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Original Research Article

Characterization of pseudobasophilia on Sysmex-XT 1800i automated hematology analyser

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ABSTRACT

Background: Pseudobasophilia is a common automation related phenomenon which requires manual peripheral smear study in an era of complete automation. This study has attempted to evaluate the reasons for pseudobasophilia and in-turn suggest measures to eliminate the errors.
Methods: A sample size of 207 cases showing pseudobasophilia on automation were studied by manual peripheral examination to categorize the possible cause for its occurrence. Descriptive and inferential statistical analysis was carried out. Results on continuous measurements are presented on Mean SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Student t test has been used to find the significance of study parameters on continuous scale within each group.
Results: Atypical/ reactive lymphocytes were present in 86.5% cases contributing to pseudobasophilia phenomenon on automation, which also showed falsely increased absolute basophil count with more percentage of lymphocytes showing reactive changes. Temperature and storage effects did not contribute to their occurrence in this study. Another finding was an associated pseudomonocytosis with pseudobasophilia on automation which was statistically significant ($p<0.001$).
Conclusions: Pseudobasophilia, and pseudomonocytosis are automation related phenomenon. Atypical/ reactive lymphocytes, which are cytoplasmic strip resistant, contribute to their occurrence. Hence, newer modalities like multicolour flow cytometry coupled with antibody tagging, multiangle polarised scatter separation and volume conductivity scatter may reduce the chances of pseudobasophilia, thereby reducing the overall turnaround time.

Keywords: Automation, Pseudobasophilia, Reactive lymphocytes

INTRODUCTION

Automated analysers offer precise and fast results in hematology.[1] However, pseudobasophilia and pseudomonocytosis are a commom problem. Of these, basophil count on automation and manual smear examination showed least correlation across various instruments.[2]

Study faced a similar problem with basophil count on Sysmex XT 1800i (Sysmex Corporation, Kobe, Japan) which performs WBC analysis by optical detector block based on flow cytometry method using a semiconductor laser.[3] Hence, the study was formulated to evaluate the causes of pseudobasophilia.

The objective of this study was to Isolate all cases showing basophilia on Sysmex XT 1800i automated hematology analyser and

To characterize the mimics of basophilia on peripheral smear study.

METHODS

All K2-EDTA anticoagulated blood samples which showed absolute basophil count >0.15 $X10^9$/L or basophil percentage >1% on Sysmex XT 1800i automated hematology analyser were isolated and included in the study.

Cases wherein automated report and peripheral smear examination were concordant with the diagnosis of basophilia were excluded from the study.

A study sample size of 207 cases were isolated applying the above criteria. For all these cases, Leishman stained peripheral smears were obtained. Smears were studied for the mimics of basophils and an attempt to categorize the causes of pseudobasophilia was done based on the presence of:

- Reactive / atypical lymphocytes
- Large granular lymphocytes
- Toxic granules in neutrophils
- Eosinophilia
- Giant platelets
- Platelet clumps
- Blasts
- Malarial parasites.

Further, the reactive lymphocytes were graded as follows:

1+ = <30% lymphocytes showing reactive changes
2+ = 30-60% lymphocytes showing reactive changes
3+ = >60% lymphocytes showing reactive changes

Statistical software

The statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Descriptive and inferential statistical analysis was carried out in the present study. Results on continuous measurements are presented on Mean SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance.

Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale with in each group.

Significant figures

*Moderately significant (P value: 0.01<P<0.05)

**Strongly significant (P value: P<0.01).

RESULTS

In the present study, a total of 207 cases were included. Of the 207 cases, 203 cases had platelet count less than 1.5 lakh/mm^3 amounting for 98.06% cases.

Table 1: Comparison of differential counts in counter and manual methods.

DC	Counter differential count	Manual differential count	difference	t value	P value
Neutrophil	40.30±12.99	41.62±13.00	-1.419	-2.363	0.019*
Lymphocyte	44.91±12.06	52.01±12.52	-6.943	-11.037	<0.001**
Monocyte	9.21±2.77	4.45±1.87	4.741	23.140	<0.001**
Eosinophil	1.66±2.28	1.79±2.52	-0.136	-1.642	0.102
Basophil	3.99±1.81	0.03±0.18	3.961	31.708	<0.001**

The differential counts obtained by automation and manually when compared showed discrepancies in lymphocyte, monocyte and basophil percentages as shown in Table 1.

