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High serum levels of endothelial cell adhesion molecules and their shedding enzymes do not discriminate between early onset sepsis and healthy newborns in Suriname.

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Manuscripts

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4 2 **High serum levels of endothelial cell adhesion molecules and their shedding enzymes do**
5 3 **not discriminate between early onset sepsis and healthy newborns in Suriname.**
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28 **Short title:** Adhesion molecule shedding in Surinamese newborns

29 **Funding source:** The Thrasher Research Fund (TRF13064) and Tergooi Hospitals, Blaricum,
30 The Netherlands.

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3 31 **What is already known on this topic?**
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- 5 32 • Sepsis is associated with an Angiotensin (Ang)-1 and Ang-2 serum level disbalance
6
7 33 and increased shedding of soluble endothelial adhesion molecules (sCAMs).
8
9 34 • Recently, we established an association of the Ang-1/Ang-2 disbalance with blood
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11 35 culture positive early onset sepsis (EOS) in newborns.
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14 36 **What this study adds?**
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- 16 37 • The Ang-1/Ang-2 disbalance in blood culture positive EOS is not paralleled by
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18 38 increased levels of sCAMs and their sheddases.
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20 39 • Levels of sCAMs and their sheddases are high after birth and do not discriminate EOS
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22 40 from healthy newborns.
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3 56 **ABSTRACT**

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5 57 **Background:** Early Onset Sepsis (EOS) is defined as onset of sepsis within 72 hours after
6
7 58 birth. Leukocyte-endothelial interactions play a pivotal part in EOS pathophysiology.

8
9 59 Endothelial cell adhesion molecules orchestrate these interactions and their soluble isoforms
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11 60 (sCAMs) are released into the vasculature by enzymes called sheddases.

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13 61 **Purpose:** This study was undertaken to explore further the pathophysiology of EOS and to
14
15 62 investigate the potential of sCAM and their sheddases as potential biomarkers for EOS.

16
17 63 **Methods:** Soluble CAMs sP-selectin, sE-selectin, vascular cell adhesion molecule-1
18
19 64 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1) and platelet and endothelial cell
20
21 65 adhesion molecule-1 (sPECAM-1), sheddases matrix metalloproteinase-9 (MMP-9) and
22
23 66 neutrophil elastase (NE), and sheddase antagonist tissue-inhibitor of metalloproteinases-1
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25 67 (TIMP-1) were measured simultaneously in serum of 71 Surinamese newborns suspected of
26
27 68 EOS and 20 healthy newborns, all included within 72 hours after birth.

28
29 69 **Results:** Six (8.5%) newborns had a positive blood culture. At start of antibiotic treatment
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31 70 and after 48-72 hours no differences were found in levels of sCAMs and sheddases between
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33 71 blood culture positive EOS and controls.

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35 72 **Conclusions:** Our data indicate that endothelial CAM shedding is not increased in EOS and
36
37 73 that levels of sCAMs and sheddases remain unchanged in early life in newborns, suggesting
38
39 74 not a role in the pathophysiology of EOS. Therefore, these markers have limited clinical
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41 75 utility as biomarkers for EOS.

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48 77 **Keywords:** newborns; early onset sepsis; adhesion molecules; shedding; Suriname.
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78 INTRODUCTION

79 Early onset sepsis (EOS) in newborns within 72 hours after birth remains a clinical challenge
80 with high morbidity and mortality [1-3]. The majority of global neonatal deaths due to EOS
81 occur in developing countries [4]. The diagnosis of EOS is complicated, resulting in late
82 recognition, or overtreatment of newborns with antibiotics. These dilemmas arise because the
83 pathophysiology of EOS is poorly understood.

84 A hallmark of sepsis pathophysiology is endothelial cell activation followed by
85 leukocyte recruitment into tissues [5]. The current model describes the occurrence of a shift in
86 balance in Tie2 receptor ligands Angiopoietin (Ang)-1 and Ang-2 affecting endothelial
87 integrity, and increased expression of endothelial cell adhesion molecules, in particular P-
88 selectin, E-selectin, vascular cell adhesion molecule (VCAM-1), and intercellular adhesion
89 molecule (ICAM-1) to facilitate this recruitment [6,7]. These endothelial cell adhesion
90 molecules orchestrate leukocyte rolling on, adhesion to, and diapedesis across the
91 endothelium [7,8]. Also, platelet and endothelial cell adhesion molecule (PECAM-1),
92 expressed at endothelial cell junctions has a function in facilitating paracellular transmigration
93 of leukocytes across the endothelium [9]. After intravenous administration of endotoxin in
94 healthy adults as a sepsis model, peak levels of Ang-2 prelude the release of soluble isoforms
95 of cell adhesion molecules (sCAMs) into the systemic circulation [10]. Endothelial cell
96 adhesion molecules are released through ectodomain shedding by enzymes called sheddases,
97 in particular matrix metalloproteinase-9 (MMP-9) and neutrophil elastase, released from
98 granules in neutrophils [7,11]. Both MMP-9 and neutrophil elastase prepare the extracellular
99 matrix for transmigration of leukocytes into inflammatory sites [12]. MMP-9 activity is
100 balanced by sheddase antagonist tissue-inhibitor of metalloproteinases-1 (TIMP-1) [12-14].

101 Recently, we showed in a cohort of near term and term Surinamese newborns that a
102 systemic circulation dysbalance in Ang-2/Ang-1 levels was associated with blood culture

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3 103 positive EOS [15]. This study was undertaken to examine if this dysbalance is paralleled by
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5 104 increased levels of sCAMs and sheddases in this cohort of newborns with EOS to explore
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7 105 further the pathophysiology of EOS and to investigate their potential as biomarkers for EOS.
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9 106 We hypothesized that sCAMs and sheddases circulate at higher levels in blood culture
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11 107 positive EOS in newborns and that they are useful as biomarkers for EOS.
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15 109 **MATERIALS & METHODS**

16 110 **Study design, subjects and clinical protocol**

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18 111 For this study, we used a Surinamese cohort of 20 healthy newborns and 71 newborns with
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20 112 suspected EOS from an earlier reported study (Supplemental Table 1, previously published)
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22 113 [15]. All newborns were included between April 1 2015 and May 31 2016. Included were
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24 114 newborns with a gestational age equal to or above 34 weeks in whom antibiotics were started
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26 115 within the first 72 hours of life for suspected EOS. Informed consent was obtained from at
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28 116 least one parent for the use of residual serum and clinical information. The study protocol was
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30 117 made available on clinicaltrials.gov (NCT02486783) and was approved by the Surinamese
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32 118 Medical Ethical Board (VG-021-14A) including permission of one parent.
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37 119 The management of these patients was described before [15]. In short, healthy control
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39 120 newborns and newborns suspected of EOS were included (t=0) within 72 hours after birth and
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41 121 clinically reevaluated 48-72 hours later (t=48-72h). At t=0 and t=48-72h blood was drawn for
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43 122 separation and storage of serum. This time point was chosen because the result of blood
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45 123 culture became available. Controls were newborns without signs of infection receiving blood
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47 124 draws for hyperbilirubinemia (n=20). Newborns with suspected EOS receiving treatment with
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49 125 intravenous antibiotics were divided in two groups based on result from blood culturing:
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51 126 blood culture negative EOS (n=65) and blood culture positive EOS (n=6).
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128 **Sample collection, preparation and analysis**

129 At t=0 blood samples were collected during the insertion of a venous cannula and after 48-72
130 hours of treatment with antibiotics a second blood sample was obtained using capillary
131 collection. After clotting at room temperature and centrifugation at 2,300xg for 8 minutes the
132 serum was harvested and the residual sample was stored at -80°C until further analysis.
133 Measurement of sP-selectin, sE-selectin, sVCAM-1, sICAM-1, and sPECAM-1 was
134 performed on serum samples using the Human Magnetic Bead Adhesion 6-plex panel
135 performance assay (LHC0016M, Thermo Scientific, Waltham, MA USA) according to the
136 manufacturer's instructions. ELISA was used on aliquots of the same samples for
137 measurement of neutrophil elastase (HK319-02, Hycult Biotech, Uden, The Netherlands),
138 MMP-9 (Quantikine DMP900, R&D systems, Minneapolis, MN USA), and TIMP-1
139 (Quantikine DTM100, R&D systems), each according to the manufacturers' instructions. For
140 each molecule, a standard curve was established via which concentrations in neonatal serum
141 were determined. Levels below or above the linear part (for MMP-9 n=11 (7.7%) samples, for
142 TIMP-1 n=2 (1.4%) samples, and for neutrophil elastase n=9 (6.3%) samples) of this standard
143 curve were reported as the lowest or highest value of the standard curve, respectively. We
144 measured intra-assay variation between plates used in the same assay by calculating
145 coefficient of variation between levels of each molecule in samples from the same patient
146 divided over those plates and accepted a maximum of 20%.

