CELL POPULATION DATA IN BACTERIAL INFECTION AND SEPSIS

Latin American Hematology Webinar Series
WHAT ARE BACTERIA?

What are the main sub-types and how they can do us harm.
(d) Staphylococci

(a) Streptococci
So, what happens when these guys enter the body?

- Stepwise and controlled immune response.
- Step 1: Macrophages phagocyte bacteria.
- Step 2: Macrophages RELEASE CYTOKINES.
- Step 3: Cytokines activate circulating WBC (neutrophils).
- Step 4: Cytokines stimulate bone marrow.
- Step 5: Released granulocytes must stay in the circulation and reach the site of infection.
- Step 6: Bone Marrow releases more granulocytes in blood, some of which are immature (left shift).
Steps 1 and 2 – Macrophage phagocytosis and cytokine release

Different cytokines promote various immune responses:

- B-cell stimulus: leads to Ab production.
- T-cell stimulus: leads to activation of cytotoxic lymphs who kills cells with phagocytosed bacteria.
- Neutrophil stimulus: toxic granules contain toxic enzymes that kill bacteria opsonized by Ab.
- Bone marrow stimulus: more granulocytes released in circulation.
Extracellular bacteria
Fungi
Autoimmunity

IL-21
IL-17a
IL-17f
IL-22
(IL-10)

TH-17
RORγt/Stat3

TGFβ (IL-1)+
IL-6,21,23

IFNγ+IL-12

TH-1
T-bet/Stat4

IFNγ
IL-2
LTα
(IL-10)

GATA-3/Stat5

IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10

Foxp3/Stat5

TGFβ
IL-35
IL-10

iTreg

Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses

Extracellular parasites
Allergy and asthma

For Research Use Only
Not for use in diagnostic procedures
Step 3

Step 4

- trabecular bone
- granulocytes
- megakaryocyte
- erythroid island
Step 5: Leucocytosis

Step 6: Left Shift

Neutrophil ("band")
Blood smear
Step 5: Leucocytosis  Step 6: Left Shift

THIS IS WHAT WE USE TODAY TO DIAGNOSE INFECTION. THE LAST STEPS IN THE ENTIRE PROCESS !!!!!!!!!!
And the problems begin...

- Hypoplastic bone marrow:
  - elderly, chemotherapy, myelodysplasia
  - neonates (hypercellular but **immature** bone marrow).
Other possible interfering factors:

- Active neutrophil pooling in lungs or infection site.
  - increased total neutrophils in body.
  - but most concentrated in one area.
  - end result: normal circulating neutrophils (nl CBC-diff).

- Significant individual variation in baseline values for WBC

- Time-lag between infection and marrow response.
Normal Granulocytic Maturation

• Typically occurs in the Bone Marrow, and more mature forms are released into the circulation.

• As cells mature, N/C ratio decreases, nuclei get convoluted and lobated, chromatin gets more condensed, cytoplasm becomes more granular.
Normal Granulocytic Maturation

- Maturation is a continuum, not a step by step approach.

- Intermediate forms can and do occur, and classifying them is often difficult.

- If only it was as easy as in the book...
Challenges in counting immature granulocytes

- Is this a band or a neutrophil? If there are 5 of these cells, and I call bands when they were neutrophils, I would change the neutrophil % from say, 69 to 64.
- What would be the clinical impact of this?
- Now imagine the same difficulty between a metamyelocyte and a myelocyte?
- The same mistake would increase the myelocyte count from 1 to 6.
- A much more significant clinical impact.
And the same happens to hematology analyzers...

There are no “gaps” in the granulocytic maturation, confirming its continuous characteristic.
So what does happen in real life?

IG as a Better Predictor of Infection

The ROC curve for the immature granulocyte count overlaps with that of the Absolute Neutrophil count (ANC).

Since the ANC is part of a regular CBC-diff made in any analyzer, why changing for a new parameter if it has exactly the same performance?
In theory it makes sense, but what about in real life?

The ROC curve for the immature granulocyte count overlaps with that of the Absolute Neutrophil count (ANC).

Since the ANC is part of a regular CBC-diff made in any analyzer, why changing for a new parameter if it has exactly the same performance?

**IG as a Better Predictor of Infection**


The ROC curve for the immature granulocyte count overlaps with that of the Absolute Neutrophil count (ANC).

