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Effects of Remifentanil and Fentanyl on the Cell-mediated Immune Response in Patients Undergoing Elective Coronary Artery Bypass Graft Surgery

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This prospective randomized pilot study compared the influence of fentanyl-based versus remifentanil-based anaesthesia on cytokine responses and expression of the *suppressor of cytokine signalling (SOCS)-3* gene following coronary artery bypass graft surgery. Forty patients were assigned to receive anaesthesia with either intravenous remifentanil (0.3 – 0.6 µg/kg per min; *n* = 20) or intravenous fentanyl (5 – 10 µg/kg per h; *n* = 20). Levels of interleukin (IL)-6, IL-10, tumour necrosis factor-α and interferon-γ (IFN-γ) and the expression of *SOCS-3* were measured pre- and post-operatively. The data from 33 of the patients were analysed. The IFN-γ/IL-10 ratio after concanavalin A stimulation in whole blood cells on post-operative day 1 and *SOCS-3* gene expression on post-operative day 2 were significantly lower in the remifentanil group than in the fentanyl group. The time in the intensive care unit was also significantly lower in the remifentanil group. These findings suggest that remifentanil can attenuate the exaggerated inflammatory response that occurs after cardiac surgery with cardiopulmonary bypass. Further clinical trials are required to define the influence of choice of intra-operative opioid on post-operative outcome.

KEY WORDS: Opioid; Inflammation; Cardiopulmonary bypass; Suppressor of cytokine signalling-3; Cell-mediated immune response

Introduction

Cardiac surgery with cardiopulmonary bypass (CPB) may induce a systemic inflammatory response syndrome (SIRS) associated with an unbalanced release of cytokines.[1,2] Cytokines are one of the dominant factors guiding the development of T-helper type 1 and 2 (Th1 and Th2) cells,[3,4] which play a major role in inflammatory responses.[4,5] An early Th1 response after cardiac surgery has been hypothesized to support the inflammatory reaction by increasing the cytokines interleukin (IL)-2, IL-12 and interferon-γ

(IFN-γ).[4] Furthermore, suppressor of cytokine signalling (SOCS)-3 protein is induced by cytokines and acts as a feedback inhibitor of cytokine receptor signalling.[5,6] It also can act as a negative regulator of $CD4^+$ T-cell differentiation.[7]

An exaggerated and prolonged activation of the immune system after cardiac surgery can lead to an increased risk of post-operative complications and a prolonged intensive care unit (ICU) stay.[1,8] Up to 36% of cardiac surgery patients require prolonged ICU care, which is associated with multi-organ failure and a higher mortality.[9] Hein *et al.*[10] demonstrated a significantly increased ICU and in-hospital mortality rate in patients with ICU stays > 3 days.

Apart from their neurohumoral effects, opioids are known to stimulate cell-mediated immune responses via opioid receptors on different immune cells.[11] Modulation by μ-receptor agonists has been demonstrated in a range of immune cells, including macrophages, monocytes, natural killer cells and T-cells.[12,13] Morphine inhibits both *in vivo* and *in vitro* T-cell proliferation and reduces IL-2 cytokine synthesis significantly. Exposure to morphine can result in modulation of expression of a wide range of specific genes, in particular down-regulation of pro-inflammatory gene expression.[13] These effects are associated with a significant reduction in the expression of IL-2 mRNA, but the exact mechanism is unknown.[13] Furthermore, Murphy *et al.*[14] demonstrated that morphine suppressed several components (IL-6, CD11b, CD18 and post-operative hyperthermia) of the inflammatory response to cardiac surgery and CPB compared with fentanyl. For anaesthesia during cardiac surgery the μ-agonists, fentanyl and remifentanil, are commonly used; remifentanil is widely used for fast-track cardiac surgery due to its short context-sensitive half-life, permitting more rapid emergence than fentanyl.[15]

Although fentanyl and remifentanil have been studied individually, the cell-mediated immune response and cytokine signalling effects of these two opioids have not been compared in a clinical study. The primary aim of this study was, therefore, to investigate the influence of fentanyl-based and remifentanil-based anaesthesia on the Th1/Th2 ratio, cytokine levels and *SOCS-3* gene expression in patients undergoing cardiac surgery with CPB. The secondary aim was to evaluate whether any of these immune parameters might be of clinical relevance with respect to the post-operative infection rate or the duration of ICU stay.

