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Alternatives to allogeneic blood transfusions

Andreas Pape*

Dr. med.

Clinic of Anaesthesiology, Intensive Care Medicine and Pain Management, J. W. Goethe University Hospital Frankfurt am Main, Theodor Stern Kai 7, 60590 Frankfurt am Main, Germany

Oliver Habler

Professor Dr. med. Department Head

Clinic of Anaesthesiology, Surgical Intensive Care Medicine and Pain Management, Nordwest-Krankenhaus, Steinbacher Hohl 2-26, 60488 Frankfurt am Main Germany

Inherent risks and increasing costs of allogeneic transfusions underline the socioeconomic relevance of safe and effective alternatives to banked blood. The safety limits of a restrictive transfusion policy are given by a patient's individual tolerance of acute normovolaemic anaemia. Iatrogenic attempts to increase tolerance of anaemia are helpful in avoiding premature blood transfusions while at the same time maintaining adequate tissue oxygenation. Autologous transfusion techniques include preoperative autologous blood donation (PAD), acute normovolaemic haemodilution (ANH), and intraoperative cell salvage (ICS). The efficacy of PAD and ANH can be augmented by supplemental iron and/or erythropoietin. PAD is only cost-effective when based on a meticulous donation/transfusion plan calculated for the individual patient, and still carries the risk of mistransfusion (clerical error). In contrast, ANH has almost no risks and is more cost-effective. A significant reduction in allogeneic blood transfusions can also be achieved by ICS. Currently, some controversy regarding contraindications of ICS needs to be resolved. Artificial oxygen carriers based on perfluorocarbon (PFC) or haemoglobin (haemoglobin-based oxygen carriers, HBOCs) are attractive alternatives to allogeneic red blood cells. Nevertheless, to date no artificial oxygen carrier is available for routine clinical use, and further studies are needed to show the safety and efficacy of these substances.

Key words: blood; transfusion; alternatives; anaemia tolerance; donation; haemodilution; cell salvage; artificial O_2 carriers; blood substitutes.

* Corresponding author. Tel.: +49 69 6301 83627; Fax: +49 69 6301 83768.
E-mail address: a.pape@em.uni-frankfurt.de (A. Pape).

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Table 1. Incidences of potential risks associated with allogeneic blood transfusions.

Risk factor		Incidence
Mistransfusion	Acute haemolytic reaction	1:6000–1:33,000
	Delayed haemolytic reaction	1:2000–1:11,000
Infections (viral)	HIV	1:20 million
	Hepatitis A	1:1 million
	Hepatitis B	1:63,000–1:320,000
	Hepatitis C	1:1.2–1:11 million
	Cytomegalovirus (CMV)	1:10–1:30
	Epstein–Barr virus (EBV)	1:200
Infections (Bacterial)	*Yersinia enterocolica*, *Serratia marcescens*, *Pseudomonas*, enterobacteria	1:200,000–1:4.8 million
Immunological	Transfusion-related lung injury (TRALI)	1:4000
	Alloimmunization	1:16,000
	Immunosuppression	1:1
	Allergic transfusion reaction	1:2000

Although safer than ever before, the transfusion of allogeneic blood is still associated with risks for the recipient (cf. Table 1), the most serious of which are allergic reactions, transfusion-related lung-injury (TRALI), accidental mistransfusions ('clerical error'), and the transmission of viral and bacterial infections (hepatitis, HIV, cytomegalovirus, Epstein–Barr virus).[1,2] Indeed, the results of several prospective clinical studies indicate that a restrictive transfusion regimen is associated with lower morbidity and mortality than a liberal transfusion policy.[3–7]

Moreover, public health systems are facing a cost explosion resulting from transfusion-related morbidity as well as from continuously rising costs of the blood products themselves; because of the growing imbalance between the decreasing rate of blood donation and the continuously increasing demand, the costs of blood products are expected to double until 2030.[8,9]

To control both the inherent risks as well as the increasing costs, allogeneic blood transfusions should be either completely avoided or at least reduced to an absolute minimum during surgical procedures.

This chapter reviews the following topics in connection with alternatives to allogeneic blood transfusions: (1) the tolerance of acute normovolaemic anaemia, including the acceptance of low intraoperative haemoglobin (Hb) concentrations; (2) the employment of autologous transfusion techniques, including supportive administration of erythropoietin; and (3) the potential of artificial oxygen carriers as substitutes for allogeneic red blood cells (RBCs).

TOLERANCE OF ACUTE NORMOVOLAEMIC ANAEMIA

The initial treatment of intraoperative blood loss always consists in the maintenance of normovolaemia by the infusion of crystalloid (3:1) and colloidal solutions (1:1). This acellular fluid replacement implies the dilution of the cell mass remaining in the vasculature (haemodilution), resulting in a dilutional anaemia (acute normovolaemic anaemia).

