XN series

Case interpretation

Gebruikersdag Vlaanderen- 6 oktober 2016
Fluorescence flow cytometry
Case 1
Case 1: Initial measurement

Patient details: 69-year-old male with myocardial infarction 10 years ago; now obvious splenomegaly and palpable lymph nodes.

Conv. units:
- HGB: 11.3 g/dL
- MCH: 48.6 pg
- MCHC: 40.6 g/dL
Case 1: Reflex measurement

Patient details:
69-year-old male with myocardial infarction 10 years ago;
now obvious splenomegaly and palpable lymph nodes

Conv. units:
HGB: 11.3 g/dL
MCH: 48.6 pg
MCHC: 40.6 g/dL
Smear results

**Differential Results**

<table>
<thead>
<tr>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg neutrophils</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td>99</td>
</tr>
<tr>
<td>Monocytes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Basophils</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Bands</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Metamyelocytes</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Myelocytes</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Promyelocytes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Nucleated red blood cells</td>
</tr>
</tbody>
</table>

**Comments**

» 12 smudge cells (excl. from the differential count as per recommendation)
Case 1
B-CLL with extreme leukocytosis

presence of uniformly round to somewhat irregular CD5 and CD23 positive B-cells in the peripheral blood
In addition:

B-CLL with extreme leukocytosis

- The RBC histogram is interfered by lymphocytes and the count falsely elevated.
- Correct the RBC, MCV, HCT and MCHC

Parameter correction:

\[
\begin{align*}
\text{RBC}_{\text{corr.}} &= S-\text{RBC} = 1.88 \times 10^{12}/\text{L} \\
\text{MCV}_{\text{corr.}} &= S-\text{MCV} = 113.1 \text{ fL} \\
\text{HCT}_{\text{corr.}} &= \frac{(S-\text{RBC} \times S-\text{MCV})}{10} = \frac{(1.88 \times 113.1)}{10} = 21.3 \% \\
\text{MCHC}_{\text{corr.}} &= \frac{(\text{HGB} : \text{HCT}_{\text{corr.}})}{100} = \frac{(7.0 : 21.3)}{100} = 32.9 \text{ g/dL}
\end{align*}
\]
In addition: ALL patient with „typical“ shark vin

Patient details:
19-year-old boy diagnosed with ALL
Case 2
Case 2: Initial measurement

Patient details:
19-year old man with fever, tiredness and frequent headaches.

Conv. units:
HGB: 13.0 g/dL
MCH: 32.38 pg
MCHC: 33.0 g/dL
Case 2: Reflex WPC channel

Patient details:
19-year old man with fever, tiredness and frequent headaches.

Conv. units:
- HGB: 12.9 g/dL
- MCH: 32.38 pg
- MCHC: 31.9 g/dL
Case 2: Smear results

Differential Results

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seg neutrophils</td>
<td>60</td>
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<tr>
<td>Lymphocytes</td>
<td>31</td>
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<tr>
<td>Monocytes</td>
<td>3</td>
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<tr>
<td>Eosinophils</td>
<td>4</td>
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<tr>
<td>Basophils</td>
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<tr>
<td>Bands</td>
<td></td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>1</td>
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<tr>
<td>Myelocytes</td>
<td></td>
</tr>
<tr>
<td>Promyelocytes</td>
<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
<td></td>
</tr>
</tbody>
</table>

Nucleated red blood cells

Comments

» 60% atypical lymphocytes suspected reactive
Case 2: Infectious mononucleosis

Comments
- 60% atypical lymphocytes suspected reactive
- EB VCA G rat +++
- EB VCA GM rat +++
- EB NA IgG +++
- EB VCA IgG +++
- Alk phos 100
- GGT 110
- ASAT 334
- ALAT 458
- LD 622
- ASLO 233
- CRP 9.4
In addition:
European consensus nomenclature

Conspicuous lymphatic cells

- Atypical lymphocytes
  - Atypical cells, suspect reactive
  - Atypical cells, suspect neoplastic

XN-Series flagging

Conspicuous lymphatic cells

Analyser flagging

- ‘Atypical lympho?’
- ‘Abnormal lympho?’

Meaning

- Atypical cells, suspect reactive
- Atypical cells, suspect neoplastic
In addition:

**Cell-mediated vs humoral immune response**

- Within the adaptive immune response, the cell-mediated immune response is activated during the early stages of an infection while the humoral immune response is typically the sign of a recovering infection.