Since the ANC is part of a regular CBC-diff made in any analyzer, why changing for a new parameter if it has exactly the same performance?
IG# as a better predictor for infection????????????

- The data clearly shows that the diagnostic performance of the IGs is equivalent to that of traditional CBC parameters, such as the ANC, which is available in any instrument in the market. This is because of the continuous nature of the granulocyte population making it difficult to place a cut-off appropriately.
The Hematology Laboratory in the detection of sepsis.

- Not all septic patients have immature granulocytes.
- Immature granulocytes are present in many other diseases beyond sepsis.
- In sepsis, mature neutrophils also have typical morphologic changes. If you rely only on immature granulocytes, you will miss all these critical morphologic changes.
- The ability of evaluate these morphologic changes in mature cells is even more important than counting immature granulocytes.
Cell Population Data

HYDRODYNAMIC FOCUSING

Sheath Stream → Flow Cell → Cell Stream
Cell Population Data

**Volume**

As opposed to using Ø light loss to estimate cell size, VCS utilizes the Coulter Principle of (DC) Impedance to physically measure the volume that the entire cell displaces in an isotonic diluent. This method accurately sizes all cell types regardless of their orientation in the light path.

**Conductivity**

Alternating current in the radio frequency (RF) range short circuits the bipolar lipid layer of a cell's membrane, allowing the energy to penetrate the cell. This powerful probe is used to collect information about cell size and internal structure, including chemical composition and nuclear volume.
Cell Population Data

Scatter

When a cell is struck by the coherent light of a LASER beam, the scattered light spreads out in all directions. Using a proprietary new detector, median angle light scatter (MALS) signals, are collected to obtain information about cellular granularity, nuclear lobularity and cell surface structure.

Simultaneous Measurements:
VCS is the only single channel analysis that uses 3 independent energy sources to probe approximately 8,192 cells in their near native state. Working in concert with each other, these three measurements are taken simultaneously, each providing 256 channels of resolution -- over 16,700,000 channels in all.
Cell Population Data

**Parameter Values**
- **WBC**: 5.9
- **NE %**: 69.9
- **LY %**: 18.7
- **MO %**: 10.0
- **EO %**: 1.2
- **BA %**: 0.2
- **NRBC %**: 0.0

**Mean and SD Values**
- **NE**: 159, SD: 26.68
- **LY**: 121, SD: 11.72
- **MO**: 129, SD: 5.01
- **EO**: 156, SD: 3.55

**Temperature**: 77.50°F

For Research Use Only
Not for use in diagnostic procedures
Atypical Lymphocytes

Hypersegmented Neutrophils

Myelodysplastic Syndrome

Acute Promyelocytic Leukemia
Neutrophil Morphologic Changes During Acute Infection

Cytoplasmic Vacuolization

Band Forms
Neutrophil Morphologic Changes During Acute Infection

MANY ARE IMMEDIATE CELLULAR RESPONSES AFTER CITOKINE RELEASE
STEP 3 IN THE IMMUNE RESPONSE TO INFECTION!!!!!!

Dohle Bodies
Toxic Granulation

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So we started asking some questions…

- Since the CPD are MORPHOLOGICAL parameters able to discriminate among the different types of leukocytes, will they also be able to detect morphologic changes seen within the same cell population in certain disease states?

- Using sepsis as a prototype, could the CPD also be used as additional diagnostic parameters, increasing the sensitivity, specificity, PPV and NPV of current lab tests for acute bacterial infection?

- Would the CPD be helpful in those populations for whom diagnosing infection in most important, and yet most difficult?
CELL POPULATION DATA IN BACTERIAL INFECTION AND SEPSIS

PUBLISHED LITERATURE
Comparison of Neutrophil CPD parameters in \textit{blood culture proven} acute bacterial infection and controls.

