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Monocytes-neutrophils-ratio as predictive marker for failure of first induction therapy in AML

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ABSTRACT

Introduction: Acute myeloid leukemia (AML) is, if untreated, a fatal hematologic neoplasia. Failure of the first induction chemotherapy is a hallmark for a poor prognosis. Early recognition of therapy failure is crucial for planning further therapies. Therefore, international guidelines recommend a bone marrow biopsy around day 14 after the beginning of induction therapy. Hypocellular bone marrow on day 14 is still gold standard for therapy assessment and further therapy strategy. Despite this, non-invasive ways for the evaluation of induction therapy were looked for in the past years.

Methods: We collected peripheral blood cell counts and routine laboratory values of patients treated with "7 + 3" induction therapy. Ratios of absolute cell counts of monocytes and neutrophils (MNR) were calculated daily, and the values were compared in patients with failure of the first induction therapy and patients with therapy response.

Results: 54 patients were included, 12 of which had failure of first induction therapy. The MNR following therapy was highly correlated with the bone marrow results. With the right cut-off, the MNR provides a valid and reliable tool for identification of patients with failure of first induction therapy with a sensitivity of 83.3% and a specificity of 87.8% on day 18.

Conclusions: We propose a novel and non-invasive method for detection of failure of first induction therapy in patients with de novo AML and "7 + 3" induction therapy. The MNR is free of cost since the required cell counts are performed routinely for each patient undergoing intensive chemotherapy.

1. Introduction

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults. Approximately 20,000 people in the United States are diagnosed with AML each year [1].

Developments in the management of AML have led to significant improvements in prognosis, particularly for younger patients. Nevertheless, it has one of the lowest survival rates of all types of leukemia.

Approximately 90% of patients who remain alive 3 or 5 years after induction had achieved complete remission (CR) after their initial therapy [2]. The current definition of morphological CR requires a morphological leukemia-free state with < 5% blasts in the bone marrow aspirate sample with an absolute neutrophil count > 1.0 · 10^9/l and platelet count > 100 · 10^9/l in the absence of any evidence of extramedullary disease [3,4]. The achievement of CR has long been considered as a fixed landmark for succeeding long-term survival in patients with AML [2]. According to results of contemporary co-operative group studies, approximately 70% to 75% of younger patients (age < 65 years) with newly diagnosed AML will reach CR if treated with standard induction therapy containing anthracyclines [5,6]. Thus, a substantial ratio of patients, approximately 20% to 25%, will survive induction therapy without achieving an initial remission.

Unfortunately, in contrast to CR, there has been no consensus for the definition of primary refractory AML at all [4,7]. The latest ELN Guidelines propose a definition of primary refractory AML as a failure to achieve complete remission after 2 cycles of induction therapy [4]. The significance of this definition for clinical use and management of

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patients not in CR after a first '7 + 3' induction in general is uncertain. Approximately half of patients do not receive a second '7 + 3' regime after failure of the first induction, but alternative chemotherapy or allogeneic stem cell transplantation [8]. Despite the diverse definitions in different trials, primary refractory AML reveals a worse prognosis with a 4-year survival rate of 23% and recent studies emphasize the importance of an initial therapy response [7,9,10]. However, the early identification of patients with therapy failure after first induction therapy is an unfulfilled clinical need and can be a decisive factor for the survival of patients. It is usually recognized between days 14 and 21 after the beginning of the induction therapy and the onset of bone marrow regeneration. International Guidelines recommend a bone-marrow assessment around day 14 after beginning of standard '7 + 3' regime and propose early reinduction therapy [11]. Hypocellular bone-marrow is a known predictor achieving CR, but recently there is upcoming disagreement how to interpret residual blast count and the routine performance of the 'day 14 marrow' [12–14]. The mandatory bone marrow puncture is an invasive, painful, and uncomfortable procedure for the patients with possibly severe complications including infection, hemorrhage, nerve and organ injuries in very rare cases [15,16].

Within the scope of our project, we evaluated the option to early, reliably, and non-invasively identify a failure of the first induction therapy just based on blood count or more precisely on the machine-generated count of the monocytes and neutrophil granulocytes. The idea was founded on the assumption that an increase of neutrophil granulocytes is associated with bone marrow regeneration and an increase of monocytes is partially associated with circulating blasts, which are counted and misinterpreted as monocytes in machine-generated blood count by most automated blood cell analyzers [17,18].

