Perfusion.com Journal. 2008

The Journal of The International Perfusion Association

What is the Role of Different Cardioplegic Solutions in the Perioperative Immune Response During Cardiac Surgery?

Wolfgang Lante, MD; Axel Franke, MD; Christian Weinhold, MD; Andreas Markewitz, MD;

Department of Cardiovascular Surgery

Bundeswehr Central Hospital, Koblenz, Germany

Running Head: cardioplegia and immune response in cardiac surgery patients

Keywords: inflammatory response, cardiac surgery, cardiopulmonary bypass, cardioplegic solutions

Words: 2933

Address reprints request to:

W. Lante, MD

Dept. of Cardiovascular Surgery

German Army Central Hospital

Ruebenacher Str.170

D 56072 Koblenz, Germany

Tel.: +49 261 281 3710

Fax: +49 261 281 3702

E-mail: wolfgang.lante@t-online.de

wolfganglante@bundeswehr.org

This research was conducted with financial support from the Bundeswehr Medical Service.

Abstract

Background

The extent of the immune response following cardiac surgery with cardiopulmonary bypass (CPB) has been successfully correlated with the incidence of postoperative complications. We tested the hypothesis that surgical trauma is the main factor determining the perioperative immune response and that, therefore, the use of different cardioplegic solutions, as previously discussed, did not influence the perioperative immune response.

Methods

430 patients who underwent uncomplicated elective cardiac surgery were included retrospectively. (Bretschneider's-solution (HTK) n=118; St. Thomas'-solution (ST) n=137; blood cardioplegia (BC) n=175). Serum levels of interleukin (IL)-6, IL-8, tumour necrosis factor (TNF)-alpha, , IL-10, and acute phase proteins, (i.e. C-reactive protein or CRP), lipoprotein-binding protein (LBP) and procalcitonin (PCT) were assayed preoperatively (d0), immediately (dx), 6 and 12 hours (dx+6, dx+12), and on the 1st (d1), 3rd (d3) and 5th (d5) day after surgery.

Results

An intergroup comparison of IL-6, IL-8, TNF-alpha, CRP, and LBP levels revealed no differences. All parameters increased postoperatively and remained elevated until d5. CRP and LBP peaked at d3 and remained elevated until d5. IL-10 levels increased to a maximum at dx and stayed elevated until d1. PCT serum levels increased postoperatively with a peak at d1. We documented higher levels for both parameters in the HTK group compared to BC patients. ST patients showed the lowest levels of these mediators.

Conclusions

The use of different cardioplegic solutions caused only minor clinical or immunological differences. We can therefore conclude that the extent of surgical trauma is the main factor

influencing the release of pro-inflammatory cytokines and the subsequent acute phase response.

Introduction

Previous studies suggest that the heart rather than the use of cardiopulmonary bypass (CPB) plays the crucial role in the release of pro-inflammatory cytokines during and after cardiac surgery. [1, 2, 3] This has been shown in studies that investigated cytokine levels in coronary sinus and peripheral arterial blood samples taken during cardiac surgery and in patients undergoing cardiac surgery with or without CPB. [2, 3, 4, 5]

High pro-inflammatory cytokine levels after cardiac surgery can contribute to myocardial dysfunction, haemodynamic instability, postoperative complications or delayed recovery. [6, 7, 8]

For this reason, recent studies have investigated whether it is possible to reduce pro-inflammatory cytokine levels by optimising surgical procedures. [5, 9, 10, 11, 12, 13] None of these studies, however, has showed a major influence of these efforts on the perioperative immune response.

In addition, more recent studies have demonstrated that the use or non-use of CPB or the type of surgical approach in thoracic surgery does not influence the extent of the perioperative immune response. There is thus a growing body of evidence which suggests that surgical trauma itself represents the major immune stimulus. [14, 15, 16, 17]

We performed a retrospective study on patients who underwent elective cardiac surgery and had an uneventful clinical course in order to investigate the hypothesis that there is no influence of the cardioplegic solution on the perioperative systemic release of pro- and anti-inflammatory cytokines and the subsequent acute phase response. We analysed serum levels of pro-inflammatory interleukin (IL)-6, IL 8 and tumour necrosis factor (TNF)-alpha, anti-inflammatory IL-10, and acute phase proteins (C-reactive protein or CRP), lipoprotein-binding protein (LPB) and procalcitonin (PCT)).

Patients and methods

Patients

All patients gave their written informed consent to have their medical records analysed for our anonymous study. In addition, serum sample material that was not needed for routine laboratory tests was frozen at –20°C and assayed for the relevant immunological parameters. Data and samples were collected randomly from patients who underwent elective cardiac surgery between January 2000 and February 2005.

