



SENSITIVE ASSESSMENT OF WHITE BLOOD CELL FUNCTIONALITY BY NOVEL HAEMATOLOGICAL PARAMETERS

**Assoc. prof. d-r Milena Velizarova,
Medical University- Sofia,
Bulgaria**

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Significance of advanced WBC parameters

- Haematology disorder suspicions are often based on an initial cell blood count and differential counting
- Additional information, provided by the analyzers, improves the screening and diagnosis of many conditions and diseases
- Modern analyzers perform 5- to 10-part differential count of WBC by using different analytical methods.

A closer look at methodologies for WBC differential count

Methodologies

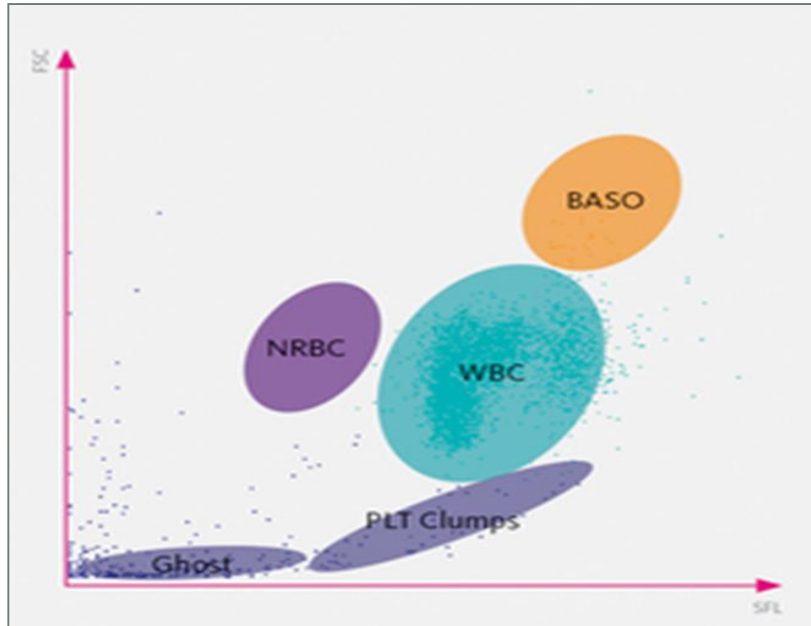
- Impedance technology
- Conductivity (radio frequency)
- Optical light scatter
- Cytochemistry
- Fluorescence detection
- Multi-channel system
- Single-channel system

Additional parameters

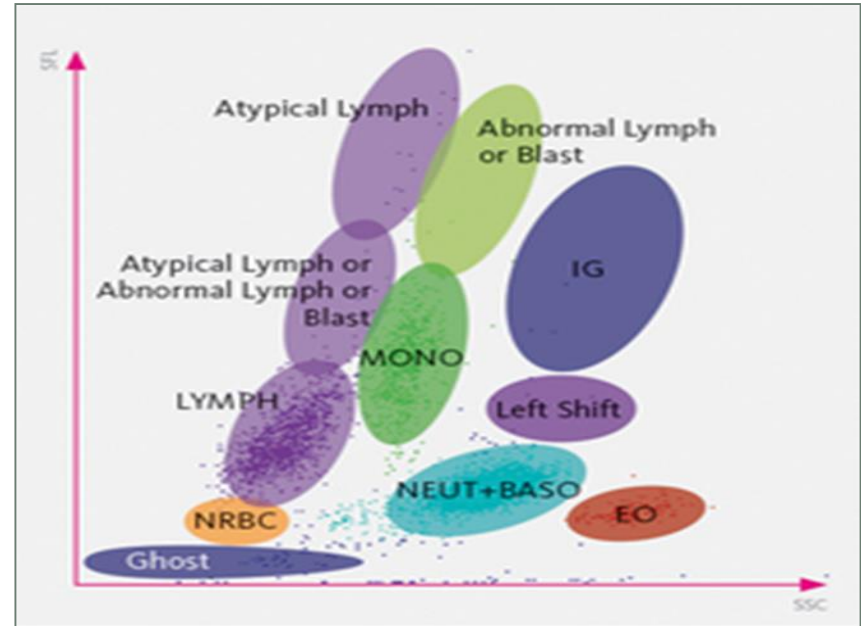
- Shape and distribution of cell populations and LYMP-index (Beckman Coulter-LH)
- Granularity index (Sysmex XE)
- Large unstained cells, LUC (Advia Systems)
- LYPM-indices, NEUT-indices, Parasites (Sysmex XN)

