

# Hemolysis in Cardiac Surgery Patients Undergoing Cardiopulmonary Bypass: A Review in Search of a Treatment Algorithm

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Presented at the 5th Intercept Meeting, Milan, Italy, April 10–12, 2008.

**Abstract:** Hemolysis is a fact in all extracorporeal circuits, as shown in various studies by the increasing levels of plasma-free hemoglobin (PfHb) and decreasing levels of haptoglobin during and after cardiopulmonary bypass (CPB). Beside complete red blood cell (RBC) destruction or hemolysis, RBCs can also be damaged on a sublethal level, resulting in altered rheological properties. Increased levels of free RBC constituents together with an exhaust of their scavengers result in a variety of serious clinical sequela, such as increased systemic and pulmonary vascular resistance, altered coagulation profile, platelet dysfunction, renal tubular damage, and increased mortality. Sublethal RBC damage is characterized by decreased microperfusion and hypoxic RBCs, leading to end organ dysfunction caused by cellular ischemia. Isolated extracorporeal circuit components can be considered non-hemolytic if used according to recommendations, but extracorporeal circuit composition and management during CPB can still be optimized, avoiding cell damaging mechanical forces. Although most RBC destruction in standard CPB re-

mains within the capacity of the endogenous clearing mechanisms, in some cases, levels of PfHb do substantially rise, and precautionary measures need to be taken. Higher degree of hemolysis can be expected in young children, after extensive surgery, and in prolonged support as in patients supported by ventricular assist devices (VADs) or extracorporeal membrane oxygenation (ECMO). These patients are especially susceptible to the toxic influences of unscavenged RBC constituents and the loss of rheologic properties of the RBCs. Considering the high percentage of neurologic and renal sequela in post-cardiotomy patients, all imbalances possibly contributing to these morbidities should be focused on and prevented, if not treated. Considering the severity of the consequences of RBC damage, the high incidence of this complication, and especially the lack of interventional strategies in cases of suspected or confirmed RBC damage, there may be a need for a treatment algorithm for this phenomenon. **Keywords:** cardiopulmonary bypass, complications, blood conservation. *JECT. 2008;40:257–267*

During cardiopulmonary bypass (CPB), forces on the formed elements of the blood induced through mechanical stress and other environmental factors result in different degrees of damage to these cells (1).

Over the past decade, there has been an increasing concern towards the preservation of platelets and handling of leukocytes during extracorporeal circulation. On the other hand, beside visual observation of reddening of urine or plasma, little attention has been spent toward the integrity of the red blood cells (RBCs). Calculation of the hemolysis index, which refers to hemoglobin being released from ruptured RBCs into the plasma, is mostly used in terms to evaluate the hemolytic characteristics of isolated extracor-

poreal components. Once these components are approved for clinical use, plasma-free hemoglobin (PfHb) is often no longer a concern for clinicians during CPB. Nevertheless, although most isolated extracorporeal circuit components can be considered non-hemolytic if used according to prescription, substantial cellular damage can be inflicted by the way the extracorporeal circuit is composed and managed (2).

Mechanical forces during extracorporeal circulation can cause complete destruction of the RBC, immediate or delayed, but are also known to cause significant changes in the mechanical properties of RBCs: decreasing their deformability and surface charge and increasing their fragility and aggregability (3).

## RED BLOOD CELLS

There are some interesting truths about RBCs that clarify the clinical complications that are the consequence of RBC damage.

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The senior author has stated that authors have reported no material, financial, or other relationship with any healthcare-related business or other entity whose products or services are discussed in this paper.

The first is the fact that the mature RBC does not contain a nucleus, which makes them easier to deform and allows them to enter the smallest capillaries. Being anucleate also allows the RBC more space to carry hemoglobin and makes them lighter, therefore reducing the workload of the heart by 15% (4). Enucleation also gives these RBCs their interesting biconcave shape, which enables them to have maximal contact with the surrounding tissues, facilitating the exchange of O<sub>2</sub> and CO<sub>2</sub> (4).

A second fact is that the two major characteristics of RBCs, deformability and aggregability, greatly contribute to the viscosity of the blood (5). Thus, the visco-elastic profile of blood is not only influenced by plasma viscosity, which is mainly a function of the concentration of macromolecules, temperature, and RBC count, but additionally, a modest decrease in RBC deformability results in a significant increased viscosity. Consequently, there is a significant higher blood pressure required for these rigid RBCs to enter the microcirculation (6).

A third phenomenon is that the major RBC responsibility, which is transportation of O<sub>2</sub> and CO<sub>2</sub> between the lung and tissues, is not a function of the RBC itself but of its main constituent: hemoglobin (Hb).

Approximately 97% of a dry RBC consists of hemoglobin, which is an assembly of four protein subunits, each of them containing one heme group with one iron molecule, and each of the heme groups being capable of binding one molecule of oxygen.

