Original Article

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Autoanalyzer Generated Spurious Basophilia in Adolescents and Adults

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Abstract

Background: Failure of hematology auto analyzers to ascertain accurate basophil count is not uncommon. In a few instances, a report of basophilia is given by the automated counters in case of those patients who neither have these diseases nor true basophilia.

Objectives: This study tries to countercheck the basophil counts given by the auto analyzers: Beckman Coulter AcT.5Diff and Beckman Coulter HmX, by peripheral smear examination and to find the significance of association between the spurious basophilia and the hematological and technical factors.

Materials and Methods: Samples of 130 cases and an equal number of controls matched for age group, sex, automated instrument used for processing the sample, and the day on which the sample was processed, were analyzed. For every case, 500 cell differential count was made. In 48 of 130 cases, manual absolute basophil count was done using toluidine blue. Technical details such as the machine model used, sample processing time, sample related parameters such as the basophil percentage, automated and manual basophil absolute count, abnormality flagged by the machine, and abnormalities detected on peripheral smear examination were analyzed. Statistical analyses include formation of frequency tables with percentages, summary statistics such as mean, standard deviation, standard error of the mean, 95% confidence intervals, tests such as Mann-Whitney U Test, Chi-square test, Binomial test, and independent samples *t*-test.

Results: Significance value was <0.05 in cases processed in AcT.5Diff, standing time >1 h, hemoglobin level of ≥15 g/dl, nucleated red blood cells (nRBC), leukopenia, leukocytosis, neutrophilia, neutrophilic shift to left, reactive lymphocytes with/without relative lymphocytosis, thrombocytopenia.

Conclusion: The auto analyzer gives a significantly higher percentage of cases of spurious basophilia. The association of spurious basophilia is significant with prolonged standing time, hemoglobin concentration ≥15 g/dl, nRBC, leukopenia, leukocytosis, neutrophilia, neutrophilic shift to left, reactive lymphocytes with/without relative lymphocytosis, thrombocytopenia.

Key words: Basophils, Hematology, Leukocyte count, Leukocyte disorders

INTRODUCTION

Basophils in peripheral blood are known to be increased in allergic reactions, myeloproliferative diseases, inflammatory conditions, etc.[1,2] Failure of hematology auto analyzers to ascertain accurate basophil count is not uncommon.[3] In a few instances, a report of basophilia is given by the automated counters in case of those patients who neither have these diseases nor true basophilia. If the report is issued to the patient, without being counterchecked, it may result in an unnecessary evaluation of the patient. Hence, it is essential to scrutinize every case of basophilia with meticulous microscopic examination of the peripheral smear. Thus, in some cases, there may be discordance between the basophil counts generated by the automated instrument and the peripheral smear examination.[4–6] Though basophils are easily recognized in routine Romanowsky stains, their low percentage makes the usual visual differential counts made up to 100 white blood cell (WBC), imprecise.[7,8] Hence, in this study,

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we counted 500 WBC for differential counting and for doing the absolute basophil count manually we used the metachromatic stain toluidine blue for highlighting the granules of the basophils. Since the hematological values differ between the subjects belonging to the age groups of ≤9 years and ≥10 years, we studied both the groups separately. Our experience with the pediatric population has already been documented.[9]

Objectives

This study tries to countercheck the basophil counts given by the auto analyzers: Beckman Coulter AcT.5Diff and Beckman Coulter HmX by peripheral smear examination, and to find the significance of association between the spurious basophilia and the hematological and technical factors.

MATERIALS AND METHODS

Cases of age ≥10 years, whose basophil percentage on automated results is ≥2% of WBC on two consecutive readings, in addition to a basophil differential count of <2%, done on 500 cells on peripheral smear by two independent observers and controls matched for age group, sex, automated instrument used for processing the sample, and the day on which the sample was processed, which show basophil percentage of <2% on both the automated results and the peripheral smear examination were included in the study. The cases and the matched controls whose automated results and the peripheral smear examination are concordant with the diagnosis of basophilia were excluded.

