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ORIGINAL ARTICLE

Perioperative application of L-alanyl-L-glutamine in cardiac surgery: effect on the polarized T cell cytokine expression

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Abstract At risk patients undergoing cardiac surgery with cardiopulmonary bypass have increased rates of postoperative infectious morbidity. Postoperatively, after cardiac surgery, an immunosuppression in the form of a polarization of T helper (Th) cells with a decreased Th1 response (IL-2 and IFN-γ) and an increased Th2 response (IL-4 and IL-10) is recognized. Therapeutic strategies to modulate the immunological response include special key nutrients such as the amino acid glutamine favoring the Th2 response. There is no information available concerning its effect in patients undergoing cardiac surgery. The aim of this clinical study was to evaluate the effects of a perioperative infusion of glutamine on the polarized lymphocyte T cell cytokine expression and on infectious morbidity in cardiac surgery patients at risk of infection. Seventy-eight patients were included in the study undergoing elective cardiac surgery with a lymphopenia less than 1.2 giga/l. One or more of the following criteria had to be met: age older than 70 years, ejection fraction less than 40%, or mitral valve replacement. We randomly assigned patients to receive infusions of either high-dose L-alanyl-L-glutamine dipeptide [0.5 g/(kg day) glutamine] dissolved in an amino acid solution or an isonitrogeneous, isocaloric, isovolemic nutritional solution. An additional group with normal saline served as control to eliminate any nonspecific nutritional effect. We started the infusion after induction of anesthesia with 1,000 ml/24 h and continued it for 3 days. The primary endpoint was intracellular T cell cytokine expression (including the description in tertiles) on the first postoperative day (pod 1). Secondary endpoints were postoperative infection rate, mortality rate, cardiovascular circulation ventilation time, and renal function. A high-dose perioperative glutamine application leading to mean plasma levels of 1,177 μM had only a minor influence on the polarized intracellular T cell cytokine expression. On pod 1 there was a polarization of T cells, i.e., an augmented Th2 response with an increased number of IL-6 and IL-10 producing cells. On the other side the Th1 response with IL-2 and TNF-α declined on pods 1 and 2. Only the intracellular IL-2 response in the lower tertile of IL-2 production was improved with glutamine indicating a small influence. We did not observe any effects on the numbers of postoperative infections; on mortality rate; on cardiovascular circulation; on ventilation time or on renal function. The elevation of glutamine plasma levels by a perioperative intravenous infusion of L-alanyl-L-glutamine influenced the intracellular expression of IL-2 in the lower tertile only slightly. However, mean glutamine values in the other groups remained above or close 500 μM, thus suggesting that glutamine supply to the immune cells was still adequate in most patients, and that glutamine deficiency, if it occurred, was marginal. In the event of a severe glutamine deficiency the observed effect on cytokine production could be more pronounced. Furthermore, we could not observe any obvious clinical advantage in this at risk cardiac surgical patient population. A glutamine supplementation for patients undergoing cardiac surgery without a clear glutamine deficiency is not recommended.

Keywords Glutamine · Cardiac surgery · T helper cells · Infection

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Introduction

Patients undergoing cardiac surgery with cardiopulmonary bypass have increased postoperative morbidity and mortality. The host defense is compromised by nutritional deficiencies, hypoperfusion, hypothermia, anesthesia, and surgical trauma. This reservation is caused by a changed reaction of cell-mediated components (Markewitz et al. 1993a, b). In this process lymphocytes play a special role. Cytokines produced by T helper (Th) cells are of critical importance for the outcome of many infectious diseases. Th1 cells, which produce interferon (IFN)-γ and interleukin (IL)-2, favor cell-mediated immunity and phagocyte-dependent inflammation. Th2 cells, which secrete IL-4 and IL-10, evoke humoral immunity, but inhibit several functions of phagocytic cells. Both environmental and genetic factors act in concert to determine the Th1 or Th2 polarization (Iwasaka and Noguchi 2004; Romagnani 2000).

A polarization of Th activity with a clear shift in Th2 direction may lead to immunosuppression and a decreased eradication of infectious agents (Iwasaka and Noguchi 2004). Postoperatively after cardiac surgery a decreased Th1 response with diminished IL-2 and IFN-γ secretion was observed (Markewitz et al. 1996). Simultaneously an increased Th2 response with IL-4 and IL-10 production was found (Muret et al. 2000).