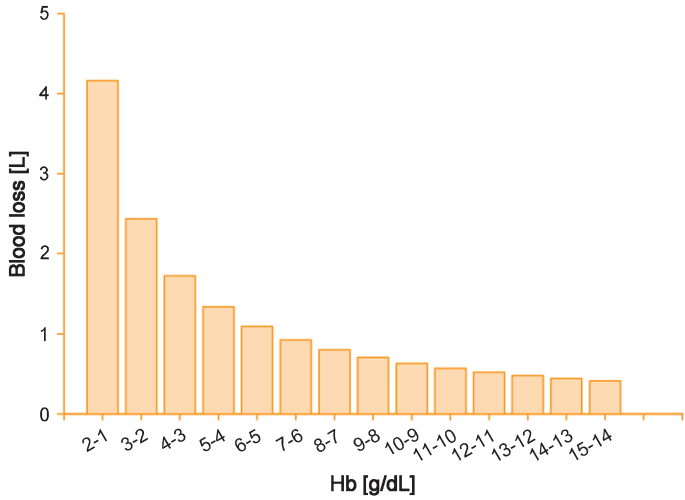

Figure 1. Extent of normovolaemic exchange of blood for acellular fluids necessary to decrease haemoglobin by 1 g/dL, exemplarily calculated for a man (body weight 80 kg, height 1.8 m, blood volume 6000 mL). X-axis: stepwise decrease in haemoglobin concentration by 1 g/dL. Y-axis: blood loss necessary to realize the respective drop in haemoglobin by maintenance of normovolaemia with cell-free solutions during acute blood loss. The lower the starting haemoglobin, the greater the blood loss necessary to decrease the haemoglobin concentration by 1 g/dL.

In the context of alternatives to allogeneic blood transfusion, the term 'anaemia tolerance' is used to refer to the patient's physiological ability to tolerate acute normovolaemic anaemia as well as the anaesthesiologist's intention to accept low haemoglobin concentrations. Hence, the omission of any avoidable transfusion represents the simplest but also the most important alternative to allogeneic blood transfusion. Indeed, the acceptance of low haemoglobin values offers two incentives: (1) the more diluted the patient – i.e. the lower the intravascular haemoglobin concentration – the less the red cell mass lost/mL blood loss (Figure 1); and (2) postponing the transfusion until after surgical haemostasis has been achieved increases the percentage of transfused red blood cells which remain within the vasculature rather than being spilled out with uncontrolled blood loss.

Compensatory mechanisms of dilutional anaemia

Regardless of the fact that arterial oxygen content (CaO_2) decreases proportionally with haematocrit (Hct), it has been known for a long time that normal oxygen supply and tissue oxygenation do not depend on a normal haemoglobin concentration, always presuming that normovolaemia is maintained.[10,11]

Initially, dilutional anaemia is essentially compensated by an increase in cardiac output (CO), which at first is caused exclusively by an increase in left ventricular stroke volume. In more profound stages of normovolaemic anaemia, this is accompanied by an increase in heart rate (HR). Oxygen delivery to the tissues (DO_2) begins to decrease beyond baseline level at Hct values lower than ~25%, so that haemodilution

to Hct ~25% (corresponding to a haemoglobin concentration of ~8 g/dL) occurs without a net decrease in DO_2.

At Hct values below ~25%, the compensation for dilutional anaemia via CO increase becomes exhausted, and DO_2 starts to fall below the baseline level. To main tain tissue oxygen demand – as reflected by total body oxygen consumption (VO_2) – the decreasing DO_2 is further compensated by: (1) utilization of 'luxury DO_2' (under normal conditions, DO_2 exceeds VO_2 by a factor of 3–4); (2) a haemodilution-related increase in nutritive organ blood flow; (3) homogenization of local DO_2; and (4) an increase in tissue oxygen extraction.[12] Therefore, VO_2 initially remains unchanged despite falling DO_2 (oxygen-supply-independency of VO_2, see Figure 2a).