In addition to the isolation of hemoglobin from the plasma by the red blood cell membrane, there is also an efficient mechanism ready to clear the free hemoglobin from the plasma upon hemolysis. This safe encapsulation suggests the toxicity of this hemoglobin molecule once freed in the plasma.

### **LETHALLY DAMAGED RBCs AND THE CLINICAL CONSEQUENCES**

Upon destruction of the RBC membrane, PfHb and heme enter the circulation. PfHb is normally cleared by the Hb scavengers, haptoglobin and CD163. Heme, the pro-oxidant and pro-inflammatory oxygen-binding component of Hb, is transported to the liver by hemopexin and degraded through heme oxygenase into carbon monoxide, biliverdin, and iron. These products, together with the interleukin (IL)-10 release induced by the Hb clearing mechanism, exert an antioxidant, anticoagulant, and vasodilating effect in the circulation, thus compensating for the adverse effects caused by PfHb and heme.

An increasing level of PfHb circulating in the plasma always indicates hemolysis, whereas normal values are not indicative of a healthy RBC.

First, the clearing mechanisms of hemolysis-derived end products only become saturated and exhausted in case of

excessive hemolysis. Second, haptoglobin, essential in scavenging PfHb, exists in three different phenotypes (Hp 1-1, Hp 1-2, and Hp 2-2), all possessing varying antioxidant capacities and varying abilities to link up with Hg, which result in different levels of circulating PfHb (7). The Hp 1-1 allele has a much stronger antioxidant and PfHb scavenging capacity than the Hp 1-2 and Hp 2-2 phenotype. The distribution of these phenotypes show geographical differences, with whites showing the highest prevalence of the Hp 2-1 and Hp 2-2 type (8). The Hp 2-2 phenotype is associated with a significantly higher degree of atherosclerotic disease and higher percentage of diabetes mellitus (9). The prevalence of the Hp 1-1 phenotype is considerably increased in the elderly, indicating a higher life expectancy for the Hp 1-1 allele carrier (10). Consequently, one can assume the highest prevalence of Hp2-2 in our adult cardiac surgery population, resulting in the lowest protection from increasing PfHb levels and the lowest antioxidant capacity.

Because haptoglobin is synthesized in the liver, patients with impaired liver function show a decreased haptoglobin production. However, low levels in these patients should not be indicative of hemolysis but a decrease in the protection from PfHb in case RBCs do lyse (11). On the other hand, haptoglobin acts as an acute-phase protein, with its plasma concentration increasing in response to a variety of stimuli, such as infection, acute myocardial infarction (AMI), and inflammatory reaction. Therefore, monitoring of hemopexin, which is a non-acute phase protein, should be preferred over haptoglobin (10).

When the capacity of the intravascular scavenging mechanisms has been saturated, levels of Hb and heme increase in plasma and urine. These are associated with adverse clinical signs and symptoms, most often not recognized as the underlying phenomenon of hemolysis.

Clinical consequences of excessive PfHb levels include dystonias involving the gastrointestinal, cardiovascular, pulmonary, and urogenital systems, as well as clotting disorders (12). It was reported that PfHb directly impairs left ventricular function and coronary blood flow in neonatal rabbit hearts, especially when ischemia and reperfusion were involved (13).

Many of the clinical sequela of intravascular hemolysis are readily explained by Hb-mediated NO scavenging. On saturation of free Hb scavengers, PfHb binds NO derived from the endothelium in a fast and irreversible way. NO plays a major role in vascular homeostasis and has been shown to be a critical regulator of smooth muscle tone and platelet activation and aggregation. It has been shown that quantities of plasma Hb > 10 mg/dL can potentially inhibit NO vasodilatation *in vivo* (14).

NO depletion results in elevated systemic vascular resistance (SVR); elevated pulmonary vascular resistance (PVR); increased thrombin formation, fibrin deposition,

platelet activation, and aggregation; organ dysfunction; and is accompanied by an elevated mortality rate (12).

Iron, freed on hemolysis, is transported to the body's iron storage proteins (ferritin and hemosiderin) by iron-transporting proteins (transferrin and lactoferrin). The iron-binding protein transferrin is normally ~30% loaded with iron, retaining a considerable iron-binding capacity (10). During CPB, iron release is increased, partly as a result from red cell lysis, resulting in iron overload in ~20% of adult patients (15) and even a higher percentage in neonates. Children >5 years of age rarely show signs of iron overload, probably because of higher plasma transferrin levels (16). Iron is a pro-oxidant, and iron overload is accompanied with acute renal failure and increased lung permeability (17).

Early postoperative hyperbilirubinemia after CPB surgery has been associated with a high mortality rate. This hyperbilirubinemia is a multifactorial process, of which the increased bilirubin production from hemolysis plays a substantial role (18).

Considering all this, one should not be worried about the relative small loss of functional RBC mass, which has no clinically significant impact on the primary function of the RBC, which is gas transport, but should be worried about the damaging effects of the RBC constituents once the body's natural scavenging mechanism is exhausted.

