

# ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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## Blood Product Transfusion and Cancer Prognosis

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### H&O What are some reasons that cancer patients might need blood products?

Cancer patients may need blood products either because of their inability to produce enough blood, due to the treatment of their disease, or the disease itself. In some tumors such as leukemia, patients can have a bone marrow replacement of the tumor. Sometimes, the bone marrow is infiltrated by a secondary or metastatic disease; sometimes the patient stops eating, which gives them a nutritional reason why they cannot produce enough blood. There could be a derangement in normal physiology, where the cancer creates factors that repress normal protein and bone marrow production (Table 1). Sometimes the bone marrow reacts to the cancer or its treatment, or patients get myelofibrosis, where less blood is made. Additionally, chemotherapy or radiotherapy can cause myelosuppression. Most chemothera-

peutic agents cause leukopenia and thrombocytopenia (acutely around 9–10 days with nadir counts at 14–18 days), which usually lasts for approximately 1 month and then tends to recover. Certain cancers can induce the peripheral destruction of blood and blood products. Treatments of cancer, such as surgery, can also cause an acute blood loss.

There are many different types of blood products. However, we physicians often tend to conveniently group them all together and assume that you only get what is on the label, which is not always true. A unit of packed cells is assumed to be all red blood cells, but it is important to note that there still is contamination with other cells, cell fragments, and bioactive mediators. Sometimes these contaminants can cause an acceleration of tumors growth and spread.

### H&O What are the risks of a transfusion?

There are many different ways of classifying these risks: local or systemic, immediate or late, or definitive, probable, or even potential (Table 2). Blood group mismatching can cause transfusion risks. Contamination of the

**Table 1.** Why the Cancer Patient May Need a Blood Transfusion

#### Causes of Anemia, Thrombocytopenia, and Leukopenia in Cancer

- Bone marrow replacement by primary tumor (eg, leukemia)
- Bone marrow involvement by metastatic tumor (eg, breast, prostate)
- Bone marrow reaction (eg, fibrosis)
- Myelosuppression by chemotherapy or radiotherapy
- Peripheral destruction (eg, immune hemolysis, disseminated intravascular coagulation, splenomegaly)
- Derangement of normal physiology
- Nutritional deficiency (eg, folate, Fe<sup>2+</sup>, negative N<sub>2</sub> balance)
- Abnormal feedback (eg, stimulation/inhibition of hematopoiesis)
- Blood loss—surgery/erosion of great vessel by disease or its treatment

**Table 2.** Common Risks of Blood Product Infusions

| <b>Some of the Risks of Transfusion</b>   |
|---|
| <ul style="list-style-type: none"> <li>• Febrile nonhemolytic</li> <li>• Acute transfusion reaction from mismatch</li> <li>• Acute hemolytic</li> <li>• Delayed hemolytic</li> <li>• Allergic               <ul style="list-style-type: none"> <li>– Anaphylactic</li> <li>– Human leukocyte antigen sensitization</li> <li>– Red blood cell allosensitization</li> <li>– Graft-versus-host-disease</li> </ul> </li> <li>• Clotting disturbances</li> <li>• Electrolyte disturbances               <ul style="list-style-type: none"> <li>– Volume overload in the young and elderly</li> </ul> </li> <li>• Transfusion-related acute lung injury</li> <li>• Peri-operative infection susceptibility</li> <li>• Blood borne infections- viral (HIV, hepatitis), bacterial prions, parasites (malaria)</li> <li>• Increased tumor recurrence from peri-operative transfusion</li> <li>• Worsened cancer prognosis from peri-operative transfusion</li> </ul> |