148 **Statistical analysis**

149 Categorical variables were presented as numbers and percentages with 95% CI and
150 continuous variables, due to the nonparametric nature of the data, as median with interquartile
151 range (IQR). The Chi-square test was used to compare categorical variables. A Mann-
152 Whitney U test and Kruskal-Wallis test with Dunn's correction for multiple comparisons

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3 153 were used for analysis of continuous variables. Because timing of inclusion after birth varied
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5 154 between groups (Supplemental Table 1), we investigated whether postnatal sampling day (i.e.,
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7 155 for both t=0 and t=48-72h between day 1 and 6 after birth) correlated with sCAM and
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9 156 sheddase levels and calculated Spearman's *rho*. P-values <0.05 were considered statistically
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11 157 significant. All analyses were done using Prism version 7.0a (Graphpad Software Inc., San
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13 158 Diego, CA USA).

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17 160 **RESULTS**

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20 161 Demographic variables of the study cohort (n=91) are given in Supplemental Table 1. Blood
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22 162 culture results revealed that 6 of 71 newborns with suspected EOS (8.5%; 95% CI 3.9-17.2)
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24 163 had a positive blood culture with gram-negative pathogens *Klebsiella pneumoniae* (n=2),
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26 164 *Enterobacter cloacae* (n=2) and *Escherichia coli* (n=2). One newborn had EOS due to a
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28 165 spontaneous bacterial peritonitis. For n=4 others cause of EOS was unknown, but they
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30 166 presented with neonatal jaundice (n=1), perinatal asphyxia (n=1), meconium aspiration (n=1),
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32 167 and hypoglycaemia (n=1). We included 20 control newborns without signs of infection
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34 168 receiving blood draws for hyperbilirubinemia.

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38 170 **Serum levels of soluble endothelial cell adhesion molecules and their sheddases**

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41 171 Serum samples (n=142) were available of all 91 newborns at t=0 and of 51 at t=48-72h. Due
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43 172 to the limited amount of serum available, not all molecules could be measured in all samples.
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45 173 Measurement of levels of MMP-9 and TIMP-1 was performed in n=90 and n=51 of newborns
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47 174 at t=0 and t=48-72h, respectively. We were able to measure sCAMs and neutrophil elastase
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49 175 levels in n=80 and n=36 newborns at t=0 and 48-72h, respectively.

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52 176 We found no differences between median levels of sCAMs between blood culture
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54 177 positive EOS, blood culture negative EOS, and control groups at either t=0 or t=48-72h

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3 178 (Table 1). Median levels of sCAMs within a group did also not change between t=0 and t=48-
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5 179 72h. Of all sCAMs only median levels of sP-selectin in pooled (n=115) samples correlated
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7 180 negatively with later sampling day (*rho* -0.21; 95% CI -0.38 to -0.02; P=0.03). No differences
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9 181 were found in median levels of MMP-9, TIMP-1, and neutrophil elastase between blood
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11 182 culture positive EOS, blood culture negative EOS, and controls at either t=0 or t=48-72h.
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13 183 Also, no differences between median levels of the sheddases within a group between t=0 and
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15 184 t=48-72h were found. No correlation was found between levels of the sheddases and sampling
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21 22 187 **DISCUSSION**

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24 188 In this study we investigated whether sCAMs and their sheddases circulate at higher levels in
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26 189 newborns with blood culture positive EOS. We serially measured levels of sCAMs and
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28 190 sheddases in a cohort of near and at term newborns. In contrast to our hypothesis, none of the
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30 191 molecules showed any difference in serum levels between blood culture positive EOS, blood
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32 192 culture negative EOS, and controls, neither at start of antibiotic treatment nor after 48-72
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34 193 hours. These data indicate that levels of sCAM and sheddases are not associated in the
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36 194 pathophysiology of EOS and have no clinical utility as early biomarkers for EOS in
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39 195 newborns.

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41 196 Previously, we found evidence for endothelial cell activation in blood culture positive
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43 197 EOS in the same newborns used for this study, represented by a dysbalance in Ang-2/Ang-1
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45 198 ratio [15]. Since the current data demonstrate that this dysbalance was not paralleled by
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47 199 increased release of sCAM or sheddases in EOS we conclude that endothelial cell adhesion
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49 200 molecule shedding is not or to a lesser extent involved in the pathophysiology of EOS. For
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51 201 interpretation of our data we reviewed and summarized available data on sCAMs and
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54 202 sheddases in newborns with sepsis in Supplemental Table 2 [16-34]. Comparison of our
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3 203 results with other existing data is complicated because of heterogenic make up of chosen
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5 204 cohorts. Only one study reported a comparable cohort of near and at term newborns with
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7 205 suspected EOS within 72 hours after birth, in whom increased levels of sICAM-1 and
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9 206 neutrophil elastase levels were associated with blood culture positive EOS [19]. Other earlier
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11 207 studies compared levels of sCAMs in heterogenic cohorts consisting of newborns with
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13 208 different gestational and postnatal ages, either having EOS (based on varying definitions), or
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15 209 sepsis after 72 hours after birth (i.e., late onset sepsis). This variation in inclusion criteria is an
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17 210 important confounding factor in the interpretation of the observed levels in septic and healthy
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19 211 newborns. Overall, our results are in line with these studies that show that clinical utility of
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21 212 levels of sCAMs and sheddases in EOS is very limited.

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23
24 213 In an earlier review by our group we pooled published data on sCAM levels in
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26 214 newborns [7]. Soluble CAM levels in the current study corresponded well with levels
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28 215 discussed in our review and those established in earlier studies in uninfected healthy
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30 216 newborns with similar gestational and postnatal age [7, 23,24,29-32]. However, MMP-9,
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32 217 TIMP-1, and neutrophil elastase levels were different and up to 4, 2, and 10-fold higher,
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34 218 respectively, than those reported in earlier studies [17,18,21,22,25,30], which may have been
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36 219 due to other methods used (see limitations). Furthermore, our earlier review and earlier data
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38 220 indicated that significant age-related discrepancies exist in sCAM levels between newborns,
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40 221 children and adults. As an example, in at term newborns sVCAM-1 concentrations in the first
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42 222 postnatal week were almost twice the levels in healthy adults, and equally high compared to
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44 223 septic adults, suggesting that sVCAM-1 levels start of high in early newborn life and then
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46 224 decrease with increasing age [7,31]. In our study, levels of sCAMs and sheddases during the
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48 225 first 6 days of life in our study remained stable for 7 out of analyzed 8 molecules, which was
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50 226 in contrast with earlier work in healthy newborns showing that sE-selectin decreased, and
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52 227 sICAM-1 and sVCAM-1 increased between day 1 and 5 after birth, while sPECAM-1 levels
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3 228 did not change [29-32]. Even though some discrepancies with earlier reports exist, overall one
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5 229 can conclude that these and our data indicate that levels of sCAMs and sheddases measured
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7 230 within 72 hours after birth are high and do not discriminate between septic and healthy
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9 231 newborns, which limits their use as biomarkers for early identification or exclusion of EOS.

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11 232 Our and pre-existing data suggest that overall high sCAM and sheddase levels in
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13 233 newborns are the result of other perinatal factors than EOS. Several pathophysiological
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15 234 processes may explain this premise. Birth may induce a 'pro-adhesive' state of the
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17 235 endothelium leading to increased endothelial cell adhesion molecules expression on, and
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19 236 shedding from, its surface. Additionally, the increase in overall leukocyte numbers and
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21 237 inflammatory activation of subsets associated with human birth, which was shown to be
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23 238 positively associated with increased perinatal stress [35-37], may cause higher intensity of
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25 239 leukocyte-endothelial interactions and subsequent increases endothelial cell adhesion
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27 240 molecules shedding. Aberrant adhesion of activated leukocytes to activated endothelium is
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29 241 associated with endothelial dysfunction and increased vascular permeability [38,39].
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31 242 Shedding of endothelial cell adhesion molecules may then result in prevention of aberrant
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33 243 leukocyte adhesion on two complementary levels, namely 1) to lower endothelial cell
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35 244 adhesion molecules density to prevent adhesion or promote de-adhesion of already adhering
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37 245 leukocytes and 2) to release circulating sCAMs that act as 'decoy receptors' to capture
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39 246 leukocytes in the vasculature to limit leukocyte-endothelial interactions [7,10]. Whether this
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41 247 occurs in real life and what the contribution is to sCAM and sheddase levels in newborns
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43 248 remains unknown and could be studied in neonatal animal models [40-42].

