p < 0.05$). The one-segment peak systolic strain was 0.8 (0.4, 1.3) % lower and the peak systolic strain rate 0.18 (0.15, 0.22) /s lower than the two-segment indices obtained in the same images earlier³³ ($p < 0.05$).

Table 3 One-segment strain and strain rate measurements. See text for details.

	Asphyxiated neonates	Non-asphyxiated neonates	p-value
All walls			
Peak systolic strain (%)	-17.8 (0.6)	-21.2 (0.3)	<0.001
Peak systolic strain rate (/s)	-1.43 (0.08)	-1.61 (0.03)	<0.001
Early Diastolic strain rate (/s)	1.72 (0.11)	2.00 (0.11)	0.002
Strain rate during the atrial systole (/s)	1.92 (0.17)	2.27 (0.10)	<0.001
Left, septum and right group of walls			
Peak systolic strain (%)			
-Left walls	-17.2 (1.2)	-19.9 (0.9)	0.001
-Septum walls	-14.2 (0.9)	-15.9 (0.7)	0.007
- Right walls	-21.3 (1.7)	-24.6 (0.7)	<0.001
Peak systolic strain rate (/s)			
-Left walls	-1.40 (0.12)	-1.59 (0.06)	0.002
-Septum walls	-1.34 (0.25)	-1.35 (0.06)	0.881
- Right walls	-1.65 (0.15)	-1.75 (0.07)	0.187
Early Diastolic strain rate (/s)			
-Left walls	1.78 (0.19)	2.24 (0.18)	0.007
-Septum walls	1.27 (0.13)	1.43 (0.11)	0.098
- Right walls	2.01 (0.21)	2.22 (0.14)	0.134
Strain rate during the atrial systole (/s)			
-Left walls	1.86 (0.21)	2.00 (0.14)	0.305
-Septum walls	1.39 (0.11)	1.60 (0.11)	0.036
- Right walls	2.26 (0.28)	2.76 (0.14)	0.001

Values are mean (standard error of the mean)

Left, septum and right groups of walls

Measurements are shown in table 3. The peak systolic strain was significantly decreased in the asphyxiated neonates in all wall groups. The peak systolic strain rate and early diastolic strain rate were significantly lower in the left walls. The strain rate during the atrial systole was significantly lower in the septum and the right walls. The difference between left, septum and right walls were significant for all indices within both the asphyxiated and the non-asphyxiated neonates ($p < 0.05$), but by post-hoc tests the differences in peak systolic strain rate between the left walls and septum in the asphyxiated neonates and in early diastolic strain rate between the left and right walls in both the asphyxiated and the non-asphyxiated neonates were not significant.

Cardiac troponin T

The highest cardiac troponin T value (1116 nanogram per litre) was assessed in the one asphyxiated neonate that did not survive. The maximal value in the other asphyxiated neonates ranged 30 - 380 nanograms per litre. The maximal value was within the normal range in seven and elevated in twelve neonates. There were no statistically significant differences in the strain or strain rate indices between the two groups of asphyxiated neonates except for the strain rate during the atrial systole (normal 1.65 (1.45, 1.85), elevated 2.04 (1.82, 2.26) /s, $p=0.022$). The fractional shortening was similar in the asphyxiated neonates with normal and with elevated cardiac troponin T (normal 30.4 (27.1, 33.6), elevated 28.6 (25.2, 32.1), $p>0.05$).