2. Material and methods

2.1. Study design

This survey is a monocentric, retrospective study. Based on ICD codes, we selected all patients diagnosed with AML within 10.5 years from 01.01.2007-30.11.2018 in our department. The responsible Ethical Committee had approved the data acquisition and processing (permission no. 17-6142, Date of permission: 09/05/17).

2.2. Patient acquisition

Only patients with de novo primary AML and intensive induction chemotherapy who received complete doses of the standard '7 + 3' scheme of Cytarabin and Daunorubicin were included. Patients with FLT3 mutation who received the FLT3 inhibitor Midostaurin in addition to the '7 + 3' scheme were included as well. Patients with induction therapies other than the '7 + 3', with dose-reduced '7 + 3', with incomplete blood counts or incomplete results of bone marrow puncture were excluded. We excluded Patients with secondary AML a priori. Patients did not receive Granulocyte-Colony Stimulating Factor (G-CSF) to speed up hematologic recovery.

2.3. Data generation and definition of terms

Clearance of blasts was defined as a morphological leukemia-free state with $\leq 5\%$ blasts in the bone marrow aspirate sample. Patients with clearance of blasts were termed to have therapy response. Bone marrow puncture was performed during day 15–21 after beginning of induction therapy. Patients with $> 5\%$ blasts were termed to have failure of first induction therapy.

We evaluated the daily machine generated peripheral cell counts and intermittent routine laboratory values which might be associated with AML disease activity like lactate dehydrogenase (LDH) of all patients included. Between 2007 and 2014, the blood cell counts were generated by Sysmex XE2000 ™ Hematology System and from 2015 onwards with Sysmex XN2000 ™ Hematology System.

Due to the assumption, that neutrophil granulocytes increase as a marker for bone narrow regeneration and monocytes increase as a marker for occurrence of leukemic cells in blood, we calculated Monocyte-Neutrophil-Ratios (MNR) by division of absolute cell counts of monocytes and absolute counts of neutrophils.

2.4. Statistical analysis

Owing to zero cell counts occurring, a procedure for dealing with division by zero was necessary for further calculations. Here, the ratio was set to 1 if both cell counts were equal to zero (0/0), and to ∞ if only the count of neutrophils was zero (x/0).

Because of the appearance of infinite values calculating the MNR, mean values with standard deviation could not be used for statistical analysis and graphics, since every mean value would be infinite, too. We used median values instead.

The Wilcoxon rank sum test was performed to compare the MNR between patients with and without clearance of blasts. To correct for multiple testing the p-values for the different days were adjusted. Additionally, LDH values as well as age of the different groups were compared; the independence of sex was tested.

Receiver operating characteristic curves (ROC curves) and the corresponding areas under the curves (AUC) were computed to assess the diagnostic ability of the different variables as a binary classifier. Best cut-offs were chosen to maximize the sum of sensitivity and specificity (Youden criterion). Sensitivity was defined as the proportion of correctly identified patients with failure of first induction therapy, specificity as the proportion of correctly identified patients with therapy response, and accuracy as the overall proportion of correctly identified patients. The negative predictive value was calculated assuming a prevalence of 25% for failure of first induction therapy.

To avoid overestimation, the best cut-offs were not chosen based on the whole dataset. Instead, the dataset was divided in half into a training dataset and a test dataset keeping the proportion of the two patient groups. ROC curves were built, and the best cut-offs chosen on the training set, whereas accuracy, sensitivity and specificity were calculated on the test dataset. The random split into training and test dataset was repeated 100 times and median values over the repetitions are reported.

Additionally, as another method to assess the variable importance, random forest models [19] were fitted. As measure for importance, the mean decrease in accuracy was used.

The statistical analysis was conducted using R version 3.4.1, graphs were designed with Graph Pad Prism Version 6.

3. Results

3.1. Baseline characteristics

We have screened 228 patients of which 54 were included (Fig. 1).