44
45 249 Our study has some limitations. First, sample size at t=48-72h was relatively small due
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47 250 to limited clinical need for additional blood draws in controls and death of patients. As a
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49 251 result, logistic regression analysis of other factors, such as maternal perinatal factors or
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51 252 method of birth, potentially influencing levels of sCAMs and sheddases, was precluded.
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3 253 Larger studies in countries such as Suriname, where the incidence of EOS is relatively high in
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5 254 comparison to Western countries, are necessary and can contribute to better insight in the
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7 255 vascular pathophysiology of EOS. Second, the use of serum in our study may have caused
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9 256 release of stored pools of MMP-9, TIMP-1, and neutrophil elastase from disrupted leukocytes
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11 257 during the clotting process, which could have accounted for higher levels of these molecules
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13 258 than reported in earlier studies.

15 259 In conclusion, our data indicate that serum levels of sCAMs and sheddases are not
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17 260 increased during EOS in Surinamese near and at term newborns. Other mechanisms, such as
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19 261 perinatal stress during birth, may drive overall high levels in all newborns which precludes
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21 262 discrimination between septic and healthy newborns based on levels of these molecules. For
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23 263 these reasons sCAMs and sheddases studied have no utility as biomarkers for EOS.
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28 265 **ABBREVIATIONS AND DEFINITIONS**

30
31 266 EOS = Early onset sepsis

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33 267 ICAM-1 = Intercellular adhesion molecule-1

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35 268 VCAM-1= Vascular cell adhesion molecule-1

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37 269 PECAM-1= Platelet and endothelial cell adhesion molecule-1

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39 270 MMP-9 = Matrix metalloproteinase-9

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41 271 TIMP-1 = Tissue-inhibitor of metalloproteinases-1

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45 273 **ACKNOWLEDGEMENTS**

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3 277 the Central Laboratory of Suriname, Paramaribo, Suriname, for assistance with sample
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5 278 storage, handling and transport.

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9 280 **CONFLICTS OF INTEREST**

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11 281 The authors declare no conflicts of interest

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15 283 **REFERENCES**

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3 407 **FIGURE LEGENDS**

4
5 408 **Figure 1.**

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7 409 **Circulating levels of endothelial adhesion molecules sP-selectin, sE-selectin, sVCAM-1,**
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9 410 **sICAM-1, and sPECAM-1 in Surinamese newborns. A: sP-selectin B: sE-selectin C:**
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11 411 **soluble vascular cell adhesion molecule-1 (sVCAM-1); D: soluble intercellular adhesion**
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13 412 **molecule-1 (sICAM-1); E: soluble platelet and endothelial cell adhesion molecule-1**
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15 413 **(sPECAM-1). Data report levels in serum sampled at t=0 (white bars) and t=48-72h (grey**
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17 414 **bars) and are analyzed with a Kruskal-Wallis test between all groups at t=0 ($P_{t=0}$) and at t=48-**
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19 415 **72 ($P_{t=48-72}$). $P<0.05$ is considered statistically significant. Bars represent median values and**
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21 416 **error bars interquartile range.**

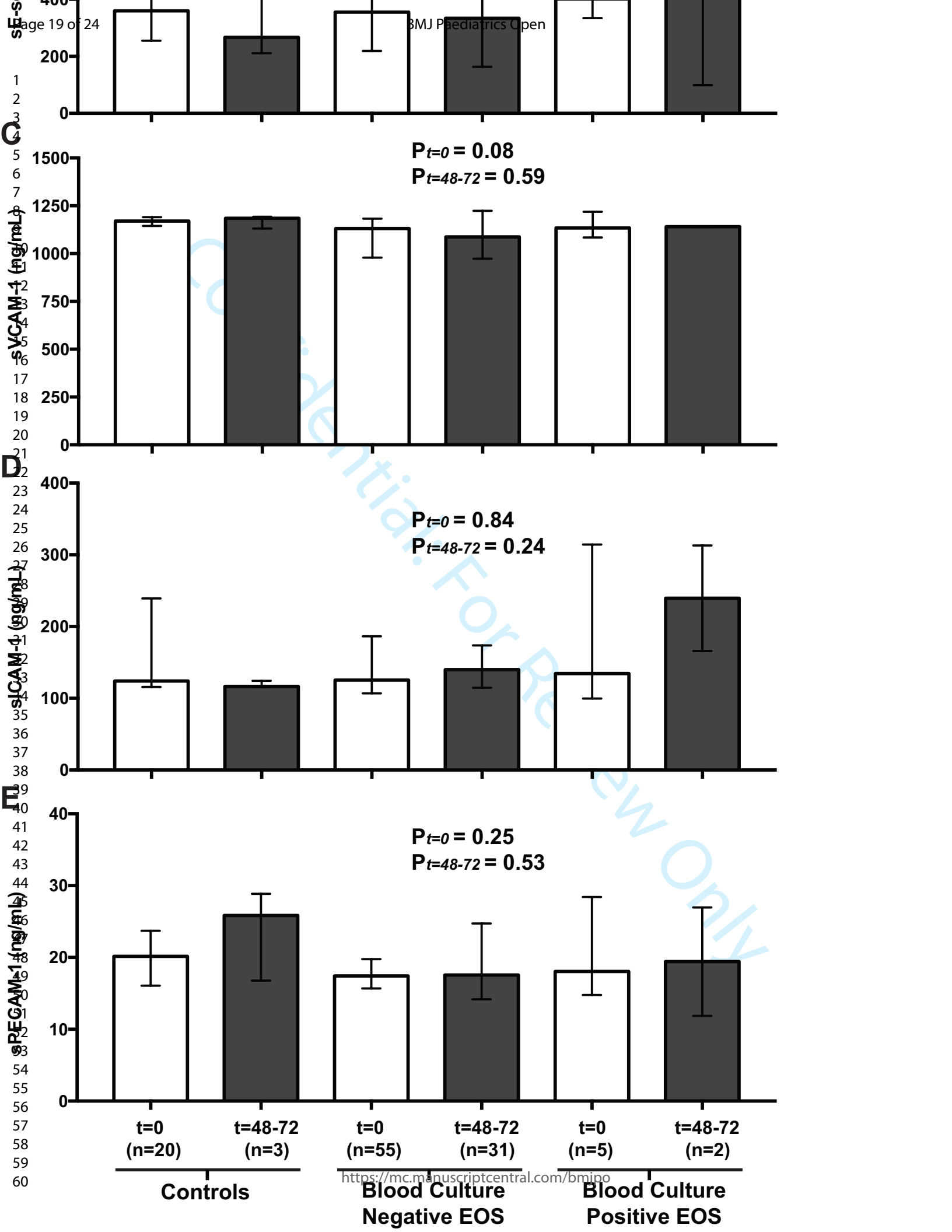
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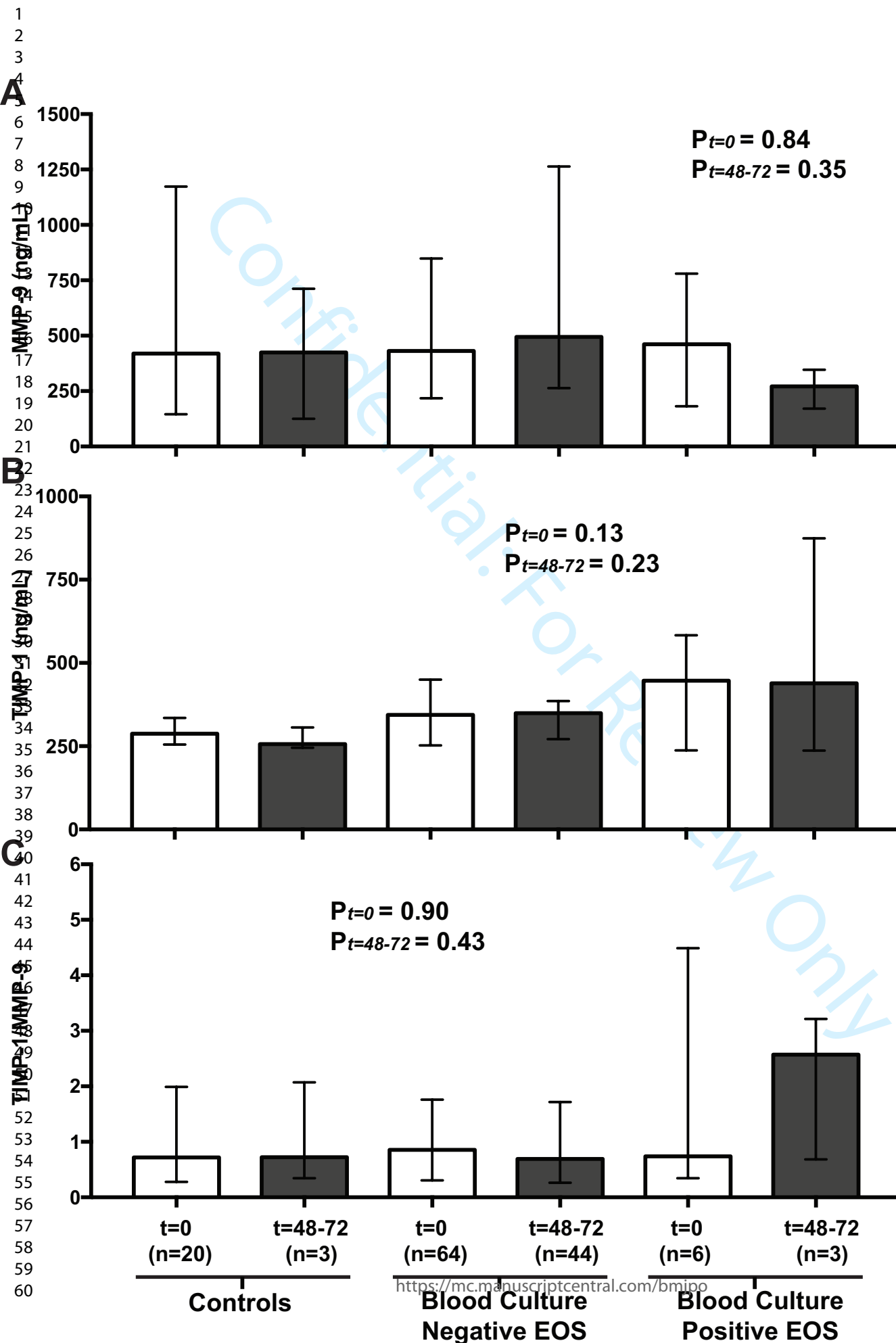
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26 418 **Figure 2. Circulating levels of MMP-9 and TIMP-1, and TIMP-1/MMP-9 ratios in**
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28 419 **Surinamese newborns. A: Matrix metalloproteinase-9 (MMP-9); B: Tissue inhibitor of**
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30 420 **metalloproteinase (TIMP-1); C: TIMP-1/MMP-9 ratios. Data report levels in serum sampled**
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32 421 **at t=0 (white bars) and t=48-72h (grey bars) and are analyzed with a Kruskal-Wallis test**
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34 422 **between all groups at t=0 ($P_{t=0}$) and at t=48-72 ($P_{t=48-72}$). $P<0.05$ is considered statistically**
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36 423 **significant. Bars represent median values and error bars interquartile range.**

