sysmex | Together for a better healthcare journey

HAEMATOLOGY WHITE PAPER | April 2024*

White blood cell functionality

Sensitive assessment of WBC functionality and greater workflow efficiency

Differentiate with confidence between suspected malignant and reactive samples

Failing to identify malignant conditions is one of clinical laboratories' greatest concerns as it has serious implications for patients' health. Avoiding false negative results and detecting all malignant samples – with the greatest possible sensitivity – is therefore crucial. Relying on manual smear reviews is not recommended as this introduces high variability in interpreting lymphocyte morphology. There is also high statistical variation at low counts. Automated haematology analysers can help out here.

Yet it's not just about great sensitivity. Once a malignant condition is suspected, laboratories need to perform time-consuming and expensive follow-up tests. As such, analysers also have to exclude false positive results so one can support diagnoses faster and keep costs under control. By combining results from the DIFF analysis and the white precursor and pathological cell (WPC) channel, XN-Series and XR-Series (Sysmex analyser) deliver both highly sensitive and specific detection of reactive and suspected malignant samples.

Combining two analysis channels using fluorescence flow cytometry inside a single analyser supports you to detect suspected malignant samples sensitively and specifically. This is achieved by detecting the differences in cell functionality of the different white blood cells.

The DIFF measurement

In the white blood cell differential (WDF) channel, fluorochrome labelling depends on the white blood cells' membrane composition and cytoplasmic content. The lipid membrane composition of activated or immature cells is different to that of non-reactive and mature cells.

A unique combination of reagents (lysis and labelling) and incubation time permits to separate different cell populations. First, the lysis reagent perforates cell membranes, whereby the extent of membrane damage depends on the lipid composition, which in turn depends on the cell type (maturity level) and the state of the cell (activation status).

* Revision of the original article published in March 2017.

Next, the fluorochrome marker labels mostly RNA in the cytoplasm (Fig. 1). The intensity of the resulting fluorescence signal depends on the degree of membrane perforation (lipid composition) and the total amount of RNA in the cytoplasm. The information about membrane composition and cytoplasmic RNA (fluorescence), cell volume (forward scatter) and intracellular structure (side scatter) is analysed with proprietary algorithms that deliver sensitive detection of reactive, immature or pathological cells in a blood sample.

The white precursor and pathological cell (WPC) channel

The WPC channel's lysis reagent has a greater effect on the membrane lipids due to a different surfactant and a longer incubation time compared to the WDF channel. In addition, the fluorescence reagent has a higher polymethine concentration and, consequently, the DNA of the nucleus is labelled.

An example of how membrane lipid composition is affected by a cell's functionality or activation status is the presence of so-called 'lipid rafts'. Lipid rafts are cholesterol- and glycosphingolipid-rich microdomains in the cellular plasma membranes that play important roles in protein trafficking and cellular signalling. Lipid rafts are more ordered and tightly packed than the surrounding membrane bilayer, but float freely in this bilayer.

Elevated levels of lipid rafts in the cell membrane have been reported in more active cells in extracellular communication (e.g. malignant and activated mature cells) compared to resting mature cells and immature cells [2, 3]. The greater permeabilisation of some cell types, such as abnormal lymphocytes, leads to cytoplasmic loss and a smaller cell size (forward scatter signal). Therefore, while the WDF channel hints mostly at cytoplasmic activity, the WPC channel detects abnormal cells by their membrane composition, resulting in differences in size (shrinkage of some cell types) and higher access to the DNA content, which gets labelled more intensely (Fig. 1).

By combining both channels – and their respective sets of reagents and reaction conditions – both the sensitivity and specificity for detecting reactive and malignant cells is optimised. As illustrated in Fig. 2, the WDF channel can identify most of the negative and some of the reactive samples. Some samples are suspected of containing either malignant or normal cells (Fig. 2: 'Malignant or negative?'), while others are suspected of containing either malignant or reactive cells (Fig. 2: 'Malignant or reactive?'). Samples that fall into either of these two categories are then further classified by an automated reflex measurement in the WPC channel.

The WPC channel can classify suspect samples into one of three clearly defined categories (reactive, suspected malignant or negative). This lets laboratories classify all samples into one of those categories and characterise reactive conditions further, once a suspected malignant condition has been excluded.