Patients and methods

PATIENTS

Patients undergoing elective coronary artery bypass graft (CABG) surgery aged > 18 years, with a left ventricular ejection fraction > 40% on pre-operative transthoracic echocardiography, a left ventricular end-diastolic pressure < 17 mmHg and no pre-existing pulmonary disease (determined by clinical examination, chest radiography, lung function tests and blood gas analysis) were enrolled in the study. All patients were treated pre-operatively with β-blockers. Exclusion criteria included pregnancy, unstable angina pectoris or acute myocardial infarction requiring emergency surgical intervention, any history of impairment of the immune system, immunosuppressive therapy, signs of pre-existing infection (white blood cell count > 12 000/μl, body temperature > 38 °C, C-reactive protein > 5 mg/dl), liver insufficiency (> Child B) or end-stage renal disease. Additionally, patients who required off-pump cardiac surgery or hypothermic bypass, patients with increased

catecholamine requirements before, during or after surgery (noradrenaline > 0.15 µg/kg per min, dopamine > 10 µg/kg per min), patients who received massive transfusions (> 4 units of allogeneic red cells) as well as patients requiring re-thoracotomy and CBP for a second time were excluded from the data analysis.

Written informed consent was obtained from all the study participants. The study protocol was approved by the institutional review board of the Charité-Universitaetsmedizin Berlin (Berlin, Germany).

ANAESTHESIA

All patients received oral pre-medication with 1 – 2 mg flunitrazepam the evening before surgery and 0.07 – 0.1 mg/kg of oral midazolam 30 min before transfer to the operating theatre. Anaesthesia was induced with midazolam (1 – 4 mg), fentanyl (4 – 7 µg/kg) and etomidate (0.15 – 0.3 mg/kg), with pancuronium bromide (0.1 – 0.15 mg/kg) for muscle relaxation. Anaesthesia was maintained with sevoflurane 1.0 – 2.0 vol% in all patients, the depth of anaesthesia being guided by the bispectral (BIS) index.[16] BIS electrodes (BIS™, Aspect Medical Systems Inc., Natick, MA, USA) were applied according to the manufacturer's instructions and BIS™ A1050 monitoring commenced prior to induction of anaesthesia.

Patients were randomized to receive either fentanyl or remifentanil. Fentanyl (5 – 7 µg/kg per h) and remifentanil intravenous infusions (0.3 – 0.6 µg/kg per min) were started after induction of anaesthesia. The fentanyl infusion was stopped at the end of surgery, whereas remifentanil infusion continued at a dose of 0.05 – 0.15 µg/kg per min in the ICU for at least 2 h after surgery. All patients remained intubated and mechanically ventilated for transfer and for at least 2 h in the ICU in accordance with our published standard operating procedures for cardiac surgery.[17] During this time, patients in both groups were sedated using a propofol infusion (2 – 3 mg/kg per h). The infusion was titrated to a BIS index of 55 – 75. In both groups, post-operative analgesia was provided by piritramide 3 – 5 mg intravenously and metamizol (2 g in a short intravenous infusion). A 5 mg piritramide intravenous bolus was administered 30 min before stopping the remifentanil infusion.

INTRA-OPERATIVE PROCEDURES

Intra-operatively patients were ventilated with a tidal volume of 6 ml/kg, with the respiratory rate adjusted to achieve an end-tidal carbon dioxide level of 32 – 34 mmHg. Normoventilation was confirmed by hourly arterial blood gas tension measurements and continuous measurement of the end-expiratory carbon dioxide concentration. Prophylactic cefuroxime (1.5 g) was administered after induction of anaesthesia, after weaning from CPB and 6 h after admission to the ICU in accordance with standard practice. Quadrox® membrane oxygenators (Jostra, Hirlingen, Germany) were used for normothermic, non-pulsatile CPB as described elsewhere.[18]