The multi-channel system: optical light scatter and fluorescence detection

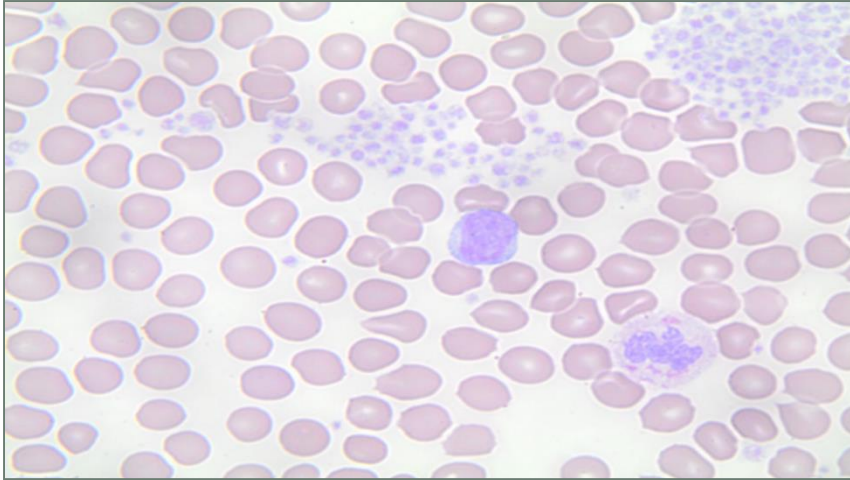
Light scatter measurement



Fluorescence detection



Reasons for manual smear reviews



- WBC flag: 37%
- Immature cell flag: 26%
- RBC flag: 13%
- PLT flag: 5.9%
- Physician request
- Scan for platelet verification
- Others

Cell functionality

- Extended haematological parameters offer added value exceeding the classical haematology analysis.
- Cell functionality assessment is based on determination of:
 1. the maturity of cells
 2. the malignancy of cells
 3. the activation state of cells

Extended inflammatory parameters

Neutrophils

- Activated neutrophils (↑NEUT-RI и NEUT-GI)
- Immature granulocytes (IG)

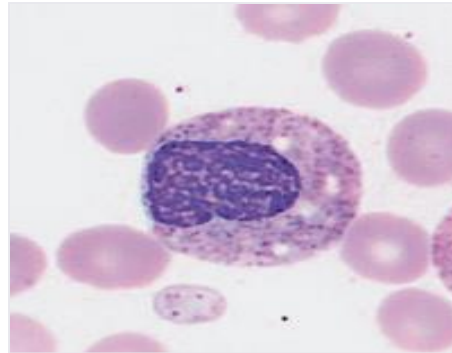
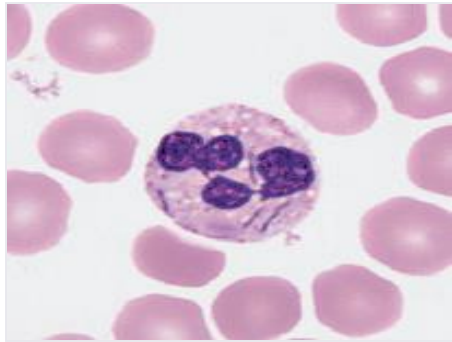
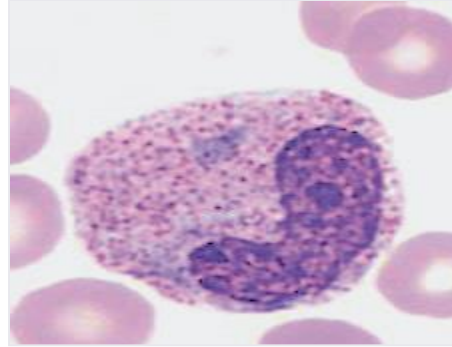
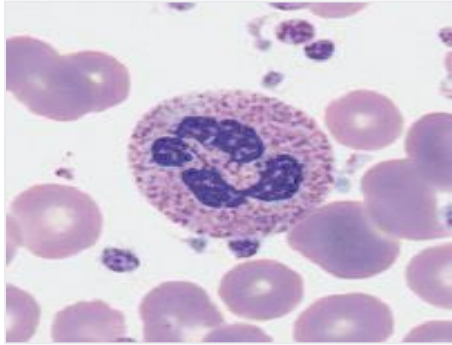
Lymphocytes

- Reactive lymphocytes (RE-LYMP)
- Antibody synthesizing lymphocytes (AS-LYMP)

Activation status of neutrophils

- Activated NEUTs have:
 - ✓ different membrane lipid composition
 - ✓ greater activity in the cytoplasm
 - ✓ morphological changes (size, shape and cytoplasm)
 - ✓ greater intensity of the fluorescence signal
- NEUTs use at least two strategies to fight pathogens:
 - ✓ Phagocytosis
 - ✓ Secretion (cytokines and antibacterial substances)

