The absolute recommendation of chamber Neubauer method for platelets counting instead of indirect methods in severe thrombocytopenic patients

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A absoluta recomendação de se usar o método direto de contagem de plaquetas em câmara de Neubauer em pacientes intensamente plaquetopênicos

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key words

platelet counts

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quality control

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abstract

Accurate and precise platelet counting is crucial for recommending platelets transfusion for thrombocytopenic patients, principally when platelet counts are bellow 30,000/μl. As most laboratories still use the indirect methods for confirming low automated platelet counts, this work compared two indirect methods used in practice (Fonio and Nosanchunk *et al.*) with the International Committee for Standardization in Hematology recommended direct method (Brecher and Cronkite). The obtained data show that the indirect methods present low precision and accuracy, and that the direct method should always be employed in severe thrombocytopenic samples thanks to its high precision.

unitermos

contagem de plaquetas

plaquetopenia

controle de qualidade

transfusão de plaquetas

resumo

A contagem precisa e exata de plaquetas é crucial para se indicar ou não a transfusão de plaquetas em pacientes plaquetopênicos, principalmente quando as contagens estão abaixo de 30.000/μl. Como muitos laboratórios ainda utilizam as contagens indiretas em esfregaço para confirmar baixas contagens obtidas por automação, este trabalho se propôs a comparar dois métodos indiretos muito usados (Fonio e Nosanchunk et al.*) com o método direto indicado pelo Comitê Internacional de Estandardização em Hematologia (ICSH) (Brecher e Cronkite). Os dados obtidos demonstram que os métodos indiretos em esfregaço apresentam baixa precisão e exatidão e que o método direto de referência deve ser usado para confirmar as contagens em amostras intensamente plaquetopênicas.*

Introduction

Platelet counting is an important tool used in clinical investigation to avoid bleeding risks. There is a general agreement that automation brought precision and accuracy for platelet counts in healthy individuals. Nevertheless, reliable platelet counts in thrombocytopenic patients is still controversial (6, 11, 15, 19).

Based on the studies of Gaydos *et al*. (1962), most of the institutions use 20.000/μl (7) as threshold for platelet transfusion, whereas others employ 10.000/μl (3, 9, 10, 14, 16, 17).

The Brecher and Cronkite (4, 5) direct manual method has been recommended by the International Committee for Standardization in Hematology ICSH (1984/1988).

Unfortunately the majority of hematology laboratories still employ indirect manual methods (blood smears) for confirming low platelet counting obtained in automated counters. The objective of this work was to evaluate the most used indirect methods and compare them with the manual direct standard method.

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Methods

Peripheral blood samples of 43 thrombocytopenic patients with platelet counts lower than 30,000plt/μl were analyzed. They were collected at the Hematology Service of the Hospital do Servidor Público Estadual (São Paulo-SP).

Blood smear indirect method 1: Nosanchung *et al*. (18) and Apibal *et al*. (1). Blood smear indirect method 2: Fonio (8).

ICSH (1984/1988) (12, 13) direct reference method: the Brecher and Cronkite method (4, 5), which requires Neubauer improved counting chamber.

The indirect platelet counting methods were performed in triplicate. Each count was obtained as the mean of 10 oil immersion fields (1000x amplification), in a total of 30 fields.

The ICSH method was also analyzed by triplicate counts. For every counting a sample was diluted in triplicate. The minimum of 200 platelets at Neubauer chamber was taken as standard procedure. The mean, standard deviation, and coefficient of variation for the manual (direct and indirect) platelet counts were compared with the direct method. Analysis of variance statistical test was employed.

Results

The results are shown in **Tables 1** and **2**. They indicate that indirect manual methods with variation coefficients higher than the upper acceptable threshold (15%) have lower precision and accuracy when compared to the direct reference method (Tables 1 and 2).

Table 1 Analysis of variance, with Tukey test, between the indirect methods and reference method (ICSH)

Method	ICSH (CV = 10.43%)	Ind. met. 1 (CV = 26.02%)	Ind. met. 2 (CV = 26.02%)
ICSH	–	< 0.001	< 0.001
Ind. met. 1	–	–	1.0

ICSH: Brecher-Cronkite reference method; ind. met. 1: indirect method 1; ind. met. 2: indirect method 2.
The variable is the coefficient of variation (CV) in patients with less than 30,000plt/μl (n = 43).

Table 2 Precision analysis on thrombocytopenic subgroups according to thresholds established by literature for platelet transfusion

Method	≤ 10,000plt/μl	10,000 to 20,000plt/μl CV(%)	> 20,000plt/μl
ICSH	11.8	12	8.2
Ind. met. 1	38.6	24.7	19.5
Ind. met. 2	36.7	24	18.7

CV: coefficient of variation; ICSH: Brecher-Cronkite reference method; $p < 0.001$; ind. met. 1: indirect method 1; ind. met. 2: indirect method 2.

Discussion

According to Berkson (2), the specific error of hemocytometer depends on the number of cells which are counted in each area of the reticulum. Using the formula proposed by this author in similar conditions to this study, the chamber error itself would be of CV = 7.59%. Therefore, as 200 cells were always counted, these experiments disclosed CV = 10.49% for platelet counts less than 30,000/μl, and are similar to the data obtained by Dickerhof *et al*. (6) for platelets around 10,000/μl (CV = 14.7%). Hanseler *et al*. (11) results show that the correct use of the Neubauer chamber presents a satisfactory precision (CV < 15%) in counts above 4,000plt/μl.

The results listed in Tables 1 and 2 indicate that the direct hemocytometer method is much more precise than the indirect methods in thrombocytopenic patients. For levels lower than 40,000plt/μl, the results obtained by Lawrence (16) (CV = 30.4%, $p < 0.001$) confirm the lower precision of indirect methods.

Based on the threshold established for prophylactic platelet transfusion (10,000 or 20,000plt/μl), the results obtained by the indirect methods show that even triplicate counts, of 10 fields each, may jeopardize the clinical decisions when prophylactic or even therapeutic platelet transfusion are in discussion.

Conclusion

The direct Neubauer chamber method for levels lower than 30,000plt/μl seems to be the most accurate and precise method for patients presenting bleeding risks.

When precision is required for taking decisions regarding platelet transfusion indication, the blood smear methods are not helpful and may even impair them.

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