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Usefulness of NEUT-X determination in routine diagnostic procedures: application to myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are common malignant disorders with a poor prognosis. Early diagnosis is warranted for optimal benefit from treatment, but MDS diagnostic features are polymorphic and non-specific. They include anaemia in the majority of cases. The standard parameters given by an automated analyser rarely bring any diagnostic argument towards the diagnosis of MDS. The aim of this study was to investigate if some structural parameters not routinely provided by blood analysis with the Sysmex XE-2100 analyser could help in the diagnosis of MDS in routine practice.

We could demonstrate that the neutrophil parameter NEUT-X, the mean value of Side Scatter measurement of the neutrophil population and representative of the structure of the neutrophils, could be used for this purpose. We converted NEUT-X into a semi-quantitative parameter, the granularity index (GI). Negative GI in the context of anaemia was highly suggestive of MDS; when considering the association of isolated anaemia and a low GI as an additional criterion for slide review, the percentage of MDS cases that were submitted to slide review increased from 67 to 96%. Moreover, we confirmed that this rule was not leading to a large excess of unfounded slide review among non-MDS patients, as only 2% of controls were reviewed in excess. The inclusion of the GI in the routine parameters of the blood counts provided by Sysmex analysers could be a major help for non-specialised routine laboratories in flagging MDS and addressing these cases to slide review.

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Introduction

Myelodysplastic syndromes (MDS) are common malignant disorders with a poor prognosis. They are a heterogeneous group of bone marrow disorders united by a common feature, the ineffective production of mature red blood cells. MDS is classified into six different groups, listed below, according to the WHO criteria.

- 5q minus syndrome
- Refractory anaemia (RA)
- Refractory anaemia with excess of blasts (RAEB)
- Refractory anaemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- RCMD with ring sideroblasts (RCMD-RS)

These syndromes can affect people of any age, but the risk of developing MDS increases with advancing age. The median age of patient diagnosed with MDS is in the mid-sixties. A diagnosis of MDS implies a risk of developing acute myeloid leukaemia (AML) in 20–30% of patients, which is nearly always lethal. Other fatal complications of MDS are blood cytopenias which are frequently associated with infections.

Until recently MDS was treated with supportive therapy only, such as blood transfusions, but there have been advances in treatment of high risk MDS and this makes the early and correct diagnosis of the type of MDS crucial. The immunosuppressive drug Lenalinomid™ has proven to be very effective in a subset of MDS with 5q deletions. Early supportive care, such as erythropoietin treatment, is effective and reduces the need for blood transfusions during the course of the disease. The presence of MDS may make the treatment of other concomitant diseases, which are common in older patients, less effective.

Unfortunately the diagnostic features of MDS are polymorphic and non-specific. They include anaemia in the majority of cases, but one third of all the blood counts in our laboratory are also anaemic. The anaemia associated with MDS is usually normocytic or macrocytic. 40% of patients with MDS are neutropenic at diagnosis and there is thrombocytopenia in one third of cases. These abnormalities may be present on their own or as a combination. These features are very nonspecific and the standard parameters reported by the haematology analyser do not give any information to help in the differential diagnosis of the disease. The ultimate diagnosis depends on the morphological examination of the peripheral blood and bone marrow.

Study aims

To investigate some structural parameters of red and white blood cells and platelets which are measured by the Sysmex XE-2100. These parameters are, at present, for research only and therefore not routinely reported but could help with the diagnosis of MDS.

Patients and control group

- 184 patients with MDS.
- 196 patients with anaemia due to various causes other than MDS.
- 3,545 unselected patient samples from the routine haematology workload acted as the control group. These patients did not have MDS but did not necessarily have normal results for the blood count.
- 1053 normal healthy samples to establish the reference range for the structural parameters.

The patients with MDS were diagnosed by bone marrow aspirate examination, enumeration of blast cells and the degree of dysplasia. A marrow karyotype was also performed in all cases. The cases were stratified according to the WHO classification.

