Reduction of the Platelet Review Rate Using the Two-Dimensional Platelet Method

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Abstract

Accurate platelet enumeration is critical for optimal treatment of patients with platelet and bleeding disorders, leukemias, and other neoplasias. The majority of automated hematology analyzers count platelets by size differentiation alone, which may result in falsely elevated platelet counts for samples containing interfering particles such as RBC fragments, microcytes, and cell debris. Most analyzers flag questionable platelet counts, necessitating review of results with confirmation by an alternative method, thus increasing the cost of performing platelet counts and delaying results.

We studied the effect of a new platelet analysis method, based on measurement of size and refractive index, on the laboratory review rate for platelet counting. We demonstrated that this method yields higher accuracy for platelet counts in samples with interferences, especially for platelet counts less than 50 × 10^3/µL (<50 × 10^9/L). As a result of the 2-dimensional analysis, the review rate for platelet counts was reduced by 65% in our institution, resulting in substantial savings.

Accurate and reliable platelet counts are essential for the proper treatment of patients, particularly those with thrombocytopenia. There are limitations to the determination of platelet counts using manual reference methods or automated analyses. These limitations include substantial imprecision of the manual method and the inability of automated methods using either impedance or optical measurements to reliably distinguish platelets from interfering particles in the size range of platelets, leading to inaccurate platelet counts for some blood samples. Various sample flagging and review criteria have been devised by clinical laboratories to verify suspect platelet counts.1 Such flagging, coupled with abnormality of patient population, creates a persistent need to review a subset of specimens (review rate) to validate the reportable results. This is costly in terms of limited resources and delays in turnaround time, and it affects laboratory efficiency.

A new strategy for better platelet discrimination has been described.2 In this approach, both volume and refractive index of platelets are determined simultaneously using 2 angles of laser light scatter (2-dimensional analysis) to obtain improved discrimination between platelet and nonplatelet particles. The improved platelet discrimination in turn should result in higher platelet count accuracy with reduced flagging and platelet review rates. The present study was undertaken to determine the effect of the new 2-dimensional technology on platelet review rates in a large tertiary care center.

Materials and Methods

Instrumentation

The Bayer ADVIA 120 (Bayer, Tarrytown, NY) hematology system is an automated, CBC count/differential/
reticulocyte analyzer that incorporates 2-dimensional analysis of platelets based on the size and refractive index of each platelet. The method has been described in detail elsewhere. The Bayer H*3 (Bayer) hematology analyzer, like most other currently used analyzers, uses 1-dimensional analysis to discriminate platelets based on size alone. Figure 1A and Figure 1B illustrate a comparison of platelet analysis using the H*3’s 1-dimensional method compared with the ADVIA 120’s 2-dimensional method. The ADVIA 120 and the H*3 systems were used to analyze, in duplicate, all specimens in the study. Both instruments were maintained and operated according to the manufacturer’s instructions.

Platelet Enumeration Methods

Manual Reference Method

In the present study, phase-contrast microscopy was used as the reference method for platelet counts on samples with potential or suspected interferences, as well as for thrombocytopenic samples. In this method, a whole blood sample was diluted with a 1% ammonium oxalate solution (BD Unopette, Becton Dickinson, Franklin Lakes, NJ) that lyses RBCs and leaves platelets and WBCs intact. Platelets then were counted under phase-contrast illumination according to the standard method recommended by the International Committee for Standardization in Haematology. Platelet counts for each sample were correlated with a Wright-stained peripheral blood smear to confirm discrepancies between automated platelet counts and manual phase counts and to document RBC morphologic features.

Automated Platelet Enumeration

Platelet counts on all blood samples were determined using the ADVIA 120 and the H*3 systems. Both of these systems use software-generated flags to identify specimens that meet defined criteria indicating either analytic failures or specimen-specific interferences. Platelet flags on H*3 systems are generated for the following specimens: samples with platelet counts less than $35 \times 10^3/\mu\text{L}$ ($<35 \times 10^9/\text{L}$), samples in which the presence of either large platelets or small RBCs is detected, samples for which there is an increased number of signals (noise) in the platelet channel that do not fit within the log-normal platelet size distribution, and samples for which a log-normal fit of the platelet volume histogram cannot be generated. On the ADVIA 120 system, platelet flags are generated for the following samples: samples with platelet clumps, samples in which large platelets (platelet volume > 20 fL) constitute more than 10% of the platelet count, and samples in which the presence of large platelets is detected in association with microcytic and/or anisocytic RBCs.

Blood Sample Collection

Specimens of tripotassium EDTA-anticoagulated blood were obtained from patients at our university hospital. For this study, we chose 101 patient blood samples for which the H*3 system generated platelet flags based on the aforementioned criteria. Specimens included blood samples from patients with RBC disorders (ie, iron deficiency anemia, thalassemia, hemolytic anemia) that are known to exhibit microcytes and RBC fragments; patients diagnosed with various leukemias and neoplastic diseases with associated low platelet counts; and other miscellaneous diagnoses, such as coronary disease, pregnancy, fever or infection, and organ transplantation.

![Figure 1A](image1a.png) and ![Figure 1B](image1b.png) illustrate a comparison of platelet analysis using the H*3’s 1-dimensional method compared with the ADVIA 120’s 2-dimensional method. The ADVIA 120 and the H*3 systems were used to analyze, in duplicate, all specimens in the study. Both instruments were maintained and operated according to the manufacturer’s instructions.