In accordance with the institutional standard operating procedures of the Department of Anaesthesiology and Intensive Care (Charité-Universitaetsmedizin, Campus Mitte, Berlin, Germany) dopamine and glycerol trinitrate were used as required during weaning from CPB. Red blood cell transfusions were given to maintain haematocrit levels above 23 – 24% during CPB. Continuous three-lead (II, aVL and V5) automated ST segment analysis was used to detect intra-operative myocardial ischaemia, defined as ST segment depression > 1 mm or elevation > 2 mm at 60 ms after

the J point and persisting for ≥ 2 min. Transoesophageal echocardiography was used according to our published standard operating procedures.[17]

POST-OPERATIVE PROCEDURES

Patients were weaned from mechanical ventilation as soon as haemodynamic stability and normothermia had been established and blood loss was satisfactory (< 100 ml/h), in accordance with our published standard operating procedures.[17] Patients were extubated once awake, co-operative and successfully weaned.[19]

Heart rate, mean arterial pressure, central venous pressure, oxygen saturation and blood temperature were monitored continuously. A 12-lead electrocardiogram was performed on admission to the ICU and daily for 2 days after surgery.

SIRS was diagnosed according to the recommendations of the American College of Chest Physicians/Society of Critical Care Medicine.[20] The Acute Physiology and Chronic Health Evaluation (APACHE) II score,[21] the Sepsis-related Organ Failure Assessment (SOFA) score[22] and the length of stay in the ICU or intermediate care unit were recorded. A prolonged ICU stay was defined as > 72 h, as previously described.[10] All infections were diagnosed according to criteria recommended by the Centers for Disease Control and Prevention.[23,24]

BIOCHEMICAL ANALYSIS

Routine laboratory parameters, including haemoglobin, haematocrit, white blood cell count, C-reactive protein, bilirubin, creatinine, platelet count and coagulation were measured pre-operatively and on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery). Procalcitonin (PCT) plasma levels were measured 2, 6 and 12 h after surgery as well as on post-operative days 1 and 2.

MEASUREMENT OF CYTOKINE RELEASE

Blood samples were drawn pre-operatively and on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery).

Ex vivo *lymphokine secretion by whole blood cells*

To determine cytokine secretion by whole blood cells, peripheral mononuclear cells (1×10^6 cells/ml) in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine were stimulated with 100 µg/ml whole blood concanavalin A (Con A) (Serva, Heidelberg, Germany) for 24 h. Levels of IFN-γ, TNF-α, IL-2 and IL-10 were then measured in the supernatant by enzyme-linked immunosorbent assay (ELISA; Biosource, Camarillo, CA).

Plasma cytokines

In addition, plasma cytokines IL-6 and IL-10 were analysed using a sandwich ELISA kit (Immunotech [Beckman Coulter Company], Marseille, France). Detection limits (ethylenediaminetetraacetic acid [EDTA] – plasma) were as follows: IL-6, 3 pg/ml (4.6% and 12.1% intra- and inter-assay variation coefficient); IL-10, 5 pg/ml (3.0% and 7.0% intra- and inter-assay variation coefficient).

MEASUREMENT OF *SOCS-3* GENE EXPRESSION

Blood samples for *SOCS-3* gene expression analysis were collected into PAXgene™ blood RNA tubes (Qiagen, Valencia, CA, USA) before induction of anaesthesia and on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery).

Total RNA was prepared using the PAXgene™ blood RNA commercial kit and QIAshredder (Qiagen), and extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. RNA (15 µl)

and oligo-dT primer (2 µl) were used to generate cDNA using TaqMan® reverse transcription reagents (Applied Biosystems, Foster City, CA, USA) according to the internal protocol of the Institute for Medical Immunology, Charité-Universitaetsmedizin Berlin. The cDNA was amplified by real-time quantitative reverse transcription–polymerase chain reaction (RT–PCR) and detected using fluorogenic probes on an ABI Prism® 5700 sequence detection system (Perkin-Elmer, Waltham, MA, USA).

