

The estimation of platelet count from a blood smear on the basis of the red cell: platelet ratio

Kan yaymasında eritrosit ve trombosit oranı incelenerek trombosit sayısının tayini

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Abstract

Objective: The estimation of platelet count from blood smears is a daily routine laboratory test, which should be systematic each time the automated count is erroneous.

In our laboratory, we estimate the platelet count indirectly by using the automated red blood cell (RBC) and calculating the platelet count on the basis of the red cell: platelet ratio in a stained blood film. In this study, we attempted to verify the reliability of this technique.

Material and Methods: One hundred ninety-one platelet counts were executed by two laboratory methods: an automated count using an impedance cell counter and then a manual method by reviewing microscopic blood smears. The number of platelets per 1000 erythrocytes was multiplied by the automated RBC ($\times 10^6$ cells/ μ l) to give an approximate manual count ($\times 10^3$ cells/ μ l). Two paired t-test was used for comparison of the two methods.

Results: The regression analyses for the entire data set collected in our study with the two laboratory methods gave the following least squares equation by comparing the automated (y) to the manual method (x): $y=0.8548x + 12.013$ ($r=0.908$). The paired t-test showed no significant difference between the two methods ($p>0.05$) and the Intra-class Correlation Coefficient (ICC) was equal to 0.905.

The plot of the differences between the automated and manual values against their means according to Band and Altman design showed that the difference mean was 3.209 with a standard deviation $SD=46.331$.

We noticed that 93% of the differences were within the agreement limits ($\text{mean} \pm 2SD$), and that 77% of the differences were less than 20,000 platelets/ μ l.

Conclusion: Estimating platelet count on the basis of the red cell: platelet ratio is a reliable technique and it should be proposed as a method of reference. (*Turk J Hematol* 2009; 26: 21-4)

Key words: Estimation of platelet count, blood smear, red cell: platelet ratio

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Özet

Amaç: Kan yaymalarından trombosit sayısının tahmin edilmesi günlük rutin bir laboratuvar testi olup, otomatik sayım hatalı olduğunda her seferinde sistematik olmalıdır. Biz laboratuvarımızda otomatik KK sayımını kullanarak ve boyalı kan örneğindeki kırmızı kan hücresi:trombosit oranına dayalı şekilde trombosit sayısını hesap ederek, dolaylı olarak trombosit sayısını tahmin ediyoruz. Bu çalışmada bu tekniğin güvenilirliğini doğrulamaya çalıştık.

Yöntem ve Gereçler: İki laboratuvar yöntemi ile 191 trombosit sayımı yapıldı; önce impedans kan sayım cihazının kullanıldığı otomatik sayım ve sonra mikroskobik kan yaymalarının incelendiği manuel bir metot.

Yaklaşık bir manuel sayım sonucu vermek için 1000 eritrosit başına düşen trombosit sayısı ($\times 10^3$ hücre/ μ l) ölçümü otomatik yapılan Kırmızı Kan Hücreleri Sayısı ($\times 10^6$ hücre/ μ l) ile çarpıldı. İki metodun karşılaştırılmasında eşleştirilmiş t-testi kullanıldı.

Bulgular: İki laboratuvar yöntemi ile çalışmamızda elde edilen tüm veri kümesi için otomatik (y) ve manuel (x) yöntemini karşılaştırıldığı regresyon analizi aşağıdaki en küçük kareler denklemini verdi: $y=0.8548x \pm 12.013$ ($r=0.908$).

Eşleştirilmiş t-testi iki yöntem arasında anlamlı bir farklılık göstermedi ($p>0.05$) ve Sınıf-İçi Korelasyon Katsayısı (ICC) 0.905'e eşitti. Band ve Altman'ın tasarımına göre otomatik ve manuel değerler arasındaki farka karşılık ortalama değerler için çizilen grafik, fark ortalamasının 46,331 standart sapma (SS) ile 3.209 olduğunu gösterdi.

Farklılıkların %93'ünün kabul edilen limitler içine dahil edildiğini (ortalama \pm 2SS) ve farklılıkların %77'sinin 20.000 trombosit/ μ l'in altında olduğunu gördük.

Sonuç: Kırmızı kan hücresi:trombosit oranına dayanarak, trombosit sayısının tahmin edilmesi güvenilir bir tekniktir ve referans bir yöntem olarak önerilmelidir. (*Turk J Hematol 2009; 26: 21-4*)

Anahtar kelimeler: Trombosit sayısının tahmin edilmesi, kan yayması, Kırmızı kan hücresi:trombosit oranı

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The estimation of platelet count from blood smears must be systematic each time the automated count is erroneous because even the most expensive and most effective machine is not able to replace human judgement [1-3].