blood product may release pyrogens, causing patients to become febrile or have rigors. Sometimes the blood product can unfortunately expose the patients to infectious agents such as viruses (eg, HIV, hepatitis B and C), bacteria and prions, or in rare cases, fungal and parasitic infection. Patients may also have an increased incidence of peri-operative infections. Sometimes the blood transfusion can cause clotting or electrolyte disturbances and, in some cases, volume overload (very young, old, and renal/cardiopulmonary impairment). Definitive risks of blood transfusions are nonhemolytic febrile transfusion reactions, cytomegalovirus (CMV) infection, and alloimmunization. Probable risks are immunomodulatory effects, which will be discussed further in our research where we found that sometimes with the transfusion of blood products, cells are also transfused, and these cells naturally contain substances that can modulate either tumor growth or the immune system. Last but not least, there are reperfusion risks when a patient who has been critically shut down is over transfused, and all those bioactive mediators go into the central circulation (Table 3). All these risks may be quantified to inform us and our patients; for example, an allergic reaction can happen in 1–4% of patients, fortunately all these risks change as our technology to minimize them advances.

### **H&O** What studies have associated blood transfusion with cancer reduced prognosis?

Most studies have been carried out on peri-operative blood transfusions for ablative resections, rather than

**Table 3.** Leukocyte Effects

| <b>Adverse effects associated with donor white cell contamination</b>  |
|--|
| <p><b>Definitive</b></p> <ul style="list-style-type: none"> <li>• Nonhemolytic febrile transfusion reactions</li> <li>• Transmission of leukocyte-associated viruses</li> <li>• Cytomegalovirus, Epstein-Barr virus, human T cell leukemia virus type 1</li> <li>• Alloimmunization</li> </ul> |
| <p><b>Likely</b></p> <ul style="list-style-type: none"> <li>• Immunomodulatory effects</li> <li>• Cancer recurrence</li> <li>• Postoperative infections</li> </ul>   |
| <p><b>Theoretical</b></p> <ul style="list-style-type: none"> <li>• Reperfusion injury</li> <li>• Transfusion storage time for red blood cells and platelets</li> <li>• Transfusion-related acute lung injury</li> <li>• Transfusion-associated graft-versus-host disease</li> </ul>            |

on only cancer patients who received blood transfusions (Figure 1). In the peri-operative setting, there is a defined indication to transfuse (eg, acute blood loss), and the number of units of blood given is more easily quantified and documented. On the other hand, ongoing care protocols with the general cancer patient may differ between institutions where the transfusion effect may be more insidious, progressive, and harder to demonstrate in smaller studies. Logistically, it is difficult to acquire accurate data.

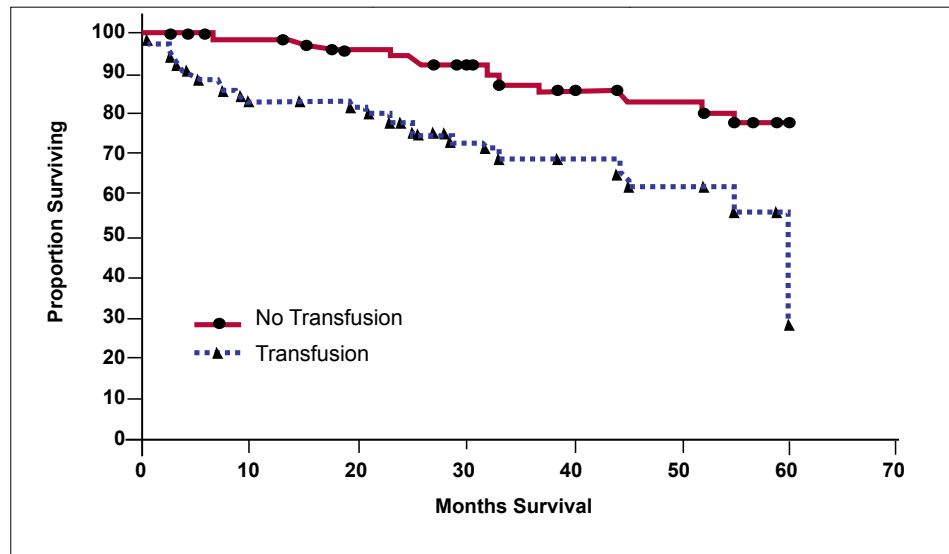
In summary, many of these previous studies were carried out examining peri-operative transfusion for ablative resection because:

