Activated or inactivated? Transfusing the right platelets

David Wild

August 2019—In the blood bank, all platelets in bags appear to be the same, whether resting or activated, and because they look the same, they may be used as if they're equal. But they are not.

Elisabeth Maurer, PhD, and Joel N. Kniep, MD, in a recent CAP TODAY webinar, explained that despite all that is done to keep platelet activation to a minimum, activation rates are still high. "And this means a lot of potential impact on patients," said Dr. Maurer, clinical associate professor, University of British Columbia, and founder of LightIntegra Technology, Vancouver.

The webinar (www.captodayonline.com) was made possible by a special educational grant from LightIntegra, which makes ThromboLux, a class I device for measuring the quality and quantity of microparticles in blood and blood products.

"I think there's a big benefit to be had from what I like to call a personalized platelet transfusion," Dr. Maurer said.

A lot of effort goes into minimizing platelet activation to improve efficacy for hematology/oncology patients, she said. For actively bleeding trauma and surgery patients, activated platelets are potentially preferable because they're hemostatically more active.

While it is possible to determine which platelets are nonactivated, or resting, based on their "discrete" and recognizable shape, "we can't have scanning electron micrographs of all our platelets, and what we deal with in the blood bank are bags that usually look very much the same," Dr. Maurer said. As a result, there's a notion that they are the same.

A large number of activated platelets are in circulation, she said. A study Dr. Maurer and others conducted at six tertiary care centers in which 13,000 platelet transfusions were tested found 36 percent to be activated despite proper storage. The findings are to be submitted to *Transfusion*.

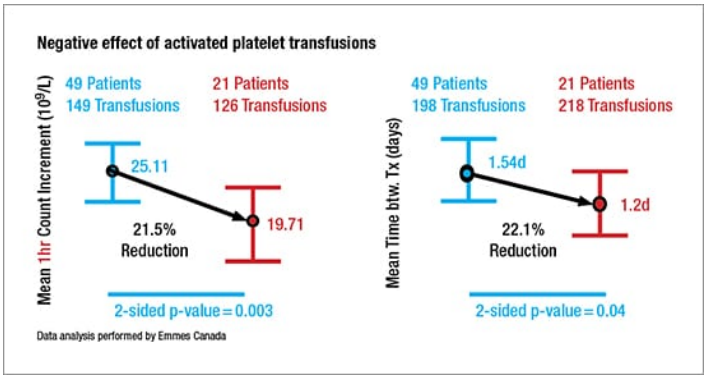
Dr. Kniep, assistant medical director of transfusion services, University of Coloraado Hospital, and assistant professor, University of Colorado, and his colleagues have documented some of the impact on patients.

"We have a very busy transfusion service supporting the emergency department, the bone marrow transplant service, and the operating rooms," Dr. Kniep said, adding that the number of platelet products transfused rose from 486 in July 2017 to 725 by June 2018. The hospital receives about 15 pathogen reduction technology platelets; the remainder are conventional platelets. "We primarily reserve the PRT platelets for our bone marrow transplant patients," he said. He and his colleagues have found that 33 percent of their platelet stock consists of activated platelets.

They studied platelet activation in 2018 in an analysis of patients with hematologic malignancies. "We had six months of baseline platelet transfusion data, and then we did platelet testing for three months and looked at nonactivated platelet transfusions" in a retrospective review. Patients with acute lymphocytic leukemia, acute myeloid leukemia, and myelodysplastic syndromes received the most platelets during the study.

An analysis of data from 149 nonactivated platelet transfusions given to 49 patients, and 126 activated platelet transfusions administered to 21 patients, revealed that activated platelet transfusions were less effective than nonactivated platelets. Mean one-hour platelet count increments declined from 25.11×10^9/L after nonactivated platelets were administered to a mean 19.71×10^9/L after transfusion of activated platelets ($P = 0.003$) (see "Negative effect of activated platelet transfusions").

Data from 198 nonactivated platelet transfusions and 218 activated platelet transfusions in the same patient groups found that use of activated platelets shortened the time between transfusions by 22.1 percent, from a mean 1.54 days (37 hours) when nonactivated platelets were administered to a mean 1.2 days (28 hours) after activated platelets were administered ($P = 0.04$).



Dr. Kniep recounted one case, that of a 66-year-old female with AML. The patient was receiving azacitidine and CD33 antibody treatment before undergoing a sibling allogeneic stem cell transplant with fludarabine/melphalan conditioning.