After getting approved by the Institutional Ethics Committee, the study was done on the samples of 130 cases and equal number of controls submitted in the hematology laboratory in Chettinad Hospital and Research Institute for routine investigations during the period of 14 months, from December 2012 to January 2014. There was no need for obtaining consent from the patients/control subjects because of complete anonymization of the samples. The blood samples anticoagulated with Ethylenediaminetetraacetic acid were used, and they were processed in automated hematology counters Beckman Coulter AcT.5Diff and Beckman Coulter HmX. Peripheral smears of the cases were examined by Dr. Femela Muniraj and Dr. Vijay Amritraj independently, and differential counts on 500 cells were made for each case. In 48 of the 130 cases, absolute counts of basophils were done manually using a diluting/staining fluid composed of 10 ml of 0.05% toluidine blue in 0.9% saline, 2.75 ml of 95% ethyl alcohol, and 0.25 ml of solution of saponin saturated in 50% ethyl alcohol. This fluid was used by Mitchell and is a modification of that used by Moore and James.[8,10,11] One volume of the anticoagulated blood sample was diluted with 10 volumes of the fluid mentioned above. Both the chambers of the improved Neubauer chamber were charged simultaneously with this diluted sample and absolute basophil count, and total leukocyte count (TLC) were done simultaneously on the 4 corner squares of each chamber, altogether making up 8 corner squares, each having 16 smaller squares. Absolute basophil count/TLC was calculated using the formula n × 12.5 per cubic mm, derived from (n × 10) ÷ (8 × 0.1), wherein, n = number of basophils/total leukocytes in 8 corner squares, 10 is the dilution factor, 8 is the number of squares counted, 0.1, i.e., 1 mm × 1 mm × 0.1 mm is the dimension of each square.

Both the automated instruments were used in accordance with the standard operating procedures of the laboratory. Once every day, the commercial quality control samples were processed in each instrument, and the internal sample integration was checked. The quality of the results had been assured by periodic calibration of the machines AcT.5Diff and HmX on prescribed dates; ensuring the results of the commercial quality control samples to be within the prescribed range. The coefficient of variation (CV%) for AcT.5Diff and HmX was 2.46% and 0.00%, respectively.

For both the cases and the controls, technical details such as the machine model which is used, sample processing time, sample related parameters such as the basophil percentage, automated and manual (for cases only) basophil absolute count, abnormality flagged by the machine, if any, and abnormalities detected on peripheral smear examination were analyzed. Statistical analyses used in the present study include formation of frequency tables with percentages, summary statistics such as mean, standard deviation, standard error of the mean; 95% confidence intervals (CI) tests such as Mann-Whitney U Test, Chi-square test, Binomial test, and independent samples *t*-test. All statistical analyses were carried out using the International Business Machines Statistical Package for the Social Sciences (IBM SPSS) Version 21 software.

RESULTS

Descriptive Analysis of Basophils (Table 1)

The percentage of basophils given by the automated counter ranged from 2% to 22.9% for the cases and from 0% to 1.7% for the controls; the mean was 5.815% (95% CI = 5.070-6.560) for the cases, and 0.535% (95% CI = 0.468-0.602) for the controls; $P < 0.001$. The basophil percentage for the cases on peripheral smear ranged from 0% to 1.6%; the mean was 0.205% (95% CI = 0.148-0.262). The absolute count of basophils as per counter ranged from 60 to 3400 per cubic mm for the cases and from 0 to 120 per cubic mm for the controls; the mean was 466.462 per cubic mm (95% CI = 388.536-544.388) for the cases and 42.692 per cubic mm (95% CI = 37.802-47.582) for the

Table 1: Descriptive analysis of basophils and standing time of samples

Parameter	Group						Independent samples *t*-test	
	Cases			Control				
	Mean	SD	SE	Mean	SD	SE	*t*-value	*P*
Percent of basophils as per automated counter	5.815	4.331	0.380	0.535	0.391	0.034	13.844	0.000
Absolute count of basophils as per counter (per cubic mm)	466.462	453.306	39.758	42.692	28.442	2.495	10.638	0.000
Percent of basophils on P. S.	0.205	0.329	0.029	-	-	-	-	-
Manual absolute count of basophils (per cubic mm)	27.344	23.865	3.445	-	-	-	-	-
Standing time of sample (minutes)	94.669	70.827	6.212	38.046	18.185	1.595	8.829	0.000

P. S.: Peripheral smear, SD: Standard deviation, SE: Standard error

controls; $P < 0.001$. The basophil count calculated manually for the 48 cases ranged from 0 to 87.5 per cubic mm; the mean was 27.344 per cubic mm (95% CI = 20.592-34.096).