DISCUSSION

Reproducibility measurements

As the one-segment intra- and inter-rater analyses were performed by the same researchers in images selected from the same patient group as for the two-segment analyses performed earlier^{32;33}, we consider the one- and two-segment repeatability indices to be comparable. The indices showed a better reproducibility for the one-segment than two-segment analyses and the 95% limits of agreement in the Bland-Altman plots were of a magnitude that indicates that one-segment analyses are feasible for assessing clinically relevant differences in daily routine care. The one-segment intra-class correlation coefficients were higher than for deformation indices in adults.⁴² The mean errors in the one-segment analyses were similar to the mean errors both for deformation indices and tissue velocities in adults.⁴³ The one-segment coefficients of variance were lower than what has been found for strain analyses by use of a strain length of 10mm in neonates³³ and in tissue velocity measurements in neonates.²⁹

The large acoustic windows in neonates give rise to good image quality. The good image quality holds the potential for high reproducibility, while a short segment length will tend to

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3 decrease the signal-to-noise ratio and hence the reproducibility. The segment length can be
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5 increased by increasing the length of the region-of-interest and by increasing the strain length.
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7 It has previously been shown that increasing the segment size by increasing the strain length
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9 improved the signal-to-noise ratio more than by increasing the length of the region-of-
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11 interest.³⁷ In the two-segment analyses performed earlier^{32;33} the segment length was 11
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13 millimetres and in the present analyses the segment length was 21 millimetres. The segment
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15 length was increased by changing the strain length from 10 millimetres to 20 millimetres while
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17 the size of the region-of-interest was unchanged. The regional value was calculated as the
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19 mean for all points within the region-of-interest. The one-segment and two-segment values
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21 were therefore calculated from a similar number of points. The value for each point was
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23 calculated by **linear regression** of all velocity measurements along the strain length, a line
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25 parallel to the ultrasound beam, centred in the point and reaching half the strain length
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27 towards the probe and half the length away from the probe. Therefore, the indices for each
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29 point within the region-of-interest were based on twice as many velocity measurements in the
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31 one-segment than the two-segment analyses. Assuming an even velocity gradient throughout
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33 the segment, the velocity differences along the strain length were twice as large in the one-
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35 segment as in the two-segment analyses. The uncertainty in each velocity measurement was
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37 similar in the one- and two-segment analyses, as the analyses were performed in the same
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39 images. Hence, the improved reproducibility in the one-segment analyses was probably caused
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41 by both an increased number of velocity measurements in the gradient estimation and by an
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43 increased difference between these velocities.
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49 **Longitudinal strain and strain rate**

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52 To our knowledge, this is the first study of one-segment longitudinal strain and strain rate in
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54 neonates and the first study of strain rate during the ventricle diastole in asphyxiated
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56 neonates.
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3 All indices were lower in the asphyxiated neonates, despite no difference in fractional
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5 shortening.³³ Fractional shortening is not always reduced in perinatal asphyxia^{23-25;34} and both
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7 tissue Doppler velocities of the AV-plane^{25;34} and two-segment strain and strain rate³³ have
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9 been shown as more sensitive indices for impaired myocardial performance.

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11 Strain and strain rate have the **potential** advantage over tissue velocities and atrioventricular
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13 annulus excursions^{44;45} that they are size independent indices. Strain rate is annulus velocity
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15 normalized for heart size, and **might** therefore more readily be compared between different
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17 hearts sizes and during growth. One-segment strain is the AV-plane excursion normalised for
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19 heart size and **might have** the same advantage over AV-plane excursion indices such as mitral
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21 annulus excursion⁴⁴ and tricuspid annular plane systolic excursion.⁴⁵ As the one-segment
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23 deformation indices are based on tissue velocities from a larger sample area than the AV-plane
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25 motion indices they might be less disturbed by clutter and reverberation.
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29 Deformation analyses by tissue Doppler have in general a low signal-to-noise ratio. **The ratio is**
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31 **improved** in large sample areas^{37;38} (figure 1). The one-segment peak systolic indices were
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33 lower than the two-segment indices assessed previously, possibly because the indices were
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35 assessed at false high local peaks caused by random noise. As the signal-to-noise ratio would
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37 be improved in large segments, these peaks were probably smaller and their impact on the
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39 measurements less pronounced in one-segment analyses. Strain measurements are less noisy
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41 due to time smoothing.^{37;38} In accordance, the differences between one- and two-segment
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43 values were relatively smaller for peak systolic strain than for peak systolic strain rate.
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48 **Left, septum and right walls**