- a. In these instances there may be an acute blood loss and a defined indication to transfuse
- b. It was easier to document transfused units rather than in ongoing care where many institutions may have treated the patient (data capture is inadequate; eg, unknown amounts may have been given or documented) and where transfusion effect may be more insidious and progressive.
- c. Surgical ablative procedures could release many growth factors and even shed tumor cells, which may help highlight the direct adverse effect of blood transfusion, or these operations serve to swamp the local protective mechanisms and cause a worsened effect.
- d. Peri-operative blood transfusions are associated with several confounding variables that by themselves predict an overall worse outcome. Some of these variables include the difficulty of the operative procedure, skill of the surgeon, extent of tumor invasion and resection,

**Figure 1.** The 5-year survival Kaplan Meir Curves in patients with squamous cell carcinoma

Note: 334 patients with squamous cell carcinoma who underwent peri-operative blood product transfusion in our institution; there was a significant difference between the survival in those who did and did not receive a peri-operative blood transfusion.

( $P < .001$ ; hazard ratio=0.33)



and the overall health of the patient. In most observational studies, cancer recurrence rates were evaluated in surgical patients who received allogeneic blood transfusions as compared with patients who received no blood transfusions. Despite multivariate statistical analyses, it is possible that confounding variables that were unaccounted for could have influenced subsequent outcomes.

### H&O What are the possible explanations for blood transfusion being associated with worsened cancer prognosis?

Worsened cancer prognosis can be due to cancer, host, or external factors that cause cancer growth and dissemination (eg, host immunomodulators, extracellular matrix eliminators, or dissemination accelerators, etc.) Also, a reduction in the host immunity, which can be caused by the delivery of exogenous or autogenous growth factors to the patient, can cause tumor growth.

Researchers have made the general assumption that the function of blood is just to deliver oxygen; whereas in fact, blood is much more multi-factorial. Growth factors are contained within red cells (eg, vascular endothelial growth factors [VEGF], fibroblastic growth factor [FGF], epidermal growth factor [EGF]) and these factors leach from the blood product as it ages and upon cell membrane disruption (eg, occurs on hemolysis from transfusion mismatching or mechanical disruption). It is important to remember that red blood cells do not contain a nucleus. Therefore, as the red blood cell ages, its machinery within itself breaks down; the membrane becomes porous, releasing various factors contained within red blood cells, such

as VEGF. It is now known that as soon as red blood cells reduce their ATP content by 30%, they undergo these membrane and metabolic changes.<sup>1,2</sup> The accumulation of these mediators can occur to the extent that they become biologically significant and widespread.

It seems reasonable that factors to promote endothelial repair and integrity are contained within the cells that accumulate upon endothelial disruption (eg, platelets, red cells, etc.) These leached growth factors have multiple effects; for example, VEGFs have a growth effect and promote vasculogenesis, angiogenesis, immunodepression as well as membrane permeability. It would also explain the association of various phenomena such as peri-operative infection and 'shock lungs' with blood transfusion, which may be due in part to VEGF (and other factors) released from stored blood. Stored VEGFs can have a direct stimulatory growth effect only on those tumors which express the VEGF growth factor receptor such as colonic adenocarcinoma, prostate carcinoma, and head and neck squamous cell cancer.

Our group has done studies looking into the supernatant of blood that was stored for various amounts of time, and we found progressive accumulation of these released factors.<sup>3-6</sup> We also found that there is a linear curve: the longer the blood is stored, the more factors are released; it becomes biologically significant after 2 weeks of storage. Most blood is 3–5 weeks stored when given to the patient (Figure 2).

When we assayed these factors on a bioassay using collagen, we found that these factors actually directly promote tumor growth. However, we also found that by using antibodies (eg, VEGF-A165), this growth promotion effect is reduced by about 60% (Figure 3). We have