Clearance of blasts after induction therapy was achieved in 77,7% of all cases (42 of 54). Men and women were nearly evenly spread in both groups (20:22 in the response group, 5:7 in the failure of first induction therapy group). Median age of the response group was 48.7 years vs. 51.8 years in the induction failure group.

Of these 54 patients, 8 patients (15%) had leukopenia and $< 30\%$ monocytes in peripheral blood count at the time of diagnosis, indicating aleukemic leukemia.

3.2. Analysis of monocytes and neutrophils cell count

We found an increasing neutrophil count beginning at day 19–20 after the start of induction therapy. Both cell types, neutrophils as well as monocytes, had an exponential progress in patients with therapy

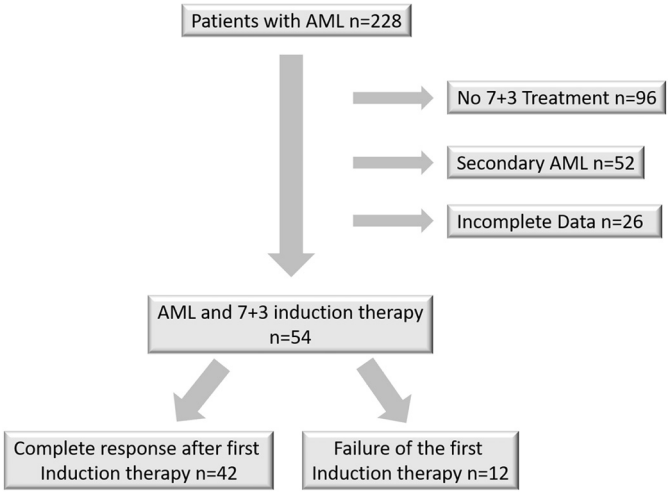

Fig. 1. Patients with acute myeloid leukemia (AML) between 01.01.07 – 30.11.2018.

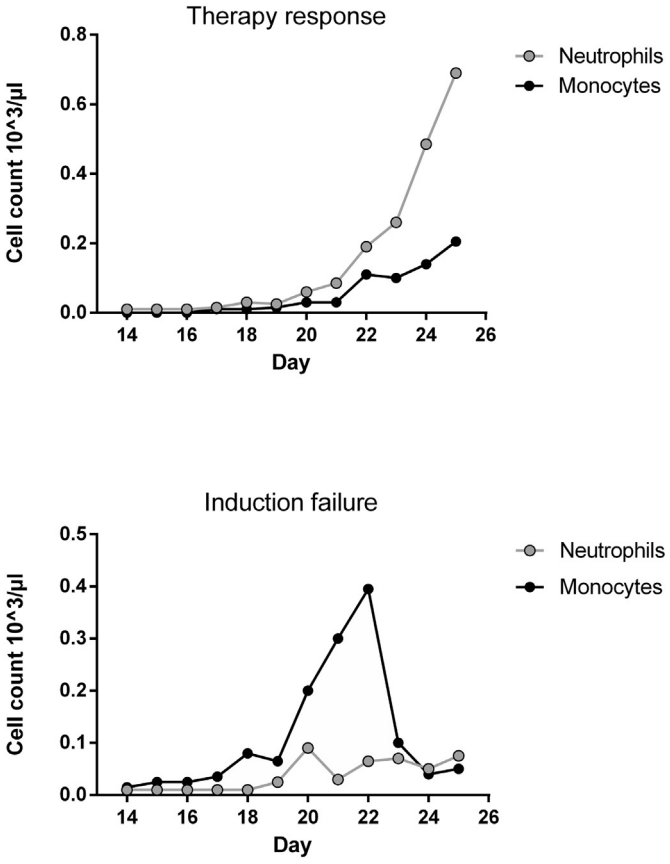

Fig. 2. Median cell counts of neutrophils and monocytes of patients with therapy response and failure of first induction therapy. For a better visualization the interquartile range is not shown.

response. Patients without blast clearance showed a median neutrophil count not > 100/µl, which stopped rising in run of the curve. Monocyte cell count apart was already smoldering at day 14 and increased quickly in patients with induction failure (Fig. 2). For graphs with median values and interquartile range please see the supplementary (**Fig. S 1**).

