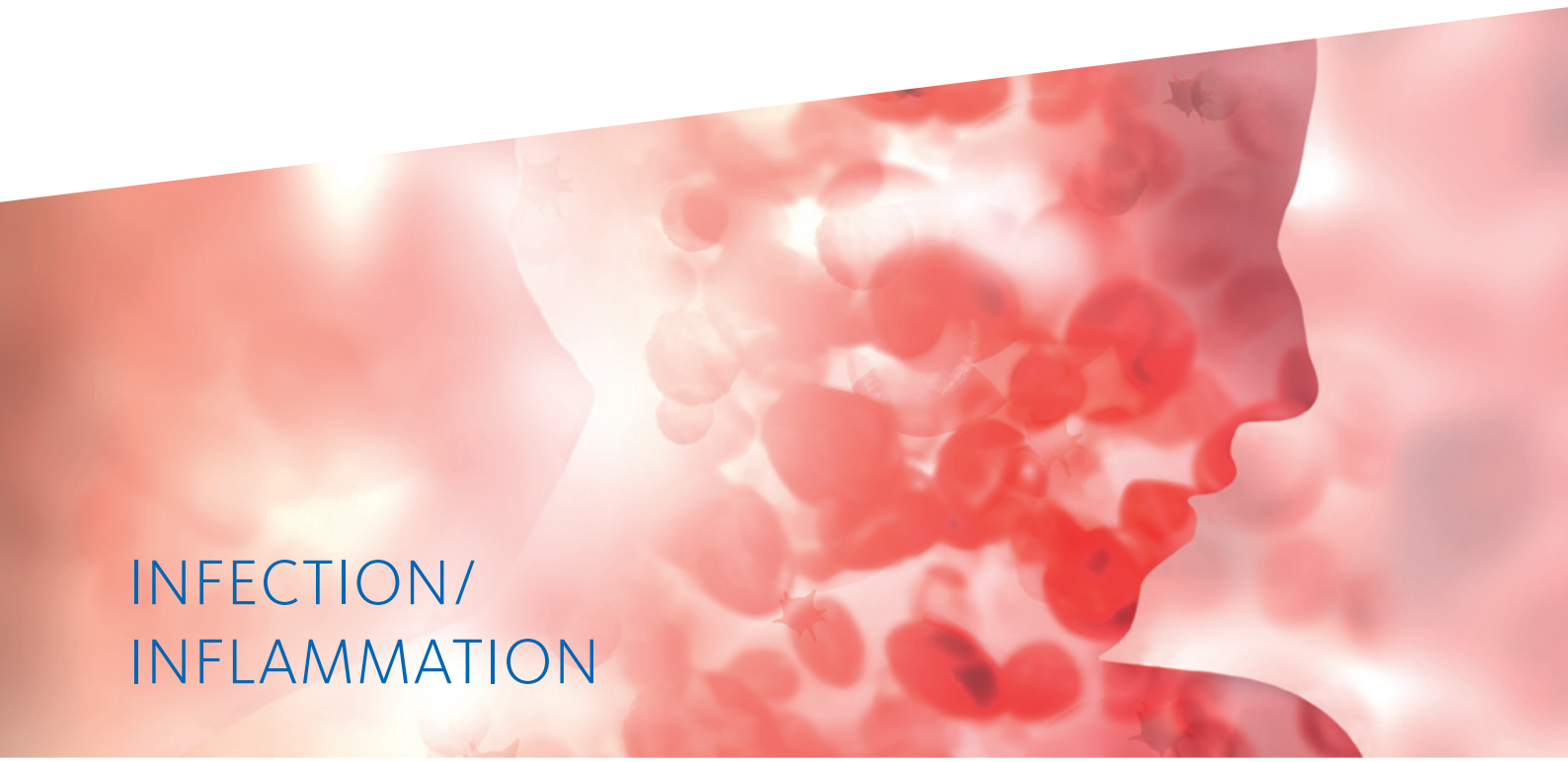
WHITE PAPER



INFECTION/ INFLAMMATION

Novel haematological parameters for rapidly monitoring the immune system response

Patients with inflammatory disease are common on hospital wards. When patients are suspected of having an inflammation, it is important to rapidly differentiate between various possible conditions. You need to distinguish between inflammations caused by infections and those that are not, and determine the responsible pathogen and the patient's immune response status in case of an infection. Next, treating physicians need to determine the appropriate therapy for their patients and avoid the overuse of antibiotics.