These categories translate into analyser flags that have the following meaning: 'suspected malignant' means the triggering of either 'Blasts?' and/or 'Abn Lympho?' flags, whereas 'reactive' refers to the flag 'Atypcial Lympho?'. In doing so, the Sysmex analysers support the idea of classifying lymphocytes according to the European consensus report on blood cell identification, which suggests, for grouping atypical lymphocytes, the use of the groups 'Atypical lymphocytes, suspect reactive' and 'Atypical lymphocytes, suspect neoplastic' [4].

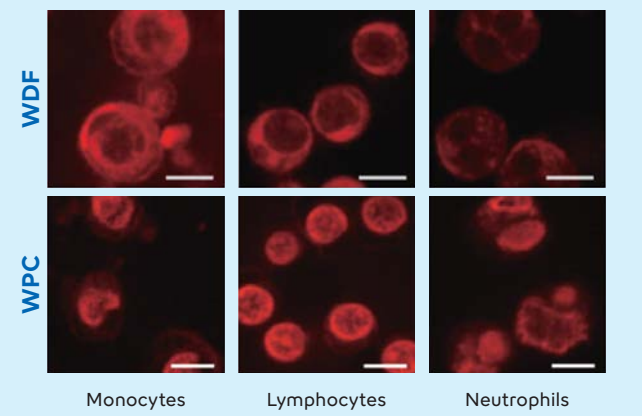

Fig. 1 Fluorescence micrographs of three cellular populations after labelling with WDF and WPC reagents. The WDF fluorochrome marker labels mostly the RNA in the cytoplasm whereas the WPC fluorochrome marker labels mostly the DNA in the nucleus. Bar width = 5 µm. Adapted from Kawauchi et al. [1].

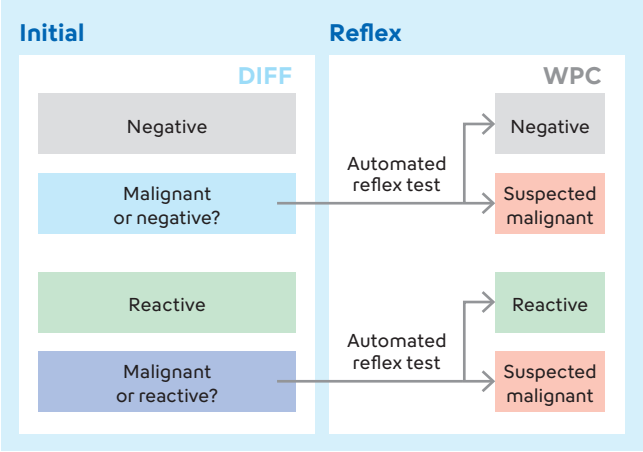

Fig. 2 The Sysmex analysers' dual-level approach to classify samples into three different, well-defined categories: negative, reactive (flag 'Atypical Lympho?') and suspected malignant (either flag 'Blasts?' and/or flag 'Abn Lympho?').

A study by Bruegel *et al.* [5] showed that the XN-Series has a superior sensitivity for blasts and abnormal lymphocytes in a large inter-instrument comparison of pathological flags in 349 samples taken randomly from routine analysis (Table 1). Another study [6] found very good performance of the XN-Series in detecting leucocytosis of neoplastic and reactive origin (Table 2). The authors concluded that the XN-Series analyser has a sensitivity and specificity similar to morphological slide review.

Other studies revealed excellent flagging sensitivity and specificity of Sysmex analysers in various patient populations for blasts, abnormal and atypical lymphocytes [7–12] as compared to manual peripheral blood smear review.

Table 1 Sensitivity, specificity, positive and negative predictive value for flagging pathological samples on five different analysers, using 349 samples taken randomly from routine analysis. Adapted from Bruegel *et al.* [5].

