Intraoperative Blood Salvage in Bacterial Contaminated Surgical Site – an in vitro Study

Christoph Lenzen

Mund-, Kiefer- und Gesichtschirurgie / Plastisch-Ästhetische Operationen, St. Josefshospital Krefeld, Germany

Key Words

Intraoperative autotransfusion · Cell salvage · Orthognathic surgery · Blood loss · Bacterial elimination · Endotoxin adsorption

Summary

Background: Cell saving and autogenic retransfusion is a well established procedure in orthopedic, cardiac and abdominal surgery. But it is contraindicated at a bacterially contaminated surgical site such as the oral cavity. **Materials and Methods:** In 2003 and 2004 a clinically oriented in vitro study was processed in 45 enlarged operations for dysgnathia to recycle the intraoperatively collected blood contaminated with microorganisms of the oral cavity. Cell salvage, leukocyte depletion, antimicrobial medical treatment, and a selective endotoxin adsorption were used as single cleaning procedures. Hematological, coagulative, immunologic and microbiological examinations were carried out, including the measurement of endotoxin before, during and after the procedure. In addition, the individual cleaning steps were changed in their succession to determine the most effective one. **Results:** Already cell salvage and leukocyte depletion could eliminate a considerable part of the oral cavity germs. However, only extracorporeal addition of antibiotic and antimycotic agents guaranteed a reproducible bacterial removal. Endotoxin could be eliminated up to $99.9 \pm 0.1\%$ by all purification steps, including a specific endotoxin adsorber. At the end of all procedures hematocrit was increased by a renewed washing centrifugation. The hemolysis rate at the end was $0.2 \pm 0.1\%$. Cytokines and fibrin degradation products were eliminated by more than 90%. Because of the multistage purification procedure, 50% of intraoperative blood loss could be compensated. **Conclusion:** The combination of cell salvage, leukocyte depletion, antimicrobial medical treatment and selective endotoxin adsorption allows for the production of autogenic red blood cell concentrates free of germs, saliva and endotoxin also from a bacterially contaminated surgical site.

Schlüsselwörter

Intraoperative Autotransfusion · Maschinelle Autotransfusion · Orthognathe Chirurgie · Blutverlust · Bakterielle Elimination · Endotoxinadsorption

Zusammenfassung

Hintergrund: Die maschinelle Autotransfusion ist ein etabliertes Verfahren in der orthopädischen, abdominalen und Kardi-chirurgie. Aber sie ist bisher noch nicht zugelassen für die Anwendung in einem bakteriell kontaminierten Operationsgebiet, z.B. der Mundhöhle. **Material und Methoden:** In den Jahren 2003 und 2004 haben wir an unserer Klinik eine klinisch orientierte In-vitro-Studie durchgeführt. Dabei wurde bei 45 ausge-dehnten chirurgischen Eingriffen wegen Dysgnathie das mit Speichel und Bakterien der Mundhöhle kontaminierte Blut gesammelt. Mit Hilfe einer Waschzentrifugation, einer Leukozyten-depletion, einer antimikrobiellen medikamentösen Behandlung sowie einer selektiven Endotoxin-Adsorption wurde das gesammelte Wundblut aufbereitet, gereinigt und zu einem autogenen Erythrozytenkonzentrat aufkonzentriert. Zur Untersuchung der Qualität wurden hämatologische und gerinnungsphysiologi-sche, immunologische und mikrobiologische Parameter analy-siert. Zusätzlich wurde die Endotoxin-Konzentration vor, wäh-rend und am Ende der Aufbereitungsprozedur bestimmt. Die Reihenfolge der einzelnen Reinigungsschritte und die Häufig-keit ihres Einsatzes wurden mehrfach geändert, bis die effektiv-ste Sequenz gefunden war. **Ergebnisse:** Durch den Einsatz des Cell-Savers sowie die Leukozytendepletion konnte ein beträcht-licher Anteil der gesammelten Keime der Mundhöhle eliminiert werden. Allerdings war nur durch die extrakorporale Zugabe von Antibiotika und Antimykotika eine reproduzierbare Bakte-rielenelimination möglich. Endotoxine konnten durch die einzel-nen Aufbereitungsschritte einschließlich des spezifischen En-dotoxin-Adsorbers bis auf $99,9 \pm 0,1\%$ reduziert werden. Die Hämolyserate betrug am Ende $0,2 \pm 0,1\%$. Zytokine und Fibrin-spaltprodukte wurden zu mehr als 90% eliminiert. Die zugesetz-ten antimikrobiellen Substanzen wurden mit Raten zwischen $95,8 \pm 11,7\%$ und $92,0 \pm 15,4\%$ wieder eliminiert. Aufgrund der mehrstufigen Aufbereitungsprozedur hätte ein intraoperativer Blutsverlust von 50% kompensiert werden können. **Schlussfol-gerung:** Durch die Kombination aus Cell-Saver, Leukozytende-pletion, antimikrobieller medikamentöser Behandlung und se-lektiver Endotoxin-Adsorption konnte ein autogenes Erythrozy-tenkonzentrat aus einem bakteriell kontaminierten Operations-gebiet reproduzierbar keimfrei hergestellt werden. Endotoxine konnten bis auf einen absoluten Gehalt von 1 ± 1 ng eliminiert werden.