Correctly diagnosing suspected inflammations and infections by clinical examination, biochemical markers and microbiological blood cultures is costly and time-consuming. A fast initial indication would be beneficial since this can point out the appropriate diagnostic tests, avoid unnecessary follow-up tests, and help start or modify treatment faster. Haematological inflammation parameters obtained from a routine blood count on Sysmex's XN-Series analysers can provide quantitative information about the inflammatory reaction of the patient's immune system.

The clinical value of haematological inflammation parameters in managing inflammatory diseases

Sysmex's XN-Series analysers offer a set of haematological inflammation parameters that lets one quantitatively assess the activation status of neutrophils (NEUT-RI, NEUT-GI), immature granulocytes (IG) and activated lymphocytes (RE-LYMP, AS-LYMP).

The innate immune system is an initial, non-specific line of defence against pathogens. Its main function is to identify and remove foreign substances by specialised white blood cells (WBC) and further activate the adaptive immune system through a process of presenting the pathogens' antigen. Typically, activated neutrophils (increased NEUT-RI, increased NEUT-GI), immature granulocytes (IG), reactive lymphocytes (RE-LYMP) and T cell-independent plasma cells (AS-LYMP) are found in this phase of infection. Generally, the change in value of these parameters depends on the nature of the inflammatory stimulus, severity and stage of the infection.

* Revision of the original article published in March 2017

The innate immune response triggers the adaptive immune response, which can be divided into an early cell-mediated immune response and a later humoral immune response. The cell-mediated response is characterised by an increase in activated T lymphocytes and NK-cells. The humoral response is typically characterised by activated B lymphocytes (plasma cells). Activated B lymphocytes can be quantified with the parameter AS-LYMP (antibody-synthesizing lymphocytes). All activated lymphocytes (including plasma cells) are quantified with the parameter RE-LYMP (total reactive lymphocytes).

The combination of the RE-LYMP and AS-LYMP parameters provides additional information about the cellular activation of the innate and adaptive immune response. The increased fluorescence values of these cell populations recorded during analysis indicate both increased cellular activity and changes in the membrane composition, and so indicate whether there is a cell-mediated or humoral immune response to pathogens. This supports differentiation between viral and bacterial infections, or between acute and subsiding infections, or whether there is an inflammatory condition without an infection.

Table 1 summarises the haematological inflammation parameters with their respective units. The parameters let one quantify:

- activated lymphocytes,
- immature granulocytes and
- the activation status of neutrophils.

Several recent studies have shown that these parameters are valuable for detecting and monitoring infections and inflammations [1 – 8]. The structural neutrophil parameters NEUT-RI and NEUT-GI obtained from the XN-Series could predict the appearance of later-stage infection markers such as the presence of immature granulocytes, suggesting that these neutrophil parameters can be used to detect early-stage bacterial infections [1]. Furthermore, an ongoing study (manuscript in preparation) found that both RE-LYMP and AS-LYMP counts were mainly increased in viral infections [2]. RE-LYMP counts were only increased in some bacterial infections and AS-LYMP counts were only mildly increased in bacterial infections (unspecific T-independent plasma cells). Another study on children younger than five years found that NEUT-RI was increased in patients with

Table 1 *A summary of haematological inflammation parameters with their respective immunological interpretation, units and reference intervals.*

Cell populations and / or their characteristics	Description	Immunological interpretation	Parameter	Unit	Reference interval
Total reactive lymphocytes	This includes activated B and T lymphocytes recognised by an increased fluorescence intensity compared to that of common lymphocytes.	Increased in innate and adaptive cell-mediated immune response	RE-LYMP# RE-LYMP%[i]	Cells/L %	0 – 0.5 x 10^9/L 0 – 5%
Antibody-synthesizing lymphocytes[ii]	These are exclusively activated B lymphocytes recognised by the markedly increased fluorescence intensity compared to that of common lymphocytes.	Increased in innate and adaptive humoral immune response	AS-LYMP# AS-LYMP%[i]	Cells/L %	0 Cells/L 0%
Granularity of neutrophils	A measure of the cytoplasmic granularity of the neutrophil population, representing their response to inflammatory processes.	Increased in early innate immune response	NEUT-GI: Neutrophil Granularity Intensity	Scatter Intensity (SI)	142.8 – 159.3 SI [1]
Reactivity of neutrophils	A measure of the fluorescence intensity of the neutrophil population, representing their metabolic activity.	Increased in early innate immune response	NEUT-RI: Neutrophil Reactivity Intensity	Fluorescence Intensity (FI)	39.8 – 51.0 FI [1]
Immature granulocytes	The total of metamyelocytes, myelocytes and promyelocytes are counted as a single population, separately from the common neutrophils.	Indicates the severity of the early innate immune response	IG# IG%[i]	Cells/L %	0 – 0.06 x 10^9/L 0 – 0.6% [9]

