Body Fluids at Copenhagen University Hospital

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Agenda

• Introduction
• BF’s analysed on SYSMEX XN
• Cytospin: Preparation and DM96
• Case stories
Rigshospitalet, Copenhagen
Accreditation:

RH: Den Danske Kvalitetsmodel (DDKM).

• Dept. of Clinical Biochemistry is accredited according to ISO 15189

• Number of analyses/year: >5 million
Amount and type of BF’s in 2013:

- **CSF** 3220
- Peritoneal Fluid (ASC) 6
- Synovial Fluid 3
- Pleural Fluid 64

**Total:** 3293

Average per week: 63

RBC - manual count: 153 (in 4 months)
BF’s can be sent to:

- Dept. of Clinical Biochemistry
  *(24 hours/day)*
- Dept. of Pathology
- Dept. of Microbiology
- Dept. of Clinical Immunology (Flowcytometry)
- Other special laboratories
A useful guide:

Body Fluid Analysis for Cellular Composition; Approved Guideline

• “…Use properly prepared cyto-centrifuge slides optimally stained with Romanowsky stains (May-Grünwald-Giemsa).”

• “…In the nucleated differential, all cells derived from the Hematopoietic system should be included. The term mononuclear cell should be avoided, since the term does not adequately distinguish monocytes from lymphocytes, a distinction that has diagnostic significance.”
From July 1 2013 we have used SYSMEX XN APP: **Body Fluid (BF) mode**
From June 2014 we have used the complete SYSMEX system: 3 Sysmex XN, 1 SP-10, 1 TS 2000 and (1 Tosoh G8).
UNITS

In BF cell-count results, we use the unit

- cells X $10^6$/L
- \(\sim\) million cells/L

for both nucleated cells (WBC) and RBC.
## Reference intervals:

<table>
<thead>
<tr>
<th>NPU</th>
<th>Name</th>
<th>Reference-interval KB3011 $10^6$/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPU 28838</td>
<td>Cerebrospinal fluid—Nucleated cells and RBC.</td>
<td>&lt; 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 5</td>
</tr>
<tr>
<td>NPU 28840</td>
<td>Pleural fluid—Nucleated cells</td>
<td>&lt; 1000</td>
</tr>
<tr>
<td>NPU 28837</td>
<td>Peritoneal fluid—Nucleated cells</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>NPU 28839</td>
<td>Synovial fluid—Nucleated cells</td>
<td>&lt; 200</td>
</tr>
</tbody>
</table>

The Body Fluid application was validated... and a report written according to SOP.

- Method comparison: Counting chamber method vs. Sysmex XN.
- For CSV: Nucleated cells (WBC) and RBC
- For other BF’s: Nucleated cells (WBC)
Lower detection limit - nucleated cells (WBC)

• Lower limit (Sysmex): 0 x 10^6/L

• Own analysis
  • 4 CSF-samples with low WBC-konc. (<5 x 10^6/L)
  • Every sample analysed 10 times
  • Average value and SD (intra-assay) calculated
  • Detection limit is set to 0 + 5 SD
  • Conclusion: SD: 0,49 x 10^6/L

• Our lower WBC- Detection limit: 3 x 10^6/L
Lower RBC-detection limit (Acc. Sysmex): 1000 x 10^6/L, which was unacceptably high for us.

If data from RBC-BF2 (Research) is used, an additional decimal is added.

10^9/L x 1000 = 10^6/L
Example: 0.5 x 1000 = 500 x 10^6/L
Lower detection limit RBC:

• Analysis of 5 samples with low count of erythrocytes (<500 x 10^6/l)
• Detection limit is set to 0 + 5SD

• Average SD: 52,7 x 10^6/L
• Lower detection limit: 264 x 10^6/L = 300 x 10^6/L

• A detection limit of 300 x 10^6/L is higher than the current limit, but it is clinically acceptable … or so we thought …
# Alarm system Sysmex XN:

<table>
<thead>
<tr>
<th>Alarms:</th>
<th>Next to result or instead of result</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>@</td>
<td>Next to result</td>
<td>Dilute – or analyze as blood.</td>
</tr>
<tr>
<td>WBC Abn Scattergram</td>
<td>IP message</td>
<td>Check number of nucleated cells by manual count</td>
</tr>
<tr>
<td>” * ” (STAR) or … (3 dots)</td>
<td>Next to result – or instead of result</td>
<td>Very, very rare – but check number of nucleated cells by manual count</td>
</tr>
</tbody>
</table>
Conclusion:

• *The method fulfils all of the established quality demands.*

• And we started using the Sysmex XN for BF’s.