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41 425 **Figure 3. Circulating levels of neutrophil elastase in Surinamese newborns. Data report**
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43 426 **levels in serum sampled at t=0 (white bars) and t=48-72h (grey bars) and are analyzed with a**
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45 427 **Kruskal-Wallis test between all groups at t=0 ($P_{t=0}$) and at t=48-72 ($P_{t=48-72}$). $P<0.05$ is**
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47 428 **considered statistically significant. Bars represent median values and error bars interquartile**
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49 429 **range.**

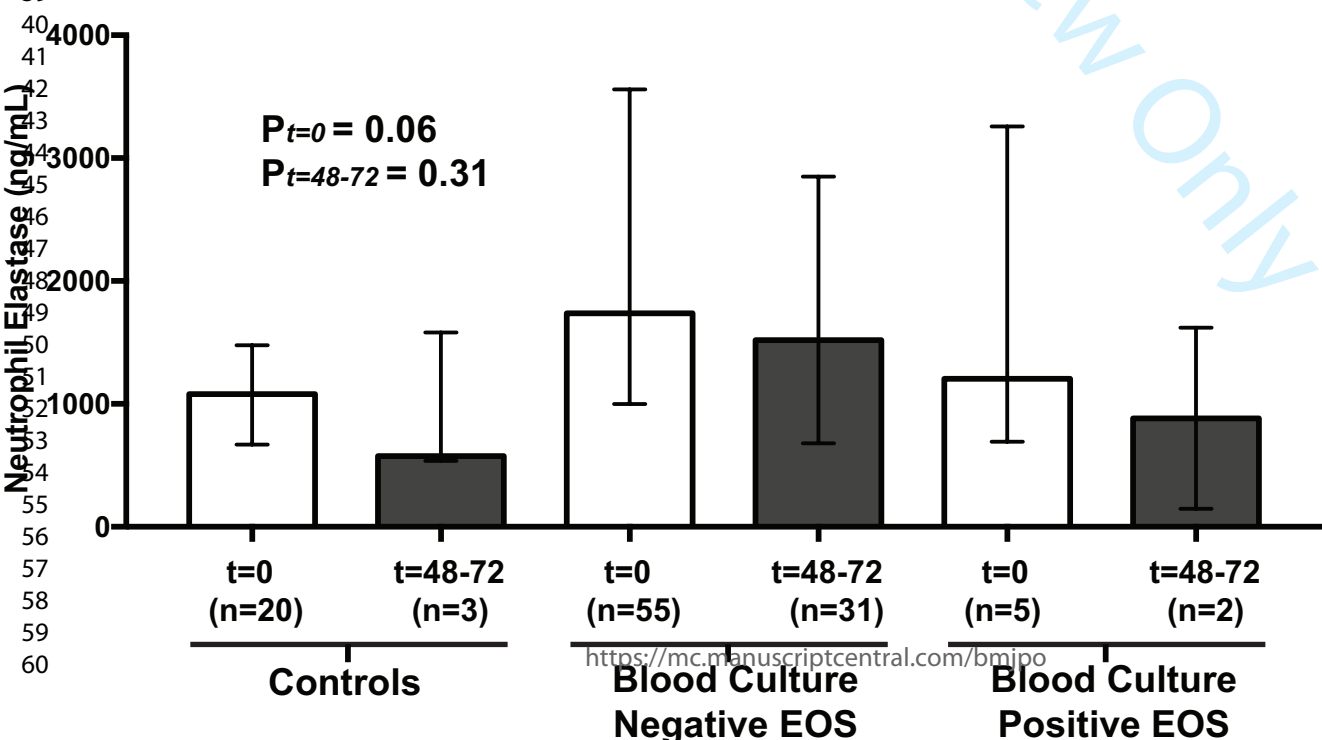
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Supplemental Table 1: Descriptive statistics of the study group (n=91)

		Controls (n=20)	Early Onset Sepsis		P-value
			Blood Culture Negative (n=65)	Blood Culture Positive (n=6)	
Pregnancy, n (%)	Complications ^a	3 (15)	16 (25)	1 (17)	0.63
	Chorioamnionitis ^b	0	18 (28)	0	
Mode of delivery, n (%)	Vaginal	12 (60)	46 (75)	4 (67)	0.54
	Caesarean	8 (40)	19 (25)	2 (33)	
Sex, n (%)	Male	9 (45)	29 (45)	5 (83)	0.19
	Female	11 (55)	36 (55)	1 (17)	
Ethnicity, n (%)	Maroon and Creole	12 (60)	44 (68)	4 (67)	0.61
	Hindo-Surinamese	3 (15)	14 (21)	1 (17)	
	Other ^c	5 (25)	7 (11)	1 (17)	
Gestational age, n (%) (weeks)	34-37	1 (5)	22 (34)	0	0.06
	37-40	14 (70)	30 (46)	4 (67)	
	≥40	5 (25)	13 (20)	2 (33)	
Apgar score, n (%)	<5	0	5 (8)	2 (33)	0.03
Birth weight, Median (IQR) (grams)		3130 (700)	2840 (835)	3500 (906)	0.02
Age at presentation, n (%) (hours)	<24	4 (20)	43 (66)	2 (33)	<0.01
	24-48	7 (35)	13 (20)	1 (17)	
	48-72	9 (45)	9 (14)	3 (50)	
Clinical course (at 48-72h), n (%)	CPAP	0	9 (14)	0	<0.001
	Mechanical Ventilation	0	7 (11)	2 (33)	
	Cardiotonics	0	5 (8)	1 (17)	
	Mortality	0	3 (5)	2 (33)	

This table was previously published in reference 15. CPAP = continuous positive airway pressure; N/A = not applicable.

