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Research Article

Automated Analysis of Bone Marrow Aspiration Fluid Using Siemens ADVIA 2120i Hematology Analyzer

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Abstract

Background: Bone marrow examination plays a central role in clinical management of many primitive and secondary hematological diseases. We conducted a systematic evaluation of Siemens ADVIA2120i analytical performance pertaining to automated analysis of bone marrow aspirate fluids.

Methods: 88 samples of bone marrow fluids were analyzed with ADVIA2120i and compared with a flow cytometry assay for total nucleated cells and manual microscopy for differential counts.

Results: Regarding total nucleated cells, ADVIA2120i showed a strong and significant correlation with flow cytometric counts, with a positive systematic bias due to interference by lipid particles. The myeloperoxidase reaction allowed ADVIA2120i to obtain a strong and significant correlation with the elements of the neutrophilic lineage. The same was observed between myeloperoxidase negative events in the PEROX channel and manual counts of NRBCs, lymphocytes and plasma cells. Moreover, scatter-plots were found to be an important source of information for the study of bone marrow aspirates.

Conclusion: Automated cytometric analysis with ADVIA 2120i could be potentially useful for bone marrow examination.

Introduction

Bone marrow examination (aspirate and biopsy) plays a central role in the clinical management of many primitive and secondary hematological diseases [1]. While cytological analysis of peripheral blood is carried out mainly with automated hematology analyzers [2], cellular analysis of bone marrow aspirates still heavily relies upon manual microscopic examination.

Bone marrow samples differ from peripheral blood mainly because of greater cellular heterogeneity (given the presence of precursors normally absent from peripheral blood, as well as small quantities of non-hematopoietic cells) and the presence of lipid particles of stromal origin [3]. Despite these differences, in the past decades some Authors have tried to analyze bone marrow aspirates with automated hematology analyzers designed for peripheral blood, sometimes using different pre-treating techniques on samples in order to eliminate interference from lipid particles [4]. From the first efforts published at the beginning of 1960s [5] and thanks to the analytical improvements these instruments have witnessed during all these decades, automated hematology analyzers proved to be of potential use in cellular analysis of bone marrow samples for the enumeration of total nucleated cells and discrimination of certain differential populations (with different performances based on the analytical principles used); moreover, some Authors showed scatter-plot review could reveal important information during analysis of bone marrow samples [6-19].

Among all the modern hematology analyzers, Siemens ADVIA2120i (Siemens Healthineers, Erlangen, Germany) received little attention from studies investigating bone marrow analysis with automated cell counters. Indeed, apart from two studies that used animal samples [20,21], only four articles conducted on humans with this analytical technology (Siemens ADVIA or predecessor instruments manufactured by Technicon/Bayer) are available and pertain mainly to the study of bone marrow specimens obtained from patients suffering from acute leukaemia. In particular, Jang MJ et al. [22] and D'Onofrio G et al. [23] focused respectively on quantitative parameters and scatter-plot review using Siemens ADVIA120 for the differential diagnosis of different types of acute leukaemia, whereas Den Ottolander GJ et al. [24] studied diagnostic accuracy of many quantitative parameters and morphological flags produced by Bayer/Technicon H1 for the identification of relapsed disease during follow-up of patients. The fourth and final paper published on the topic with this technology refers to a study by Lesesve JF et al. [25] that described the appearance of the scatter-plots produced by Bayer/Technicon H2 in the presence of lipid particle interference.

Given these premises, we realized a systematic evaluation of Siemens ADVIA2120i analytical performances pertaining to cell counts and differentials for the automated analysis of bone marrow

aspirate fluids. Moreover, we offer some examples of how the careful revision of scatter-plots could reveal useful and important information for the study of clinical cases.

Materials and Methods

To evaluate the analytical performance of Siemens ADVIA2120i, its total nucleated cell counts and differentials were compared with a flow cytometry assay for the quantification of nucleated cells and with manual differential counts, respectively.