Various proposals have been made for a reference method for platelet estimation [4-10].

Although platelet count is a daily routine laboratory test, the estimation techniques seem to have not been validated. This is due to the fact that the methods of validation of the diagnostic tests were finalized during the second half of the 20th century and researchers are tempted to validate the new methods first, especially the less widespread [11].

The estimation technique used in our laboratory was proposed by Thelml and other researchers [7-10] and is outlined herein with an attempt to verify its reliability.

Material and Methods

Blood Samples

Blood samples were obtained from 191 patients, less than 15 years of age, who were receiving an anti-cancer chemotherapy, as part of routine hematologic investigation or disease monitoring in our department.

All venous blood specimens were collected into tubes containing ethylenediaminetetraacetic acid (K_2 or K_3 EDTA) and then were stored at room temperature until analyzed within four hours.

Notation was made if clots were seen in the blood sample or if the amount of blood in the tube was grossly inadequate such that a disproportionately high concentration of EDTA would be present; these samples were excluded from the study.

Automated Method

After thorough mixing of each blood sample on an automated mixer for 10 min, a complete automated blood count was performed using an impedance cell counter (Coulter A^{CT}), which was maintained and calibrated as recommended by the manufacturer.

Manual Method

Thin air-dried blood smears made after thorough mixing of each sample were stained manually with a May-Grünwald-Giemsa stain and examined under light microscopy with a X100 oil-immersion lens.

The slides were entirely scanned for platelet aggregates and/or macrothrombocytes and, if any, the samples were excluded from the study.

If neither aggregates nor macrothrombocytes were found, the red cell: platelet ratio was calculated in the monolayer zone of the smear as follows:

The number of erythrocytes observed in a quarter of the oil-immersion field was multiplied by four instead of counting all the erythrocytes in the field, which is a laborious and time-consuming method. Then all the platelets in the same field were counted.

Other fields were examined in the same way until we reached a minimum number of 1000 erythrocytes.

The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count (RBC) ($\times 10^6$ cells/ μ l) to give an approximate manual count ($\times 10^3$ cells/ μ l).

Statistical Method

Simple linear regression and difference plots were used to compare the manual platelet counts with the automated platelet counts [12].

The Shrout and Fleiss Intra-class Correlation Coefficient (ICC) was calculated in order to identify the degree of correspondence and the agreement between the two methods [13,14]. The ICC value is measured on a scale of 0 to 1, and in accordance with Portney and Watkins, good reliability was generally assumed as an ICC>0.75 [14].

A paired t-test was performed in order to assess the match between platelet count results by both methods [15]. In this evaluation, a statistically significant difference in platelet level was set at a level of $p=0.05$.

Results

The report of evaluation on all 191 individual samples with the two laboratory methods gave the following least squares equation by comparing the automated (y) to the manual method (x): $y=0.8548x + 12.013$ ($r=0.908$) (Figure 1).

The paired t-test showed no significant difference between the two methods ($p>0.05$).

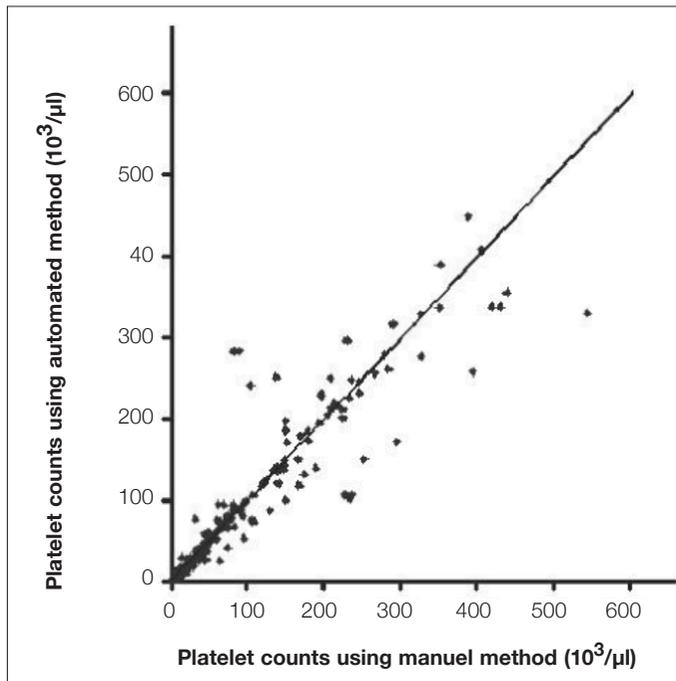


Figure 1. The regression analyses for the entire data set collected in our study with the line of equality

