Plasma sE-selectin in Premature Infants: A Possible Surrogate Marker of Retinopathy of Prematurity

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PURPOSE. To prospectively study plasma levels of soluble E-selectin (sE-selectin) in premature infants and to identify their relationship to retinopathy of prematurity (ROP) on the background of known clinical risk factors.

METHODS. Eighty-five sE-selectin plasma samples from 42 preterm infants born at 23 to 32 weeks of gestational age (GA) were analyzed. Twenty-two of the infants did not have ROP, eight had stage I, seven stage II, and five stage III. Infants having no ROP or stage I were designated as the no-ROP group, and infants with stage II or III formed the ROP group.

RESULTS. In ROP infants, sE-selectin levels were significantly increased, with a median plasma level of 74.7 ng/mL (range, 28.5–222.0) compared with that in the no-ROP infants, with a median sE-selectin plasma level of 39.7 ng/mL (range, 11.9–130.0, P = 0.005). Children with ROP were born with lower birth weight and at lower GA. They were ventilated and needed surfactant therapy more often. However, multivariate analysis identified only sE-selectin level and GA as independent predictors. An increase of 10 ng/mL in sE-selectin increased the risk of ROP 1.6-fold. Receiver operating characteristic curve analysis confirmed the clinical usefulness of sE-selectin plasma levels in the prediction of ROP.

CONCLUSIONS. Elevated sE-selectin plasma levels are associated with the development of ROP and are an independent risk predictor in addition to other known risk factors. A score based on the infant’s GA and sE-selectin plasma concentrations would improve ROP prediction. Plasma concentrations in premature infants should be assessed 2 to 3 weeks after birth. (Invest Ophthalmol Vis Sci. 2010;51:3709–3713) DOI:10.1167/iovs.09-4723

Retinopathy of prematurity (ROP) is a significant cause of blindness worldwide. In the United States it is the second most frequent cause of childhood blindness after cortical visual impairment.1 ROP is caused by delayed and abnormal retinal vascular growth after premature birth. Hypoxia-induced synthesis and secretion of angiogenic factors lead to abnormal neovascularization at the boundary of the vascularized retina, which may cause retinal detachment. Currently, the main treatment option is the inhibition of neovascularization by laser ablation of the hypoxic peripheral retina. However, it is only partially effective in preventing visual impairment.2

Vascular endothelial growth factor (VEGF) and its receptors have been shown to play a key role in the pathogenesis of ROP.3–7 Only recently, members of the selectin family have also been implicated in angiogenesis—one of them being E-selectin, an inducible endothelial leukocyte adhesion molecule expressed on the surface of endothelial cells. Beyond its role in inflammation, where it mediates the migration of leukocytes into the tissue after activation by inflammatory cytokines,8 E-selectin has been found to be directly involved in angiogenesis and capillary morphogenesis.9–11 The soluble form of E-selectin (sE-selectin), which is detectable in plasma correlates with its cellular expression.12

Very little is known about serum and plasma concentrations of sE-selectin in infants. In newborn infants, levels have been shown to be highly elevated in comparison to that in adults.13 A significant decrease in plasma sE-selectin concentrations occurs between the second and fifth postnatal days, reflecting its transient expression, which peaks and begins to decline before the appearance of CD35+ cells.15,14 Term-born infants have higher levels than do premature newborns.14,15 The time at which adult plasma levels are reached has not been investigated.

Elevated levels of sE-selectin have been found in vasoproliferative disorders such as rheumatoid arthritis and in tumor growth.11,16,17 Also, ocular vasoproliferative processes such as proliferative diabetic retinopathy have been associated with elevated sE-selectin levels.18,19 A possible relation between sE-selectin and ROP has not been studied so far. In this prospective study, we therefore investigated the relationship of plasma levels of sE-selectin in premature infants at risk of ROP. We used multivariate analysis to determine the predictive value of sE-selectin for ROP development, taking into account other clinical factors such as gestational age, birth weight, days of intubation, and the presence of bronchopulmonary dysplasia (BPD).