For proprietary information, see the text.
Cost Analysis

The platelet review rate (percentage of platelet counts that require confirmatory testing) for the H*3 compared with the ADVIA 120 was used for evaluating the potential savings of the improved platelet enumeration method. The historic platelet review rate of 8% on the H*3 at our institution was validated by evaluation of more than 2,000 consecutive platelet counts before the collection of the 101 study samples. The reduction in review rate was multiplied by the total number of platelet counts per year that historically have required review to calculate the potential annual impact. The annual reduction in the number of samples requiring review then was multiplied by the incremental cost per test to determine potential savings. The average incremental cost of $2.60 per test for our laboratory includes labor and supplies required for performing platelet confirmatory procedures (repeated testing, smear scan, and/or manual phase platelet count).

Results

Comparison of Methods

We analyzed 101 blood samples that were flagged for review by the 1-dimensional strategy for platelet analysis (H*3 system) on the ADVIA 120 system using the 2-dimensional strategy. Only 35 of 101 specimens were flagged for review using this method. The 35 specimens flagged by the ADVIA 120 were from a variety of diagnostic classes, including neoplastic disease (8), fever or infection (7), anemia (3), coronary disorders (3), pregnancy (2), transplantation (2), and miscellaneous disorders (10).

The lower platelet review rate on the ADVIA 120 for these 101 samples was related in part to the improved ability of the 2-dimensional strategy to discriminate platelets from nonplatelet particles and in part to the more accurate enumeration of platelet counts. Indeed, for the subset of these specimens that had been shown previously to contain interfering particles (n = 77), a higher correlation was noted between ADVIA 120 platelet counts and manual phase counts ($r^2 = 0.9645$) than that noted between H*3 platelet counts and manual phase counts ($r^2 = 0.8967$) \( \text{Figure 2A} \) and \( \text{Figure 2B} \). Similarly for the subset of specimens in which the platelet count was less than $50 \times 10^3/\mu L$ (<50 $\times 10^9/L$; n = 68), a higher correlation was noted between ADVIA 120 platelet counts and manual phase counts ($r^2 = 0.6803$) than that noted between H*3 platelet counts and manual phase counts ($r^2 = 0.5019$) \( \text{Figure 3A} \) and \( \text{Figure 3B} \). Furthermore, the H*3 system consistently overestimated the platelet count in these thrombocytopenic samples, most likely resulting from the presence of interfering particles.

Cost Analysis

Based on an annual platelet count volume of about 120,000 and an H*3 platelet review rate of 8%, 9,600 confirmatory procedures were performed per year in our laboratory. A 65% reduction in the review rate with the ADVIA 120 translates to approximately 6,240 fewer confirmatory procedures required per year, with a potential cost savings of
approximately $16,224 (6,240 procedures × $2.60 incremental cost per test).

**Discussion**

Accurate and timely reporting of platelet counts is an ongoing issue in major medical centers with large hematology, oncology, and bone marrow transplantation services. Recent improvements in platelet enumeration technology using 2-dimensional analysis with refractive index and volume measurements (ADVIA 120 system) theoretically should improve the accuracy of platelet counts and turnaround time, if less review and confirmatory testing are required. The purpose of the present study was to determine how the platelet count review rate would be affected by using the 2-dimensional analysis on the ADVIA 120 as opposed to the 1-dimensional analysis on the H*3.

When 101 samples flagged by the H*3 system for platelet count confirmation were reanalyzed using the ADVIA 120 system, 65% could be reported without additional testing. Platelet counts performed on the ADVIA 120 were more accurate, as shown by the higher correlation noted between the manual phase counts and the ADVIA 120 than with the H*3 system.

Higher accuracy of platelet counts reported by the 2-dimensional method is a result of the more accurate discrimination of platelets from nonplatelet particles. The interfering particles in the size range of platelets, such as RBC fragments, microcytes, and cell debris, can falsely elevate platelet counts. Using the 2-dimensional approach in which platelets are discriminated from similar cells based on cell size and cell granularity results in substantially improved platelet counts.

In our large, tertiary care hospital laboratory, the improved platelet counting substantially reduced the workload. The study indicated a potential reduction of approximately 6,240 confirmatory procedures per year with a cost saving of $16,224. After completion of the study and after incorporation of the ADVIA 120 into our laboratory, the overall platelet review rate on the ADVIA 120 again was determined by reviewing more than 2,000 consecutive platelet counts. The ADVIA 120 review rate in this follow-up study was 3% (vs 8% for the H*3), which corroborates the 65% reduction in review rate noted on the 101 study samples.

Samples also may require additional smear review for confirmation of WBC flags (blast, left shift/immature granulocytes, large unstained cells, and nucleated RBCs). Therefore, potential savings with the new platelet method may be reduced by the percentage of samples that would require such review. Of the 66 samples in the study not requiring platelet review on the ADVIA 120, 36 samples would have required WBC review. If a differential count were ordered on all of these samples, the additional review would reduce savings to approximately $7,500. However, in our laboratory, WBC differential counts are ordered on only 50% of our samples, and WBC flags are confirmed only when a WBC differential count is ordered. Therefore, we estimate our total savings at approximately $10,000 annually.
As clinical laboratories are continually seeking ways to improve efficiency, the marked reduction in the platelet review rate using a new platelet analysis strategy has favorably affected workflow and laboratory costs. In addition, patient care is improved with more timely reporting of accurate results.

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References


