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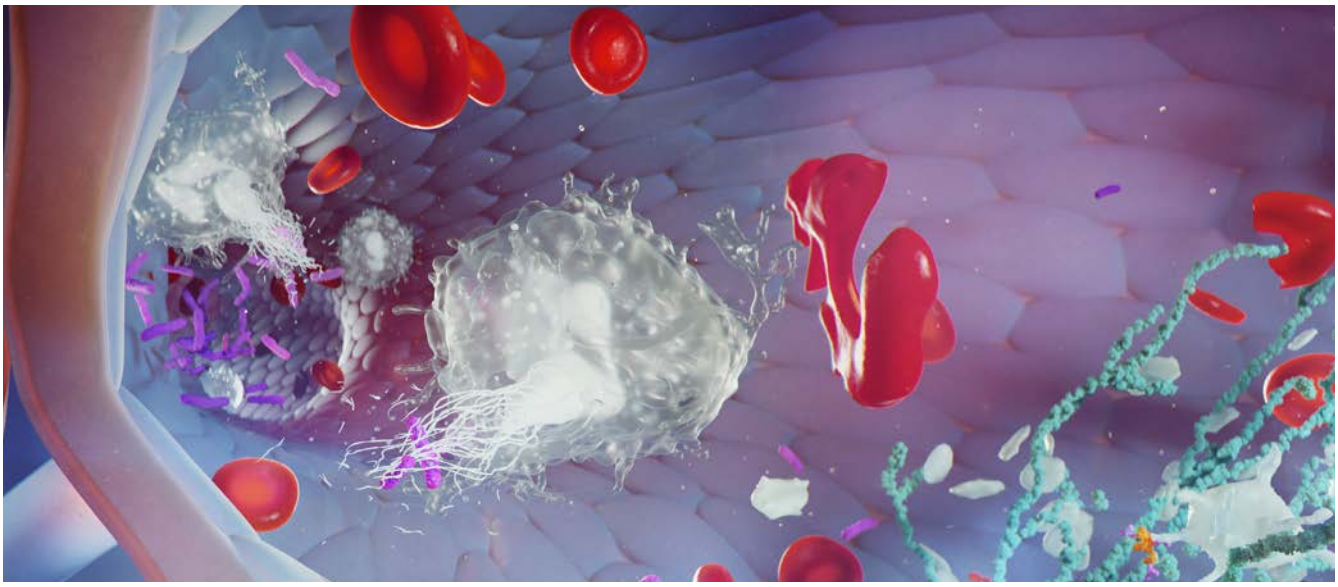
HAEMATOLOGY | April 2023

CASE REPORT

Infection

A patient with erysipelas – Delta-H*e* reflecting changes in iron availability due to an infection



The following case study has been provided and compiled by Priv.-Doz. Dr Mathias Zimmermann and describes diagnostic parameters and uses of scores that are not validated by Sysmex. Details on the Intended Use can be found in the Sysmex Instructions For Use (IFU).

The classic use of a haematology system is to establish precise results for complete blood counts and cell differential, including screening for anomalies that are usually defined more precisely in a stained blood smear. Modern analytical systems, such as Sysmex XN-10 and XN-20*, however, have the potential to provide some additional data that can be cost-efficient and quickly support the diagnosis or monitoring of treatment in specific patient cohorts.

Case description

An 82-year-old female patient visited urgent care with erysipelas and a related large-scale skin damage. The suspected diagnosis was a systemic bacterial infection with clear signs of infection, such as accelerated pulse, increased respiratory rate and increased temperature, blood pressure was reduced and the patient met the criteria for shock. The following pre-existing conditions were listed: hypertension, spinal canal stenosis, thrombosis with thrombophlebitis, renal disease, supraventricular tachycardia.

Laboratory results

Initially, CRP was significantly elevated, and the CBC showed leucocytosis. Only on the third day after admission were reticulocytes ordered: here Delta-H*e* exhibited a value in the negative range (Delta-H*e* = difference between RET-H*e* and RBC-H*e*). Reticulocyte haemoglobin (RET-H*e*) was reduced (corresponding to the negative Delta-H*e*) – a sign of an already ongoing iron sequestration due to an acute phase reaction [1].