^a Presence of pregnancy-induced hypertension, preeclampsia or diabetes mellitus;

^b Defined as intrapartum fever or administration of antibiotics;

^c Includes: Javanese, Chinese, Caucasian and Amerindian.

Supplemental Table 2: Studies reporting levels of endothelial cell adhesion molecules and shedding enzymes in newborns

Study (ref.), year	CAM or shedding enzyme	Cohort Characteristics	Gestational age (weeks)	Postnatal age	Main Results	Analysis Method	Healthy (ng/mL) ¹	Septic (ng/mL) ¹
Fatah et al. (16), 2017	sE-selectin	EOS and LOS	NS	NS	sE-selectin elevated in BCPS	ELISA	148.9 ± 7.9	177.1 ± 3.5
Weitkamp et al. (17), 2016	MMP-9	EOS and LOS	25-36	< and ≥ 3 days	MMP-9 levels lower in BCPS	Multiplex bead assay	NS	NS
Wynn et al. (18), 2015	MMP-9	Chorioamnionitis	25-36	NS	MMP-9 levels lower in chorioamnionitis	Multiplex bead assay	NS	NS
Sugitharini et al. (19), 2013	sICAM-1, NE	EOS	34-42	0-72 hours	sICAM-1 and NE elevated in EOS	ELISA Antibody array	sICAM-1/ NE: NS	sICAM-1: NS NE: 499.2 ± 22.0
Edgar et al. (20), 2010	sICAM-1, sE-selectin	EOS and LOS	24-41	NS	sICAM-1 and sE-selectin elevated	ELISA	sICAM-1: 165 (130-290) sE-selectin: 71 (51-118)	sICAM-1: 405 (252-666) sE-selectin 158 (94-207)
Fukanaga et al. (21), 2009	MMP, TIMP-1	Uninfected newborns	<30	Cord blood	No difference	ELISA	MMP-9: 22 (16-48) TIMP-1: 122 (86-249)	NS
Sunagawa et al. (22), 2009	MMP-9, TIMP-1	Uninfected newborns	35-41	1-2 days	NA	ELISA	NS	NA
Figueras et al. (23), 2007	sICAM-1, sVCAM-1, sP-selectin,	EOS and LOS	32-40	1-32 days	sICAM-1 and sVCAM-1 increased over time.	ELISA	sICAM-1: 156 (150-194) sVCAM-1: 856 (742-960) sP-selectin: 272 (152-288)	sICAM-1: 394 (342-600) sVCAM-1: 1153 (726-1307) sP-selectin: 244 (170-324)
Sitaru et al. (24), 2005	sP-selectin	Chorioamnionitis	25-40	Cord blood	sP-selectin elevated in chorioamnionitis	ELISA	104 ± 71	222 ± 128
Schulz et al. (25), 2004	MMP-9, TIMP-1	Uninfected newborns	25-40	1-28 days	MMP-9 highest in preterm TIMP-1 highest in at term	ELISA	NS	NA
Edgar et al.	sICAM-1	EOS and LOS	24-42	NS	sICAM-1 elevated in	ELISA	205 (146-343)	406 (345-1180)

(26), 2002					BCPS			
Apostolou et al. (27), 2002	sICAM-1	EOS and LOS	25-42	NS	sICAM-1 elevated in BCPS	ELISA	358.4 ± 28.9	710.7 ± 56.6
Dollner et al. (28), 2001	sICAM-1, sE-selectin	EOS and LOS	30-42	1-7 days	sICAM-1 and sE-selectin elevated in BCPS	ELISA	sICAM-1: 244.0 (92.5-500) sE-selectin: 91.4 (<2.0-217.8)	sICAM-1: 357.4 (141.6-500) sE-selectin: 151.7 (37.0-362.2)
Malamitsi et al. (29), 2000	sVCAM-1, sPECAM-1	Uninfected newborns	37-40	1-5 days	No change between day 1 and 5	ELISA	sVCAM-1: 1340 ± 58.3 sPECAM-1: 17.5 ± 0.7	NA
Giannaki et al. (30), 2000	sE-selectin	Uninfected newborns	At term	1-5 days	sE-selectin decreases between day 1 and 5	ELISA	139 ± 48	NA
Giannaki et al. (31), 1999	sICAM-1, sVCAM-1	Uninfected newborns	At term	1-5 days	sICAM-1 and sVCAM-1 increase between day 1 and 5	ELISA	sICAM: 179 ± 56.1 sVCAM-1: 1125.0 ± 281.0	NA
Phocas et al. (32), 1998	sICAM-1	Uninfected newborns	35-42	1-30 days	sICAM-1 increases between day 1, 5 and 30	ELISA	137.3 ± 62.0	NA
Berner et al. (33), 1998	sICAM-1	EOS	26-42	0-96 hours	sICAM-1 lower in EOS. sICAM-1 increases over time	ELISA	421 (291-459)	446 (171-534)
Austgulen et al. (34), 1997	sICAM-1, sVCAM-1, sE-selectin	EOS and LOS, pneumonia	24-42	0-162 hours	sE-selectin and sICAM-1 elevated in infected neonates	ELISA	sE-selectin: 84.2 (21.6-231.3) sICAM-1: 2131.3 (1449.5-3500.0) sVCAM-1: 237.0 (122.0-500.0)	NS

CAM = endothelial cell adhesion molecule; sVCAM-1 = soluble Vascular Cell Adhesion Molecule-1; sICAM-1 = soluble Intercellular Adhesion Molecule-1; NE = neutrophil elastase NA = Not available; NS = Not specified; EOS = Early Onset Sepsis; LOS = Late Onset Sepsis; BCPS = Blood Culture Positive Sepsis.

^a Levels are in mean ± SD, mean ± SEM, median (interquartile range), or median (range).

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Serum concentrations of endothelial cell adhesion molecules and their shedding enzymes and early onset sepsis in newborns in Suriname

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3 35 **Short title:** Adhesion molecule shedding in Surinamese newborns
4 36 **Funding source:** The Thrasher Research Fund (TRF13064) and Tergooi Hospitals, Blaricum,
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6 37 The Netherlands.
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3 38 **ABSTRACT**

4
5 39 **Background:** Early Onset Sepsis (EOS) is defined as onset of sepsis within 72 hours after
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7 40 birth. Leukocyte-endothelial interactions play a pivotal part in EOS pathophysiology.

8
9 41 Endothelial cell adhesion molecules (CAMs) orchestrate these interactions and their soluble
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11 42 isoforms (sCAMs) are released into the vasculature by enzymes called sheddases.

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13 43 **Purpose:** This study was undertaken to explore further the pathophysiology of EOS and to
14
15 44 investigate the potential of sCAM and their sheddases as potential biomarkers for EOS.

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17 45 **Methods:** Stored serum aliquots were used from 71 Surinamese newborns suspected of EOS
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19 46 and 20 healthy newborns from an earlier study. Serum had been collected within 72 hours
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21 47 after birth and six (8.6%) newborns had a positive blood culture with gram-negative
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23 48 pathogens. Concentrations of sCAMs sP-selectin, sE-selectin, vascular cell adhesion
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25 49 molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1) and platelet and
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27 50 endothelial cell adhesion molecule-1 (sPECAM-1), sheddases matrix metalloproteinase-9
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29 51 (MMP-9) and neutrophil elastase (NE), and sheddase antagonist tissue-inhibitor of
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31 52 metalloproteinases-1 (TIMP-1) were measured simultaneously with Luminex and ELISA.

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33 53 **Results:** MMP-9 and TIMP-1 levels were measured in serum of n=91 newborns and sCAMs
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35 54 and NE levels in serum of n=80 newborns, respectively. We found no differences in median
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37 55 concentrations of sCAMs, MMP-9 and TIMP-1, or NE between blood culture positive EOS,
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39 56 blood culture negative EOS, and control groups at start of antibiotic treatment.

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41 57 **Conclusions:** Our data indicate that serum concentrations of sCAMs and their sheddases have
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43 58 no clinical utility as biomarkers for EOS.

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50 60 **Keywords:** newborns; early onset sepsis; adhesion molecules; shedding; Suriname.

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3 63 **KEY MESSAGES**
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7 65 **What is already known on this topic?**
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9 66 • Recently, we established an association of the Ang-1/Ang-2 disbalance with blood
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11 67 culture positive early onset sepsis (EOS) in newborns.

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13 68 • The relationship between this Ang-1/Ang-2 disbalance and serum levels of sCAMs
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15 69 and their sheddases is unclear.
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18 70 **What this study adds?**
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20 71 • The Ang-1/Ang-2 disbalance in blood culture positive EOS is not paralleled by
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22 72 increased levels of sCAMs and their sheddases.