The *SOCS-3* gene was amplified using comparative threshold cycling and the *hypoxanthine phosphoribosyltransferase* (*HPRT*) housekeeping gene was used as an internal standard. The primers used for amplification of the *SOCS-3* gene (based on the sequence 5′-CCAGCGCCACTTCTTCACGCTCAG-3′) were as follows: forward, 5′-CTTTCTGATCCG CGACAGCT-3′; reverse, 5′-TCACACTGGATG CGCAGGT-3′. All primers and probes were designed and assays validated at the Institute for Medical Immunology, Charité-Universitaetsmedizin Berlin. Gene expression was quantified using a GeneAmp® 5700 Sequence Detection System (Applied Biosystems). The amplification took place in a two-step PCR (40 cycles, 15 s denaturation step [95 °C] and 1 min annealing/extension step [60 °C]). The mean cycle threshold (C_T) values for the housekeeping gene and the genes of interest were calculated from double determinations and samples were considered negative if C_T exceeded 40 cycles, as described previously.[25] Investigated mRNAs were measured in duplicate for each sample. Amplification efficiencies of almost one were achieved for all panels, hence specific gene expression was calculated relative to the housekeeping gene, *HPRT*, as follows: ΔC_T = mean C_T (*SOCS 3*) – mean C_T (*HPRT*), where C_T is the cycle threshold. Analysis of the relative gene expression data of *SOCS-3* was performed using the $2^{-\Delta\Delta C_T}$ method of Livak and Schmittgen.[26] Changes in gene expression parameters were validated if values were 2.5-fold higher than baseline.

STATISTICAL ANALYSIS

All data were expressed as medians and 25 – 75% quartiles. Multivariate data such as repeated measurements (longitudinal data) were evaluated using a non-parametric analysis for longitudinal data[27] for independent groups. *Post-hoc* analyses within groups were performed using the univariate Wilcoxon matched-pairs signed-rank test.

The IFN-γ/IL-10 ratio after Con A stimulation was calculated and analysed with respect to time using the non-parametric multivariate analysis of covariance for repeated measurements in a two-factorial design (first factor, fentanyl versus remifentanil; second factor, time) with the baseline as covariate.[28] Univariate analysis of *SOCS-3* gene expression between groups was performed using the Mann–Whitney *U*-test.

The predictive performance of the risk model was assessed by determining the area under the receiver–operating characteristic (ROC) curve.

For all data a P-value < 0.05 was considered to be statistically significant. Numerical calculations were carried out using SAS version 8.02 (SAS Institute Inc., Cary, NC, USA) for Windows® and SPSS® version 13 (SPSS Inc., Chicago, IL, USA).

Results

A total of 40 patients were enrolled in the study and randomly allocated to intravenous infusion of fentanyl (n = 20) or remifentanil (n = 20). Three of these patients were subsequently excluded due to haemodynamic instability or it being the

second time CPB was carried out and, in another four patients, measurement of immune parameters was not possible due to technical problems. Consequently, 33 patients (18 who received fentanyl and 15 who received remifentanil) were included in the final analysis (Fig. 1).

The two groups of patients did not differ with respect to demographic or clinical characteristics (Table 1). Intra-operative BIS levels and haemodynamic parameters did not differ significantly between the two groups (data not shown). The rate of transfusion was comparable in both groups. In total, 11 patients received allogeneic transfusion (< 4 units) during CPB (seven from the fentanyl group and four from the remifentanil group). Leucocyte and lymphocyte counts as well as the level of C-reactive protein did not differ significantly between the two groups (data not shown).

In the remifentanil group, the IFN-γ/IL-10 ratio in whole blood cells after Con A stimulation was significantly lower on post-

