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Prevalence of discordant microscopic changes with automated CBC analysis

Prevalência de alterações microscópicas discordantes com análise automatizada do hemograma

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ABSTRACT

Introduction: The most common cause of diagnostic error is related to errors in laboratory tests as well as errors of results interpretation. In order to reduce them, the laboratory currently has modern equipment which provides accurate and reliable results. The development of automation has revolutionized the laboratory procedures in Brazil and worldwide. **Objective:** To determine the prevalence of microscopic changes present in blood slides concordant and discordant with results obtained using fully automated procedures. **Materials and method:** From January to July 2013, 1,000 hematological parameters slides were analyzed. Automated analysis was performed on last generation equipment, which methodology is based on electrical impedance, and is able to quantify all the figurative elements of the blood in a universe of 22 parameters. The microscopy was performed by two experts in microscopy simultaneously. **Results:** The data showed that only 42.70% were concordant, comparing with 57.30% discordant. The main findings among discordant were: Changes in red blood cells 43.70% ($n = 250$), white blood cells 38.46% ($n = 220$), and number of platelet 17.80% ($n = 102$). **Discussion:** The data show that some results are not consistent with clinical or physiological state of an individual, and cannot be explained because they have not been investigated, which may compromise the final diagnosis. **Conclusion:** It was observed that it is of fundamental importance that the microscopy qualitative analysis must be performed in parallel with automated analysis in order to obtain reliable results, causing a positive impact on the prevention, diagnosis, prognosis, and therapeutic follow-up.

Key words: microscopy; automation; CBC.

INTRODUCTION

The development of automation has revolutionized the laboratory procedures in Brazil and worldwide. About 40 years ago, fully automated methodologies are been assembled from the basic and simplest to techniques that are more complex. Undoubtedly, this advent ensured a major step regarding the reliability and speed in delivery of results⁽⁵⁾.

Despite all this progress, numerous automated hematology analyzers indicate the extreme need of counter analysis for a qualitative assessment of CBC, regardless ensuring a high-resolution quantitative analysis. This analysis allows us to distinguish important changes to diagnose due to the peculiarity presented by some blood diseases⁽¹⁾.

The most common cause of diagnostic error is related to errors in laboratory tests, as well as interpretation errors of results. In order to reduce these errors, currently the laboratory has modern equipment with accurate and reliable results⁽⁸⁾.

Most laboratories uses a selective procedure for slides reading, taking into account several criteria, such as age, sex, sample origin, and, mainly *flags* (alerts) provided by the analyzer equipment itself⁽¹²⁾.

However, there is no ground for dispensing with qualitative analysis of blood figurative elements in parallel with automated analysis, since this analysis allows the observation of changes that can not be obtained in automation, and are often not flagged by the automated study, but may be clinically or biologically relevant⁽⁶⁾.

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OBJECTIVE

The aim of this study was to determine the prevalence of microscopic changes present in blood smear in microscope slide concordant and discordant with results obtained using fully automated procedures.

MATERIALS AND METHOD

In the period from January to July 2013, 1.000 slides were examined, and the following parameters were analyzed: white blood cells (leukocytes and leukocyte subpopulations), red blood cells (CBC, hemoglobin, hematocrit, and red blood cell), and number of platelet. The automated analysis was performed on last generation equipment, which methodology is based on electrical impedance, and is able to quantify all the figurative elements of the blood in a universe of 22 parameters. Two technicians performed blood smear microscopy at the same time.

RESULTS

The data showed that only 42.70% were concordant, comparing with 57.30% discordant (**Figure 1**). The main findings among discordant were changes in red blood cells 43.70% ($n = 250$), white blood cells 38.46% ($n = 220$), and number of platelet 17.80% ($n = 102$) (**Figure 2**).

In red blood cells, white blood cells, and number of platelet, several discordant changes were observed, as shown in **Tables 1**, **2** and **3**, respectively.