**Table 4.** White Cell Filter Systems Used to Reduce White Cell Contamination

| Generation | Pore Size      | Mechanism       | Purpose   |
|------------|----------------|-----------------|---|
| First      | 170–260 mm     | Screen filter   | “standard” blood filter                           |
| Second     | 20–49 mm       | Screen filter   | Micro-aggregate filter; 90% leukocyte filtration. |
| Third      | Not applicable | Adhesion filter | Absorption filter; 99.9% leukocyte filtration.    |

experimental evidence for the accumulation of these factors (eg, VEGF) over time. Furthermore, by the use of VEGF antibodies, we were able to specifically reduce the direct growth effect of these accumulated factors on tumor and vascular endothelial cell lines. This effect is not complete, suggesting there are other untested factors that are also leached from stored blood products and have a growth-enhancing effect. We have shown that after 2 weeks of storage, there is a significant accumulation of extracellular VEGF sufficient to cause tumor growth and migration. This effect can again be abrogated by the use of anti-VEGF antibodies.

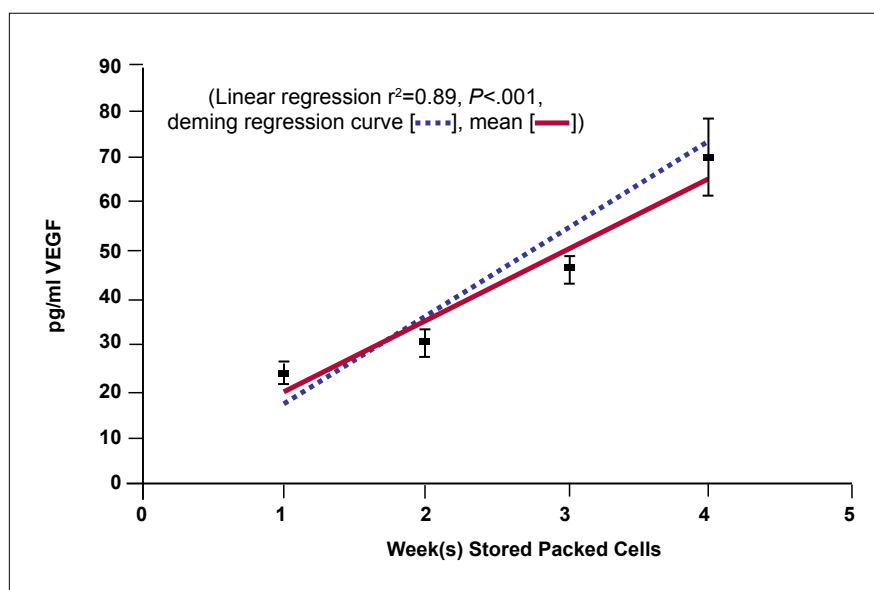
Therefore, we predict that if you give younger blood to tumor patients, they will probably be fine.<sup>2</sup> Unfortunately, since most blood for predicted operations tends to be stored, and most of the storage facilities prefer to use their old blood before it expires, it is not good for the tumor patient. However, if a patient

was having a heart operation and needed VEGF, then it is good for him or her to receive slightly older blood. The release of VEGF causes increased vascularization, meaning that capillary beds will be revascularized (ie, heart transplants will do better). Therefore, for different procedures, one may want to use blood products of different ages to reduce waste.

Unfortunately, no blood product is absolutely pure (Table 4). Most blood products used in relevant studies, even after leukodepletion,<sup>2</sup> contain  $10^6$  to  $10^7$  white blood cells, white cell fragments, and platelets, which are known to carry many growth factors. The presence of leukocytes in blood components reduces glucose availability, and leukocyte lysis leads to the release of cytokines that reduce red blood cell survival.<sup>1</sup> White blood cells are notorious because they have a nucleus, survive longer, and can release their bioactive mediators, such as VEGF. This issue is even worse in platelets, and packed cells are contaminated with platelets as well. We found that 60% or more of this worsened cancer prognosis effect is due to VEGF, and we found that there are even more VEGF in platelets, causing a deranged growth of tumors. For this reason, I would tend to try to avoid platelet transfusions for cancer patients, especially in the peri-operative setting.

