Innovative assessment practices of inflammation

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Abstract

Inflammation is a basic response to noxious stimuli: pathogens, chemical substances or irradiation. It is a fundamental defence mechanism. Currently, the assessment of inflammation involves serum assay of the following markers: C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), procalcitonin, inflammatory interleukins etc. But new parameters, which will allow to perform quick and easy inflammation assessment and its aetiology, are still searched for. New aspects in this area are primarily new haematological parameters, sometimes referred to as Enhanced Inflammatory Parameters (EIP), e.g.: AS-LYMPH (B-lymphocytes which make antibodies), RE-LYMPH (activated T-lymphocytes), NEUT-RI, NEUT-GI and NEUT-WY (activated neutrophils).

Key words:

Inflammation, Haematology, Lymphocytes, Neutrophils, Flow Cytometry

Inflammation

Inflammation is most commonly associated with five basic symptoms: rubefaction, pain, oedema, heat and lack of lost tissue function. Some of them were mentioned in the 1st year A.D. by Cornelius Celsus in his work titled "*De medicina*", in which he wrote about "redness and swelling with heat and pain" (lat. rubor et tumor cum colore et dolore) [1]. Inflammation is a human body response to external or internal noxious stimuli such as pathogens, toxins, irradiation, etc. [2]. Development of its clinical symptoms results from activating various inflammatory mediators, whose task is to neutralise noxious factor and, as a result, restoring homeostasis. Ability to initiate, sustain and mute an inflammatory reaction is an essential body self-defence mechanism. An acute inflammatory response itself can sometimes initiate healing

process. Unfortunately, persistent inflammation can lead to increasingly greater tissue damage [2].

Over many years numerous mechanisms for activating inflammatory response have been detected. These include an ability to activate cells involved in inflammatory response by pathogen-associated molecular patterns (PAMP) and danger-associated molecular patterns (DAMPS) [3]. In cell receptors recognising PAMP and DMAPS (PRRs – *Pattern Recognition Receptors*) are included i.a. all TLR-receptors (*Toll-like receptors*). Stimulation of these receptors leads to activation of extremely significant intracellular pathways. One of them is connected with transcription factor NF-κB, which as a result of activation of TLR-receptors e.g. by bacterial LPS or haemagglutinin of influenza virus undergoes nuclear translocation and affects production of inflammatory cytokines and recruitment of cells involved in inflammatory response [4]. Stimulation of TLR-receptors by osmotic stress, thermal shock or IL-6 can lead to activation of an inflammatory pathway dependent on mitogen-activated protein kinases (MAPK). MAP kinases induce an inflammatory reaction, activate endothelial cells and monocytes as well as they participate in T cell differentiation [2,5]. The JAK-STAT signalling pathway has also a significant role in stimulation of body's inflammatory response [2].

Knowledge concerning importance of inflammatory response process is so extensive that it might appear that there is nothing else to study. But nothing could be further from the truth. What is necessary is to search for clear inflammatory markers, which could be financially useful for assessment and monitoring intensification of body's response to noxious stimulus.

Currently used inflammatory markers

As inflammatory markers most commonly used in laboratory practice can be categorised in particular: Erythrocyte Sedimentation Rate (ESR), CRP levels, PCT levels, total number of white blood cells (WBC) and other blood count parameters, levels of pro- and anti-inflammatory cytokines or microbiological examinations. Performing these assays is necessary for assessment of inflammation but it's not always sufficient to determine its aetiology.

Erythrocyte Sedimentation Rate (ESR) and acute-phase CRP protein are basic as well as the only ones of the most commonly assayed parameters used for inflammation assessment. As a result of pro-inflammatory cytokines' activities synthesis of acute-phase proteins such as CRP is activated in human body. Fibrinogen and immunoglobulins, which belong to them, increase relative permittivity of blood initiating rouleaux formation of erythrocytes and increasing sedimentation speed of red blood cells, consequently causing ESR increase [6]. ESR and CRP offer high sensitivity but low specificity in differentiating inflammation. Their values increase in inflammation with different aetiologies and are very sensitive to interferences by age, sex, underlying diseases or pregnancy. There are so-called bedside tests (POCT – *point-of-care-tests*) available on the market, which on the basis of CRP levels measured in patient's capillary blood allow to differentiate between bacterial aetiology of an inflammation and viral aetiology. The National Institute for Health and Care Excellence recommends that all the patients who are at risk of inflammation and its noxious factor has no clear and known origin should undergo this test. If CRP levels equal less than 20 mg/l, then viral aetiology of an inflammation is more probable. However, if CRP levels equal more than 20 mg/l, an antibiotic therapy should be considered due to high risk of bacterial infection [7].