Patient group inclusion criteria:
- true infection, not contaminant bacteria
- VCS parameters drawn within 2 days of blood culture
- no underlying hematologic disorders

Control group inclusion criteria:
- unremarkable CBC
- no underlying hematologic disorders
<table>
<thead>
<tr>
<th>Patients</th>
<th>1. Controls</th>
<th>2. Group I</th>
<th>3. Group II</th>
<th>4. Group III</th>
<th>All Groups</th>
<th>1 vs 2</th>
<th>1 vs 3</th>
<th>1 vs 4</th>
<th>2 vs 3</th>
<th>2 vs 4</th>
<th>3 vs 4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n = 35</td>
<td>n = 31</td>
<td>n = 18</td>
<td>n = 20</td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td>MNV</td>
<td>143 ± 4.8</td>
<td>152 ± 13.5</td>
<td>157 ± 15.8</td>
<td>161 ± 9.1</td>
<td>0.0001</td>
<td>0.015</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.339</td>
<td>0.027</td>
<td>0.757</td>
</tr>
<tr>
<td>MNC</td>
<td>142 ± 2.6</td>
<td>141 ± 4.4</td>
<td>142 ± 3.10</td>
<td>141 ± 3.8</td>
<td>0.392</td>
<td>0.445</td>
<td>0.999</td>
<td>0.868</td>
<td>0.506</td>
<td>0.949</td>
<td>0.858</td>
</tr>
<tr>
<td>MNS</td>
<td>146 ± 7.3</td>
<td>139 ± 12.7</td>
<td>143 ± 6.90</td>
<td>139 ± 7.2</td>
<td>0.015</td>
<td>0.021</td>
<td>0.801</td>
<td>0.045</td>
<td>0.391</td>
<td>1</td>
<td>0.521</td>
</tr>
</tbody>
</table>

Group 1: WBC < 11,000/uL
Group 2: WBC >= 11,000, but <= 15,000/uL
Group 3: WBC > 15,000/uL
• Chart review of 242 adult patients, divided in 3 groups:
  - No infection – *Group 1*
  - Localized infection (UTI, decubitus ulcer) – *Group 2*
  - Generalized infection (sepsis, pneumonia, meningitis)  
    *Group 3*
  - No underlying hematological disorder.
  - Comparison of MNV, NDW, and commonly used tests in the diagnosis of infection.
• The MNV and the NDW were the ONLY PARAMETERS able to discriminate localized infection from controls.

• Other tests only showed a statistically significant difference between SEVERE, GENERALIZED infection and controls.
ROC Curve

Source of the Curve
- CRP
- BANDS
- WBC
- NEUTR%
- A-NEU
- MNV
- NDW
- Reference Line

Sensitivity vs. 1 - Specificity

Diagonal segments are produced by ties.

<table>
<thead>
<tr>
<th>Source</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0.648</td>
</tr>
<tr>
<td>BANDS</td>
<td>0.743</td>
</tr>
<tr>
<td>WBC</td>
<td>0.737</td>
</tr>
<tr>
<td>NEUTR %</td>
<td>0.672</td>
</tr>
<tr>
<td>A-NEU</td>
<td>0.746</td>
</tr>
<tr>
<td>MNV</td>
<td>0.879</td>
</tr>
<tr>
<td>NDW</td>
<td>0.844</td>
</tr>
</tbody>
</table>

Patients divided in 3 groups:
- True septic: patient had a positive blood culture
- Probably septic: negative blood culture but clinical evidence of sepsis
- Non septic: asymptomatic infant

Comparison of MNV, NDW, and commonly used tests in the diagnosis of neonatal sepsis.
## Results

<table>
<thead>
<tr>
<th>Test(s)</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0.9 (mg/dl)</td>
<td>65</td>
<td>95</td>
<td>76.5</td>
<td>93.2</td>
<td>90.1</td>
</tr>
<tr>
<td>I/T ratio</td>
<td>0.2</td>
<td>55</td>
<td>89</td>
<td>41.2</td>
<td>87.4</td>
<td>71.4</td>
</tr>
<tr>
<td>Ne #</td>
<td>1.8-7.0 (x10³/µL)</td>
<td>55</td>
<td>66</td>
<td>24.4</td>
<td>88</td>
<td>66.7</td>
</tr>
<tr>
<td>NeDW</td>
<td>27.5</td>
<td>80</td>
<td>52</td>
<td>25</td>
<td>92.8</td>
<td>75.4</td>
</tr>
<tr>
<td>MNeV</td>
<td>148</td>
<td>95</td>
<td>88</td>
<td>61.3</td>
<td>98.9</td>
<td>92.9</td>
</tr>
<tr>
<td>WBCs</td>
<td>4.8-10.8 (x10³/µL)</td>
<td>60</td>
<td>59</td>
<td>22.6</td>
<td>88</td>
<td>51.8</td>
</tr>
<tr>
<td>MNeV &amp; CRP</td>
<td>148 – 0.9</td>
<td>95</td>
<td>97</td>
<td>86.4</td>
<td>99</td>
<td>95.7</td>
</tr>
</tbody>
</table>
Automated determination of neutrophil VCS parameters in diagnosis and treatment efficacy of neonatal sepsis

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METHODS: Peripheral blood samples from 304 newborns, 206 in group I (76 proven and 130 clinical sepsis) and 98 in group II (control group), were studied on diagnosis, 3rd day, and at the end of the treatment.