* Including WPC channel (white precursor and pathological cells channel)

The transitory increase in transferrin despite the acute phase – transferrin is an anti-acute-phase protein – was most likely caused by the temporary iron deficiency during iron sequestration. Increased procalcitonin at admission additionally indicated a systemic bacterial infection.

During the course of treatment, CRP remained significantly elevated, RET-H*e* continued to decrease and Delta-H*e* dropped from -2.1 pg to -8 pg. The ICIS* (Intensive Care Infection Score), an RUO application on *Extended* IPU, was already elevated and reached a maximum of 9.0 on day 10 (an ICIS score › 5 indicates bacterial sepsis [2]). The patient's antibiotics were administered systemically and was changed three times in total, most recently when diarrhoea began and bacterial overgrowth of *Clostridioides difficile* in the intestine or an infection with *salmonellae/shigellae/yersiniae* was suspected. However, microbiological cultures never succeeded in detecting a pathogen. Several tests for SARS-CoV-2, influenza A, influenza B and RSV were negative too.

Between day 10 and day 13, Delta-H*e* began to normalise and showed positive values (Delta-H*e* 1.4 pg). Similarly, at day 13, the ICIS decreased to 4.0 indicating that the immune response weakens and systemic bacterial infection had already resolved [2]. However, WBC and CRP continued to remain high.

Considering Delta-H*e* and RUO ICIS results, the antibiotics could have been stopped on day 13 already. But as CRP is an established parameter for inflammatory conditions despite its delayed kinetics, antibiotics were continued.

Table 1 *Selected patient results during the course of treatment: persistent leucocytosis until day 24. Negative Delta-He and decreased RET-He available for the first time on day 3; worsening on day 5. The ICIS, which currently is available on the laboratory level, reacted similarly. An antibiotic treatment (or switching the antibiotic treatment) occurred on days 3, 5 and 17, normalisation of Delta-He and ICIS on day 13 allows the conclusion that the systemic bacterial infection had already resolved, even though CRP only dropped several days later (day 24) due to delayed kinetics.*

		06 Jan.	07 Jan.	08 Jan.	10 Jan.	13 Jan.	15 Jan.	18 Jan.	22 Jan.	25 Jan.	29 Jan.
Parameters	**Unit**	**Day 1**	**Day 2**	**Day 3**	**Day 5**	**Day 8**	**Day 10**	**Day 13**	**Day 17**	**Day 20**	**Day 24**
HGB	g/dL	12.1	11.6	11.1	11.6	10.3	9.8	10.1	9.0	8.9	8.7
WBC	10^3/µL	15.8	18.4	20.7	15.2	38.8	20.5	19.0	19.7	10.3	11.8
IG #	10^3/µL	0.15	n/a	0.18	0.72	n/a	1.24	0.95	0.38	0.22	n/a
IG%	%	0.90	n/a	0.90	4.70	n/a	6.10	5.00	1.90	2.10	n/a
RET-He	pg	n/a	n/a	30.7	23.6	26.0	27.6	33.4	32.8	33.7	36.3
Delta-He	pg	n/a	n/a	-2.1	-8.1	-6.1	-4.0	1.5	1.8	2.7	4.5
CRP	mg/L	18.4	36.2	na	22.3	22.5	14.6	18.2	14.4	10.0	3.5
PCT	ng/mL	n/a	n/a	23.2	n/a	2.5	n/a	n/a	n/a	n/a	n/a
Transferrin	g/L		3.08			1.7					
ICIS		n/a	n/a	7.0	8.0	n/a	9.0	4.0	4.0	1.0	2.0