### H&O What rationale can be given to explain the conflicting findings of research in this area?

One main reason for the many varying reports is because many studies failed to accurately document the transfusion nature or circumstances. Some did not reveal the timing of the peri-operative transfusion—whether it was

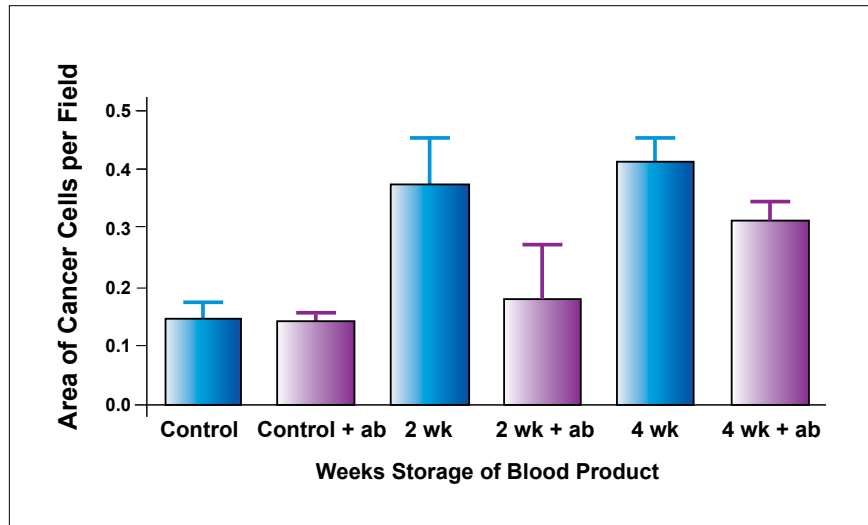


**Figure 2.** The release of VEGF from leukocyte depleted packed cells stored for varying times, showing the accumulation of VEGF in the supernatant of blood products stored from 1–4 weeks (the usual range available from the blood transfusion service).

Note: Leaching is significantly correlated to length of storage (wks) with a correlation coefficient of 0.89,  $P < .001$ . (VEGF detected by ELISA, blood products obtained from the local UK blood transfusion service and stored under standard conditions).

**Figure 3.** The effect on growth in area of a head and neck cancer cell line by supplementation of supernatant from stored bloods of different ages (weeks), and abrogating effect of eg, anti-VEGF antibody is also shown.

Note: The leuko-depleted blood products were obtained from the local UK blood transfusion service and stored under standard conditions. There is a statistically significant difference ( $P < .01$ ) between control, 2 week- and 4 week-old blood products, as well as the effect of the antibody ( $P < .01$ ).



just before the operation, during the operation, a week later, etc.—the definition of peri-operative is very blurred. Some studies did not indicate the nature of the transfusion product—whether it was red cells, whole blood, packed cells, leukocyte-depleted cells, or even platelets. Many studies used different leukocyte-depleting protocols and followed the patients for different amounts of time. Some studies even had poorly matched controls. Most of these studies were retrospective and observational, and the patient numbers were small.

The types of tumors that have been studied and tend to be documented for this sort of risk are: colonic adenocarcinoma, head and neck squamous cell carcinoma, some neurologic tumors, prostate carcinoma, and some breast carcinomas. However, when people conducted general studies, they used heterogeneous tumor groups or tumor groups where this risk or association has not been found, which is another reason why there are many conflicting results. Investigators often erroneously concluded that if something holds true for one tumor group, it applies to all tumor groups.

Our group has found that the association is very tumor-specific because distinct tumors express receptors to the chemicals released by the blood product. Different tumors express VEGF to different extents on different substrates. Some past experiments used tumors that tended not to express VEGF, so it was not affected by the blood transfusions.

In previous in vitro laboratory studies, many used plastic for their cell line substrate, which changes the receptor expression profile (eg, reduces the expression of VEGF) and may give results that vary from clinical findings. Current knowledge suggests that the use of Type 4 collagen as a cell line substrate more accurately reflects

the natural cytoskeletal attachment to the extra-cellular matrix or ‘in-vivo’ state.

Many studies in the past appear to compare what are, in effect, apples to pears. They did not document the type of blood product, the timing of blood transfusion, and many did not appreciate that different tumors have different growth factor receptors on their surface.