Introduction

The more elective an operation, the greater is the necessity of preventing hemophilic complications. One of the most elective operations in cranio-maxillofacial surgery are surgical corrections of an inborn or acquired skeletal deformity of the upper and/or lower jaw with incongruence of the bite plane. These surgical procedures were carried out exclusively from an intraoral approach. During maxillary or mandibular orthognathic surgery unexpected, excessive blood loss may occur due to a reduced activity of individual coagulation factors or disturbances of thrombocyte function [1, 2].

Intra- and postoperative hemorrhagic complications may be due to thrombopathy, coagulopathy or vascular disorders. Congenital as well as acquired disorders may affect all three systems involved in coagulation. Congenital disorders of thrombocytes include the various diseases associated with thrombocytopenia and hemorrhage.

However, acquired disorders leading to excessive intraoperative blood loss are more frequent. In particular drug-induced thrombopathy resulting from administration of acetylsalicylic acid or non-steroidal antiphlogistics may bring the patient into a hemodynamically dangerous situation during or following orthognathic surgery. Acquired, drug-induced coagulopathies almost exclusively result from administration of anticoagulants, e.g. cumarine derivatives. Acquired vascular disorders are observed in vitamin C deficiency, dysproteinemia as well as serious infections. Prevention of coagulation complications as well as therapy of intra- or postoperative bleeding requires management on a systemic and a local level.

There are a lot of general measures to reduce intraoperative blood loss. These include acute normovolemic hemodilution, controlled hypotension, placing the patient in such a way that the head is higher than the heart region, and intensive intraoperative hemostasis which however is rather difficult in bony structures [3]. In the past few years it has been possible to avoid allogeneic blood transfusion in orthognathic surgery at the Krefeld department of maxillofacial plastic surgery by using autogenic blood donated preoperatively [17]. Under certain conditions erythropoietin was administered preoperatively, and a rather low postoperative hemocrit was also accepted [3, 4].

So far intraoperative autotransfusion is contraindicated in a bacterially contaminated surgical site such as oral cavity. The oral cavity of healthy individuals is contaminated with more than 700 bacterial species, i.e. a mixture of Gram-positive and Gram-negative aerobic and anaerobic germs [5], but washing centrifugation is not able to eliminate bacterial contamination totally [6]. *Candida albicans* is most frequently found species in the oral cavity which could be isolated of 2–71% of healthy individuals [7]. Odds [8] reported a mean colonization rate of 26% in immunocompetent individuals. Transfusion of centrifuged shed red blood cell (RBC)concentrates resulted not only in elevated cytokine levels but also in fever and transient

Table 1. Origin of coagulation restriction in 105 patients of the Department for Cranio-Maxillofacial and Plastic-Esthetic Surgery, Krefeld

Origin of coagulation restriction	Patients, %
Acetylsalicylic acid-induced thrombopathia	14.3
Idiopathic thrombopathia	3.8
Coagulation factor activity between 40 and 70%	28.6
Coagulation factor activity below 40%	2.9

bacteremia involving the pathogens previously detected in centrifuged shed red blood cell concentrates [9, 10].