Patients with known immune or central nervous system dysfunction as well as congestive heart failure, exogenous hormone therapy, malnutrition, infection or inflammation were not enrolled. In order to ensure a high level of homogeneity, we also excluded patients who died during the first 5 postoperative days as well as those with a complicated clinical course, i.e. patients who were ventilated for more than 12 hours, stayed in the intensive care unit (ICU) for more than 2 days, needed a postoperative dose of inotropic or vasoactive drugs (ephinephrine or norepinephrine) of more than 0.5 mg/h, or required the use of an intra-aortic balloon pump (IABP) as well as patients with renal failure who required haemofiltration. Clinical characteristics of the patients are provided in Table 1.

Clinical management

Cardiac surgery with CPB: Etomidate, midazolam, fentanyl, pancuronium, and nitrous oxide were used as anaesthetic drugs; isoflurane was given if necessary. Cefazolin was used for antibiotic prophylaxis. The cardiopulmonary bypass equipment included roller pumps and disposable membrane oxygenators (Affinity™, Avecor, Bellshill, UK). The pump was primed with 1.5 L of a standard electrolyte solution, 0.5 L of a colloid solution (Biseko™, Biotest Pharma GmbH, Dreieich, Germany), 37.5 g of mannitol, and 5000 IU heparin; blood was added, if necessary. Moderate hypothermia (32°C) was used in all cases and pump flow rates were maintained at 2.2-2.4 L/min per m^2. After the aorta was crossclamped, the patients received St. Thomas' Hospital, Bretschneider's HTK, or blood cardioplegic solution.

The cardioplegic solution was chosen preoperatively on the day of surgery depending on findings. All patients were operated on by the same team of seven cardiac surgeons during the aforementioned period.

Sampling

Serum samples were taken preoperatively (**d0**), immediately after surgery (**dx**), 6 and 12 hours after surgery (**dx+6** and **dx+12**), and on the 1st (**d1**), 3rd (**d3**) and 5th (**d5**) postoperative days at 8:00 a.m.

Parameters

We measured **IL-6, IL-8** and **TNF-alpha** in order to assess the pro-inflammatory immune response and **IL-10** to determine the anti-inflammatory immune response. These cytokines are released primarily by monocytes and macrophages and indicate activation of the non-specific immune system due to cellular contact with endothelial surfaces in the traumatised tissue (lung and myocardium) or extracorporeal surfaces.

Nearby several other mediators of different inflammatory cascades, they stimulate local immune responses and induce systemic immune responses, which, depending on the extent of clinical symptoms, are known as systemic inflammatory response syndrome (**SIRS**).

C-reactive protein (**CRP**), lipoprotein-binding protein (**LBP**) and procalcitonin (**PCT**) were used as parameters of the acute phase response. They indicate the extent to which pro- and anti-inflammatory responses result in a systemic immune response.

Serum levels of the pro-inflammatory cytokines (**IL-6, IL-8** and **TNF-alpha**), anti-inflammatory cytokines (**IL-10)** and **LBP** were measured using the commercially available Immulite® system (DPC-Biermann, Bad Nauheim, Germany), which is a luminometric immunoassay with a sensitivity of 5pg/mL for each parameter.

CRP serum levels were measured using a nephelometer (Behring BN II Analyzer, Dade Behring, Marburg, Germany) and the N High Sensitivity Reagent Kit (sensitivity: 0.175mg/L, Dade Behring, Marburg, Germany).

Serum concentrations of **PCT** were assayed using the commercially available BeriLux® Analyzer 250 system (Dade Behring, Marburg, Germany), which is an immunoluminometric assay (PCT LUMItest®, Brahms AG, Hennigsdorf, Germany) with a sensitivity of 0.1 ng/mL.

The results were not corrected for haemodilution, since we intended to document *in vivo* levels and used comparable perioperative management procedures (e.g. transfusion guidelines) in the study groups.

Statistical analysis

Data were stored and analysed using standard computer software (Statview 5.0, Abacus concepts, Berkeley, CA). The analysis of variance (ANOVA) test was used to identify differences within groups (time points) and between groups (surgical procedures). When the ANOVA test revealed significant group differences, Fisher's PLSD was used for pair-wise comparisons. If ANOVA assumptions were violated, unpaired Student's t-tests were used for comparisons within groups (time points) and between groups (surgical procedures). A p-value of less than 0.05 was considered significant. The results are expressed as mean ± standard error of the mean, unless otherwise indicated.

Results

Clinical results

Operative and postoperative data are given in Table 2. There was no operative mortality or morbidity.

There were only minor differences in the preoperative data collected for the 430 patients who took part in the study. Patients who received Bretschneider's HTK cardioplegic solution had the poorest left ventricular function. There were significant differences between the groups in the duration of surgery, which was shortest in the ST group. CPB and cross-clamp times were shorter for patients who received St. Thomas' Hospital cardioplegic solution (ST) than for those who received blood cardioplegia (BC). The longest CPB and cross-clamp times were observed in the HTK group.