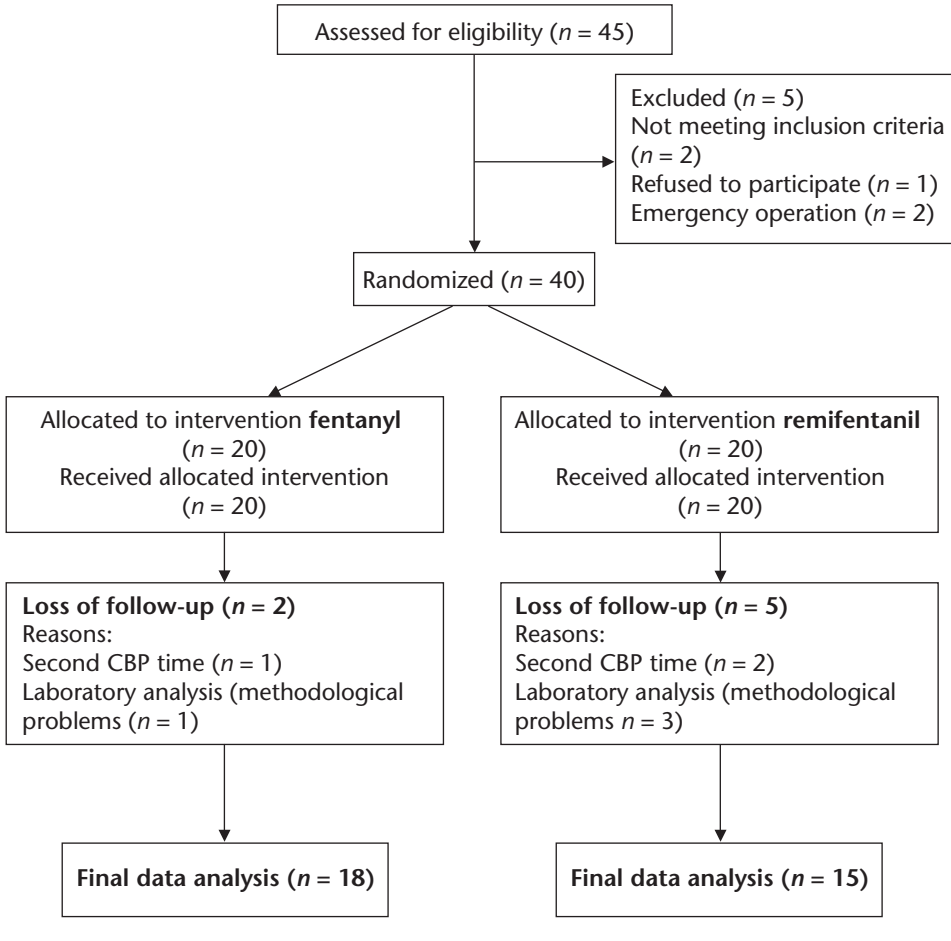

FIGURE 1: Flow diagram showing progress through the various stages of the trial for elective coronary artery bypass graft surgery patients selected and then randomized to receive fentanyl or remifentanil anaesthesia (CBP, cardiopulmonary bypass)

TABLE 1:
Demographic characteristics, comorbidities and surgery-relevant data for patients undergoing elective coronary artery bypass graft surgery with either fentanyl or remifentanil anaesthesia

	Fentanyl group (n = 18)	Remifentanil group (n = 15)
Age (years)	65 (61 – 73)	66 (62 – 70)
Body mass index (kg/m²)	26 (25 – 27)	27 (24 – 29)
Ejection fraction (%)	53 (50 – 60)	53 (50 – 65)
Acute coronary syndrome[a] (n)	10	6
Arterial hypertension (n)	18	15
Chronic obstructive pulmonary disease (n)	2	0
Diabetes mellitus (n)	7	4
Smoker/ex-smoker (n)	1/6	2/3
Obesity (n)	7	10
Operation time (min)	180 (165 – 195)	180 (160 – 205)
Cardiopulmonary bypass time (min)	71 (59 – 93)	82 (59 – 90)
Cross-clamping time (min)	39 (35 – 51)	44 (33 – 60)

Values given are the median and 25 – 75% quartiles (except for where n is shown). There are no statistically significant between-group differences (Mann–Whitney U-test $P > 0.05$).
[a]Acute myocardial infarction or unstable angina with first emergency hospitalization < 4 weeks prior to surgery.

operative day 1 (24 h after surgery) compared with the fentanyl group (P = 0.032; Fig. 2). IFN-γ levels in whole blood cells after Con A stimulation fell significantly (P < 0.001) in the remifentanil-group (median 286 pg/ml, 25 – 75% quartiles 135 – 540 pg/ml) compared with the fentanyl-group (median 925 pg/ml, 25 – 75% quartiles 301.5 – 1783.5 pg/ml) on post-operative day 1 (24 h after surgery). Levels of TNF-α and IL-2 after Con A stimulation did not differ significantly between the groups (data not shown).