Lipopolysaccharides (LPS) being released from the cell wall of Gram-negative bacteria act as endotoxins. They are responsible for the occurrence of fever and septic symptoms. Intravenous injections of LPS lead to fever and septic symptoms as it is well known from hyperthermia therapy [11–15].

Because formerly investigations in our department have shown that a lot of the normally young and healthy patients undergoing orthognathic surgery have disorders of platelet function or reduced coagulation factor activity, it would helpful to have autogenic RBC concentrates in case of excessive intraoperative blood loss. These former tests revealed thrombocytopenia with values lower than $100,000 \times 10^{-9}/l$ in 2.9% of the cases. In 18.1% of the cases, thrombocytopenia with reduced function and thus an increased risk of intra- or postoperative bleeding was found preoperatively. The case histories revealed that in 78.9% of these patients the intake of acetylsalicylic acid at least 14 days prior to the analysis of the coagulation status caused the reduction of thrombocyte function (14.3% of all patients). A reduced activity of coagulation factors ($>70\%$ of normal value) was observed in 31.4% of the patients – in 28.6% of the patients the activity of at least one coagulation factor was reduced to only 40–70%, and in 2.9% of the patients the activity of one or more coagulation factors even did not reach 40% of normal values (table 1) [1]. Because investigations of the coagulation status are not included in clinical routine, unexpected bleeding complications can result and lead to a necessity of transfusion [1, 2, 16–23].

However, the transfusion of allogeneic RBC concentrates is afflicted with significant risks, e.g. transmission of HIV, HBV and HCV or other viruses. Also bacterial contamination, confusion of RBC concentrates, and immunomodulating effects can endanger patients receiving allogeneic transfusions during these elective operations [25–28]. Lowe [27] stated that the risk for a complication following allogeneic blood transfusion is 1:25,000 and for dead 1:100,000. Some of these dangers can be prevented by preoperative autogenic blood donation. However, secondary bacterial contamination or mix-up of blood samples may also occur in autogenic RBC concentrates [28]. Moreover, there is a considerable number of mostly female patients in whom preoperative autogenic blood donation cannot be done due to a low hemoglobin value.

Fig. 1. Six-stage purification sequence for manufacturing an abacterial autogenic RBC concentrate from wound blood of the oral cavity.

Materials and Methods

At the Department of Cranio-Maxillofacial and Plastic Surgery, Krefeld, 352 orthognathic operations of the upper and/or lower jaw were performed in the years 2003 and 2004. In 45 of these patients, blood was collected intraoperatively for analysis of microbiological contamination. All patients got an antibiotic treatment with ampicillin/sulbactam 3 g before starting the operation. Swaps were taken from the oral cavity at the beginning and at the end of surgery. This was performed to identify the origin of bacteria in the blood collecting trunk (BCT) later on. Furthermore, the endotoxin level of patients' blood was examined in 10 patients before operation, 1 h after start of the operation, at the end of operation and 24 h after surgery. The endotoxin detection limit was 0.00625 ng/ml.

Different purification systems were used to clean the collected blood saliva mixture from microbiological contamination and endotoxin load. These systems include the cell saver System CATS™ (Fresenius AG, Bad Homburg, Germany), the leukocyte depletion filter WBF3™ (Pall GmbH, Dreieich, Germany), and antimicrobial addition of 4.5 g/l piperacillin/tazobactam (Tazobac™, Wyeth Pharma GmbH, Münster, Germany), 240 mg/l gentamicin (ratiopharm GmbH, Ulm, Germany) and 1,600 mg/l fluconazol (Diflucan™, Pfizer Pharma GmbH, Karlsruhe, Germany). This combination and dosage of antimicrobial drugs has been shown to effectively eliminate germs of the oral cavity. Moreover, an endotoxin adsorber which is based on immobilized human serum albumin (iHSA) (MATISSE™; Fresenius AG) was used. This system was shown to efficiently remove LPS from blood during direct hemoperfusion [29].

