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Haematologic malignancies

Early identification of haematologic malignancies using a complete blood count

Every 35 seconds somewhere in the world an individual is diagnosed with blood cancer. Being diagnosed with haematologic malignancy actually happens either because the patient presents with certain symptoms or – as is becoming increasingly common – there are incidental findings during routine blood screening on modern haematology analysers [1-3].

Symptoms that suggest a possible underlying haematologic malignancy are various and may relate to the effect of the malignancy on bone marrow function, invasion of the lymph nodes and spleen, destruction of tissue (bone) and increased metabolism. Although these symptoms need to be considered in the clinical context, they may be a first sign of possible haematologic malignancy.

However, in case of chronic, asymptomatic conditions it becomes increasingly common for patients to be diagnosed with haematologic malignancy based on incidental findings obtained from routine screening. A raised white blood cell (WBC) count found via a routine blood analysis performed, for example, as part of a health screen may indicate the presence of a yet undiscovered chronic leukaemia. A blood count may reveal significant abnormalities and lead to an urgent referral to a specialist; however, with some malignancies the basic WBC differential may not necessarily appear abnormal – e.g. in patients presenting with lymphoma [1].

The complete blood count (CBC, also referred to as 'full blood count – FBC') is the most frequently ordered laboratory test in both the inpatient and outpatient medical setting. Modern automated haematology analysers are used for quantitative high-throughput analysis. Moreover, they are widely used for the sensitive identification of pathological samples by different alerts for subsequent smear review (e.g. using microscopy).

The latest generation of Sysmex XN-Series analysers enables detecting pathological cells from a blood sample with a high degree of sensitivity. However, especially from the perspective of the laboratory's workflow and costs, keeping the number of unnecessary follow-up tests to a minimum is equally important. Thus, the ideal modern haematology analyser detects neoplastic cells sensitively and specifically.

The following text describes how a complete blood count measured on Sysmex XN-Series analysers may reveal significant abnormalities and improve the incidental findings of haematologic malignancies or the relapse of cancer under treatment early on.

Sensitive detection of nucleated red blood cells can point towards malignancies early on

Nucleated red blood cells (NRBC) are immature red blood cell precursors that are not present in the circulation of healthy adults. For already several years the counting of these cells has been available on haematology analysers. However, only with the introduction of the XN-Series it has become possible to determine NRBC routinely and accurately with each measurement.

NRBC are associated with many haematologic disorders including haematologic malignancies, and the finding of NRBC should prompt the evaluation of a peripheral blood smear (see Figure 1) [3, 4]. One of the reasons why NRBC may become present in peripheral blood is damage or stress to the bone marrow, which is often the case in haematologic malignant diseases. Table 1 summarises relevant publications on NRBC.

For example, in a study of 478 patients with haematologic diseases, the frequency of NRBC positivity at diagnosis was highest in patients with chronic myeloid leukaemia (100 %), acute leukaemia (62 %), and myelodysplastic syndromes (45 %) [5]. NRBC can also be seen in peripheral blood, for example in case of bone marrow metastasis or extramedullary haematopoiesis. Even the screening of children for increased NRBC has revealed – besides the highest probability of underlying disorders being hypoxia (49 %) – malignancies (8 %) to be an important cause of normoblastaemia [6]. The comprehensive performance evaluation of the NRBC count of five haematology analysers found the best precision for Sysmex XN-Series with a very low limit of quantification (LoQ) of 0.029 $\times 10^9$/L [7]. A study by Bruegel M *et al.* also showed the best NRBC count for Sysmex XN-Series [8]. Due to such excellent performance in samples even at very low concentrations of NRBC, a case study report using the XN-Series analyser showed the presence of NRBC in the peripheral blood of a patient more than one year before the diagnosis of primary myelofibrosis, which highlights the value of routine NRBC analysis of blood samples when screening for many relevant haematologic malignancies in an early subclinical stage [2].

The NRBC count is part of the CBC even if no WBC differential has been ordered. However, ordering the differential count provides clinicians not only with the quantitative information on different WBC subpopulations, but also enhances the analytical depth in order to sensitively detect possible pathological cells circulating in the blood of the patient very early on.

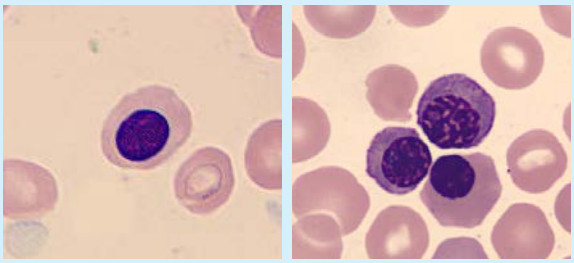

Fig. 1 *Random selection of nucleated red blood cells in the peripheral blood smear. Among other pathological conditions the incidental finding of solitary NRBC can give a sign of various malignancies early on.*

