Counting platelets at transfusion threshold levels: impact on the decision to transfuse. A BEST Collaborative - UK NEQAS(H) International Exercise

M. Lozano,¹ A. Mahon,² P. F. van der Meer,³ S. Stanworth,⁴ J. Cid,¹ D. Devine,⁵ M. K. Fung,⁶ B. de la Salle,² N. M. Heddle² & on behalf of Biomedical Excellence for Safer Transfusion (BEST) Collaborative

¹University Clinic Hospital, Barcelona, Spain
²UK National External Quality Assessment Scheme for General Haematology, Watford, UK
³Sanquin Blood Supply, Amsterdam, the Netherlands
⁴John Radcliffe Hospital, National Blood Service, Oxford, UK
⁵Canadian Blood Services, Vancouver, BC, Canada
⁶Fletcher Allen Health Care, Burlington, VT, USA
⁷McMaster University, Hamilton, ON, Canada

Received: 1 July 2013, revised 8 October 2013, accepted 9 October 2013

Background and Objectives Obtaining accurate and precise platelet enumeration in automatic platelet analysers at low platelet counts is a challenge. To explore the performance of current haematology analysers in counting platelet concentrations usually used as platelet transfusion threshold.

Material and Methods An international exercise where four blood samples with platelet levels near usual platelet transfusion thresholds was prepared and distributed.

Results The samples shipped had a platelet count of 6.3, 13.3, 21.6 and 53.0 × 10⁹/l according to the international reference method. We received 82 sets of results from nine countries. Instruments from six different manufacturers were represented. Although the mean count for each of the four samples was very similar to the values, according to the reference method (9.0, 16.2, 23.0 and 57.6 × 10⁹/l), significant variability in the results was found. Assuming that these were patient samples and the result of the count used to indicate a prophylactic platelet transfusion, undertransfusion would have occurred for 24.5% of the LP1 samples at a transfusion threshold of 10 × 10⁹/l and, at a threshold of 20 × 10⁹/l, undertransfusion would have occurred for 7.2% of the LP1 and 16.2% of the LP2 samples and overtransfusion would have occurred with 23.1% of the LP3 samples.

Conclusion The results suggest that significant inaccuracy exists in counting low levels of platelets and that this inaccuracy might have a significant impact in under- and overtransfusion of platelet concentrates to patients.

Key words: haematology analysers, low platelet count, platelet transfusion.

Introduction

Platelet transfusion therapy continues to be the mainstay of the treatment of patients suffering from quantitative and qualitative platelet disorders. In 2009, about 2.8 million platelet transfusions were performed in Europe and USA [1]. The most frequent indication for transfusing
platelet concentrates in most hospitals is the prophylaxis of spontaneous bleeding in the haematology/oncology patient population. Some recent studies have challenged the rationale behind this common practice but without apparent success [2, 3].

There are data suggesting that the transfusion threshold for indicating a platelet transfusion to prevent bleeding for patients with hypoproliferative bone marrow, with no other bleeding risk, can go as low as 10\( \times 10^9/l \) [4]. Even some guidelines recommend that threshold could be reduced further to 5\( \times 10^9/l \) in the absence of fever >38°C or fresh minor haemorrhage [5]. If some other bleeding risks are present, most of the prophylactic transfusion protocols increase the transfusion threshold to 20\( \times 10^9/l \). Although the evidence is scarce, most of the guidelines recommend that before an invasive procedure or if the patient is bleeding, a platelet count above 50\( \times 10^9/l \) should be maintained [4].

Automated haematology analysers are the standard methodology used for enumerating platelets in EDTA anticoagulated blood samples collected from patients, leaving the manual method of counting platelet in a haemocytometer with phase contrast microscopy, as a historical reminiscence in most clinical laboratories around the world. However, for obtaining accurate, precise and reliable platelet counts as a reference for the calibration of haematology analysers, the method recommended by the International Council for Standardization in Haematology (ICSH) is based on an indirect platelet count. This method involves the counting of specifically labelled platelets relative to the red blood cells (RBC) with a fluorescence flow cytometer together with an accurate RBC count determined using a semi-automated, single-channel, aperture-impedance particle counter (International Reference Method, IRM) [6]. Recently, the Biomedical Excellence for Safer Transfusion (BEST) Collaborative has developed a method for counting platelets in platelet concentrates where the content of RBC is extremely low, so the ICSH method cannot be applied. Instead of using RBC for reference, the BEST method uses fluorescence beads in commercially available tubes (BD TruCount, BD Biosciences, San Jose, CA, USA) as reference in the flow cytometer [7].