Technical Parameters versus Spurious Basophilia

The automated counter AcT.5Diff had given the spurious basophilia report in the majority of the cases (89.23%) (116/130); $P = 0.000$ (Table 2a). The average standing time of the sample was 94.669 min for the cases (95% CI = 82.493-106.845) and 38.046 min for the controls (95% CI = 34.920-41.172); $P < 0.001$ (Table 1). The standing time of the sample was ≥2 h in 26.92% (35/130) cases and none of the controls; $P < 0.001$. The standing time was ≥1 h but <2 h in 33.08% (43/130) cases, 11.54% (15/130) controls; $P < 0.001$ (Table 2b).

Table 2a: Descriptive analysis of technical parameters-Machine model

Machine model	Cases		Binomial
	N	%	*P*
AcT.5Diff	116	89.23	0.000
HmX	14	10.77	

Table 2b: Descriptive analysis of technical parameters-Standing time of sample

Standing time	Group				Mann-Whitney U-test	
	Cases		Control			
	N	%	*N*	%	U-value	*P*
≥2 h					6175.000	0.000
Yes	35	26.92	0	0.00		
No	95	73.08	130	100.00		
≥1 h, <2 h					4092.5	0.000
Yes	43	33.08	15	11.54		
No	87	66.92	115	88.46		

Hematological Parameters versus Spurious Basophilia (Table 3)

Hemoglobin was ≥17 g/dl in 25.38% (33/130) cases, 1.54% (2/130) controls; $P < 0.001$. It was ≥15 g/dl but <17 g/dl in 18.46% (24/130) cases and 8.46% (11/130) controls; $P = 0.018$. Red blood cells were normocytic normochromic in 87.69% (114/130) cases and 85.38% (111/130) controls; microcytic hypochromic in 2.31% (3/130) cases and 7.69% (10/130) controls; macrocytic normochromic in 10% (13/130) cases and 6.92% (9/130) controls; $P = 0.690$ (Tables 3 and 4). Nucleated red blood cells were present in 5.38% (7/130) cases and none of the controls; $P = 0.007$. The TLC was normal in 51.54% (67/130) cases and 80% (104/130) controls; leukocytosis was observed in 26.92% (35/130) cases and 16.92% (22/130) controls; leukopenia in 21.54% (28/130) cases and 3.08% (4/130) controls; $P < 0.001$ (Tables 3 and 4). Neutrophilia was present in 21.54% (28/130) cases and 12.31% (16/130) controls; $P = 0.048$. Neutrophilic left shift was present in 22.31% (29/130) cases and none of the controls; $P < 0.001$. Eosinophilia was noted in 6.15% (8/130) cases and 14.62% (19/130) controls; $P = 0.026$. Absolute lymphocytosis was present in one case and one control (0.77% each); $P = 0.317$. Lymphocytic preponderance was seen in 21.54% (28/130) cases and 6.92% (9/130) controls; $P = 0.001$. Reactive lymphocytes were observed in the peripheral smear in 61.54% (80/130) cases and 3.85% (5/130) controls; $P < 0.001$. Platelet count was normal in 60% (78/130) cases and 86.92% (113/130) controls; thrombocytosis in 2.31% (3/130) cases and 1.54% (2/130) controls; thrombocytopenia in 37.69% (49/130) cases and 11.54% (15/130) controls; $P < 0.001$ (Tables 3 and 4).