Of the 430 patients undergoing elective cardiac surgery, 377 had coronary bypass grafting. In 91 patients, coronary bypass grafting was combined with aortic valve replacement. A total of 53 patients underwent aortic valve replacement only.

In 377 patients, aortocoronary vein bypass interventions were performed (1 graft: n=113, 2 grafts: n=148, 3 grafts: n=86, 4 grafts: n=21). A total of 300 patients also received left internal mammary artery (LIMA) grafts. A total of 15 patients received only a LIMA graft, whereas 3 patients received a left and right IMA graft. Operative data are given in Table 1.

Pro-inflammatory cytokines

IL-6

In all patient groups, serum levels of IL-6 significantly increased at dx, reached a maximum at dx+6 and remained significantly increased until d5. (Figure 1) There were no statistical intergroup differences.

IL-8

Like IL-6, serum levels of IL-8 also increased at dx and reached a maximum between dx and dx+6. Preoperative levels were reached at d3 in the ST group. The BC group showed serum levels that remained elevated even at d5. (Table 2) An intergroup comparison showed that IL-8 levels were significantly lower at dx+12 in the ST group than in the BC group.

TNF-alpha

In all groups, TNF-alpha serum levels showed two postoperative peaks. The first peak occurred immediately after surgery (dx) and the second peak at d3. TNF-alpha levels were significantly elevated in all groups at dx, d3 and d5. Wide inter-individual variation was the reason why the differences between baseline values and measurement results in some groups did not reach the significance level at dx+6, dx+12 and d1. (Table 2) A comparison of the patient groups at d5 showed that TNF-alpha levels were significantly higher in the ST group than in the BC and HTK groups.

Anti-inflammatory cytokines

IL-10

In all patient groups, IL-10 serum levels increased and reached a maximum immediately after surgery (dx). They returned to preoperative levels at d3. (Figure 2) An intergroup comparison revealed that IL-10 levels at dx were higher in the HTK group than in the BC group. In addition both groups showed significantly higher levels than the ST group. At dx+6, we measured significantly higher levels for the HTK patient group than for the ST group.

Acute phase response

CRP

Six hours after surgery (dx+6), CRP levels began to increase and reached a maximum at d3. Serum levels remained elevated at d5 when compared to preoperative levels. (Table 2) An intergroup comparison revealed higher levels in the HTK group than in the BC group immediately after surgery.

LBP

In all groups of patients, the same patterns were observed for LBP as for CRP serum concentrations. (Table 2) An intergroup comparison revealed higher levels in the HTK group than in the ST and BC groups immediately after surgery.

PCT

In all patient groups, PCT serum levels began to increase 6 hours after surgery (dx+6) and reached a maximum either at dx+12 or d1. They remained significantly elevated until d5 compared to preoperative levels. (Figure 3) A comparison of the different groups showed that the patients in the HTK group tended to show higher levels than the other patients from dx+6 to d1. However, the differences between the ST group and the HTK group at dx+6, between the ST group and the HTK group at d1 and between the BC group and the HTK group at d1 reached a level of significance of $p<0.05$.

Discussion

We hypothesized that surgical trauma is the main factor determining the perioperative immune response, irrespective of which surgical procedure (with or without CPB) is performed and which cardioplegic solution is used. For this reason, we retrospectively analysed serum samples from patients who had an uncomplicated course after elective cardiac surgery.

Apart from and in spite of the differences between the study groups in preoperative and perioperative data, there were only minor differences in the course and extent of the perioperative immune response. In all patient groups, **IL-6**, **TNF-alpha**, **LBP**, and **CRP** serum levels showed similar synthesis patterns, with no significant differences among the groups in the perioperative period from dx+6 to d3.

These findings are in contrast to other studies, which reported differences in the release of pro-inflammatory cytokines after the use of different cardioplegic solutions. [2, 3, 11, 12, 13, 18] In these studies, the authors used coronary sinus blood samples, which can probably reveal a relationship between mediator levels and reperfusion injury in a more specific way and thus may explain the different results. Due to their complex methods, however, these studies used much smaller patient populations than we did. This may offer a further explanation for the differences between their tests and ours.

Our results for **IL-6**, **TNF-alpha**, **LBP** and **CRP** suggest that the use of different cardioplegic solutions has only a minor, if any, influence on the perioperative immune response. This confirms the results of other studies on small patient populations [19, 20] and emphasises the role of the surgical trauma and the perioperative stress reaction. Whereas similar results were obtained for IL-6, TNF-alpha, LBP and CRP, different synthesis patterns were found for the other three parameters investigated.