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50 Septum values were lower than left heart values and right heart values were higher than left
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52 and septum values in most cases in this study, as in the two-segment systolic analyses.³³ A
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54 global impact from birth asphyxia on the myocardial performance was found, as has been
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56 shown in other studies both functionally^{25;32-34} and histologically.⁸
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Cardiac Troponin T

The myocardial injury can be assessed biochemically by measuring cardiac troponin T,^{17;24;46;47} which has shown to have a high positive predictive value in the retrospective diagnosis of asphyxia.⁴⁸ Cardiac troponin T values are higher in healthy neonates than in healthy adults, 97 nanograms/ml has been suggested as a cut-off point between normal and elevated levels.³⁹ As all indices except the strain rate during the atrial systole were similar in the asphyxiated neonates with elevated cardiac Troponin T as in the asphyxiated neonates with normal values, we speculate that the reduced myocardial performance following birth asphyxia might in part be caused by factors not directly related to myocardial injury.

Should one-segment or two-segment analyses be used in neonates?

The one-segment analysis procedure is fast and easy. More importantly, the reproducibility is significantly better than for two-segment analyses, and the differences between the asphyxiated and the healthy neonates were found similar by the present one-segment analyses as by the two-segment analyses previously performed in the same images.³³ However by one-segment analysis, differences between the apical and basal segments within each wall, within group of walls or within the heart at gross cannot be assessed. Such differences were found in the right group of walls by two-segment analyses. In our opinion, the ease of the one-segment procedure and the benefit of improved repeatability outweigh the loss of spatialisation, especially in situations where the myocardial impairment is expected to affect each heart wall globally and in situations where the global myocardial performance is of interest. The one-segment analysis enables regional differences still to be assessed. We suggest that one-segment strain and strain rate can be used as indices of myocardial performance after perinatal asphyxia.

Assessment of deformation indices by different modalities

Deformation analyse by tissue Doppler carries a high temporal resolution due to a high frame rate, in our study typically 170-220/s. The main limitation of these measurements is that they are one-dimensional and hence angle-dependent, as only the deformation parallel to the ultrasound beam can be obtained. Deformation analyses based on speckle-tracking have been used in neonates.^{35;36} The main advantage of the speckle-tracing is that the deformation analyses are to a certain degree two-dimensional, i.e. the measurements are independent of the angle between the ultrasound beam and deformation within the two-dimensional ultrasound plane. However, this depends on the line density, and is poorer in the base, as well as being reduced with increased frame rate (which is obtained by reducing the number of lines). Even at the highest frame rate, however, the temporal resolution is lower as it is determined by the frame rate of the grey-scale image (typically 70-100/s), and the deformation analyses are not totally independent of angle, as deformation perpendicular to the ultrasound plane cannot be assessed. Only in three-dimensional datasets the deformation can be assessed independent of angle. Such three-dimensional datasets can be obtained by ultrasound⁴⁹ and by MRI.⁵⁰ The temporal resolution is low (frame rate by MRI typically 30/s and by 3D-echocardiography typically 20/s), in addition 3D ultrasound has extremely low line density and such deformation analyses have so far to our knowledge only been used in adults. The utility of using 3D ultrasound for deformation analysis with speckle tracking in view of this low temporal resolution is still unclear.

Limitations

As an unselected group of asphyxiated neonates was studied in order to evaluate the sensitivity of the method for asphyxia in general, the study was not design for quantifying the myocardial performance after perinatal asphyxia. The degree of asphyxia varied widely, and measurements from mechanically ventilated neonates were combined with measurements

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3 from asphyxiated neonates breathing spontaneously. To study in more detail the impact from
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5 different degree of asphyxia on the myocardial performance a larger study should be
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7 conducted.

10 Further studies

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12 A larger study is needed for assessing the myocardial impairment in perinatal asphyxia.