Determination of pro- and anti-inflammatory cytokines such as TNF-α, IL-1 and IL-6 in assessment of an inflammatory response is definitely more crucial [8]. Cytokines are released from immune cells, particularly from monocytes, macrophages and lymphocytes [2]. This kind of determination is usually made by flow cytometry or immunoenzymatic assays (e.g. ELISA –*Enzyme-Linked Immunosorbent Assay*). As a result, it is possible to identify the stage of inflammation and assess whether the immune response to the injurious stimulus is healthy and intact.

Procalcitonin is one of the most frequently applied biomarkers to determine the cause of inflammation. It is a very sensitive indicator, but it has also its limitations. Procalcitonin is actually only useful

in assessment of severe bacterial infections in which there is high risk of developing sepsis [8].

An assay carried out on a routine basis in assessment of inflammation is Complete Blood Count. This extremely important assay can provide enormous amount of clinical status information of a patient. Not only do today's haematological analysers allow to determine absolute values and percentages of particular fractions of cells, which belong to leucocyte and erythrocyte system as well as to thrombocytes but also, as a result of recent technological advances, it is possible to perform an analysis of totally new parameters. Their determination on a routine basis can make it possible to clearly confirm presence of an inflammation identified as acute, sub-acute and chronic condition or even to identify its aetiology.

New inflammatory markers

From a clinical perspective, assessment of new laboratory parameters is of fundamental importance for assessment of infection aetiology because of the need to decide about proper treatment initiation [7]. The perfect inflammatory marker would be a parameter that could allow to differentiate an inflammation from a proper condition with a great sensitivity and simultaneously to determine origin of an infectious stimulus. In spite of many currently used inflammatory markers there are still some markers needed, which can be easily interpretable, financially accessible as well as be of good sensitivity and specificity.

Haematological parameters, which confirm the activation of the immune response and are determined using the SYSMEX haematology analysers, are very likely to become such markers in near future. They allow to determine quantity of parameters of inflammatory cells, i.e. assess activated RE-LYMP and AS-LYMP lymphocytes and also confirm the activation of the NEUT-RI, NEUT-GI and NEUT-WY neutrophils. These parameters reflect respectively the presence of reactive lymphocytes emitting antibodies and neutrophils at different stages of activation in the fraction peripheral blood. They allow to differentiate between an inflammation and an infection as well as between a factor causing (viral or bacterial) infection and type of immune response (early, innate, cell-mediated or humoral). To fully exploit the potential of Enhanced Inflammatory Parameters (EIP) development of cancer must be excluded. When neutrophils and lymphocytes are activated with various intracellular pathways of inflammatory response, reactive lymphocytes (RE-LYMP) – NEUT-RI, NEUT-GI, NEUT-WY – grow, so do also antibody-secreting lymphocytes (AS-LYMP). Change of these parameters depends on the nature of an inflammatory stimulus, intensity and stage of infection [9].

Specifying these new haematological parameters during Complete Blood Count can be useful in differentiating bacterial infection from viral infection. It may be assumed that using these parameters during laboratory assessment on a routine basis could significantly accelerate inflammation assessment and establishing its aetiology.

Neutrophils and inflammation

Neutrophils, i.e. neutrophilic granulocytes (polynuclear cells – polymorphonuclear neutrophils [PMN]) are the body's first line of defence against pathogenic invasion such as bacteria and parasites. In healthy people they constitute over a half of white cells in circulation. Together with monocytes and macrophages they are commonly known as "well-trained" phagocytes and are part of the initial immune response [10].

Neutrophils use various strategies against pathogens, e.g. phagocytosis, secretion and releasing Neutrophil Extracellular Traps (NET). In response to various stimuli, e.g. TNF-α, GM-CSF, IL-8 and IFNs [11], neutrophils are able to release many inflammatory stimuli, cytokines and antibacterial substances, which are accumulated in infected or inflamed spot (Fig. 1). In these spots they encounter signals causing bacterial killing. They operate also as Antigen-Presenting Cells activating immune response.