In all study groups, **PCT** and **IL-10** showed very similar time courses. Their absolute levels, however, tended to be higher in the study groups with a longer duration of surgery, extracorporeal circulation or cross-clamping. Since we found no significant differences between the patients who received cold cristalloid cardioplegia (ST) or blood cardioplegia (BC), the type of cardioplegic solution (crystalloid versus blood) thus again appears to be of minor importance. This confirms the findings of other studies that report a similar relationship between the degree of trauma and the absolute serum levels of the investigated parameters. [21, 22, 23]

Only **IL-8** secretion followed a different pattern. IL-8 levels reached a peak at dx. The peak increased with the duration of surgery. In other words, the lowest level was noted for the ST group and the highest level for the HTK group.

The most probable factor influencing IL-8 release appears to be the myocardium since we and other authors were able to show that IL-8 levels did not increase after cardio-thoracic surgical procedures or sternotomy alone. [16] Our data thus show that there appear to be immune mediators (such as IL-8) that show specific responses to different surgical approaches.

The clinical role of the investigated parameters requires further clarification. It is undisputed that pro- and anti-inflammatory cytokines are released during surgical trauma and induce both local and systemic immune responses. In addition, especially IL-6 and TNF-alpha appear to be able to directly mediate organ dysfunction. [8, 24, 25, 26] This has provided the basis for an approach to reducing the incidence of postoperative complications by decreasing the intraoperative and postoperative serum levels of relevant cytokines through medication and other measures. [27, 28, 29, 30] In this context, contradictory results have been reported in the literature and emphasise the need for comprehensive studies that investigate these aspects in a large patient population. As a result, the role of pro- and anti-inflammatory cytokine levels continues to be the subject of ongoing discussions.

In addition, we believe that IL-6, PCT, IL-10 and similar parameters are potentially promising indicators of postoperative complications that, however, require further validation in the context of cardiac surgery. Our data appear to justify a comparison of patients and the kinetics of postoperative complications irrespective of which cardioplegic solution was used.

Limitations of the study

We are well aware of the fact that the relatively long measurement intervals and the collection of blood samples from peripheral sites limit the ability of our study protocol to document the direct release of mediators from the myocardium during reperfusion after cardioplegic arrest. There are, however, a growing number of studies which suggest that the levels of systemic mediators play a decisive role in the recovery of immune function and organ dysfunction in the postoperative course after cardiac surgery. [25] We conducted this research in order to provide a comprehensive study investigating pro-inflammatory, anti-inflammatory and acute phase responses after different types of cardiac surgical procedures in a large population of patients. In addition, we investigated only patients with an uneventful clinical course in order to make our patient population more homogeneous. The results of this study therefore apply only to low risk patients. Further studies targeting higher risk patients are thus needed.

It should be noted that further analyses or measurements would not have been justified since our laboratory findings did not reveal any adverse effects on the clinical course. Last but not least, it should be stressed that the study was not designed to document postoperative myocardial function. It is therefore impossible to draw conclusions on the pros and cons of different cardioplegic solutions and their contribution to myocardial preservation.

Conclusions

We were able to show in our study that the three different cardioplegic solutions that were investigated in elective cardiac surgery patients with an uneventful postoperative course did not influence the systemic inflammatory response and the subsequent acute phase response. As far as the immune response is concerned, the choice of cardioplegic solution should depend on factors other than immunological considerations.

Acknowledgements

The authors gratefully acknowledge the excellent assistance provided by B. Isenberg, L. Herter and Y. Wyss.

References

1. Valen G, Paulsson G and Vaage J Induction of inflammatory mediators during reperfusion of the human heart. Ann Thorac Surg 2001; 71: 226-232.

2. Liebold A, Keyl C and Birnbaum DE The heart produces but the lungs consume proinflammatory cytokines following cardiopulmonary bypass. Eur J Cardiothorac Surg 1999; 15: 340-345.

3. Liebold A, Langhammer T, Brunger F, et al. Cardiac interleukin-6 release and myocardial recovery after aortic crossclamping. Crystalloid versus blood cardioplegia. J Cardiovasc Surg (Torino) 1999; 40: 633-636.

4. Schulze C, Conrad N, Schutz A, et al. Reduced expression of systemic proinflammatory cytokines after off-pump versus conventional coronary artery bypass grafting. Thorac Cardiovasc Surg 2000; 48: 364-369.

5. Czerny M, Baumer H, Kilo J, et al. Inflammatory response and myocardial injury following coronary artery bypass grafting with or without cardiopulmonary bypass. Eur J Cardiothorac Surg 2000; 17: 737-742.

6. Franke A, Lante W, Fackeldey V, et al. Proinflammatory and antiinflammatory cytokines after cardiac operation: different cellular sources at different times. Ann Thorac Surg 2002; 74: 363-370; discussion 370-361.

7. Rothenburger M, Soeparwata R, Deng MC, et al. Prediction of clinical outcome after cardiac surgery: the role of cytokines, endotoxin, and anti-endotoxin core antibodies. Shock 2001; 16 Suppl 1: 44-50.