13 Assessment of the myocardial performance by other ultrasound indices such as AV-plane
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15 motion and excursion should be compared against deformation indices in neonates.
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23 CONCLUSIONS

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25 One-segment strain and strain rate analyses by tissue Doppler is feasible and reliable in term
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27 neonates and can be used for assessing the reduced myocardial performance in asphyxiated
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29 neonates with significantly improved reproducibility as compared to two-segment analyses.
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31 One-segment analyses are therefore more feasible than two-segment analyses for assessment
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33 of myocardial performance in birth asphyxia.
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37
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39
40 participated in image acquisition.
41
42

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44
45 Health and Rehabilitation and by Renée and Bredo Grimsgaard's Foundation.
46

47 **The contribution of each author:** EN participated in the planning of the study, participated in
48
49 image acquisition, analyzed the images, performed the statistics and prepared the final draft.
50
51 AS participated in the planning of the study, advised on technical issues, performed the inter-
52
53 rater measurements and contributed in the preparation of the final draft.
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3 DF participated in the planning of the study, recruited most patients, participated in image
4
5 acquisition and contributed in the preparation of the final draft.
6

7 **Competing interests:** None
8
9

10 **Data sharing statement:** The complete dataset is available from the corresponding author at
11
12 nestaas@hotmail.com pending approval from the Vestfold Hospital Trust and Oslo University
13
14 Hospital, Ullevål. Consent for data sharing was not obtained from parents or carers of
15
16 neonates, but the presented data are anonymised and risk of identification is low.
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Figure legends:

Figure 1: One-segment and two-segment strain rate analysis. Upper row: Greyscale image showing the one-segment (left) and two-segment (right) analysis of the right lateral wall (apical 4-chamber view). Mid row: Strain rate curves for three heart cycles showing the lower irregularity in the one-segment analysis (left) than the two-segment analysis (right). Lower row: Strain curves for three heart cycles showing the lower irregularity in the one-segment analysis (left) than the two-segment analysis (right). S: Peak systolic strain rate. E: Early diastolic strain rate. A: Strain rate during the atrial systole. P: Peak systolic strain.

Figure 2. Bland Altman plots of the repeatability measurements for one-segment and two-segment^{32,33} measurements. See text for details.

X-axes: Absolute mean of the repeated measurements.

Y-axes: Difference between the repeated measurements.

Dashed line: Mean difference in the repeated measurements.

Solid lines: 95% limits of agreement.

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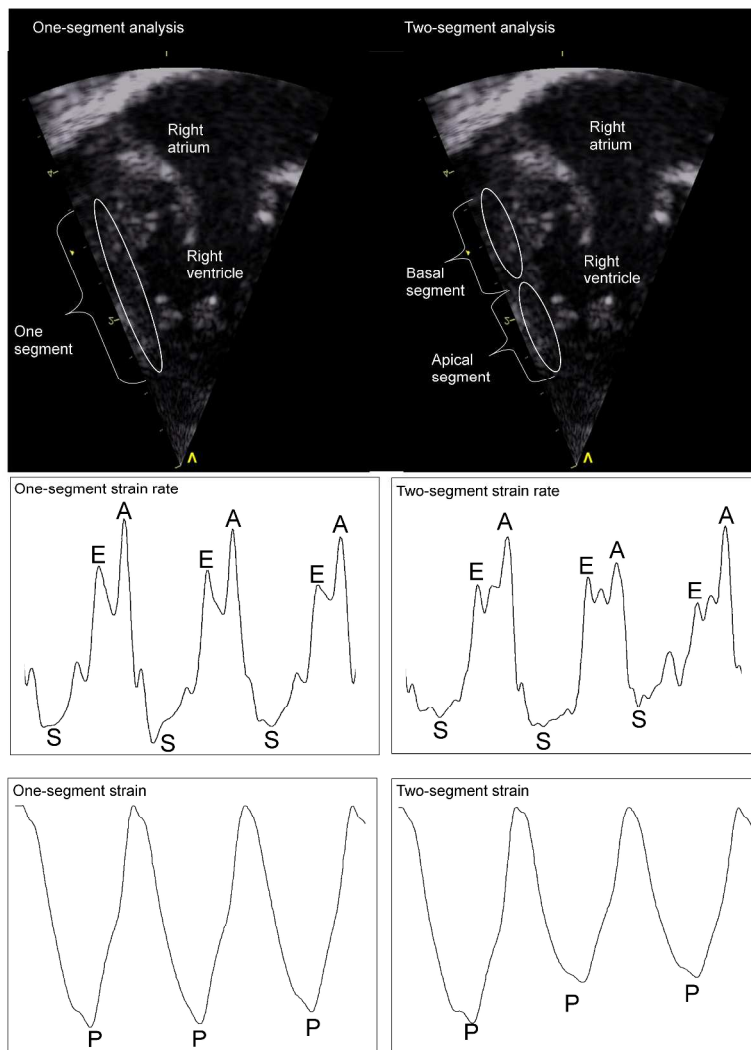


