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Letter to the Editor

Neut-X can be successfully used as diagnostic and prognostic tool in MDS

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow disorders with the common feature of ineffective production of mature blood cells [1]. Alas, diagnostic features of MDS are polymorphic and non-specific. 40% of patients with MDS are neutropenic at diagnosis and the degree of neutropenia has prognostic impact [2]. Final diagnosis still depends on morphological examination of peripheral blood (PB) and bone marrow (BM). One of the most important morphologic features is hypogranulation of granulocytes in peripheral blood often leading to further investigation of BM. More than 80% of MDS patients show signs of dysplasia in granulopoiesis [3]. Although an experienced investigator can assess MDS with its typical features, judging the degree of hypogranulation via morphological assessment is limited. Most cytogenetic and molecular markers, although useful in the diagnosis and prognostication of MDS, are not specific for MDS. These reasons have obvious implications for therapeutic decision making and patient counseling and require further diagnostic methods [4]. Flow cytometry may provide additional information on dysplasia of granulopoiesis [5].

2. Materials and methods

All blood samples were examined with the Sysmex XE-5000 system which utilizes impedance technology and fluorescence flow cytometry. By using a stable red diode laser and a polymethine-based fluorescent dye three different signals are produced: forward-scattered light, providing information on cell size, side-scattered light, providing information on internal cell structure and granularity of the cell, and side-fluorescence light, providing information on DNA/RNA content. Neut-X depicts the mean value of side scatter measurement [6].

We performed 9226 complete blood counts of 980 individual patients treated in our outpatient department, 99 of them diagnosed with MDS including some cases with AML with myelodysplasia-related changes (the former RAEB-T) and some CMML cases. 881 patients with different types of leukemia, lymphoma, plasma cell dyscrasia, myeloproliferative diseases, solid tumors, MDS after allogeneic stem cell transplantation and other hematologic diseases were included for analyses which in total accounted for 8506 Neut-X values. 798 healthy controls were examined from a pool of university employees of both genders and all ages. Patient characteristics of MDS patients including WHO 2016 type, IPSS, age, time of diagnosis, and survival time were available in the Duesseldorf MDS registry.

For interindividual comparison the two-sided unpaired Student's *t*-test was used. To analyze differences between patient gender and age, the non-parametric Mann-Whitney-U test was applied. For intraindividual analyses Root Mean Square Error was used to measure within-patient standard deviation. Survival was analyzed by use of the Kaplan–Meier method. A p value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows (Version 22 Inc. Chicago, IL).

3. Results

3.1. Morphologic hypogranulation versus Neut-X values

Of all 99 MDS patients analyzed, 5% showed hypogranulation morphologically. The Sysmex XE-5000, however, was able to measure a low Neut-X value in 73% of all MDS patients

3.2. Patients with MDS had lower Neut-X values than healthy controls and Neut-X values were lower in all MDS subtypes

We compared Neut-X values of healthy individuals with MDS patients. MDS patients had significantly lower median values of Neut-X than healthy individuals (Healthy: 134 vs. MDS: 128) ($p < 0.05$). The range of Neut-X values of healthy individuals was significantly smaller with the lowest value being higher than the median values of MDS patients (Healthy: 130-142 vs. MDS: 75-143). Neut-X values were analyzed within high-risk and low-risk MDS-types according to the WHO 2016 classification. All MDS subtypes showed lower Neut-X values than healthy individuals ($p < 0.05$) (Fig. 1a). MDS patients with multi-lineage dysplasia showed statistically significantly lower Neut-X values than patients with single-lineage dysplasia. When we analyzed MDS patients with Neut-X values lower than the tenth percentile we could not find correlations to WHO type, IPSS category, or cell counts.