8. Deng MC, Dasch B, Erren M, et al. Impact of left ventricular dysfunction on cytokines, hemodynamics, and outcome in bypass grafting. Ann Thorac Surg 1996; 62: 184-190.

9. Eising GP, Schad H, Heimisch W, et al. Effect of cardiopulmonary bypass and hemofiltration on plasma cytokines and protein leakage in pigs. Thorac Cardiovasc Surg 2000; 48: 86-92.

10. Menasche P, Peynet J, Lariviere J, et al. Does normothermia during cardiopulmonary bypass increase neutrophil-endothelium interactions? Circulation 1994; 90: II275-279.

11. Wan S, Izzat MB, Lee TW, et al. Avoiding cardiopulmonary bypass in multivessel CABG reduces cytokine response and myocardial injury. Ann Thorac Surg 1999; 68: 52-56; discussion 56-57.

12. Wan S and Yim AP Cytokines in myocardial injury: impact on cardiac surgical approach. Eur J Cardiothorac Surg 1999; 16 Suppl 1: S107-111.

13. Wan S, Yim AP, Arifi AA, et al. Can cardioplegia management influence cytokine responses during clinical cardiopulmonary bypass? Ann Thorac Cardiovasc Surg 1999; 5: 81-85.

14. Wehlin L, Vedin J, Vaage J, et al. Peripheral blood monocyte activation during coronary artery bypass grafting with or without cardiopulmonary bypass. Scand Cardiovasc J 2005; 39: 78-86.

15. Vedin J, Antovic A, Ericsson A, et al. Hemostasis in off-pump compared to on-pump coronary artery bypass grafting: a prospective, randomized study. Ann Thorac Surg 2005; 80: 586-593.

16. Franke A, Lante W, Fackeldey V, et al. Pro-inflammatory cytokines after different kinds of cardio-thoracic surgical procedures: Is what we see what we know? Eur J Cardiothorac Surg 2005; accepted for publication 07/2005.

17. Wehlin L, Vedin J, Vaage J, et al. Activation of complement and leukocyte receptors during on- and off pump coronary artery bypass surgery. Eur J Cardiothorac Surg 2004; 25: 35-42.

18. Wan S, Arifi AA, Wan IY, et al. Cytokine responses to myocardial revascularization on cardiopulmonary bypass: intermittent crossclamping versus blood cardioplegic arrest. Ann Thorac Cardiovasc Surg 2002; 8: 12-17.

19. Wan S, Yim AP, Wong CK, et al. Expression of FHL2 and cytokine messenger RNAs in human myocardium after cardiopulmonary bypass. Int J Cardiol 2002; 86: 265-272.

20. Wan IY, Arifi AA, Wan S, et al. Beating heart revascularization with or without cardiopulmonary bypass: evaluation of inflammatory response in a prospective randomized study. J Thorac Cardiovasc Surg 2004; 127: 1624-1631.

21. Neuhof C, Wendling J, Dapper F, et al. Endotoxemia and cytokine generation in cardiac surgery in relation to flow mode and duration of cardiopulmonary bypass. Shock 2001; 16 Suppl 1: 39-43.

22. Zimmermann AK, Simon P, Seeburger J, et al. Cytokine gene expression in monocytes of patients undergoing cardiopulmonary bypass surgery evaluated by real-time PCR. J Cell Mol Med 2003; 7: 146-156.

23. Varan B, Tokel K, Mercan S, et al. Systemic inflammatory response related to cardiopulmonary bypass and its modification by methyl prednisolone: high dose versus low dose. Pediatr Cardiol 2002; 23: 437-441.

24. Diwan A, Tran T, Misra A, et al. Inflammatory mediators and the failing heart: a translational approach. Curr Mol Med 2003; 3: 161-182.

25. Sablotzki A, Friedrich I, Muhling J, et al. The systemic inflammatory response syndrome following cardiac surgery: different expression of proinflammatory cytokines and procalcitonin in patients with and without multiorgan dysfunctions. Perfusion 2002; 17: 103-109.

26. Murphy GJ and Angelini GD Side effects of cardiopulmonary bypass: what is the reality? J Card Surg 2004; 19: 481-488.

27. Cagli K, Ulas MM, Ozisik K, et al. The intraoperative effect of pentoxifylline on the inflammatory process and leukocytes in cardiac surgery patients undergoing cardiopulmonary bypass. Perfusion 2005; 20: 45-51.

28. Greilich PE, Brouse CF, Whitten CW, et al. Antifibrinolytic therapy during cardiopulmonary bypass reduces proinflammatory cytokine levels: a randomized, double-blind, placebo-controlled study of epsilon-aminocaproic acid and aprotinin. J Thorac Cardiovasc Surg 2003; 126: 1498-1503.