Levels of the plasma cytokines IL-6 and IL-10 did not differ significantly between the two groups (data not shown). The PCT plasma levels were significantly higher in the fentanyl group 12 h after surgery (P = 0.017) and on post-operative day 1 (24 h after surgery) (P = 0.048) compared with levels in the remifentanil group (Fig. 3).

SOCS-3 gene expression increased significantly (over 2.5-fold compared with the pre-operative level) on post-operative day 1 (24 h after surgery) in both groups (P < 0.05). On post-operative day 2, *SOCS-3* expression in the fentanyl group remained significantly increased compared with baseline (P < 0.05) and was also significantly higher than in the remifentanil group (P = 0.025; Fig. 4).

The APACHE II score on admission to the ICU, the SOFA score and the number of patients with SIRS or post-operative nosocomial pneumonia or wound infection did not differ significantly between the remifentanil and fentanyl groups (Table 2). The overall ICU stay, however, was significantly shorter in the remifentanil group compared with the fentanyl group (P = 0.039; Table 2). In addition, ROC curve analysis showed that *SOCS-3* expression on post-operative day 2 was predictive for a prolonged ICU stay of > 72 h (Fig. 5).

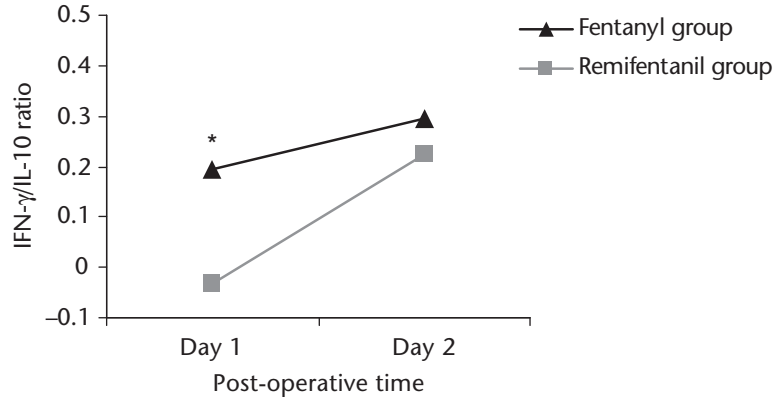

FIGURE 2: Interferon-γ/interleukin-10 (IFN-γ/IL-10) ratio in whole blood from patients undergoing elective coronary artery bypass graft surgery with either fentanyl or remifentanil anaesthesia, presented as the adjusted relative effects of the two treatments on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery) (*P = 0.032 for fentanyl versus remifentanil [multivariate analysis of covariance with baseline as covariate])