The primary aim of this in vitro study were to eliminate the bacterial contamination and the endotoxin load of the intraoperatively collected blood. The secondary aim was to create an autogenic RBC concentrate having a hematologic quality comparable to that of allogeneic RBC concentrates. To reach this the four different single steps were combined in various orders. To identify the most effective sequence, the four purification steps described above were applied in varying order. After each purification step, the following parameters were measured: hemoglobin, hematocrit, erythrocytes, leukocytes, thrombocytes, mean corpuscular volume, mean corpuscular hemoglobin, lactate dehydrogenase, D-dimer, potassium, calcium, protein, amylase, heparin, free hemoglobin, haptoglobulin, tat complex, TNF- α , IL-1 β , IL-6, rate of hemolysis, activated complement 3, LPS, tazobactam, piperacillin, gentamicin, fluconazol, number of germs, num-

ber of Gram-positive germs, number of Gram-negative germs, number of fungi, concentration of germs.

All data, except for hemoglobin concentration, hematocrit and leukocyte concentration, were calibrated to eliminate dilution factors.

All data were documented as single measures and as means $\pm$ standard deviation. Significance of differences was tested using Student's t test, with the level of significance being defined as $p < 0.05$.

Results

Most Effective Purification Sequence for Bacterially Contaminated Shed Blood

With respect to the primary and secondary aims, the following order of the processing steps was shown to be the most efficient (fig. 1): First, the collected blood, contaminated with saliva of the oral cavity and saline fluid of the drill cooling system while plate fixation, was cleaned and concentrated using the cell saver system CATS. Then the persistent leukocyte concentration was reduced by a special white blood cell filter. After this, the antimicrobial agents piperacillin/tazobactam, gentamicin and fluconazol were added. To increase the antimicrobial effect, the samples were incubated for at least 2 h under agitation. Leukocyte depletion was performed once more to reduce remaining white blood cells with possibly phagocytized bacteria. Then, the endotoxin adsorber MATISSE was applied. Before use the endotoxin adsorber must be activated by a large volume of saline solution (8 l). To compensate for dilution by the addition of antimicrobial agents endotoxin adsorber treatment and to finally get an RBC concentrate, repeated CATS application was performed. Only with this setting, a complete bacterial elimination could be achieved.

Table 2. Microorganisms most commonly found in the oral cavity preoperatively and postoperatively and in the BCT of 45 patients

	Oral cavity		BCT
	preoperatively	postoperatively	
<i>C. albicans</i>	18	18	18
Beta-hemolytic streptococci	15	17	14
Alpha-hemolytic streptococci	11	8	6
<i>Staphylococcus aureus</i>	2	5	5
<i>Klebsiella pneumoniae</i>	5	3	5
<i>Streptococcus viridans</i>	5	3	3
<i>Streptococcus dysgalactiae</i> , subsp. <i>equisimilis</i>	3	3	3
<i>Hafnia alvei</i>	3	2	3
<i>Corynebacterium</i> sp.	2	2	3

Table 3. Quantity of different groups of the 25 microorganisms detected in the oral cavity preoperatively and postoperatively and in the BCT of 45 patients

Gram	Form	Milieu	Oral cavity		BCT
			preoperatively	postoperatively	
+	coccus	aerobic	36	35	32
+	bacillary	aerobic	2	2	3
-	coccus	aerobic	8	5	2
-	bacillary	aerobic	15	12	14
+	coccus	anaerobic	2	0	0
+	bacillary	anaerobic	2	4	0
-	bacillary	anaerobic	8	3	0
	fungus		18	18	18

Fig. 2. Number of different detected microorganisms in each purification step.

Fig. 3. Changes of the LPS concentration over the whole purification procedure.

Measurements of Bacterial Reduction and Endotoxin Elimination

The oral cavity of healthy individuals is contaminated with more than 700 Gram-positive and Gram-negative aerobic and anaerobic bacterial species [5]. However, not all are detectable because of their small count. Only bacteria above 10^2 CFU/ml (CFU = colony forming units) can be detected by standardized swaps.