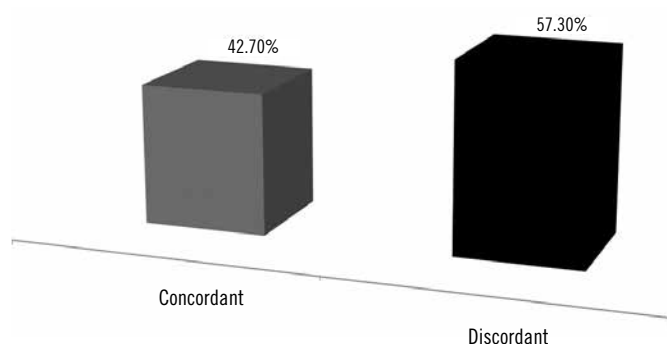


FIGURE 1 – Concordant and discordant results between the automated counting and microscopy examination ($n = 1,000$). We highlight the prevalence of discordant exams

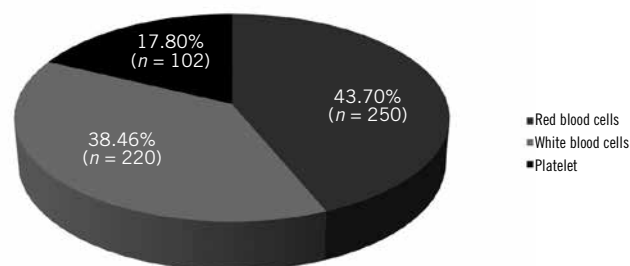


FIGURE 2 – Discordant changes ($n = 572$). We highlight the prevalence of changes observed among the discordant analyzes

TABLE 1 – Discordant changes observed in red blood cells ($n = 250$)

Microscopic analysis	Automation		
	Parameter	Mean	%
Macrocytosis	MCV	84.6 (fl)	60 ($n = 150$)
Microcytosis	MCV	83.2 (fl)	20.8 ($n = 52$)
	MCH	28 (pg)	
Hypochromia	MCHC	33.3 (g/dl)	19.2 ($n = 48$)

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

TABLE 2 – Discordant changes observed in white blood cells ($n = 220$)

Microscopic analysis	Automation	
	%	Flag (alert)
Atypical lymphocytes	62 ($n = 136.4$)	10% ($n = 13.64$)
Coarse grits	18 ($n = 39.6$)	SNF
Immature cells	16 ($n = 35.2$)	33% ($n = 11.61$)
Multi-segmentation	4 ($n = 8.8$)	SNF

SNF: showed no flag (alert).

TABLE 3 – Discordant changes observed in number of platelets ($n = 102$)

Microscopic analysis	Automation		
	Parameter	Mean	%
Macroplatelets	MPV	9.7 (fl)	80 ($n = 81.6$)
Platelet clumps	SNF	SNF	20 ($n = 18.4$)

MPV: mean platelet volume; SNF: showed no flag (alert).

DISCUSSION

Several studies demonstrate efficiency, sensitivity, and accuracy of the automated systems used in hematology laboratories⁽¹³⁾. However, numerous records show that some results are not consistent with clinical or physiologic state of an individual. In addition, some data cannot be explained because they have not been investigated, which may compromise the final diagnosis.

Failace (2004) found in analysis of 149 slides that only one concordant showed result without clinical significance. According to this author's data, result release criteria are satisfactory⁽²⁾.

However, in this study, we observed a prevalence of 57.30% ($n = 572$) of discordant tests, among them the red blood cells presented the most relevant prevalence (43.70%; $n = 220$). This may be related to the fact that the red blood cells are influenced by both the pathophysiological state and the adaptive state.

We observed that numerous changes are not highlighted nor flagged by the automated methods, i.e., poikilocytosis (elliptocytes, codocytes, dacriocytes, acanthocytes, echinocytes, stomatocytes, and drepanocytes), coarse granules in neutrophils, erythrocyte inclusions, polychromasia, erythrocyte *rouleaux*, accurate and reliable amount of immature or abnormal cells that are specific or indicative data of pathological process, and able to direct a diagnostic line^(9,13).