Reference based on microscopy	N	Analyser	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Blasts ('Blasts?' flag)	34	Sapphire	76	93	55	97
		DxH 800	74	95	63	97
		Advia 2120i	65	97	65	97
		XE-5000	65	98	79	96
		XN-2000	**97**	**96**	**70**	**100**
Lymphoma cells ('Abn Lympho?' flag)	25	Sapphire	56	94	44	96
		DxH 800	64	94	47	97
		Advia 2120i	72	88	31	98
		XE-5000	80	95	54	99
		XN-2000	**80**	**95**	**59**	**98**
Neoplastic cells ('Blasts?' and/or 'Abn Lympho?' flags)	57	Sapphire	74	95	72	95
		DxH 800	81	95	75	96
		Advia 2120i	77	94	71	96
		XE-5000	75	96	80	95
		XN-2000	**96**	**94**	**75**	**99**

Table 2 Performance of the XN-Series for detecting white blood cells of reactive and neoplastic origin. Adapted from Schuff-Werner *et al.* [6].

Reference based on microscopy, immune phenotyping and clinical diagnosis	N	Analyser	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Blasts ('Blasts?' flag)	30	Morphology	93	99	90	99
		XE-2100	90	39	17	97
		XN-2000	**93**	**96**	**74**	**99**
Lymphoma cells ('Abn Lympho?' flag)	18	Morphology	89	99	89	99
		XE-2100	78	62	14	97
		XN-2000	**89**	**97**	**70**	**99**
Neoplastic cells ('Blasts?' and/or 'Abn Lympho?' flags)	48	Morphology	92	98	92	98
		XE-2100	85	41	25	92
		XN-2000	**94**	**93**	**75**	**99**
Reactive lymphocytes ('Atypical Lympho?' flag)	35	Morphology	91	100	97	100
		XE-2100	63	77	31	98
		XN-2000	**86**	**98**	**86**	**99**

Confident characterisation of reactive conditions by quantitative parameters

When reactive cells are present, the patient is suspected of having an inflammation with or without an infection, so that it is important to rapidly differentiate between various reactive conditions. For example, clinicians need to determine the appropriate treatment for their patients and avoid the overuse of antibiotics, e.g. in case of viral infections.

Correctly diagnosing suspected infections based on clinical examination, biochemical markers and microbiological blood cultures is both costly and time-consuming. However, if the laboratory has the possibility of a fast initial indication, the right follow-up test can be performed and consequently, the clinician can start, change or adapt treatment faster.

The 'Extended Inflammation Parameters' let one quantify activated lymphocytes and neutrophils, and the results can be applied once a suspected malignant condition has been excluded. The combination of the RE-LYMP and AS-LYMP parameters, which quantify the numbers of all reactive lymphocytes and antibody-synthesizing lymphocytes, respectively, provides additional information about the cellular activation of the innate and adaptive immune response. Furthermore, the granularity and reactivity of neutrophils (NEUT-GI and NEUT-RI, respectively) reflect innate immune response to bacterial infections [13].

Even though RE-LYMP and AS-LYMP are measured in the WDF channel, they are of limited use without the WPC channel analysis, since malignancies cannot be excluded by the WDF channel for approximately 60 % of reactive samples. For example, in a dataset consisting of 7,782 CBC+DIFF samples from a regional hospital, out of 255 reactive samples (confirmed with the 'Atypical Lympho?' flag from the WPC channel) 148 were given the flag combination 'Atypical Lympho?' and 'Blasts/Abn Lympho?' in the initial DIFF measurement. For these 148 samples, the values of RE-LYMP and AS-LYMP were unreliable due to suspected malignant conditions. So for only 107 samples out of this dataset of 255 samples the 'Atypical Lympho?' flag was triggered as a single flag, which would have permitted the use of these parameters straight away. The Extended Inflammation Parameters and their clinical use are explained in our white paper 'Novel haematological parameters for rapidly monitoring the immune system response'.

Workflow implications

Improved workflow thanks to fewer false positive malignant samples

High specificity is important for reducing the number of false positive malignant samples. Smear reviews to confirm the presence of malignant cells can be reduced significantly when running analyses that combine the WDF and WPC channels. For example, in the dataset mentioned before (7,782 CBC+DIFF samples from a regional hospital), 51 samples (8 %) out of 665 samples with the flag 'Blasts/Abn Lympho?' from DIFF analysis and subsequent WPC measurement could be reclassified as 'negative' by the WPC channel and 60 samples (9 %) could be reclassified as 'reactive'. Thus, in total, 111 samples (17 %) with 'Blasts/Abn Lympho?' flag could be reclassified as 'non-malignant'. Table 3 below summarises the reduction in the number of suspected malignant samples from several studies using different patient populations.