During tissue infection it comes to morphological changes in neutrophils (size, shape and structure) and motility changes (chemotaxis and migration), which prolong their vitality and change their molecular properties, enabling them to perform a number of functions. Neutrophil intercellular activities can

be specified as presence of toxic granulation, Döhle bodies and vacuolation. Presence of vacuoles in cytoplasm indicate an enhanced neutrophil phagocytosis in response to bacterial infection. Presence of Döhle bodies is also their activation marker, which is released by an inflammatory factor [12].

Neutrophils can also kill extracellular bacteria by "Neutrophil Extracellular Traps (NETs)". This kind of network is developed in response to the Gram-positive bacteria, Gram-negative bacteria, fungi, protozoons and viruses. Trapped inside these chromatine structures (NET) pathogens are exposed to various substances, including cationic serine proteases (proteinase 3, cathepsin G, neutrophil elastase – NE), myeloperoxidase (MPO), BPI protein (bactericidal/permeability-increasing protein), lactoferrin, gelatinase B, cathelicidin (LL-37 or CAP-18 [cathelicidin antimicrobial peptide 18]) and histone proteins (core and linker histones H1) and tryptase [13]. This network consists of proteolytic enzymes, DNA and other cell nucleus components. This mechanism of programmed cell death leads to decondensation of chromatin in the nucleus of a cell, disintegration of cell organelles, mixing of their ingredients and cell membrane lysis. As a result, binding, disarming and extracellular killing of microorganisms appears regardless of phagocytosis.

Neutrophils play also an important role in inflammation and sepsis. Although they actively fight with invasion of pathogens during an infection, they contribute to septic shock and insufficiency of many organs because of their excessive inflammatory activation. Zonneveld et al. [14] observed morphological changes in neutrophils (size, composition, shape, motility, deformability) in the course of sepsis. Neutrophils become then bigger, contain fewer granules, their migration from blood to tissues is reduced and they are more resistant to mechanical impacts compared to neutrophils in healthy volunteers. These changes allow to make a difference between a sepsis phenotype and a phenotype developing simple infection. During inflammation deformation of neutrophilic granulocytes is reduced, size of cells and is increased and it comes to endothelial adhesion, which causes microvascular involvement [15].

New parameters of neutrophils

Parameter NEUT-RI reflects neutrophil reactivity intensity expressed in the unit FI (fluorescence intensity). Light scattered at 90 degrees from WBC differential channel (SFL detector – *Side-Fluorescence Light*), provides information about cell density and consequently about their metabolic activity. Parameter NEUT-GI expressed in the unit SI (scatter intensity) provides information about internal cell structure (SSC detector – *Side Scatter*). Parameter NEUT-WY informs on the other hand about size of an analysed cell (FSC detector – *Forward Scatter*) (Fig. 2) [16,17].

Zonneveld et al. [14] proved, using AHA to assess morphology of the neutrophils in sepsis, that neutrophilic granulocytes in patients with sepsis are bigger and are prone to scattering, which indicates granulation of neutrophils and their immature fractions (*Immature Granulocytes*, IG). Cornet et al. [18] used analyser XN-1000 (Sysmex Corporation ™, Hyogo, Japan) in order to assess intensity of neutrophils in granulation (NEUT-GI) and reactivation of neutrophils (NEUT-RI) in improvement of laboratory process and to control IG number. A group of 222 patients (3,082 blood samples collected for K2-EDTA) were examined for assessing scope of the standards for these parameters and their potential influence on IG number interpretation in blood samples collected from healthy volunteers. Statistically significant difference occurred between NEUT-RI and infection ($p < 0.05$). NEUT-WY was two times higher in infected patients while NEUT-RI was, on the contrary, 2.5 times higher in non-infectious causes. It means that the both situations have different immunological responsiveness. NEUT-WY was a good marker to suspect an infection and consequently useful in differentiation between non-infectious and infectious causes, which is of great clinical relevance.