A bone marrow biopsy conducted about one month after the transplant showed full donor chimerism. After the biopsy she was admitted to acute rehabilitation. Following a 10-day stay in rehab, the patient was readmitted to the hospital for worsening diarrhea and was switched from her original regimen of tacrolimus and methotrexate for graft-versus-host disease prophylaxis to sirolimus and methylprednisolone.

A subsequent bone marrow biopsy showed hypocellular marrow without evidence of relapsed leukemia. Due to ongoing cytopenias, the patient was started on eltrombopag about two weeks later. It was several days after that that she received the first of 14 platelet transfusions administered over a three-month period.

Using LightIntegra's ThromboLux to screen for activation status, Dr. Kniep's team found that the ninth transfusion met the definition of activated platelets, which, according to the manufacturer, is defined as a microparticle concentration greater than 15 percent.

A review of the patient's outcomes showed that, prior to the ninth platelet transfusion, the average time between transfusions was slightly more than 63 hours. That interval narrowed following transfusion of the activated platelets, dropping to an average of 47 hours between the remaining transfusions, Dr. Kniep said.

In addition, prior to transfusion of the activated platelets, the average platelet count increment was slightly above 60×10^9/L, but it fell to 41×10^9/L after transfusion of the activated platelets.

"One of the questions you may have is, Is there anything that might have happened during that time period either before or around the activated platelet [transfusion] that might confound or possibly could have contributed to the poorer platelet recovery?" Dr. Kniep said.

Dr. Kniep

At about the time of the activated platelet transfusion, the patient had an episode of hypertension and became unresponsive, requiring resuscitation with albumin and intravenous fluids. "She responded to treatment but then became unresponsive again," Dr. Kniep said.

A stroke was called and she was transferred to the hospital's ICU where she was intubated and arterial and central lines were placed. Blood cultures revealed Gram-negative rods, and the patient was given additional antibiotics.

Chest x-ray revealed bilateral pleural effusions. The patient was placed on stress-dose steroids.

“Overall,” Dr. Kniep said of his group’s study, “we found there was a decrease in count increment and time between transfusion. Clinically that can translate into additional platelet transfusions for the patient. And that’s additional money out of the blood bank budget in a time when we are constantly being asked to do more with less and to try to minimize transfusions throughout the hospital.”

Dr. Maurer describes the Thrombo-Lux device as a “small benchtop instrument that is easy to use and can, in a very small, almost toothpick-sized, sample from a platelet concentrate or a platelet-rich plasma, characterize the platelets in that sample.” It detects platelet activation by the fragments of cells, called microparticles.

Performed in about five minutes, the test uses laser light to illuminate a sample and identify the shape of platelets as well as the microparticles, “maybe even small aggregates,” she said. As the light bounces, or scatters, off the platelets and particles, it provides information on the speed of the particles based on their movement in suspension.

“It’s very similar to the radar technology the police use to detect speeding cars, and in our situation the speeders are the microparticles.” The microparticles are very small, “and therefore they are fast compared to the platelets or maybe the even larger activated and microaggregate platelets combined.”

The device plots the size distribution of the particles using the scattered light information. If the microparticle concentration in the sample is less than 15 percent, it is classified as nonactivated, or resting. If it is composed of more than 15 percent microparticles, it is considered activated.

There are many ways to test for platelet activation, one of which is to use the microscope, which Dr. Maurer called “subjective and cumbersome.” CD62 expression on the surface is a research test. Blood cell counters cannot detect the microparticle sizes that the ThromboLux can, she said.

Flow cytometers can identify the smaller particles, Dr. Maurer noted, but a number of factors should be considered, including “gating and setting thresholds,” which is currently not standardized. And not every flow cytometer can be used to identify microparticles, she said. “The International Society for Extracellular Vesicles does say one needs very specific flow cytometers to identify particles as small as microparticles, which have an average diameter of about 200 nm.

“But we have done comparative studies with ThromboLux and flow cytometers, and we did see high correlations, even though the very small microparticles might not be detected with a flow cytometer,” she said (Xu Y, et al. *Transfusion*. 2011;51[2]:363–370; Labrie A, et al. *Transfus Med Hemother.* 2013;40[2]:93–100). “But in terms of seeing whether platelets are activated or not based on the fragmentation, it still works on flow cytometers.”