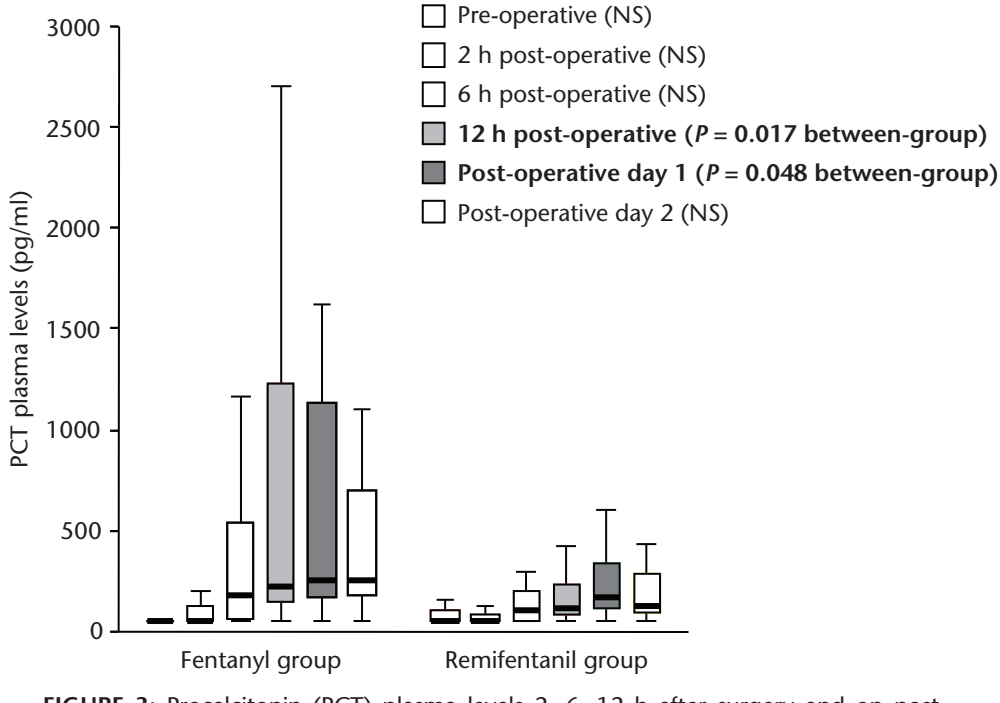

FIGURE 3: Procalcitonin (PCT) plasma levels 2, 6, 12 h after surgery and on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery) in patients undergoing elective coronary artery bypass graft surgery with either fentanyl or remifentanil anaesthesia (data shown are the median, 25 – 75% quartiles and range; NS, no statistically significant between-group differences $P > 0.05$)

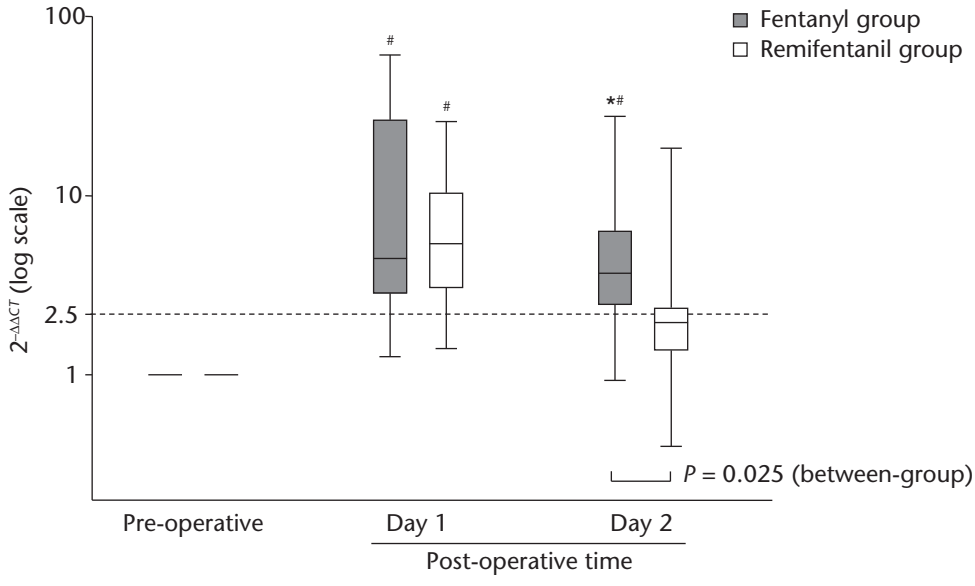

FIGURE 4: Levels of *suppressor of cytokine signalling (SOCS)-3* gene expression pre-operatively and on post-operative days 1 and 2 (24 and 48 h, respectively, after surgery) in patients undergoing elective coronary artery bypass graft surgery with either fentanyl or remifentanil anaesthesia (data shown are the median, 25 – 75% quartiles and range; $^{\#}P < 0.05$ compared with the pre-operative value [intra-group Wilcoxon rank sum test]); $^{*}P = 0.025$ (inter-group, Mann–Whitney *U*-test)