In conclusion, smear reviews to confirm the presence of malignant cells can typically be reduced by approximately 10–35 % (routine haematological laboratory) and over 40 % (specialised laboratory with a high proportion of positive samples) when combining the WDF and WPC channels for analysis.

Table 3 Summary of published results on reducing suspected malignant samples with a combined analysis in Sysmex's WDF and WPC channels.

Publication	Number of patients	Patient population	Reduction of suspected malignant samples with Sysmex's WDF and WPC channels
Seo *et al.* [14]	1005	Adults – malignancies	63 % compared to XE-2100*
Schuff-Werner *et al.* [6]	253	Adults – malignancies and reactive conditions	41% compared to XE-2100
Briggs *et al.* [15]	1000	Routine blood samples, university hospital	49 % compared to XE-2100*
Noordegraaf *et al.* [16]	1778	All routine blood samples, general hospital	26 % compared to DIFF alone**
Schapkaitz *et al.* [10]	275	Routine adult and paediatric blood samples, university hospital	34 % compared to DIFF alone
Sejrup *et al.* [17]	117	Routine blood samples, university and regional hospital	18 % compared to DIFF alone
Schoorl *et al.* [18]	2011	All routine blood samples, general hospital	27 % compared to DIFF alone
Blomme *et al.* [11]	630	Routine blood samples (including children and infants), university hospital	12 % compared to DIFF alone
Dedeene *et al.* [12]	1889	Routine blood samples, university hospital	16 % compared to DIFF alone

** Reported smear reduction based on samples with malignant and reactive flags ('Blasts?', 'Abn Lympho?', 'Atypical Lympho?')
** Based on the reported data (221/847).

WPC channel and laboratory cost implication

As explained in the paragraph above the use of the WPC channel leads to a significant reduction of microscopic blood smears. Apart from the support of morphologists in smear review with a focus on specific cell types and pathologies, there is an obvious associated cost-related question of whether the reduction in smears due to WPC measurement counterbalances the investment of the analysis module with the WPC channel and associated reagent costs with that. As the degree of reduction of unnecessary blood smears by the WPC channel is dependent on the tested patient group and various other factors such as e.g. depreciation period of the analyser, cost of smear and smear review, etc. there is only one published result from such cost-associated study [16]. However, Sysmex performed a comprehensive analysis by calculating the costs and benefits based on broad statistical data and variables from different laboratories.

The internal analysis confirmed that the WPC channel leads to a significant reduction in smear reviews. When factoring in the costs for manual smear reviews, a minimum of 300 CBC+DIFF samples per day would be typically needed to compensate the costs of the WPC channel. The break-even point was calculated from the fixed and operational costs of the WPC channel versus the costs of saved smear reviews in the laboratory (Fig. 3).

In conclusion, depending on the WPC associated laboratory costs and laboratory sample composition, 300 CBC+DIFF samples or more per day would typically lead to a reduction in the overall laboratory costs.

Focused slide review

The information from the WPC channel can also help morphologists as confirmed suspected malignant samples are further classified into clear categories: samples containing blasts ('Blasts?' flag) and samples containing abnormal, neoplastic lymphocytes ('Abn Lympho?' flag). This lets morphologists focus on specific cell types and pathologies in a follow-up smear review. Fig. 4 summarises the possibilities for improving the workflow with the WPC channel.

The examples of three clinical cases distinguished by the use of the WPC channel (reactive sample, neoplastic lymphocytosis, and neoplastic disease with blasts) are shown in Fig. 5.

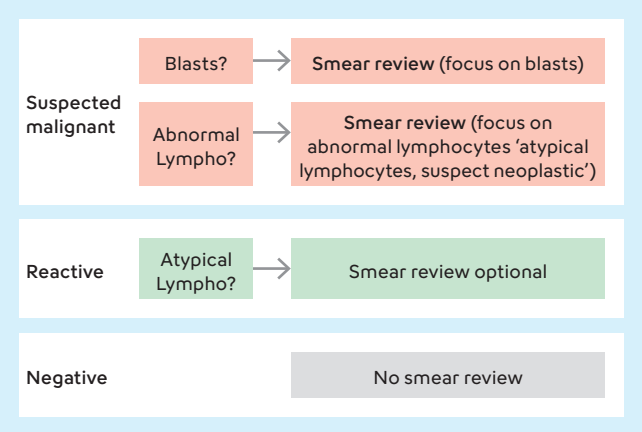

Fig. 4 Suggested smear workflow based on the information from WPC analysis. Suspected malignant samples are categorised, enabling one to focus on specific cell types in a follow-up smear review. For samples classified as 'negative' or 'reactive', unnecessary smear reviews can be avoided.