Therapeutic strategies to modulate the immunological response include specific interventions but also special key nutrients are possible. It was speculated (Laffey et al. 2002) that the use of glutamine supplementation to improve patients outcome after cardiac operations could be beneficial. However, the beneficial role of glutamine has been established in other groups of postoperative and critically ill patients. There is no information available concerning the effect in patients undergoing cardiac surgery.

Glutamine, a nonessential amino acid, can be synthesized in the cytosol of most of the cells of the organism and is the most abundant free amino acid in the body. After major surgery (Jensen et al. 1996), after multiple trauma and in sepsis (Roth et al. 1982) decreased glutamine plasma levels were observed (van der Hulst et al. 1993). Decreased glutamine levels are associated with an increased infectious morbidity and at levels below 420 μM with higher hospital mortality (Oudemans-van Straaten et al. 2001). There is a speculation that this can be explained (among other things) by a diminished supply of glutamine to lymphocytes and macrophages. A relationship between glutamine concentration and function of lymphocytes and macrophages has been described (Newsholme and Calder 1997). Glutamine had no effect on the production of IL-1, but influenced the production of IL-2 and IFN-γ in an experimental setting (Rohde et al. 1996; Yaqoob and Calder 1997) such suggesting an amelioration of the Th2 response after surgery.

The aim of this clinical study was to evaluate the effects of a perioperative infusion of glutamine on the polarized lymphocyte T cell cytokine expression (IL-2, IFN-γ, IL-4, IL-10) in at risk cardiac surgical patients. Furthermore, we observed the consequences on the incidence of infection.

Patients and methods

We included patients undergoing elective cardiac surgery with cardiopulmonary bypass and a lymphopenia less than 1.2 giga/l. One or more of the following criteria has to be met: age older than 70 years, ejection fraction less than 40%, or mitral valve replacement. Exclusion criteria were: age below 21 years, pregnancy, corticoid or immunosuppressive therapy, nonsteroidal antiinflammatory medication, autoimmune disease, malignancy, severe hepatic (GOT $>$ 150 U/l, GPT $>$ 150 U/l, CHE $<$ 1,000 U/l) or renal failure (creatinine $>$ 2 mg/dl, urea $>$ 180 mg/dl), and diabetes mellitus. All patients gave their written consent. Approval for this study was obtained from the local ethics committee (Az 41/03).

Patients were randomly and blindly assigned to receive parenterally either high-dose glutamine [0.5 g/(kg day); L-alanyl-L-glutamine dipeptide, Dipeptamin®, Fresenius Kabi Pharmaceuticals, Bad Homburg) dissolved in an amino acid solution or an isonitrogeneous, isocaloric, isovolemic nutritional solution (Aminoplasmal®-10%, B. Braun Melsungen AG) (Table 1). Randomization and preparation of the solutions were done by a person not involved in the study. We started the administration after induction of anesthesia with a dosage of 1,000 ml/24 h. Infusion was continued until recovery from the acute phase after 3 days. A group of 20 patients with normal saline served as a control to eliminate any nonspecific nutritional effect.

The primary endpoint was the intracellular T cell cytokine expression on the first postoperative day (pod 1) (including the description in tertiles because production varies between healthy persons due to genetic origin).

Table 1 Composition of perioperative nutritional diets

	Glutamine	Amino acids	Control
Total Protein (g/kg)	0.835	0.835	–
Glutamine (g/kg)	0.5	–	–
Alanine (g/kg)	0.248	0.114	–
Aminoplasmal®-10% (g/kg)	0.1	0.835	–
Glucose (g/kg)	0.4	0.4	–
Volume (ml)	1,000	1,000	1,000

Secondary endpoints were postoperative infection rate, mortality rate, length of hospital stay (hospital LOS), and renal function (creatinine, urea).

After oral premedication with 2 mg flunitrazepam and with 30 mg morphine, intravenous induction of anesthesia was performed with sufentanil (0.2–0.3 μg/kg), midazolam (0.05–0.1 mg/kg) and pancuronium (0.1 mg/kg). All patients were orotracheally intubated and ventilated with volume-control (Servo 900C, Siemens, Erlangen, Germany) with a $paCO_2$ of 34 and an orotracheal pressure of 44 mmHg. For maintenance of anesthesia, propofol and sufentanil were given continuously. An antibiotic prophylaxis was carried out with 2 g cephazoline. The patients were anticoagulated with 300 U/kg heparin before the beginning of cardiopulmonary bypass (CPB). CPB was performed with moderate hypothermia (rectal temperature 33°C) and non-pulsatile perfusion [2.4 l/(min m^2)] with a monoatriale non-pulsatile canula. Systemic blood pressure was set at 50–80 mmHg. Inotropes were not administered routinely but only when indicated either based on the observation of reduced cardiac contractility during or after coming off bypass by direct visual inspection of the right ventricle and/or transoesophageal echocardiography or after measuring a reduced cardiac index [<2.0 l/(min m^2) measured by thermodilution], or both.