### **SUBLETHALLY DAMAGED RBCs AND THE CLINICAL CONSEQUENCES**

Beside complete cellular destruction, damage can also be induced to the RBC membrane on a sublethal level, resulting in decreased deformability and increased aggregability. This phenomenon is reversible to a certain extent depending on the time and intensity of damage (19).

This sublethal damage of the RBC membrane is hard to measure, but can become clinically very important because these changes can result in the early removal of the RBC from the circulation by the spleen, or more seriously, in altered rheological properties of the blood. The decrease in cell deformability results in a reduced capacity to enter small capillaries and a reduced contact of the cell surface with the surrounding vessel wall, all compromising efficient microcirculation and oxygen delivery to the surrounding tissues (20). Rigidified RBCs have lower oxygen content than their healthy colleagues, as a result of a decreased oxygen uptake in the lungs (6). Thus, the clinical consequences of sublethal RBC damage are a function of the loss of rheologic properties of the RBC and of the potential of the organism to compensate for the increased need and loss of vasodilatation and the need for higher perfusion pressures. Sublethal RBC damage can result in end organ dysfunction caused by cellular ischemia.

### **RBC DAMAGE AND CPB**

Data from different studies consistently show an increase in plasma Hb concentrations and a decrease in haptoglobin levels during CPB, indicating progressive destruction of RBCs (21–24).

Results of in vitro studies regarding the sublethal damage caused by CPB are sometimes inconsistent or even contradictory (25–27). The major problem is the absence of standard methods of measurement of RBC deformability or other parameters characterizing sublethal blood trauma. Nevertheless, a recent study did indicate that RBCs exposed to certain uniform shear stresses for prolonged time (>20 minutes) show statistically significant changes in their deformability without lysis (25).

One study showed delayed hemolysis after CPB: ~15% of the RBCs subjected to the extracorporeal circulation were irreversibly damaged and removed during the 24-hour post-infusion period (28).

Considering the clinical consequences of both RBC destruction and decreased RBC deformability, it is obvious that all efforts should be made to minimize RBC aggression during CPB.

The first step in the reduction of hemolysis during CPB is the identification of all factors responsible for RBC damage. Second, a maximal hemo-compatible system should be composed and customized for each patient, and CPB management must be optimized toward gentle blood handling. Third, high-risk groups, such as small patients and complex surgery cases, need to be identified and paid special attention to minimize hemolysis-related morbidity.

The mechanisms of mechanical cell damage during CPB have been described by different investigators (29,30), identifying positive pressure, wall impact forces, blood non-endothelial surfaces, negative pressure, blood-air interface, and shear stress as responsible forces for blood trauma. It seems that RBCs have a high tolerance to most forces, except for shear stress (30). Physiologic values of shear stress range from 1 to 50 dynes/cm<sup>2</sup>, sublethal damages are reported to appear from 210 to 430 dynes/cm<sup>2</sup> onward, lethal damage to RBCs occur from 1500 dynes/cm<sup>2</sup> onward, and the threshold for platelets and leukocyte activation is, respectively, ~100 and 75 dynes/cm<sup>2</sup> (22,31,32).

Beside shear stress, time of exposure seems to be another impacting factor related to cellular damage (31).

### **RBC DAMAGE AND CPB: ISOLATED CPB COMPONENTS**

The magnitude and duration of shear stress will be different in the various components of the CPB circuit but will always be present to some extent. A high value with short duration will be found in arterial cannula, but lower

magnitudes of shear stress with longer duration can be found in oxygenators and venous reservoirs.

By analyzing the cardiopulmonary system, we can locate different areas where RBC damage can occur.

### **Tubing/Connectors**

To minimize blood trauma in tubing and connectors, one should strive to have smooth, hydrophobic inside walls of non-toxic materials, to avoid velocities >100 cm/s, and to avoid exceeding the critical Reynolds number ( $Re_{crit}$ ). The critical Reynolds number indicates the transition from laminar to turbulent flow.

One also needs to minimize the gradient necessary to propel the blood along the tubing. The selection of wide tubing aids in achieving these objectives, but on the other hand, greatly increases the priming volume. Keeping the tubing as short as possible will reduce prime volume, pressure gradient, and blood trauma (33).

Disposable polycarbonate connectors with smooth, hydrophobic hemo-compatible inner surfaces that make smooth junctions with plastic tubing, to minimize turbulence, are desirable. Smooth curves rather than sharp-angled bends will minimize turbulence.

Silastic rubber, latex rubber, and polyvinyl chloride (PVC; e.g., Tygon) tubing have been used in the raceway. The latter type is an integral part of the circuit, is durable, and is associated with acceptable rates of hemolysis. It is not preferred as raceway tubing material because it gets stiff during hypothermia. All are subject to spallation and tubing wear when used as raceway material (34). Latex rubber is associated with greater hemolysis, whereas Silastic rubber or silicone has less hemolysis when the pump was completely occlusive. In a study comparing different types of tubing, silicone and PVC tubing were both shown to be non-hemolytic; in contrast, heparin-coated PVC tubing showed a significant higher hemolysis level than those of the other tubes. However, the degree of hemolysis remains very low, and the material is therefore not considered hemolytic (35).