METHODS

In this study, sE-selectin was measured prospectively in 50 preterm infants at high risk of ROP (gestational age [GA] between 23 and 32 weeks) admitted to the neonatal intensive care unit of the University of Freiburg. One child died before eye examination, two were discharged without a complete ophthalmic screening, and five had no measurements because of hemolytic samples; thus, 42 infants were analyzed. ROP staging was performed by two experienced pediatric ophthalmologists (CP, FB) by dilated indirect ophthalmoscopy. Ophthalmic examinations started 5 weeks after birth and were repeated after 2 to 14 days, depending on the stage of ROP, until retinal maturation was completed. Infants with no or with stage I ROP are referred to as the no-ROP group, and infants who had stage II (independent of the presence of plus disease) or stage III ROP formed the ROP group. To
avoid excessive blood loss in this vulnerable cohort, we used only residues of blood samples withdrawn for other clinical purposes in the analyses. Scheduled blood withdrawals for study purposes were not authorized by the local ethics committee. Sample collection started as early as 2 days after birth and continued up to 15 weeks, depending on length of hospitalization. A total of 85 plasma samples were collected. One to five samples were available per infant: 20 infants had one, 10 had two, 6 had three, 3 had four, and 3 had five. A minimal volume of 50 μL of plasma was necessary for repeated ELISAs. Samples were gathered in serum tubes (Vacutte; Greiner Bio-One, Kremsmuenster, Austria) containing EDTA and were centrifuged at 23,000×g at room temperature for 10 minutes. The clear supernatant was immediately separated and frozen at −20°C until analyzed. A sandwich enzyme immunoassay was performed with factor-specific monoclonal mouse antibodies (R&D Systems, Wiesbaden-Nordenstadt, Germany). sE-selectin levels were determined in 100 μL 1:20 diluted plasma per data point. The minimum detectable concentration of sE-selectin was 2.12 ng/mL. Information regarding clinical parameters that have been associated with ROP, such as GA, birth weight, oxygen supplementation, respiratory distress syndrome,20 BPD, sepsis, and cerebral hemorrhage,21,22 and other parameters was obtained from the medical records of all the children and analyzed. Our study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Albert-Ludwigs-University Freiburg, Germany. Written informed consent was obtained from the parents or guardians of all the children.

To examine the dependence of sE-selectin plasma levels on GA kernel, we applied smoothing to fit a nonlinear curve to all sE-selectin measurements obtained by using a Gaussian kernel with a bandwidth (SD) of approximately 2.1 days (2σ² = 9).23 For statistical evaluation of differences between infants with and those without ROP, only the first plasma sample collected in each child was used.

For group comparisons, we evaluated median plasma concentrations for each group by univariate analysis and tested for significance with the Mann-Whitney test for nonparametric data. The following clinical data were assessed in a univariate analysis with respect to the ROP groups: GA, birth weight, arterial umbilical cord pH, days of mechanical ventilation, need for surfactant therapy, days of FiO₂ > 0.4 per month, occurrence of BPD (by definition of Bancalari et al.24), vasopressor treatment, sepsis, defined as clinical signs and interleukin-6 > 100 pg/mL or CRP > 20 mg/L, mode of ductus botalli closure, necrotizing enterocolitis (NEC, if surgery was performed), and intraventricular hemorrhage (any grade by Papile’s definition). Differences were considered significant when P < 0.05. Univariate analysis was performed (SPSS ver. 15.0; SPSS, Chicago, IL), and multiple regression analysis and the receiver operating characteristic (ROC) curve were calculated (PROC logistic procedure, SAS; SAS Institute, Cary, NC). The models were tested in forward, backward, and stepwise selections. Correlation coefficients were calculated by Spearman rank order correlation.

### RESULTS

#### Patient Characteristics

Of the 42 children 30 did not (n = 22) have ROP or had only stage I (n = 8). They were born at a mean (±SD; range) GA of 28.8 weeks (±1.7; 25.6–32.6 weeks) and had a mean birth weight of 1129 g (±322; 490-1990) g. The 12 infants who had moderate to severe ROP (stages II and III) were born at a mean GA of 25.5 weeks (±1.6; 23.1–29.3) with a mean birth weight of 739 g (±177; 450-1020) g. Seven infants had stage II ROP and five had stage III. Four of these five had threshold retinopathy and underwent laser photoocoagulation.25 Clinical characteristics are further detailed in Table 1.

