Endothelial Progenitor Cells (EPC) In Neonatal- Perinatal Medicine

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Disclosure

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- I do not intend to discuss an unapproved/investigative use of a commercial product/device in my presentation.
Overview

• Introduction to Stem Cells
• Endothelial Progenitor Cells (EPC) and Vasculogenesis
• EPC in Cord blood
• EPC in Neonatal Peripheral Blood
• EPC and ROP
• Scope of collaboration/research
I keep six honest serving-men
(They taught me all I know);
Their names are What and Why and When
And How and Where and Who.
I send them over land and sea,
I send them east and west;
But after they have worked for me,
I give them all a rest. I let them rest from nine till five,
For I am busy then,
As well as breakfast, lunch, and tea,
For they are hungry men.

But different folk have different views;
I know a person small-
She keeps a million serving-men,
Who get no rest at all! She sends'em abroad on her own affairs,
From the second she opens her eyes-
One million Hows, two million Wheres,
And seven million Whys!
Early Development

Fertilized egg

Totipotent stem cells

Totipotent: Can become any cell in body or placenta

Pluripotent stem cells

Pluripotent: Can become any cell in body

Multipotent stem cells

Multipotent: Can become any cell within a specific germ layer or cell lineage

Blastocyst

Fate Decision

Implantation

Gastrulation

Primary Germ Cells
Endoderm (inner)
Mesoderm (middle)
Ectoderm (outer)

Embryonic stem cells come from inner cell mass of blastocyst
Embryonic stem cells
are those removed from the blastocyst before the fate decision from pluripotentiality to multipotentiality.

Adult stem cells
multipotential cells that persist in fully developed tissues. These cells never differentiated into the mature cell types of the tissues in which they reside.
Background

- Endothelial progenitor cells (EPC) are bone marrow derived stem cells
- Exhibit characteristic endothelial and hematopoietic surface markers
- Vasculogenesis: potential to form new blood vessels
Vascular System - Mesoderm

- Vasculogenesis: vascular progenitors
- Angiogenesis
  - Sprouting, intussusceptive; from preexisting vessels
  - Extraembryonic vasculogenesis: yolk sac, blood islands, vascular plexus
  - Intraembryonic vasculogenesis: AGM region – Aorta-Gonad-Mesonephros (AGM)
- Proangiogenic factors: VEGF, bFGF, angiopoietins
- Maturation and stabilization: TGFβ and PDGF
- Anti-angiogenic: MMP inhibitors, Endostatin
Mesodermal Origin
(Muscle, Mesenchyme, endothelium, blood cells)
Stem Cell Ontogeny

Endothelial Progenitor Cells (EPC)

- Reflection of angiogenic activity / endothelial dysfunction
- Correlation with disease state and response to therapy
- Target of therapy
EPCs Role in Cardiac Repair

- CD34+, CD133+, and VEGF2R+
- Circulate in blood stream
- Contribute to repair of vascular or myocardial injury and collateral formation

EPCs and RDS

Circulating EPCs and BPD

Early EPCs and ROP

- Machalinska A *et al* from Poland:
  - Demonstrated that the number of early EPCs in the peripheral blood was significantly greater in the preterm infants with ROP than in the preterm infants without ROP

Hypothesis

- Human umbilical cord blood of preterm newborns have higher proportion of EPC compared to term newborns

- Intrauterine environment affects the proportion of cells with vasculogenic potential
Study Design

• Inclusion Criteria:
  – All deliveries where cord blood available between 6 am and 3 pm (Monday – Friday)

• Exclusion Criteria:
  – Major congenital malformation

• Chart review for Perinatal data
Definitions

- **Intra Uterine Growth Restriction (IUGR): Hadlock Criteria**
  - Antenatal Ultrasound: Fetal Weight <10%ile

- **Pregnancy Induced Hypertension (PE): ACOG Criteria**
  - Systolic BP > 140 +/- Diastolic BP > 90 mm Hg with evidence of proteinuria
Sample Preparation

• Cord Blood was processed using Ficoll-Paque™ gradient centrifugation to obtain mononuclear cells

• RBC lysis using standard protocol

• Cells were transferred in media containing 5% bovine serum for flowcytometry
Flowcytometry

- COULTER® EPICS® XL-MCL™ Flow Cytometer EXPO-32 Software (Coulter, Krefeld, Germany)
- CD 45 - ECD (BD Pharminogen)
- CD 133 - PE (Miltenyi Biotec)
- CD 34 - PC5 (BD Pharminogen)
- VEGFR-2 (KDR) - CFS (R&D Systems)
- IgG 1 - CFS (BD Pharminogen)
Methodology