Obtaining accurate and precise platelet enumeration using automatic platelet analysers for patient samples with low platelet counts is challenging but clinically important as the decision to give prophylactic platelet transfusions is based on the result obtained. The difficulties in discriminating the platelet signals from those of debris and spurious noise and the potential of interference with the platelet count by substantially more numerous RBCs could result in an over- or underestimate of the count affecting the decision to transfuse [8]. Evidence exists that at low platelet levels, platelet counts using automated analysers are overestimated in 67% of specimens when compared with the IRM. In addition, the coefficient of variation (CV) increases as the platelet count decreases, particularly once the platelet counts fall below 10\( \times 10^9/l \) [9]. For instance, De la Salle et al. reported a CV ranging from 15 to 43% for several different analyser at platelet levels between 5 and 10\( \times 10^9/l \) [10].

To get more insight into the accuracy and the precision of the current haematology analysers used for counting platelets at levels usually used for indicating a prophylactic or therapeutic platelet transfusion, we performed an international sample sent around survey to hospital laboratories who agreed to perform the platelet counts according to a pre-established protocol. To our knowledge, this is the first study of its kind performed and this report summarizes its findings.

### Materials and methods

#### Participating centers

The exercise was announced through BEST Collaborative members to hospital clinical laboratories and research laboratories in different countries. No attempt was made to obtain a representative or proportionate sample based on hospital type, country or analyser. Demographic information about the type of laboratory (hospital or research) and, in the case of hospital laboratories, the number of beds of the institution was recorded. Analyser manufacturer, model and day of counting were also captured. Some centres counted the samples using more than one analyser model. The study was not intended to compare results between different analysers.

#### Survey material

Survey material was prepared from leucodepleted, human whole blood, anticoagulated with CPD that was obtained from healthy blood donors who had consented to the use of their blood for external quality assurance purposes, and supplied by UK National Health Service Blood and Transplant. Buffy coat residues were added to leucodepleted whole blood to give a master specimen pool with a platelet count of approximately 50\( \times 10^9/l \), as measured on a Sysmex XE2100 automated haematology analyser (Sysmex Corporation, Kobe, Japan).

The master specimen pool was divided into four aliquots, one was retained without further manipulation (L4 – platelet count approximately 50\( \times 10^9/l \)). The remaining three aliquots were diluted with leucodepleted ABO identical whole blood to give survey material pools with approximate platelet counts of 5\( \times 10^9/l \) (pool L1), 10\( \times 10^9/l \) (pool L2) and 20\( \times 10^9/l \) (pool L3). Antibiotics were added

© 2013 International Society of Blood Transfusion

*Vox Sanguinis* (2013)
to the four survey material pools before partial fixation, according to the protocol used for the preparation of the United Kingdom National External Quality Assessment Scheme for General Haematology (UK NEQAS (H)) Full Blood Count Scheme survey material. The pools were dispensed into vacutainer tubes using bespoke Hook and Tucker Zenyx Beeline dispensing equipment. The specimens were labelled LP1, LP2, LP3 or LP4, corresponding to the survey material pool.

**Specimen distribution**

Each laboratory received a set of four specimens (L1, L2, L3 and L4 samples). Specimen packages were dispatched by first-class post to participant laboratories in the UK and by courier delivery to laboratories outside the UK. Sets of specimens were also distributed to four centres in the UK for platelet counting by the ICSH IRM [6]. These centres analysed each sample in triplicate. For each set of results, the mean and the coefficient of variation (CV) were calculated. The mean of the replicates and the CV were further analysed.

**Counting protocol**

Laboratories were instructed to store the specimens on receipt at 2–8°C until the prespecified analysis date. Each specimen was to be analysed six consecutive times in succession on the chosen instrument. The results were documented on a standardized work sheet returned by email or fax to the co-ordinating centre for analysis. For each set of results, the mean and a coefficient of variation were calculated.