Infections were defined according to the criteria of the US Centers for Disease Control and Prevention (Garner et al. 1988). Mortality was defined as hospital mortality rate. At 8:00 a.m. plasma creatinine and urea was measured in the central lab.

Blood samples were collected preoperatively prior to anesthesia, postoperatively at the end of surgery, on pod 1, on pod 2, and on pod 3 at 8:00 a.m. Whole blood was collected in sodium heparin tubes, processed and analyzed directly with a FACS fluorescence-activated cell sorter. Each blood sample was diluted at 1:1 in a glutamine-free RPMI-1640 culture medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Whole blood was stimulated with phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and ionomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in the presence of a secretion inhibitor, Brefeldin A (BFA, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After a stimulation period of generally six hours at 37°C in humidified atmosphere and 5% CO2, the lymphocytes were fixed and erythrocytes lysed simultaneously. The cells were then washed and permeabilized. Finally, intracellular antigens (IL-2, IFN-γ, IL-4, IL-10) were stained with fluorescence conjugated monoclonal antibodies (Becton-Dickinson, Heidelberg, Germany), followed by flow cytometry analysis with FACSCalibur (Becton-Dickinson, Heidelberg, Germany). Lymphocytes were gated by their specific localization in the forward/sideward scatter diagram. The T cell population was identified by the expression of CD3, verification of T cell activation by expression of CD69 before and 6 h after activation with PMA and ionomycin. Data from at least 10,000 T cells were gathered for each sample and fluorescence intensity was calculated using using CELLQUEST TM software (Becton-Dickinson, Heidelberg, Germany).

Blood for glutamine estimation was immediately immersed in melting ice (4°C) and centrifuged at 1,500 r.p.m for 10 min and stored at −80°C until glutamine analysis. Samples were prepared and glutamine concentrations were measured as described earlier (Mühling et al. 1999) using a fluorescence high-performance liquid chromatography system (F-HPLC).

Statistics

The IL-2 production on pod 1 and the infection rate were taken as the principal end points. In order to refute the null-hypothesis for both variables, the sample size has to be the maximum of the estimation for IL-2 production and for the infection rate. We proceeded from the assumption that the mean fluorescence intensity (MFI) of IL-2 was 45 and a relevant difference was 20%. At an observed standard deviation of 10, an α of 0.05, and a power of 0.8 we calculated an $n = 27$ for each group.

The descriptive presentation of the results was performed using arithmetic mean, standard deviation, and tertiles. Data was tested for normal distribution with the Shapiro–Wilk-Test. Comparisons between the groups were examined using ANOVA with post-hoc Scheffé test as well as the χ^2 test. Questions about the timeline were answered using repeated measurements ANOVA. A P value less 0.05 corrected according to Bonferroni was considered significant. The statistical analysis was performed with the programm SPSS for windows version 11.0.1 in the Statistics Bureau MoreData, Giessen, Germany.

Results

Seventy-eight patients were included in the study after testing for the inclusion and exclusion criteria (Fig. 1). Table 2 shows the baseline characteristics of the patients. All patients received all of their prescribed nutritional solution. The perioperative nutrition was well tolerated. There were no signs of excessive hyperglycemia or of increased insulin demand. Also creatinine and urea revealed no adverse effects. Table 3 shows operation characteristics of the patients.