DISCUSSION

The modern era hematology analyzers combine various techniques such as electronic impedance, conductivity, absorption spectrometry and flow cytometry for cell counting, and differential analysis.[4] However, still the automated hematology analyzers are known to give erroneous basophil counts, which is confirmed by interpretation of peripheral smear/cytogram/flow cytometry.[4,7,12,13] The machine Beckman Coulter HmX is superior to Beckman Coulter AcT.5Diff in giving a lesser frequency of spurious basophil counts, as is documented in our previous study.[9] Beckman Coulter HmX employs volume, conductivity and scatter to differentiate the leukocytes, whereas Beckman Coulter AcT.5Diff employs volume and cytochemistry for differential analysis without staining the basophil granules. Differential scattergram is

Table 3: Descriptive analysis of hematological parameters

Parameter	Group				Mann-Whitney U-test	
	Cases		Control			
	N	%	*N*	%	U-value	*P*
Hb≥17 g/dl					6435.000	0.000
Yes	33	25.38	2	1.54		
No	97	74.62	128	98.46		
Hb≥15<17 g/dl					7605.000	0.018
Yes	24	18.46	11	8.46		
No	106	81.54	119	91.54		
RBC predominant morphology					8306.500	0.690
Normocytic normochromic	114	87.69	111	85.38		
Microcytic hypochromic	3	2.31	10	7.69		
Macrocytic normochromic	13	10.00	9	6.92		
nRBC					7995.000	0.007
Present	7	5.38	0	0.00		
Absent	123	94.62	130	100.00		
TLC					5807.000	0.000
Leukopenia	28	21.54	4	3.08		
Leukocytosis	35	26.92	22	16.92		
Normal count	67	51.54	104	80.00		
Neutrophilia					7670.000	0.048
Present	28	21.54	16	12.31		
Absent	102	78.46	114	87.69		
Neutrophilic shift to left					6565.000	0.000
Present	29	22.31	0	0.00		
Absent	101	77.69	130	100.00		
Eosinophilia					7735.000	0.026
Present	8	6.15	19	14.62		
Absent	122	93.85	111	85.38		
Absolute lymphocytosis					8385.000	0.317
Present	1	0.77	1	0.77		
Absent	129	99.23	129	99.23		
Relative lymphocytosis					7215.000	0.001
Present	28	21.54	9	6.92		
Absent	102	78.46	121	93.08		
Reactive lymphocytes on P.S.					3575.000	0.000
Present	80	61.54	5	3.85		
Absent	50	38.46	125	96.15		
Platelets					6148.500	0.000
Thrombocytopenia	49	37.69	15	11.54		
Thrombocytosis	3	2.31	2	1.54		
Normal count	78	60.00	113	86.92		

nRBC: Nucleated red blood cells, TLC: Total leukocyte count, P. S.: Peripheral smear

Table 4: Descriptive analysis of RBC morphology, TLC and platelet count

Parameter	Cases		Chi-square test	Control		Chi-square test
	N	%	*P*	*N*	%	*P*
RBC predominant morphology						0.000
Normocytic normochromic	114	87.69	0.000	111	85.38	
Microcytic hypochromic	3	2.31		10	7.69	
Macrocytic normochromic	13	10.00		9	6.92	
TLC						0.000
Leukopenia	28	21.54	0.000	4	3.08	
Leukocytosis	35	26.92		22	16.92	
Normal count	67	51.54		104	80.00	
Platelet count						0.000
Thrombocytopenia	49	37.69	0.000	15	11.54	
Thrombocytosis	3	2.31		2	1.54	
Normal count	78	60.00		113	86.92	

RBC: Red blood cells, TLC: Total leukocyte count

sensitive and is known to reduce the chances of spurious results, which explains the superiority of Beckman Coulter HmX.[3,14] Delayed processing time is a proven cause of spurious basophilia.[15] A delay of even 1 h is significantly associated with spurious basophilia in our study.

Cases having a hemoglobin level of ≥15 g/dl and cases having nucleated red cells are significantly associated with spurious basophilia. This is because the differential leukocyte count is done after lysing the red cells, and when there is incomplete lysis of the red blood cells (RBC), in the presence of high hemoglobin concentration, nucleated RBC (nRBC), it results in spurious elevation of the basophil count.[3] Abnormality in RBC morphology does not have any significant association with spurious basophilia. The majority of the cases, as well as the controls, had a normal RBC morphology.