- Flowcytometry:
  - Analysis was carried out using a Beckmann Coulter EPICS flow cytometer. Forward (FSC) and side scatter (SSC) voltages and threshold were set to exclude cell debris.
Flowcytometry Sequence

Viable cell gate

CD45dim gate

CD34+/CD133+ gate

VEGFR-2 Expression (KDR/Flk-1)
Statistics

- Cells: Number and percentage to total MNC were obtained
- Data was analyzed using SPSS statistical package version 18.0
- Mann-Whitney U Test and Pearson Correlation Coefficient were used as appropriate
### Gestational Age Group Description

<table>
<thead>
<tr>
<th>Characteristic (n=46)</th>
<th>Preterm (n=19)</th>
<th>Term (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black (n=33)</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>White (n=13)</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=17)</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td><strong>Chorioamnionitis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>IUGR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=8)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><strong>PE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=14)</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td><strong>Birth Weight</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2019 ± 383</td>
<td>3014 ± 436</td>
</tr>
<tr>
<td><strong>Gestational Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>32 ± 3</td>
<td>38 ± 1</td>
</tr>
</tbody>
</table>
Results

• Difference in number or percentage of any of the EPC subtypes across gestational ages was not statistically significant
<table>
<thead>
<tr>
<th>CD 45 (Dim) Cells</th>
<th>Preterm (n= 19)</th>
<th>Term (n= 27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD 34+ 133-</strong></td>
<td>0.89 (0.12-8.75)</td>
<td>0.87 (0.87-22.00)</td>
<td>0.746</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 133+34-</strong></td>
<td>0.21 (0.01-19.2)</td>
<td>0.18 (0.01-1.07)</td>
<td>0.220</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 34+ 133+</strong></td>
<td>1.04 (0.01-31.00)</td>
<td>0.30 (0.03-20.70)</td>
<td>0.173</td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 34+ 133+ VEGFR-2+</strong></td>
<td>0.10 (0.00-0.53)</td>
<td>0.12 (0.00-0.79)</td>
<td>0.733</td>
</tr>
</tbody>
</table>
# PE Effect on EPC

<table>
<thead>
<tr>
<th>CD 45 (Dim) Cells</th>
<th>PE (n=14)</th>
<th>No PE (n=32)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD 34⁺ 133⁺</strong></td>
<td>0.01</td>
<td>1.74</td>
<td>0.005</td>
</tr>
<tr>
<td>Percentage</td>
<td>(0.00-2.6)</td>
<td>(0.00-3.1)</td>
<td></td>
</tr>
<tr>
<td>[Median(Range)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 133⁺ 34⁻</strong></td>
<td>0.43</td>
<td>0.12</td>
<td>0.002</td>
</tr>
<tr>
<td>Percentage</td>
<td>(0.06-1.38)</td>
<td>(0.01-1.92)</td>
<td></td>
</tr>
<tr>
<td>[Median(Range)]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PE and EPC

The graph shows the percentages of EPC (endothelial progenitor cells) phenotypes in PE (preeclampsia) and No PIH (preeclampsia without hypertension) groups. The x-axis represents different EPC phenotypes: CD34+ CD133+ and CD133+ CD34-. The y-axis indicates the percentages of EPC. The graph compares the PIH (red) and No PIH (blue) groups.
## IUGR and EPC

<table>
<thead>
<tr>
<th>CD 45 (Dim) Cells</th>
<th>IUGR (n=8)</th>
<th>No IUGR (n=38)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD 34⁺ 133⁺</strong></td>
<td>0.02 (0-2.46)</td>
<td>1.74 (0-31)</td>
<td>0.012</td>
</tr>
<tr>
<td>Percentage [Median(Range)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 133⁺ 34⁻</strong></td>
<td>292 (28-1328)</td>
<td>55 (2-1420)</td>
<td>0.011</td>
</tr>
<tr>
<td>Number [Median(Range)]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Subgroup Analysis

- Differences in EPC types associated with PE and IUGR were present only in term and not in preterm CB
## Subgroup Analysis