29. Chew MS, Brix-Christensen V, Ravn HB, et al. Effect of modified ultrafiltration on the inflammatory response in paediatric open-heart surgery: a prospective, randomized study. Perfusion 2002; 17: 327-333.

30. Yilmaz M, Ener S, Akalin H, et al. Effect of low-dose methyl prednisolone on serum cytokine levels following extracorporeal circulation. Perfusion 1999; 14: 201-206.

Tables

Table 1: Clinical data of patients (n = 430)

Parameters	HTK [n = 118]	ST [n = 137]	BC [n = 175]
Male [n]	85	110	138
Female [n]	33	27	37
Age [years]	66.2 ± 0.9	65.2 ± 0.8	65.5 ± 0.7
Weight [kg]	81.9 ± 1.4	82.5 ± 1.4	83.9 ± 1.3
Height [cm]	171.4 ± 1.0	171.7 ± 1.1	170.3 ± 1.0
Preoperative ejection fraction [%]	57.5 ± 2.9 # versus ST	64.8 ± 1.7	62.9 ± 2.2
Coronary artery bypass grafting (CABG) [n]	37	136	166
CABG and aortic valve replacement [n]	32	-	9
Isolated aortic valve replacement [n]	49	1	-
Duration of surgery [min]	210 ± 5	195 ± 4 # versus HTK, BC	211 ± 3
Duration of extracorporeal circulation [min]	106 ± 3 ## versus ST, BC	82 ± 2	93 ± 2 # versus ST
Cross-clamp time [min]	74 ± 2 ## versus ST, BC	52 ± 2	59 ± 2 # versus ST
Preoperative haemoglobin level [mg/dL]	13.7 ± 0.11	13.7 ± 0.17	13.6 ± 0.12
Postoperative haemoglobin level [mg/dL]	10.6 ± 0.09	10.3 ± 0.06	10.2 ± 0.07
Number of units of packed red blood cells transfused intraoperatively	1.0 ± 0.1 # versus ST	0.6 ± 0.1	1.0 ± 0.1 # versus ST
Number of units of packed red blood cells transfused postoperatively	2.3 ± 0.2 # versus ST	1.7 ± 0.2	2.1 ± 0.1 # versus ST
Mean number of distal anastomoses [n]	1.5 ± 0.1 ## versus ST, BC	2.9 ± 0.1	2.9 ± 0.1
Length of stay in ICU [days]	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1

Results are displayed as mean ± standard error of the mean.
HTK – Bretschneider's cardioplegic solution
ST – St. Thomas' Hospital cardioplegic solution
BC – blood cardioplegia
Grey boxes indicate significant differences between the study groups.
# indicates p<0.05

Table 2: IL-8, TNF-alpha, CRP and LBP serum levels (n = 430)

Time point	IL-8 [pg/mL]			TNF-alpha [pg/mL]			CRP [mg/L]			LBP [pg/mL]		
	HTK [n = 118]	ST [n = 137]	BC [n = 175]	HTK [n = 118]	ST [n = 137]	BC [n = 175]	HTK [n = 118]	ST [n = 137]	BC [n = 175]	HTK [n = 118]	ST [n = 137]	BC [n = 175]
d0	27.6 ± 3.4	28.3 ± 5.0	20.7 ± 1.9	6.6 ± 0.5	7.8 ± 0.8	6.7 ± 0.6	7.0 ± 1.4	7.9 ± 1.9	6.0 ± 0.9	11.6 ± 0.7	11.3 ± 0.7	10.3 ± 0.5
dx	120.7 ± 22.1 **	99.6 ± 16.3 **	111.4 ± 12.2 **	16.1 ± 2.0 **	15.1 ± 1.1 **	19.5 ± 3.0 **	11.4 ± 3.3	7.7 ± 1.9	5.4 ± 0.8 # BC *vs.* HTK	13.8 ± 1.4	12.2 ± 0.8	11.0 ± 0.7 # BC *vs.* HTK
dx+6	92.7 ± 15.4 **	74.1 ± 6.3 **	87.6 ± 7.3 **	11.3 ± 0.8 **	11.5 ± 0.6 *	11.9 ± 0.7 **	20.6 ± 3.5 *	20.1 ± 2.5 **	16.6 ± 1.3 **	27.1 ± 1.7 **	28.0 ± 1.6 **	25.8 ± 1.5 **
dx+12	71.2 ± 7.9 **	55.6 ± 3.9 **; # ST *vs.* BC	77.8 ± 8.4 **	8.7 ± 0.5 *	9.0 ± 0.5	8.5 ± 0.4 *	56.2 ± 4.9 **	58.1 ± 3.8 **	57.1 ± 2.3 **	47.5 ± 3.1 **	49.5 ± 2.7 **	47.1 ± 2.6 **
d1	54.5 ± 4.4 **	44.3 ± 2.9 *	52.2 ± 3.7 **	47.8 ± 19.6 *	43.0 ± 19.0	19.4 ± 7.7	112.8 ± 6.6 **	115.5 ± 4.6 **	114.3 ± 3.7 **	52.7 ± 3.4 **	56.1 ± 3.0 **	54.3 ± 2.9 **
d3	41.7 ± 6.0 *	34.7 ± 7.4	38.2 ± 5.6 *	16.5 ± 4.8 *	17.8 ± 4.5 *	16.3 ± 3.4 *	181.8 ± 4.7 **	186.4 ± 7.0 **	178.5 ± 6.3 **	56.6 ± 3.0 **	60.5 ± 3.6 **	60.2 ± 3.1 **
d5	36.6 ± 8.2	36.7 ± 5.4	34.3 ± 3.8 *	8.8 ± 0.5 *	12.9 ± 2.3 * # ST *vs.* HTK, BC	8.9 ± 0.4 *	96.2 ± 5.9 **	91.7 ± 4.3 **	92.3 ± 4.9 **	40.4 ± 2.3 **	39.9 ± 2.0 **	41.2 ± 2.3 **