3.3. Patients with MDS had lower Neut-X values than patients with other hematologic diseases both malignant and non-malignant

We compared Neut-X values in healthy controls, MDS patients, myeloproliferative neoplasms, plasma cell dyscrasia, and other hematologic diseases. MDS patients showed significantly lower Neut-X values than all other entities ($p < 0.05$) (Fig. 1b). No other entity showed such low Neut-X values. Neut-X ranges were smaller in all entities when compared to MDS. Neut-X values were lower in MDS patients when compared to other malignant hematologic diseases. Medians of Neut-X values in patients with leukemia, lymphoma, multiple myeloma, and solid tumors did not differ significantly and their ranges were similar. Their lowest Neut-X values were higher than the median of the Neut-X values of MDS patients ($p < 0.05$) (Fig. 1c).

3.4. Neut-X values normalize after allogeneic stem cell transplantation

We performed Sysmex analyses with MDS patients before and more than 1 year after allogeneic stem cell transplantation. Blood counts

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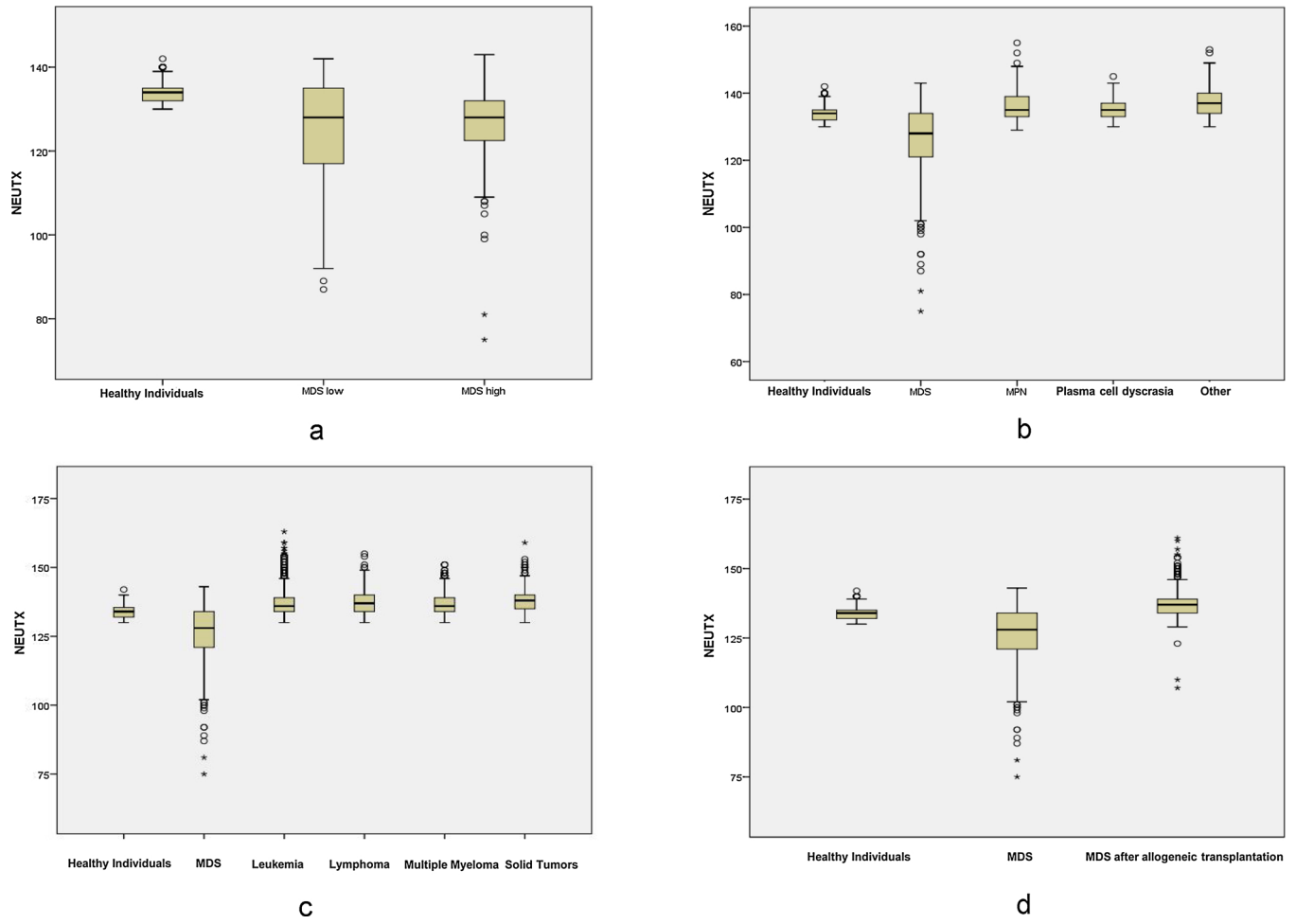