MDS patients frequency

Anaemia plus Neutropenia and/or Thrombopenia	Anaemia plus Macrocytosis	Isolated Anaemia Isolated Macrocytosis	Neutropenia and/or Thrombopenia	Normal without Anaemia
50%	5%	32%	12%	1%

Table 1 *Frequency of abnormal quantitative parameters in patients with MDS*

Methods

All blood counts were performed on the Sysmex XE-2100. A diagnosis of MDS was considered using the following parameters.

- Cut off values for the following quantitative parameters:
  - Haemoglobin <1 g/dL
  - Platelets <120 x 10^9/L
  - Neutrophils <1.5 x 10^9/L
  - MCV >110 fL
- In addition to the usual parameters structural and maturation parameters were included:
  - RET-Y and IRF for red cells
  - NEUT-X and NEUT-Y for neutrophils
  - P-MFV for platelets

RET-Y is measured in the reticulocyte channel, using a polymethine dye and analysis of forward scatter light. The XE-2100 provides a measurement of the approximate size of the mature red cells and reticulocytes; these are expressed as RBC-Y and RET-Y. IRF is measured by the reticulocyte fluorescence index and it represents the immature fraction of the reticulocytes.

NEUT-X and NEUT-Y are the mean values for both side scatter diffraction and fluorescence of the neutrophil population. NEUT-X represents the structure of the neutrophils, while NEUT-Y represents the fluorescence.

P-MFV is the peak of the distribution curve for the platelets. It is a useful parameter because unlike the median value, it is available even when there is severe thrombocytopenia.

196 blood films from patients with MDS and those with anaemia with other causes were examined by two experts. A semiquantitative scale was set to evaluate the granularity of the neutrophils. This ranged from 0 (hypergranular) to 5 (agranular). The score was then compared to the NEUT-X value.

In the routine laboratory not all abnormal quantitative parameters would lead to the examination of a blood film. Samples with an isolated haemoglobin of less than 11 g/dL or an isolated macrocytosis would not have blood films made. However, neutropenia and thrombocytopenia, whether as isolated abnormalities or concomitant with other abnormalities, would have a blood film examined.

Results

Quantitative parameters in MDS

The frequency of abnormal parameters in MDS is presented in table 1.32% of patients with MDS have an isolated anaemia or macrocytosis and so therefore would not routinely have a blood film reviewed in our laboratory. Other parameters are needed to identify these patients.

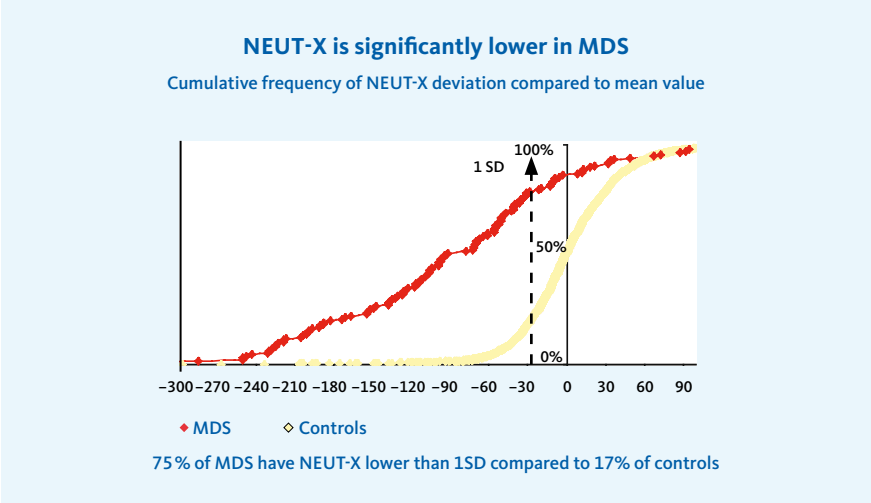

Figure 1 *Plot showing the cumulative frequency of deviation of NEUT-X from the mean normal value in the control group and patients with MDS.*

Structural parameters in the reference group and those with MDS

1053 control samples with normal values for haemoglobin, white blood cell count, neutrophil count, platelet count were used to determine the median normal values and standard deviation (SD) for the parameters. Results are shown in table 2.