### **Arterial Cannula**

Cannula can cause RBC damage if blood flow exceeds its size capacity. However, this damage may not be directly related to the increased pressure inside the cannula, because investigators have shown that static positive pressures up to 600 mmHg did not result in hemolysis (36,37). Some investigators showed static pressures as high as 2300 mmHg as safe in regard to RBC membrane rupture. Increasing static pressure is only related to hemolysis when high shear rates are applied to the erythrocytes (38).

In cannula, shear stresses is in relation to the radius and length of the cannula and pressure drop over the cannula (pressure drop <100 mmHg is considered to be non-hemolytic) (39). Shear stress is also a function of canula

design, and studies have shown that there is less shear stress in uniform sized cannulae and that straight designs are less hemolytic than angled designs (40).

For any cannula, there exists a fluid flow limit beyond which laminar flow cannot be maintained, and the flow becomes at first disturbed and then, at increasing flow rates, it becomes turbulent. This point can be identified by plotting pressure against flow and observing the change of slope at the point of disturbance, which theoretically corresponds to a Reynolds number of 2000. At higher flow rates, local fluid velocities are unpredictable but higher than the mean forward velocity (30).

Although the underlying mechanisms of turbulence-induced trauma are not clear, it has been shown that turbulent stresses contribute strongly to blood trauma, generating exponential increases of hemolysis at higher Reynolds numbers in turbulent flows. At identical shear stress, turbulent flow produces far more blood trauma than laminar flow (41). Therefore, hydrodynamic flowcharts should be based on *in vitro* tests, where blood is used instead of water. Blood is less susceptible to turbulent flow than water, but only in the blood tests is the transition of laminar flow to turbulent flow apparent. This transition is indicated by a break in the curve, thus better guiding the choice of the canula size as a function of laminar flow (42).

The choice of arterial canula is especially important in long-term settings, where the high shear stresses may increase RBC damage to an extent that exhausts scavengers of hemolysis-derived end products.

### **Venous Cannula**

Venous cannulae are most often large enough for the required flow to remain laminar; therefore, turbulence is not a factor influencing hemolysis in this zone.

In contrast, air trapped in the venous blood, either created through cavitation or aspirated through uncovered holes, may result in increased damage to the RBC.

### **Open/Closed Venous Reservoirs**

In case of an open venous reservoir when cavitation occurs in the presence of an air interface, this interface acts as a major source for bubble formation. A larger air interface results in more bubbles and, therefore, more blood trauma when they collapse. However, studies have indicated no difference in hemolysis between open venous reservoirs and closed venous reservoirs in cases of separated suction (43).

On the other hand, investigators showed that pumping high blood flows with low venous reservoirs significantly increase air microbubble passage through the lines, which would theoretically cause some degree of damage to the blood elements (44).

### **Roller/Centrifugal Pumps**

Hemolysis caused by roller pumps is mainly because of the occlusive settings of the rollers; data indicate the dif-

ference between barely non-occlusive and just occlusive to be a major cause of RBC damage (45). Recently, it has been suggested to set the pump as non-occlusive as possible to decrease tubing spallation, increase tubing life and minimizing hemolysis. A suggested method of testing pump occlusively is the dynamic method; here, the pump speed is set at 5 rpm and the pressure is maintained at 150–250 mmHg. This way, the error of different roller positions with varying occlusively is eliminated. The increase in pump speed, to compensate for roller incompetence and decreased pump flow, shows no increase in hemolysis (46).

There are conflicting data concerning the superiority of rollers vs. centrifugal pumps with regard to hemolysis. In summary, it can be said that hemolysis levels in non-occlusive roller pumps are similar or lower than those seen with the centrifugal pumps. The roller pump with the standard occlusion setting (just non-occlusive) caused hemolysis that was the same or higher than that of the centrifugal pump (47).

Blood damage in centrifugal pumps is primarily caused by shear stress, time, negative pressure, and turbulent flow and can differ according to different designs. The Jostra Rotaflow Quadrox (Jostra, Hirrlingen, Germany) and Cobe Revolution pump (Cobe Cardiovascular, Arvada, CO) are found to be less hemolytic than the Medtronic Biomedicus pump (Medtronic, Minneapolis, MN) (48). The latter is thought to be more hemolytic because of local heat generation by mechanical friction between shaft and seal, which causes thrombus formation around the central bearing on which the pump rotates (49).

Heat is generated when there is a lasting disagreement between flow and rotations, preventing efficient cooling of the pump rotor and indicating persistent high negative pressures or high afterload. These situations are often seen in long-term, closed circulation settings as in ECMO or VAD, illustrated by the exponentially increasing hemolysis indicators that disappear on the isolated change-out of the clotted pump (50). In these long-term applications, incorporating a negative pressure feedback system to the pump reduces greatly the risk of pump overheating and clotting, as well as air entrapment in tubing by cavitation.