All procedures performed in this study were in accordance with the ethical standards of the ethics committee of the University Hospital of Modena and with the 1964 Helsinki declaration and its later amendments.

Bone marrow samples

88 consecutive bone marrow aspirate fluids received at the Flow Cytometry Unit of the Clinical Laboratory, Policlinico di Modena were included in the study. Only coagulated or scarce (<0.5 mL) specimens were excluded. All the specimens were obtained from iliac crests of patients during routine diagnostic procedures and contained about 2 mL of fluid, aspirated after the first mL (for morphological analysis). Bone marrow fluid was collected in BD Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing K_3-EDTA as anticoagulant. Within 1 hour from collection, samples were transported to the laboratory at room temperature, where they were analyzed with Siemens ADVIA2120i, flow cytometry and manual microscopy without any pre-treating technique.

Automated cell analysis with siemens ADVIA2120i

Briefly, Siemens ADVIA2120i is an optical hematology analyzer that provides leukocyte analysis by means of two different analytical channels, measuring cell size and myeloperoxidase staining (PEROX channel) or nuclear lobulation (BASO/lobularity channel): the result is a 6-part differential consisting of neutrophils, lymphocytes, monocytes, eosinophils, basophils and Large Unstained Cells (LUC) [26]. Likewise, nucleated red blood cells are identified and counted based on cell size and negative peroxidase reaction (PEROX channel) or nuclear density (lobularity channel).

All specimens were analyzed in duplicate. Total Nucleated Cell Counts (TNCC×10^9/L) were obtained adding Nucleated Red Blood Cells (NRBC) to white blood cell counts; likewise, the uncorrected differential counts (%; i.e. percentages that include NRBCs) were taken for the analysis.

For each case, scatter-plots and quantitative counts were reviewed along with clinical history and other laboratory data (including cytology, immunophenotyping and histology, if available).

During the study, the analytical performances of the analyzer were monitored daily with internal quality controls and periodically by means of external quality assessments.

Flow cytometry assay for total nucleated cells count

In the absence of a reference measurement procedure for total nucleated cells in bone marrow aspirate fluids, we applied to bone marrow a flow cytometry assay derived from candidate reference methods for nucleated cells quantification on peripheral blood [27,28].

Briefly, 50μL of bone marrow fluid were incubated for 15 minutes at room temperature and in the dark with 10μL of SYTO16 (Invitrogen – Thermo Fisher Scientific, Waltham, MA) and 10μL of anti-CD45 monoclonal antibody conjugated with PerCP-Cy5.5 (Becton Dickinson). SYTO16 is a fluorescent cell-permeant probe that shows a large increase in quantum yield (and hence, fluorescence enhancement) upon binding intracellular nucleic acids. After incubation, 1mL of BD Pharm Lyse, a lysing solution for red blood cells, was added. After another 15 minutes in the dark and at room temperature, the specimens were acquired on a BD FACS Canto II (or, for a maximum delay of 60 minutes, the samples could be preserved in the dark at 4°C). Samples were prepared in BD Trucount tubes, containing known amounts of lyophilized fluorescent beads: comparing the number of cells and fluorescent beads acquired from the same specimen enabled us to obtain total nucleated cell concentrations in a given sample. Indeed, given the proportion [B]:[C]=B:C, it follows that [C]=([B]*C)/B, where [B] and B refer to beads concentration and acquired absolute count, respectively, and [C] and C are total nucleated cells concentration and absolute count of pertinent events acquired, respectively.

Gating strategy is fully depicted in Figure 1.