3.3. Monocytes-neutrophils-ratio

The observation of the homogenous ascent of both monocytes and neutrophils in patients with a therapy response provided us with another rationale for generating a ratio. As bone marrow recovers from the toxic effect of chemotherapy, a healthy population of all kinds of hematopoietic cells gains from it in an equal manner.

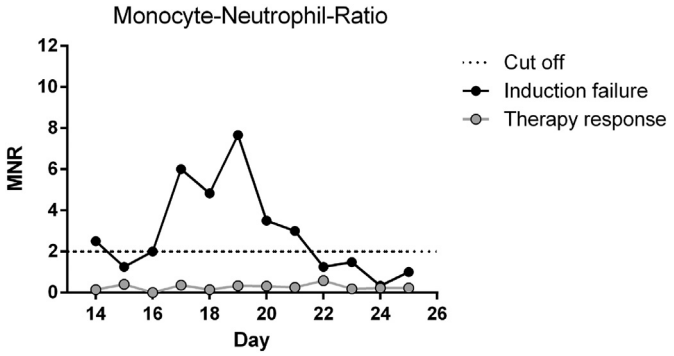

Fig. 3. Median Monocyte-Neutrophil-Ratio of all patients with therapy response and failure of first induction therapy pictured on each day after beginning of induction chemotherapy. The cut-off line was set to 2. Regarding the appearance of infinite high values, the interquartile range is not shown.

In patients with failure of first induction therapy, there was a rising population of monocytes and partial blasts, which were falsely counted as monocytes in machine generated cell counts. We found that the MNR increased much higher than in patients with therapy response (Fig. 3). Graphs showing the MNR of each patient group with interquartile range are presented in the supplementary (**Fig. S 2**).

We compared the MNR of responders and patients with failure of first induction therapy on each day (14 to 25). There were significant differences of the MNR between the two groups on day 16–18 in our observation. The lowest p-value (p = 0.005) was obtained on day 18. Using the described repeated splitting into a training and a test data set, the median AUC was the highest on day 18 with a value of 0.83 (**Table S1**). The calculated median optimal cut-off of the MNR was 1.174 on day 18 for the prediction of complete therapy response in the study population. Summarized over the 100 repetitions the best cut-offs on day 18 led to a high median accuracy (0.849), sensitivity (0.833) and specificity (0.857). Compared to the other days, the MNR on day 18 seems to be the best for predicting therapy response. For further details please see supplemental material (**Table S1**).

For a better handling in practice, we think the threshold should be an integer number. Due to a higher accuracy and specificity, we have chosen two instead of one as a cut-off value for the following analysis.

Using the integer cut-off, day 18 was still the best day for predicting therapy response with a high accuracy (0.868), specificity (0.833) and the best sensitivity (0.878) (Table 1). The AUC was 0.837, the highest

Table 1
Accuracy, sensitivity and specificity correspond to the cut-off 2. The p-values belong to the Wilcoxon rank sum test for comparing the MNR between the two patient groups. They were adjusted for multiple testing using the Bonferroni-Holm procedure.

Day	AUC	Accuracy	Sensitivity	Specificity	p-Value
14	0,6860	0,7885	0,5833	0,85	0,0513
15	0,5750	0,7692	0,4545	0,8537	0,1880
16	0,7663	0,8	0,5833	0,8684	0,0020
17	0,6750	0,8039	0,6364	0,85	0,0382
18	0,8373	0,8679	0,8333	0,878	0,0005
19	0,6333	0,6981	0,6667	0,7073	0,0763
20	0,7024	0,7692	0,5455	0,8293	0,0797
21	0,6476	0,7358	0,6364	0,7619	0,1880
22	0,6294	0,7255	0,4167	0,8205	0,2386
23	0,6581	0,78	0,4545	0,8718	0,1880
24	0,6308	0,8163	0,2727	0,9737	0,2386
25	0,7487	0,875	0,4	1	0,1152