Park et al. [19] using Sysmex XN-2000 haematological analysers examined peripheral blood collected for EDTA from 280 healthy volunteers and 130 patients with sepsis. Parameters NEUT-RI and NEUT-WY showed greater AUC values (*Area Under*

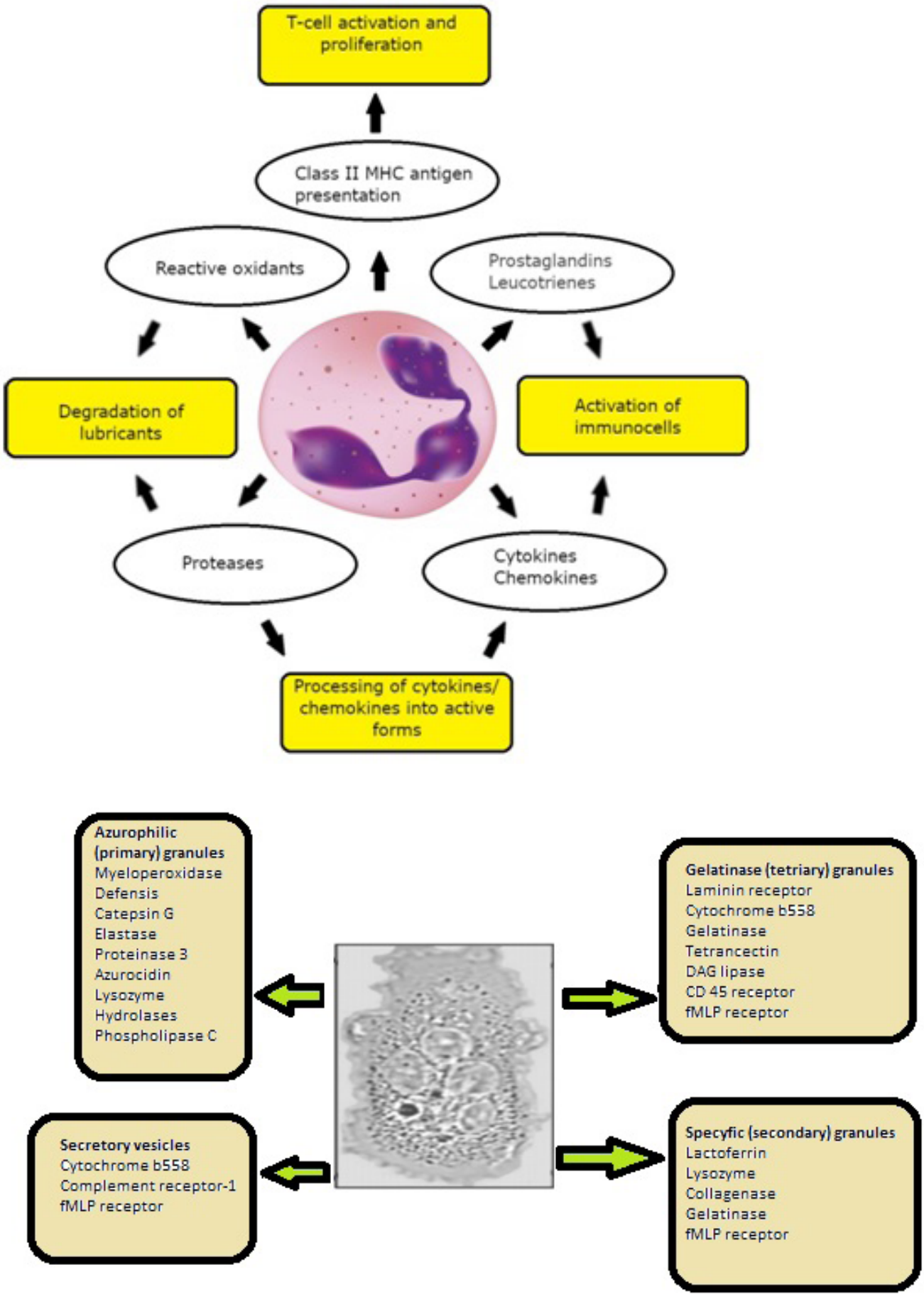

Fig. 1.
Properties of neutrophils (A), granularity of neutrophils (B) [1].