Figure 1:

One-segment and two-segment strain rate analysis. Upper row: Greyscale image showing the one-segment (left) and two-segment (right) analysis of the right lateral wall (apical 4-chamber view). Mid row: Strain rate curves for three heart cycles showing the lower irregularity in the one-segment analysis (left) than the two-segment analysis (right). Lower row: Strain curves for three heart cycles showing the lower irregularity in the one-segment analysis (left) than the two-segment analysis (right). S: Peak systolic strain rate. E: Early diastolic strain rate. A: Strain rate during the atrial systole. P: Peak systolic strain.

399x500mm (300 x 300 DPI)

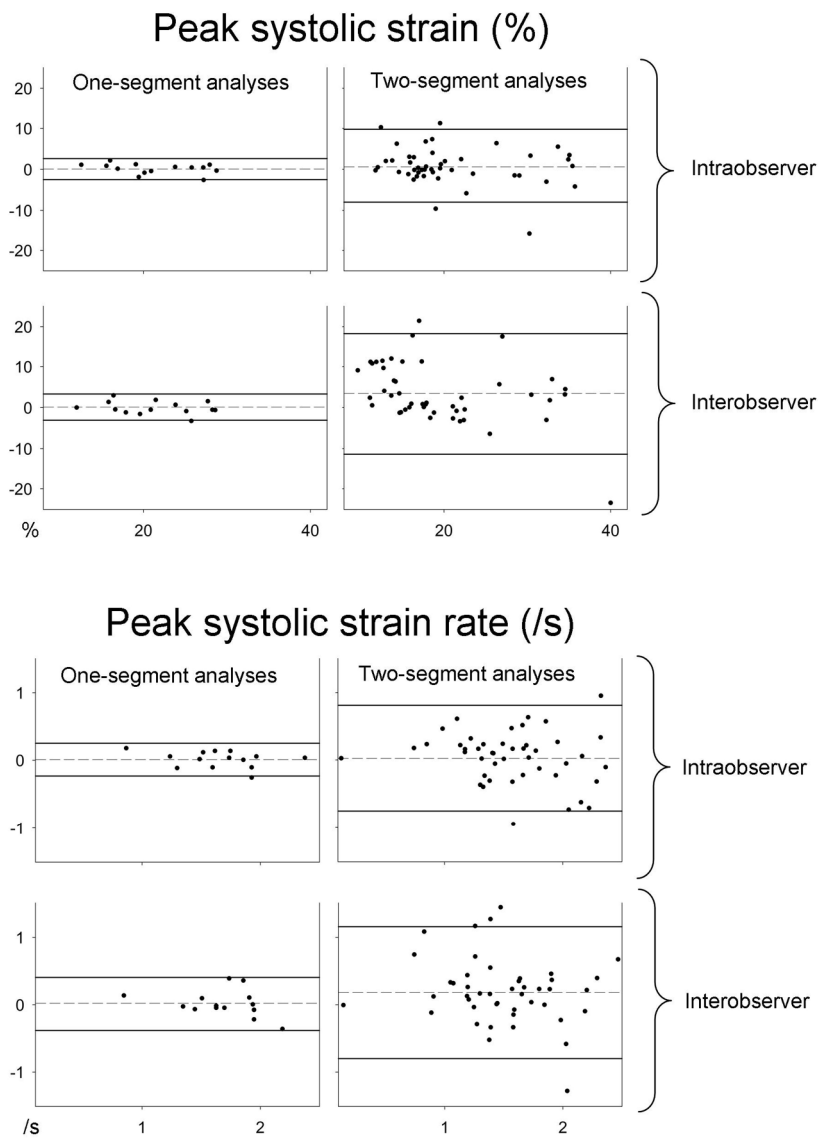


Figure 2
Bland Altman plots of the repeatability measurements for one-segment and two-segment(32;33) measurements. See text for details.

X-axes: Absolute mean of the repeated measurements.

Y-axes: Difference between the repeated measurements.

Dashed line: Mean difference in the repeated measurements.

Solid lines: 95% limits of agreement.

221x311mm (300 x 300 DPI)

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Strobe statement for the manuscript

Myocardial performance assessment in neonates by strain and strain rate by tissue Doppler is improved by one-segment analysis – a quality improvement cohort study

Submitted to BMJ Open June 2012

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract OK
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found OK
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported OK
Objectives	3	State specific objectives, including any prespecified hypotheses OK
Methods		
Study design	4	Present key elements of study design early in the paper OK
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection OK
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up OK (there were no follow-up in our study)
		(b) For matched studies, give matching criteria and number of exposed and unexposed Not relevant to our study
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable OK
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group OK, data assessment and analyses procedures are described, as well as assessment methods in both groups
Bias	9	Describe any efforts to address potential sources of bias The same protocols and equipment were used, to minimize the sources of bias. This is reported in the materials and methods section.