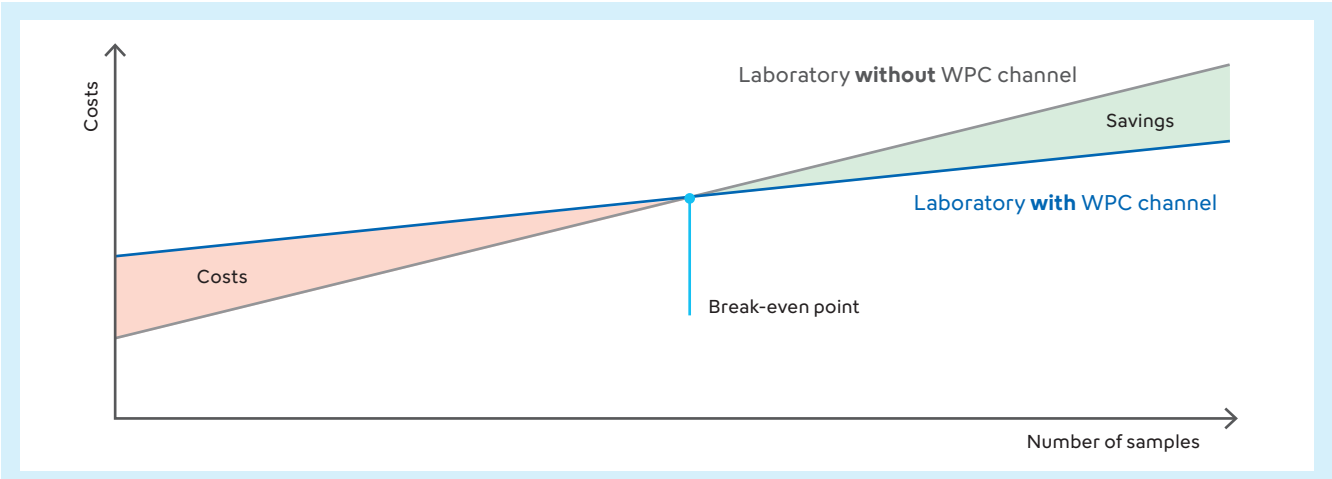

Fig. 3 Illustration of cost analysis for a laboratory equipped with the WPC channel compared to a laboratory without the WPC channel. The grey line represents the total costs of the analysis for a given number of samples (X-axis) for a laboratory equipped with Sysmex analysers without the WPC channel. The blue line represents the total costs of the analysis for a given number of samples for a laboratory equipped with the WPC channel. Even though the initial investment costs for the WPC channel are higher, at a certain total number of analysed samples the break-even point is reached. The break-even point is the point at which the savings from skipped smears equals the invested WPC hardware and increased reagent costs.