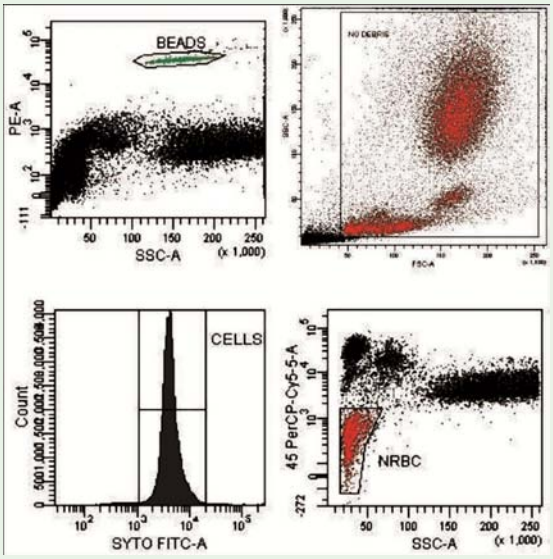

Figure 1: Gating strategy of the flow cytometry assay for total nucleated cells count. Fluorescent beads are counted and excluded from all subsequent analysis in a side scatter (SSC) vs PE plot; in a forward scatter (FSC) vs SSC plot all the events representing cellular debris are excluded from the analysis; all the SYTO-16-positive events, representing nucleated cells, are identified and counted in the FITC channel; in a SSC vs PerCP-Cy5.5 plot the percentage of nucleated red blood cells (CD45-negative events) over all nucleated cells is obtained.

Slides preparation and manual microscopic review

For every sample, two slides were produced with the automated Siemens ADVIA Autoslide, using a May-Grunwald-Giemsa staining protocol. In the absence of a reference method for bone marrow differentials, we integrated guidelines from the International Council for Standardization in Haematology regarding standardization of bone marrow specimens [1] and the evaluation of blood cell analyzers [29] with standards from Clinical and Laboratory Standards Institute

Table 1: Clinical reasons for bone marrow examination and number of patients.

Clinical reasons for bone marrow examination	Number of patients
Acute leukaemia, diagnosis or follow-up	45
Non-Hodgkin lymphoma, diagnosis or follow-up	11
Suspicious for hematological malignancy	8
Monoclonal gammopathy/plasma cell dyscrasia	6
Myeloproliferative neoplasm	5
Thrombocytopenia	3
Myelodysplasia	3
Clinical trial (G-CSF in peripheral neuropathy)	2
Hypereosinophilia	1
Total	84

G-CSF: Granulocyte Colony Stimulating Factor.

for reference leukocyte differential counts on peripheral blood [30]. In particular, an expert reviewer counted 500 cells on both sides of each sample. To facilitate comparisons with the classes of cells identified by the analyzer, manual differentials were obtained for the following groups of cells: mature neutrophils (including "band cells"), immature neutrophils (promyelocytes, myelocytes, metamyelocytes), lymphocytes, monocytes (including promonocytes), eosinophils (mature and immature forms), basophils (mature and immature forms), blast cells, nucleated red blood cells, plasma cells, other cells (macrophages, megakaryocytes, osteoblasts, endothelial cells etc.).

Statistical analysis

R software and additional packages have been used to analyze the data. In particular, descriptive statistics have been computed with R default packages; Kolmogorov-Smirnov test has been conducted with the "nortest" package; to calculate Spearman's coefficient of correlation and pertinent confidence intervals the additional packages "Hmisc" and "RVAideMemoire" have been used; the package "mcr" has been used to calculate Passing-Bablok regression analysis.

Results

The 88 samples included in this study were obtained from 84 patients, 35 males and 49 females, median age 59 years (interquartile range: 46-67 years; minimum-maximum: 19-81 years). In Table 1 the clinical reasons for bone marrow examination of all the patients are summarized; one patient suffering from myelodisplasia and three patients during follow-up for acute leukaemia had each two samples obtained during the study. Cases include three morphological relapses of acute leukaemia and one new diagnosis of acute promyelocytic leukaemia, 3 cases of bone marrow involvement by non-Hodgkin lymphomas (1 marginal zone lymphoma, 1 B-cell chronic lymphocytic leukaemia and 1 peripheral T-cell lymphoma, not otherwise specified) and 4 patients with multiple myeloma with medullary involvement. 5 cases of myeloproliferative neoplasms include 2 cases of primary myelofibrosis, 1 case of essential thrombocytosis, 1 case of chronic myelogenous leukaemia and 1 case of polycythemia vera with the JAK2 V617F mutation.