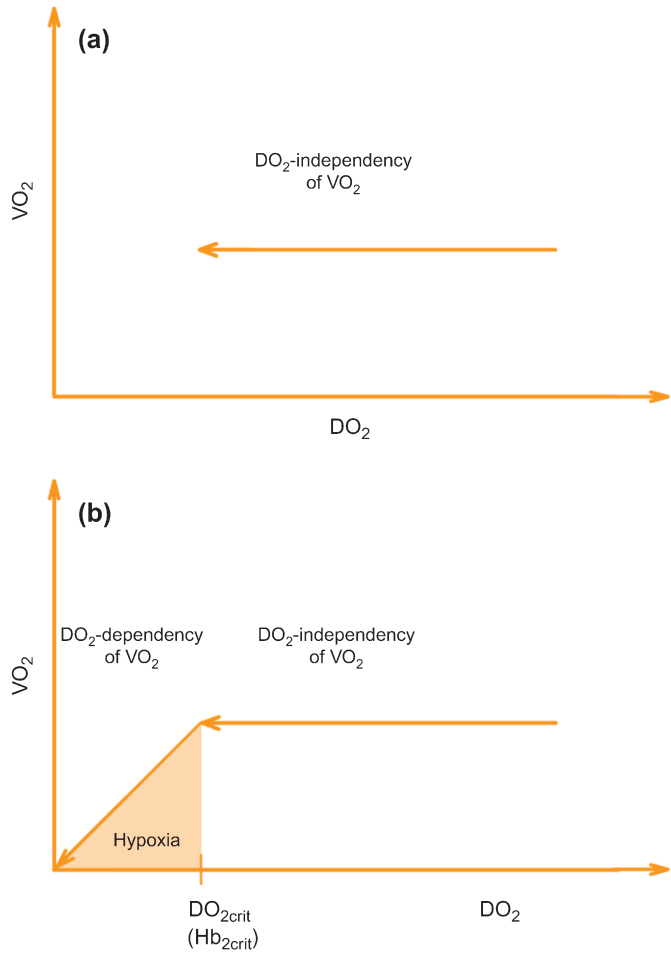

Figure 2. The relationship between oxygen consumption (VO_2) and oxygen delivery (DO_2). Physiologically, DO_2 is three or four times higher than VO_2. (a) Over a long period, VO_2 remains independent of DO_2 despite the anaemia-related decrease of DO_2 (oxygen-supply-independency of DO_2). (b) When a critical haemoglobin concentration (Hb_{crit}) is reached, DO_2 falls short of the actual oxygen demand and VO_2 begins to decrease (onset of oxygen-supply-dependency of VO_2).

Limits of dilutional anaemia – the concept of critical Hct

At extreme degrees of dilutional anaemia, DO_2 falls below a critical value (DO_{2crit}). The amount of oxygen delivered to the tissues becomes insufficient to meet their oxygen demand, and VO_2 starts to decline (oxygen-supply-dependency of VO_2, cf. Figure 2b).[13] This indirectly indicates the onset of tissue hypoxia. The haemoglobin value that corresponds to the inflection of VO_2 is called 'critical haemoglobin' (Hb_{crit}) and reflects the physiological limit of dilutional anaemia. In a standardized experimental protocol, it could be demonstrated that the persistence of DO_{2crit} without any treatment finally leads to death in less than 3 hours.[14]

Both DO_{2crit} and Hb_{crit} vary within and between individuals and are influenced by different physiological circumstances (see below). In previous experimental studies, Hb_{crit} values between 2 and 3 g/dL were found. In clinical observations in anaesthetized patients, extremely low haemoglobin concentrations (3.0 ± 0.8 g/dL in children undergoing major spine surgery[15] and 1.1 g/dL in an unexpected massive blood loss[16]) have been tolerated without meeting the DO_{2crit} (Table 2).

Table 2. Physiological limits of acute normovolaemic anaemia in different species.

Author	Species	Anaesthesia	FiO_2	Plasma substitute	Identification of Hb_{crit}	Hb_{crit} (g/dL)
Fontana et al[15]	Man (child)	Isoflurane Sufentanil Vecuronium	1.0	Albumin	Decay of VO_2	2.1
Van Woerkens et al[99]	Man (84 years)	Enflurane Fentanyl Pancuronium	0.4	Gelatin	Decay of VO_2	4
Zollinger et al[16]	Man (58 years)	Propofol Fentanyl Pancuronium	1.0	Gelatin	ST-segment depression	~1.1
Cain et al[13]	Dog	Pentobarbital	0.21	Dextran	Decay of VO_2	3.3
Meier et al[14]	Pig	Propofol Fentanyl	0.21	HES	Decay of VO_2	3.1 ± 0.4
Pape et al[24]	Pig	Propofol Fentanyl Midazolam Pancuronium	0.6	HES	Decay of VO_2	1.5 ± 0.4
Kemming et al[100]	Pig	Midazolam Morphine Pancuronium	0.21	HES	ST-segment depression	2.6 ± 0.3
Meisner et al[101]	Pig	Diazepam Morphine Pancuronium	0.21	Albumin	ST-segment depression	2.0 ± 0.8
Meier et al (unpublished data)	Pig	Propofol Fentanyl Pancuronium	0.21	HES	Decay of VO_2	2.6 ± 0.4

Hb_{crit}, critical haemoglobin level; HES, hydroxyethyl starch.

These data demonstrate that the tolerance of acute normovolaemic anaemia is high in anaesthetized subjects. However, the presented concept of DO_{2crit} refers to a critical limitation of total body oxygen supply. The limiting factor of anaemia tolerance is the oxygenation of the myocardium as the motor of haemodynamic compensation: when DO_{2crit} is reached, a deterioration in myocardial performance represents imminent breakdown of total body oxygenation.

Since myocardial oxygen extraction is already maximal under rest conditions, increased myocardial oxygen demands can only be met by utilization of the coronary flow reserve.[17] In contrast, other vitally important organs (i.e. brain, intestine, kidneys) can increase oxygen extraction to compensate for acute anaemia.[18] However, what degree of dilutional anaemia may result in a critical limitation of oxygen delivery to these organs has not yet been completely elucidated. Further research is necessary to identify organ-specific limits of anaemia tolerance.

