Arginase as a new concern in blood transfusion

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Dear Sir,

Blood transfusion is a life-saving process for anaemic, surgical, oncological and severely injured patients. However, an increasing number of reports suggest that transfusion of stored human blood may also pose some risks and can be associated with adverse clinical outcomes, including infections and tumour recurrence, which, in some studies, have been found to be more frequent as blood storage time increases. Nevertheless, retrospective analyses on large numbers of patients do not support the notion that increasing median duration of red blood cell storage is associated with an enhanced risk of postoperative mortality. Appropriate randomised, controlled clinical trials will be crucial to address the controversial issue of the effect of the duration of blood storage on outcomes. The proposed detrimental effects of prolonged blood storage have been attributed in part to haemolysis of packed erythrocytes stored for a prolonged period, which leads to an increased oxyhaemoglobin concentration and nitric oxide (NO) scavenging. Indeed, the haemoglobin level in the storage bag supernatant was found to be higher after 40 days of storage than after 3 days of storage, indicating increased haemolysis, and infusion of 40-day packed erythrocytes was recently suggested to lead to increased NO production from endothelial nitric oxide synthase (NOS), as a compensatory mechanism for reduced NO bioavailability caused by plasma oxyhaemoglobin scavenging of NO. NOS and arginase use L-arginine as a common substrate and the presence of increased levels of these enzymes could lead to L-arginine depletion and its ensuing physiological effects, including inhibition of T-cell function, as L-arginine is required for T-cell activation and proliferation. Human erythrocytes express an active and functional endothelial-type NOS as well as arginase. Thus, increased haemolysis might be an important aspect in blood transfusion, leading to elevated levels of arginase and NOS, and, thereby, to L-arginine depletion that could eventually elicit adverse outcomes. We took 10-mL samples of units of blood after different periods of storage and centrifuged them at 1,200 rpm for 12 min at room temperature. Free arginase activity and the haemolytic index were determined using standard methods of measuring the conversion of arginine into ornithine and urea (arginase activity) and the absorbance of free haemoglobin in the plasma (haemolytic index). We found statistically significant increases in haemolysis and free arginase activity along the storage time of the packed erythrocyte bags (Table I). Because transfusion of contaminating leucocytes is now considered to be potentially harmful, leucocyte reduction, by which white blood cells are removed before transfusion, has been incorporated into the practice of most medical centres around the world. However, although leucoreduced units of packed red blood cells contain fewer than 5×106 white blood cells, it should be noted that neutrophils, which undergo spontaneous cell death, constitutively express large amounts of arginase and even small contamination of packed red cell bags by neutrophils might, therefore, produce significant levels of arginase activity, apart from that derived from the red cells themselves. This is a possible explanation for the relatively greater increase in arginase level than in haemolytic index (Table I). Arginase is gaining momentum as a key player in the control of L-arginine availability and the regulation of several physiological processes, including the immune system. Thus, high levels of free arginase following blood transfusion could underlie many of the deleterious outcomes, including immunosuppression and infectionrelated processes associated with transfusion of blood stored for long periods. Although arginase has a short half-life of only a few hours in human blood, it might act in early stages of immunosuppression. Our finding of raised levels of free arginase in blood stored for long periods could have implications for patients in whom immunosuppression is a major challenge.

Of course, in vitro findings cannot be translated automatically to clinical outcomes and further investigation is required. Current Food and Drug Administration regulations allow transfusion of packed red blood cells stored for up to 42 days. Nevertheless, our data draw attention to the fact that levels of free arginase increase in packed red blood cell units with increasing storage time, which could be a concern when transfusing patients, particularly those such as cancer patients who are immunodepressed, given the potential adverse effects.

Table I - Free arginase activity and haemolytic index in units of human packed red blood cells at different days of storage.

Days of storage	Arginase activity (mU/mL)	Haemolytic index
6-10	62.1±15.5	43.7±8.9
11-15	55.0±8.0	55.5±8.9
16-20	88.3±33.4	53.4±10.5
21-25	220.6±64.5**	135.5±55.7*
26-30	402.9±52.6**	147.2±37.3**

Data are means ± SD (n ≥7). Asterisks indicate values that are significantly different from those of the values for 6-10 days of storage, according to a Student's t-test. *P <0.01.