Administration of L-alanyl-L-glutamine effected a significant increase of plasma glutamine levels at the end of surgery and on pod 1 ($P = 0.001$). Plasma levels in this

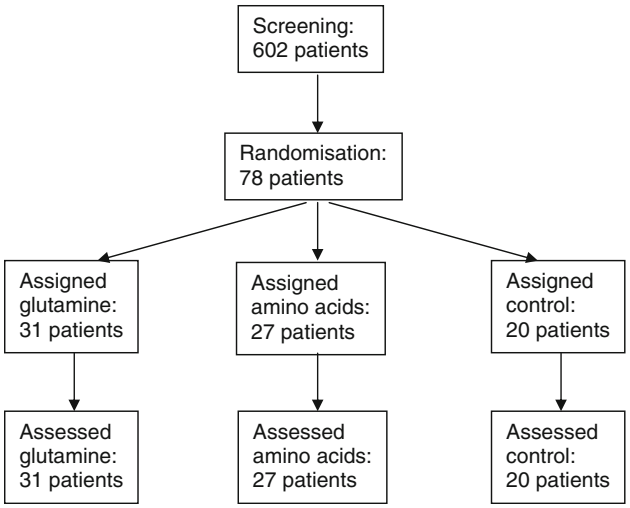

Fig. 1 Trial profile

group were higher postoperatively until pod 3 compared to the both other groups (Fig. 2).

At the beginning of the study there was no difference in the measured intracellular interleukines between the groups. Th1 response represented by IL-2 producing cells prior to anesthesia was: glutamine 44.3 ± 18.2, amino acids 44.8 ± 15.0, and control 45.4 ± 17.9. Between the groups, the means showed no significant differences over the five perioperative time points (Fig. 3a). However, on pod 1 the values decreased significantly ($P < 0.001$) to 38.5 ± 17.2 (glutamine), 35.1 ± 13.8 (amino acids), and 33.9 ± 12.1 (control). A recovery of the function was observed until pod 3. Considering the lower tertile of IL-2 production the values decreased on pod 1 in all groups. However, with glutamine IL-2 producing T cells were higher (34.1 ± 7.3) compared to amino acids (23.2 ± 3.9) and control (21.7 ± 7.6) (Fig. 3b). In the middle and higher tertile of IL-2 production no differences between groups were observed.

The Th1 response with IFN-γ showed no differences between groups with glutamine, amino acids and control. Furthermore over time, no changes to preoperative IFN-γ production was seen (Fig. 4). The lower, middle, and higher tertiles revealed no differences.

The Th2 response with IL-4 showed no differences between groups (Fig. 5). A decrease in the glutamine group from 5.8 ± 1.5 to 4.6 ± 1.6, in the amino acids group from 5.8 ± 2.4 to 4.8 ± 1.3, and in the control group from 5.8 ± 1.6 to 4.8 ± 1.2 at pods 2–3 was obvious. The testing of the tertiles revealed no differences between the groups.

Furthermore the Th2 response with IL-10 showed no differences between groups (Fig. 6). From the end of the surgery to pod 1 IL-10 producing cells increased significantly: glutamine to 5.8 ± 2.1, amino acids to 5.8 ± 2.0, and in the controls to 5.7 ± 2.3. On pod 2 the glutamine values decreased to 5.1 ± 2.2, amino acids to 5.0 ± 2.5, and control to 5.0 ± 2.0. The tertiles revealed no differences between the groups.

In the glutamine group one out of 31 patients suffered from a pulmonary infection, however, none of the 27 patients with amino acids did so (Table 4). In the control group two out of 20 patients had bronchopulmonary infectious problems. One patient died in the control group because of myocardial insufficiency with development of multiorgan failure. There was no difference in the three groups concerning hospital LOS and the renal function (Table 4).

Discussion

Our results demonstrate that a high-dose perioperative glutamine administration has only a minor influence on the polarized intracellular T cell cytokine expression in at risk patients undergoing cardiac surgery. We could not observe any effect on the numbers of postoperative infections, on mortality rate, on hospital LOS, or on renal function.

L-Alanyl-L-glutamine is hydrolyzed to the single amino acids in circulating blood by the activity of plasma and membrane bound hydrolases. In this way glutamine plasma levels can be significantly increased. Glutamine plasma levels were with L-alanyl-L-glutamine postoperatively (1,062 ± 434 μM) and on pod 1 (866 ± 379 μM) significantly higher than in the amino acid (502 ± 145 μM) or the control group (498 ± 117 μM).