• BUT…
We received an e-mail:

- It turns out that normal atraumatic spinal fluids are now given with erythrocyte numbers <300 x 10^6/L. Previously, they were given as <3 x 10^6/L with normal spinal fluids.
- This is of considerable importance to our department, as a certain number of **RBC >10 x 10^6/L equals a traumatic** puncture in our protocols. For leukaemia, a traumatic lumbar puncture at the time of diagnosis **means an increased risk of spread to CNS**, and this will often trigger extra intrathecal chemotherapy.
- At present, we thus find ourselves unable to distinguish between these situations (traumatic versus atraumatic lumbar puncture).
Change of handling – CSF RBC < 300 x 10^6/L

- With samples from certain wards (children, oncology and haematology), we count RBC manually if the concentration of RBC in CSF is < 300 x 10^6/L (counted on SYSMEX XN).

- SYSMEX is working on solving the problem.
New NPU-codes and parameter names for BF cell count and diff count?

- We do not believe that WBC and other nucleated cells can be separated from each other by automated or manual methods.
- (Tumour cells, erythroblasts, fungi, microorganisms, and more). This can only happen with manual differential count in the microscope/Cellavision DM96.
- Therefore, we decided to change the name in all BF’s so instead of WBC-concentration, from July 1 2013 we have used:

  **E.g.:** **CSF-Nucleated cells; conc.** (Unit: x 10^6/L)  
  IFCC-IUPAC-code: NPU028838

... and we will use the total count *(TC-BF#)* as result.
WBC-BF: 264 $\times 10^6$/L
TC-BF: 265 $\times 10^6$/L

We always use TC-BF#.
Cytocentrifuge-preparation (we have used this for many years)

Shandon Cytospin:
Optimal amount WBC’s per pellet:
Approximately 5000.
Optimal amount of liquid:
300-500 µl (Max. 500 µl).
We always add:
1 drop of 20 % Albumin.
Speed:
1000 (700) RPM.

(Optimized in 2008)
Manual diff count of BF’s

If the concentration of nucleated cells is

\[ > 10 \times 10^6/L \]

we will make a differential count.
The Cytospin slides are stained in Sysmex SP-10

Staining method: May-Grünwald – Giemsa
NOTE!: BLUE cassette for body fluids.

CellaVision DM96 for BF-diff’s since 2008

Loading of the preparation:

CSV-Cytospin-prep. stained in SP1000i:
Overview of pellet
BF differential count in CellaVision DM 96 - screenprint where 3 cell classes are shown.

The neural network pre-validates cells, and Lab Technician checks and moves cells if necessary - and signs.

- Neutrophils
- Monocytes/Macrophages
- Lymphocytes
Eos
Plasmacells

OBS. Are placed in "Other"
## Differential count of body fluids:

<table>
<thead>
<tr>
<th></th>
<th>Previously (Manually)</th>
<th>Since Dec. 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of cells:</strong></td>
<td>100 (3)</td>
<td>200 (5)</td>
</tr>
<tr>
<td><strong>Cell classes:</strong></td>
<td><strong>Neutrocytes</strong></td>
<td><strong>Neutrocytes</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Lymphocytes and monocytes</strong></td>
<td><strong>Lymphocytes</strong></td>
</tr>
<tr>
<td></td>
<td><strong>”Other”</strong></td>
<td><strong>Monocytes and macrofages</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Eosinophiles</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Other</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Unidentified)</td>
</tr>
</tbody>
</table>
Smudge cells?

- Are a problem.
- We ”include” them in the result if they are > 20%-
- We place them in ”Other” and give a comment.
- ”Other” we use to so many other cell-classes: Mesothelia-cells, blasts, basofiles, plasmacells ....
So: New cell type:

- DNK35265 Plv(spec.)—Smudge celler; antalk. = ? x 10^6/L
- DNK35264 Csv—Smudge celler; antalk. = ? x 10^6/L
- DNK35266 Asc—Smudge celler; antalk. = ? x 10^6/L
- DNK35267 Ledv(spec.)—Smudge celler; antalk. = ? x 10^6/L
- DNK35269 B—Smudge celler; antalk. = ? x 10^9/L

"Celler" = Danish word for cells.
Training? Blood-Diff.: 22 technologists
CSV-Diff.: 32 technologists

CellaVision Competency software/ Proficiency Software (BF-test)
Cases:
CSF Case 1: Dept. of Neurosurgery