Table 1 *Summary of publications on NRBC related to sensitivity performance and clinical relevance to haematologic malignancies*

Clinical relevance of NRBC to haematologic malignancies	Performance of the NRBC count on XN-Series
Buoro S *et al.* (2016) ■ Case study that shows excellent performance in samples of very low NRBC concentrations and highlights the value of routine NRBC analysis of blood samples in an early subclinical stage [2].	**Da Rin G *et al.* (2017)** ■ Sysmex XN-Series shows best precision amongst five haematology analysers with a very low limit of quantification (LoQ) of 0.029 × 10^3/L [7].
Danise P *et al.* (2011) ■ The frequency of NRBC positivity at diagnosis was highest in patients with chronic myeloid leukaemia (100 %), acute leukaemia (62 %), and myelodysplastic syndromes (45 %) [5].	**Bruegel M *et al.* (2015)** ■ Best NRBC count on Sysmex XN-Series [8].
Sills RH *et al.* (1983) ■ Increased NRBC revealed, besides underlying disorders with hypoxia (49 %), malignancies (8 %) as an important cause of normoblastaemia [6].	

Sensitive recognition of immature precursor cells for the detection of malignant samples

In peripheral blood the appearance of immature granulocytes (IG) is quite a common finding in infections, inflammations, haematologic malignancies or other factors that stimulate the bone marrow. Circulating immature precursor cells often indicate malignant diseases such as acute myeloid or lymphoid leukaemia, but also other pathologies such as myelodysplastic syndromes or myeloproliferative neoplasms. Based on a unique measuring technology and reagents in combination with proprietary algorithms, modern haematology analysers will recognise samples with specific pathological cell types and abnormal populations of WBC, including immature granulocytes, blasts and abnormal lymphocytes.

Table 2 summarises interesting publications on the detection and recognition of immature WBC.

Table 2 *Publications on the detection of immature WBC*

Detection and recognition of immature WBC
Blomme S *et al.* (2020) ■ Excellent sensitivity (99 %) for pathological cells using WPC channel analysis and WPC reflex testing led to a reduction of the blood smear review rate by 12 % [9].
Schuff-Werner P *et al.* (2016) ■ Very good performance of the XN-Series in detecting leucocytosis of neoplastic and reactive origin [10].
Bruegel M *et al.* (2015) ■ XN-Series showed superior sensitivity for the presence of blasts, abnormal lymphocytes and IG in an inter-instrument comparison and outperformed other analysers in flagging, especially blast flagging was found to have significantly better sensitivity [8].

Blomme S *et al.* evaluated the diagnostic performance of the XN-Series analyser as well as the workflow impact in the laboratory using a total of 630 preselected abnormal patient blood samples. The testing showed excellent sensitivity (99 %), important for an excellent screening device, but low specificity (29 %). This means when abnormal cell populations are present, the XN-Series analyser can detect them and provide more information on the abnormal cell type reported, helping morphologists classify them into clear categories: 'Blasts?' or 'Abnormal lymphocytes?'. This is especially relevant to newly discovered haematologic malignancies where XN-Series technology remains important for its additional information on the abnormal cell types [9].

A study by Bruegel M *et al.* showed that the XN-Series has a superior sensitivity for the presence of blasts, abnormal lymphocytes and IG in a large inter-instrument comparison of pathological flags in 349 samples taken randomly from routine analysis [8]. Importantly, the XN-Series outperformed the other analysers in flagging, while specificity was comparable between analysers and thus not increasing the number of false positive samples leading to an increased smear review burden for the laboratory. An essential role of modern routine haematology analysers in clinical practice beyond the complete blood count is to screen sensitively for samples potentially containing pathological cells. For blast flagging, which is one of the clinically most relevant warning messages, a significantly better sensitivity was found for XN-Series in comparison with other analysers [8]. Another study found a very good performance of the XN-Series in detecting leucocytosis of neoplastic and reactive origin [10].

Timely detection of potentially unrecognized cases of chronic myelomonocytic leukaemia (CMML)

It is important to know whether the cause of monocytosis is an underlying reactive condition or a malignant haematologic disease (see Figure 2). Typically, the laboratory smear review criteria for monocytosis and the recommended WHO diagnosis criteria show a gap which could lead to unrecognised cases of CMML. Recently, Schillinger F *et al.* developed and validated a 'Mono-dysplasia score' based on Sysmex XN's monocyte count and structural parameters and found this to be a reliable tool for the timely recognition of CMML cases out of all monocytosis cases [11]. The score was tested on a validation cohort of 1,809 samples in which 26 cases of CMML were present and yielded a sensitivity of 92.3 % and a specificity of 93.6 %. Using this new tool enables a confident recognition of CMML at any time and irrespective of the experience level of the laboratory staff, and thus supports a faster diagnosis of CMML.