Statistical analysis confirmed a significant difference between the ROP and the no-ROP groups for the following clinical parameters: GA, birth weight, time of intubation, surfactant application, cerebral hemorrhage, and BPD.

#### Longitudinal Data

To correlate sE-selectin plasma levels with ROP over the entire investigated age range, we applied kernel smoothing to all sE-selectin values obtained (data not shown). sE-selectin concentrations were constantly higher in children with ROP than in those with no ROP. Longitudinal median values for the different GAs are shown in Table 2.

Further, we performed regression analysis with only one measurement per child. The first sample collected in each

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### Table 1. Clinical Data of All Infants

<table>
<thead>
<tr>
<th></th>
<th>ROP (n = 12)</th>
<th>no-ROP (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA, wk</td>
<td>25.5</td>
<td>28.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>7.39</td>
<td>11.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH, umbilical cord</td>
<td>7.3</td>
<td>7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of intubation, d</td>
<td>22</td>
<td>4</td>
<td>0.04</td>
</tr>
<tr>
<td>Days of FiO₂ &gt;0.4</td>
<td>2.7</td>
<td>1.1</td>
<td>NS (0.69)</td>
</tr>
<tr>
<td>Vasopressor treatment, %</td>
<td>58</td>
<td>19</td>
<td>NS (0.08)</td>
</tr>
<tr>
<td>Surfactant application, %</td>
<td>83</td>
<td>38</td>
<td>0.03</td>
</tr>
<tr>
<td>Sepsis, %</td>
<td>50,</td>
<td>15</td>
<td>NS (0.09)</td>
</tr>
<tr>
<td>Ductus botalli, medical treatment, %</td>
<td>67</td>
<td>19</td>
<td>NS (0.07)</td>
</tr>
<tr>
<td>Ductus botalli, surgical treatment, %</td>
<td>25</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>NEC, surgical treatment, %</td>
<td>33</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>BPD %</td>
<td>67</td>
<td>23</td>
<td>0.045</td>
</tr>
<tr>
<td>Cerebral hemorrhages, %</td>
<td>58</td>
<td>15</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are the minimum, maximum, and mean values and percentages of affected infants.

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### Table 2. Longitudinal Data

<table>
<thead>
<tr>
<th>sE-selectin (ng/mL)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level in GA Groups (wk)</td>
<td>ROP (n = 30)</td>
</tr>
<tr>
<td>≤30</td>
<td>133.0</td>
</tr>
<tr>
<td>31–33</td>
<td>72.1</td>
</tr>
<tr>
<td>34–36</td>
<td>74.7</td>
</tr>
<tr>
<td>37–39</td>
<td>71.8</td>
</tr>
<tr>
<td>≥40</td>
<td>103.2</td>
</tr>
</tbody>
</table>

Data are the median sE-selectin plasma concentrations at different GAs in infants with and without ROP.
child was included. There was no significant correlation between sE-selectin and GA, and the slope of the two regression lines did not differ significantly in ANCOVA testing (Fig. 1).

**sE-selectin Levels and ROP**

To analyze whether higher levels of sE-selectin are associated with an increased risk of ROP, we used only the first plasma sample collected in each child. Table 3 shows that the median sE-selectin level in the ROP group was nearly two times that in the no-ROP group.

On the basis of the significant clinical differences between the ROP and no-ROP groups shown in Table 1, we searched for possible covariates that might explain the difference in sE-selectin levels between the two groups. First, as sE-selectin plasma levels have been associated with BPD, we searched for a possible correlation in our patient cohort. There was a significant correlation between the duration of ventilation and sE-selectin plasma levels (r = 0.31; P = 0.048). However, there was only a trend of higher sE-selectin levels in patients with BPD (73 ± 49 ng/mL) compared with those in children without BPD (47 ± 51 ng/mL, P = 0.07). There was also a significant correlation between the GA and sE-selectin plasma levels (r = 0.31; P = 0.047). No significant correlation was found between sE-selectin levels and the other clinical parameters assessed. Second, we used multivariate analysis to test all variables that showed a significant association with ROP in univariate analysis (Table 1), to identify those that have an independent predictive effect. In forward, backward, and stepwise analyses, only sE-selectin and GA were significant. The results are summarized in Table 4. Thus, an increase of 10 ng/mL in the sE-selectin level increased the risk of ROP 1.6-fold, whereas being born with 1 week less of GA increased the risk 5-fold. Other parameters, especially the occurrence of BPD and the duration of ventilation did not show an independent effect beyond sE-selectin and GA.