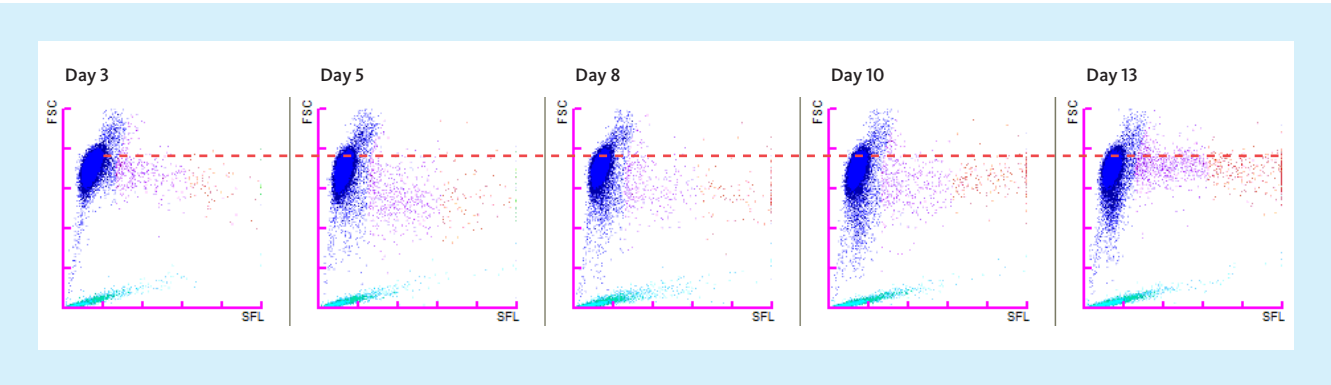


Fig 1 *RET scattergrams during the course of treatment. The RET scattergram on day 3 shows a reticulocyte cloud with reduced reticulocyte haemoglobin of the immature reticulocytes newly released bone marrow (HFR right side of the scattergram). On day 10, an improvement in erythropoiesis quality and quantity can be seen. On day 13, the reticulocyte cloud and RET-He are already back in the reference intervals. The red dotted line shows the mean of reticulocytes of a healthy individual.*

* ICIS: For research use only. Any *in vitro* diagnostic purpose has not been established by the manufacturer.

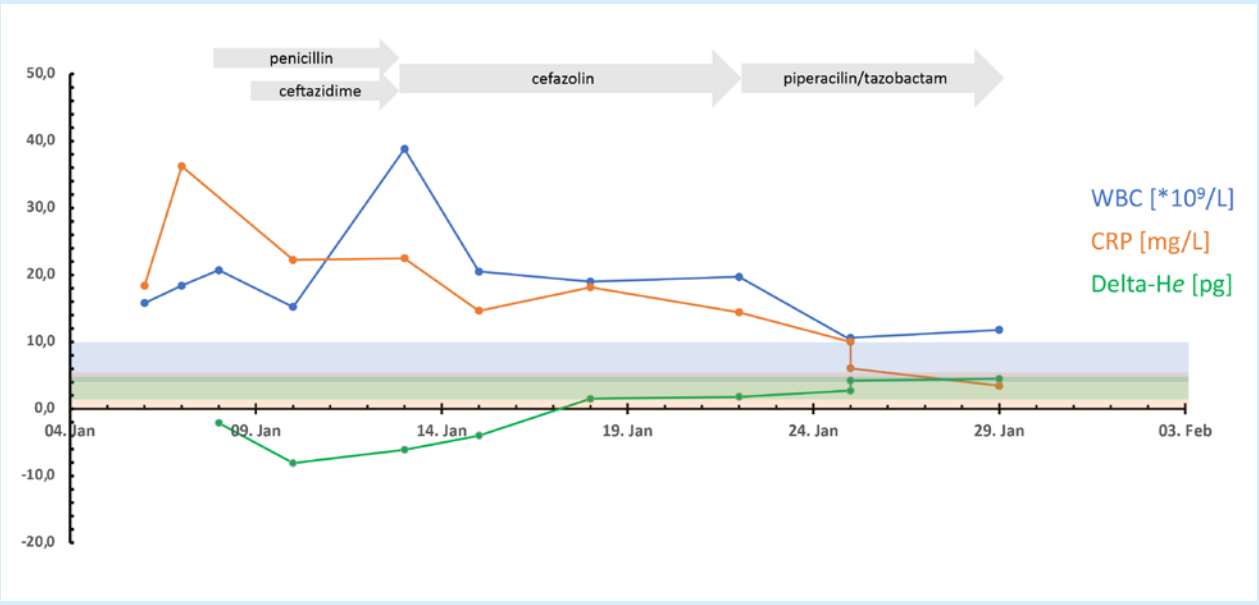


Fig 2 *Visual comparison of WBC, CRP and Delta-He. With recovery of the value, Delta-He indicates significantly earlier that the acute phase has been overcome, while CRP and WBC only react later.*

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Delta-H*e* – an indicator of changes in haemoglobin concentrations

Delta-H*e* is the difference of the measured average haemoglobin concentration of reticulocytes (RET-H*e*) and mature red blood cells (RBC-H*e*), measured in the RET channel (reference interval: 1.2 – 3.6 pg [3]).