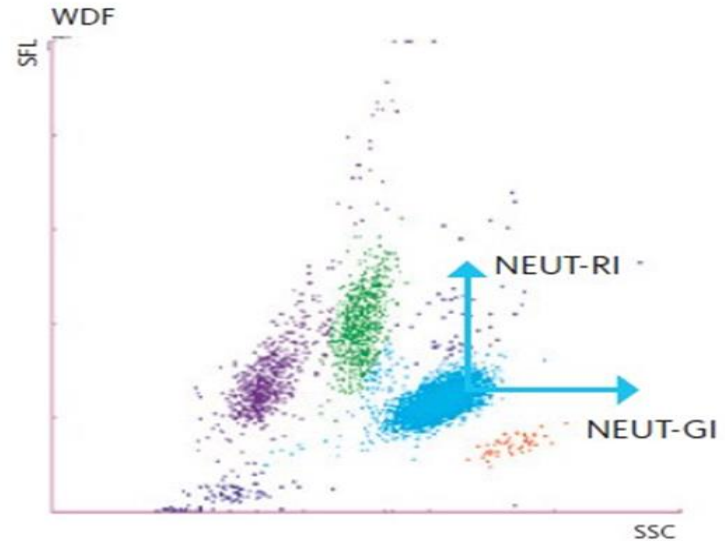
Neutrophil activation



Neutrophil activation (NEUT-RI, NEUT-GI)

- Extended inflammation parameter derived from the NEUT-X
- Measures the fluorescent intensity of neutrophil population.
- NEUT-RI is the mean value of high angle diffraction
- Represents the complexity of the neutrophils (nucleus lobularity, granulations)
- Reflects neutrophil metabolic activity (cytokines)

NEUT- Reactive Intensity (NEUT-RI)

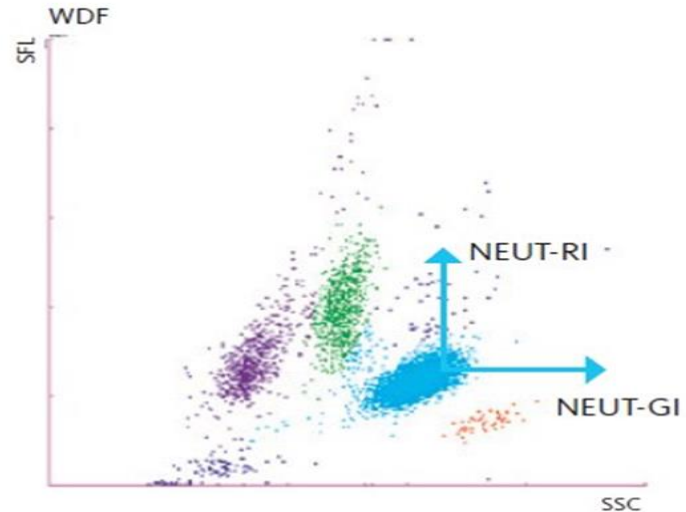


SI (scatter intensity)

Neutrophil activation (NEUT-RI, NEUT-GI)

- NEUT-GI is derived from NEUT-Y
- Provides information about cell density or complexity → represent cell granularity
- Toxic granulation and vacuolisation affect position of the neutrophil cloud in the scattergram
- Is related to dysplasia of neutrophils

NEUT- Granularity Intensity (NEUT-GI)



SI (scatter intensity)

