WHITE PAPER

WHITE BLOOD CELL FUNCTIONALITY

Sensitive assessment of WBC functionality *and* greater workflow efficiency

Differentiate with confidence between malignant and reactive conditions

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 deliver diagnoses faster and keep costs under control. By combining results from the XN-DIFF analysis and the white precursor and pathological cell (WPC) channel, Sysmex XN-Series delivers both highly sensitive *and* specific detection of reactive and malignant samples.

Combining two analysis channels using fluorescence flow cytometry inside a single analyser lets you detect malignancies sensitively *and* specifically. This is achieved by using the differences in cell functionality of the different white blood cells.

The XN-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).

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 intensively (Fig. 1).

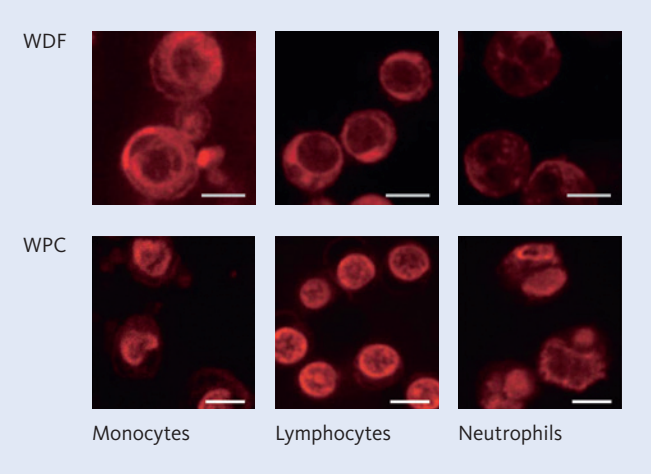

Fig. 1 *Fluorescence micrographs of three cellular populations after labelling with WDF and WPC reagents. The WDF fluorochrome marker labels mostly the RNA in the cytoplasm whereas the WPC fluorochrome marker labels mostly the DNA in the nucleus. Bar width = 5 µm. Adapted from Kawauchi et al. [1].*

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 finally 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 malignant condition has been excluded.

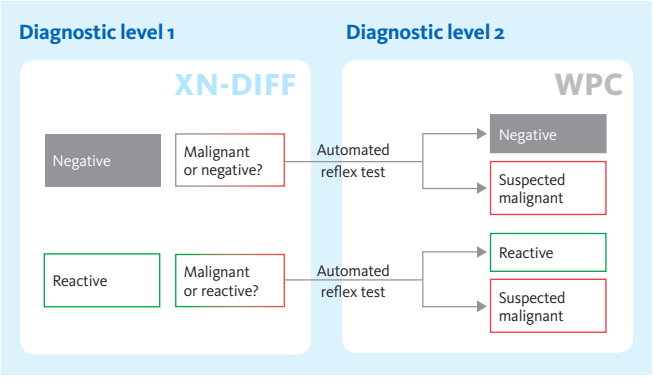

Fig. 2 *The Sysmex XN-Series' dual-level approach to classify samples into three different, well-defined categories: negative, reactive (flag 'Atypical Lympho?') and suspected malignant (either flag 'Blasts?' or flag 'Abn Lympho?').*

These categories translate into analyser flags that have the following meaning: 'suspected malignant' means the triggering of either 'Blasts?' or 'Abn Lympho?' flags, whereas 'reactive' refers to the flag 'Atypcial Lympho?'. In doing so, the XN-Series 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].

A recent study [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 recent 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.

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 novel 'Extended Inflammation Parameters' let one quantify activated lymphocytes and neutrophils, and the results can be applied once a malignancy 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) support differentiation between early and advanced bacterial infections.

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 XN-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 suspected 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 above (7,782 CBC+DIFF samples from a regional hospital), 51 samples (8 %) out of 665 samples with the flag 'Blasts/Abn Lympho?' from XN-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.

* Unpublished: Samples were measured on XN-Series with software version 16; results were re-analysed with software version 21.11.

Table 3 *Summary of published results on reducing suspected malignant samples with a combined analysis in the XN-Series' WDF and WPC channels.*

Publication	Number of patients	Patient population	Reduction of suspected malignant samples with XN-Series' WDF and WPC channels
Seo *et al.* [7]	1005	Adults – malignancies	63 % compared to XE-2100*
Jones *et al.* [8]	150	Children – malignancies	46 % compared to XN-DIFF alone
Schuff-Werner *et al.* [6]	253	Adults – malignancies and reactive conditions	41 % compared to XE-2100
Briggs *et al.* [9]	1000	Routine samples, university hospital	49 % compared to XE-2100*

* Reported smear reduction based on samples with malignant and reactive flags ('Blasts?', 'Abn Lympho?', 'Atypical Lympho?')