The Curie) respectively 0.909 and 0.905 compared with routine parameters such as haematocrit, haemoglobin, RBC, RDW, IG, number of lymphocytes and number of neutrophils. NEUT-RI and NEUT-WY in patients with sepsis compared to healthy patients were statistically significant p<0.001 (respectively: 57.56 +/− 949 vs. 47.19 +/− 2.64 and 827.14 +/− 314.50 vs. 611.81 +/− 45.49). Patients with sepsis showed relevant changes in number of RBC, neutrophils, lymphocytes and thrombocytes in comparison to group of healthy volunteers. Increase of parameters NEUT-RI and NEUT-WY can indicate immaturity of neutrophils or their activation and can be useful in detection of patients with sepsis in combination with current sepsis biomarkers. Luo et al. [20] used Sysmex XE-5000 haematological analyser for the purpose of assessment of toxic granulation and nuclear maturity of neutrophilic granulocytes by parameters NEUT-GI, NEUT-RI, CRP and PCT in 130 healthy volunteers and 113 patients with sepsis. NEUT-GI, NEUT-RI and CRP were significantly higher in patients with septicaemia than in healthy volunteers. It indicates possibility to use NEUT-GI and NEUT-RI as simple auxiliary markers in sepsis assessment. Urrechaga [21] examined in his study 215 patients with fever (bacterial or viral) and 212 healthy persons and observed that parameter NEUT-WY reflects an increase in content of neutrophils' nucleic acid in response to bacteria and this assay is reliable for diagnosing acute bacterial infection, which compared to clinical data can be helpful in differentiating influenza aetiology.

Study was conducted on children under 5 years and showed that NEUT-RI was elevated in patients with bacterial infections in comparison to control group [9], while RE-LYMP and AS-LYMP were significantly more elevated in patients with viral infections than in patients with bacterial infections. Stiel et al. [22] proved that parameter NEUT-RI has high sensitivity and specificity to diagnosing disseminated intravascular coagulation in patients with septic shock.

Lymphocytes and inflammation

Lymphocytes constitute a group of cells responsible for activating inflammatory response, namely specific body's immune response to noxious stimulus. In physiological state they should be 25-35% of all leukocytes present in peripheral blood. They develop from CLP cell (Common Lymphocyte Progenitor) in the bone marrow and subsequently they mature in the thymus (T lymphocytes) or in the red bone marrow (B-lymphocytes) [23].

Due to the presence of TCR or BCR receptors, clusters of differentiation (CD) and HLA class I antigens on the surface of lymphocytes and also because of substances secreted by these cells and their functions it was possible to divide i.a. T lymphocytes into numerous subpopulations [24].

Non-activated lymphocytes are morphologically indistinguishable under the light microscope. They are small cells with big nucleus and surrounding a thin layer of cytoplasm deficient in cell organelles. Under influence of noxious stimulus and via the immune synapse produced due to formation of BCR or TLR receptor complex with antigen occurs activation of lymphocytes [25,26]. Activated lymphocyte is much different from a cell in physiological state. B-lymphocyte transformation into plasmocytes with strongly basophilic cytoplasm occurs and this cytoplasm has characteristic increased lucency while T-lymphocytes are transformed into bigger polymorphic effector T cells with abnormal nuclei, which are sometimes visible and there is cytoplasm with azurophilic granules [26].

New lymphocyte parameters

Due to the afore-mentioned differences some of the haematological analysers can make it possible to differentiate activated lymphocytes among all leucocytes (RE-LYMP) and to differentiate lymphocytes producing antibodies which are activated B-lymphocytes (AS-LYMP). This process is carried out by fluorescence flow cytometry (FFC) based on the assessment of differences in signals produced by sending

cells with different functionality for SSC detectors (which analyses granularity of cells) and FSC detectors (which analyse cell size) (Fig. 3) [9,27].

Assessment of these two new haematological parameters is very crucial for assessment and specifying aetiological factors of inflammation in adults as well as in children. Patients with developed inflammatory response and especially with viral aetiology are prone except for increase in WBC to increase in neutrophil-lymphocyte ratio (NEUT/LIMF) and to increase in the percentage of reactive lymphocytes (RE-LIMF defined in percentage) in relation to WBC. What is more, in viral infections there is increase in antibody-secreting lymphocytes (AS-LIMF defined in percentage) [9]. Henriot and his co-workers [9] found in their study that in pediatric patients these parameters can be used together with PLT number and total number of lymphocytes LIM as auxiliary markers in inflammation assessment, although in conducted studies statistically significant results were not achieved. The same research team proved that new haematological parameters are useful in differentiating viral from bacterial aetiology based on comparison of ROC Curves of procalcitonin in relation to AS-LIMF % and RE-LIMF %.