[i] As a percentage of all WBC
[ii] When antibody-synthesizing lymphocytes (AS-LYMP) are present, they are also included in the total reactive lymphocytes (RE-LYMP).

bacterial infections compared to controls [6], whereas only RE-LYMP and AS-LYMP counts were significantly higher in patients with viral infections than in patients with bacterial infections. Moreover, in this study the AS-LYMP parameter provided the same discrimination power between viral and bacterial infections as procalcitonin. Stiel *et al.* (2016) showed that the NEUT-RI parameter has a high sensitivity and specificity for diagnosing disseminated intravascular coagulation in patients with septic shock [7]. Oehadian *et al.* (2015) studied the differential diagnostic possibilities of atypical lymphocyte research parameters (RE-LYMP and AS-LYMP are derived from them) and found that they can help in the differentiation of dengue from leptospirosis and enteric fever [8].

Case study: Early innate immune response to intracellular bacteria

Case history

A 23-year old man with an intermittent fever visited his physician three days after the initial onset of fever. The man reported the following symptoms: shortness of breath, productive cough, abdominal pain, diarrhoea, night sweats and malaise. Considering the man's symptoms, the physician suspected pneumonia and ordered a complete blood count with white blood cell differential analysis to investigate the possible cause of infection.

Laboratory results

Table 2 *An overview of the laboratory results obtained from the Sysmex XN-Series haematology analyser.*

WBC parameters	Data
WBC (10^9/L)	2.98
NEUT# (10^9/L)	2.50*
LYMPH# (10^9/L)	0.29*
MONO# (10^9/L)	0.17*
EO# (10^9/L)	0.01*
BASO# (10^9/L)	0.01
IG# (10^9/L)	0.02*
RE-LYMP# (10^9/L)	0.03
AS-LYMP# (10^9/L)	0.02
NEUT%	84.0*
LYMPH%	9.7*
MONO%	5.7*
EO%	0.3*
BASO%	0.3
IG%	0.7*
RE-LYMP%	1.0
AS-LYMP%	0.6
NEUT-GI (SI)	145.5
NEUT-RI (FI)	60.7

RBC parameters	Data
RBC (10^{12}/L)	3.96
HGB (g/L)	102
HCT (L/L)	0.312
MCV (fL)	78.8
MCH (pg)	25.8
MCHC (g/L)	327
RDW-SD (fL)	42.2
RDW-CV (%)	14.6
NRBC# (10^9/L)	0.00
NRBC%	0.0
MicroR (%)	8.3
MacroR (%)	3.3
HYPO-H_e (%)	1.6
HYPER-H_e (%)	0.3
RET# (10^9/L)	22.6
RET%	0.57
IRF (%)	5.1
RET-H_e (pg)	29.8
Delta-H_e (pg)	3.8
FRC# (10^{12}/L)[§]	0.0851
FRC%[§]	2.15

PLT parameters	Data
PLT-I (10^9/L)	244
PLT-F (10^9/L)	231
PDW (fL)	11.4
MPV (fL)	11.2
P-LCR (%)	30.7
PCT (L/L)	0.0027
IPF# (10^9/L)	4.2
IPF (%)	1.8

WBC Flag(s)
Lymphopenia
Left Shift?
Atypical Lympho?

* result marked as unreliable
§ research parameter

Case interpretation

The XN analysis results of the young man with fever and clinical focus on the lungs revealed a leucopenia with a relative increase in neutrophils (NEUT/LYMPH = 8.5). The neutrophils showed an increased activation – NEUT-RI = 60.7 FI – and combined with the low concentrations of AS-LYMP (0.6 %), the results indicate an early innate immune response to intracellular bacteria.

The differential diagnosis in such pneumonia cases aims to distinguish the underlying cause, which can be either extracellular or intracellular bacteria, a viral infection or an inflammation from a non-pathogenic source. The results presented showed a decrease in the absolute neutrophil count. The activated neutrophils and a decreased lymphocyte count – of both relative and absolute values – excluded a viral infection from the differential diagnosis in this case. Usually, if pneumonia is caused by extracellular bacteria, it would cause an increased absolute neutrophil count (together with an increased IG count) and decreased monocyte count. This would characterise an acute phase infection and would usually also be accompanied by thrombocytopenia, which was not observed here. An inflammation without infection would result in neutrophilia without the activation of neutrophils. The low numbers of AS-LYMP in the differential white blood cell count are T cell-independent plasma cells, which are circulating B cells producing unspecific antibodies after direct activation by lipopolysaccharides. These can be released from the cell walls of certain bacteria, and bind to the B cell receptor.