In the oral cavity, we found 20 different species of microorganisms preoperatively and 18 species postoperatively. In the BCT 15 different microorganisms were still detected. The most common microorganisms were *C. albicans*, beta-hemolytic streptococci and alpha-hemolytic streptococci (tables 2, 3).

On average we have preoperatively found 2.2 ± 0.8 different germs in the oral cavity of our young and healthy patients. Postoperatively 2.1 ± 0.7 different bacteria species were detected. Their count was more than 10^6 /ml, independent of the intravenous antibiotic treatment at the beginning of the operation. In the BCT 1.0 ± 0.6 different bacterial microorganisms were found. All detected species in the BCT were also found formerly in the oral cavity. So a secondary contamination during the purification procedure was excluded. Bacterial contamination data was given in CFU/ml. In BCT we found a mean concentration of $2.8 \times 10^4 \pm 3.3 \times 10^4$ CFU/ml, with a maximum of 10^5 CFU/ml. After the first CATS application, 1.6 ± 0.7 different microorganism species were identified. Their concentration was $2.6 \times 10^4 \pm 3.3 \times 10^4$ CFU/ml, with a maximum of 10^5 CFU/ml. After the first leukocyte depletion, we found

Fig. 4. Changes of the LPS content over the whole purification procedure.

Fig. 5. Changes of the hemoglobin concentration over the whole purification procedure.

Fig. 6. Changes of the hematocrit over the whole purification procedure.

Fig. 7. Changes of the hemolysis rate over the whole purification procedure.

0.4 ± 0.7 bacteria. Bacterial contamination decreased to $6.4 \times 10^3 \pm 2.3 \times 10^4$ CFU/ml, with a maximum of 10^5 CFU/ml. After application of the antimicrobial agents no bacterial contamination could be detected anymore. This means that the bacterial concentration is reduced below 10^2 CFU/ml (fig. 2). The portions of Gram-positive and Gram-negative pathogens were similar (40 ± 9 vs. $40 \pm 5\%$). The bacterial elimination of the single steps was not significant. But we found a highly significant reduction after the addition of antibiotics with respect to the start concentration in BCT ($p < 0.01$).

C. albicans was found in the oral cavity of 40% of our patients pre- and postoperatively. In 35% of our BCTs, *C. albicans* was found too. But in all cases it could not be detected after first leukocyte depletion and in all further purification steps. The concentration of *C. albicans* in BCT was $3.6 \times 10^4 \pm 4.0 \times 10^4$ CFU/ml, $4.0 \times 10^4 \pm 4.5 \times 10^4$ CFU/ml after the first CATS application and 0.0 ± 0.0 CFU/ml after the first leukocyte depletion. The maximum detected concentration of fungi was 10^5 CFU/ml.

LPS was detected in the BCT and during all further purification steps up to the final RBC concentrate in different concentrations: LPS concentration in BCT 15.6 ± 6.3 ng/ml, after the first CATS application 31.5 ± 26.7 ng/ml, after the first leukocyte depletion 22.0 ± 26.8 ng/ml, after antimicrobial treatment 81.0 ± 237.6 ng/ml, after the second leukocyte depletion 1.4 ± 2.0 ng/ml, after selective endotoxin adsorption 0.7 ± 0.7 ng/ml, and after second CATS centrifugation 0.04 ± 0.05 ng/ml. The final concentration of endotoxin corresponded to a total content of 1.0 ± 1.0 ng in the whole RBC concentrate (fig. 3, 4).

Measurements of Hematological Parameters during Purification

Hemoglobin

The hemoglobin concentration in the BCT was 5.1 ± 6.1 g/dl and increased to 15.5 ± 7.6 g/dl after the first CATS application. Leukocyte depletion reduced the hemoglobin content to 11.0 ± 6.5 g/dl. After dilution by antimicrobial agents the hemoglobin concentration was 5.0 ± 3.2 g/dl, after the second leukocyte depletion 4.5 ± 1.3 g/dl, and after endotoxin adsorption 2.4 ± 0.8 g/dl. The second CATS application increased the hemoglobin content again to 13.3 ± 4.6 g/dl. The hemoglobin contents in the BCT and after second CATS are significantly different ($p < 0.05$) (fig. 5).