<table>
<thead>
<tr>
<th></th>
<th>Preterm CB</th>
<th></th>
<th></th>
<th>Term CB</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PE (n= 6)</td>
<td>No PE (n= 13)</td>
<td>PE (n= 8)</td>
<td>No PE (n= 19)</td>
<td></td>
</tr>
<tr>
<td><strong>CD 45 (Dim)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>CD 34^+ 133^-</strong></td>
<td></td>
<td>0.58 (0.14-5.81)</td>
<td>1.18 (0.12-8.75)</td>
<td>0.735 (0.26-22.00)</td>
<td>1.82 (0.03-13.7)</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.508</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 133^+34^-</strong></td>
<td></td>
<td>0.72 (0.29-1.38)</td>
<td>0.10 (0.03-2.07)</td>
<td>0.37 (0.06-1.00)</td>
<td>0.12 (0.03-0.43)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.630</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD 34^+ 133^+</strong></td>
<td></td>
<td>0.14 (0.01-31.00)</td>
<td>1.15 (0.03-20.70)</td>
<td>0.001 (0.00-29.70)</td>
<td>3.08 (0.00-26.00)</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.852</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VEGFR-2^+ CD 34^+ 133^+</strong></td>
<td></td>
<td>0.05 (0.03-0.53)</td>
<td>0.1 (0.00-0.46)</td>
<td>0.095 (0.00-0.54)</td>
<td>0.16 (0.02-0.79)</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Correlation Between Cell Phenotypes

<table>
<thead>
<tr>
<th>Percentage of Cells</th>
<th>CD 34⁺</th>
<th>CD 133⁺</th>
<th>CD34⁺ CD133⁺</th>
<th>CD34⁺ CD133⁺ VEGFR-2⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD34⁺</strong> Coefficient /P value</td>
<td>xx</td>
<td>0.024</td>
<td>0.608</td>
<td>0.432</td>
</tr>
<tr>
<td><strong>CD 133⁺</strong> Coefficient /P value</td>
<td>0.024</td>
<td>0.876</td>
<td>xx</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>CD34⁺ CD133⁺</strong> Coefficient /P value</td>
<td>0.096</td>
<td>0.013</td>
<td>0.934</td>
<td>0.377</td>
</tr>
<tr>
<td><strong>CD34⁺ CD133⁺ VEGFR-2⁺</strong> Coefficient /P value</td>
<td>0.432</td>
<td>0.043</td>
<td>0.377</td>
<td>xx</td>
</tr>
</tbody>
</table>
Limitations

- Significant inter-patient variability
- Limited sample size
Conclusion

- PE and IUGR may influence the EPC milieu hence the vasculogenic potential

- Longer exposure to PE was associated with significant changes in EPC populations

- Additional studies are warranted to explore physiology of EPC
Post-natal EPC

- Preterm vs Term
Hypothesis

• Peripheral blood of preterm newborns has a higher percentage of Endothelial Progenitor Cells compared to term newborns
Objective

• To compare the percentage of different EPC subtypes in the peripheral blood of preterm and term newborns
Sample Size Calculations

- No baseline data to calculate required sample size

- Sample size calculated using mean and SD values for CD34+CD133+VEGFR+ cells in preliminary data

- For type 1 error of 0.05 and desired power of 0.85, we required 23 infants in each group
Inclusion Criteria

• All neonates born at the gestational age of 23 weeks and above admitted to Hutzel NICU, Special Care Nursery or Perinatal Floor and CHM NICU within first 24 hours of life, in whom parental consent could be obtained
Exclusion Criteria

- Neonates with major congenital malformations
- Neonates who received blood transfusions or exchange transfusions before sample collection
Methods/Procedures

• Prospective study with IRB approval and informed parental consent

• 0.5 ml of peripheral blood was collected from enrolled infants in an EDTA tube under complete aseptic precautions between 0-24 hours of life
Laboratory Experiments

- Peripheral blood specimens were centrifuged to separate plasma from cellular components

- Nucleated Cells were then separated from RBCs using ficoll gradient centrifugation

- Cells separated by ficoll column separation were then subjected to staining using fluorescent antibodies against CD45, CD34, CD133 and VEGFR2

- Percentage of different EPC subtypes were determined using flowcytometric analysis
Flowcytometry of Mononuclear Cells

Viable cell gate

CD45dim gate

CD34+/CD133+ gate

SS Log

CD45-ECD

CD34-PC5

VEGFR-2 Expression (KDR/Flk-1)

VEGFR2-CFS
Statistical Analysis

- All analyses performed using SPSS for Windows, Version 18.0 (SPSS Inc., Chicago, Illinois)

- Patient groups compared using:
  - chi-square test for categorical variables
  - t-test for normally distributed continuous variables
  - non-parametric test (Mann-Whitney U) for non-normally distributed EPC subtype percentages
Results