In Table 2 distributions of the parameters obtained with Siemens ADVIA2120i, manual microscopy and flow cytometry are reported. None but automated neutrophil counts showed a normal distribution (as suggested by Kolmogorov-Smirnov test's P-value, with statistical significance level α=0.05, reported in Table 2).

Table 2: Distribution of automated, manual and flow cytometry results.

	Median	Interquartile range	Min-max	Kolmogorov-Smirnov test (P)
Siemens ADVIA2120i				
TNCC (WBC+NRBC) (10^9/L)	10.03	6.46-14.91	0.71-52.84	0.047
NRBCs (%)	3.62	0-7.84	0-36.12	<0.001
Neutrophils (%)	56.04	47.31-63.78	16.70-85.71	0.307
Lymphocytes (%)	15.84	12.66-23-36	2.40-77.4	<0.001
Monocytes (%)	10.46	7.83-14.11	0.33-34.61	0.002
Eosinophils (%)	1.61	1.08-2.21	0.09-7.98	<0.001
Basophils (%)	2.34	1.52-3.12	0.3-30.95	<0.001
LUC (%)	3.01	2.41-4.18	0.55-30.66	<0.001
Blasts (%)	0.80	0.55-1.21	0.08-6.95	<0.001
Manual microscopy				
Mature neutrophils (%)	57	50.08-64.2	1.3-87.7	0.003
Immature neutrophils (%)	8.69	5-12.1	0-69.8	<0.001
Lymphocytes (%)	10.75	8.05-17.64	0.6-88.7	<0.001
Monocytes (%)	4.07	2.7-5.8	0-25.9	<0.001
Eosinophils (%)	1.75	0.7-3.1	0-11.3	<0.001
Basophils (%)	0.3	0.1-0.53	0-2.26	<0.001
Blasts (%)	0.7	0.3-1.1	0-29	<0.001
NRBCs (%)	9	4.43-13.82	0-38.4	0.020
Plasma cells (%)	0	0-0.1	0-45.8	<0.001
Other cells (%)	0	0-0	0-0.5	<0.001
Flow cytometry				
TNCC (10^9/L)	8.55	4.46-12.86	0.61-52.98	0.008
NRBCs (%)	8.6	4.43-14.55	0.2-32.7	0.001

TNCC: Total Nucleated Cells Count; WBC: White Blood Cells; NRBC: Nucleated Red Blood Cells; LUC: Large Unstained Cells.

Spearman's coefficients of correlations and the results of non-parametric Passing-Bablok regression analysis conducted between Siemens ADVIA2120i automated parameters and microscopy or flow cytometry are reported in Table 3, while in Figure 2 some regression plots are depicted.

Discussion

In this study, we evaluated how Siemens ADVIA2120i analyzes bone marrow aspirate fluids. To do so, we compared its automated total nucleated cell counts and differentials with a flow cytometry assay designed for nucleated cells quantification and with manual differential counts, respectively.

Regarding total nucleated cells, a very strong and significant correlation emerged between the counts obtained with ADVIA and the flow cytometry assay designed for the purpose, with regression analysis showing however a significant positive systematic bias for the automated hematology analyzer (Figure 2): this could be easily explained by the presence of interfering lipid particles, that are erroneously counted as cells by the optical apparatus of Siemens ADVIA2120i, as can be seen from the scatter-plots (Figure 3).

