

Advancements in laboratory diagnostics: an invaluable tool for assessing quality of blood transfusions

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The interesting article by Willy A. Flegel that recently appeared in this journal provides valuable insights into an ongoing debate on the use of fresher material for blood transfusions¹. Regardless of important considerations about the delicate balance between selection of units and blood supply, an innovative approach for defining the quality of transfused components is increasingly supported by recent advancements in laboratory diagnostics, which would enable to determine whether leucocytes, platelets and red blood cells are suitable for transfusion regardless of the ageing of the stored material. Several examples can be given, whereby routine, automatic flow haemocytometry combined with additional technical tools provides valuable information.

As regards platelets, the loss of function during storage is mirrored by disc-to-sphere transformation, extension of pseudopodia, alteration of the canalicular system and granules, up to loss of storage granules². Some haemocytometers already provide a variety of activation-related information about these blood elements. For example, in Advia 2120 (Siemens Diagnostics, Tarrytown, New York, United States of America), the intensity of light scattered by platelets is assessed at two different angles (i.e., 2 to 3° and 5 to 15°). Mean platelet volume and mean platelet component are then calculated and from these paired measures. The latter parameter, in particular, has been shown to be a reliable index of platelet degranulation, since it is directly related to CD62 expression, which is highly indicative of platelet activation and ageing³. The same instrument, along with others such as the PENTRA 60C (Horiba-ABX Diagnostics, Montpellier, France), provide specific flags or alerts in the presence of degranulation of polymorphonuclear leucocytes⁴, which is a hallmark of functional impairment⁵. More specifically, the Advia 2120 also enables reliable quantification of the number of degranulated neutrophils in specific clusters of the peroxidase scattergram based on cell volume and intracellular myeloperoxidase concentration. Additional morphological changes that characterise gradual deterioration of leucocytes in stored blood include a more homogeneous staining of nuclei, the separation of the nuclear lobes or irregular lobulation and vacuolisation, which can be reliably identified in

the scattergram of modern haemocytometers such as those of the Sysmex XE series, in which the intensity of side scatter is dependent on the complexity of nuclear lobulation and granules within the cytoplasm⁶.

It is also noteworthy that several changes occur to red blood cells during medium- and long-term storage in blood bags, including reduced deformability, altered adhesiveness and aggregability, and decreased 2,3-diphosphoglycerate and ATP content, to the point of frank injury and spurious haemolysis⁷. Specific macromolecular remodelling, along with lipid peroxidation and/or oxidative damage to the membrane, are mainly responsible for the onset of irreversible morphological abnormalities and loss of function. Most of these physicochemical changes, usually referred to as the "storage lesion", can be identified by time-consuming or expensive analytical techniques such as flow cytometry, time-resolved phosphorescence anisotropy and microscopy, which are unsuitable for rapid examination of a large number of blood bags⁸. Nevertheless, the presence of red blood cell crenation or fragmentation -and finally haemolysis- can now be reliably and rapidly identified by specific erythrocyte indices on automated flow haemocytometers, such as the "immature platelet fraction" on XE-2100 (Sysmex, Kobe, Japan), or "RBC ghosts" on Advia 2120⁹. Another interesting approach is ThromboLUX™ (LightIntegra Technology Inc., Vancouver, British Columbia, Canada), which uses the principle of dynamic light scattering -also known as photon correlation spectroscopy or quasi-elastic light scattering- to identify and quantify the particles that may be present in a platelet concentrate, as well as showing how they respond to temperature stress. The instrument finally generates a quality score on a scale ranging from 0 to 40, with platelet concentrates scoring from 13 to 40 containing mostly discoid and functional platelets. Recent data show that the information from ThromboLUX™ correlates adequately with that from flow cytometry and microscopy and may, therefore, be suitable for quality assessment of platelet concentrate samples¹⁰.

Several technical advances have occurred in laboratory diagnostics over the past decades. These have not only improved the global quality of testing,

but have also enabled reliable application to transfusion medicine for the assessment of the quality of transfused components, as attested by some of the examples previously provided. The modern, fully-automated haemocytometers -which are characterised by high accuracy and precision, notable throughput and full integration with information systems- can also provide a kaleidoscope of cellular indices. Most of these novel parameters are still partially unexploited, but it is conceivable that they may be reliably used to detect artefactual changes in leucocytes, platelets and red blood cells, which may be suggestive of less functional, defective or even apoptotic cells, and finally establish as to whether cellular elements within blood bags are still viable for transfusion. Future studies should be focused on the evaluation of the usefulness of these parameters in the setting of transfusion medicine.

The Authors declare no conflicts of interest.

References

- 1) Flegel WA. Fresh blood for transfusion: how old is too old for red blood cell units? *Blood Transfus* 2012; **10**: 247-51.
- 2) Klinger MH. The storage lesion of platelets: ultrastructural and functional aspects. *Ann Hematol* 1996; **73**: 103-12.
- 3) Kratz A, Wood MJ, Siegel AJ, et al. Effects of marathon running on platelet activation markers: direct evidence for in vivo platelet activation. *Am J Clin Pathol* 2006; **125**: 296-300.
- 4) Guerti K, Vertessen F, Daniëls L, Van Der Planken M. Performance evaluation of the PENTRA 60C+ automated hematology analyzer and comparison with the ADVIA 2120. *Int J Lab Hematol* 2009; **31**: 132-41.
- 5) Lane TA, Lamkin GE. The effect of storage on degranulation by human neutrophils. *Transfusion* 1985; **25**: 155-61.
- 6) Walters J, Garrity P. Performance evaluation of the Sysmex XE-2100 Hematology Analyzer. *Lab Hematol* 2000; **6**: 83-92.
- 7) Sowemimo-Coker SO. Red blood cell hemolysis during processing. *Transfus Med Rev* 2002; **16**: 46-60.
- 8) Karon BS, Van Buskirk CM, et al. Temporal sequence of major biochemical events during Blood Bank storage of packed red blood cells. *Blood Transfus* 2012; **10**: 453-61.
- 9) Lippi G, Pipitone S, Gennari D, Franchini M. Identification of spurious hemolysis in anticoagulated blood with Sysmex XE-2100 and Siemens Advia 2120. *Clin Lab* 2012; **58**: 801-4.
- 10) Xu Y, Nakane N, Maurer-Spurej E. Novel test for microparticles in platelet-rich plasma and platelet concentrates using dynamic light scattering. *Transfusion* 2011; **51**: 363-70.

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