Factors influencing anaemia tolerance

DO_{2crit} and Hct_{crit} are influenced by a couple of physiological variables. The basic requirement for the efficacious compensation of dilutional anaemia is normovolaemia. During hypovolaemic haemodilution the total body oxygen demand increases due to the release of catecholamines and other stress hormones, and the 'critical' oxygen delivery ($DO_{2\ crit}$) is met at higher values than under normovolaemia. Myocardial performance is another variable that determines anaemia tolerance. During haemodynamic compensation of dilutional anaemia, increased myocardial oxygen demand is met by a coronary vasodilation and an increase in coronary blood flow (coronary flow reserve, see above). In patients with restricted coronary reserve (e.g. coronary artery disease), limited ventricular performance (e.g. congestive heart failure) and cardiodepressive medication, anaemia tolerance is reduced.[19]

Anaemia tolerance is also influenced by the depth of anaesthesia and muscular relaxation. In high doses most of the anaesthetics attenuate the cardiac output response during haemodilution and thus reduce anaemia tolerance.[20] In contrast, neuromuscular blockade increases anaemia tolerance, since skeletal muscle mass represents about 30% of total body mass, so that reduction in muscular oxygen demand significantly decreases total body oxygen consumption.[21] In an experimental study in anaesthetized pigs, deep neuromuscular block using rocuronium significantly increased anaemia tolerance (Hb_{crit} 2.4 ± 0.5 g/dL versus 3.2 ± 0.7 g/dL in animals without relaxation; personal unpublished data).

Moreover, body temperature modulates anaemia tolerance. In experimental models mild hypothermia has been shown to increase anaemia tolerance due to a reduction in total body oxygen demand.[22] The opposite should be postulated for hyperthermia.

Finally, anaemia tolerance can also be increased by ventilation with high inspiratory oxygen fraction (FiO_2, hyperoxic ventilation). The amount of oxygen physically dissolved in the plasma increases proportionally with arterial partial pressure of oxygen (paO_2). In profound anaemia, the plasma compartment is significantly increased and becomes an important source of oxygen.[23] In experimental studies, the positive effect of hyperoxic ventilation on anaemia tolerance has been demonstrated repeatedly (Table 3).[14,24–26]

The omission of any avoidable transfusion is the most important alternative to the application of allogeneic blood. In the best case, permissive anaemia can be sustained

Table 3. Factors influencing anaemia tolerance.

Factor	Effect on anaemia tolerance
Hypovolaemia	↓
Coronary arterial stenosis	↓
Hyperoxaemia	↑
Muscular relaxation	↑
Hypothermia	↑
Depth of anaesthesia	↓
Choice of infusion fluid	↔
Hypoxaemia	↔
Sepsis	↓
Polytrauma	↓
Pregnancy	↔
Chronic anaemia	↔

until surgical bleeding is under control[27], which may allow saving of blood products which would get lost immediately after transfusion via the ongoing bleeding. The patient's individual tolerance towards acute normovolaemic anaemia reflects the mar gin of safety, in between which any restrictive transfusion policy will not be associated with an increased the risk of tissue hypoxia. Both the optimization of anaemia tolerance and the choice of an adequate transfusion trigger (cf. Chapter 2 by B. Vallet) enable the implementation of a safe and effective blood-sparing strategy.

AUTOLOGOUS TRANSFUSION TECHNIQUES

Autologous transfusion techniques are generally intended to replace as many allogeneic RBC transfusions as possible by (re)transfusion of autologous blood. Autologous blood is either harvested a couple of weeks before (preoperative blood donation, PAD) or immediately before surgery (acute normovolaemic haemodilution, ANH). The concept of intraoperative cell salvage (ICS) implies the collection and reprocessing of shed blood for autologous retransfusion.

Preoperative autologous blood donation (PAD)

In the course of PAD, autologous whole blood is collected weekly within 4–6 weeks prior to surgery. The final donation must not be performed later than 72 hours before surgery.[28] Whole blood units are separated into red blood cells and plasma, and subsequently classified according to the ABO and rhesus systems and clearly allocated to the donor.

Usually, PAD is suitable when a blood loss of 500–1000 mL is anticipated in at least 5–10% of the cases, or when the estimated transfusion probability exceeds 50%, respectively. The minimum acceptable haemoglobin concentration for PAD is 11 g/dL.[28] In the presence of lower preoperative haemoglobin levels the supportive administration of iron and/or recombinant erythropoietin (rhEPO) may encourage PAD anyway (see below).

PAD is contraindicated in patients with elevated cardiac risk, i.e., patients with unstable angina, myocardial infarction within the previous 3 months, coronary artery

main stem stenosis, congestive heart failure, and significant aortic valve stenosis (gradient >70 mmHg).[29]

The adequate number of PAD units has to be calculated prospectively for each individual case, the type of surgery, the probability of a transfusion requirement, and the time left until the date of surgery being taken into consideration. A helpful tool may be the maximum surgical blood ordering schedule (MSBOS), which is based on a specific institutional analysis of the mean number of blood units transfused per type of surgical intervention and individual surgeon.[30,31]

The cost-efficacy of PAD decreases with the number of blood units discarded[11], which underlines the necessity to exactly calculate the individual number of blood donations.[32] Indeed, PAD is not cost-effective if only one unit of allogeneic blood must be transfused despite previous PAD, or if more than 15% of donated blood is discarded.[33]

Major risks associated with PAD consist in contamination during storage and – as in the transfusion of allogeneic blood – in the potential clerical error with consecutive mistransfusion.[34]

Whereas the blood-sparing potential of PAD had been documented in some previous studies[29], a recent meta-analysis indicates that PAD is actually associated with a higher overall transfusion rate.[35] Overall it can be assumed that other blood-conservation techniques will increasingly replace PAD in elective surgical procedures.