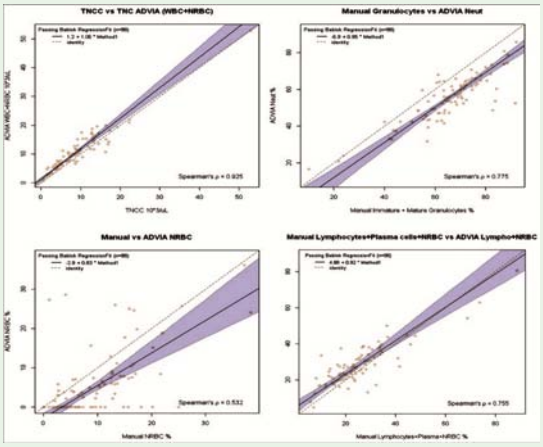

Figure 2: Passing-Bablok regression plots. Top left: total nucleated cell counts from ADVIA and flow cytometry. Top-left: automated Neutrophil counts (events with high peroxidase activity) and manual neutrophil granulocytes (mature and immature). Bottom left: nucleated red blood cell counts from ADVIA and manual cytology. Bottom right: myeloperoxidase negative events in ADVIA's PEROX channel and manual counts of NRBCs, lymphocytes and plasma cells.

Regarding flow cytometry, a proof for the accuracy of the assay was obtained confronting its nucleated red blood cell counts (as CD45-negative and SYTO16-positive cells) with those obtained with manual microscopy: data analysis revealed the two methods showed a very strong and significant correlation with no bias observed.

To evaluate Siemens ADVIA2120i differentials we compared its counts to those obtained by manual microscopy. Predictably, the use of the myeloperoxidase reaction for leukocyte analysis resulted in a strong and significant correlation between highly positive cells (counted as Neutrophils by the instrument) and cellular elements belonging to the neutrophilic lineage (ρ=0.703 with mature neutrophils and ρ=0.775 with mature and immature neutrophils combined). Since Neutrophil area shows relatively little interference from lipid particles in the PEROX scatter-plot (Figure 3), Siemens ADVIA2120i showed a tendency to underestimate neutrophilic granulocytes compared to other cell classes (Figure 2): this also explains why this negative tendency paradoxically disappear confronting ADVIA results with only mature neutrophils. For the same reasons, eosinophil counts were strongly correlated with manual counts but showed a significant underestimation.

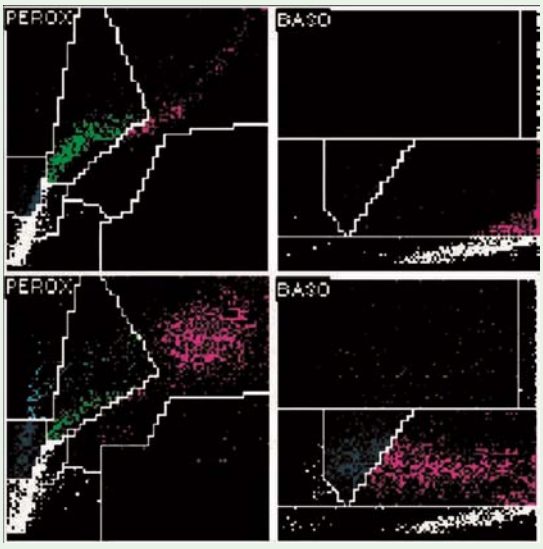

Figure 3: ADVIA2120i scatter-plots of bone marrow aspirate fluids. At the top: PEROX and BASO scatter-plots of a bone marrow sample obtained from a patient after administration of salvage therapy for relapsed acute myeloid leukaemia; the flow cytometric analysis for nucleated cells revealed a severe cytopenia (0.06*10^3 cells/μL) and the events depicted on the plots are almost only lipid particles of stromal origin. At the bottom: scatter-plots from a normocellular bone marrow sample show the same lipid interference along with normal cluster of cells.

Since the monocyte region in the PEROX channel is the most interested by lipid particles interference, it is not surprising that ADVIA showed only a moderate correlation with manual counts of monocytes (ρ=0.537) and a significant positive systematic error (Table 3).