TC-BF: 25 x 10^6/L

RBC: 1300 x 10^6/L

88 % MN
(LY: 28% and MO: 60 %)

12 % PMN
Neut: 14%
Lymf: 33.5%
Mono/Macro: 52.5%

Diagnosis:
Glioblastoma in occipital region.
Infection in shunt?
# CSF Case 2

<table>
<thead>
<tr>
<th>WBC</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>28</td>
<td>11.8</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Macrophage</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Other</td>
<td>269</td>
<td>87.8</td>
</tr>
<tr>
<td>Total</td>
<td>238</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-WBC</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smeadage cell</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>Artifact</td>
<td>27</td>
<td>-</td>
</tr>
</tbody>
</table>

12 % Lymph

88 % Other
# CSF Case 2

<table>
<thead>
<tr>
<th>WBC</th>
<th>Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Macrophage</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Other</td>
<td>288</td>
<td>87.8</td>
</tr>
<tr>
<td>Total</td>
<td>331</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-WBC</th>
<th>Count</th>
<th>%</th>
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</tr>
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<td>-</td>
</tr>
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</table>

**BF comment**

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**Image Description:**
- **Microscopic Images:** A series of microscopic images are displayed, showcasing various cellular structures. The images are arranged in a grid format, with each cell appearing to be stained with a purple hue, indicative of a microscopic examination of cells.
- **Staining and Preparation:** The cells are likely stained with hematoxylin and eosin (H&E) or a similar staining method, which is common in neurocytology to highlight cellular components.
- **Clinical Relevance:** The cells shown are likely neurons or glial cells, which are fundamental in assessing neurological conditions such as those involving the central nervous system. The presence of macrophages and other cellular elements is noted, which can provide insights into inflammation or immune responses in the central nervous system.
CSV- WBC (nucleated cells): 188
CSV-RBC: 35

A note to "Leukocyttter (uspec.) – "OTHER" :
"Dominated by immature, mononuclear cells. Mitosis is detected."
Case 2: NK-T-cell lymphoma with CNS-infiltration. (Confirmed by dept. of pathology)
CSF Case 3:

Male, 19:

• 2007: Pre B-ALL
• April 2010: Finished treatment
• August 2010: Isolated CNS
Case 3: CSF-sample with blasts. Sysmex XN-print (2012):

- TC-BF#: 265
- MN: 95%
- PMN: 5%
Case 3: Same sample with blasts (CellaVision DM96-screenshot)

- 15% Lymf
- 80% Blaster (confirmed by Dept. of Pathology)
- 5% mono/macro

CNS-recurrence and later: allogen transplantation
Case 4: Blood diff (girl, 7) - AML

WBC = 2,1 x 10⁹/L
WBC: $232 \times 10^6$/L

RBC: $<3 \times 10^6$/L
Case 4: CSF diff (girl, 7) - AML

• Our comment:
  • Other: Large, immature cells

• Saturday evening: We made 9 Cytospin-slides and sent them to Dept. of Pathology

• Their result was:
  • Cell-rich cytospin with many large cells, primarily blast cells and early myeloid precursors. Few RBC’s. CNS-leukaemia
Case 5:
Diagnosis: Brain tumour (Neuroepitelium)

CSF RBC: 931 x 10^6/l
CSF WBC: 50 x 10^6/l

Comment to "other":
Immature, "blast-like" cells.
Case 6: CSF

WBC-BF: 4783-4916
TC-BF: 5079-5264
RBC: 14900

Lots of debris – no alarm (Abn. Scattergram)
Case 6:
Case 6:

Lots of bacteria and some smudge cells.
Case 6:

- Patient from Dept. of Neurosurgery.
- 1 month previously: Surgical removal of infected bone plate.
- Dept. of Microbiology found: A few neutrophils, numerous Gram-positive rods in clusters.

**Problem: No alarm (abn. Scattergram). Sysmex is involved, and are at the moment looking into the problem in Japan.**
Case 7:
ASC: Nucleated cells: 1130 x 10^6/L
Case 7: Patient with chronic Hepatitis C (alcoholic)

Dept. of Pathology:
Blood – and mesothelia cells. No malignancy.

Other = Dominated by Mesothelia cells
From CAP

ISBN: 0930304918
THANK YOU FOR YOUR ATTENTION.
Mesothelial cells:

"The layer of flat cells of mesodermal origin that lines the embryonic body cavity and gives rise to the squamous cells of the peritoneum, pericardium, and pleura."