- 82 mothers were approached.
- 56 (68%) consented to participate
- 4 samples discarded due to clot formation during transport to lab
- 52 infants (23 preterm and 29 term) included in the final analysis
## Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Preterm (n=23)</th>
<th>Term (n=29)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GA (weeks)</strong></td>
<td>31 ± 4</td>
<td>39 ± 1</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td><strong>B. Wt. (gm)</strong></td>
<td>1515 ± 728</td>
<td>3306 ± 488</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td>15 (65%)</td>
<td>19 (66%)</td>
<td>0.605</td>
</tr>
<tr>
<td><strong>AA Race</strong></td>
<td>22 (96%)</td>
<td>21 (72%)</td>
<td>0.248</td>
</tr>
<tr>
<td><strong>C-Section</strong></td>
<td>16 (70%)</td>
<td>18 (62%)</td>
<td>0.395</td>
</tr>
<tr>
<td><strong>Hist. Chorio</strong></td>
<td>11 (48%)</td>
<td>15 (52%)</td>
<td>0.500</td>
</tr>
<tr>
<td><strong>SGA</strong></td>
<td>5 (22%)</td>
<td>2 (7%)</td>
<td>0.126</td>
</tr>
<tr>
<td><strong>PIH</strong></td>
<td>10 (44%)</td>
<td>13 (45%)</td>
<td>0.573</td>
</tr>
<tr>
<td><strong>Preeclampsia</strong></td>
<td>4 (17%)</td>
<td>2 (7%)</td>
<td>0.229</td>
</tr>
<tr>
<td><strong>Prolonged ROM</strong></td>
<td>5 (22%)</td>
<td>6 (21%)</td>
<td>0.595</td>
</tr>
<tr>
<td><strong>GBS +ve</strong></td>
<td>9 (39%)</td>
<td>6 (21%)</td>
<td>0.256</td>
</tr>
<tr>
<td><strong>Prophylaxis for GBS</strong></td>
<td>6 (26%)</td>
<td>4 (14%)</td>
<td>0.222</td>
</tr>
</tbody>
</table>
## Comparison of Different EPC Subtypes

<table>
<thead>
<tr>
<th>EPC Subtype (%) (CD45 dim cells)</th>
<th>Preterm (n=23) [Median (IQR)]</th>
<th>Term (n=29) [Median (IQR)]</th>
<th>P Value (NP test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+</td>
<td>0.50 (0.34-1.04)</td>
<td>0.39 (0.19-0.73)</td>
<td>0.163</td>
</tr>
<tr>
<td>CD133+</td>
<td>0.32 (0.18-0.63)</td>
<td>0.29 (0.15-0.58)</td>
<td>0.780</td>
</tr>
<tr>
<td>CD34+CD133+</td>
<td>0.30 (0.17-0.61)</td>
<td>0.25 (0.12-0.54)</td>
<td>0.402</td>
</tr>
<tr>
<td>CD34+CD133+VEGFR2+</td>
<td>0.025 (0.0099-0.053)</td>
<td>0.0079 (0.0020-0.024)</td>
<td>0.012*</td>
</tr>
<tr>
<td>CD34+VEGFR2+</td>
<td>0.021 (0.0083-0.046)</td>
<td>0.0062 (0.0016-0.023)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>
Conclusions

- Circulating VEGFR2 expressing EPC subtypes are significantly higher in peripheral blood of preterm newborns as compared to term newborns
Discussion

- It is hard to differentiate whether the higher percentage of EPCs in preterm blood relates to normal growth of immature vasculature or abnormal vascular pathology associated with various prematurity complication such as ROP etc.

- Further prospective studies with large cohort size are required to delineate this association.
EPC and RoP
Hypothesis

- Peripheral blood of preterm newborns have higher proportion of EPC compared to term newborns

- Infants with Retinopathy of Prematurity (RoP) will have higher proportion of cells with vasculogenic potential
Study Design

• Inclusion Criteria:
  – Prospective study with IRB approval and informed consent from parents after delivery
  – 23.0/7 weeks to 32.6/7 weeks GA
  – 0.5 ml of PB collected from enrolled infants in an EDTA tube under aseptic precautions between 36.0/7 and 40.0/7 PMA

• Exclusion Criteria:
  – Major congenital malformation
Sample Size Calculations

- No baseline data to calculate required sample size
- Sample size calculated using mean and SD values for CD45dCD34+CD133+VEGFR+ cells in preterm and term infants from preliminary data
- For type 1 error of 0.05 and desired power of 0.85, we required 23 infants in each group
Sample Preparation

- **Lymphocyte Separation:**
  - Ficoll-Paque™ gradient centrifugation to obtain mononuclear cells
  - The tube is centrifuged @ 2000 rpm for 10 min
  - As a result we get 3 layers, the middle layer which is white is separated into a separate tube.