Table 2 Characteristics of patients

	Glutamine, $n = 31$	Amino acids, $n = 27$	Control, $n = 20$	P-value
Age (years)	71.3 ± 7.4	69.5 ± 9.1	69.5 ± 7.5	0.343
Sex				
Male	21	15	17	0.488
Female	10	12	4	0.509
Body mass Index (kg/m^2)	26.4 ± 3.2	26.2 ± 3.5	27.1 ± 4.3	0.676
ASA	3.1 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	0.101
EF (%)	57.4 ± 11.8	59.5 ± 11.6	53.4 ± 17.3	0.295

ASA Score of the American Society of Anesthesiologists, *EF* ejection fraction

Table 3 Operation characteristics

	Glutamine, $n = 31$	Amino acids, $n = 27$	Control, $n = 20$	P-value
Operation time (min)	213 ± 67	222 ± 70	229 ± 67	0.795
Extracorporeal circulation time (min)	104 ± 42	113 ± 46	120 ± 41	0.414
Aortic cross clamp time (min)	68 ± 23	71 ± 30	72 ± 28	0.851
Operation				
CABG (*n*)	15	13	15	0.996
AVR (*n*)	8	5	2	0.173
MVR (*n*)	0	1	0	0.991
ACB + AVR (*n*)	5	4	4	0.996
ACB + MVR (*n*)	3	2	0	0.263
AVR + MVR (*n*)	0	2	0	0.497
Blood glucose (maximum) (mmol/l)	8.8 ± 2.7	9.5 ± 3.3	8.1 ± 2.7	0.261
Blood lactate (maximum) (mmol/l)	2.2 ± 1.1	2.3 ± 1.3	1.7 ± 0.5	0.127
Insulin (I.U.)	0.6 ± 2.4	2.3 ± 6.0	2.7 ± 5.3	0.219
RBC (*n*)	1.6 ± 1.8	1.7 ± 2.3	1.2 ± 1.7	0.659
Fresh frozen plasma (*n*)	0.6 ± 1.2	0.8 ± 1.7	0.4 ± 1.1	0.604
Aprotinine (Mio E.)	0.9 ± 0.9	0.8 ± 0.8	1.0 ± 1.0	0.744
Cristalloids (ml)	1,758 ± 693	1,685 ± 574	2,109 ± 887	0.105
Colloids (ml)	387 ± 264	350 ± 268	435 ± 265	0.548
Fluid balance (ml)	984 ± 1,067	950 ± 1,119	1,302 ± 970	0.466

CABG coronary artery bypass graft, *AVR* aortic valve replacement, *MVR* mitral valve replacement, *RBC* red blood cells

The high-dose perioperative glutamine administration has no significant influence on IL production. On pod 1 there was a polarization of T cells in that there was an augmented Th2 response with an increased number of Il-4 and IL-10 producing cells. On the other side the Th1 response with IL-2 and IFN-γ declined predominantly on pod 1. Only the intracellular IL-2 response in the lower tertile of IL-2 production was improved, with glutamine indicating a small influence.

Other authors observed distinct effects of glutamine on IL production. Isolated lymphocytes were incubated in culture medium, either with no glutamine or a supraphysiological concentration of 2,000 µM (Newsholme 2001; Yaqoob and Calder 1997). The influence was mainly on IL-2 (Newsholme 2001). Also an additional influence on IFN-γ and IL-10 was noticed with 100 or 300 µM as opposed to a glutamine-free medium (Rohde et al. 1996). The main differences in our results were: that these were studies with isolated cells of healthy persons in an in vitro investigation. The physiological glutamine concentration in blood of 500–800 µM was not considered. Isolation of the cells can potentially stimulate the cells ex vivo leading to a change in the percentage of different parts of the cell subpopulations (Bossuyt et al. 1997). Therefore, false high cytokine levels may be measured. Also, the stimulating agents may play a crucial role. Depending on the method and duration of the stimulation the results of the measured IL can differ (Murphy et al. 2003). The combination of PMA and ionomycin is considered as reliable stimulation for the lymphocytes (Murphy et al. 2003).