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24 73 • Levels of sCAMs and their sheddases are high after birth and do not discriminate EOS
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26 74 from healthy newborns.
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76 INTRODUCTION

77 Early onset sepsis (EOS) in newborns within 72 hours after birth remains a clinical challenge
78 with high morbidity and mortality [1-3]. The majority of global neonatal deaths due to EOS
79 occur in developing countries [4]. The diagnosis of EOS is complicated, resulting in late
80 recognition, or overtreatment of newborns with antibiotics. These dilemmas arise because the
81 pathophysiology of EOS is poorly understood.

82 A hallmark of sepsis pathophysiology is endothelial cell activation followed by
83 leukocyte recruitment into tissues [5]. The current model describes the occurrence of a shift in
84 balance in Tie2 receptor ligands Angiopoietin (Ang)-1 and Ang-2 affecting endothelial
85 integrity, and increased expression of endothelial cell adhesion molecules, in particular P-
86 selectin, E-selectin, vascular cell adhesion molecule (VCAM-1), and intercellular adhesion
87 molecule (ICAM-1) to facilitate this recruitment [6,7]. These endothelial cell adhesion
88 molecules orchestrate leukocyte rolling on, adhesion to, and diapedesis across the
89 endothelium [7,8]. Also, platelet and endothelial cell adhesion molecule (PECAM-1),
90 expressed at endothelial cell junctions has a function in facilitating paracellular transmigration
91 of leukocytes across the endothelium [9]. After intravenous administration of endotoxin in
92 healthy adults as a sepsis model, peak concentrations of Ang-2 prelude the release of soluble
93 isoforms of cell adhesion molecules (sCAMs) into the systemic circulation [10]. Endothelial
94 cell adhesion molecules are released through ectodomain shedding by enzymes called
95 sheddases, in particular matrix metalloproteinase-9 (MMP-9) and neutrophil elastase (NE),
96 released from granules in neutrophils [7,11]. Both MMP-9 and NE prepare the extracellular
97 matrix for transmigration of leukocytes into inflammatory sites [12]. MMP-9 activity is
98 balanced by sheddase antagonist tissue-inhibitor of metalloproteinases-1 (TIMP-1) [12-14].

99 Recently, we showed in a cohort of near term and term Surinamese newborns that a
100 systemic dysbalance in Ang-2/Ang-1 concentrations was associated with blood culture

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3 101 positive EOS [15]. This study was undertaken to examine if this dysbalance is paralleled by
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5 102 increased serum concentrations of sCAMs and sheddases in this cohort of newborns with
6
7 103 EOS to explore further the pathophysiology of EOS. We hypothesized that sCAM and
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9 104 sheddases concentrations measured at start of antibiotic treatment for suspected EOS are
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11 105 higher in newborns with blood culture positive EOS than in healthy controls.
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16 107 **MATERIALS & METHODS**

17 108 **Study design, subjects and clinical protocol**

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20 109 For this study, we used a Surinamese cohort of 20 healthy newborns and 71 newborns with
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22 110 suspected EOS from an earlier reported study [15]. All newborns were included after
23
24 111 admission between April 1 2015 and May 31 2016 to the neonatal care facility of Academic
25
26 112 Pediatric Center Suriname at the Academic Hospital Paramaribo in Suriname. Included were
27
28 113 newborns with a gestational age equal to or above 34 weeks in whom antibiotics were started
29
30 114 within the first 72 hours of life for suspected EOS. Suspicion of EOS was based on the
31
32 115 attending physicians decision to start antibiotic treatment. Informed consent was obtained
33
34 116 from at least one parent for the use of residual serum and clinical information. The study
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36 117 protocol was made available on clinicaltrials.gov (NCT02486783) and was approved by the
37
38 118 Surinamese Medical Ethical Board (VG-021-14A) including permission of one parent.
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41 119 The management of these patients was described before [15]. In short, healthy control
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43 120 newborns and newborns suspected of EOS were included at start of antibiotic treatment
44
45 121 within 72 hours after birth. At start of antibiotic treatment blood was collected for separation
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47 122 and storage of serum. Controls were newborns without signs of infection receiving blood
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49 123 draws for hyperbilirubinemia (n=20). Newborns with suspected EOS receiving treatment with
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51 124 intravenous antibiotics were divided in two groups based on result from blood culturing:
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53 125 blood culture negative EOS (n=65) and blood culture positive EOS (n=6).
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5 127 **Sample collection, preparation and analysis**

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7 128 In the previous study, serum had been collected from whole blood collected after insertion of
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9 129 a venous cannula in newborns suspected of EOS and after capillary collection in controls
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11 130 [15]. Frozen serum samples had been transported on dry ice to the Netherlands and aliquoted
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13 131 and frozen again upon arrival. A stored aliquot was used for the measurement of sP-selectin,
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15 132 sE-selectin, sVCAM-1, sICAM-1, and sPECAM-1 using the Human Magnetic Bead
16
17 133 Adhesion 6-plex panel performance assay (LHC0016M, Thermo Scientific, Waltham, MA
18
19 134 USA) according to the manufacturer's instructions. ELISA was used on the same aliquots for
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21 135 measurement of neutrophil elastase (HK319-02, Hycult Biotech, Uden, The Netherlands),
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23 136 MMP-9 (Quantikine DMP900, R&D systems, Minneapolis, MN USA), and TIMP-1
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25 137 (Quantikine DTM100, R&D systems), each according to the manufacturers' instructions. For
26
27 138 each molecule, a standard curve was established via which concentrations in neonatal serum
28
29 139 were determined. Levels below or above the linear part of this standard curve were reported
30
31 140 as the lowest or highest value of the standard curve, respectively. We measured intra-assay
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33 141 variation between plates used in the same assay by calculating coefficient of variation
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35 142 between levels of each molecule in samples from the same patient divided over those plates
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37 143 and accepted a maximum of 20%.

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44 145 **Statistical analysis**

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46 146 A Kruskal-Wallis test with Dunn's correction for multiple comparisons were used for analysis
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48 147 between the blood parameters and the three groups (blood culture positive EOS, blood culture
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50 148 negative EOS, and control groups). P-values <0.05 were considered statistically significant.
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52 149 All analyses were done using Prism version 7.0a (Graphpad Software Inc., San Diego, CA
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54 150 USA).

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152 RESULTS

153 Demographic variables of the whole study cohort (n=91) can be found in Table 1 of reference
154 15. Of baseline characteristics birth weight, age at presentation (between 0 and 72 hours after
155 birth), and Apgar score at 5 minutes were distributed unevenly amongst the three groups
156 (P<0.05). Blood culture results revealed that 6 of 70 newborns with suspected EOS (8.6%;
157 95% CI 1.9-15.3%) had a positive blood culture with gram-negative pathogens *Klebsiella*
158 *pneumoniae* (n=2), *Enterobacter cloacae* (n=2) and *Escherichia coli* (n=2). One newborn had
159 EOS due to a spontaneous bacterial peritonitis. For n=4 others cause of EOS was unknown,
160 but they presented with neonatal jaundice (n=1), perinatal asphyxia (n=1), meconium
161 aspiration (n=1), and hypoglycaemia (n=1).

162

163 Serum concentrations of soluble endothelial cell adhesion molecules and their sheddases

164 Due to the limited amount of serum available, not all molecules could be measured in all
165 samples. We were able to measure MMP-9 and TIMP-1 levels in serum of n=90 newborns
166 and sCAMs and NE levels in serum of n=80 newborns, respectively. We found no differences
167 in median concentrations of sCAMs (Figure 1), MMP-9 and TIMP-1 (Figure 2), or NE
168 (Figure 3) between blood culture positive EOS, blood culture negative EOS, and control
169 groups at start of antibiotic treatment within 72 hours after birth.

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171 DISCUSSION

172 In this study we investigated whether sCAMs and their sheddases circulate at higher
173 concentrations in near and at term newborns with blood culture positive EOS at start of
174 antibiotic treatment. In contrast to our hypothesis, none of the molecules showed any
175 difference in serum concentrations between blood culture positive EOS, blood culture

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3 176 negative EOS, and controls within 72 hours after birth. Our data indicate that serum
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5 177 concentrations of sCAMs and their sheddases have no clinical utility as biomarkers for EOS
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7 178 nor to guide the start of antibiotic treatment.