Results of Henriot's research team were confirmed in 2019 by Prodjosoewojo and his co-workers [28]. They performed an analysis of AS-LIMF % and RE-LIMF % parameters in patients diagnosed with tropical diseases. It was concluded that characteristic for appearance of dengue fever was an increase in AS-LIMF % and RE-LIMF % parameters. In patients with intracellular bacterial infections caused by rods of Salmonella spp. or murine typhus caused by Rickettsia typhi it was typical that parameter RE-LIMF % was significantly more elevated than in patients with other bacterial infections (salmonellosis vs. leptospirosis P = 0.006, murine typhus vs. leptospirosis P = 0.007 and murine typhus vs. cosmopolitan bacterial infections P <0.0001) [28].

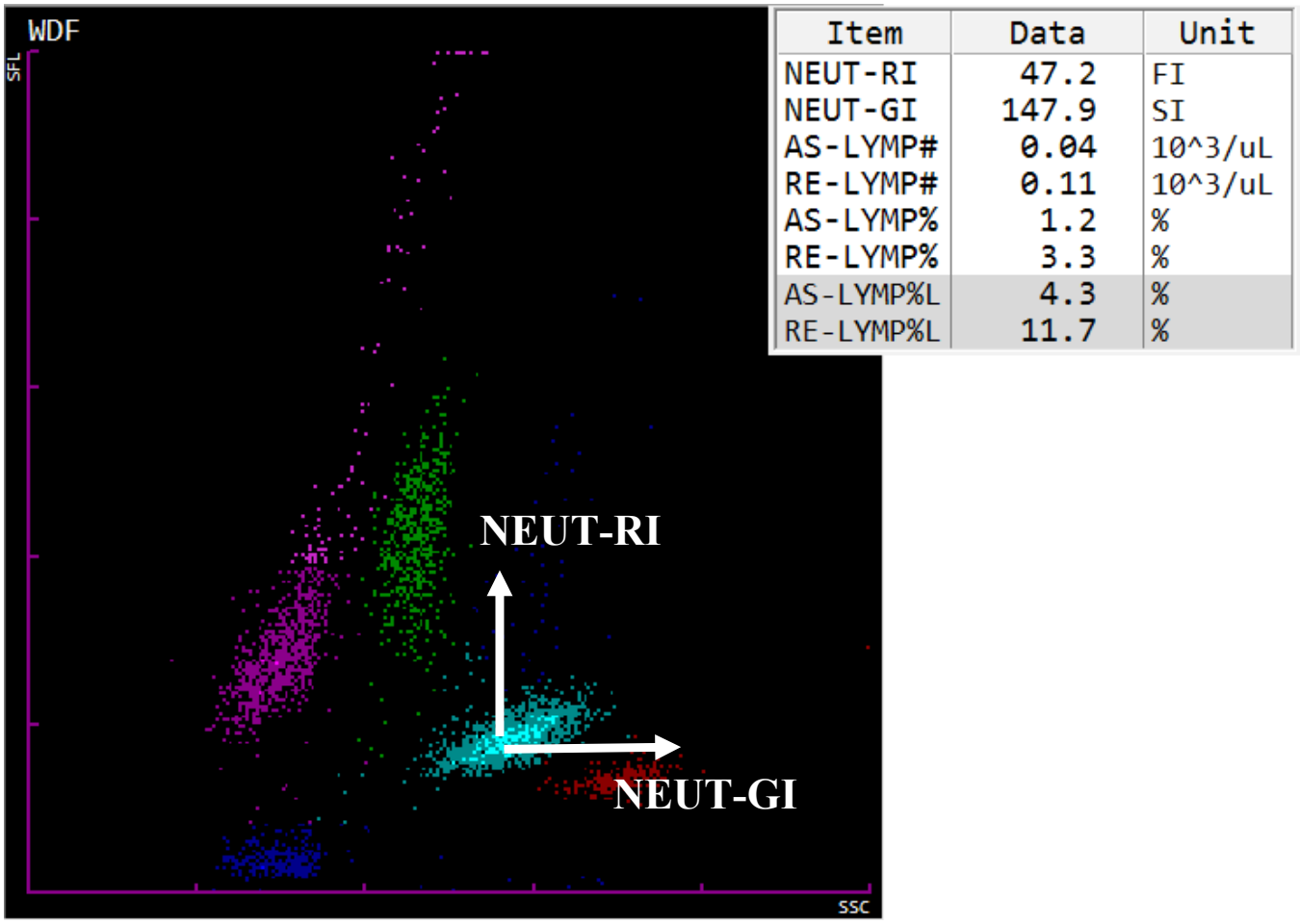

Fig. 2.
Graph of the SFL/SSC response curve corresponding to parameters NEUT-RI and NEUT-GI

Summary

In conclusion, numerous morphological characteristics such as size, shape, granulation, maturity and type of cells can accelerate assessment procedure using automatic SYSMEX haematological analysers. Using new integration techniques in Complete Blood Count, mechanical properties and motility of neutrophilic granulocytes can result in more specific diagnosis and improved sepsis surveillance [14]. What is more, performed analyses can be a crucial factor in obtaining a clearer understanding of an individual therapy and reveal new specific treatment options at the right time but they can also show how to determine therapeutic effects of new therapies.