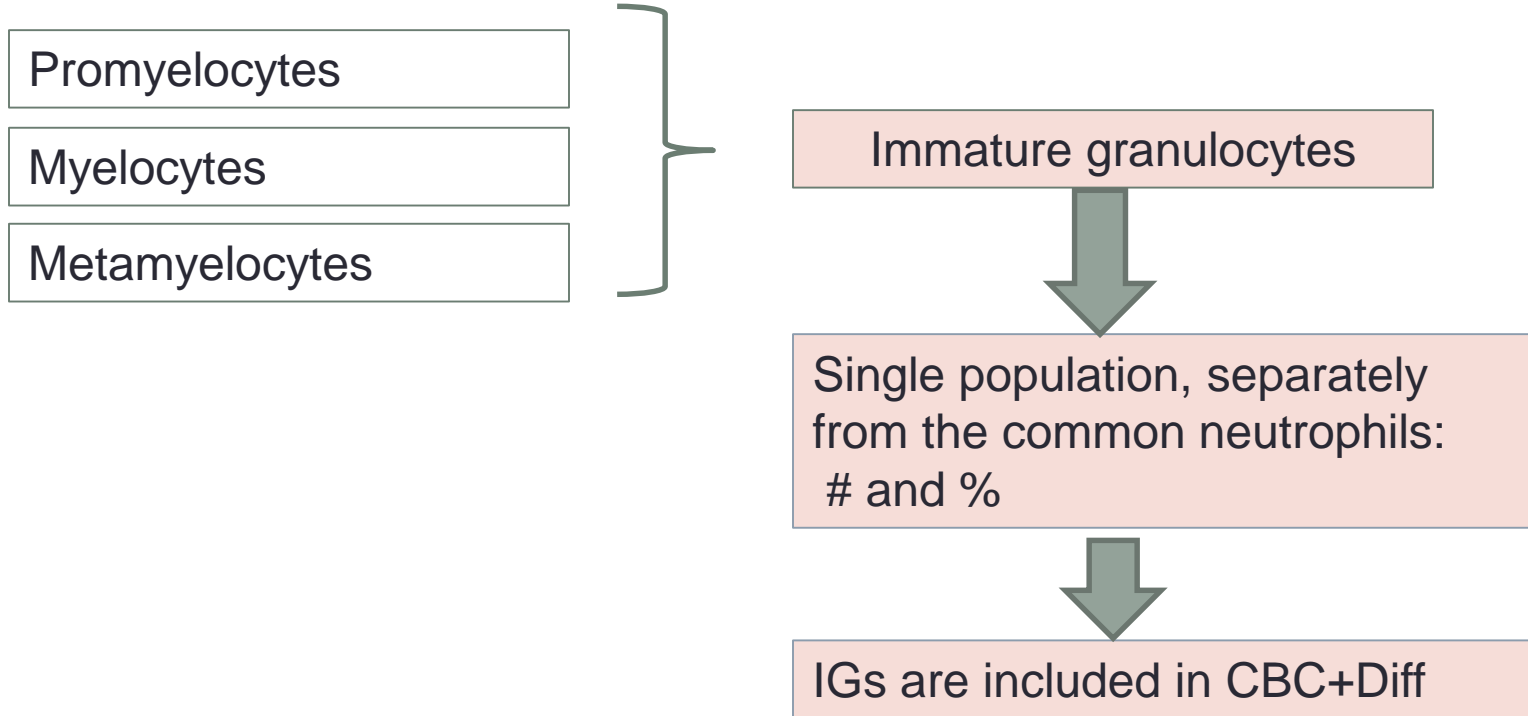
Immature Granulocytes (IG)

- Additional marker in differential count of WBC
- Found in:
 - haematological malignant diseases: myeloproliferative syndrome, myelodysplastic syndrome (MDS) or chronic myelomonocytic leukaemia
 - infectious (septic) diseases
 - noninfectious reactive (inflammatory) conditions
- A persistently increased IG (>10%) in the case of infection is considered as a grave criterium for sepsis.

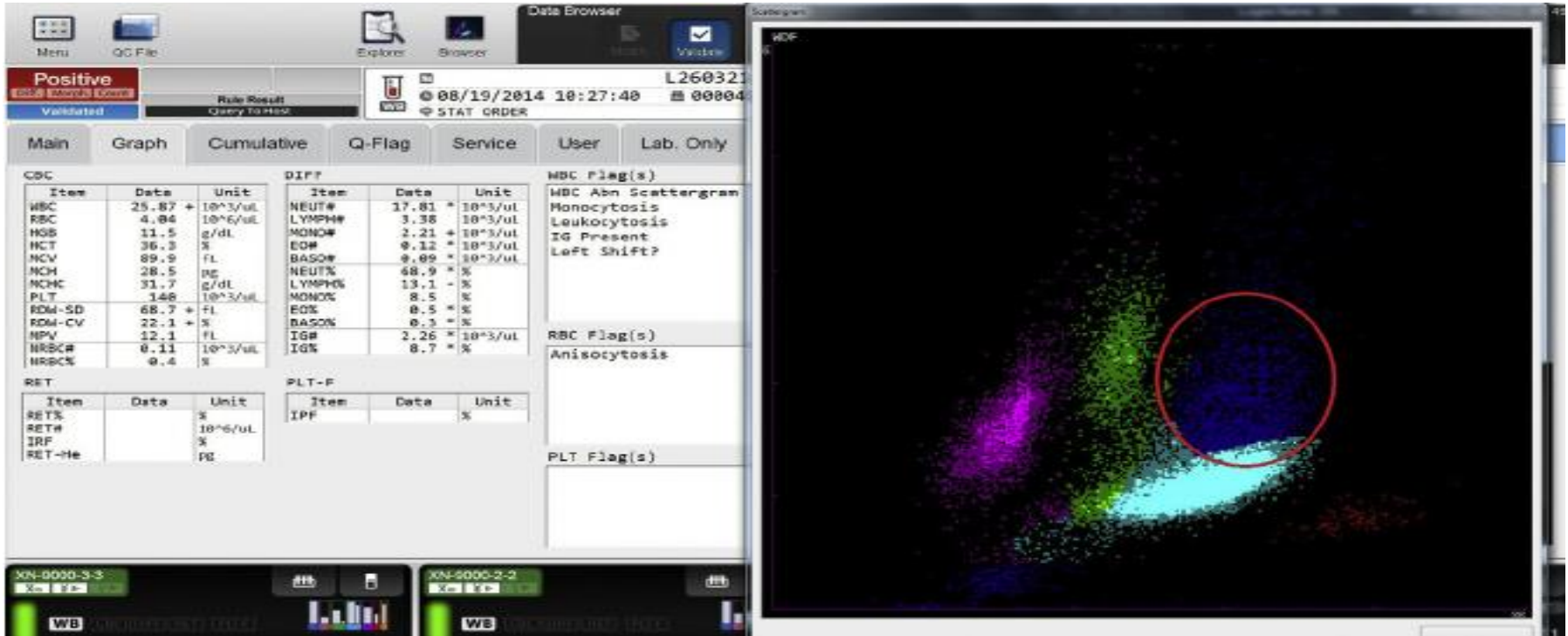
Immature Granulocytes (IG)