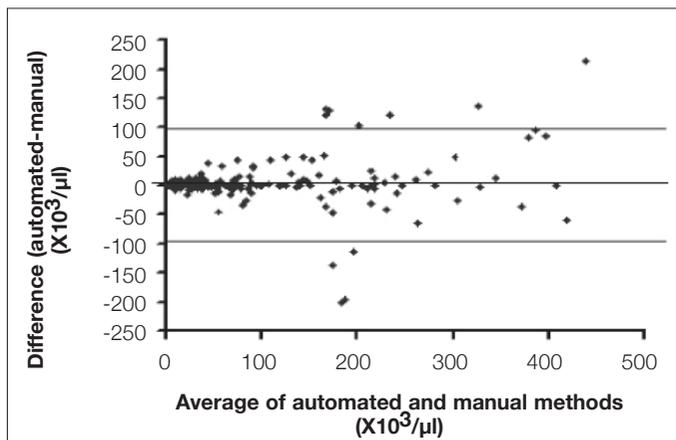


Figure 2. Difference versus mean plots for automated and manual platelet counts according to Bland and Altman design. The middle solid line is the mean of the difference; the outer solid lines are the upper and lower limits of agreement ($\text{mean} \pm 2\text{SD}$)

The ICC was equal to 0.905.

The plot of the differences between the automated and manual values against their means according to Bland and Altman design showed that the difference mean was 3.209 with a standard deviation $\text{SD} = 46.331$ (Figure 2) [12].

We noticed that 93% of the differences were within the agreement limits ($\text{mean} \pm 2\text{SD}$), and that 77% of the differences were less than 20,000 platelets/ μl .

Discussion

Obtaining an accurate platelet count by using an automated hematology analyzer may be complicated by the presence

of particles of similar size and/or light scatter properties (red cell fragments, microcytic red cells, apoptotic white blood cell fragments) and by giant platelets and platelet clumps [16,17].

Even the most expensive and accurate hematology analyzers are not designed to eliminate peripheral blood film evaluation, and microscopic validation of platelet counts is an important component of the blood smear review.

Some authors recommend calculating the average number of platelets counted in 10 immersion fields; the adequate values are included between 8 to 20 platelets per field [4-6]. The average number of platelets is then multiplied by a factor of 20,000 for wedge preparations or 15,000 for monolayer preparations in order to obtain and estimate the platelet count per micro litter, but this method is approximative and does not give the real number of platelets.

In our laboratory, we estimate the platelet count indirectly by using the automated RBC and calculating the platelet count on the basis of the red cell: platelet ratio in a stained blood film.

This technique had been cited in the literature but to the best of the authors' knowledge, there are no indications of its eventual validation [7-10].

The ICC was calculated in order to identify the reliability of the manual technique in comparison to the automated method [13]. The ICC is a "reliability coefficient that is calculated using variance estimates obtained through analysis of variance; it reflects both degree of correspondence and agreement among ratings" [14]. The ICC value is measured on a scale of 0 to 1, and in accordance with Portney and Watkins, good reliability was generally assumed as an $\text{ICC} > 0.75$ [14].

In our study, the ICC was equal to 0.905, which is widely greater than this limit.

A plot of the differences between the automated and manual values against their means was drawn in order to assess agreement between the two methods, and this plot showed that the mean difference was equal to 3.209 platelets/ μl , which is clinically acceptable, and 93% of the differences were situated in the limits of agreement ($\text{mean} \pm 2\text{SD}$) [12].

Theml [7] recommends estimating the number of platelets relative to 1000 red cells but counting 1000 erythrocytes constitutes a laborious and time-consuming method, especially in the laboratories with a heavy work load.

In our laboratory, the number of erythrocytes is estimated by multiplying by four the number of erythrocytes observed in a quarter of the oil-immersion field.

We suggest to the technicians to execute two counts per patient; if the difference between the two counts exceeds the 20,000 platelets / μl , a third count is desirable. The average of these counts is considered as the final result.

Even if the manual platelet numeration, using a counting chamber, remains the technique of reference, it consumes more time and requires a phase-contrast microscope, which is not always available in routine laboratories [8,18]. In addition, it is worth remembering the important risk of error estimated up to 10-20% by some authors [18]. That is why we prefer the proposed method, since it is faster, taking only five minutes on average per patient, while demonstrating good precision.

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