Including new haematological parameters in routine analysis gives the possibility to easily diagnose an inflammation and divide it into bacterial and viral infections in acute responses (Table 1). Here it should be noted that in cases of chronic inflammation activation of humoral and cellular responses can occur, and this would result in changes of the following dependencies.

Determination of these parameters is quick, low-cost and one-stage. The same blood sample which is used for the Complete Blood Count can also be used for other assays to determine levels of other markers which helps to avoid additional costs. They allow to make the assessment procedure more efficient and they improve inflammation aetiology assessment without need to perform specialised and expensive assessment procedure.

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Conflict of Interest

No competing financial interests or other conflict of interests exist for any of the authors.

Tabela 1.
Differentiating inflammation aetiology based on fundamental and new inflammatory markers [12, 27].

Parameter	Bacterial aetiology	Viral aetiology
CRP (<5 mg/l)	Higher values (approximately more than 26.6 mg/l)	There is an increase but lower than in cases with bacterial diseases In some cases of viral aetiology CRP does not rise
PCT (< 0.5 ng/ml)	Very low numbers only in bacterial infections In 1/3 patients no increase in spite of bacterial infection	No increase
AS-LIMF %: 0 % #: 0 cells/l	No statistically significant increase	Higher increase than in cases with bacterial diseases
RE-LIMF %: 5 % #: 0 – 0.5 x 10^3/µl		
NEUT-GI 142.8 – 159.3 SI	Higher increase than in cases with viral diseases	No statistically significant increase
NEUT-RI 39.8 – 51.0 FL		