The frequency of occurrence of various parameters thought to be associated with pseudobasophilia are shown in Table 2.

Of the various parameters, reactive lymphocytes were present in 86.5% of the cases followed by giant platelets which was seen in 54 cases amounting to 26.1%. The other variables studied accounted for <12% independently.

The absolute basophil count was compared to the grading of reactive lymphocytes (table 3). 48 of the 59 (81.4%) cases showing 3+ reactive lymphocytes and 42 of the 56 cases (75%) showing 2+ reactive lymphocytes showed increased absolute basophil count.

Blast flag and atypical lymphocyte flag was enumerated from the automated hematology analyser reports (Table 4). On manual smear study, blasts were absent in 100% cases as against 92.3% showing blast flag on automation.

Table 2: Occurrence of various parameters (possible mimics of pseudobasophilia).

Criteria	Criteria	No. of patients (n=207)	%
Platelet clumps	Absent	198	95.7
	Present	9	4.3
Eosinophilia	Absent	197	95.2
	Present	10	4.8
Reactive lymphocytes	Absent	28	13.5
	Present	179	86.5
Toxic granules	Absent	184	88.9
	Present	23	11.1
Large granular lymphocytes	Absent	192	92.8
	Present	15	7.2
Giant platelets	Absent	153	73.9
	Present	54	26.1
Blasts	Absent	207	100
	Present	0	0
Malarial parasites	Absent	207	100
	Present	0	0

Table 3: Absolute basophil count (ABC) correlation with grading of reactive lymphocytes (N=179).

ABC	Grading of reactive lymphocytes			
	Absent	**1+**	**2+**	**3+**
<150/ cc.mm	13	25	14	11
>150/cc.mm	15	39	42	48

Table 4: Blast flag and atypical lymphocyte flag enumeration.

Criteria	Criteria	No. of patients (n=207)	%
Blast Flag	Absent	16	7.7
	Present	191	92.3
Atypical lymphocyte flag	Absent	192	92.8
	Present	15	7.2

Table 5: Comparison of automation results with peripheral smear examination for blast and atypical lymphocyte flag.

Criteria	Blast	Blast %	Atypical lymphocyte	Atypical lymphocyte %
Automation	191	92.3	15	7.2
Manual smear study	0	0	179	86.5

DISCUSSION

Basophils are granulocytes which usually constitute less than 1% of the total leukocyte count. The reference method for the basophil count is by microscopic slide examination as described in the H20-A2 standard from the Clinical and Laboratory Standards Institute (CLSI). But when counting the recommended 400 leukocytes, one might expect to find only 0 to 4 basophils, which leads to poor precision. This problem is also recognized by the CLSI, which states that the manual count is not suitable as a reference method for cells less frequent than 5% of the total leukocyte count.[4]

Haematology instruments count some thousand leukocytes and, therefore, may potentially offer more reliable estimates of the basophil count. Sysmex (Sysmex, Kobe, Japan) instruments count basophils as cells resistant to acidic stripping of the cytoplasm.[4] In some samples, there are pathologic cells that are lysis-resistant, which leads to a falsely elevated basophil count (pseudobasophilia).[4]

The cell types most frequently associated with pseudobasophilia include abnormal lymphocytes in HIV infected patients, myeloblasts, promyelocytes in acute promyelocytic leukemia, lymphoblasts, dysplastic neutrophils, atypical lymphocytes, plasma cells or myeloma cells, lymphoma cells, nucleated red cells and neutrophils in Maroteaux-Lamy syndrome.[5-7]

Reactive/ atypical lymphocytes are characterized by large size, abundant basophilic cytoplasm that is often vacuolated, diffuse or partially condensed chromatin with nucleoli, and large, often irregularly shaped nuclei.[8] In our study, reactive lymphocytes were present in 86.5% of the cases accounting for major cause of pseudobasophilia followed next in frequency by giant platelets (26.1%). Platelet clumps, eosinophilia, toxic granules in neutrophils and large granular lymphocytes accounted for <12% occurrence independently. Blasts and malarial parasites were not present in a single case. The findings were similar to a study by Jacomo R where pseudobasophilia almost always meant presence of atypical lymphocytosis.[1]

Another potential contributing factor for the occurrence of pseudobasophilia is that of coincidence, whereby multiple particles (clumps of cells or bare nuclei) enter the flow cell simultaneously, giving the appearance of a single, large cellular event. This phenomenon is more likely to occur in samples with high cellular concentrations, such as the frequently encountered marked leucocytosis of leukemic patients.[5] In present study, the leucocyte count was within normal limits in 67% (136/207) cases with 620 cells/mm^3 being the least and 15,310 cells/mm^3 being the highest value recorded. No case in our study had total leucocyte count in leukemic range, hence pseudobasophilia from coincidence was ruled out.