Acute normovolaemic haemodilution (ANH)

ANH entails the isovolumic exchange of whole blood for acellular fluids (colloids and/or crystalloids) directly prior to surgery.[36] Usually, 3–4 units of blood are withdrawn and are stored at the bedside in the operating room. In terms of the safe application of ANH, it is essential to know the physiological changes that occur during dilutional anaemia (see above), and to evaluate the patient's individual anaemia tolerance (i.e. the lowest, safely tolerable haemoglobin level).[37] The benefit of ANH consists in a - reduction of net RBC loss related to dilutional anaemia (see above) and the availability of fresh whole blood, including coagulatory factors and platelets, for autologous retransfusion.

The blood-sparing efficacy of ANH depends on the baseline haemoglobin level, the target haemoglobin after ANH, and the dimension of blood loss measured as a fraction of circulating blood volume.[38] ANH should therefore target as low a haemoglobin concentration as possible while still leaving an adequate margin of safety for tissue oxygenation (i.e. haemoglobin 6–7 g/dL in otherwise healthy patients and 9–10 6–7 g/dL in patients with cardiovascular comorbidity).

While the efficacy of ANH in reducing perioperative allogeneic transfusion could be demonstrated in several clinical trials (abdominal, vascular, orthopaedic, urological and maxillofacial surgery)[39–43], the same effect could not be confirmed in some meta-analyses.[44,45] However, the heterogeneity of transfusion managements between different institutions (e.g. choice of transfusion triggers) complicates the comparability of the different patient populations.

ANH is contraindicated with unstable angina, coronary artery disease with significant main-stem stenosis or myocardial infarction within the past 6 months, high-grade aortic valve and carotid artery stenosis, renal insufficiency, and manifest bacteraemia, but not with malignant disease.

All in all, ANH should be preferred to PAD, since: (1) ANH is less expensive than PAD ($28 versus $226 per unit)[46] because travel expenses, costs for staff, material,

and processing and testing devices can be omitted; and (2) the performance of ANH allows for a more flexible scheduling of the date of surgery since the complex logistics necessary for PAD can be omitted. As a blood conservation method, ANH has been readopted in the practice guideline for perioperative blood transfusion of the American Society of Anesthesiologists (ASA).[47]

Supportive administration of iron and/or recombinant human erythropoietin (rhEPO)

During the perioperative phase, iron and/or rhEPO are administered either alone[48] or in combination with PAD and/or ANH[11], both allowing for the implementation of a restrictive transfusion protocol. In particular, anaemic patients seem to benefit from preoperative substitution of iron[48], whereas in non-anaemic patients undergoing orthopaedic surgery the isolated administration of iron did not decrease the perioperative transfusion rate.[49] In combination with PAD, the substitution of iron (e.g. 100–200 mg/day orally) is recommended anyway for treatment of PAD-related anaemia.[29]

The administration of rhEPO (e.g. 100–150 U/kg subcutaneously twice a week) should always be accompanied by iron substitution in order to achieve an effective stimulation of erythropoesis.[50] In these low dosages, the costs of rhEPO are comparable with those of allogeneic blood.[51] The augmentation of haematopoiesis alone has already been proven to reduce allogeneic blood transfusions, since a low preoperative haemoglobin level is a relevant predictor of allogeneic RBC transfusion.[11] Moreover, an increase in preoperative haemoglobin levels using rhEPO also increases the efficacy of ANH by allowing for a more extensive exchange of blood for acellular fluids.

Intraoperative cell salvage (ICS)

In surgical interventions with a blood loss of at least 800–1000 mL, autotransfusion of RBCs salvaged from shed blood is a highly effective method for reducing allogeneic blood transfusions. Basically, shed blood is aspirated via a heparinized suction tube into a collection reservoir. Erythrocytes are salvaged by differential centrifugation and washing in 0.9% saline, while contaminants such as fibrin, cell debris, microaggregates, bone fragments, fat, haemoglobin and heparin are eliminated. Depending on the washing program, the haematocrit of the autologous RBC concentrate is 55–80%.[52] The quality of salvaged blood is excellent compared with stored pRBCs; fresh salvaged blood has a lower oxygen affinity related to a more physiological pH and a higher content of ATP and 2,3-diphosphoglycerate (2,3-DPG). However, an extensive list of contraindications to ICS is traditionally proposed by the manufacturers of ICS devices (Table 4).

To a certain extent, these contraindications have been challenged by recent literature. Only the potential bacterial contamination of collected wound blood represents an absolute contraindication for autotransfusion in patients undergoing replacement surgery (i.e., implantation of vascular grafts or joint prostheses, cardiac valve replacement).[33]

In the case of definite contamination of shed blood with bacteria or malignant cells, some authors advocate the use of PAL leukocyte-depleting filters in addition to the centrifugation and washing process of the cell saver.[53] A recent study performed in a South African trauma centre even suggests that ICS without PAL filters in 44 patients with penetrating abdominal trauma significantly reduced the need for allogeneic blood

Table 4. Proposed contraindications to intraoperative cell salvage.