Results are displayed as mean ± standard error of the mean.
HTK – Bretschneider's cardioplegic solution
ST – St. Thomas' Hospital cardioplegic solution
BC – blood cardioplegia
Grey boxes indicate significant differences between the study groups:
# indicates p<0.05; **##** indicates p<0.001
***** indicates p<0.05 same group versus d0; ****** indicates p<0.001 same group versus d0

Legends

Figure 1: IL-6

IL-6 levels (mean ± standard error of the mean) in serum samples taken from patients undergoing cardiac surgery with CPB and cardioplegic arrest [**ST** – St. Thomas' Hospital cardioplegic solution, **BC** –blood cardioplegia, **HTK** – Bretschneiders' cardioplegic solution] on the first 5 postoperative days (**d0** – preoperatively; **dx** – immediately after surgery, **dx+6** – 6 hours after surgery, **dx+12** – 12 hours after surgery, **d1** – first postoperative day; **d3** – third postoperative day; **d5** – fifth postoperative day). (* indicates $p<0.05$ in intergroup comparisons, # indicates $p<0.05$, ## indicates $p<0.001$ versus d0)

Figure 2: IL-10

IL-10 levels (mean ± standard error of the mean) in serum samples taken from patients undergoing cardiac surgery with CPB and cardioplegic arrest [**ST** – St. Thomas' Hospital cardioplegic solution, **BC** –blood cardioplegia, **HTK** – Bretschneiders' cardioplegic solution] on the first 5 postoperative days (**d0** – preoperatively; **dx** – immediately after surgery, **dx+6** – 6 hours after surgery, **dx+12** – 12 hours after surgery, **d1** – first postoperative day; **d3** – third postoperative day; **d5** – fifth postoperative day). (* indicates $p<0.05$ in intergroup comparisons, # indicates $p<0.05$, ## indicates $p<0.001$ versus d0)

Figure 3: PCT

PCT levels (mean ± standard error of the mean) in serum samples taken from patients undergoing cardiac surgery with CPB and cardioplegic arrest [**ST –** St. Thomas' Hospital cardioplegic solution, **BC** –blood cardioplegia, **HTK** – Bretschneiders' cardioplegic solution] on the first 5 postoperative days (**d0** – preoperatively; **dx** – immediately after surgery, **dx+6** – 6 hours after surgery, **dx+12** – 12 hours after surgery, **d1** – first postoperative day; **d3** – third postoperative day; **d5** – fifth postoperative day). (* indicates $p<0.05$ in intergroup comparisons, # indicates $p<0.05$, ## indicates $p<0.001$ versus d0)