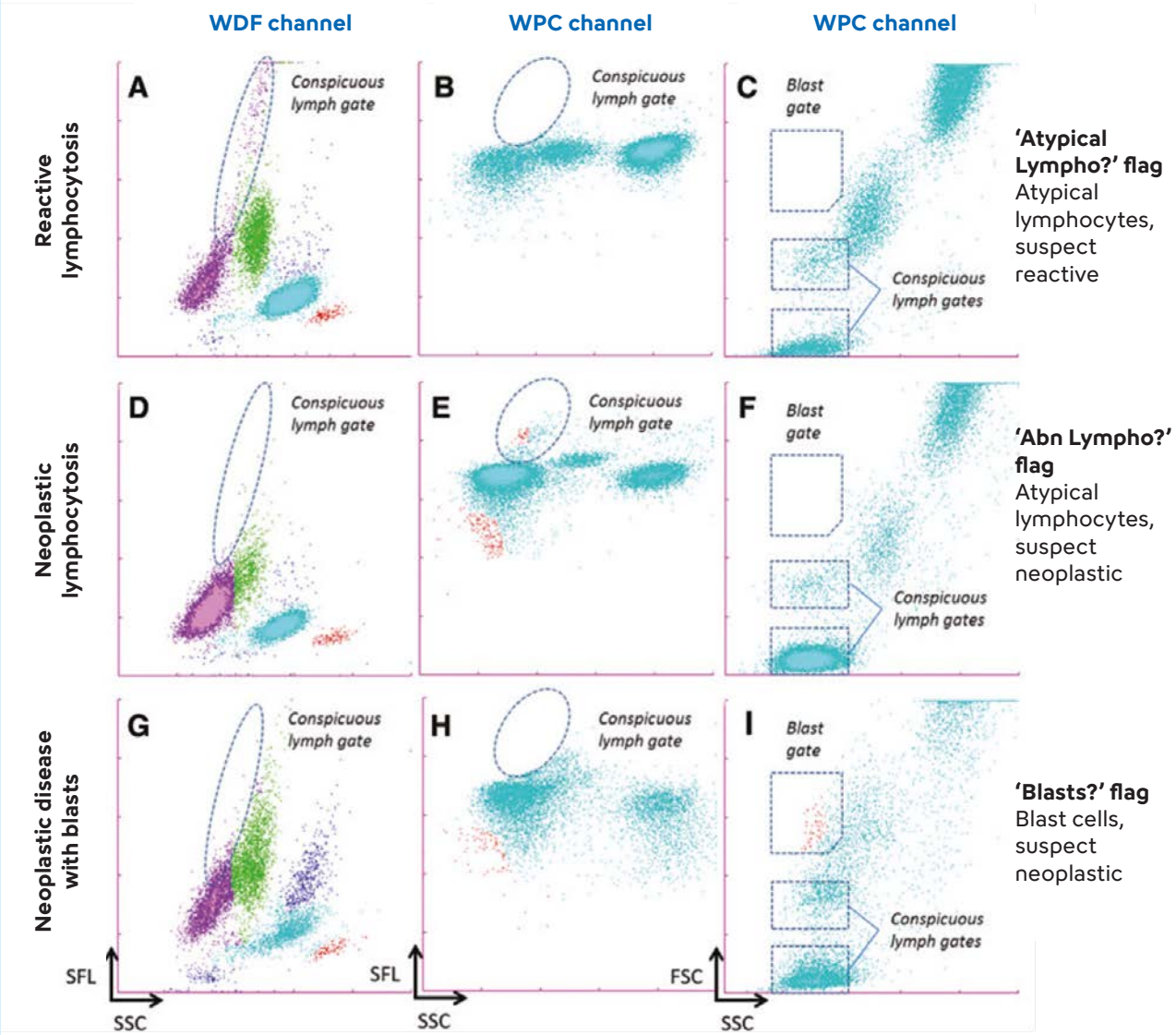

Fig. 5 Examples of the WDF and WPC scattergrams for three clinical scenarios. Reactive lymphocytosis (recovery after a cytomegalovirus infection) (A–C), neoplastic lymphocytosis (B-CLL) (D–F), and neoplastic disease with blasts (AML M4) (G–I). Adapted from Schuff-Werner *et al.* [6].

How to use quantitative information on reactive conditions to improve your workflow

As described above, the Extended Inflammation Parameters can provide quantitative information about the status of immune system activation, which allows laboratories to create new triggers for smear management and consequently to improve their workflow by decreasing clinically irrelevant smears.

Typically, laboratories face high numbers of reactive and negative samples and only a small fraction of samples comes from patients with undiscovered, new malignancies. This means that smear reviews to follow up on suspicious cell counts, for example in case of a monocytosis, lymphocytosis or the presence of immature granulocytes (IG), can be significantly reduced because most of the time these findings are associated with reactive conditions. The cell counts of reactive origin can be reported to clinicians straight away.

Taking the presence of IG as an example, they are typically present in a reactive sample and there is no added clinical value of confirming their morphology or count in a known patient's blood smear review. On the other hand, a chronic myelocytic leukaemia patient may have IG in his/her peripheral blood too, but in this case any follow-up test is unrelated to the IG count; rather, it is focused on other cells' morphology and arriving at a diagnosis.