High specificity and low sensibility in cases with infections → we can not use them for infection disease screening; useful for treatment monitoring.

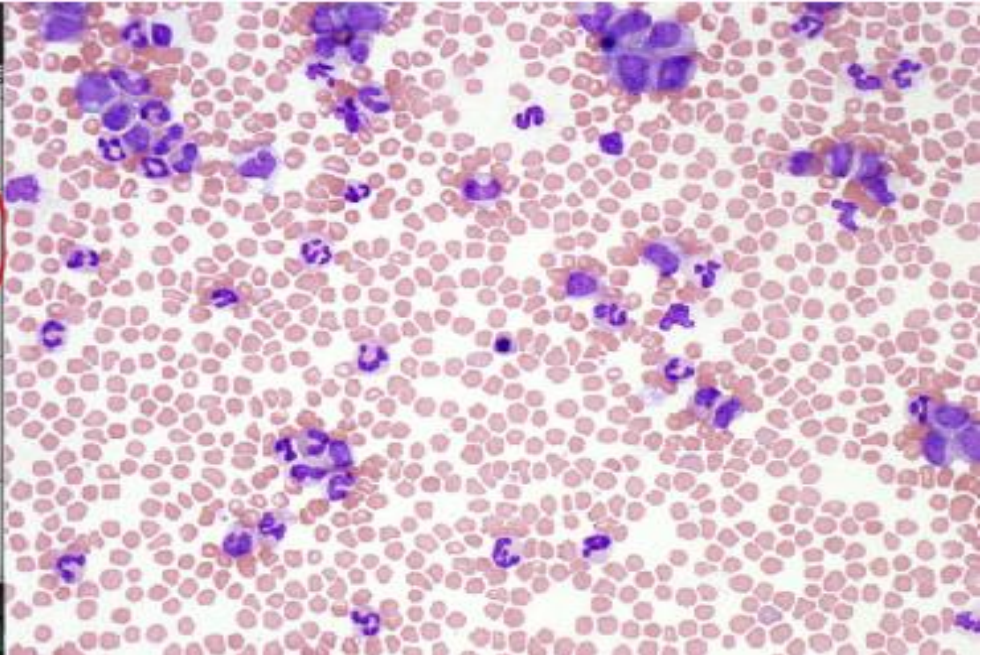
Immature Granulocytes (IG)

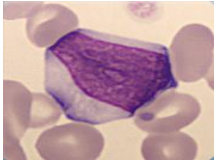


Immature Granulocytes (IG)



Immature Granulocytes (IG)