One study in neonates found data indicating not only higher hemolysis levels in the roller pump vs. centrifugal pump group but also a sustained elevation after CPB in the roller pump group, indicating a higher sublethal damage in this group (51).

### Oxygenators

The hemolytic characteristics associated with oxygenators are not yet well defined. Different types of oxygenators cause different amounts of hemolysis based on shear stress and blood exposure time.

In addition, the relationship between the value of he-

molysis and pressure drop in the oxygenator is not always clear, some studies show a close relationship, whereas others report a weak correlation, and others found no statistical differences (52,53). The reason for this poor correlation is that the dimensions of the oxygenator (radius and length) have to be included in the calculation of shear stresses from pressure drop. An oxygenator with high pressure drop over a long blood path length may have smaller shear stress than an oxygenator with a low pressure drop over a short length. Nevertheless, the overall shear stress levels in most actual oxygenators are far below the threshold for hemolysis and most often below the threshold for sublethal RBC damage (54).

Hemolysis from the oxygenator is also dependent on other physical factors or surface characteristics, such as surface roughness, coating, and membrane material.

Although some studies could not find data to support the advantage of coated systems related to RBC rupture (55), the opposite has been shown in other studies (56). These investigators also showed that, in uncoated systems, albumin prime reduces the degree of RBC damage. The increased hemolysis in uncoated oxygenators might be related to the increased shear stress and transient high pressure drop resulting from the packing of platelets to the uncoated fibers, which can be prevented by albumin coating. Data from other studies indicate that heparin coating also reduces RBC rheologic or sublethal damage significantly in patients undergoing CPB. However, the clinical significance is not yet clear (57).

Considering all of the above, one can conclude that most isolated CPB components, if used according to the manufacturer's instructions, can be considered relatively non-hemolytic.

### RBC DAMAGE AND CPB: CPB COMPOSITION AND MANAGEMENT

More important in respect to RBC integrity is the composition of the CPB system and CPB management.

#### Assisted Venous Drainage

Hemolysis in a gravity-assisted venous drainage system (GAVD) was reported to be approximately three times higher when air was entrained with the blood than when no air was allowed to enter the system (58).

In dynamic conditions, increasing negative pressures led to an increase in the number of microbubbles transmitted, and this air–blood contact, causing high shear stresses, is a major factor in the damage to the RBCs.

Increasing negative pressures also result in increased shear stress by increasing turbulence. Increasing suction beyond a critical point, reported to be  $-70$  mmHg, will cause the vessels to collapse, cavitation to occur, and the flow to stagnate (58).

Suction pressures below  $-40$  mmHg can be considered as safe and do not significantly increase gaseous microemboli activity and can therefore be considered as non-hemolytic (58).

Apart from the level of negative pressure, studies indicate that the way of augmenting venous drainage can have an influence to the degree of damage; *in vitro* and *in vivo* studies showed no significant increase in RBC damage in VAVD compared with the classical GAVD (59–61).

Although venous drainage can improve significantly when assisted with a vacuum, the use of a VAVD increases the risk of air embolism, and negative pressure exceeding  $-50$  mmHg can result in a significant overestimation of the delivered blood flow, probably caused by the collapse of the raceway tubing (62).

Some investigators have found a higher degree of hemolysis in VAVD in comparison to KAVD. They state that the direct application of vacuum to the venous reservoir causes an augmentation of turbulence and shear stress to the RBCs (59).

Cavitation can occur in venous drainage lines when blood is exposed to negative pressure, thus forming bubbles causing shear stress to the RBCs. When these bubbles collapse, the high local pressures impulses and high-velocity microjets that are created damage the surrounding cells. In absence of an air interface, the bubbles formed from the gas dissolve in the blood. The number and the size of the bubbles depend on the amount of gas the blood contains (29).

### Separated Suction/Cardiotomy Suction

The cardiotomy suction reservoir has been found to be a major source of hemolysis, particulate and gaseous microemboli, fat globule formation, cellular aggregation, and platelet injury and loss. A major contributor to these adverse effects is the amount of air that is aspirated along with the blood. The severe trauma caused by air entrapment in cardiotomy suction has been explained by some through the fact that air has a much lower viscosity than blood; therefore, air flows at a much higher velocity than the blood when subjected to the same applied force. This causes turbulence and high shear stresses that injure both RBCs and platelets (63).

The entrapped gaseous microemboli within open cardiac chambers are particularly hard to dissipate because they mainly contain nitrogen, which is quite insoluble. For this reason, the insufflation of  $\text{CO}_2$  flush in the surgical field has been suggested (64).