Since reticulocytes circulate only hours up to a few days in the peripheral blood, the median haemoglobin concentration of reticulocytes – RET-H*e* – is considered a real-time marker of iron availability [4,5]. Using Delta-H*e*, a change in iron availability becomes more obvious. Thus Delta-H*e* supports the monitoring of treatment – both for absolute iron deficiency and for functional iron deficiency in the context of inflammation [6].

In a healthy person, Delta-H*e* exhibits positive values [1]. If haemoglobinisation is lower in reticulocytes, the Delta-H*e* value shifts into the negative range. Delta-H*e* as a difference can quickly indicate changes, if hepcidin is released as a result of interleukin-6 production during the acute phase of an infection. Hepcidin inhibits iron release and absorption by blocking ferroportin (Fe sequestration in the cells of the RES or RE system) and can cause production of hypochromic reticulocytes within a few hours.

When inflammatory processes are turned down again, the hepcidin level normalises and erythropoiesis quality can recover, e.g. when antibiotics are successful and the infection has resolved.

Delta-H*e*

- Reflects haemoglobinisation of RBC and thus the iron availability. Considering hepcidin signalling pathways Delta-H*e* supports conclusions about inflammatory processes.
- Allows for fast and cost-effective support in infection diagnostics.

Unlike mature red blood cells, reticulocytes contain residual nucleic acids that are stained to different degrees depending on the concentration. Thus, reticulocytes can be divided into three levels: LFR (low fluorescence ratio) corresponds to rather mature reticulocytes, while MFR (medium fluorescence ratio) and HRF (high fluorescence ratio) are assigned to immature reticulocytes. The parameter IRF (immature reticulocyte fraction) combines the parameters HRF and MFR.

In addition to the fluorescence concentration in cells, the forward scatter light is viewed at a specific angle. The reticulocyte haemoglobin equivalent RET-H*e* arises from the median forward scatter light of the reticulocytes, RBC-H*e* arises from the median forward scatter light of the red blood cells. Delta-H*e* is calculated from these two parameters (Delta-H*e* = RET-H*e* – RBC-H*e*).