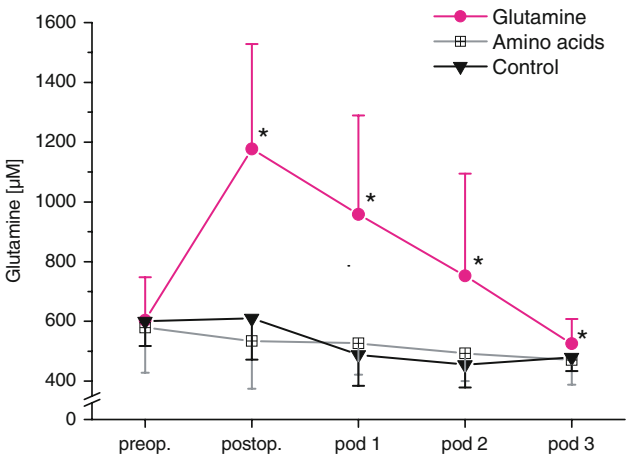

Fig. 2 Perioperative glutamine plasma levels. *$P \leq 0.05$

The effects of glutamine on lymphocyte function were explained in that this amino acid is a main energy supplier. However, only a small amount of glutamine is completely oxidized even though all the components of the citrate cycle are available. This partial oxidation may be essential to deliver a high turnover of precursors for the purine and pyrimidine synthesis and ultimately for DNA and RNA synthesis (Newsholme and Parry-Billings 1990). When activated, the immune cells are able to react more quickly for protein synthesis, e.g., for building of cytokines.

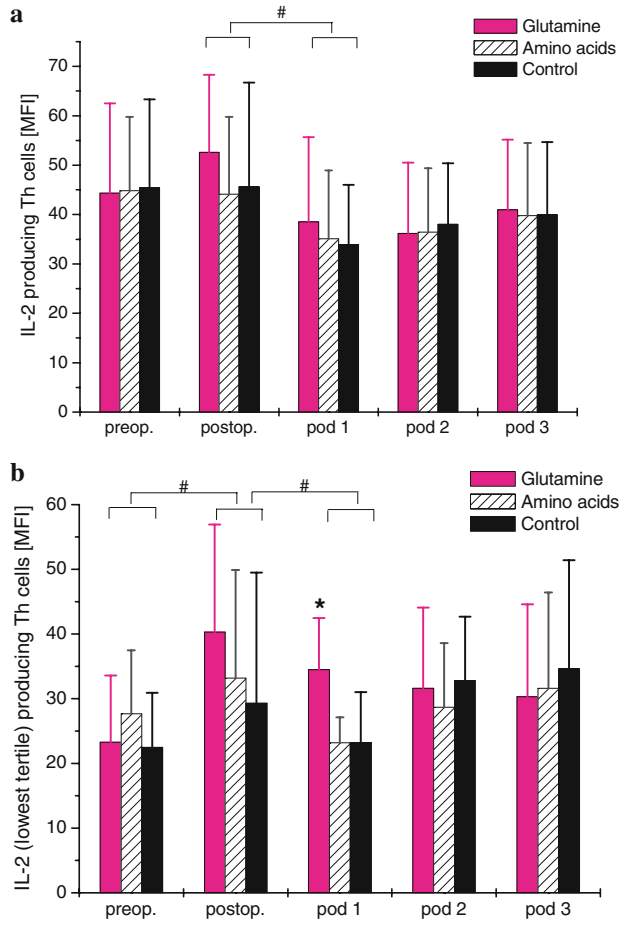

Fig. 3 **a** IL-2 producing Th-cells. #$P \leq 0.05$ (in course). **b** IL-2 (lowest tertile) producing Th-cells. *$P \leq 0.05$ (between groups) #$P \leq 0.05$ (in course)

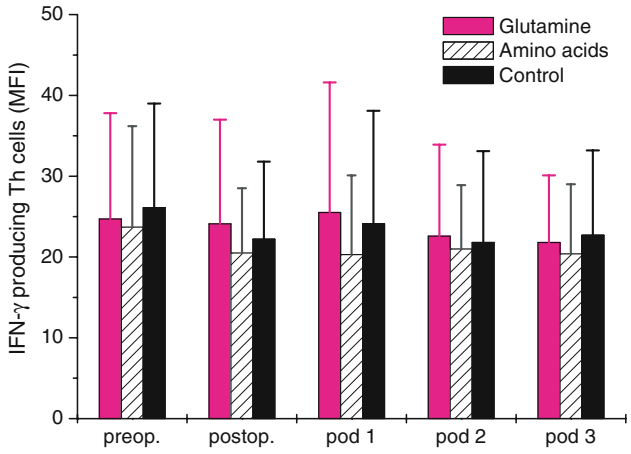

Fig. 4 IFN-γ producing Th-cells

The Th2 response in cardiac surgery could be interpreted as physiological as most of the products of extracorporeal circulation represent extracellular antigens which are eliminated by a Th2 mediated response (Romagnani 1997).