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9 179 Previously, we found evidence for endothelial cell activation in blood culture positive
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11 180 EOS in the same newborns used for this study, represented by a dysbalance in Ang-2/Ang-1
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13 181 ratio [15]. Since the current data demonstrate that this dysbalance was not paralleled by
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15 182 increased release of sCAM or sheddases in EOS we conclude that endothelial cell adhesion
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17 183 molecule shedding is not or to a lesser extent involved in the pathophysiology of EOS. For
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19 184 interpretation of our data we reviewed and summarized available data on sCAMs and
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21 185 sheddases in newborns with sepsis in Supplemental Table 1 [16-34]. Comparison of our
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23 186 results with other existing data is complicated because of heterogenic make up of chosen
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25 187 cohorts. Only one study reported a comparable cohort of near and at term newborns with
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27 188 suspected EOS within 72 hours after birth, in whom increased concentrations of sICAM-1
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29 189 and neutrophil elastase were associated with blood culture positive EOS [19]. Other earlier
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31 190 studies compared concentrations of sCAMs in heterogenic cohorts consisting of newborns
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33 191 with different gestational and postnatal ages, either having EOS (based on varying
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35 192 definitions), or sepsis after 72 hours after birth (i.e., late onset sepsis). This variation in
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37 193 inclusion criteria is an important confounding factor in the interpretation of the observed
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39 194 concentrations in septic and healthy newborns. Overall, our results are in line with these
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41 195 studies that show that clinical utility of sCAMs and sheddases in EOS is very limited.

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43
44 196 In an earlier review by our group we pooled published data on sCAM concentrations
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46 197 in newborns [7]. Soluble CAM concentrations in the current study corresponded well with
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48 198 concentrations discussed in our review and those established in earlier studies in uninfected
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50 199 healthy newborns with similar gestational and postnatal age [7, 23,24,29-32]. However,
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52 200 MMP-9, TIMP-1, and neutrophil elastase concentrations were different and up to 4, 2, and
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3 201 10-fold higher, respectively, than those reported in earlier studies [17,18,21,22,25,30], which
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5 202 may have been due to other methods used (see limitations). Furthermore, our earlier review
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7 203 and earlier data indicated that significant age-related discrepancies exist in sCAM
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9 204 concentrations between newborns, children and adults. As an example, in at term newborns
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11 205 sVCAM-1 concentrations in the first postnatal week were almost twice the concentrations in
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13 206 healthy adults, and equally high compared to septic adults, suggesting that sVCAM-1
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15 207 concentrations start of high in early newborn life and then decrease with increasing age
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17 208 [7,31]. In our study, concentrations of sCAMs and sheddases during the first 3 days of life in
18
19 209 our study remained stable, which was in contrast with earlier work in healthy newborns
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21 210 showing that sE-selectin decreased, and sICAM-1 and sVCAM-1 increased between day 1
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23 211 and 5 after birth, while sPECAM-1 levels did not change [29-32]. Even though some
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25 212 discrepancies with earlier reports exist, overall one can conclude that these and our data
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27 213 indicate that concentrations of sCAMs and sheddases measured within 72 hours after birth are
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29 214 high and do not discriminate between septic and healthy newborns, which limits their use as
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31 215 biomarkers for early identification or exclusion of EOS.
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35 216 Our and pre-existing data suggest that overall high sCAM and sheddase concentrations
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37 217 in newborns are the result of other perinatal factors than EOS. Several pathophysiological
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39 218 processes may explain this premise. Birth may induce a ‘pro-adhesive’ state of the
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41 219 endothelium leading to increased endothelial cell adhesion molecules expression on, and
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43 220 shedding from, its surface. Additionally, the increase in overall leukocyte numbers and
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45 221 inflammatory activation of subsets associated with human birth, which was shown to be
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47 222 positively associated with increased perinatal stress [35-37], may cause higher intensity of
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49 223 leukocyte-endothelial interactions and subsequent increases endothelial cell adhesion
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51 224 molecules shedding. Aberrant adhesion of activated leukocytes to activated endothelium is
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53 225 associated with endothelial dysfunction and increased vascular permeability [38,39].
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3 226 Shedding of endothelial cell adhesion molecules may then result in prevention of aberrant
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5 227 leukocyte adhesion on two complementary levels, namely 1) to lower endothelial cell
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7 228 adhesion molecules density to prevent adhesion or promote de-adhesion of already adhering
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9 229 leukocytes and 2) to release circulating sCAMs that act as ‘decoy receptors’ to capture
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11 230 leukocytes in the vasculature to limit leukocyte-endothelial interactions [7,10]. Whether this
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13 231 occurs in real life and what the contribution is to sCAM and sheddase concentrations in
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15 232 newborns remains unknown and could be studied in neonatal animal models [40-42].

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18 233 Our study has some limitations. First, sample size was relatively small. As a result,
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20 234 logistic regression analysis of other factors, such as maternal perinatal factors or method of
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22 235 birth, potentially influencing levels of sCAMs and sheddases, was precluded. Larger studies
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24 236 in countries such as Suriname, where the incidence of EOS is relatively high in comparison to
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26 237 Western countries [43], are necessary and can contribute to better insight in the vascular
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28 238 pathophysiology of EOS. Second, the use of serum in our study may have caused release of
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30 239 stored pools of MMP-9, TIMP-1, and neutrophil elastase from disrupted leukocytes during
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32 240 the clotting process, which could have accounted for higher levels of these molecules than
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34 241 reported in earlier studies. Last, repeated freeze-thaw cycles may have affected quality of
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36 242 serum samples with regards to reproducibility of NE concentrations.

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39 243 In conclusion, our data indicate that serum concentrations of sCAMs and sheddases
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41 244 are not higher in Surinamese newborns with EOS versus controls at start of antibiotic
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43 245 treatment. Although concentrations may still increase significantly more in newborns with
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45 246 EOS, other mechanisms, such as perinatal stress during birth, may drive overall high
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47 247 concentrations in all newborns which precludes discrimination between septic and healthy
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49 248 newborns.

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3 251 **ABBREVIATIONS AND DEFINITIONS**

4
5 252 EOS = Early onset sepsis

6
7 253 ICAM-1 = Intercellular adhesion molecule-1

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9 254 VCAM-1= Vascular cell adhesion molecule-1

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11 255 PECAM-1= Platelet and endothelial cell adhesion molecule-1

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13 256 MMP-9 = Matrix metalloproteinase-9

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15 257 TIMP-1 = Tissue-inhibitor of metalloproteinases-1

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20 259 **ACKNOWLEDGEMENTS**

21
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29
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31
32 265 the statistical analysis for the final version of this paper.

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37 267 **CONFLICTS OF INTEREST**

38
39 268 The authors declare no conflicts of interest

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44 270 **AUTHOR CONTRIBUTIONS**

45
46 271 RZ, MvM, GM, and FBP conceived and designed the study. RZ and AJ collected clinical data

47
48 272 and collected the samples. RZ, RMJ, and MvM prepared the samples and performed the

49
50 273 sample analysis. RZ and MvM analyzed the final database. RZ, MvM, GM, and FBP drafted

51
52 274 the manuscript. All authors co-authored and approved the final manuscript.

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3 403 **FIGURE LEGENDS**

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5 404 **Figure 1.**

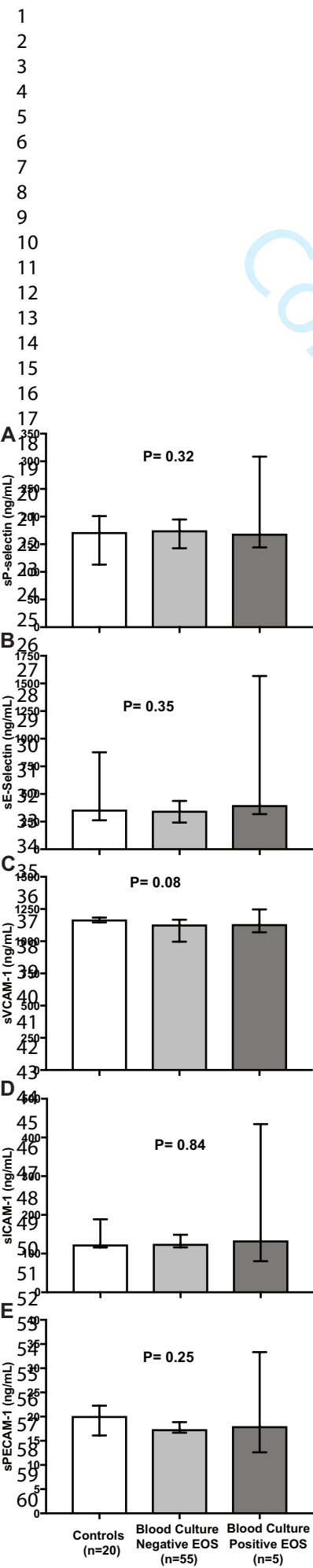
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7 405 **Circulating levels of endothelial adhesion molecules sP-selectin, sE-selectin, sVCAM-1,**
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9 406 **sICAM-1, and sPECAM-1 in Surinamese newborns. A: sP-selectin B: sE-selectin C:**
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11 407 **soluble vascular cell adhesion molecule-1 (sVCAM-1); D: soluble intercellular adhesion**
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13 408 **molecule-1 (sICAM-1); E: soluble platelet and endothelial cell adhesion molecule-1**
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15 409 **(sPECAM-1). Bars represent median values and error bars 95% confidence intervals. P-values**
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17 410 **<0.05 were considered statistically significant.**