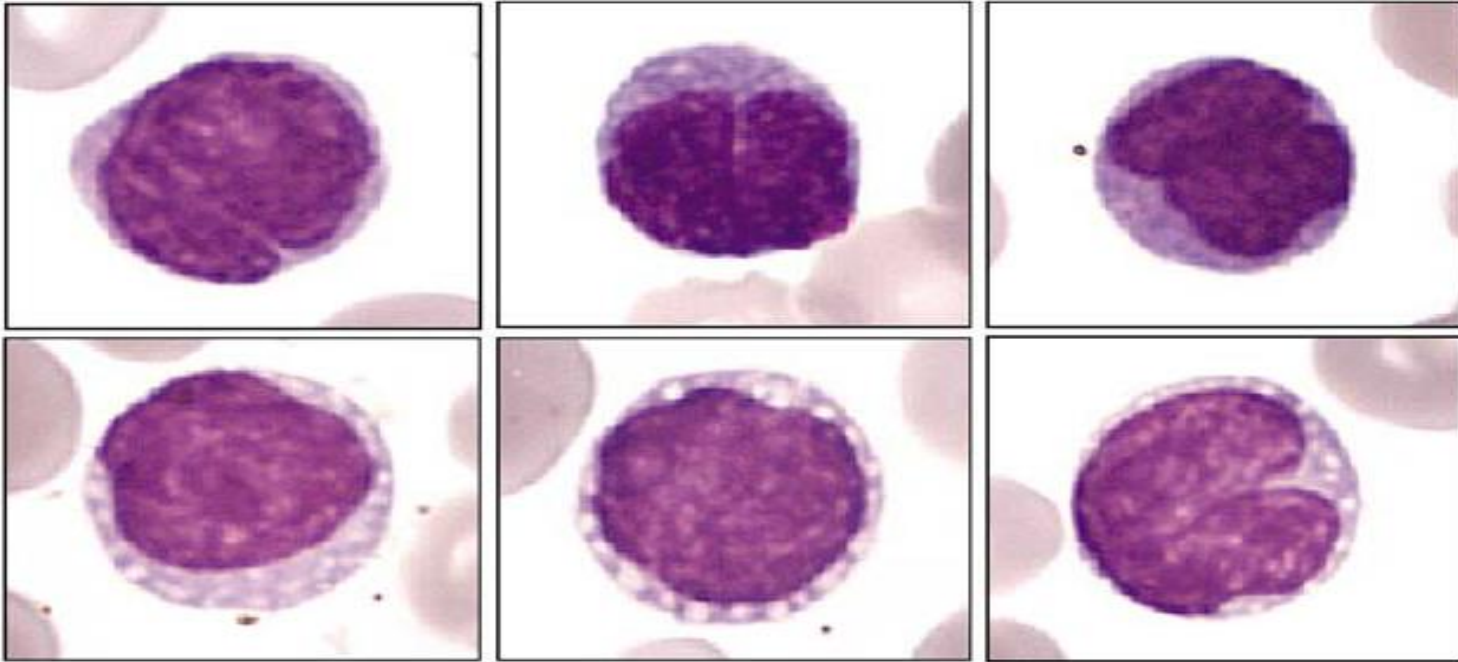




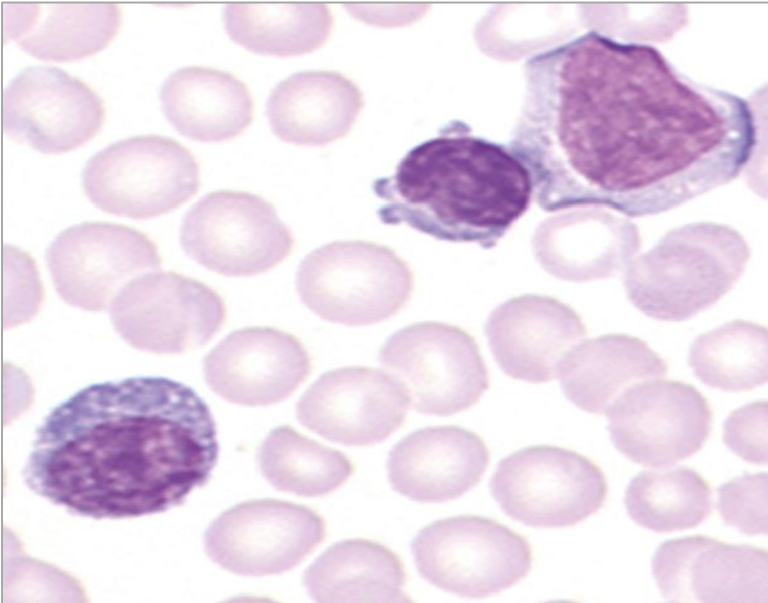
Variant or Atypical Lymphocytes

- Lymphocytes showing morphologic features different from normal lymphocytes:
 - larger than normal lymphocytes due to antigen stimulation
 - nuclei can be round, elliptic, indented, cleft, or folded
 - nucleoli- absent or up to 1-4
 - cytoplasm is often abundant and can be basophilic
 - vacuoles and/or azurophilic granules present

