sysmex | Together for a better healthcare journey

HAEMATOLOGY WHITE PAPER | March 2024*

Infection/Inflammation

Novel haematological parameters for investigation of the immune system response

Introduction

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 using 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 haematology analysers can provide quantitative information about the inflammatory reaction of the patient's immune system.

Haematological inflammation parameters and the immune response

Sysmex's haematology analysers offer a set of haematological inflammation parameters that makes it possible to quantitatively assess the activation status of neutrophils (NEUT-RI, NEUT-GI) and count 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 using 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, as well as the 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)'. In the previous analyser generation, these were combined in the research parameter 'high fluorescence lymphocyte count (HFLC)'.

The combination of the RE-LYMP and AS-LYMP parameters provides additional information about the cellular activation of the innate and adaptive immune response [1]. 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 the clinician in differentiating 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 make it possible to quantify:

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

Several research studies have shown that these parameters are increased in patients with infections and inflammations [2–9]. The structural neutrophil parameters NEUT-RI and NEUT-GI obtained from the XR-Series or XN-Series could predict the appearance of later-stage infection markers such as the presence of immature granulocytes [2]. Furthermore, studies found that both RE-LYMP and AS-LYMP counts were mainly increased in viral infections [3, 5]. 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). In a study cohort of children younger than five years, NEUT-RI was found to be increased in patients with bacterial infections compared to controls [5], whereas only RE-LYMP and AS-LYMP counts were significantly higher in patients with viral infections than in patients with bacterial infections. Moreover, the AS-LYMP parameter provided

Table 1 A summary of haematological inflammation parameters with their respective immunological interpretation, units and reference intervals [11].

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%[1]	Cells/L %	0.03–0.17 × 10^9/L 0.4–2.5 %
Antibody-synthesizing lymphocytes[2]	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%[1]	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)	143–157 SI
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)	42.0–50.6 FI
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%[1]	Cells/L %	0.01–0.07 × 10^9/L 0.2–1.0 %

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

the same discrimination power between viral and bacterial infections as procalcitonin in this study setting. In a cohort of bacterial infection patients with and without HIV, Lemkus and colleagues also identified NEUT-RI exhibiting the best performance and found a significant correlation with CD64, a marker for activated neutrophils [6]. 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, 8]. Oehadian *et al.* (2015) studied the high fluorescence lymphocyte count (HFLC) and found that this lymphocyte activation parameter can aid in the differentiation of dengue from leptospirosis and enteric fever [9].

Case study: Early innate immune response in a case of infection with intracellular bacteria

Case history

A 23-year-old man with an intermittent fever visited his physician three days after the initial onset of the 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 and reticulocyte 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-He (%)	1.6	+
HYPER-He (%)	0.3	–
RET# (10^9/L)	22.6	–
RET%	0.57	–
IRF (%)	5.1	
RET-He (pg)	29.8	
Delta-He (pg)	3.8	+
FRC# (10^{12}/L)§	0.0851	+
FRC%§	2.15	+

PLT parameters	Data
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 analysis results of the young man with fever and clinical focus on the lungs revealed leucocytopenia with a relative increase in neutrophils (NLR = 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 were in line with the pattern of an early innate immune response, as described for infections with intracellular bacteria [10].

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 [3]. 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 were 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 [10].

The overall results excluded extracellular bacterial infection, non-pathogenic inflammation and viral infection. The final diagnosis of suspected tuberculosis was made by a 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 a positive Ziehl-Neelsen stain sputum culture for acid-fast bacilli.

How to measure these parameters on a haematology analyser

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

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.

Conclusion

The diagnostic parameters described in this white paper can support physicians in diagnosing patients with inflammatory diseases. The haematological inflammation parameters provide additional information about an activation of the immune response. They help to recognise different types of immune response using the quantitative assessment of activation 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.

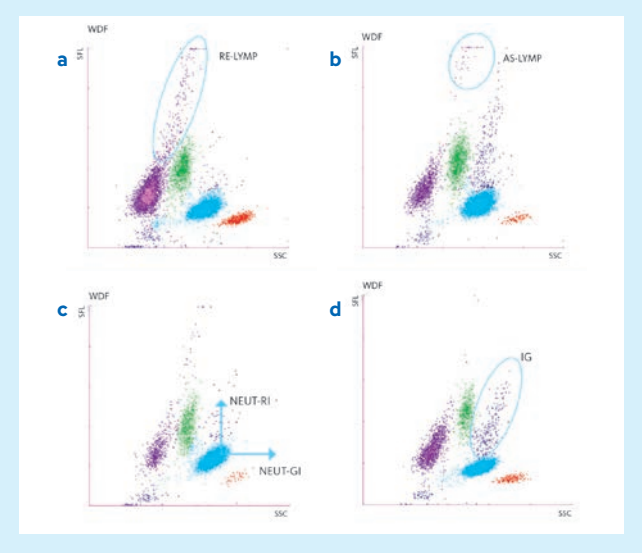

Fig. 1 Haematological inflammation parameters describing activated cell populations that appear within the course of the immune response. The scattergrams are plotted using intracellular structure (side scatter: SSC) on the x-axis and the presence of bioactive materials (side fluorescence signal: SFL)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

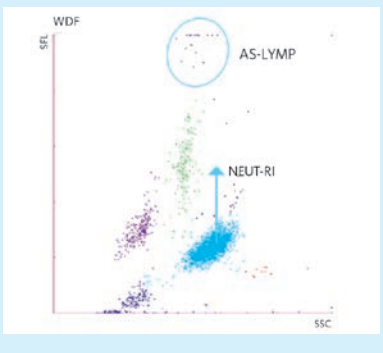

Fig. 2 Scattergram of the patient case above.

References

[1] **Linssen J *et al.* (2007):** Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/secreting cells using the automated routine hematology analyzer XE-2100. Cytometry B Clin Cytom. 72(3): 157–66.

[2] **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.

[3] **Prodjosoewojo S *et al.* (2019):** 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 13(3): e0007183.

[4] **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.

[5] **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.

[6] **Lemkus L *et al.* (2019):** The utility of extended differential parameters as a biomarker of bacteremia at a tertiary academic hospital in persons with and without HIV infection in South Africa. PLoS One. 17(2): e0262938.

[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–e1136.

[8] **Delabranche X *et al.* (2017):** Evidence of Netosis in Septic Shock-Induced Disseminated Intravascular Coagulation. Shock. 47(3): 313–317.

[9] **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): 861-8.

[10] **Muñoz Carrillo JL *et al.* (2017):** 6. Physiology and Pathology of Innate Immune Response Against Pathogens. In Rezaei N (Editor), Physiology and Pathology of Immunology (DOI: 10.5772/intechopen.70556).

[11] **L van Pelt J *et al.* (2022):** Reference intervals for Sysmex XN hematological parameters as assessed in the Dutch Lifelines cohort. Clin Chem Lab Med. 60(6): 907–920.

You can download our white papers from our website:
www.sysmex-europe.com/whitepapers