Some authors have analyzed the ability of various commercial cardiotomy reservoirs to remove bubbles from aspirated blood and have described desirable features: direct injection of blood into the defoamer, avoidance of turbulence at the inlet, ensuring that all blood passes through the defoamer, avoiding free fall of blood into the reservoir, and incorporation of a micropore filter (66). Storing

the blood in the cardiotomy reservoir for as long as possible rather than letting it continuously flow into the main circuit will reduce the number of gaseous microemboli and will reduce the air-to-blood ratio in the transferred blood. Although static negative pressures up to  $-300$  mmHg failed to induce RBC damage, the threshold values for blood damage by negative pressure in dynamic conditions, under which conditions the blood will experience negative pressure during CPB, are possible at around  $-120$  mmHg (66).

The amount of blood damage can be minimized by avoiding or minimizing coaspiration of air (the sucker tip should be kept below the blood level and the field not sucked “dry”), using the slowest flow rates and largest suction tips possible, and avoiding generation of high degrees of negative pressure by not occluding the sucker tip and using a controlled vacuum suction rather than a roller pump. It has been reported that using a vacuum pump device to generate negative pressure, instead of a roller pump, results in significantly less hemolysis, possibly because less foaming occurred with vacuum suction (67). The use of controlled vacuum suction, however, is somewhat more complicated and requires a closed system, increasing the risk of developing positive pressure in the cardiotomy reservoir and thereby risking systemic air embolism.

The avoidance of air entrapment has been exploited in the design of several automatic cardiotomy suction systems that switch the suction pump on and off depending on the presence of blood or air in the suction catheter.

These systems reduce blood trauma, but the beneficial effects do not match up to expectations in clinical practice because of a continuing problem of air aspiration each time the pump switches off with a negative pressure residing within the suction catheter (68–70). This problem may be solved by the control of the pump speed as shown in another suction system (71). In this device, a sensor at the tip of the suction catheter records the depths of the pool of blood and also the rate of blood flow into the pool.

Consequently, the speed of the suction pump is always matched to the volume of the blood that has to be pumped and air intake is close to zero. Here, even at very low suction rates, the difference in blood trauma between manual and automatic suction is clear and applies to platelet trauma and to RBC destruction. Another controlled suction system has a jet-driven aspirator separating and removing air from blood immediately within the suction tip (72). This device suctions blood at rates from 100 to 700 mL/min and separates and removes 80–100% of aspirated air, resulting in significantly less hemolysis.

An alternative avoiding PFHb during CPB is the separation of the cell-damaged suctioned blood in a separate cardiotomy reservoir and to discard this blood. Nevertheless, if large amounts of blood are collected, it may be

necessary to subsequently process the blood in a red cell washer and return it to the patient. In this case, the way the suctioned blood has been handled is of major importance for the quality of the recuperated RBCs (21).

### Left Ventricular Venting

The left ventricular (LV) vent is classically attached to a suction device, either a roller pump or regulated wall suction, and the blood is returned to the cardiomy reservoir. When the tip of the vent is in the ventricle, the degree of suction must be constantly adjusted to avoid excess suction (with collapse and trauma of the ventricle and the risk of aspirating air) or inadequate suction (with overdistension). Minimizing RBC trauma during venting implements avoiding air and excessive negative pressures. Incorporation of suction relief or one-way vent valves in the vent line prevents excessive negative pressures but allows air.

The "Gentle Vent" pump header (Medtronic) allows the pump header tube to collapse, preventing build-up of negative pressure and does not allow air into the circuit. This header controls negative pressures from  $-150$  to  $-250$  mmHg and has a valve incorporated to prevent backflow (73).

To avoid excessive air, some surgeons use gravity drainage instead of suction. This requires that the drainage reservoir be placed well below the patient, and it may not provide adequate drainage if large volumes of blood are returning to the left heart. To avoid this problem, they Y the line coming from the ventricular vent so that either gravity or suction drainage can be used.

Venting the left ventricle could also be performed with a pressure-controlled suction system with a feedback mechanism to the roller pump. This way the roller pump stops in case of increasing negative pressure, avoiding aspiration of air by cavitation or through pressure relieve valves present in the venting lines.

### Autotransfusion

The harmful effects of autotransfusion that contribute to RBC damage are analogous to these of the separated suction, yet the identification of the damaging forces somehow differs among different investigators. Most agree that the combination of negative pressure with air interface causes the greatest range in change in PfHb followed by negative pressure alone and then air interface alone (29,74–76).

Data from several studies support the idea that the lowest vacuum pressure compatible with a clear surgical field should be used during intraoperative blood salvage and that the suctioning of air should be avoided as much as possible (77). RBC lysis is shown to occur from negative pressures of  $-120$  mmHg onward, but suction can be increased up to  $-300$  mmHg if required by the rate of bleed-

ing without causing excessive hemolysis, in case there is no air aspiration (29).

Significant reduction of blood damage can also be obtained by diluting blood with normal saline while suctioning it from the surgical field (78).

Being a cause of hemolysis itself, autotransfusion devices have the capacity of removing up to 90% of the free hemoglobin. Processed blood should preferably be kept aside until the bypass is terminated, because a lot of these cells are sublethally damaged and have therefore a much lower threshold for lysis than normal RBCs.