In conclusion, smear reviews to confirm the presence of malignant cells can typically be reduced by approximately 20 % (routine haematological laboratory) and over 40 % (specialised laboratory with a high proportion of positive samples) when combining the WDF and WPC channels for analysis [6 – 9].

Focused slide review

The information from the WPC channel can also help morphologists as confirmed 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. 3 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. 4.

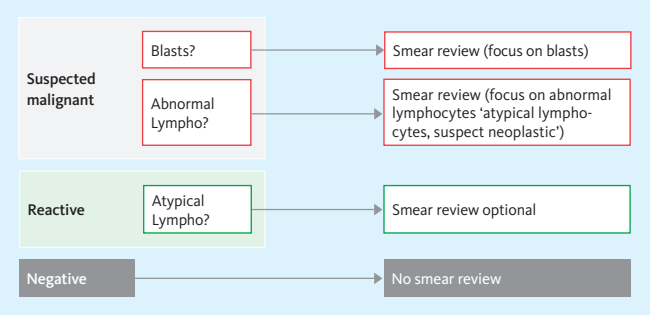

Fig. 3 *Suggested smear workflow based on the information from WPC analysis. Suspected malignant samples are categorised, enabling one to focus on specific cell types in a follow-up smear review. For samples classified as 'negative' or 'reactive', unnecessary smear reviews can be avoided.*