Fig. 1. (a) Neut-X values of different MDS-subtypes according to the WHO 2016 classification.
Left to right: Healthy Individuals (n = 789), Patients with low-risk MDS (WHO type MDS-SLD, MDS-MLD, MDS-RS-SLD/-MLD, MDS with isolated Del5q, n = 70), and patients with high-risk MDS (WHO type MDS-EB-1 & -2, AML with myelodysplasia-related changes (the former RAEB-T), CMML, n = 29).
(b) Neut-X values of patients with different hematologic entities.
Left to right: Healthy Individuals (n = 789), MDS (n = 99), MPN (Essential Thrombocythemia, Polycythemia vera, Osteomyelofibrosis, Chronic myeloid leukemia, n = 103), Plasma cell dyscrasia (MGUS & Smoldering Myeloma, Waldenstrom macroglobulinemia, Amyloidosis, n = 52) and other hematologic diseases (Iron Deficiency Anemia, Hemolytic Anemia, Aplastic Anemia, Immune Thrombocytopenic Purpura, Hemophagocytosis, n = 40).
(c) Neut-X values of patients with different malignant hematologic entities.
Left to right: Healthy Individuals (n = 789), MDS (n = 99), Leukemia (AML, ALL, CLL, n = 117), Lymphoma (Burkitt-Lymphoma, Diffuse large cell B-cell lymphoma, Follicular lymphoma, Hodgkin lymphoma, Mantle cell lymphoma, Marginal zone B-cell lymphoma, n = 202), Multiple Myeloma (n = 251), Solid tumors (Non-small cell lung cancer, Small cell lung cancer, Seminoma, Renal cell carcinoma, Ovarian cancer, Breast cancer, n = 58).
(d) Neut-X values of MDS patients before and after allogeneic stem cell transplantation.
Left to right: Healthy Individuals (n = 789), MDS-patients (n = 99), and MDS-patients after allogeneic stem cell transplantation (n = 58).

during aplasia were excluded. We observed initially low Neut-X values in MDS patients to normalize after allogeneic stem cell transplantation with a median comparable to the control group ($p < 0.05$). Notably, the median of Neut-X values in the group of patients who underwent allogeneic stem cell transplantation was higher than in healthy controls (Fig. 1d).

3.5. MDS patients with lower Neut-X values have a shorter median survival

Based on survival data from the Duesseldorf MDS registry we could not find an association between Neut-X values and prognosis below the median of 127. When we examined patients with a slightly lower Neut-X value, however, we observed a massive difference in survival. Patient with Neut-X < 126 had a median survival of only 5 months as compared to 13.5 months in patients with Neut-X > 126 ($p < 0.05$) (Fig. 2). Neut-X values were slightly lower in females than in males but not statistically significantly different. Gender did not impact median survival. Age, however, did: median Neut-X values showed no difference in patients younger than 75 years of age versus those older than 75 years. Patients with low Neut-X values aged over 75 years, however, showed significantly shorter survival ($p < 0.05$). In a multivariate analysis, Neut-X < 127 had independent impact on prognosis when tested together with the IPSS.

4. Discussion

Although hypogranulation is frequently observed in peripheral blood samples of MDS patients, its pathophysiology is not well understood. Light microscopy is often unable to measure either presence or degree of hypogranulation [7]. Of all diseases examined in our study, only MDS patients showed lower Neut-X values determining it as a unique feature in MDS. Although other myeloid malignancies are akin to MDS and share morphological features, low Neut-X values seem highly specific for MDS.