Erythrocyte indices are calculated by the computer from the determinations carried out by the equipment^(10,12), thus, it is common to obtain values that do not match the microscopic analysis (Table 1), since the calculated values may assume an average, and, when compared with reference values may be normal. Therefore, it is possible to observe the morphological profile of the cells, using the microscopic analysis, and not only to estimate changes from the mean.

The RBC indices are of utmost importance for the differential diagnosis of anemia, therefore, we should emphasize the need for reliable and safe results^(4, 7, 13). The microscopic analysis shows that it is possible to critically evaluate these values or even add a change not evidenced by calculation.

The automated analysis gives greater accuracy and precision for the results⁽⁴⁾, however it should be stressed that data in this study show that these characteristics are more related with quantitative than with qualitative analysis. It is possible to observe in Table 2 that only 10% of the sample presented *flag*, indicating lymphocytic atypia, compared

with 90% of the same change found when the analysis was performed with microscope. In addition, other changes with a significant rate were also found, such as coarse granules and segmented neutrophils, important data for diagnosis and clinical monitoring of patients with bacterial infections and megaloblastic anemia, respectively.

According to Santos (2004), the mean platelet volume (MPV) is a biological variable that is related to platelet function and activity as regards the platelets size and morphology. To this, it is estimated that an increased MPV is indicative of macroplatelets, which can lead to strong aggregation that may promote thrombus formation^(3,11). However, Table 3 shows that 80% of discordant in platelet number corresponds to the macroplatelets, although there was no increased in MPV in any case analyzed; such change was only evidenced by microscopic analysis.

CONCLUSION

According to this study, we could observe that it is essential that the qualitative microscopic blood smear analysis be carried out in parallel with the automated analysis. With regard to clinically relevant results, it is expected the laboratory to generate reliable data that make positive impact on prevention, diagnosis, prognosis, and therapeutic follow-up, so that it is possible to minimize errors or to refute an action taken, and not deciding whether a result is clinically important or not.

Microscopic analysis is a millenary practice and is an important tool for clinical laboratories. It requires a skilled and qualified Professional to perform it. Training and continuing education are essential for these professionals to be able to analyze and mainly to evaluate the data generated by automation, and they must to correlate them safely with microscopic data, and, where relevant, challenge them.

RESUMO

Introdução: A causa mais comum de erro no diagnóstico relaciona-se com erros de exames laboratoriais, bem como erros de sua interpretação. E com o intuito de reduzi-los, atualmente o laboratório dispõe de equipamentos modernos com resultados precisos e confiáveis. O desenvolvimento da automação revolucionou os procedimentos laboratoriais no Brasil e no mundo. **Objetivo:** Verificar a prevalência de alterações microscópicas presentes em lâminas hematológicas concordantes e discordantes com resultados obtidos por meio de procedimentos totalmente automatizados. **Materiais e método:** No período de janeiro a julho de 2013, foram analisados os parâmetros hematológicos de 1.000 lâminas. A análise automatizada foi realizada em equipamento de última geração, cuja metodologia baseia-se em impedância elétrica e é capaz de quantificar todos os elementos figurados do sangue em um universo de 22 parâmetros. A hematoscopia foi realizada por dois microscopistas ao mesmo tempo. **Resultados:** Os dados demonstraram que apenas 42,70% foram concordantes, confrontando com 57,30% discordantes. Os principais achados entre os discordantes foram alterações na série vermelha 43,70% ($n = 250$), série branca 38,46% ($n = 220$) e plaquetária 17,80% ($n = 102$). **Discussão:** Os dados comprovam que alguns resultados não são compatíveis com a clínica, nem condizem com o estado fisiológico de um indivíduo e podem não ser explicitados por não terem sido investigados, o que pode comprometer o diagnóstico final. **Conclusão:** Observou-se a importância de uma análise hematoscópica qualitativa ser realizada em paralelo à análise automatizada para que se obtenha resultados confiáveis, que cause impacto positivo na prevenção, no diagnóstico e no seguimento terapêutico.

Unitermos: microscopia; automação; hemograma.

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