	P-MFV (fL)	NEUT-X	IRF	RET-Y
MIN	7.4	1212	17	1720
MAX	13.4	1413	243	1992
MEDIAN	9.1	1330	103	1872
SD	8%	3%	53%	4%

Table 2 *Normal ranges for the structural parameters. MIN = minimum; MAX = maximum; SD = standard deviation.*

The NEUT-X in particular had a very narrow standard deviation.

Results from the control group and those from patients with MDS were compared using multivariate analysis. The neutrophil counts and NEUT-X were significantly different in patients with MDS when compared with normal samples; NEUT-X was the most sensitive parameter, having a significantly lower value in MDS. The cumulative frequency of NEUT-X deviation from the normal mean value in MDS compared to the mean normal value is shown in figure 1. About 75% of patients with MDS have NEUT-X less then 1 SD below the mean normal value compared to 17% for the control group of patients.

Relationship between NEUT-X and morphological features of the neutrophils

A low NEUT-X strongly correlates with hypogranularity in the neutrophils (R^2 = 0.867), the correlation graph is shown in figure 2. NEUT-X was not found to correlate to the neutrophil count, even in quite severe neutropenia. As the patient population with MDS was older than the control group a correlation of NEUT-X with age was determined. No correlation was found and the value of NEUT-X is not age dependent.

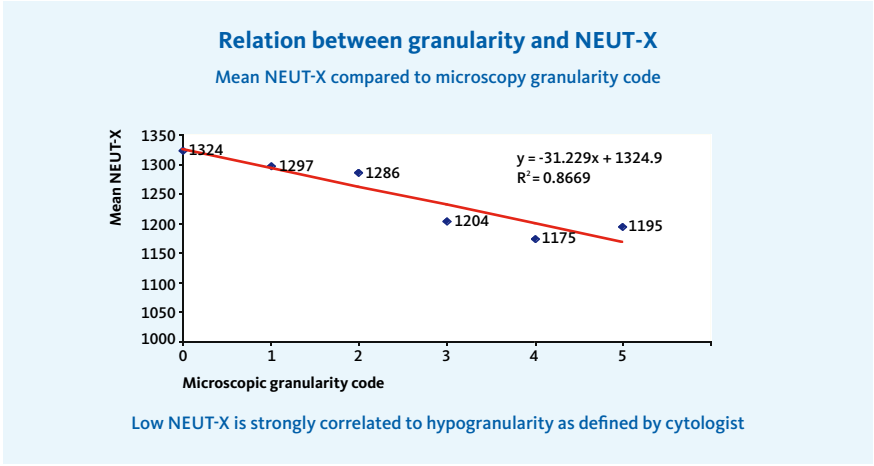

Figure 2 *Correlation of NEUT-X with granularity of the neutrophils measured by microscopic examination and the semi-quantitative scoring system.*