- Since different cell types are involved in these different stages, this is reflected in the WDF scattergram by two new parameters.
**In addition:**

Extended Inflammation Parameters – launch end 2016*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without license</th>
<th>With license</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE-LYMP#/%</td>
<td></td>
<td>Diagnostic</td>
</tr>
<tr>
<td>AS-LYMP#/%</td>
<td></td>
<td>Diagnostic</td>
</tr>
</tbody>
</table>

In case of a lymphocytosis:

→ RE-LYMP > 6% without AS-LYMP indicates a cellular immune response

→ AS-LYMP presence indicates a humoral immune response

* in combination with WPC channel
Cell-mediated immune response
Humoral immune response
Case 3
Case 3: Initial measurement

Patient details:
A 34-year old man who frequently travels to India presents with fever, nausea, headache and painful joints.

Conv. units:
HGB: 7.2 g/dL
MCH: 31.7 pg
MCHC: 33.7 g/dL
RET-He: 32.5 pg
Case 3
Smear results

Differential Results
73 Seg neutrophils
15 Lymphocytes
5 Monocytes
2 Eosinophils
Basophils
4 Bands
1 Metamyelocytes
Myelocytes
Promyelocytes
Myeloblasts

Nucleated red blood cells

Comments
» Schizonts and early schizont stages ++
» 3% atypical lymphocytes suspected reactive
Case 3

Malaria *Plasmodium vivax*
**In addition:**

**Malaria Plasmodium vivax**

- The SSC-FSC scattergram of the WDF channel showed two neutrophil populations and the WBC count from the WDF channel (WBC-D) was much higher than the count from the WNR channel (WBC-N).

- This is indicative of nucleic acid-containing cellular inclusions in RBC, which do not interfere with the WBC count in the WNR channel due to the strong lysis reagent.

- Since RBC are not completely lysed in the WDF channel, they are visible as an additional neutrophil population.

- ‘IG present’ was triggered due to the wrong clustering and the automated differential is incorrect.

**Unusual cell clustering**

<table>
<thead>
<tr>
<th>Item</th>
<th>Data</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4.61</td>
<td>10^3/uL</td>
</tr>
<tr>
<td>WBC-N</td>
<td>4.61 * 10^3/uL</td>
<td></td>
</tr>
<tr>
<td>WBC-D</td>
<td>12.06 * 10^3/uL</td>
<td></td>
</tr>
</tbody>
</table>

**Action**

Difference between WNR and WDF. Check the results. Suspect sample, check the sample.
**In addition**

**Malaria *Plasmodium vivax* ‘iRBC?’ flag**

- Without the activated ‘iRBC?’ flag, the reported values can be incorrect, such as false high neutrophil, IG or eosinophil counts, when schizonts, gametocytes and/or trophozoites are present and overlap with these cells.
- Even a small number of infected RBC with these larger forms may have a significant impact on the differential count, as the concentration of RBC is a thousand-fold higher than WBC and infected RBC are not completely lysed in the WDF channel.
- After flag activation:
  - ‘iRBC?’ is triggered and infected RBC are shown in purple in the WDF scattergram
  - WBC differential is corrected
Case 4
### Case 4: Patient 1

Heinz bodies ++++

Patient had a splenectomy

Lab checked HGB by HPLC

Result: HGB variant! Name: **HGB Köln**

The mutated HGB seems to interact (bind?)