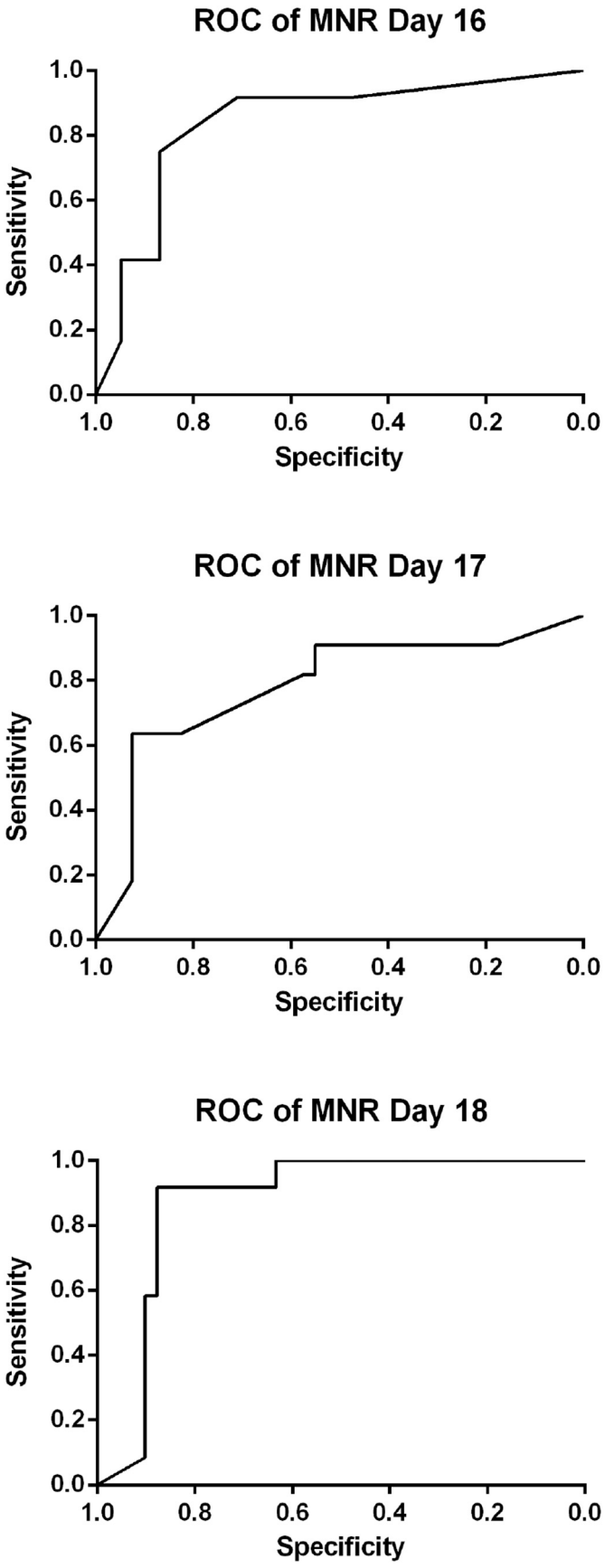

Fig. 4. Receiver operating characteristic curves of the MNR on days 16–18.

value over the twelve different days, and the negative predictive value to rule out failure of first induction therapy was 95.3%.

Additionally, random forest models identified the MNR on this day as the most valuable variable measured in this study separating patients with and without therapy response. The corresponding ROC curves based on all 54 data points on days 16–18 show an overall high predictive power of the MNR (Fig. 4).

3.4. Other laboratory values, sex and age

There were no differences in LDH values on day 18 ($p = 0.116$) between the responding and failure of first induction therapy group (data not shown). Neither Sex nor Age is a predictor for therapy response ($p = 0.75$ and $p = 1$ respectively).

3.5. Comparison between Sysmex XE2000 ™ hematology system and Sysmex XN2000 ™ hematology system

In 2015 the blood cell analyzer in our hospital was changed from the *Sysmex XE2000*™ to the *Sysmex NX2000*™. We divided the patients depending on which blood cell analyzers was used and checked the sensitivity and specificity of the MNR for each group. On day 18 with the *Sysmex XE2000* ™ the MNR had a sensitivity of 100% and a specificity of 82% ($n = 22$, $n_{\text{therapy response}} = 17$, $n_{\text{failure of first induction therapy}} = 5$). With the *Sysmex XN2000* ™ there was a sensitivity of 88% and a specificity of 71,5% ($n = 32$, $n_{\text{therapy response}} = 25$, $n_{\text{failure of first induction therapy}} = 7$). However, due to a balanced distribution of patients with therapy response and patients with failure of first induction therapy throughout both devices no bias is expected.