The percentages of leukopenia and leukocytosis, neutrophilia, neutrophilic shift to the left are found to be higher in the cases having spurious basophilia, and the percentage of normal TLC is higher in the control group. This is because the granules of the neutrophils present in the cases with or without leukocytosis, especially when exhibiting a toxic change in the setting of sepsis is interpreted as basophil granules. This has already been observed in our previous study in the pediatric population.[9] Of the 28 cases of leukopenia, 9 cases had coexisting thrombocytopenia and reactive lymphocytes. In four cases of leukopenia, there were reactive lymphocytes, and the standing time was prolonged. In three cases of leukopenia, thrombocytopenia and prolonged standing time were also seen. In nine cases, leukopenia, thrombocytopenia, reactive lymphocytes and prolonged standing time were present. In two cases, leukopenia was noted along with reactive lymphocytes. In only one case, isolated leukopenia was seen. Eosinophilia may be associated with true basophilia. However, eosinophilia does not have any association with spurious basophilia in this study. In the study by Mitchell, eosinophil count was directly proportional to the basophil count in some cases and had an inverse relationship in some cases.[8] The presence of reactive lymphocytes with/without relative lymphocytosis has a significant association with spurious basophilia. In our study, all the cases, which had relative and absolute lymphocytosis, had reactive lymphocytes on peripheral smear. Conversely, not all the cases, which showed reactive lymphocytes, had lymphocytosis. The association of spurious basophilia with the cases having abnormal leukocytes has been documented, and our observation is consistent with it.[4,12,13,16] The

proportion of thrombocytopenia is higher in the cases, and the normal count is higher among the controls. However, all the 49 cases of thrombocytopenia had coexisting abnormalities such as prolonged standing time, the presence of reactive lymphocytes, and hemoglobin concentration of ≥15 g/dl, neutrophilia and neutrophilic shift to the left. None of the cases had isolated thrombocytopenia. This explains the reason for the association of thrombocytopenia with spurious basophilia.

CONCLUSION

Our study emphasizes the importance of peripheral smear examination of the cases especially those which are flagged by the automated counter. In this study, spurious basophilia is significantly associated with the auto analyzer AcT.5Diff, sample standing time of ≥1 h, hemoglobin level of ≥15 g/dl, nRBC, leukopenia, leukocytosis, neutrophilia, neutrophilic shift to left, reactive lymphocytes with/without relative lymphocytosis, thrombocytopenia.

Based on our previous study in pediatric age group, we summarize in terms of basophil counts, the similarities and differences between pediatric and adolescent/adult age groups who had spuriously elevated basophil counts.

Pediatric (≤9 years) versus Adolescent/Adult (≥10 years) Age Groups[9]

The percentage of basophils on peripheral smear ranges from 0% to 1.2% (mean = 0.191%) in pediatric population and from 0% to 1.6% (mean = 0.205%) in adolescent/adult population. The manual basophil count is similar in both groups; range from 0 to 87.5 per cubic mm; mean is 27.344 per cubic mm. The basophil percentage on automated counter ranges from 2.1% to 27.9% (mean = 7.973%) in pediatric group and from 2% to 22.9% (mean = 5.815%) in adolescent/adult age group. The automated absolute basophil count ranges from 50 to 4590 per cubic mm (mean = 1160.143 per cubic mm) in pediatric group and from 60 to 3400 per cubic mm (mean = 466.462 per cubic mm) in adolescent/adult group. The basophil counts are almost similar in both the age groups. Beckman Coulter AcT.5Diff auto analyzer had given the maximum number of cases in both the groups. Irrespective of the age, prolonged standing time of ≥1 h, Hb ≥ 15 g/dl, neutrophilia, neutrophilic shift to left, reactive lymphocytes with/without lymphocytosis, and with/without leukopenia/thrombocytopenia are significantly associated with spurious basophilia in both the age groups. RBC morphology and eosinophilia did not have an association with spurious basophilia. The TLC and platelet count show association with spurious basophilia in adolescent/adult age group; whereas, they do not have any association with it in the pediatric population.

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