Sysmex suggests reporting the Extended Inflammation Parameters to the laboratory information system (LIS) together with the 6-part white blood cell differential count, including an IG count, and performing a microscopic blood smear review as shown in Fig. 6.

Conclusion

Overlooking malignant samples is one of the main concerns in a modern haematological laboratory. The ability to detect neoplastic cells in a blood sample with a high degree of sensitivity is therefore essential. However, from the perspective of the laboratory workflow and costs, keeping the number of unnecessary follow-up tests to a minimum is also very important.

The Sysmex analysers' dual-level approach, using results from both the WDF and WPC channels, supports exclusion of samples associated with malignant conditions with great sensitivity and specificity. It also opens up possibilities for better aid in diagnosis and measurement of reactive cells without the need for clinically irrelevant follow-up tests. The WPC channel can reclassify a significant fraction of samples that are suspected by the DIFF analysis of being malignant as 'reactive' or 'negative'.

The combination of both channels can also be a very useful support tool for morphology classifications, especially in samples containing conspicuous lymphocytes that are difficult to recognise. The interplay between the WDF and WPC channels can significantly improve the smear review rate and add new clinical value with the reportable Extended Inflammation Parameters.

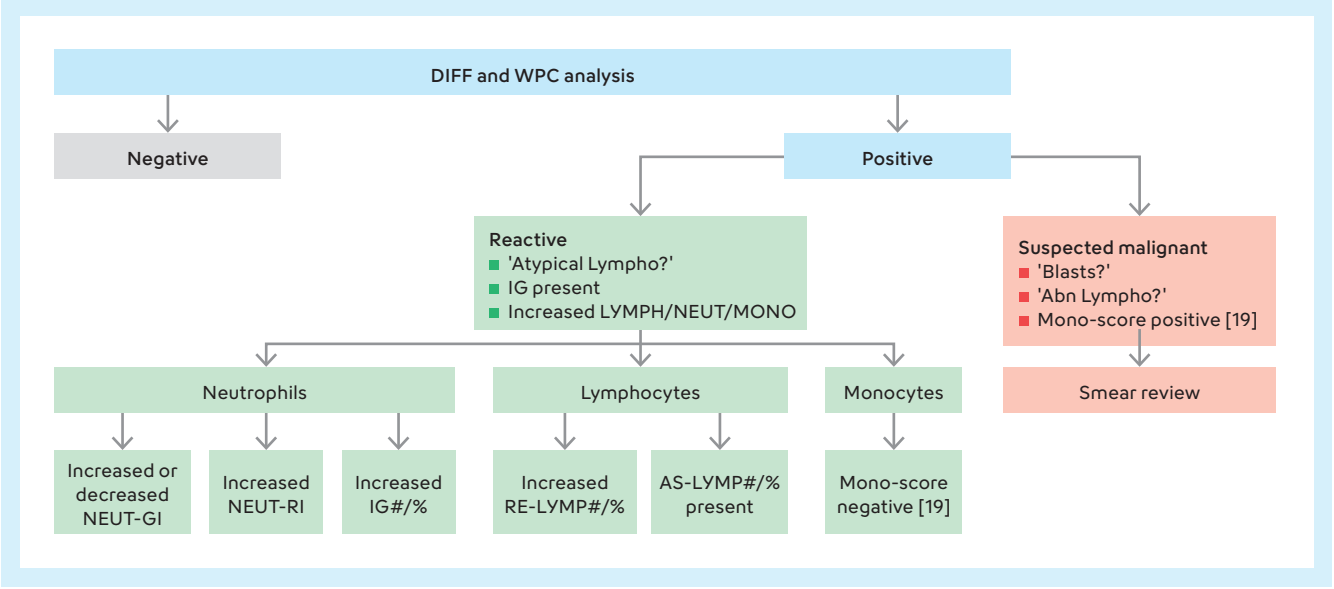

Fig. 6 Possible (user-defined) workflows for reducing the number of smears when monitoring disease using the reactive cells' count parameters – IG, RE-LYMP and AS-LYMP – and the NEUT-GI and NEUT-RI parameters. Red: smear review mandatory; green: smear review optional.