Pharmacological agents	Clotting agents
	Irrigating solutions meant for topical use
	Methylmethacrylate
Contaminants	Urine
	Bone chips
	Fat
	Bowel contents
	Infection
	Amniotic fluid
Malignancy	
Haematological disorders	Sickle-cell disease
	Thalassaemia
Miscellaneous	Carbon monoxide (electrocautery smoke)
	Catecholamines (phaeochromocytoma)
	Oxymetazoline

From Waters (2004, *Transfusion* **44:** 40S–44S) with permission.

transfusions. These patients received prophylactic antibiotics, and no differences to the control group were apparent regarding the incidence of sepsis or overall mortality.[54]

As a highly effective method for completely eliminating contaminating tumour cells, Hansen and co-workers propose the irradiation of RBC concentrates salvaged from operating fields in cancer surgery.[55] Moreover, irradiation of salvaged blood has been demonstrated to mitigate the release of inflammatory mediators, which may provide an additional advantage when compared with allogeneic blood.[56]

Even in obstetric surgery, ICS seems possible when combined with PAL leukocyte filters.[57] A current investigation demonstrated that these filters effectively removed squamous cells and other amniotic contaminants from washed blood salvaged during caesarean deliveries.[58] However, in the setting of a massive blood loss, the efficacy of this procedure seems questionable, since the filter interposed into the transfusion line increases the resistance of the system, resulting in a substantial reduction in transfusion velocity.

In any case, it must be borne in mind that any use of cell salvage, despite the stipulated contraindications, represents off-label use from the medico-legal point of view, and has to be based on a thorough analysis of the individual risk/benefit ratio. Further systematic research is necessary to elucidate important safety aspects of these application modalities.

The blood-sparing potential of ICS has been proven in several clinical trials and meta-analyses.[11,35,59] ICS is also accepted by Jehovah's Witnesses as long as the patient, collection system, processing unit and final blood bag form a closed circuit.[60]

ARTIFICIAL OXYGEN CARRIERS

An attractive alternative to allogeneic RBCs consists in synthetic blood substitutes (artificial oxygen carriers), which can be applied independently of blood-group typing or infectious risks. Currently, there are two types of artificial oxygen carrier under experimental and clinical investigation: (1) synthetically manufactured perfluorocar

bons (PFC), and (2) haemoglobin-based oxygen carriers (HBOCs), i.e., solutions based on isolated human or bovine haemoglobin.[61]

Perfluorocarbons (PFCs)

PFCs are simply constructed molecules (MW 450–500 D) derived from cyclic or straight-chain hydrocarbons with hydrogen atoms replaced by halogens (i.e. fluorine or bromide). PFCs are chemically and biologically inert; they are insoluble in water and therefore have to be emulsified for intravenous application. Oxygen kinetics of PFCs are characterized by a linear relationship between partial pressure of arterial oxygen and oxygen content; therefore high partial pressures of arterial oxygen are required to maximize the amount of oxygen transported by the PFC (Figure 3).[62]

Oxygen release from PFC to the tissues is almost complete in the presence of a high pO_2 gradient between arterial blood and the tissues (cf. Figure 3). At a given pO_2 gradient of 560 mmHg (arterial blood 600 mmHg, tissue 40 mmHg), 100 g of a 60% (w/v) PFC emulsion (e.g. perfluorooctylbromide, Oxygent™, Alliance Corp., San Diego, CA, USA) release 15 mL oxygen.[63] The same amount of oxygen is provided by 450 mL of whole blood with a haemoglobin concentration of 14 g/dL. Additionally, PFCs enhance tissue oxygenation by lowering the diffusion barrier between erythrocytes and the plasma ('facilitated diffusion').[61]

After intravenous infusion, PFC emulsion droplets are rapidly taken up by the reticuloendothelial system (RES). To avoid RES overload and consequent immunosuppression, the clinical application of PFC is restricted to low dosages (e.g. maximum dose of 60% Oxygent™: 2.7 g/kg).

In elective surgery with anticipated substantial blood loss, a suitable application mode of PFC is represented by the concept of augmented haemodilution (A-ANH™, patented by Alliance Corp.): prior to surgery, autologous blood is harvested by ANH. During acellular fluid replacement of surgical blood loss, the combination of hyperoxic ventilation and repetitive co-administration of low boluses of PFC maintains adequate tissue oxygenation despite further decrease of haemoglobin concentration. In the best case, retransfusion of autologous blood can be postponed until bleeding is under control.[64,65]