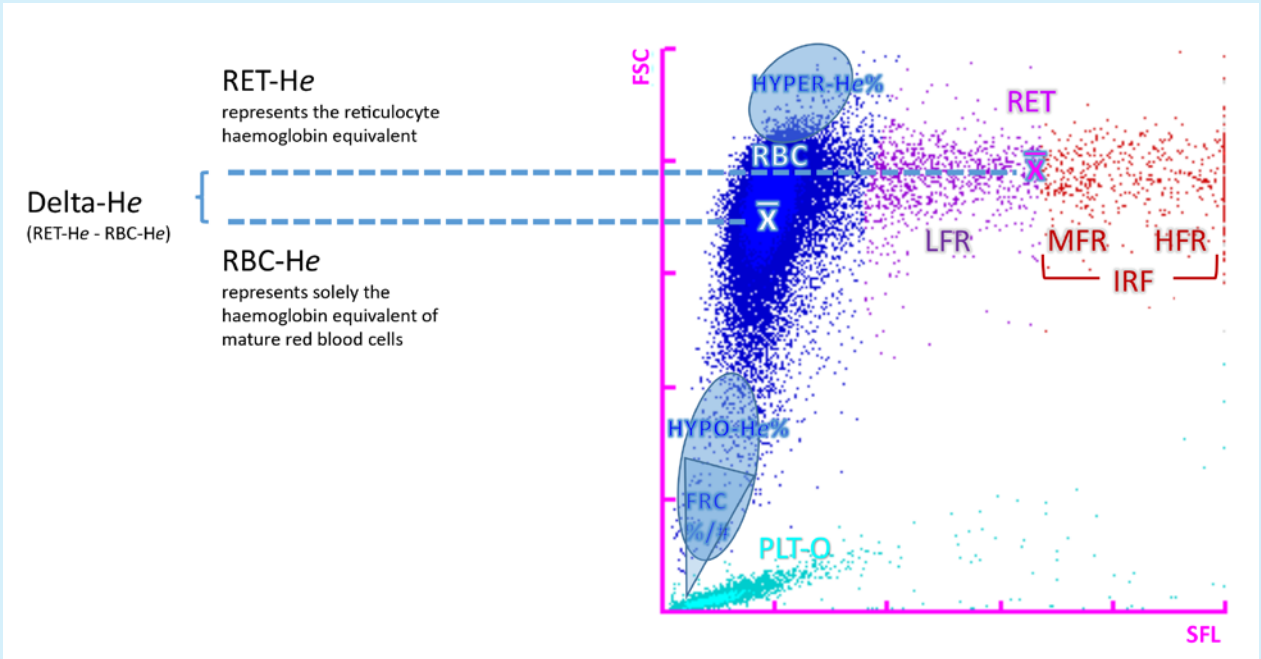

Fig 3 *Technology: RET-He/Delta-He – reticulocyte channel, overview of parameters.*

RUO Intensive Care Infection Score (ICIS)*

RUO ICIS* is a score initially developed and published by Nierhaus A *et al.* [7] to support identification of bacterial infection in critically ill patients. The score consists of parameters from the complete blood count and the reticulocyte analysis and values can range 0–20. In independent studies [2, 8], values have been shown to reflect the likelihood for bacterial infection. Beside Delta-He, the absolute count of neutrophils, immature granulocyte count (IG #), neutrophil activation measured by neutrophil reactivity index (NEUT-RI, or NEUT-SFL) and highly fluorescent lymphocytes (AS-Lymp or HFLC#) are considered parameters that record the activation status of the cells and thus potentially supports conclusions about the status of immune response.

Table 2 *Five parameters of ICIS*

Delta-He (pg)	■ Inference of iron availability ■ Indirect hepcidin marker
Neutrophils #	■ Absolute number of neutrophil granulocytes ■ Bacterial infections are associated with elevated values
IG #	■ Absolute number of immature granulocytes ■ Promyelocytes, metamyelocytes, myelocytes ■ Associated with the severity of a bacterial infection
NEUT-RI (FI)**	■ Average fluorescence intensity of mature (segmented) neutrophils ■ Marker of metabolic neutrophil activity ■ Increase in NEUT-RI reflects the production of pro-inflammatory cytokines as an early marker of the immune response
AS-Lymp #**	■ Absolute number of antibody-synthezing lymphocytes ■ Differentiated B lymphocytes, plasma cells (adaptive immune response) ■ Or T cell-independent B lymphocytes during the acute phase (IGM antibodies) of the innate immune response

** Extended Inflammation Parameters (optional). Optional application - depending on software status. An underlying malignant disease must be excluded for the evaluation of these parameters in the context of infections.

References

[1] ***Zuther M et al. (2022):*** *Improved Diagnosis of Iron Deficiency Anemia in the Critically Ill via Fluorescence Flowcytometric Hemoglobin Biomarkers. Cells. 12(1): 140.*

[2] ***Weimann K et al. (2015):*** *Intensive Care Infection Score – A new approach to distinguish between infectious and noninfectious processes in intensive care and medicosurgical patients. J. Int. Med. Res. 43(3): 435–51.*

[3] ***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): 907–20.*

[4] ***Brugnara C et al. (2006):*** *Reticulocyte hemoglobin equivalent (RET-He) and assessment of iron-deficient states. Clin Lab Haematol. 28(5): 303–308.*

[5] ***Mast AE et al. (2008):*** *Reticulocyte hemoglobin content. Am J Hematol. 83(4): 307–310.*

[6] ***Weimann A et al. (2016):*** *Delta-He, RET-He and a New Diagnostic Plot for Differential Diagnosis and Therapy Monitoring of Patients Suffering from Various Disease-Specific Types of Anemia. Clin Lab; 62(4): 667.*

[7] ***Nierhaus A et al. (2012):*** *Use of a Weighted, Automated Analysis of the Differential Blood Count to Differentiate Sepsis from Non-Infectious Systemic Inflammation: The Intensive Care Infection Score (ICIS). Inflamm. Allergy Drug Targets 11(2): 109–15.*

[8] ***van der Geest PJ et al. (2016):*** *The intensive care infection score – a novel marker for the prediction of infection and its severity. Crit. Care 20(1): 18.*

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