**Results**

Responses were received from a total of 69 different sites (see Table 1 for country distribution). Of these, 59 were hospital clinical laboratories and the remainder were research laboratories. From the hospitals laboratories, 50% of the hospital laboratories served institutions with more than 500 beds, and 29% had fewer than 250 beds.

<table>
<thead>
<tr>
<th>Table 1 Number of participating centres by country (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Country</strong></td>
</tr>
<tr>
<td>Canada</td>
</tr>
<tr>
<td>United Kingdom</td>
</tr>
<tr>
<td>The Netherlands</td>
</tr>
<tr>
<td>Spain</td>
</tr>
<tr>
<td>United States of America</td>
</tr>
<tr>
<td>Norway</td>
</tr>
<tr>
<td>Austria</td>
</tr>
<tr>
<td>Belgium</td>
</tr>
<tr>
<td>Germany</td>
</tr>
</tbody>
</table>

From the 69 participating sites, we received 82 set of results as some centres counted the samples using more than one model of analyser/method. The distribution of the set of results received by analyser/method is shown in Table 2.

The results of the four samples measured by the haematology analysers are shown in Table 3. The results of the platelet counts using the IRM (n = 4) are also provided for each of the four specimen types. Although the mean values of the results from the haematology analysers were similar to the value measured with the IRM, the variability observed was wider that that observed in the reference method and the CV decreased with the increase in the platelet level to be counted, as previously reported.

We also looked at the impact that the variation in counting might have in indicating a platelet transfusion at different thresholds (Table 4). To do so, we determined the number and percentage of platelet counts received for each of the specimens that were above or below three commonly used transfusion thresholds (10, 20 and 30).
50 $\times 10^9$/l), making the assumption that only the platelet count would have been used to indicate the necessity for a platelet transfusion. For sample LP1, (reference method platelet count of 6.3 $\times 10^9$/l), 24-59% of the results returned were higher than 10 $\times 10^9$/l. If these had been patients’ specimens, using a transfusion threshold of less than 10 $\times 10^9$/l, the patient would have not received a platelet transfusion because the count was higher than the threshold. The manufacturers of all haematology analysers were represented in the group returning counts greater than 10 $\times 10^9$/l for sample LP1, with some variations between manufacturers in the percentage of the counts greater than 10 $\times 10^9$/l observed with respect to the total of the results received of that particular manufacturer (Table 5). Even with a transfusion threshold of less than 20 $\times 10^9$/l, a platelet transfusion would not have been indicated by 7-2% of the results for specimen L1. Interestingly, about 1% of the counts received for sample LP2 (expected count 13.3 $\times 10^9$/l) and LP3 (expected count 21.6 $\times 10^9$/l) were lower than 10 $\times 10^9$/l, which would lead to overtransfusion.

A similar pattern was observed when the platelet transfusion threshold was less than 20 $\times 10^9$/l for sample LP2 (expected platelet count 13.3 $\times 10^9$/l) with 16-2% of the results giving a count higher than the threshold and thus theoretically not provoking a platelet transfusion. Interestingly, we also observed a theoretical overtransfusion in the case of sample LP3 with an expected count of 21.6 $\times 10^9$/l. In 23-1% of the results received, the count was below 20 $\times 10^9$/l and thus in theory would have resulted in a platelet transfusion if the trigger of 20 $\times 10^9$/l was used and the decision to transfuse was based on count alone.

### Discussion

This international study, performed in 69 centres with haematology analysers from six different manufacturers, in nine different countries, has found that accurate counting of platelet at the thresholds commonly used for indicating a platelet transfusion continues to be challenging for hospital laboratories.