If platelets are stored at room temperature with agitation for five to seven days, why does 36 percent of the inventory, or in the University of Colorado’s case, 33 percent, become activated? “It really comes from the donor,” Dr. Maurer said. Platelets are immune cells and hemostatic cells, “so there are a lot of things they have to do in the donor’s circulation.”

“When the donor has anything—from allergies, asthma, depression, stress—the donor might donate activated platelets, and the separation process could have an additional effect on whether or not those platelets are stressed and more activated.” In a donor whose platelets are activated, the platelets will be more vulnerable to additional stress, more fragile, and will be even more activated once in the bag. She compares it to putting glasses into a dishwasher: “Intact glasses won’t mind that stress, but a glass with a crack might actually break.”

A very healthy donor will have resting platelets that are resilient to additional stress and will remain resting after processing.

Dr. Maurer shared an example from one of the other study sites, the University of Kansas Medical Center. In one patient with AML who received seven platelet transfusions in that study, a single activated platelet transfusion with

an approximately 25 percent microparticle concentration reduced 18-hour platelet count increments from an average of about 80 × 10^9/L following the first three nonactivated platelet transfusions to a mean of about 35 × 10^9/L over the subsequent four transfusions, after administration of only a single activated platelet product.

The higher response to the resting platelets than to the activated platelets "is really interesting," Dr. Maurer said, "because we are used to thinking that if we give resting platelets, we should immediately see some positive outcome." She asks: "Is there an explanation based on the immunology of the patient and the response of the patient to transfusion?"

Atypical hemolytic uremic syndrome is an example of a situation in which a patient might have an immune response to platelets, Dr. Maurer said, pointing to a publication that outlined this process (Peerschke EI, et al. *Adv Exp Med Biol.* 2008;632:81–91).

Dr. Maurer

"A triggering event can activate the platelets, and the immediate immune response would be that the platelets get opsonized by complement and then recognized by the macrophages and therefore removed," she explained. "Interestingly, the macrophages then become antigen-presenting cells, and they can trigger T-cells and B-cells subsequently to make antibodies, which, with a subsequent trigger event, would cause the platelets to be very quickly removed."

In a situation in which activated platelets come from the donor and are transfused to a thrombocytopenic patient, they become opsonized and removed from circulation, Dr. Maurer said. "But they could also trigger this response to produce antibodies, and when antibodies already exist, the platelets get removed quickly, even if not activated. So in a situation of HLA or HPA mismatch, that could still be the case."

The meta-analysis of the four platelet activation studies conducted at the University of Kansas Medical Center, UC Health Denver, Children's Medical Center Dallas, and Vancouver General Hospital reveals that when patients receive activated platelets, their count increments are lower by an average of about 5,000 platelets/µL. While this may not seem like much, Dr. Maurer said, the fact that the time between transfusions also decreased by an average of 13 hours is an indication that the clinicians thought it to be important.

"We know that most clinical decisions are made based on the platelet count for patients who receive platelets prophylactically," she said.

Results from the four sites demonstrate that "despite the differences in platelets, despite the differences in patient population, any kind of differences in SOPs, we see a decrease in the clinical outcome both in count increment as well as time between transfusion when hematology/oncology patients receive activated platelets." (Maurer E, Noland DK, Kniep J, et al. Platelet activation affects transfusion outcomes in hematology-oncology patients: meta-analysis of data from four North American hospitals. Poster to be presented at 2019 AABB Annual Meeting; October 2019; San Antonio.)

Not surprising, she said, in that the regulatory measures put in place decades ago to minimize activation are there so the best outcome can be obtained for cancer patients. Now, knowing that a high percentage of activated platelets are in inventory, "we have to screen so that the resting platelets, as was intended, go to cancer patients while the activated platelets go to the actively bleeding patients in surgery and trauma."

With transfusion frequency increasing after activated platelets are administered, it's not only a matter of cost, Dr. Maurer said, but also workload. "It increases how many platelets have to be coming into inventory, have to be released, have to be tested." And though cost-related studies are still being done, "the prognosis is that platelets are probably 10 to 20 times more expensive in terms of their administration cost compared to the purchasing cost."

If the time between transfusions declines because they're less efficacious than they could be, Dr. Maurer said, "there's more work."

Hence the benefits from what she calls the personalized platelet transfusion. "It has been in the literature and the knowledge of transfusion medicine for a long time that resting platelets should go to cancer patients and the others to trauma and surgery patients"—to reduce usage, cost, and workload and to give the patient what is best.

David Wild is a writer in Toronto.