References

1. Medzhitov R. Inflammation 2010: New Adventures of an Old Flame. Cell 2010; 140(6): 771-6.
2. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018; 9(6): 7204–7218.
3. Gudkov AV, Komarova EA. p53 and the Carcinogenicity of Chronic Inflammation. Cold Spring Harb Perspect Med 2016; 1, 6(11).
4. Gruber BM. Transcription factor NFkB — a new perspective in anticancer therapy. Postępy Biochemii 2004; 50(2).
5. Bryk D, Olejarz W, Zapolska-Downar D. Mitogen--activated protein kinases in atherosclerosis. Postepy Hig Med Dosw 2014; 68: 10-22.
6. Costenbader KH, Chibnik LB, Schur PH. Discordance between erythrocyte sedimentation rate and C-reactive protein measurements: clinical significance. Clin Exp Rheumatol 2007; 25(5): 746-9.
7. National Institute for Health and Care Excellence. Clinical guideline [CG191]. Pneumonia in adults: diagnosis and management. December 2014.
8. Vijayan AL, Vanimaya, Ravindran S, Saikant R, Lakshmi S, Kartik R, Manoj G. Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy. Journal of intensive care 2017; 5: 51.
9. Henriot I, Launay E, Boubaya M, Cremet L, Illiaquer M, Caillon H, Desjonquères A, Gillet B, Béné MC, Eveillard M. New parameters on the hematology analyzer XN-10 (Sysmex™) allow to distinguish childhood bacterial and viral infections. Int J Lab Hematol 2016; 39(1): 14–20.
10. Wright HL, Moots RJ, Bucknall RC, Edwards SW. Neutrophil function in inflammation and inflammatory diseases. Rheumatology 2010; 49: 1618–31.
11. Martin SJ, Henry CM. Distinguishing between apoptosis, necrosis, necroptosis and other cell death modalities. Methods 2013; 61(2): 87-9.
12. Berthier E, Surfus J, Verbsky J, Huttenlocher A, Beebe D. An arrayed high-content chemotaxis assay for patient diagnosis. Integr Biol (Camb) 2010; 2(11-12): 630-8.
13. Papayannopoulos V, Zychlinsky A. NETs: a new strategy for using old weapons. Trends Immunol 2009; 30: 513–52.
14. Zonneveld R, Molema G, Plötz FB. Analyzing Neutrophil Morphology, Mechanics and Motility in Sepsis: Options and Challenges for Novel Bedside Technologies. Crit Care Med 2016; 44: 218–28.
15. Roussel M, Benard C, Ly-Sunnaram B, Fest T. Refining the white blood cell differential: the first flow cytometry routine application. Cytometry A 2010; 77(6): 552-63.
16. Alvarez DF, Helm K, DeGregori J, Roederer M, Majka S. Publishing flow cytometry data. American Journal of Physiology – Lung Cellular and Molecular Physiology 2010; 298(2): 127–130.
17. Ibrahim SF, van den Engh G. Flow cytometry and cell sorting. Adv Biochem Eng Biotechnol 2007; 106: 19-39.
18. Cornet E, Boubaya M, Troussard X. Contribution of the new XN-1000 parameters NEUT-RI and NEUT--WY for managing patients with immature granulocytes. Int J Lab Hematol 2015; 37(5): e123–6.
19. Park SH, Park CJ, Lee BR, Nam KS, Kim MJ, Han MY, Kim YJ, Cho YU, Jang S. Sepsis affects most routine and cell population data (CPD) obtained using the Sysmex XN-2000 blood cell analyzer: neutrophil--related CPD NE-SFL and NE-WY provide useful information for detecting sepsis. Int J Lab Hematol 2015; 37(2): 190–8.
20. Luo Y, Lin J, Chen H, Zhang J, Peng S, Kuang M. Utility of neut-X, neut-Y and neut-Z parameters for rapidly assessing sepsis in tumor patients. Clin Chim Acta 2013; 422: 5–9.
21. Urrechaga E, Bóveda O, Aguirre U, García S, Pulido E. Neutrophil Cell Population Data biomarkers for Acute Bacterial Infection. J Pathol Infect Dis 2018; 1(1): 1-7.
22. Stiel L, Delabranche X, Galoisy AC, Severac F, Toti F, Mauvieux L, Meziani F, Boisramé-Helms J. Neutrophil Fluorescence: A New Indicator of Cell Activation During Septic Shock-Induced Disseminated Intravascular Coagulation. Crit Care Med. 2016; 44(11): e1132–36.
23. Gołąb J, Jakóbisiak M, Lasek W, Stokłosa T. Immunologia. Warszawa: Wydawnictwo Naukowe PWN 2012.
24. Niedźwiedzka-Rystwej P, Tokarz-Deptuła B, Deptuła W. Characteristic of T limphocyte subpopulations. Postępy Hig Med Dosw 2013; 67: 371-379.
25. Woodruff MF, Reid B, James K. Effect of antilymphocytic antibody and antibody fragments

on human lymphocytes in vitro. Nature 1967; 215(5101): 591.

26. Carr JH, Rodak BF. Clinical Hematology Atlas. Third Edition. St Louis, Sounders 2009.
27. Sysmex Europe GmbH. SEED Haematology. Looking deeper into inflammatory conditions from a laboratory and clinical perspective. Sysmex Educational Enhancement and Development 2018.
28. Prodjosoewojo S, Riswari SF, Djauhari H, Kosasih H, van Pelt LJ, Alisjahbana B, van der Ven AJ, de Mast Q. A novel diagnostic algorithm equipped on an automated hematology analyzer to differentiate between common causes of febrile illness in Southeast Asia. PLOS Negl Trop Dis 2019; 13(3): e0007183.