### RBC DAMAGE AND CPB: ENVIRONMENTAL INFLUENCES

The primary cause for RBC damage during CPB is indeed mechanical aggression, but the susceptibility of RBCs to this stress can be influenced by other environmental factors during CPB.

First, and important in interpreting hemolysis-related studies, there is a difference in individual RBC fragility toward mechanical stress. Fetal RBCs show a significantly higher susceptibility toward mechanical stress than adult RBCs. Male RBCs are significantly more fragile than female RBCs, and human RBCs are significantly more fragile than RBCs from animals. RBCs show a shear stress-induced pre-conditioning; sublethally damaged RBCs lyse at a much lower shear stress threshold than healthy RBCs (79).

In vitro data showed a significant decrease in RBC deformability and increased fragility caused by either hypothermia or plasma dilution (80).

The effect of hypothermia is more pronounced in deep than in moderate hypothermia and was found to be transient. The reasons for the effect of hypothermia on the mechanical properties of RBCs remain to be identified.

It was shown by some investigators that the dilution of plasma with phosphate-buffered saline (PBS) or dextran solutions caused a significant increase in the susceptibility of human RBCs to mechanical stress. Their results indicate that hemodilution without plasma protein replacement may therefore elevate hemolysis during extracorporeal circulation. The mechanisms of a plasma protective effect on RBC fragility is not fully understood, but it may be assumed that the components of plasma that provide protection against hemolysis give a surface coating to RBCs (81).

Recent studies showed a protective effect from mechanical damage to the RBCs through innovative pharmacologic approaches.

### Pentoxifyline

Pentoxifyline (PTX), a methylxanthine derivative, has been known for its hemorheologic properties. The pri-

many pharmacodynamic effects of PTX are caused by increased RBC deformability and decreased blood viscosity. PTX treatment caused statistically significant decrements in PfHb levels during CPB. In contrast, plasma haptoglobin levels stayed higher in PTX-medicated patients during CPB compared with control subjects. PTX was shown to be an effective agent in reducing hemolysis during CPB (82).

### **Polyethylene Glycol**

Polyethylene glycol (PEG) molecules (20,000 molecular weight) have been shown to be efficient in reducing mechanical trauma to RBCs (83).

Melatonin has been shown to protect against lipid peroxidation and membrane rigidity in erythrocytes from patients undergoing CPB surgery (84).

### **Simvastatin**

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are widely used for the control of hypercholesterolemia. Among patients with hypercholesterolemia undergoing CPB surgery, use of simvastatin for 3 weeks before surgery has had significant beneficial effects on erythrocyte membrane fluidity (85).

### **Inhaled NO**

Inhaled NO (iNO) may prevent mechanical deterioration of RBCs exposed to high shear stresses by NO-mediated inhibition of potassium leakage (86).

## **DEALING WITH CPB-INDUCED RBC DAMAGE**

Separated suction is a major contribution toward minimizing RBC damage during CPB (65).

Autotransfusion devices remove up to 90% of plasma Hg and therefore could be used in processing damaged blood. If possible, a separate cardiomy reservoir should be installed in all CPB cases, and damaged blood should be processed in an autotransfusion device to eliminate lysed RBCs and PfHb (87). If the blood in the separated cardiomy reservoir is too damaged, the blood should be discarded, because it will contain sublethal damaged RBCs that will lyse later.

In case there is no separated suction and the blood is potentially too damaged because of a long and complicated run, the pump blood should preferably not be returned to the patient unless processed through an autotransfusion device (88).

Although autotransfusion devices are capable of removing most of the PfHb, this is not the case for hemofilters used perioperatively.

Various types of membranes are traditionally used for perioperative hemofiltration, all capable of removing molecules up to a molecular weight of 20,000–50,000 da. Free hemoglobin molecules have a molecular weight of ~68,000

da, and free hemoglobin dimers are ~32,000 da (89). This may lead to retention of these toxic molecules during classical hemofiltration. Studies have shown a contribution of hemofilters to additional hemolysis, especially in “rinse-free” hemofilters with no crystalloid prime before use (90).

To remove PfHb as efficiently as washing and centrifugation, a super high-flux 100,000-da hemofilter must be used (89).

There are some options for a pharmacologic approach in patients with hemolysis.

In case of exhausted endogenous clearing mechanisms for PfHb, additional haptoglobin could be administered. Some data have shown that haptoglobin treatment significantly decreases the levels of renal tubular enzymes, suggesting a protective effect on renal tubular function (23).

Comparing the prophylactic administration with the therapeutic administration of haptoglobin, both methods effectively prevented an increase of PfHb level, but prophylactic administration (priming administration) was safer and more useful considering PfHb level in filtered blood and changes of serum-free haptoglobin, free hemoglobin, and creatinine clearance during and after the operation (23,24,90).