Variant or Atypical Lymphocytes

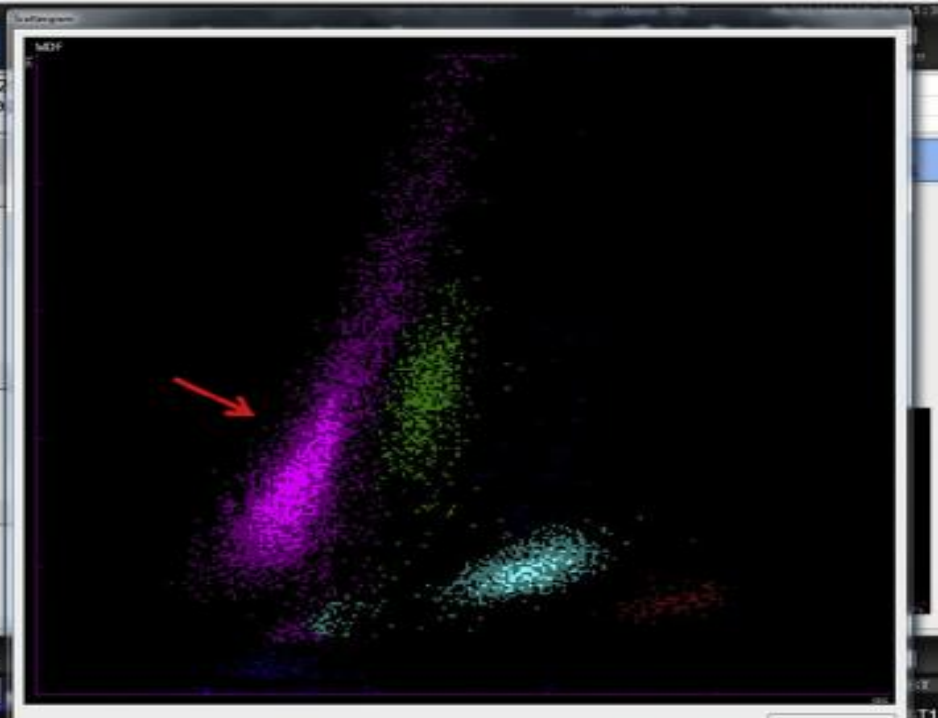


Atypical Lymphocytes

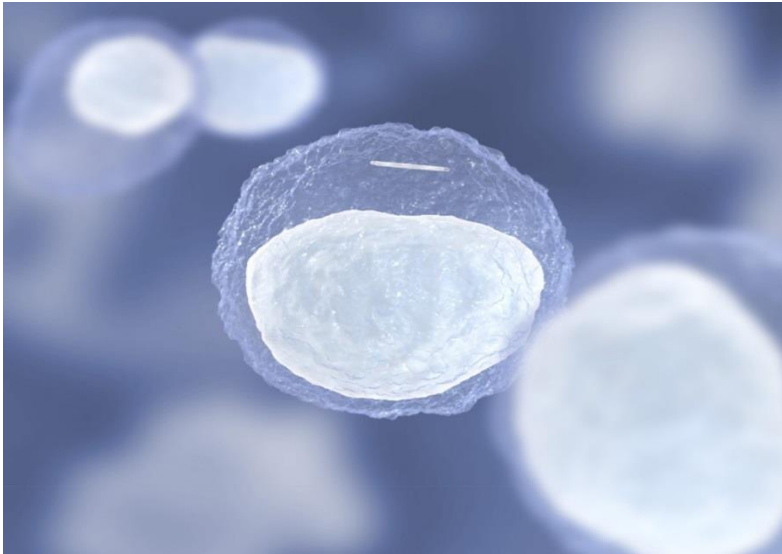


- Viral infections
- Infectious mononucleosis
- Cytomegalovirus (CMV) infection
- Viral hepatitis
- Acute leukemia
- Lymphoma
- B- or T-CLL

Atypical Lymphocytes



Can we recognize malignant cells and atypical lymphocytes?



Lipid membrane composition depends on:

- the cell type (maturity level)
- the state of the cell (activation status)

Blasts have lower lipids →

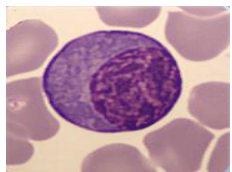
Less membrane perforations →

Less fluorochrome marker into the cytoplasm →

Weaker resulting fluorescence signal.

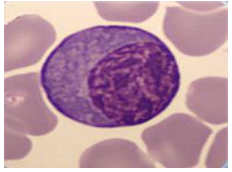
Sensitivity, specificity and predictive values for pathological cells

| Pathological cells | Analyser | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|---------------------------|----------------|-----------------|-----------------|-------------------------------|-------------------------------|
| Blasts | Sapphire | 76 | 95 | 55 | 97 |
| | DxH 800 | 74 | 95 | 63 | 97 |
| | Advia 2120i | 65 | 97 | 65 | 97 |
| | Sysmex XN-2000 | 97 | 96 | 70 | 100 |
| Lymphoma cells/ Abn Ly | Sapphire | 56 | 94 | 44 | 96 |
| | DxH 800 | 64 | 94 | 47 | 97 |
| | Advia 2120i | 72 | 88 | 31 | 98 |
| | Sysmex XN-2000 | 80 | 95 | 59 | 98 |
| Neoplastic cells | Sapphire | 74 | 95 | 72 | 95 |
| | DxH 800 | 81 | 95 | 75 | 96 |
| | Advia 2120i | 77 | 94 | 71 | 96 |
| | Sysmex XN-2000 | 96 | 94 | 75 | 99 |