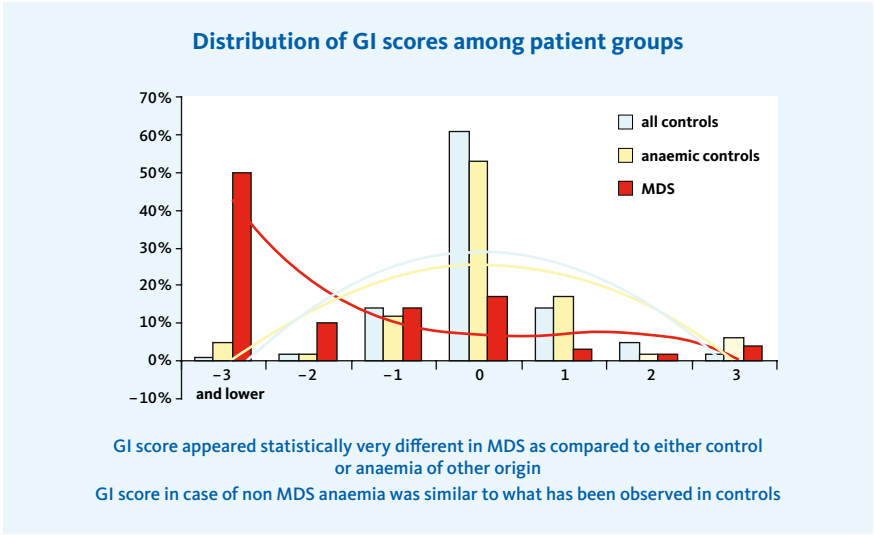

Figure 3 *Distribution of the GI score in the three groups of patients. A negative score indicates lower granularity in the neutrophils. Results of scores from a GI of 3 to –3 only are shown, scoring for MDS patients goes down to –9.*

Confirmation of the NEUT-X values in the control group of patients and the cut-off values used for the study

In order to ascertain that the median values for NEUT-X in our control group of patients was not biased by the category of patients seen in our hospital the NEUT-X range from control groups of patients from other hospitals was established. Three laboratories were selected on the basis of the different cohorts of patients seen in those hospitals. The distribution of the NEUT-X value was found to be the same from all laboratories.

The cut-off value of 30 (1 SD) below the normal value provided the best discrimination for the diagnosis of MDS, the same value found in our original study and this value has since been confirmed by independent statisticians.

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Difference of NEUT-X from reference value	> –90	–61 to –90	–31 to –60	–30 to +30	+31 to +60	+61 to +90	> +90
GI score	–3	–2	–1	0	1	2	3

Table 3 *The calculation of the granularity index based on deviations from the mean value for NEUT-X found in the normal reference and control groups of patients.*

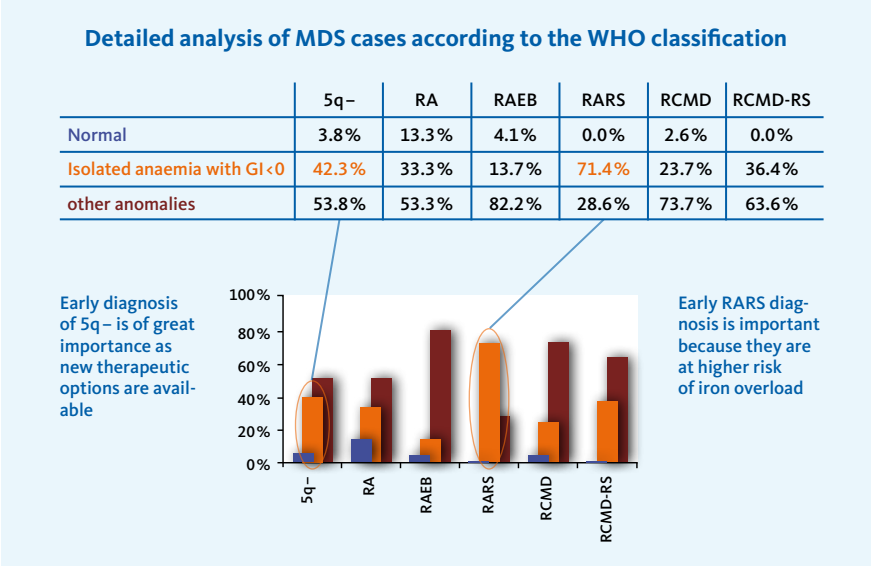

Figure 4 *Number of blood films reviewed in each of the WHO classified MDS with and without the use of the GI flag.*

Definition of the Granularity Index (GI) and its use as a screening tool for diagnosis of MDS in routine practice

As already discussed, NEUT-X is associated with the granularity of the neutrophils. This study investigated if it was possible to use the semi quantitative expression of NEUT-X as a flag for the diagnosis of MDS in the routine laboratory. The mean value of NEUT-X from the control group of patients was 1330, with a SD of 30. This was the same difference seen between the median values of those with MDS and the normal healthy reference group. It was therefore decided to attribute an index of granularity based on the number of standard deviations.