Figures

Figure 1: IL-6

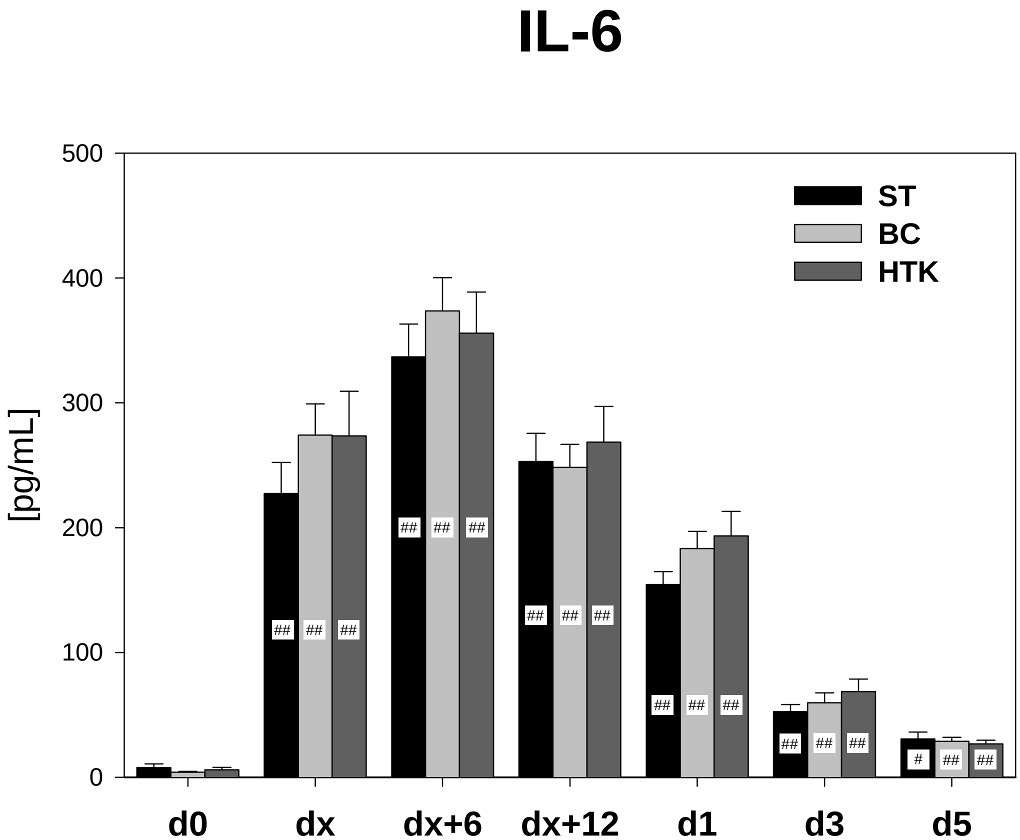

Figure 2: IL-10

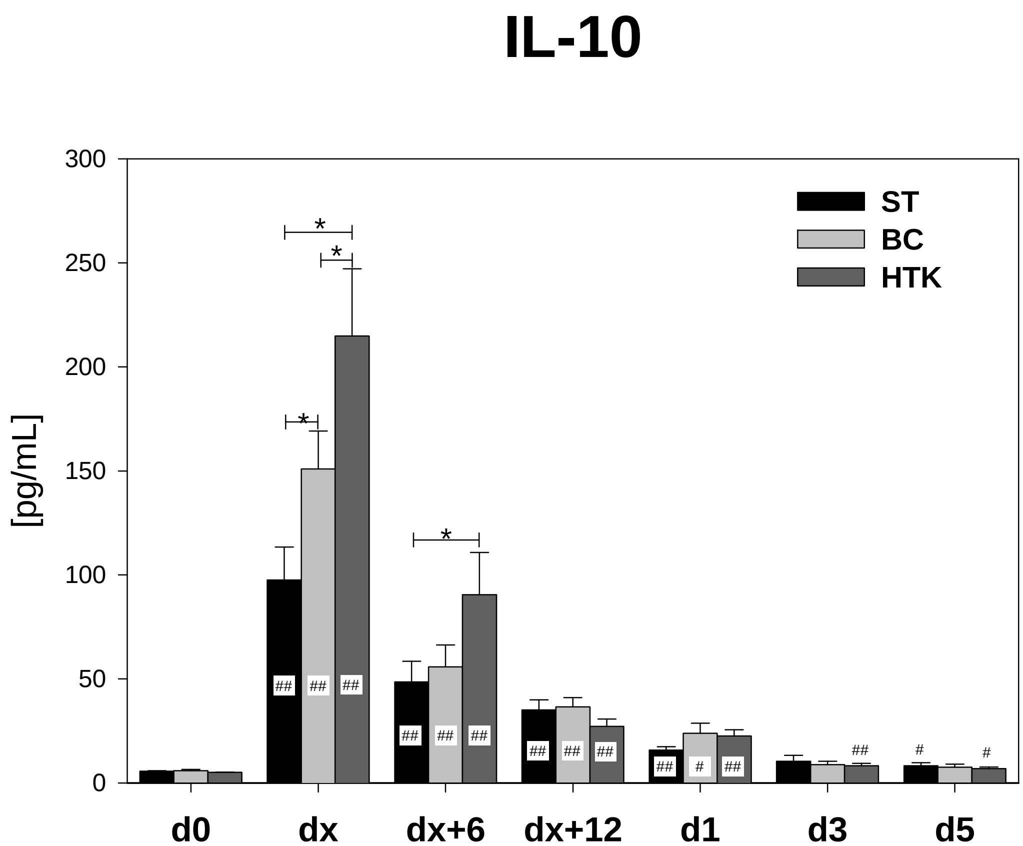

Figure 3: PCT