References

[1] **Kawauchi S *et al.* (2014):** Comparison of the Leukocyte differentiation Scattergrams Between the XN-Series and the XE-Series of Hematology Analyzers. Sysmex Journal International Vol.24 No.1.

[2] **Tuosto L *et al.* (2001):** Organization of plasma membrane functional rafts upon T cell activation. Eur J Immunol. 31(2): 345–9.

[3] **Li YC *et al.* (2006):** Elevated Levels of Cholesterol-Rich Lipid Rafts in Cancer Cells Are Correlated with Apoptosis Sensitivity Induced by Cholesterol-Depleting Agents. Am J Pathol. 168(4): 1107–18.

[4] **Zini G *et al.* (2010):** A European consensus report on blood cell identification: terminology utilized and morphological diagnosis concordance among 28 experts from 17 countries within the European LeukemiaNet network WP10, on behalf of the ELN Morphology Faculty. Br J Haematol. 151(4): 359–64.

[5] **Bruegel M *et al.* (2015):** Comparison of five automated hematology analyzers in a university hospital setting: Abbott Cell-Dyn Sapphire, Beckman Coulter DxH 800, Siemens Advia 2120i, Sysmex XE-5000, and Sysmex XN-2000. Clin Chem Lab Med. 53(7): 1057–71.

[6] **Schuff-Werner P *et al.* (2016):** Performance of the XN-2000 WPC channel-flagging to differentiate reactive and neoplastic leukocytosis. Clin Chem Lab Med. 54(9): 1503–10.

[7] **Kweon OJ *et al.* (2018):** Evaluation of the Flagging Performance of the Hematology Analyzer Sysmex XN Series on the Basis of "Q Values". Arch Pathol Lab Med. 142(1): 83–88.

[8] **Petrone J *et al.* (2019):** Blast flagging of the Sysmex XN-10 hematology analyzer with supervised cell image analysis: Impact on quality parameters. Int J Lab Hematol. 41(5): 601–606.

[9] **Querol MS *et al.* (2021):** Evaluation of the New Beckmann Coulter Analyzer DxH 900 Compared to Sysmex XN20: Analytical Performance and Flagging Efficiency. Diagnostics (Basel). 11(10): 1756.

[10] **Schapkaitz E *et al.* (2018):** Performance evaluation of the new measurement channels on the automated Sysmex XN-9000 hematology analyzer. Clin Biochem. 53: 132–138.

[11] **Blomme S et al. (2021):** The integration of Sysmex XN-9100' WPC channel reflex testing in the detection of reactive versus malignant blood samples. Int J Lab Hematol. 43(2): 191–198.

[12] **Dedeene L *et al.* (2022):** "Smart" WPC reflex testing enables optimal use of the WPC channel in the detection of malignant blood samples using Sysmex XN-9100. Int J Lab Hematol. 44(4): e153-e155.

[13] **Henriot E *et al.* (2017):** New parameters on the hematology analyzer XN-10 (SysmexTM) allow to distinguish childhood bacterial and viral infections. Int J Lab Hematol. 39 (1): 14–20.

[14] **Seo JY *et al.* (2015):** Performance evaluation of the new hematology analyzer Sysmex XN-Series. Int J Lab Hematol. 37: 155–64.

[15] **Briggs C *et al.* (2012):** Performance evaluation of the Sysmex haematology XN modular system. J Clin Pathol. 65: 1024–30.

[16] **Noordergraaf M *et al.* (2016):** Diagnostic efficiency of the Sysmex XN WPC channel for the reduction of blood smears. Clin Biochem. 49(16-17): 1292–1294.

[17] **Sejrup J *et al.* (2020):** Performance of the Sysmex White Precursor Channel to discover circulating leukemic blast cells. Int J Lab Hematol. 42(6): 734–743.

[18] **Schoorl M *et al.* (2016):** Flagging performance of the Sysmex XN2000 haematology analyser. Int J Lab Hematol. 38(2): 160–6.

[19] **Schillinger F *et al.* (2018):** A new approach for diagnosing chronic myelomonocytic leukemia using structural parameters of Sysmex XN analyzers in routine laboratory practice. Scand J Clin Lab Invest. 78(3): 159–164.