- **RBC Lysis:**
  - This tube is again centrifuged @ 2000 rpm for 7 min
  - A small pellet appears at the bottom of tube with a clear supernatant
  - Lysis done using standard protocol
  - The supernatant is discarded and the pellet is mixed thoroughly with 1 ml of 1x 5% bovine serum

- This is used for FCM
Flowcytometry (FCM)

- COULTER® EPICS® XL-MCL™ Flow Cytometer EXPO-32 Software (Coulter, Krefeld, Germany)
- CD 45 - ECD (BD Pharminogen)
- CD 133 - PE (Miltenyi Biotec)
- CD 34 - PC5 (BD Pharminogen)
- VEGFR-2 (KDR) - CFS (R&D Systems)
- IgG 1 - CFS (BD Pharminogen)
Flowcytometry Sequence

Viable cell gate → CD45dim gate → CD34+/CD133+ gate

VEGFR-2 Expression (KDR/Flk-1)

VEGFR2-CFS
Statistics

- Cells: Number and percentage to total MNC were obtained
- Data was analyzed using SPSS package version 18.0
- Mann-Whitney U Test and Pearson Correlation Coefficient were used as appropriate
### Perinatal and Postnatal Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Laser n=9</th>
<th>No Laser n=21</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt wt (g)</td>
<td>618±142</td>
<td>870±200</td>
<td>0.14</td>
</tr>
<tr>
<td>Current wt (g)</td>
<td>2087±667</td>
<td>2247±550</td>
<td>0.5</td>
</tr>
<tr>
<td>GA (wks)</td>
<td>24.8±1.3</td>
<td>26±1.75</td>
<td>0.41</td>
</tr>
<tr>
<td>PMA (wks)</td>
<td>37.44±1.6</td>
<td>36.8±1.75</td>
<td>0.7</td>
</tr>
<tr>
<td>Ventilator need (wks)</td>
<td>10.2±3</td>
<td>6.9±4.7</td>
<td>0.67</td>
</tr>
<tr>
<td>Supplemental oxygen (wks)</td>
<td>12.8±3</td>
<td>9.95±3.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Transfusions</td>
<td>7.22±5</td>
<td>4.65±5</td>
<td>0.87</td>
</tr>
</tbody>
</table>
### Results

<table>
<thead>
<tr>
<th>CD 45 Dim cells</th>
<th>Laser (n=9) Mean±SD</th>
<th>No Laser (n=21) Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+CD133- (%)</td>
<td>0.43±0.34</td>
<td>0.26±0.18</td>
<td>0.195</td>
</tr>
<tr>
<td>CD133+CD34- (%)</td>
<td>0.68±0.68</td>
<td>0.3±0.3</td>
<td>0.154</td>
</tr>
<tr>
<td>CD133+ (%)</td>
<td>0.02±0.02</td>
<td>0.031±0.04</td>
<td>0.45</td>
</tr>
<tr>
<td>CD34+133+VEGF (%)</td>
<td>0.14±0.16</td>
<td>0.11±0.15</td>
<td>0.57</td>
</tr>
</tbody>
</table>
Subgroup Analysis

- Percentages of CD34+CD133+VEGFR2+ cells were significantly higher (p=0.05) in newborns born at ≥ 26 weeks gestation (0.17 ± 0.18) (n=15) as compared to newborns born at <26 weeks gestation (0.06 ± 0.09)
Results

• EPC population difference in RoP needing laser therapy did not reach statistical significance
Limitations

- Significant inter-patient variability
- Limited sample size
- Single time point evaluation
Conclusion

• Vasculogenic potential in premature newborns may be higher

• EPC milieu with respect to severity of RoP and timing of analysis will need to be further reviewed

• Additional studies are warranted to explore physiology of EPC
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- Steven Buck MS
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- Ingrid and Ashok Sarnaik Endowment (WSU/DMC)
Future? Collaboration

- Hurley Medical Center
- Michigan State University
- Wayne State University

- Maternal Blood
- Umbilical Cord Blood
- Post-natal Blood at Birth
- Blood at 32 week PMA
- Blood at 36 week PMA

- Flowcytometry
- Biochemical Markers
- Cell Cultures

- PE
- IUGR
- BPD
- RoP
• Thank You