Hematocrit

Hematocrit was measured in BCT as $16.5 \pm 15.7\%$. It increased by the different cleaning steps up to $41.9 \pm 13.2\%$ at the end of all procedures ($p < 0.01$). The hematocrit of the final product is nearly as high as that of allogeneic or preoperatively stored autogenic RBC concentrates (fig. 6).

Hemolysis

In BCT the rate of hemolysis was $22.6 \pm 15.7\%$, but after the whole purification it was reduced to $0.2 \pm 0.1\%$, being far below the legitimate limit of hemolysis (0.8%) in allogeneic RBC concentrates (fig. 7).

Free Hemoglobin

Free hemoglobin is a marker for destruction of RBCs. In BCT the free hemoglobin concentration was $1,061.2 \pm 563.7$ mg/dl. After the first CATS application it was reduced to 379.8 ± 223.2 mg/dl. Dual application of leukocyte depletion, antimicrobial treatment, and endotoxin adsorption only marginally affects free hemoglobin. After final CATS washing a free hemoglobin concentration of 77.8 ± 34.3 mg/dl was measured. A highly significant reduction of free hemoglobin of the whole purification process was found when comparing free hemoglobin in BCT and in the final RBC concentrate ($p < 0.01$), and the elimination rate amounts to 92.7% (fig. 8).

Fig. 8. Changes of the free hemoglobin concentration over the whole purification procedure.

Leukocytes

The leukocyte concentration in BCT was $1.3 \pm 0.7 \times 10^9$ /l. After CATS it amounts to $3.4 \pm 1.8 \times 10^9$ /l. With the first white blood cell filter reduction to $0.3 \pm 0.9 \times 10^9$ /l was achieved. After dilution with antimicrobial agents we found a leukocyte concentration of $0.05 \pm 0.1 \times 10^9$ /l and after the second leukocyte depletion of $0.08 \pm 0.1 \times 10^9$ /l. Endotoxin adsorption did not change the leukocyte concentration. After final CATS treatment a leukocyte concentration of $0.12 \pm 0.1 \times 10^9$ /l was measurable. None of the changes was significant.

Cytokines

The following cytokines were measured: TNF- α , IL-1 β , and IL-6. In BCT the concentrations of TNF- α , IL-1 β and IL-6 were 143.5 ± 261.0 pg/ml, $574.3 \pm 1,551.9$ pg/ml and $668.6 \pm 1,316.4$ pg/ml, respectively. At the end of the purification the concentrations of TNF- α , IL-1 β and IL-6 were found to be 1.0 ± 2.3 pg/ml, 1.2 ± 2.6 pg/ml and 0.7 pg/ml ± 1.0 pg/ml, respectively. These reductions of cytokine concentration during purification are only tendencies without any significance, presumably because of the high standard deviations (fig. 9).

Potassium

Potassium is found extracellularly in a concentration of 3.0–5.5 mmol/l. An increase of the potassium concentration is a marker for mechanical injury of RBCs during washing. In BCT the potassium concentration was 8.0 ± 4.0 mmol/l, after antimicrobial agent addition 4.1 ± 2.9 mmol/l, and after second CATS application 0.6 ± 0.2 mmol/l. The reduction of potassium between start and end of the cleaning sequence was highly significant ($p < 0.01$). The elimination rate was 92.1% (fig. 10).

Fig. 9. Changes of the concentrations of TNF- α , IL-1 β und IL-6 over the whole purification procedure.

Fig. 10. Changes of the potassium concentration over the whole purification procedure.

Fig. 11. Changes of the amylase activity over the whole purification procedure.