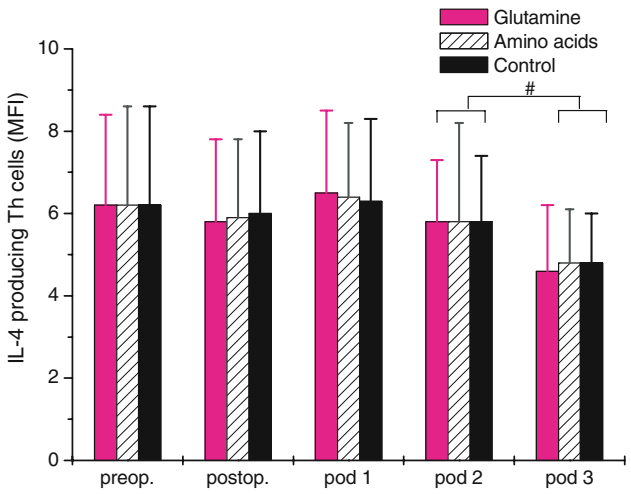

Fig. 5 IL-4 producing Th-cells. #$P \leq 0.05$ (in course)

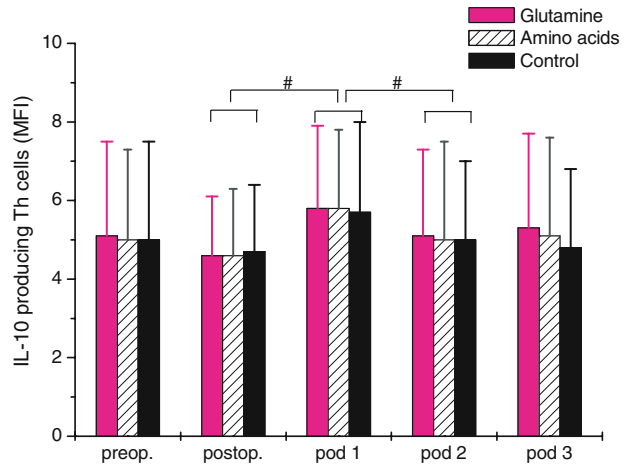

Fig. 6 IL-10 producing Th-cells. #$P \leq 0.05$ (in course)

This suggests an unremarkable clinical picture, which does not reflect the immunological changes (Markewitz et al. 1993a). A decrease of the Th1/Th2 balance with a Th2 dominance may lead to immunosuppression (Iwasaka and Noguchi 2004). This condition is significant when the Th1 response is poorly effective or exhaustively prolonged (Romagnani 1997). Host damage can be the result because a Th1-dominated responses are potentially effective in eradicating infectious agents. Infections in cardiac surgery are frequently caused by opportunistic bacteria. In 10% of cardiac surgery, the patients aggravated septic reactions up to and including multiorgan failure (Markewitz et al. 1993b).

We found neither an influence on infectious morbidity nor on mortality rate or hospital LOS and renal function. In the literature, the decrease of plasma glutamine after severe trauma or other stress and the simultaneous increased infectious morbidity raise the question of a causal relationship (Houdijk et al. 1998). On the contrary, studies

Table 4 Clinical variables

	Glutamine, $n = 31$	Amino acids, $n = 27$	Control, $n = 20$	P-value
Infections (n)	1	0	2	0.558
Mortality (n)	0	0	1	0.403
Hospital LOS (days)	9.1 ± 2.8	9.1 ± 3.2	9.2 ± 1.8	0.989
Creatinine (mg/dl)				
Preoperative	1.1 ± 0.3	1.1 ± 0.4	1.0 ± 0.2	0.469
Postoperative	1.0 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	0.441
Pod 1	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	0.999
Pod 2	1.1 ± 0.3	1.2 ± 0.8	1.2 ± 0.4	0.264
Pod 3	1.2 ± 0.4	1.4 ± 1.0	1.2 ± 0.6	0.252
Urea (mg/dl)				
Preoperative	43 ± 16	52 ± 25	42 ± 8	0.098
Postoperative	49 ± 13	53 ± 18	45 ± 9	0.154
Pod 1	58 ± 18	54 ± 24	48 ± 9	0.171
Pod 2	71 ± 24	66 ± 30	59 ± 21	0.256
Pod 3	70 ± 26	83 ± 43	63 ± 21	0.089

LOS length of stay, *pod* postoperative day

have shown that glutamine supplementation decreases infectious morbidity, hospital length of stay, and an improves long-term prognosis (Griffiths et al. 2002; Houdijk et al. 1998). For trauma patients glutamine has been shown to decrease the incidence of pneumonia, bacteremia, and sepsis (Houdijk et al. 1998). The effect of the postoperative administration is time and dose dependent (Furst 2001). An early start is important to avoid glutamine shortage. Tepaske et al. were able to improve host defense and decrease the rate of postoperative infectious complications with a preoperative oral application of a mixture of immunonutrients in cardiac surgical patients (Tepaske et al. 2001).