Participants and Definitions
Clinical findings for a diagnosis of sepsis required at least three of the following: bradycardia (<100/min), tachycardia (>200/min), hypotension, hypotonia, seizures, apnea, tachypnea, cyanosis, respiratory distress, poor skin color and perfusion, feeding difficulty, irritability, lethargy, and laboratory results showing elevated levels of IL-6 and/or CRP (19).

Patients were grouped into a sepsis group (group I), including proven and clinical sepsis, and a control group (group II): Group Ia (proven sepsis): newborns with positive blood cultures, clinical findings in agreement with the diagnosis, and elevated IL-6 and/or CRP levels during the clinical course; Group Ib (clinical sepsis): newborns with clinical findings of infection, plus a significant increase in IL-6 and/or CRP levels during the clinical course, but with negative blood cultures; Group II (control group): newborns admitted to the hospital for perinatal risk factors such as prematurity; ablation placenta; Rh isoimmunization; conditions, such as hypoglycemia; intrauterine growth restriction; transient tachypnea; and indirect hyperbilirubinemia without clinical findings of infection were used to define control levels. Infants in the control group had normal physical examination findings and were matched as far as possible in demographic characteristics to those in the proven and clinical sepsis groups.
### Table 1. Characteristics of patients according to group

<table>
<thead>
<tr>
<th></th>
<th>Group Ia (n = 76)</th>
<th>Group Ib (n = 130)</th>
<th>Group II (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>34/32</td>
<td>67/63</td>
<td>50/48</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>30 ± 5 (22–41)</td>
<td>33 ± 4 (24–42)</td>
<td>30 ± 4 (22–40)</td>
</tr>
<tr>
<td>Gestational age ≤ 32 wk (%)</td>
<td>75</td>
<td>52</td>
<td>75</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1,423 ± 828</td>
<td>1,967 ± 1,035</td>
<td>1,571 ± 626</td>
</tr>
<tr>
<td>Sepsis work-up day (median)</td>
<td>10 (1–134)</td>
<td>5 (1–80)</td>
<td>11.5 (2–62)</td>
</tr>
<tr>
<td>Late-onset sepsis (%)</td>
<td>89.5</td>
<td>63.5</td>
<td>—</td>
</tr>
<tr>
<td>Vaginal delivery (%)</td>
<td>43.4</td>
<td>39.2</td>
<td>38.5</td>
</tr>
</tbody>
</table>

### Table 2. Neutrophil volume, conductivity, and scatter parameters and distribution width results

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 206)</th>
<th>Group Ia (n = 76)</th>
<th>Group Ib (n = 130)</th>
<th>Group II (n = 98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNV (au)</td>
<td>170.2 (18.9)</td>
<td>173.4 (22.2)</td>
<td>168.4 (16.6)</td>
<td>148.4 (11.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VDW</td>
<td>40.5 (8.5)</td>
<td>42.1 (8.2)</td>
<td>39.6 (8.3)</td>
<td>33.8 (6.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MNC (au)</td>
<td>155.9 (11.9)</td>
<td>158.1 (12.9)</td>
<td>154.6 (11.1)</td>
<td>161.5 (9.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CDW</td>
<td>15.3 (4.6)</td>
<td>15.7 (5.0)</td>
<td>15.1 (4.4)</td>
<td>13.1 (3.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MNS (au)</td>
<td>125.5 (11.8)</td>
<td>127 (14.8)</td>
<td>125 (9.5)</td>
<td>129 (10.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SDW</td>
<td>17.1 (5.0)</td>
<td>17.4 (5.8)</td>
<td>16.9 (4.5)</td>
<td>17.2 (5.5)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

au, arbitrary units; CDW, conductivity distribution width; MNC, mean neutrophil conductivity; MNS, mean neutrophil scatter; MNV, mean neutrophil volume; SDW, scatter distribution width; VDW, volume distribution width. Values represent mean (SD). P value is for difference between groups I and II.