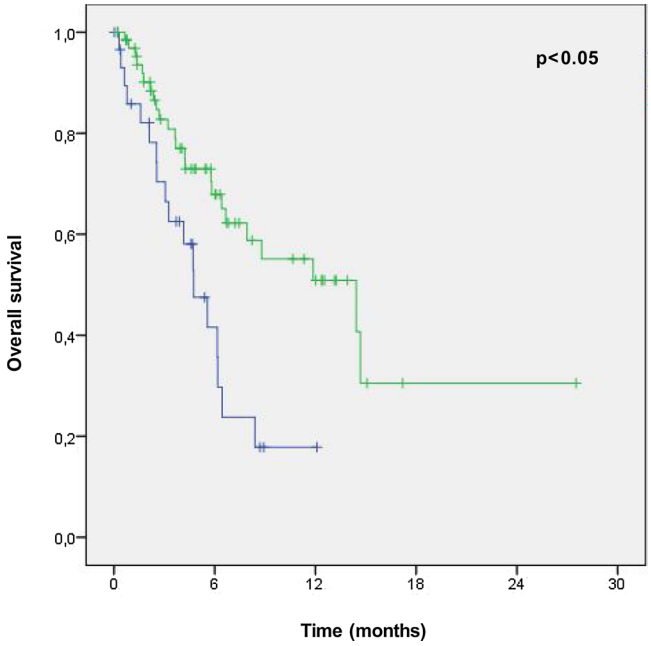

Fig. 2. Cumulative survival rates over time (months) according to median Neut-X value. Patient with Neut-X < 126 had a median survival of only 5 months (blue curve) as compared to 13.5 months in patients with Neut-X > 126 (green curve) ($p < 0.05$).

We were able to show that Neut-X values normalized after allogeneic stem cell transplantation. However, the median Neut-X value after allogeneic stem cell transplantation was higher than in healthy individuals. Neutrophils often appear hypergranulated due to severe inflammation which frequently occurs after allogeneic stem cell transplantation and has previously been corroborated with the Sysmex technology in patients with inflammatory disease [8]. Hence, monitoring Neut-X after allogeneic transplantation may serve as an early marker for MDS relapse.

Survival of MDS patients has been previously correlated with the percentage of bone marrow blasts at diagnosis, the number and severity of hematopoietic lineages affected by cytopenia, and by the presence and type of chromosomal abnormalities [9]. We were able to demonstrate a significant difference in survival for patients with Neut-X levels lower than 126 which was independent of IPSS corroborating morphologic data previously reported [6].

The standard diagnostic approach to MDS has been proposed by the European Leukemia network [10]. Multiparameter flow cytometry is not mandatory but has been shown to be instrumental to distinguish myelodysplastic syndromes from non-neoplastic types of cytopenias [11]. Scoring systems have long been established and the Ogata score is widely used [12,13]. Duetz et al have outlined the clinical implications of flow cytometry in MDS demonstrating how flow cytometry can aid in the diagnosis of cytopenic patients suspected for MDS. [14]. However, as Ogata et al have pointed out, flow cytometry is not yet used in many laboratories as routine tool as part of the diagnostic work-up. No single flow parameter is sufficient for diagnosis in patients with cytopenia and suspected MDS. Many fluorescent antibodies have been proposed making MDS flow cytometry complex requiring a high level of expertise and high cost [15].

The Sysmex system cannot replace a thorough diagnostic work-up to diagnose MDS. However, diagnosing hypogranulation during a routinely performed doctor's visit with a widely available and simple tool could prompt further investigation. This would ultimately allow an early, quick and reliable recognition of one of the most essential features of dysplasia in peripheral blood which is also highly specific for MDS. We have previously shown that less than 51% of peripheral blood cells presented morphologic dysplastic features besides anisocytosis [3]. Hence, identifying hypogranulation with a sensitivity rate of 73% may be superior for a screening method. Especially in times and areas of economic constraint the Sysmex system could serve as minitool not only in recognizing disease but also in avoiding cost-intensive procedures. In addition, monitoring patients with Neut-X during treatment could contribute to patient care in a cost-effective way but more work is necessary to establish Neut-X as a follow-up tool.

Declaration of Competing Interest

All authors declared no conflict of interest.

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