A second pharmacologic approach is the administration of indirect or direct NO donors, compensating for the decrease in NO availability caused by the binding of endogenous NO with PfHb.

A study investigating sodium nitroprusside administration during the rewarming or reperfusion period showed a decreased PfHb-induced increased pulmonary (PVR) and systemic vascular resistance (SVR). Their data also showed a protective effect on renal function (91). Another study warned of the adverse effect of sodium nitroprusside administration; it may induce accelerated free cyanide release, resulting in increased risk of cyanide toxicity.

This cyanide toxicity is hard to recognize and is accompanied by hypotension, encephalopathy, and metabolic acidosis (92).

Restoration of endogenous NO depletion could also be provided through iNO administration.

iNO converts plasma-free oxyHb to plasma-free methemoglobin, which is not an effective NO scavenger, and one study showed a 100% reversal of PfHb-induced PVR and SVR (84). In contrast, the formation of methemoglobin can induce oxidative damage because of formation of other radicals (93).

Methemoglobin has a very high affinity for oxygen and can clinically be detected during extracorporeal circulation by the appearance of chocolate-colored blood.

Data from a recent NIH-supported study suggested that nitroxyl generated by Angeli salt (sodium  $\alpha$ -oxyhypocyanite,  $\text{Na}_2\text{N}_2\text{O}_3$ ) preferentially reacts with cell-free hemoglobin compared with that encapsulated in the RBCs

under physiologically relevant conditions. Nitroxyl oxidizes oxygenated ferrous hemoglobin to methemoglobin and can convert the methemoglobin to a more stable, less toxic species: iron-nitrosyl hemoglobin. These results support the notion that Angeli salt or a similar compound could be used to effectively treat conditions associated with intravascular hemolysis (94).

Iron chelation therapy such as recombinant human transferrin or lactoferrin for neonates or desferrioxamine for adults has proven to be beneficial to avoid iron overload (17).

A recent study suggested the prophylactic alkalinization of urine with sodium bicarbonate during CPB to protect the kidney from acute kidney injury by PfHb (95).

Sublethal blood damage resulting in decreased microcirculation is harder to detect, and thus, to treat. Innovative ways of monitoring oxygen consumption in vital organs, such as non-invasive venous oxygen saturation monitoring, might help to detect hypoperfusion of the brain and abdominal organs. Once detected, efforts could be made to restore systemic vasodilation, and blood pressure could be increased to ensure microperfusion.

## DISCUSSION

In summary, it can be said that the mechanical forces and environmental damage during standard CPB are reasonably well tolerated by RBCs.

If proper component selection is made, the little RBC damage that does occur is mostly within the clearance capacity of the reticulo-endothelial system, so that the PfHb should not increase significantly during standard CPB. On the other hand, even with state of the art equipment and proper size selection, much damage can be incurred from the way a perfusionist is managing the heart lung machine.

Additionally, there are inevitable situations of more pronounced hemolysis, such as in pediatric cases and during more extensive or prolonged surgery.

This is the case for long-term support, as in VAD or ECMO support, where isolated component selection is much more important than in regular CPB. Here, mechanical RBC damage is often encouraged by the blood pump in combination with inappropriate cannula size or design. In VAD- and ECMO-supported patients, control of bleeding and coagulation is detrimental for improved outcome, low pulmonary and systemic resistance is essential for the suffering ventricle and/or lungs to be weaned off mechanical support, and compensatory mechanisms fail to compensate for the increased viscosity because of rigidified RBCs. Thus, outcome in these patients could theoretically be substantially improved by eliminating toxic hemolysis-derived end products and rheologic-altered blood cell characteristics.

In all patients with poor outcome results, potential clinical complications of sublethal and lethal RBC damage might add to increased morbidity and mortality rate, and, even more importantly, to decreased quality of life.

In patients undergoing CPB, efforts are routinely done to minimize damaging forces, although this is mainly done to protect platelets and leukocytes from activation. Perfusionists and physicians consider the detection of free hemoglobin only as a window to a greater view; because RBCs are much more resistant to mechanical stress than platelets and white blood cells, it is assumed correctly that once RBCs are damaged, other blood elements have already been affected more severely.

To summarize, besides clinical monitoring through reddening of urine or plasma in standstill blood, no further action is undertaken during or after CPB, and nowhere in the literature is there any mention of existing written protocols or algorithms toward protecting patients from this underestimated complication.

## CONCLUSION

From extensive literature, it seems that hemolysis during CPB is a well-recognized, but somewhat underestimated complication. Although efforts are made to minimize RBC damage during CPB, the potential consequences of hemolysis seem to be neglected in the perioperative management of patients undergoing CPB. Nevertheless, a growing list of clinical manifestations related to hemolysis suggests the toxicity of the pro-inflammatory and pro-oxidant RBC-derived components.

Therefore, and especially in those patient groups where there is still room to improve outcome, there may be a need for an algorithm in the detection, prevention, and treatment of RBC damage and related complications.

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