Lipid particles likely interfere in the PEROX channel even with lymphocytes, whose automatic counts were strongly correlated with manual differentials (ρ=0.666) but with a significant overestimation. Similar results were obtained including LUCs and plasma cells within ADVIA and manual counts, respectively (Table 3).

The identification and enumeration of nucleated red blood cells made by Siemens ADVIA 2120i is based only on the scatter properties of such cells [31]. In bone marrow aspirates, it can't be excluded that earlier and bigger precursors are erroneously identified as lymphocytes by the analyzer. Moreover, ADVIA does not report nucleated red blood cells if NRBC <200/μL, NRBC <2/100 WBC or WBC <3000/μL. For all these reasons, it is not surprising that the analyzer significantly underestimated NRBCs (Figure 2), with only a moderate correlation with manual counts (ρ=0.532).

A strong and significant correlation was observed between myeloperoxidase negative events in the PEROX channel (Lymphocytes + LUC + NRBC) and manual counts of NRBCs, lymphocytes and plasma cells (ρ=0.755), even in the presence of a positive systematic bias due to lipid interference probably associated to NRBC underestimation (Figure 2); moreover, even the small number of myeloperoxidase-negative non-hematopoietic cells could likely contribute to this overestimation.

Finally, basophil count by ADVIA was the only automated parameter that showed no significant correlation with manual differentials (Table 3). On the contrary, automated blast counts demonstrated a significant but poor correlation (ρ=0.345) with microscopy, showing however a tendency for underestimation at higher counts: this was probably due to the small number of samples with elevated blast counts included in the study (only 3 specimens with blasts >5%).

The review of the analyzer's scatter plots, along with quantitative results and other clinical information could provide important and immediate information for the study of clinical cases. We provide two examples of how the "morphology" of scatter plots obtained with Siemens ADVIA2120i assisted the study of two hematological cases with similar peripheral blood findings.

The first clinical case concerns a 5-year-old boy who was brought to the emergency department because of persistent fever associated with pancytopenia (WBC 2.89 × 10^9/L, hemoglobin 100 g/L, platelets 62 × 10^9/L). A peripheral blood smear was obtained and the result was negative for immature or atypical cells. However, automated cytometric analysis of bone marrow aspirate revealed high cellularity (WBC/nucleated cells 137 × 10^9/L) with augmented lymphocytes and LUCs (92.1% and 15.6% of cellularity, respectively) forming a single cluster spanning both regions of the PEROX channel. BASO channel registered an increased number of events corresponding to nuclei with dispersed chromatin, along with many events in the basophil area (8% of total nucleated cells), suggestive for the presence of lysis-resistant cells (Figure 4). Moreover, these findings were associated with the disappearance of lipid particles of stromal origins, a finding evocative of an underlying expanding disease affecting bone marrow. The diagnosis of acute lymphoblastic leukemia was immediately suspected on the basis of these automated findings and subsequently confirmed with morphological, immunophenotyping and molecular investigations.

Table 3: Spearman's coefficients of correlations and non-parametric Passing-Bablok regression analysis.