### Table 3. Cutoff levels of MNV, VDW, MNC, CDW, and MNS

<table>
<thead>
<tr>
<th></th>
<th>MNV</th>
<th>VDW</th>
<th>MNC</th>
<th>CDW</th>
<th>MNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I vs. II</td>
<td>&gt;157.15</td>
<td>&gt;37.44</td>
<td>&lt;159.3</td>
<td>&gt;12.3</td>
<td>&lt;127.5</td>
</tr>
<tr>
<td>Group Ia vs. II</td>
<td>&gt;159.65</td>
<td>&gt;36.92</td>
<td>&lt;159.3</td>
<td>&gt;15.0</td>
<td>&lt;125.15</td>
</tr>
<tr>
<td>Group Ib vs. II</td>
<td>&gt;157.15</td>
<td>&gt;37.46</td>
<td>&lt;159.3</td>
<td>&gt;12.48</td>
<td>&lt;125.1</td>
</tr>
</tbody>
</table>

CDW, conductivity distribution width; MNC, mean neutrophil conductivity; MNS, mean neutrophil scatter; MNV, mean neutrophil volume; VDW, volume distribution width.

### Table 4. Sensitivity, specificity, PPV, NPV, and area under the ROC curve for MNV, VDW, MNC, CDW, MNS, IL-6, CRP, and combination of MNV, IL-6, and CRP at the optimal cutoff levels between groups I and II

<table>
<thead>
<tr>
<th></th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNV</td>
<td>&gt;157.15 au</td>
<td>79</td>
<td>82</td>
<td>90</td>
<td>65</td>
<td>85</td>
</tr>
<tr>
<td>VDW</td>
<td>&gt;37.44 au</td>
<td>60</td>
<td>78</td>
<td>85</td>
<td>48</td>
<td>74</td>
</tr>
<tr>
<td>MNC</td>
<td>&lt;159.3 au</td>
<td>66</td>
<td>64</td>
<td>80</td>
<td>47</td>
<td>66</td>
</tr>
<tr>
<td>CDW</td>
<td>&gt;12.3 au</td>
<td>78</td>
<td>51</td>
<td>77</td>
<td>52</td>
<td>66</td>
</tr>
<tr>
<td>MNS</td>
<td>&lt;127.5 au</td>
<td>60</td>
<td>65</td>
<td>21</td>
<td>55</td>
<td>62</td>
</tr>
<tr>
<td>IL-6</td>
<td>&gt;18.9 pg/ml</td>
<td>82</td>
<td>93</td>
<td>97</td>
<td>67</td>
<td>91</td>
</tr>
<tr>
<td>CRP</td>
<td>&gt;7.6 mg/dl</td>
<td>72</td>
<td>99</td>
<td>99</td>
<td>54</td>
<td>89</td>
</tr>
<tr>
<td>IL-6, CRP, and MNV</td>
<td>94</td>
<td>88</td>
<td>95</td>
<td>86</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

au, arbitrary units; AUC, area under the curve; CDW, conductivity distribution width; CRP, C-reactive protein; IL, interleukin; MNC, mean neutrophil conductivity; MNS, mean neutrophil scatter; MNV, mean neutrophil volume; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; VDW, volume distribution width.
Additional Observations

Patients with Gram-negative microorganisms had higher MNV (>163.25 au) and VDW (>41.75 au) levels than patients with Gram-positive microorganisms ($P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>MNV</th>
<th>VDW</th>
<th>MNC</th>
<th>CDW</th>
<th>MNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis work-up day</td>
<td>170.5</td>
<td>41.0</td>
<td>155.5</td>
<td>15.1</td>
<td>125.9</td>
</tr>
<tr>
<td>Treatment day 3</td>
<td>159.7</td>
<td>37.6</td>
<td>158.0</td>
<td>14.1</td>
<td>122.3</td>
</tr>
<tr>
<td>At the end of treatment</td>
<td>150.6</td>
<td>36.8</td>
<td>157.3</td>
<td>12.8</td>
<td>129.2</td>
</tr>
</tbody>
</table>