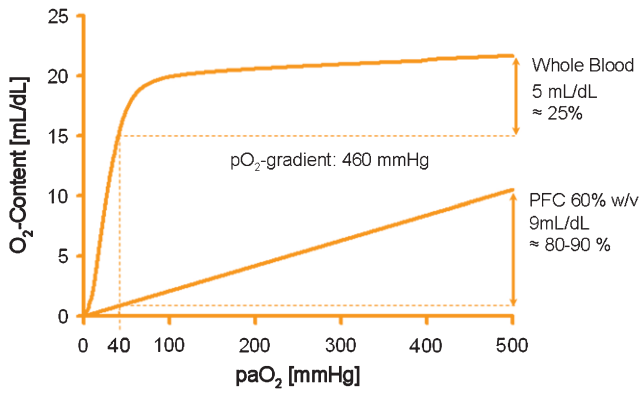

Figure 3. Oxygen dissociation kinetics of native blood (sigmoidal) and a 60% (w/v) Oxygent™ emulsion (linear). At a given tissue pO_2 of 40 mmHg, oxygen extraction from perfluorocarbon (PFC) is almost complete, in contrast to that from blood (oxygen extraction rate 80–90% PFC versus 25% blood).[63]

In splenectomized dogs, this concept allowed the extension of acute normovolaemic anaemia from Hct 21% to Hct 8% without any signs of impaired tissue oxygenation or compromised myocardial contractility.[25,66] In patients undergoing cardiac surgery, the application of 2.7 g/kg Oxygent™ provided adequate gastrointestinal tissue oxygenation at haemoglobin to 6.6 ± 0.4 g/dL.[67] In non-cardiac surgical patients (orthopaedic and general surgery), the low-dose-bolus administration of 60% Oxygent™ (0.9, 1.8 or 2.7 g/dL) allowed the transfusion of allogeneic blood to be postponed by 80 minutes.[68]

In a recent multicentre phase-III study, the number of pRBC units transfused until postoperative day 3 was significantly lower in patients treated with PFC. However, aside from typical mild side-effects of PFC (flu-like symptoms, primarily fever, chills, headache, nausea and myalgia), an increased incidence of postoperative ileus has been reported.[69] Moreover, patient enrolment in a phase-III study in cardiac surgery was suspended in 2001 due to an increased rate of neurological complications.[62] Nevertheless, the manufacturers are seeking to perform additional multicentre studies in Europe and the USA before filing for market approval.

Haemoglobin-based oxygen carriers (HBOC)

Haemoglobin used for manufacturing HBOCs originates from outdated human red cells or from bovine blood, or it is genetically engineered. Purified haemoglobin molecules are chemically modified to increase their stability and to modulate oxygen affinity. These chemical modifications include intramolecular cross-linking of α-subunits, polymerization of haemoglobin molecules using glutaraldehyde or o-raffinose, conjugation of polyethylene glycol to the surface of the haemoglobin molecule, insertion of 2,3-DPG analogues or embedding haemoglobin molecules into phospholipid vesicles(Table 5).[70]

In contrast to PFCs, HBOCs feature sigmoidal oxygen kinetics. As indicated by high p50 values, the oxygen affinity of most HBOCs is lower than that in native human blood, facilitating the offloading of oxygen to the tissues.[71] Moreover, extracellular haemoglobin possesses strong vasoconstrictive properties, the underlying mechanisms of which are: (1) scavenging of nitric oxide ('NO scavenging'); (2) augmented release of endothelin; and (3) stimulation of endothelin receptors and adrenoreceptors.[72]

Due to their oncotic properties, most HBOCs can be characterized as 'oxygen-transporting plasma expanders' suitable for fluid resuscitation from haemorrhagic shock as well as for the treatment of surgical blood loss.

During fluid resuscitation from haemorrhagic shock, hypovolaemia can be treated effectively, while arterial oxygen content is maintained despite progressive dilutional anaemia. Indeed, in experimental studies of severe hemorrhagic shock, resuscitation with HBOCs consistently effected a sustained stabilization of the haemodynamics and tissue oxygenation and significantly decreased mortality.[73–76] Moreover, the post-ischaemic interaction between leukocytes and the endothelium could be attenuated by infusion of HBOCs based on human[77,78] as well as bovine haemoglobin.[79]

Surprisingly, the long-time favourite among the HBOCs, DCLHb, was abandoned in 1998 after an interim analysis of a trauma study performed in the USA. After enrolment of 112 patients, the 24- and 48-hour mortality was significantly higher in patients treated with DCLHb.[80] Although severe deficiencies regarding design and performance of the study (under-resuscitation and over-proportional enrolment of

Table 5. Physicochemical characteristics and actual state of clinical research on haemoglobin-based oxygen carriers (HBOCs).

	Source of haemoglobin	Concentration (g/dL)	MW (Da)	P50 (mmHg)	Indication	Phase of clinical testing
PHP™	Human	8	123,000	23.6	Haemodynamic instability in septic shock	II/III
HemAssist™	Human	10	65,000	32	Reduction of perioperative transfusion rate	Up to III, stopped
r-Hb 1.1™	Recombinant	5–10	64,000	31–32	Reduction of perioperative transfusion rate	I/II, stopped
r.Hb 2.0™	Recombinant	10	320,000	31–32	Reduction of perioperative transfusion rate	I/II, stopped
Hemopure™	Bovine	13	250,000	38	Reduction of perioperative transfusion rate	III
Polyheme™	Human	10	150,000	26–32	Reduction of perioperative transfusion rate	III
Hemolink™	Human	10	120–180,000	39	Reduction of perioperative transfusion rate	III, discontinued
Hemospan™	Human	4	95,000	6	Reduction of perioperative transfusion rate	II