The overall results exclude an extracellular bacterial infection, non-pathogenic inflammation and viral infection. The final diagnosis of suspected tuberculosis was made by positive chest X-ray. Four weeks after the initial blood count and start of the antibiotic treatment the final tuberculosis diagnosis caused by *M. tuberculosis* was confirmed by positive Ziehl-Neelsen stain sputum culture for acid-fast bacilli.

Conclusion

The diagnostic parameters described in this white paper can help physicians diagnose, treat and monitor patients with inflammatory diseases. The haematological inflammation parameters provide additional information about an activation of the immune response. They support differentiation between inflammation and infection, different pathogenic causes of infection (viral versus bacterial) and different types of immune response: early innate, cellular or humoral immune response. These parameters allow a quantitative assessment of the activation status of neutrophils (NEUT-RI, NEUT-GI), immature granulocytes (IG) and activated lymphocytes (RE-LYMP, AS-LYMP). They are readily available from a routine blood laboratory test, together with the complete blood count.

References

[1] ***Cornet E et al.*** *(2015): Contribution of the new XN-1000 parameters NEUT-RI and NEUT-WY for managing patients with immature granulocytes. Int J Lab Hematol. 37(5): e123 – 6.*

[2] ***Van der Ven A et al.:*** *Manuscript in preparation.*

[3] ***Park SH et al.*** *(2015): 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. 37(2): 190 – 8.*

[4] ***Luo Y et al.*** *(2013): Utility of neut-X, neut-Y and neut-Z parameters for rapidly assessing sepsis in tumor patients. Clin Chim Acta. 422: 5 – 9.*

[5] ***Linssen J et al.*** *(2008): Automation and validation of a rapid method to assess neutrophil and monocyte activation by routine fluorescence flow cytometry in vitro. Cytometry B Clin Cytom. 74(5): 295 – 309.*

[6] ***Henriot I et al.*** *(2016): New parameters on the hematology analyzer XN-10 (Sysmex™) allow to distinguish childhood bacterial and viral infections. Int J Lab Hematol. 39(1): 14 – 20.*

[7] ***Stiel L et al.*** *(2016): Neutrophil Fluorescence: A New Indicator of Cell Activation During Septic Shock-Induced Disseminated Intravascular Coagulation. Crit Care Med. 44(11): e1132 – 36.*

[8] ***Oehadian A et al.*** *(2015): New parameters available on Sysmex XE-5000 hematology analyzers contribute to differentiating dengue from leptospirosis and enteric fever. Int J Lab Hematol. 37(6):861.*

[9] ***Pekelharing JM et al.*** *(2010): Haematology reference intervals for established and novel parameters in healthy adults. Sysmex Journal International. 20(1): Online only.*

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How can these parameters be measured on a haematology analyser?

These haematological inflammation parameters can be determined using fluorescence flow cytometry used by the XN-Series analysers. They are shown (here as examples) in the so-called 'scattergrams', which are produced during a measurement, in Fig. 1.