### **H&O** In your opinion, when should blood transfusion be considered/not considered?

The easiest way to solve this problem is to avoid using blood transfusion all together. However, blood transfusion remains an important tool in the therapeutic armamentarium. For the anemic patient, it improves the quality of life. Transfusion also improves the efficacy of radiotherapy, as radiotherapy and photodynamic therapy (PDT) work by affecting oxygen free radicals. So if a patient has good oxygenation, radiotherapy and PDT work better.

I think you have to tailor the decision to transfuse to the patient. It is entirely reasonable for a poor prognosis end-stage patient to receive a blood transfusion, just as it is for a patient with life-threatening anemia. This is because if a patient is given a transfusion, the therapeutic options are increased; and as physicians, we want to increase the options a patient has (ie, so that radiotherapy is more effective).

However, treatment in the nonacute setting for anemia can range from dietary change (eg, improving iron, folic acid intake) to the use of recombinant erythropoietin, although people are rightfully cautious in using a blood-stimulating growth factor in a situation where the patient has unregulated growth from their tumor cells. The reason why people examine peri-operative transfusions is because

in this situation, everything comes to a head and the acute blood loss and the associated hypovolemia may affect life. So when physicians deal with transfusion, they must logically look at the indications and make sure they are giving the optimal transfusion product.

In the past, surgeons used the 10–30 rule (developed at the Mayo clinic): if the hemoglobin was less than 10 g/dL, or the hematocrit was less than 30%, they would give a transfusion. Now, based on recent findings, we know that if the decision to give transfusions is based on the patient's physiologic or symptom needs, we can get away by using transfusions at a much lower level. This approach is more patient-centered and cost-effective.

We now use hemodilution or the use of nutritional supplements or recombinant erythropoietin to combat hemovolemic insult. There is also a forward process toward the use of synthetic alternative blood products. I believe that in the United States, the United Kingdom, and mostly all over the world, we have started using leukodepletion to filter white cells from the packed cells, which would lead to less growth factors in the blood that we transfuse.

What we also have to consider is that even in the most matched transfusions, there will be slight sub-group mismatches unless it is an autologous transfusion. A mismatch could cause a cellular autolysis or cellular hemolysis and the release of growth factors. Interestingly, patients who go on heart bypass machines or heart-lung machines also get a physical disruption of the red blood cells and a subsequent release of growth factors. These people, or people who have concurrent tumor operations or vascular operations, have an increased risk of tumor growth. The simple solution for these situations of peri-operative transfusions is to use younger blood, but also to try to avoid transfusion. If transfusion cannot be avoided and younger blood is not available, absorption antibody filters should be used, or the blood could simply be washed.

We have also noticed, after reviewing many studies, that transfusion is least harmful in terms of cancer survival when less than 3 units of blood is given. If a patient is given more than 3 units, there is a significant accumulative effect of the growth factors that can cause deleterious tumor growth.<sup>3</sup>

The data we have are on the effect of peri-operative transfusions for ablative tumor surgery and their effect on prognosis. The rationale and our evidence to support this have been outlined. The logical extrapolation of this to the transfusion in the non-operative setting still requires experimental support to determine its significance.

**Table 5.** Recommendations for Practice

#### Summary

- Use of revised physiologically based transfusion triggers for peri-operative transfusion.
- Use of perfectly matched or autologous blood with minimal mechanical disruption of the blood
- Use of efficient leukodepletion
- Use of antibody filters for white cells, platelets or deleterious growth factors.
- Use of washed cells
- Use of younger blood (<2 weeks) if possible stored for the minimum amount of time
- Use of less units of blood (<3 units)
- Use of synthetic blood products
- Use of marrow stimulators eg, recombinant erythropoietin (caution in giving cell growth stimulators to cancer patients)
- Use of nutritional supplements
- Use of surgical technique with an emphasis on hemostasis and atraumatic tumor removal with negative margins
- Use of hemodilution to combat hemovolemic insult
- Avoid platelet transfusions

Finally, the only mechanism available to develop data that will unequivocally settle this issue is a randomized clinical trial in which some patients receive blood transfusions of different storage durations and some do not. However, current ethical ramifications preclude the execution of such a study.<sup>3-5</sup>

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