**DISCUSSION**

This study is the first in which plasma levels of sE-selectin were quantified in premature infants at risk of ROP. We found a significant increase in sE-selectin in the plasma of infants with ROP in comparison to that in premature neonates without ROP. Elevated plasma concentrations were largely independent of other clinical parameters that may influence the development of ROP. Despite a weak association of sE-selectin with GA, sE-selectin still showed a significant independent predictive value for ROP, even after GA was taken into account in a multivariate analysis.

On the basis of the data in this prospective study, it is difficult to link increased levels of sE-selectin to a specific pathologic process, such as angiogenesis or inflammation in premature children with ROP. sE-selectin-induced angiogenesis is predominantly mediated through the Src-Pi3K pathway. Src kinases are activated by a variety of growth factors and function downstream of receptor tyrosine kinases. Src kinases, once activated, may in turn activate downstream Pi3K, which has been implicated in several cellular functions, including angiogenesis. A downstream target of Pi3K is the serine-threonine kinase Akt, which was found to play an important role in angiogenesis. Ocular neovascularization has been associated with sE-selectin, which was found to be significantly increased in the vitreous of patients with proliferative diabetic retinopathy. The plasma from patients with type 2 diabetes

**TABLE 3. Median sE-selectin Plasma Concentrations**

<table>
<thead>
<tr>
<th></th>
<th>ROP (n = 12)</th>
<th>no-ROP (n = 30)</th>
<th>Mann-Whitney U Test, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sE-selectin, ng/mL</td>
<td>74.7</td>
<td>39.7</td>
<td>0.005</td>
</tr>
<tr>
<td>GA (wk) at time of</td>
<td>32.0</td>
<td>33.6</td>
<td>NS (0.3)</td>
</tr>
</tbody>
</table>

**TABLE 4. Results of Multivariate Regression Analysis for Predicting the Occurrence of ROP**

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sE-selectin (1.1–2.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>GA (1.6–17.7)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Prediction of ROP**

We used receiver operating characteristic (ROC) analysis to evaluate the clinical usefulness of sE-selectin in the prediction of ROP. The dashed line in Figure 2 is the ROC curve for the sensitivity and specificity of sE-selectin alone in predicting the occurrence of ROP at different cutoff values. Analysis of GA alone (dotted line) also provided a good prediction of ROP. However, the most reliable prediction was achieved by the creation of a score based on sE-selectin level and GA (continuous ROC curve). The score was calculated as −1(0.54 × sE-selectin [nanograms/milliliter]) − (13.2 × GA [weeks]).

Thus, when a plasma level of >43 ng/mL is used to predict the onset of ROP, sE-selectin level has a sensitivity of 83% and a specificity of 60%. Prediction becomes more reliable when data for GA and sE-selectin plasma concentration are combined. A score below 340 predicts the development of ROP, with a sensitivity of 92% and a specificity of 85%. Therefore, on the basis of the ROC analysis sE-selectin concentrations ≥43 ng/mL or a score of ≤340 should be considered to indicate a significant risk for the development of ROP.
CONCLUSION

In addition to its role in leukocyte adhesion in inflammatory processes sE-selectin is known to be involved in angiogenesis. We were able to show significantly increased sE-selectin plasma levels, independent of other clinical parameters, in infants in whom ROP developed. Therefore, sE-selectin may serve as a surrogate marker for the development of ROP. In clinical practice it may become the first laboratory parameter applicable for ROP prediction. We recommend measuring the plasma concentrations of sE-selectin in premature infants 2 to 3 weeks after birth. An established score based on the infant’s GA and sE-selectin plasma concentrations may help to predict the development of ROP more precisely.

Acknowledgments

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References


FIGURE 2. ROC curve for the prediction of ROP on the basis of plasma levels of sE-selectin (dashed line), GA (dotted line), with a score combining the two (solid line).