A relationship between glutamine concentration and function of lymphocytes and macrophages has been described. Several other mechanisms possibly explaining the improvement of clinical outcome should be discussed, including the induction of heat-shock proteins (Wischmeyer et al. 2003), the stimulation of lung secretory immunoglobulin A (Lai et al. 2004), antioxidant effects (Heyland et al. 2007) and modulation of insulin resistance (Dechelotte et al. 2006). In addition, glutamine may improve nitrogen balance (Garcia-de-Lorenzo et al. 2003) and intestinal protein metabolism (Coeffier et al. 2003). Because enterocytes consume large amounts of glutamine in stress, a supplementation may protect the intestinal barrier from mucosal atrophy and from a consecutive increased translocation rate. Indeed, glutamine decreased intestinal permeability in stressed patients (van der Hulst et al. 1993). In this study only 0.05 g/kg glutamine was infused. A meta-analysis of Novak et al. showed (Novak et al. 2002) that a minimum of 0.2 g/(kg day) is necessary to achieve an effect. Heyland et al. (2003) suggests that glutamine below 20 g/day is insufficient to influence any clinical outcomes. Therefore, the dosage in our study does not explain the fact that no positive effects were observed.

The positive effects of glutamine in patients (improvement of infectious morbidity and reduction of length of stay) observed in the meta-analysis of Novak et al. (2002) arise exclusively from gastrointestinal major surgery with a high risk of intraoperative bacteremia. In addition, these patients are not able to eat normal food for several days postoperatively and may, therefore, have a loss of glutamine. The combination of a high infectious risk with a high protein loss is also present in burn patients. In addition, these patients seem to profit from a glutamine supplementation (Wischmeyer et al. 2001). It appears, therefore, that only those cardiac surgical patients who have a preexisting glutamine deficiency combined with a high risk of infection would benefit from perioperative glutamine supplementation. However, until now, it was not possible to define a glutamine deficiency syndrome (Tapiero et al. 2002). The validity of plasma glutamine estimation comes into question since the level is maintained as normal at the expense of the glutamine stores. Of course, a major loss in glutamine stores is eventually reflected by a decrease in plasma levels. Glutamine plasma levels below 420 μM are associated with an increased mortality rate in intensive care patients (Oudemans-van Straaten et al. 2001). In our observation mean glutamine values remained above or close 500 μM, thus suggesting that glutamine supply to the immune cells was still adequate in most patients, and that glutamine deficiency, if it occurred, was marginal. However, for the assessment of the host defense the intracellular levels are more accurate than the plasma levels (Engel et al. 2003). The circulating immune cells, for example the

neutrophils, actively increase glutamine uptake. Adaptive increases in transport were observed in glutamine-deprivation (Wasa et al. 2002). Thus, transport mechanisms on a cellular level may function sufficiently over a wide range to cover cellular requirements. Therefore, to monitor glutamine deficiency over time is both logistically difficult and expensive.

A limitation of our study is the fact that, at planning, a higher infection rate than 10% was observed. This may be due to an episode of increased sternal infections from several causes. We found no unambiguous explanation. For this reason it can be debated whether our sample size has a sufficient statistical basis. Our observed infections rate was low being less than 10% compared with 54% in the control group of another study using immunonutrition in cardiac surgical patients (Tepaske et al. 2001). The data on patients and operations in both studies were comparable.

In summary the elevation of glutamine plasma levels by a perioperative intravenous infusion of L-alanyl-L-glutamine influenced the intracellular expression of IL-2 only slightly in the lower tertile of IL-2 production. In the case of a severe glutamine deficiency this effect may be more pronounced. We could not observe any obvious clinical advantage in this group of at risk cardiac surgical patients. The infection rate, at less than 10%, was comparatively low. A glutamine supplementation for all patients undergoing cardiac surgery without a clear glutamine deficiency is not recommended.

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