The absolute basophil count (ABC) was compared with number of lymphocytes showing reactive changes. In 81.4% cases showing 3+ reactive lymphocytes and 75% showing 2+ reactive lymphocytes, higher ABCs was seen. This finding suggests a possibility of higher

percentage of reactive lymphocytes to be associated with pseudobasophilia.

Modern automated analysers are able to "flag" the presence of qualitative abnormalities that require a blood film to be examined for confirmation of the abnormality or for further elucidation. A reported increase in basophil count should generally be regarded as a flag because it often represents a pseudobasophilia, resulting from the presence of leukemia or lymphoma cells.[9] In present study involving 207 cases showing pseudobasophilia, no single case showed blasts or lymphoma cells. Further, the automated analyser also showed blast flag in 92.3% cases. The atypical lymphocyte flag was shown in only 7.2% cases as when compared to peripheral smear examination which revealed atypical lymphocytes in 86.5% cases (Table 5). This finding is similar to Jacomo R who states that the presence of the ''blast'' flag defined a subgroup with higher incidence of atypical lymphocytes.[1]

In a study titled "hematological manifestations in dengue fever- an observational study" 221 cases of sero-positive classic dengue showed thrombocytopenia (100%) and atypical lymphocytes (87%) which is similar to our study showing thrombocytopenia in 203 of 207 cases (98.06%) associated with reactive/atypical lymphocytes.[10] However, in present study, dengue serological correlation was not done.

Other hematological features observed in our cases showing pseudobasophilia was relatively higher percentage of monocytes and lower percentage of lymphocytes on automation as seen when compared to manual differential counts. These showed a statistically significant (p value<0.001) association with the presence of pseudobasophilia.

Pseudomonocytosis has been described as a common storage change by Sysmex.[11] In a study titled "Evaluation of the performance of the Sysmex XT-2000i hematology analyzer with whole bloods stored at room temperature", monocyte counts showed significant decline with time. After 24 hours, the monocyte count was no longer stable compared to the 4-hour counts or the same-day precision results. The cell size, structure, or degree of granulation may change enough with time to make it no longer recognizable as a monocyte on automation.[12] However, in present study pseudomonocytosis was observed in 71 out of 207 cases (34.3%) on automation while none of the cases showed monocytosis on manual study. Pseudomonocytosis also presented with pseudobasophilia on automated analysis and was statistically significant in their co-existence (p<0.001). Storage effect was ruled out as all samples in the study were processed within 1 hour of sample collection. The reason for pseudomonocytosis on automation similar to pseudobasophilia could be a result of reactive lymphocytes whose cytoplasm has been stripped and owing to its larger volume is interpreted as a monocyte. As reasoned above, of the 71 cases, 63 (88.7%) cases of pseudomonocytosis showed reactive lymphocytes on peripheral smear study.

Temperature is a crucial element in the cytoplasmic stripping process and it is thought that variations in temperature can induce pseudobasophilia. Samples run at temperatures less than 20-25ºC (room temperature) increased the prevalence of pseudobasophilia due to incomplete lysis of leukocytes.[5] In our laboratory, temperature was not an interfering factor as all samples are collected in-house and are processed within 1 hour. Hence, the occurrence of pseudobasophilia due to variation in temperature was ruled out.

CONCLUSION

Pseudobasophilia is an automation related phenomenon, warranting manual peripheral smear examination. Atypical/ reactive lymphocytes, which are cytoplasmic strip resistant, contributed to pseudobasophilia in present study. Hence newer modalities like multicolour flow cytometry coupled with antibody tagging, multiangle polarised scatter separation and volume conductivity scatter may reduce the chances of pseudobasophilia, thereby reducing the overall turnaround time. Pseudomonocytosis, an incidental finding in this study, needs further elucidation as there is minimal literature on the same.

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