4. Discussion

Despite improvements in treatment strategies of AML in the recent years, a significant number of patients still fail to reach CR with the initial induction chemotherapy. It is generally accepted that early clearance of blasts in bone marrow and blood is an essential milestone of therapy and assessment of the prognosis [7,20]. In a study of 449 adult patients with AML ranging in age from 16 to 76 years old, lower marrow blasts count at day 16 after the standard induction treatment with '7 + 3' regimens was associated with a higher event-free, disease-free and overall survival ($p < 0.0001$ for all endpoints) [20].

Due to invasiveness of bone marrow puncture, and the hope for a non-invasive medical treatment in the foreseeable future, we sought for a method predicting therapy response based on the count of neutrophil granulocytes and monocytes in peripheral blood.

Using a cut-off value of 2, the monocytes-neutrophils-ratio on day 18 after induction therapy demonstrates a sensitivity of 88% and a specificity of 83% for the identification of therapy failure. It shows a high negative predictive value (NPV) of 95.3% to rule out failure of induction therapy. The assumed prevalence calculating the NPV was 25% for failure of the first induction, which fits with the prevalence of the study group and literature findings [5,6]. In conclusion, we propose the MNR as an additional tool to early, cost effectively and easily perceive failure of the first induction therapy in daily clinical practice. Thus, it can be a piece in the diagnostic puzzle.

Absolute cell counts of neutrophils and monocytes are generally used to identify hematopoietic reconstitution, but they are individually different and influenced e.g. by infection and seem not to be reliable in therapy monitoring. By creating a ratio, the equal onset of leukopoiesis is monitored apart from absolute cell counts and the selectivity between responders and patients with therapy failure becomes higher. Because this approach is based on the idea of a healthy recovery of hematopoiesis, patients with secondary AML, which may still have dysplastic cells arise from myelodysplastic syndrome after induction therapy, cannot be monitored with this method. Therefore, we did not include these patients in our study a priori.

Other studies which tried to seek for a non-invasive monitoring used the clearance of blasts in peripheral blood during induction therapy for response prediction [21–23], but this approach fails in rare cases of aleukemic leukemia. Compared to the studies mentioned above, we prove the healthy regeneration of the bone marrow after therapy and not only the impact of chemotherapy itself by lowering the blast count in peripheral blood or bone marrow.

The ELN Guideline 2017 emphasizes the MRD clearance for complete remission and response criteria, which provides a high sensitivity and specificity [4]. Genetic analyses used for monitoring therapy are expensive, need time and are sometimes not informative [4]. Multiparameter Flow Cytometry (MFC) is another highly sensitive tool to assess MRD, but the exact time point to assess MRD with MFC is not clear [4,24]. In deep aplasia after induction therapy, the impact of the low cell count in peripheral blood samples on the accuracy of MFC must be considered. The MNR is suitable even with low blood counts and in case of aleukemic leukemia. Furthermore, MNR is immediately available during the daily ward round and thus having clinical impact.

Current guidelines recommend considering a second course of induction chemotherapy if the bone marrow still has more than a 5% blast count on day 14–21. Regarding these issues, we propose that it is possible to safely predict therapy response and start early on with the planning of a second induction therapy by utilizing non-invasive techniques, including the MNR. Latest publications and reviews support this hypothesis of a questionable ‘day 14 marrow’ and strengthen non-invasive monitoring [12–14,25–28].

The probability that myeloblasts appears as part of the monocyte cell count varies depending on the kind of the blood cell analyzer. This issue was addressed by multiple former studies [17,18,29–37]. The blood cell analyzers tested in our study are most frequently used and belong to the standard equipment in hematologic labs in Germany. However, it must be noted that our findings must be validated using other blood cell analyzers and cannot directly be transferred to all analyzers.

We are very much aware of the fact that data based on a population of 54 patients cannot allow us to recommend the MNR for clinical use, but it is promising enough to be reassessed and validated in a larger study. In case of successful validation, the easy-to-apply MNR would be an important additional instrument in the hands of hematologists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcmd.2019.04.008.

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