Manual (or flow cytometry) parameters	Siemens ADVIA2120i automated parameters	Spearman's coefficient of correlation (ρ) [95%CI]	Passing-Bablok regression analysis	
			Intercept [95%CI]	Slope [95%CI]
TNCC (flow cytometry)	WBC+NRBC	0.925 [0.875,0.951]	1.204 [0.377,1.883]	1.06 [0.968,1.173]
Mature neutrophils	Neutrophils	0.703 [0.545,0.814]	2.685 [-5.437,10.849]	0.922 [0.787,1.059]
Mature neutrophils + Immature neutrophils	Neutrophils	0.775 [0.645,0.868]	-6.905 [-16.688,0.040]	0.952 [0.850,1.083]
Monocytes	Monocytes	0.537 [0.334,0.688]	0.545 [-1.946,3.342]	2.44 [1.599,3.266]
Monocytes + blasts	Monocytes	0.584 [0.408,0.710]	0.472 [-2.823,3.449]	1.909 [1.219,2.8]
Eosinophils	Eosinophils	0.781 [0.634,0.878]	0.501 [0.331,0.690]	0.619 [0.54,0.724]
Basophils	Basophils	-0.03 [-0.241,0.203]	8.338 [-3.320,12.540]	-17.9 [-36.015,23.601]
Lymphocytes	Lymphocytes	0.666 [0.494,0.803]	3.882 [1.578,5.438]	1.110 [0.932,1.310]
Lymphocytes + Plasma cells	Lymphocytes + LUC	0.67 [0.483,0.813]	6.849 [4.175,8.605]	1.14 [0.96,1.362]
Lymphocytes + Plasma cells + Blasts	Lymphocytes + LUC	0.666 [0.498,0.794]	5.899 [3.493,8.458]	1.091 [0.884,1.287]
Plasma cells + Blasts	LUC	0.416 [0.230,0.597]	0.932 [0.247,1.725]	2.482 [1.355,3.837]
NRBC	NRBC	0.532 [0.344/0.703]	-2.803 [-4.947/-1.417]	0.829 [0.65/1.08]
Lymphocytes + Plasma cells + NRBCs	Lymphocytes + LUC + NRBC	0.755 [0.608,0.857]	4.888 [1.318,8.348]	0.923 [0.792,1.105]
Lymphocytes + Plasma cells + NRBCs + Blasts	Lymphocytes + LUC + NRBC	0.739 [0.593,0.846]	5.225 [1.914,8.302]	0.868 [0.765,1.014]
Blasts	Blasts	0.345 [0.114,0.541]	0.329 [0.119,0.453]	0.7 [0.465,1.072]
Manual NRBC	Flow cytometry NRBC	0.907 [0.837,0.944]	0.048 [-0.493,0.650]	0.949 [0.843,1.075]

TNCC: Total Nucleated Cells Count; WBC: White Blood Cells; NRBC: Nucleated Red Blood Cells; LUC: Large Unstained Cells.

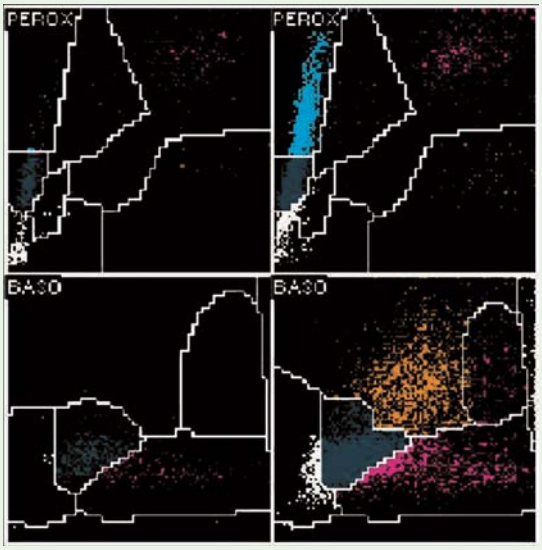

Figure 4: Scatter-plots of peripheral blood (left) and bone marrow fluid (right) from a 5-year-old boy with a newly diagnosed acute lymphoid leukemia. The bone marrow plots show increased cellularity due to a large number of myloperoxidase-negative events in the PEROX plot and lysis-resistant cells in the BASO channel. Both channels show the disappearance of lipid particles of stromal origins, a finding suggestive of an underlying expanding disease of the bone marrow.