Amylase

Amylase is an enzyme of oral saliva and blood serum. Its activity in blood is smaller than 0.100 U/ml but in saliva it ranges between 5 and about 700 U/ml. After collecting blood and saliva of the oral cavity, we found an activity of amylase of 463.3 ± 473.5 U/ml. After the first CATS washing 137.3 ± 103.4 U/ml were remaining. Leukocyte depletion led to a reduction

Fig. 12. Percentage elimination of piperacillin, tazobactam, gentamicin and fuconazol during further processing in the six-stage purification procedure.

to 72.0 ± 108.0 U/ml. The addition of antimicrobial agents further reduced amylase activity to 24.2 ± 22.9 U/ml. After the second white blood cell filter it was 70.4 ± 136.9 U/ml. Endotoxin adsorption decreased amylase activity to 6.3 ± 11.2 U/ml. After second CATS we could not detect any amylase activity. The difference between the first and the last value ($p < 0.01$) was highly significant (fig. 11).

Mean Corpuscular Volume

In all 6 steps of the purification sequence the mean corpuscular volume (MCV) of the erythrocytes increases. In BCT we found an MCV of $79.2 \pm 14.5 \mu\text{m}^3$ and at the end an MCV of $91.1 \pm 3.9 \mu\text{m}^3$. All measures are in the physiological range.

Reduction of the Utilized Antimicrobial Substances

During the addition of piperacillin/tazobactam, gentamicin and fluconazol final concentrations of 4.5 g/l, 240 mg/l and 1.6 g/l, respectively, were striven. However, since the initial volumes only could be estimated, the initial concentration measured afterwards was put as a 100% standard. After leukocyte filtration, endotoxin adsorption and centrifugation by CATS, the final concentrations of piperacillin, tazobactam, gentamicin and fluconazol amounted to $4.2 \pm 11.7\%$, $4.7 \pm 11.6\%$, $6.6 \pm 30.1\%$ and $8.0 \pm 15.4\%$, respectively. Thus, the added antimicrobial drugs could be substantially reduced but not eliminated during further processing (fig. 12).

Qualitative Analysis of the Fibrin Degradation Products

The splitting of cross-linked fibrin molecules result in so called fibrin degradation products (FDP) which can be found intravasally if coagulation takes place. Their extracorporeal presence indicates that the coagulation cascade was activated. In the present study FDP proof was done only qualitatively. FDP were found in $96.7 \pm 7.6\%$ of the samples of the BCT. After the first CATS $86.7 \pm 22.5\%$ were detected FDP-positive. First leukocyte depletion reduced FDP to $76.7 \pm 34.6\%$. After addition of antimicrobial agents, FDP were found in $80.0 \pm 28.8\%$ of the samples. After the second leukocyte depletion FDP were detectable in only $10.0 \pm 22.7\%$ and after endotoxin adsorption in $40.0 \pm 38.9\%$ of the samples. After the final CATS, FDP could be found in $6.3 \pm 7.6\%$ of the samples.

Measurements of the Perioperative Endotoxin Level in Patients' Blood

Because open blood vessels of soft tissue and bony structures of the oral surgical site had contact to bacteria of the oral cavity we measured in 10 patients the inner endotoxin blood level before operation, 1 h after start of the operation, at the end of operation, and 24 h after surgery. In neither of the patients endotoxin could be detected in the blood, indicating that the endotoxin concentration remains below 0.00625 ng/ml over the whole examination period.

Discussion

Re transfusion of an autogenic RBC concentrate which is produced by intraoperative cell salvage is contraindicated in bacterially contaminated surgical site. To produce an abacterial autogenic RBC concentrate from blood collected at such a contaminated site (oral cavity), we developed a six-stage

processing sequence including four different cleaning and purification procedures. Using this method, the initially very high bacterial concentration could reliably be reduced to at least 10^2 CFU/ml (detection limit in this study), resulting in an autogenic RBC concentrate suitable for retransfusion intra- or postoperatively.

In 2001, the German subdivision 'Microbiological Investigations in Transfusion Medicine' of the Study Group 'Blood' (Arbeitskreis Blut) has found a bacterial contamination in 0.22% of preoperatively donated autogenic and in 0.13% of allogeneic RBC concentrates [28].