Currently, there are two methods for enumerating platelets in EDTA anticoagulated blood, the impedance method and the optical method. The impedance method, based on the electrical pulse generated by a blood cell passing through an aperture, was the first to be introduced [11]. The size of the pulse is proportional to the size of the cell causing it. Most instruments enumerate cells falling between 2 and 20 fl as platelets. The optical method is based on the scatter of a source of light (in general a laser beam) produced by the cell passing in front of the light. Two sensors measure the scattered light at two different angles, providing the information to characterize the cell. In general, Beckman Coulter (Beckman Coulter Inc), Horiba ABX (Horiba Ltd, Kyoto, Japan) and Sysmex (Sysmex Corporation, Kobe, Japan), manufacturers use impedance methodology, while Siemens ADVIA (Siemens AG, Munich, Germany) uses the optical method. However, in recent years, models combining multiple light scatter parameters and/or fluorescence, rather than impedance sizing alone, have been introduced in automated haematology analysers (such as Sysmex XE-2100 and Abbott Cell-Dyn; Abbott Laboratories). The software controlling the analyser enables, depending on some patterns detected in the sample (e.g. red cell fragments or giant platelets), the selection of the optical or the impedance enumeration count as the reported platelet count. Some analysers are capable, using a special tube with fluorescent isothiocyanate-conjugated anti-CD61 monoclonal antibody, of counting platelets using an adaptation of the IRM (Cell-Dyn 4000 and Cell-Dyn Sapphire from Abbott) [12].

Unfortunately, despite these newer methods, accuracy of the platelet count remains a challenge as observed from the 82 different sets of results received. However, it is interesting to note that if we examined only the means of the platelet counts returned from the haematology analysers for each of the four samples, the figure is very close to the expected platelet count according to the IRM.

© 2013 International Society of Blood Transfusion

Var Sanguinis [2013]
But what is clinically important is the wide variability in the results received and the impact that this would have at the time of ordering a platelet transfusion if only the platelet count was used as the reason for transfusion. For instance, for sample LP1, 24% of the counts received were above 10^9/l, meaning that a theoretical patient would not have received a prophylactic platelet transfusion (undertransfusion), although the true count according to the IRM was 6.3 × 10^9/l. But the impact on transfusion is even more important if the transfusion threshold used is 20^9/l. 7.2% of the platelet counts received for samples LP1 and 16.2% of the counts received for sample LP2 were above this threshold, which would result in a theoretical undertransfusion of platelets. In contrast, in the case of LP3 with an expected count of 21.6 × 10^9/l, 23.1% of the results received were below the 20 × 10^9/l threshold, leading to a theoretical overtransfusion. A similar tendency for overtransfusion was observed in the case of sample LP4 with 9.5% of the counts being below a theoretical platelet transfusion threshold of 50 × 10^9/l.

One limitation of our study is that it was performed with shipped fixed blood samples that might affect the results of the count and, hence, the potential extrapolation of the results to the routine counts performed with blood from patients; however, several publications performed using freshly drawn blood samples from thrombocytopenic patients report similar findings. Segal et al. reported a study where samples drawn from patients with haematological malignancies were counted using routine haematology analysers at five different sites and those with platelet counts below 20 × 10^9/l were counted again using the IRM within 24 h. They found that optical platelet counting methods available were no more accurate than impedance platelet counting. Interestingly, data showed that the same type of analysers at different hospital laboratories performed differently in comparison with

<table>
<thead>
<tr>
<th>Sample</th>
<th>IRM count</th>
<th>Transfusion threshold &lt; 10 × 10^9/l</th>
<th>% samples below trigger</th>
<th>% samples above trigger</th>
<th>Transfusion threshold &lt; 20 × 10^9/l</th>
<th>% samples below trigger</th>
<th>% samples above trigger</th>
<th>Transfusion threshold &lt; 50 × 10^9/l</th>
<th>% samples below trigger</th>
<th>% samples above trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP1</td>
<td>6.3</td>
<td>24.5</td>
<td>0</td>
<td>92.8</td>
<td>7.2</td>
<td>0</td>
<td>92.8</td>
<td>7.2</td>
<td>0</td>
<td>92.8</td>
</tr>
<tr>
<td>LP2</td>
<td>13.3</td>
<td>90.2</td>
<td>0.8</td>
<td>99.2</td>
<td>16.2</td>
<td>0.4</td>
<td>99.6</td>
<td>0.4</td>
<td>16.2</td>
<td>0.4</td>
</tr>
<tr>
<td>LP3</td>
<td>21.6</td>
<td>0.8</td>
<td>0</td>
<td>99.2</td>
<td>0.8</td>
<td>0</td>
<td>99.6</td>
<td>0.8</td>
<td>0</td>
<td>99.6</td>
</tr>
<tr>
<td>LP4</td>
<td>53.0</td>
<td>0.2</td>
<td>0</td>
<td>99.8</td>
<td>0.2</td>
<td>0</td>
<td>99.8</td>
<td>0.2</td>
<td>0</td>
<td>99.8</td>
</tr>
</tbody>
</table>