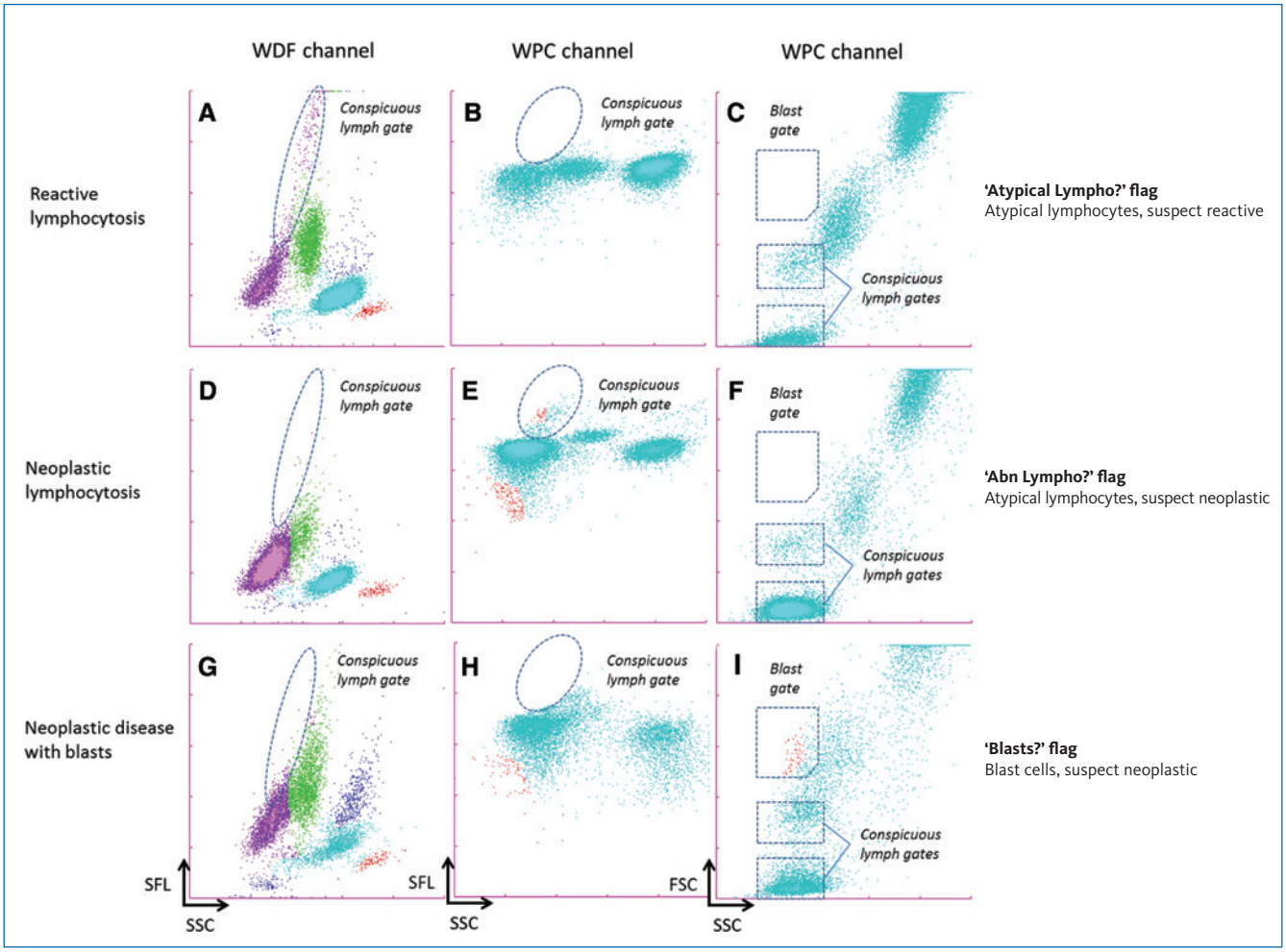

Fig. 4 *Examples of the WDF and WPC scattergrams for three clinical scenarios. Reactive lymphocytosis (recovery after a cytomegalovirus infection) (A – C), neoplastic lymphocytosis (B-CLL) (D – F), and neoplastic disease with blasts (AML M4) (G–I). Adapted from Schuff-Werner et al. [6].*

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. 5.

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 XN-Series' dual-level approach, using results from both the WDF and WPC channels, excludes malignancies with great sensitivity *and* specificity. It also opens up possibilities for better diagnosis and monitoring of reactive diseases without the need for clinically irrelevant follow-up tests. The WPC channel can reclassify a significant fraction of samples that are suspected by the XN-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 novel, reportable Extended Inflammation Parameters.

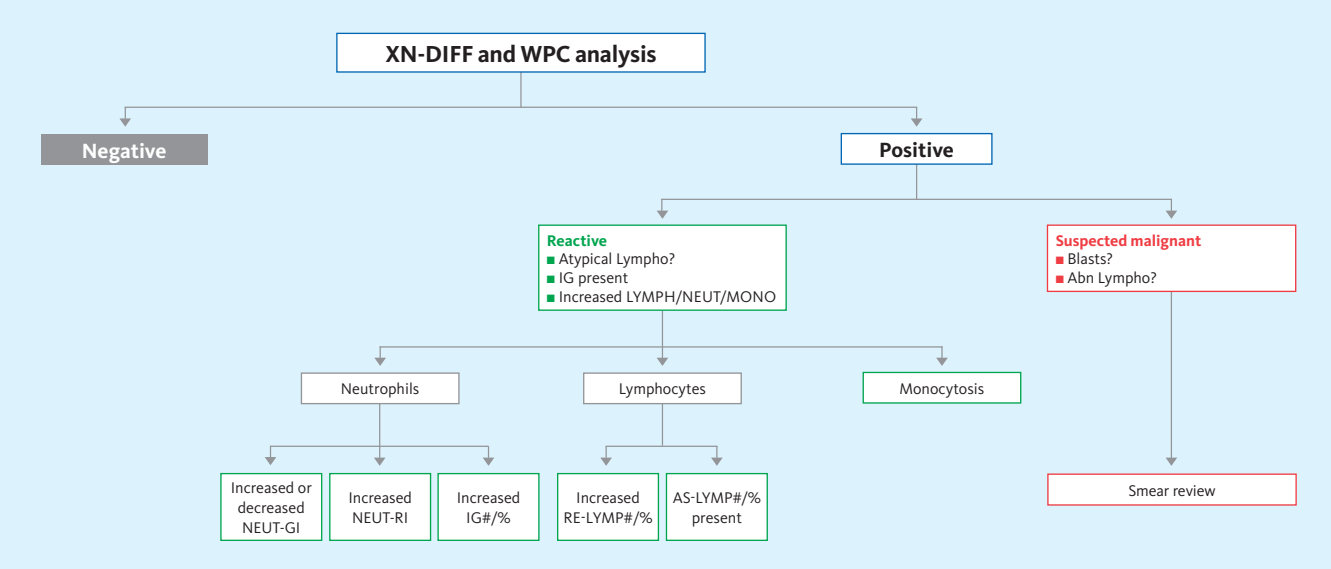

Fig. 5 *Possible (user-defined) workflows for reducing the number of smears when monitoring disease using the reactive cells' count parameters – IG, RE-LYMP and AS-LYMP – and the NEUT-GI and NEUT-RI parameters. Red: smear review mandatory; green: smear review optional.*

References

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