A remaining minor bacterial contamination in our autogenic RBC concentrates would be eliminated by routine perioperative antibiotic prophylaxis using 2×3 g/day ampicillin/sulbactam, which is given just before operation until the 3rd postoperative day. Furthermore, residual concentrations of the antibiotics piperacillin/tazobactam, gentamicin and fluconazol which had been added in vitro during the manufacturing process were also present in the RBC concentrates. Thus, the risk of bacterial contamination by retransfusion of these RBC concentrates should be very low. In a study done by Locher et al. [9] to investigate the use of the Cell Saver in transoral maxillofacial surgery described the estimated total number of inoculated bacteria retransfused was between 10^5 and more than 10^7 , resulting in an increase of body temperature to more than 39°C in all patients.

The endotoxin load was reduced in our study from 15.6 to 0.04 ng/ml on average. This corresponded to an absolute content of 1.0 ± 1.0 ng. Intravenous injection of LPS is a well established procedure in active hyperthermia therapy [1, 11]. In hyperthermia therapy, 2–4 mg LPS will be infused to increase the body temperature. e.g. in cancer therapy [9]. Rohling et al. [10] showed that retransfusion of RBC concentrates produced from blood of the oral cavity leads to an increase of interleukin and to bacteremia. According to our investigations after the first CATS application, an LPS content of 32.6 ± 16.6 mg has to be assumed in the retransfused RBC concentrates of this study. In our study the amount of retransfused LPS could be reduced to 1.0 ± 1.0 ng (0.03%). Examination of LPS in the patients' blood did not show any detectable endotoxin perioperatively. However, the detection limit for LPS is 0.00625 ng/ml. If this would circulate in 5 l total blood volume of a 70 kg patient, 31.25 ng would be undiscovered. An endotoxin load below the detection limit represents is regarded as safe. Animal experiments have shown that injection of 0.002 $\mu\text{g/kg}$ leads to an increase of body temperature, but changes in pulmonary arterial pressure, pulmonary vascular resistance, plasma thromboxane B₂, and circulating leukocyte concentration occurs even after injection of 0.02 $\mu\text{g/kg}$ [15].

Also the provable little residual concentrations of TNF- α , IL-1 β and IL-6 which merely indicate a terminated activation of the immune system have pharmacologically and immunologically to be classified as quite safe [30–35].

Our autogenic RBC concentrates produced from wound blood of the oral cavity largely fulfilled the criteria for intraoperative autotransfusion described by Hansen et al. [36]. The rate of plasma elimination, measured via activity of amylase and LDH and via concentration of cytokines, potassium and free hemoglobin, amounted to at least 92%.

The laborious production of autogenic RBC concentrates out of bacterially contaminated wound blood could only compensate 50% of the blood loss at the utmost. However, in hemodynamically critical situations of blood loss short-term available autogenic RBC concentrates could prevent life-threatening conditions and should be preferred to allogeneic transfusions in elective surgery. The method described here should provide autogenic blood also in cases of unexpected excessive blood loss during elective surgery at a bacterially contaminated surgical site. Thus, also in these cases the risks associated with transfusion of allogeneic RBC concentrates, e.g. HIV, HBV and HCV transmission, induction of immunomodulatory effects and possibly also bacterial contamination, might be avoided. Former investigations have shown that there is a considerable number of patients with preoperatively undiscovered restricted thrombocyte function and coagulation factor activity. Such patients are endangered for excessive blood loss during orthognathic surgery [1]. Perioperative blood salvage might further reduce the consumption of allogeneic blood in these patients as well as in patients (mostly females) who are not able to preoperatively donate blood due to their low hemoglobin value (<11 g/dl).

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Conclusion

Using the multistage purification procedure described above, wound blood of the oral cavity could be utilized to produce autogenic RBC concentrates fulfilling the actual quality criteria with respect to elimination of bacterial contamination and endotoxins. The hematological quality of the so obtained blood product is comparable with that of other autogenic RBC concentrates, which are produced in the context of the established intraoperative autotransfusion in orthopedic, abdominal and cardiac surgery. The retransfusion of these autogenic RBC concentrates can therefore be considered safe.

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