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19
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22 412 **Figure 2. Circulating levels of MMP-9 and TIMP-1, and TIMP-1/MMP-9 ratios in**
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24 413 **Surinamese newborns. A: Matrix metalloproteinase-9 (MMP-9); B: Tissue inhibitor of**
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26 414 **metalloproteinase (TIMP-1); C: TIMP-1/MMP-9 ratios. Bars represent median values and**
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28 415 **error bars 95% confidence intervals. P-values <0.05 were considered statistically significant.**

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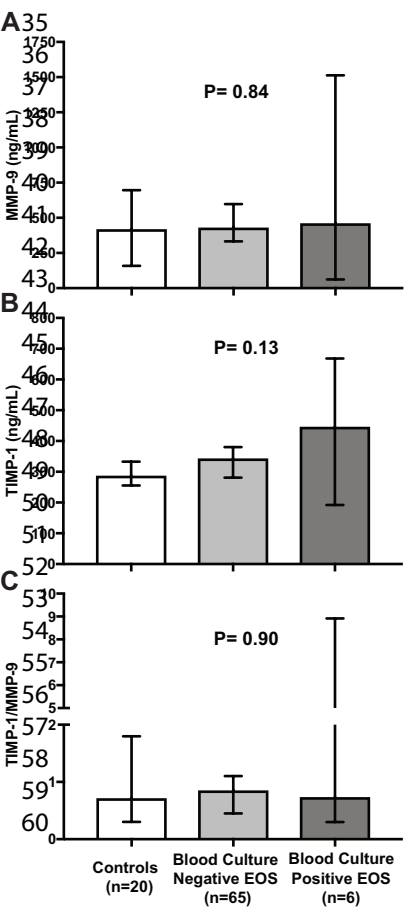
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33 417 **Figure 3. Circulating levels of neutrophil elastase in Surinamese newborns. Bars**
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35 418 **represent median values and error bars 95% confidence intervals. P-values <0.05 were**
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37 419 **considered statistically significant.**



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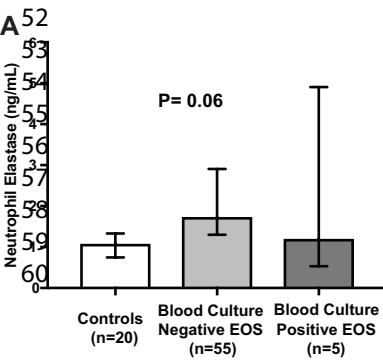
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Supplemental Table 2: Studies reporting levels of endothelial cell adhesion molecules and shedding enzymes in newborns

Study (ref.), year	CAM or shedding enzyme	Cohort Characteristics	Gestational age (weeks)	Postnatal age	Main Results	Analysis Method	Healthy (ng/mL) ¹	Septic (ng/mL) ¹
Fatah et al. (16), 2017	sE-selectin	EOS and LOS	NS	NS	sE-selectin elevated in BCPS	ELISA	148.9 ± 7.9	177.1 ± 3.5
Weitkamp et al. (17), 2016	MMP-9	EOS and LOS	25-36	< and ≥ 3 days	MMP-9 levels lower in BCPS	Multiplex bead assay	NS	NS
Wynn et al. (18), 2015	MMP-9	Chorioamnionitis	25-36	NS	MMP-9 levels lower in chorioamnionitis	Multiplex bead assay	NS	NS
Sugitharini et al. (19), 2013	sICAM-1, NE	EOS	34-42	0-72 hours	sICAM-1 and NE elevated in EOS	ELISA Antibody array	sICAM-1/ NE: NS	sICAM-1: NS NE: 499.2 ± 22.0
Edgar et al. (20), 2010	sICAM-1, sE-selectin	EOS and LOS	24-41	NS	sICAM-1 and sE-selectin elevated	ELISA	sICAM-1: 165 (130-290) sE-selectin: 71 (51-118)	sICAM-1: 405 (252-666) sE-selectin 158 (94-207)
Fukanaga et al. (21), 2009	MMP, TIMP-1	Uninfected newborns	<30	Cord blood	No difference	ELISA	MMP-9: 22 (16-48) TIMP-1: 122 (86-249)	NS
Sunagawa et al. (22), 2009	MMP-9, TIMP-1	Uninfected newborns	35-41	1-2 days	NA	ELISA	NS	NA
Figueras et al. (23), 2007	sICAM-1, sVCAM-1, sP-selectin,	EOS and LOS	32-40	1-32 days	sICAM-1 and sVCAM-1 increased over time.	ELISA	sICAM-1: 156 (150-194) sVCAM-1: 856 (742-960) sP-selectin: 272 (152-288)	sICAM-1: 394 (342-600) sVCAM-1: 1153 (726-1307) sP-selectin: 244 (170-324)
Sitaru et al. (24), 2005	sP-selectin	Chorioamnionitis	25-40	Cord blood	sP-selectin elevated in chorioamnionitis	ELISA	104 ± 71	222 ± 128
Schulz et al. (25), 2004	MMP-9, TIMP-1	Uninfected newborns	25-40	1-28 days	MMP-9 highest in preterm TIMP-1 highest in at term	ELISA	NS	NA
Edgar et al.	sICAM-1	EOS and LOS	24-42	NS	sICAM-1 elevated in	ELISA	205 (146-343)	406 (345-1180)

(26), 2002					BCPS			
Apostolou et al. (27), 2002	sICAM-1	EOS and LOS	25-42	NS	sICAM-1 elevated in BCPS	ELISA	358.4 ± 28.9	710.7 ± 56.6
Dollner et al. (28), 2001	sICAM-1, sE-selectin	EOS and LOS	30-42	1-7 days	sICAM-1 and sE-selectin elevated in BCPS	ELISA	sICAM-1: 244.0 (92.5-500) sE-selectin: 91.4 (<2.0-217.8)	sICAM-1: 357.4 (141.6-500) sE-selectin: 151.7 (37.0-362.2)
Malamitsi et al. (29), 2000	sVCAM-1, sPECAM-1	Uninfected newborns	37-40	1-5 days	No change between day 1 and 5	ELISA	sVCAM-1: 1340 ± 58.3 sPECAM-1: 17.5 ± 0.7	NA
Giannaki et al. (30), 2000	sE-selectin	Uninfected newborns	At term	1-5 days	sE-selectin decreases between day 1 and 5	ELISA	139 ± 48	NA
Giannaki et al. (31), 1999	sICAM-1, sVCAM-1	Uninfected newborns	At term	1-5 days	sICAM-1 and sVCAM-1 increase between day 1 and 5	ELISA	sICAM: 179 ± 56.1 sVCAM-1: 1125.0 ± 281.0	NA
Phocas et al. (32), 1998	sICAM-1	Uninfected newborns	35-42	1-30 days	sICAM-1 increases between day 1, 5 and 30	ELISA	137.3 ± 62.0	NA
Berner et al. (33), 1998	sICAM-1	EOS	26-42	0-96 hours	sICAM-1 lower in EOS. sICAM-1 increases over time	ELISA	421 (291-459)	446 (171-534)
Austgulen et al. (34), 1997	sICAM-1, sVCAM-1, sE-selectin	EOS and LOS, pneumonia	24-42	0-162 hours	sE-selectin and sICAM-1 elevated in infected neonates	ELISA	sE-selectin: 84.2 (21.6-231.3) sICAM-1: 2131.3 (1449.5-3500.0) sVCAM-1: 237.0 (122.0-500.0)	NS

CAM = endothelial cell adhesion molecule; sVCAM-1 = soluble Vascular Cell Adhesion Molecule-1; sICAM-1 = soluble Intercellular Adhesion Molecule-1; NE = neutrophil elastase NA = Not available; NS = Not specified; EOS = Early Onset Sepsis; LOS = Late Onset Sepsis; BCPS = Blood Culture Positive Sepsis.

^a Levels are in mean ± SD, mean ± SEM, median (interquartile range), or median (range).