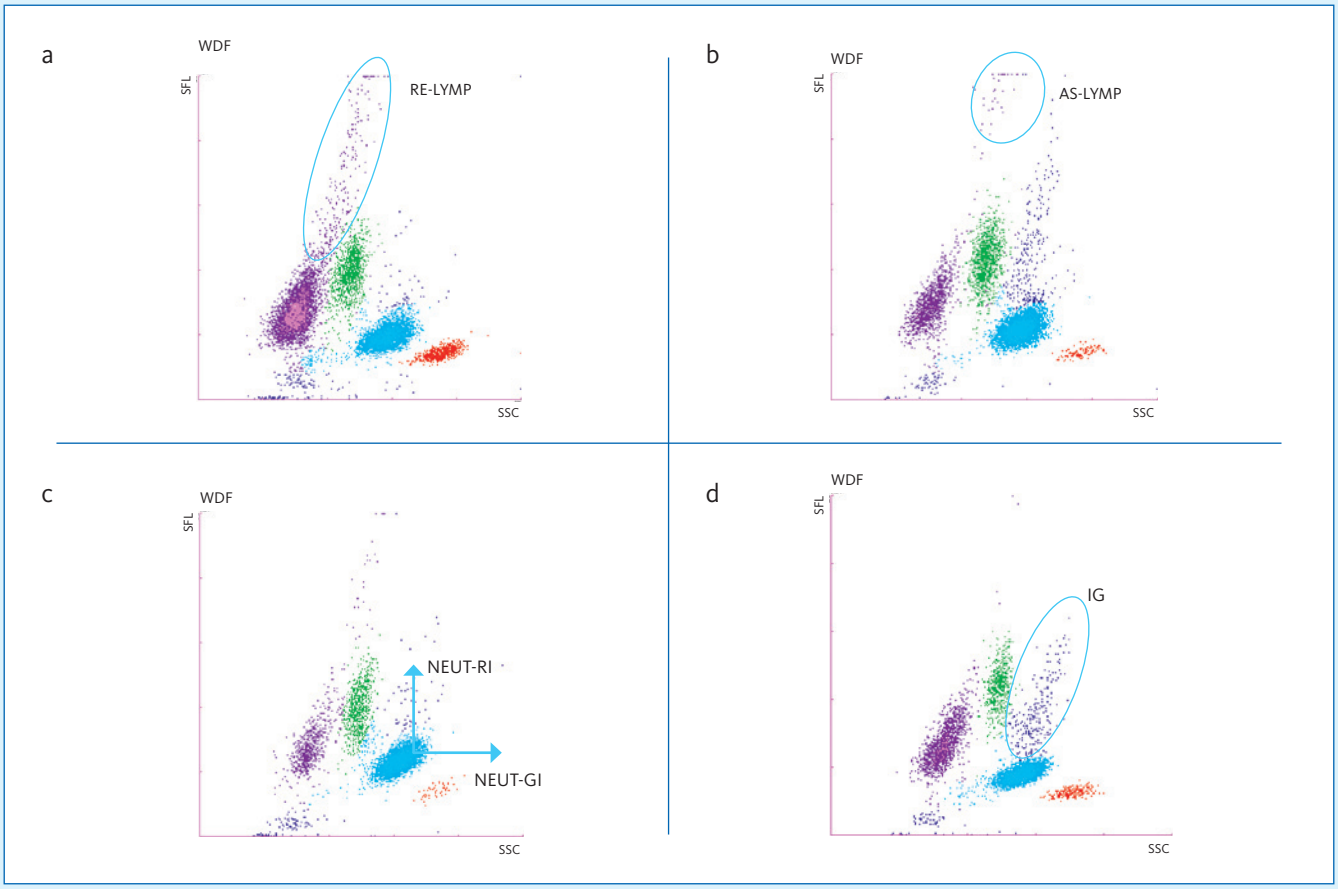

Fig. 1 *Haematological inflammation parameters describing activated cell populations that appear within the course of the immune response. The scattergrams are plotted using intracellular structure (side scatter: SSC) on the x-axis and the presence of bioactive materials (side fluorescence signal: SFL) on the y-axis. Each dot represents one cell. a: Reactive lymphocytes; b: Antibody-synthesizing lymphocytes; c: Activated neutrophils; d: Immature granulocytes.*

In the scattergram of the presented patient case (Fig. 2), the following was observed: activated neutrophils (NEUT-RI indicated by increased fluorescence intensity, light blue) and monocytes (green). Additionally, some plasma cells (AS-LYMP) were also detected.

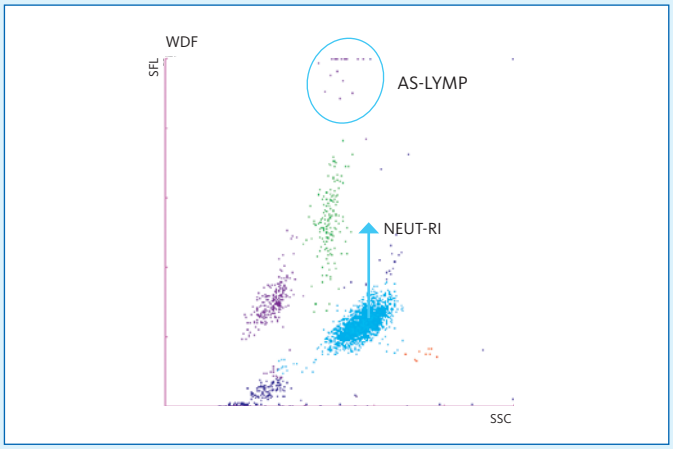

Fig. 2 *Scattergram of the patient case above*