Reactive (RE-LYMP) and antibody-synthesizing (AS-LYMP) lymphocytes

- In cases with inflammation it is important to rapidly differentiate between various possible conditions
- Support the differentiation between:
 - viral and bacterial infections,
 - acute and subacute infections,
 - inflammatory conditions without an infection
- Both RE-LYMP and AS-LYMP are mainly increased in viral infections

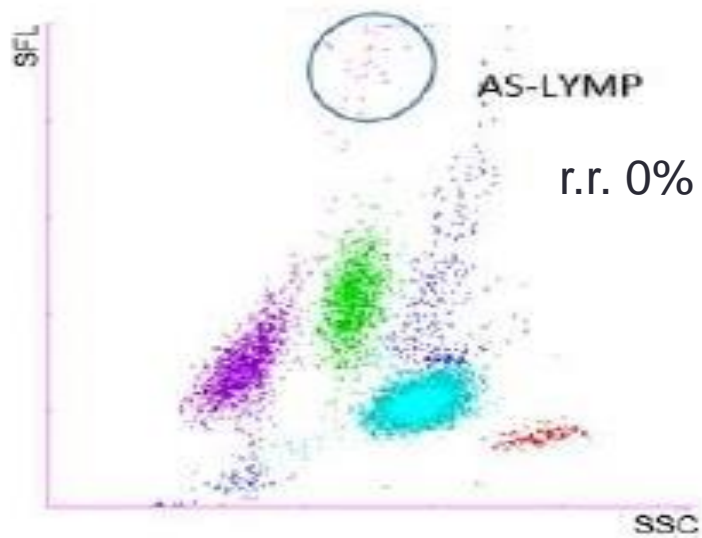


Reactive (RE-LYMP) and antibody-synthesizing (AS-LYMP) lymphocytes

- Additional information about the cellular activation of the innate and adaptive immune response
- Support the differentiation between different types of immune response:
 - early innate (\uparrow RE-LYMP and \uparrow AS-LYMP)
 - cell-mediated (only \uparrow RE-LYMP)
 - humoral response (\uparrow RE-LYMP and \uparrow AS-LYMP)

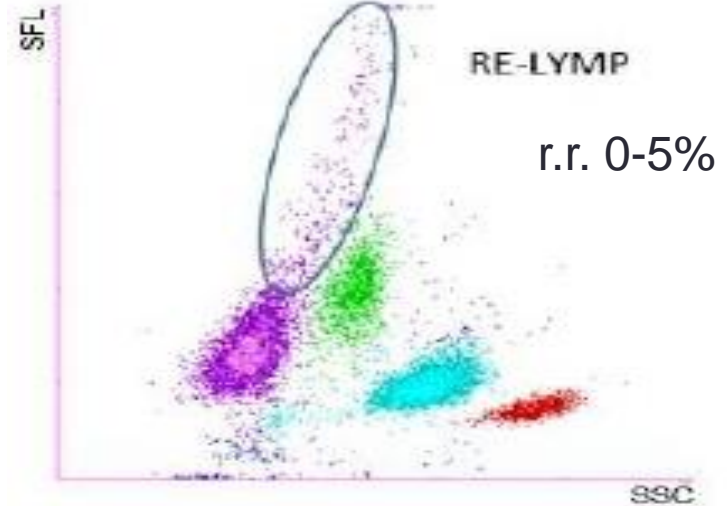
How can we identify AS-LYMP and RE-LYMP?

Activated B- lymphocytes (plasma cells), capable to synthesize antibodies



LYMP subpopulation with the highest fluorescence signals

Total reactive (activated) lymphocytes: B-, T-, plasma cells



LYMP with a higher fluorescence signal than the normal lymphocytes

In conclusion...

- Automated assessment of leucocyte functionality can provide quantitative information about the status of immune system activation.
- Advanced lymphocyte parameters are useful especially in samples containing conspicuous lymphocytes that are difficult to recognise.
- The ability of haematology analyzers to detect neoplastic cells in a blood sample with a high degree of sensitivity is essential for management of disease treatment and progression.
- Laboratory productivity can be improved through reduced manual reviews and faster turnaround times.

A microscopic view of numerous cells, likely cancer cells, with a prominent central cell in focus. The cells are rendered in shades of grey and white against a dark red background. The central cell is larger and more detailed, showing a textured, fibrous surface and a central nucleus. The text "THANK YOU" is overlaid in white, bold, sans-serif font across the center of the image.

THANK YOU