PHP™, pyridoxylated, polyethylene-glycol conjugated haemoglobin (Curacyte Health Sciences, Munich, Germany); HemAssist™, diaspirin cross-linked haemoglobin (DCLHb, Baxter Healthcare, Round Lake, USA); r-Hb 1.1, recombinant haemoglobin, version 1.1 (Somatogen Inc., Boulder, USA, later Baxter Healthcare); r-Hb 2.0, recombinant haemoglobin, version 2.0 (Baxter Healthcare); Hemopure™, polymerized bovine haemoglobin (HBOC 201, Biopure Corp., Cambridge, USA); Polyheme™, pyridoxylated, glutaraldehyde-polymerized haemoglobin (Northfiled Lab. Inc., Evanston, USA); Hemolink™, haemoglobin raffimer (Hemosol Inc., Toronto, Canada); Hemospan™, maleimide-activated polyethylene glycol-modified haemoglobin (MP4, Sangart INC, San Diego, USA).

desperate cases in the DCLHb group), the study has been terminated prematurely and has never been restarted.[81]

In contrast, PolyHeme™ proved to be an effective resuscitation fluid when 171 patients suffering massive haemorrhage were treated with this HBOC. Compared with a historical control group, 30-day mortality could be reduced significantly (64.5% versus 25%).[82] However, this report does not comment on potential side-effects of PolyHeme™. Enrolment in another pre-hospital phase-III study has recently been completed, and the first results are not expected before autumn 2006.

Aside from fluid resuscitation from haemorrhagic shock, HBOCs are also suitable for the treatment of intraoperative blood loss. During isovolaemic replacement of lost blood, the oxygen-transport properties of the HBOC allow for haemodilution to a lower Hct than do crystalloid and colloid solutions. Hence, the transfusion of

allogeneic blood can be postponed until surgical bleeding is under control. HBOCs have been tested in several clinical phase-III studies, including cardiac and non-cardiac (general, vascular, trauma) surgery.[82–90] Frequently observed side-effects consisted of increased systemic and pulmonary arterial resistances, decreased cardiac output, jaundice, and increased activities of amylase, lipase and hepatic transaminases.[86–88,90] Whether the increased enzyme activities must be judged as signs of pancreatitis or whether they may be related to interference with photometric laboratory tests has to date not been fully elucidated.[91]

However, a sustained reduction of allogeneic blood transfusion (up to postoperative day 7) attributable to the use of an HBOC has been reported by only two authors[83,88], but the blood-sparing potential was limited to only 260–600 mL pRBC. The clinical relevance of this finding has been critically discussed by the authors themselves. A reason for the finding may be the short intravascular half-life of HBOCs. The short-term application only postpones the allogeneic blood transfusion. To achieve an effective reduction of RBC transfusions, HBOCs must be infused over a longer term, theoretically until the erythropoiesis can provide a sufficient quantity of autologous RBCs. Regarding the long-term use of HBOCs, only case reports are currently available.[92,93]

Finally, the clinical impact of vasoconstrictive activity exerted by most HBOCs is not yet fully understood. Experimental data indicate that these properties may be harmful with respect to nutritional blood flow and organ function.[94,95] Therefore the availability of a non-vasoactive HBOC may be desirable. Maleimide-activated polyethylene-glycol-modified haemoglobin (Hemospan™, Sangart Corp.) represents such an HBOC featuring a low haemoglobin concentration (4 g/dL), a high oxygen affinity (p50 5.9 mmHg) and a high viscosity (2.5 cP). These characteristics, at first sight counterintuitive, have been demonstrated to provide sufficient tissue oxygenation on the microcirculatory level.[96,97] Currently, Hemospan™ has finished testing phases I and II, and a clinical phase-III trial is scheduled for 2006.[98]

To date, no HBOC with worldwide approval is available for routine clinical use. Only the bovine HBOC Hemopure™ (Biopure Inc., Cambridge, USA) had been approved by the South African Ministry of Health in April 2001. The decisive factor for this regional approval might have been the high incidence of infectious diseases among blood donors in South Africa. However, in 2002, Biopure filed approval by the FDA, the procedure is still pending.[70] The blood-sparing potential of both types of artificial oxygen carrier currently under investigation (PFC and HBOCs) has been proven in experimental as well as in clinical studies. Nevertheless, the approval of a particular synthetic oxygen carrier by the FDA is not yet not foreseeable. Further research and development activities targeting the identification of an ideal oxygen carrier suitable for clinical use remains an issue of substantial interest.

Practice points

- the indication to transfuse allogeneic blood must be based on a critical judgement of necessity
- augmentation of anaemia tolerance allows a restrictive transfusion policy to be extended
- among autologous transfusion techniques, ANH and/or ICS are the most effective

Research agenda

- although the limits of total body anaemia tolerance are well known, further research is necessary to evaluate the specific anaemia tolerance of individual organs
- contraindications of ICS should be re-evaluated on the basis of systematic research
- research and development activities in the field of artificial oxygen carriers are still mandatory to prove the safety and efficacy of these 'blood substitutes'

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