CDW, conductivity distribution width; MNC, mean neutrophil conductivity; MNS, mean neutrophil scatter; MNV, mean neutrophil volume; VDW, volume distribution width.
Indications that MNV may be more specific for bacterial infection and not just a marker of inflammation

• Most described sepsis markers increase in SIRS (markers of inflammation, not infection). Similar issue with traditional CBC parameters (i.e. WBC counts).
Increased mean cell volume of monocytes discriminates patients with infections and systemic inflammatory response and seems to be associated with mortality

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\textsuperscript{2}Inst. of Laboratory Med. and Microbiology, St. Joseph-Hospital, Bremerhaven, GERMANY

**Table 1: baseline characteristics, mortality, antibiotic use and classical parameters of infection / inflammation in study patients (\* value < 0.05 (T-Test) was considered statistically significant; N.s. not significant)**

<table>
<thead>
<tr>
<th></th>
<th>SIRS score ≤ 2 (n=49)</th>
<th>SIRS score &gt; 2 (n=49)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>66.7 (17.4)</td>
<td>63.4 (17.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sex (f/m)</td>
<td>25/24</td>
<td>22/27</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>6</td>
<td>16</td>
<td>n.s.</td>
</tr>
<tr>
<td>Antibiotic therapy (%)</td>
<td>92</td>
<td>100</td>
<td>n.s.</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>146 (115)</td>
<td>214 (136)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>157 (400)</td>
<td>1236 (3617)</td>
<td>0.04</td>
</tr>
<tr>
<td>WBC (\textsuperscript{*}10\textsuperscript{9}/mL)</td>
<td>12.9 (7.5)</td>
<td>16.8 (8.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>PCT (ng/mL)</td>
<td>7.01 (28.81)</td>
<td>10.27 (25.84)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neutrophil Volume (MNV)</td>
<td>148.4</td>
<td>150.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Signif. microbiology (%)</td>
<td>31</td>
<td>49</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

MNV – no change in infected patients with higher or smaller SIRS score
## Lymph-Index in Viral and Bacterial Infections

No Change in MNV in Viral Infections

<table>
<thead>
<tr>
<th></th>
<th>1. Normal Controls (n = 204)</th>
<th>2. Viral infection (n = 72)</th>
<th>3. Bacterial infection (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>6.28 ± 1.30</td>
<td>6.02 ± 1.55</td>
<td>11.73 ± 2.84</td>
</tr>
<tr>
<td>NE%</td>
<td>56.41 ± 7.18</td>
<td>53.80 ± 9.61</td>
<td>79.12 ± 5.06</td>
</tr>
<tr>
<td>NE#</td>
<td>3.57 ± 1.01</td>
<td>3.28 ± 1.17</td>
<td>8.58 ± 2.70</td>
</tr>
<tr>
<td>LY%</td>
<td>34.08 ± 6.25</td>
<td>36.72 ± 9.15</td>
<td>14.88 ± 4.65</td>
</tr>
<tr>
<td>LY#</td>
<td>2.12 ± 0.50</td>
<td>2.17 ± 0.72</td>
<td>1.51 ± 0.37</td>
</tr>
<tr>
<td>MNV</td>
<td>144.33 ± 3.22</td>
<td>145.36 ± 3.89</td>
<td>157.41 ± 5.64</td>
</tr>
<tr>
<td>NDW</td>
<td>19.14 ± 1.0</td>
<td>19.33 ± 1.37</td>
<td>23.34 ± 2.52</td>
</tr>
<tr>
<td>Lymph-Index</td>
<td>10.95 ± 0.96</td>
<td><strong>15.33 ± 1.76</strong></td>
<td>11.32 ± 1.06</td>
</tr>
</tbody>
</table>

Lymph-Index = LV x LV-SD ÷ LC

*Respiratory syncyntial virus (n=26), Adenovirus (n=21), Coxsachievirus (n=2), Epstein-Barr virus (n=15) and Hepatitis B virus (n=8)*
Cell Population Data: present and future

• PRESENT: - parameters can be used as FLAGS, so that cases that would be otherwise automatically released get a manual review – opportunity for diagnosing infection.
  - new capabilities built in software allow for automatic comments about WBC morphology (*neutrophil macrocytosis, neutrophil anisocytosis*).
  - need to educate clinicians on the meaning of these morphologic abnormalities.

• FUTURE: new chapter beginning.