NEUT-X +/– 1 SD corresponds to a GI of zero, a NEUT-X lower than 1 SD from the mean would give a GI of –1 and NEUT-X higher than 1 SD from the mean would give a GI of +1. GI scoring system is shown in table 3.

Distribution of GI values among the three groups of patients

The GI was examined in the normal reference group, the anaemic controls and the patients with MDS. It was found that patients with anaemia due to causes unrelated to MDS had a GI very similar to the normal group. The GI for MDS patients was statistically lower than in the other two groups. These results are demonstrated in figure 3. Controls, and patients with anaemia, show the same Gaussian distribution around the GI score of zero. The majority of samples with MDS have a GI score of –3, the scoring system goes down to a GI of –9, and 50% of MDS patients have a score of –3 or lower. Conversely neither of the control groups, unselected controls and anaemic controls, ever demonstrated a score of less than –3. A small number of MDS patients do have a GI of zero or above.

The clinical utility of a GI flag for MDS in the presence of an isolated anaemia

As already established 33% of MDS patients have a low haemoglobin as the only abnormality in the blood count results. Among these 54/58 patients had a low GI. If the GI flag had been in use then these patients would have had a blood film reviewed. At the same time, the percentage of review was only increased by 2% in non MDS cases. Therefore, the association of an anaemia alone with a low GI proves to be highly suggestive of MDS and this would be an extremely useful to flag detect new cases of MDS in routine practice, without leading to an increase of false positive blood film reviews.

GI and the differential diagnosis of MDS using the WHO criteria

We investigated the importance of low GI as diagnostic criterion for each subclass of MDS. In each category, we considered together all the anomalies leading to slide review: Neutropenia, thrombocytopenia, bi- or tricytopenia, and anaemia with macrocytosis (MCV >110 fL). Among the remaining cases, none of the above anomalies, or isolated anaemia, would not be considered for slide review. Therefore all the cases with isolated anaemia and low GI represent the gain from considering low GI in the context of isolated anaemia as fostering slide review.

As presented in figure 4, this gain was present in all categories, and ranged between 13.7% for RAEB and 71.4% for RARS. Notably, 42.3% of 5q– MDS were spotted out through this combination of anaemia + low GI.

GI in the context of isolated anaemia has a highly predictive value (97%), which means that in the absence of a low GI, the risk of MDS is close to zero.

If the GI is less than –1 it is 18 times more likely that the patient has MDS than not, the diagnosis of MDS in these circumstances is very likely.

Conclusions

- The aim of this study was to try to find some parameters provided by the Sysmex analyser that could help routine laboratories detect MDS.
- In this study, we have demonstrated that the structural neutrophil parameter, NEUT-X, can be used to detect MDS.
- NEUT-X has been converted into a semi-quantitative parameter, the granularity index, the GI.
- A negative GI in the context of anaemia proves to be highly suggestive of MDS.
- The use of isolated anaemia in association with a negative GI inreased the number of patients with MDS that will be addressed to blood film review from 67% to 96%.
- There were only 2% of controls which showed false positive results resulting in unnecessary film review.
- In the context of isolated anaemia, with a positive GI score, MDS is almost certainly not the correct diagnosis.
- A GI of less than –1 strongly suggests the diagnosis of MDS (eighteen times more likely than not).
- There are further studies on-going and preliminary findings from these suggest that NEUT-X/GI may have other applications in different diseases.

This study suggests that NEUT-X/GI should be included in the routine reportable blood count parameters provided by Sysmex analysers.

Acknowledgement

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