Table 5 Number of haematology analysers according to the manufacturers producing for sample LP1 a platelet count equal or above 10 × 10^9/l, while the count according to the international reference method was 6.3 × 10^9/l. The right column provides the percentage of the total counts performed with analysers of that particular manufacturer.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>N</th>
<th>% of the total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbot</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Sysmex</td>
<td>5</td>
<td>16.1</td>
</tr>
<tr>
<td>Horiba ABX</td>
<td>3</td>
<td>16.1</td>
</tr>
<tr>
<td>Beckman Coulter</td>
<td>2</td>
<td>14.3</td>
</tr>
<tr>
<td>Siemens</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Boule-Medonics</td>
<td>1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

© 2013 International Society of Blood Transfusion
Vox Sanguinis (2013)
the IRM, suggesting that local adjustment/calibration or maintenance might significantly affect the result of the analysis. They also found a tendency for all types of routine analysers used in that study, except ABX Pentra, to overestimate the platelet count [13].

Hong et al. studied the accuracy of three haematology analysers and compared them with the IRM with blood samples from patients. In 34 samples with a platelet count below $5 \times 10^9/l$, they reported ‘For the threshold of 10 and $20 \times 10^9/l$, the proportion of inadequate transfusion was considerable’. Undertransfusion and overtransfusion were present [14]. Cid et al. reported similar findings when comparing the impedance and optical platelet enumerations of thrombocytopenic patients’ samples in a Cell-Dyn Sapphire with those measured using an adaptation of the IRM. In the case of a $20 \times 10^9/l$ transfusion threshold, 7.3% of results measured by the optical method would result in undertransfusion and 8% in overtransfusion [12].

The IRM for platelet counting requires the availability of a flow cytometer and technical skills that are not always present in the routine haematology laboratory. However, the possibility of using the haematology analyser itself to perform the platelet count using an adaptation of the IRM in an automated way might open the door to its introduction in the routine haematology laboratory, although the cost of the count must be taken into considerations. However, if an accurate platelet count can be provided, decreasing the transfusion threshold to $5 \times 10^9/l$ might be considered. The PLADO study found that the risk of developing a WHO bleeding grade of two or higher in patients suffering from hypoproliferative bone marrow was very similar, when patients had a morning platelet count between $\geq 6 \times 10^9/l$ and $80 \times 10^9/l$, and only increased significantly at count of, or lower than, $5 \times 10^9/l$ [15]. We speculate that a more accurate platelet counts at low levels might permit a decrease in the threshold for prophylactic platelet transfusions, which in turn would reduce the number of platelet transfusions and the costs associated to it. The saving associated with the reduction in platelet transfusions could offset the increased cost associated with the addition of an immune staining platelet count to samples with very low platelet counts [16]. Obviously, a randomized, controlled clinical trial of enough power would be needed to test whether that hypothesis is correct.

In conclusion, the results of this international exercise suggest that significant inaccuracy still exists when counting low levels of platelets using routine haematology analysers and that this inaccuracy might have a significant impact on under- and overtransfusion of platelet concentrates to patients.

Acknowledgements

The authors are deeply grateful to all the colleagues in the different participating centres that with their selfless and generous collaboration made this study possible. We thank also Dr Paul Harrison for useful discussions during study design.

Author contributions

Miguel Lozano, Anne Mahon, Pieter F. van der Meer, Simon Stanworth, Joan Cid, Dana Devine, Mark K Fung, Barbara de la Salle and Nancy M Heddle designed the study and recruited participants. Anne Mahon and Barbara de la Salle prepared the counting protocol and prepared and distributed the samples. Miguel Lozano and Joan Cid collected and analysed data. Miguel Lozano drafted the manuscript that was revised and approved by all the authors.

References


© 2013 International Society of Blood Transfusion

Var Sanguinis [2013]