Study size	10	Explain how the study size was arrived at Not directly relevant to this study. All images used in the former study (the two-segment analyses) were used in the present study, to use as much data as available when comparing the two analysis modalities.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why This is described in the materials and methods section, including the rationale for the chosen cut-off value for the cardiac Troponin T
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding OK
		(b) Describe any methods used to examine subgroups and interactions OK
		(c) Explain how missing data were addressed – OK, it is reported how the values for each examination and each neonate was obtained, by averaging measurements from all walls eligible for analysis
		(d) If applicable, explain how loss to follow-up was addressed – Not relevant
		(e) Describe any sensitivity analyses – No sensitivity analyses were performed
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed – In the materials section it is now stated that

		one-segment analyses were performed in all images used for the two-segment analyses earlier
		(b) Give reasons for non-participation at each stage – Not relevant, all participants form the last study were included in the present study
		(c) Consider use of a flow diagram Not relevant
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders OK (b) Indicate number of participants with missing data for each variable of interest OK, it is reported that the Cardiac Troponin T was obtained in 19 out of 20 asphyxiated neonates. For all tissue Doppler analyses, the same images were used in the present study as in the former (two-segment) study (c) Summarise follow-up time (eg, average and total amount) OK
Outcome data	15*	Report numbers of outcome events or summary measures over time OK
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included No adjustment for confounders were done, as all analyses were done in the same images. (b) Report category boundaries when continuous variables were categorized The cut-off value for regarding the Cardiac Troponin T as “High” vs. “Normal” is reported. (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not relevant
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Measurements in the subgroups of neonates with high vs. normal Cardiac Troponin T is reported
Discussion		
Key results	18	Summarise key results with reference to study objectives OK
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias OK, limitation section
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence OK
Generalisability	21	Discuss the generalisability (external validity) of the study results OK
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based OK

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.