Fluorocell WDF
Case 4: Patient 2

Patient details:
A blood collection of 13-year boy was initiated by GP

HPLC was done: diagnosed with Hgb Koeln

Conv. units:
HGB: 18.1 g/dL
MCH: 32.3 pg
MCHC: 34.8 g/dL

### Lab. Details

<table>
<thead>
<tr>
<th>Item</th>
<th>Data</th>
<th>Unit</th>
<th>Item</th>
<th>Data</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>5.46</td>
<td>10^10/L</td>
<td>NEUT#</td>
<td>2.87</td>
<td>10^3/μL</td>
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<tr>
<td>RBC</td>
<td>5.58</td>
<td>10^12/L</td>
<td>LYMPH#</td>
<td>1.78</td>
<td>10^3/μL</td>
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<tr>
<td>HGB</td>
<td>11.2</td>
<td>mmol/L</td>
<td>MONO#</td>
<td>0.48</td>
<td>10^3/μL</td>
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<td>HCT</td>
<td>51.8</td>
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<td>EO#</td>
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<td>10^3/μL</td>
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<td>MCV</td>
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<td>BASO#</td>
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<td>10^3/μL</td>
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<tr>
<td>MCH</td>
<td>200.7</td>
<td>fl</td>
<td>NEUT%</td>
<td>52.6</td>
<td>%</td>
</tr>
<tr>
<td>MCHC</td>
<td>21.6</td>
<td>mmol/L</td>
<td>LYMPH%</td>
<td>32.6</td>
<td>%</td>
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<tr>
<td>PLT</td>
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<td>10^9/L</td>
<td>MONO%</td>
<td>8.8</td>
<td>%</td>
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<td>RDW-SD</td>
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<td>fl</td>
<td>E0%</td>
<td>5.5</td>
<td>%</td>
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<tr>
<td>RDW-CV</td>
<td>13.7</td>
<td>%</td>
<td>BASO%</td>
<td>0.5</td>
<td>%</td>
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<tr>
<td>MPV</td>
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<td>T#</td>
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<tr>
<td>P-LCR</td>
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<td>%</td>
<td>IgG</td>
<td>0.4</td>
<td>%</td>
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<td>PCT</td>
<td>42.6</td>
<td>%</td>
<td>PLT-F</td>
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<tr>
<td>NRBC#</td>
<td>0.01</td>
<td>10^6/μL</td>
<td>RET%</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>NRBC%</td>
<td>0.2</td>
<td>%</td>
<td>N#</td>
<td></td>
<td></td>
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<tr>
<td>RET</td>
<td></td>
<td></td>
<td>MFR</td>
<td></td>
<td>%</td>
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<tr>
<td></td>
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<td>HFR</td>
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<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RET-He</td>
<td></td>
<td>pg</td>
</tr>
</tbody>
</table>

### Differential Count

- **WBC Abn Scattergram**
- **RBC Flag(s)**
- **PLT Flag(s)**
Case 4: Patient 3

Patient details:
woman * 1944
send by the GP
no further
information

Conv. units:
HGB: 14.5 g/dL
MCH: 28.4 pg
MCHC: 28.4 g/dL
Case 4: Patient 3

- 80 year old patient without anaemia or other reason to think about Hgb variant
- Reason for low SFL unknown
Similar cases described…

» Published about HGB variant Ferrara and variant Leiden
» Both also caused by genetic mutation in HGB gene
» Also strongly reduce SFL in DIFF channel

“We hypothesize that the polymethine fluorescent dye used for leukocyte differentiation binds some Hb variants with affinity greater than nucleic acids or reduces the WBC permeability to the dye. In conclusion, the presence of unstable Hb variants can interfere with the DIFF channel of Sysmex XE-2100 hematology analyzer.”
Case 5
Case 5: Initial measurement

Patient details:
73-year-old male with known CLL, diagnosed 12 years ago. Now obvious splenomegaly and palpable lymph nodes.

Conv. units:
HGB: 6.0 g/dL
MCH: 26.2 pg
MCHC: 22.4 g/dL
Case 5
Smear results

Differential Results

- Seg neutrophils
- Lymphocytes: 90
- Monocytes
- Eosinophils
- Basophils
- Bands
- Metamyelocytes
- Myelocytes
- Promyelocytes
- Blasts: 10

Nucleated red blood cells

Comments

10 smudge cells (excl. from the differential count as per recommendation)
Case 5
CLL in combination with AML/acute monoblastic leukaemia

BM immunhistology
30 % CLL population, CD20+, CD79a+, CD23+ en CD5+
2/3 of BM blast CD15+ (partly) CD4+ (weak) CD68+, CD34- and CD117-
Case 6
Case 5: Initial measurement

Patient details:
22-year-old male visited his family in Nigeria. Back home he has fever and headache.

Conv. units:
- HGB: 10.5 g/dL
- MCH: 27.7 pg
- MCHC: 31.9 g/dL
Case 4
Smear results

Differential Results

- 68 Seg neutrophils
- 24 Lymphocytes
- 5 Monocytes
- 2 Eosinophils
- 1 Basophils
- Bands
- Metamyelocytes
- Myelocytes
- Promyelocytes
- Blasts

Nucleated red blood cells

Comments
6% atypical lymphocytes suspected reactive
19% plasmodium falciparum!
Case 6
Malaria falciparum infection
In addition
Malaria falciparum infection

» The high amount of plasmodia trigger the flag “Ret abn scattergram.” The reticulocyte count is interfered by the plasmodia and is not possible to use the automated reticulocyte count.

» The reticulocyte count can be used for monitoring the therapy. Already after 12 hours therapy is the reticulocyte count (including plasmodia containing RBC) about factor 3 fallen.

» The total amount of PLT is 40 10^3/ul. The result was checked by PLT-f. 12.7% of PLT are reticulated (IPF)