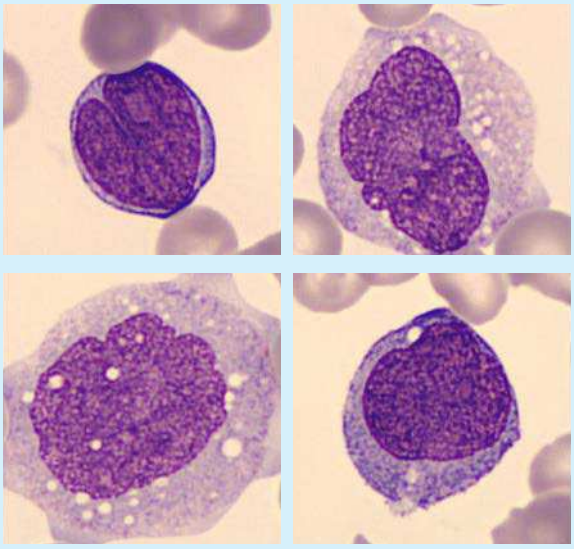

Fig. 2 *Random selection of reactive monocytes and monoblasts. Examination of the blood smear is the first step to identify cytological arguments of CMML which can be discrete: dysplastic abnormalities, immature granulocytes, promonocytes and/or few blasts. Such analysis requires experienced laboratory staff and is subject to poor inter-operator reproducibility. In reactive monocytosis, a slide review is not necessary, if no suspicion has been raised in the initial analysis on the haematology analyser [11].*

Diagnostic aid in identifying patients with a high likelihood of myeloproliferative diseases

Early diagnosis of patients with essential thrombocythaemia (ET) and polycythaemia vera (PV) is desirable in order to instigate monitoring and/or appropriate treatment to prevent thrombosis. Several studies found an association of the parameter 'immature platelet fraction' (IPF) that helps in identifying a cohort of high-likelihood patients and to refer them for haematologic review [12–14]. These findings revealed that the JAK2 V617F mutation is linked to the quantity of IPF in patients with such myeloproliferative diseases. Due to the immature platelets' higher haemostatic potential than mature platelets, they might even contribute to the prothrombotic phenotype in those patients, making the determination of the IPF parameter worthwhile in preventing adverse thrombotic conditions.

Early detection of multiple myeloma due to reliable erythrocyte sedimentation rate (ESR) measurement

Multiple myeloma (MM) is a neoplastic plasma cell disorder that is characterised by a clonal proliferation of malignant plasma cells in bone marrow. It can manifest with a myriad of non-specific signs and symptoms and thus diagnosis is frequently delayed [15–16]. Due to non-specific symptoms such as musculoskeletal pain and tiredness, these patients are more likely than those with other malignancies to have three or more general practitioner visits before a secondary care referral is initiated [17]. Delayed diagnosis of MM is associated with an increased risk of complications (e.g. bone disease, anaemia and renal failure) and reduced overall survival [15, 18]. MM is also more likely than other malignancies to be diagnosed after emergency presentation. This is considered as an indicator of delay and associated with poorer outcomes [17, 19].

The combined determination of CBC and ESR is currently recommended when multiple myeloma is suspected. Moreover, ESR has been reported as a prognostic marker for myeloma with higher values of ESR associated with a more advanced malignancy stage [20].

ESR is one of the oldest and most frequently requested blood tests. It has not changed fundamentally in the way it is carried out since Westergren described it first in 1921. In 2011, the International Council for Standardization in Hematology (ICSH) and the Clinical and Laboratory Standards Institute (CLSI) recommended the Westergren method as reference method for ESR measurement.

Using the Westergren method, Interrliner XN integrated into Sysmex haematology automation lines can process ESR measurements from the same EDTA tube in a single run together with the routine CBC. M-proteins are found in conditions such as MM or Waldenstrom's macroglobinaemia and will enhance rouleaux formation. Raijmakers MTM *et al.* investigated the effect of different M-protein classes on the ESR in Westergren and alternative ESR methods and found a large divergence of ESR values especially above 40 mm/h. They concluded that non-Westergren methods are not a good indicator for the detection of patients with an M-protein like in the case of MM disease [21]. Therefore, using the Westergren method for ESR measurement, such as on Interrliner XN, is of utmost importance for an early and reliable diagnosis of suspected MM.

Conclusion

Modern haematology analysers deliver much more than just basic cell counts and are valuable tools in identifying patients' pathologic conditions early on.

Sysmex XN-Series analysers support

- early findings of haematologic malignancies due to the sensitive detection of various pathological cells.
- indicating damage or stress to the bone marrow by a very sensitive detection of NRBC – performed with every blood count.
- sensitive recognition of immature precursor cells – with every differential blood count – considered 'best in class'.
- a new score for the timely detection of potentially unrecognized cases of chronic myelomonocytic leukaemia (CMML).
- identifying patients with a high likelihood of myeloproliferative diseases and starting the supportive care early – with the help of the IPF.
- early detection of multiple myeloma using the Westergren method of erythrocyte sedimentation rate (ESR) on the Interrliner XN.

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