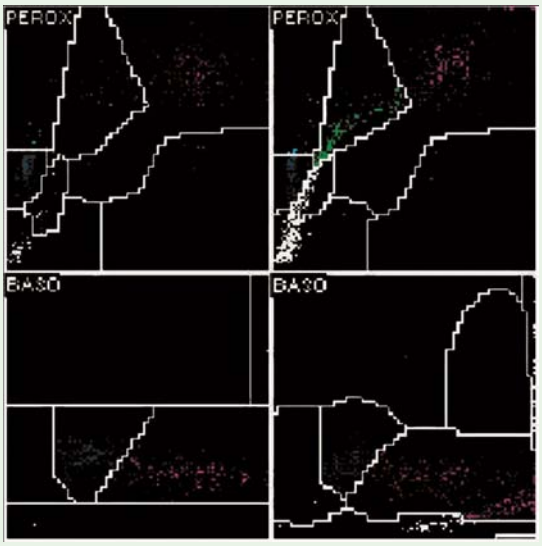

Figure 5: Scatter-plots of peripheral blood (left) and bone marrow fluid (right) from a 38-year-old man suffering from idiopathic aplastic anemia. Automated analysis of bone marrow fluid reveals reduced cellularity with evident stromal particles and no increase of LUCs or blasts.

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The second clinical case concerns a 38-year-old man who visited a clinical Hematologist because of persistent asthenia; diffuse petechiae and pancytopenia (WBC 0.96 × 10^9/L with neutrophils 0.54 × 10^9/L, hemoglobin 109 g/L, platelets 20 × 10^9/L). The peripheral blood smear was negative for immature and atypical cells; moreover, there was no appreciable lymphadenopathy or splenomegaly. Automated analysis of bone marrow fluid revealed reduced cellularity with evident stromal particles (Figure 5); no increase of LUCs or blasts was observed (3.6% and 0.4%, respectively). A final diagnosis of idiopathic bone marrow aplasia was made after reduced cellularity (<10%) was confirmed with biopsy and since secondary causes were excluded.

This study has at least two limitations. First of all, since the aspiration of increasing bone marrow volumes determines progressive dilution with peripheral blood, even if this is an erratic and unpredictable phenomenon [32], caution should be used before extending our results to different volumes and modalities of sample withdrawal. Moreover, we did not apply sample pre-treatment, but it should be interesting to evaluate ADVIA's analytical capabilities after elimination of lipid particles interference, since we found this as the main source of inaccuracy.

Conclusion

Despite automated cellular analysis of bone marrow aspirate samples is hampered by the great cytological heterogeneity and the presence of lipid particles, some Authors successfully analyzed these samples with automated hematology analyzers designed for peripheral blood testing, proving potentially useful information could be obtained regarding sample cellularity, differential cellular analysis and scatter-plot appearance. Since Siemens ADVIA2120i has never been thoroughly studied for this purpose, we decided to evaluate its analytical performance for the analysis of bone marrow aspirates. Our results reveal that Siemens ADVIA2120i could provide important automated quantitative and qualitative information regarding bone marrow aspirate fluid: for example, it could provide a rapid estimate of sample cellularity and a distribution of cellular components (myeloid vs non-myeloid). In fact, the analyzer showed excellent counting capabilities for total nucleated cells and good correlations with specific cellular populations, albeit with systematic errors mainly due to interference from lipid particles. Moreover, the presence of certain pathological cellular populations could be highlighted as well (e.g. peroxidase negative blasts in the LUC region). Finally, scatter-plots were found to be an important and rapid source of information for the study of bone marrow aspirates. In fact, one of the potential advantages of automated analysis is the great reduction in turn-around-time that could be achieved compared to more cumbersome and manual techniques: for example, Siemens ADVIA 2120i analyze a sample in less than a minute [26], while almost an hour is needed to prepare, acquire and analyze cells with flow cytometry whereas several hours/few days may be necessary to process and report a sample with manual microscopy [1].

In conclusion, automated cellular analysis of bone marrow fluid is still in its infancy and to date it should be considered an off-label application of instruments designed for peripheral blood and body fluids analysis. Nonetheless, this study provides further evidence that bone marrow cytometric analysis could be of use in selected cases and shows great potential for the development of more advanced and clinically useful applications.

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