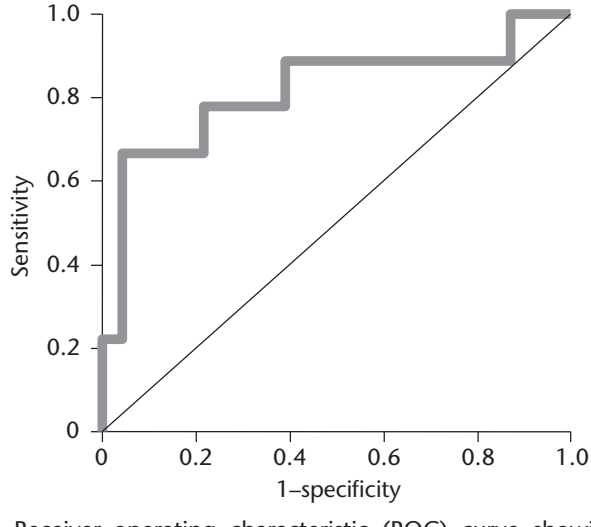

FIGURE 5: Receiver–operating characteristic (ROC) curve showing the predictive value of *suppressor of cytokine signalling (SOCS)-3* gene expression on post-operative day 2 (48 h after surgery) for a prolonged ICU stay (> 72 h) in patients undergoing elective coronary artery bypass graft surgery (median area under curve 0.83; 25 – 75% quartiles 0.66 – 0.99; $P = 0.006$)

TABLE 2:
Post-operative course, outcome and infection complications in patients undergoing elective coronary artery bypass graft surgery with either fentanyl or remifentanil anaesthesia

	Fentanyl group (*n* = 18)	Remifentanil group (*n* = 15)
APACHE II (on ICU admission)	12 (9 – 16)	11 (9 – 13)
SOFA (first 24 h)	4.5 (3.8 – 6)	3 (2 – 5)
Time to extubation (min)	450 (325 – 680)	405 (243 – 576)
Overall ICU/IMCU stay (h)	75 (46 – 116)	49 (42 – 61)[a]
SIRS (*n*)	12	10
Post-operative nosocomial pneumonia (*n*)	1	1
Post-operative wound infection (*n*)	2	3

Values given are the median and 25 – 75% quartiles (except for where *n* is shown).
APACHE, Acute Physiology and Chronic Health Evaluation score; ICU, intensive care unit; IMCU, intermediate care unit; SIRS, systemic inflammatory response syndrome.
[a]$P = 0.039$ versus the fentanyl group.

Discussion

This study demonstrates for the first time that the cell-mediated inflammatory response as well as expression of the transcription factor SOCS-3, reflecting cytokine signalling, was significantly reduced with remifentanil-based analgesia compared with fentanyl-based analgesia after CABG surgery. In addition, the overall ICU stay was significantly shorter in the remifentanil group.

In the present study, the IFN-γ/IL-10 ratio after Con A stimulation, which reflects Th1/Th2 balance, was changed to a greater extent in favour of a pro-inflammatory response in the remifentanil group on post-operative day 1 (24 h after surgery). A number of *in vitro* studies have investigated the effect of different concentrations of opioids on the differentiation of T-helper cells.[29,30] After treatment with tramadol and morphine, a dramatic dose-dependent decrease in the Th1/Th2 ratio was observed by Qian *et al.*[29] In addition, Nair *et al.*[30] reported that morphine could modulate mice T-helper cell differentiation *in vitro*, resulting in a rise in Th2 subsets that suppressed cell-mediated immunity. These findings indicate that excessively high concentrations of opioids should be avoided clinically in order to maintain a healthy Th1/Th2 balance, and that the immunosuppressive effect of opioids might help to maintain the Th1/Th2 balance in the exaggerated inflammatory response seen after cardiac surgery. Remifentanil analgesia might be particularly useful for this due to its short context-sensitive half-life. However, further studies are required to determine more precisely the influence of opioids on T-cell-mediated responses.

The present study also demonstrated that *SOCS-3* gene expression remained significantly increased compared with baseline on the second post-operative day in the fentanyl group but not in the remifentanil group. Previous experimental studies have demonstrated the involvement of opioid-receptor agonists in the modulation of transcription factors regulating the immune system and, in particular, the JAK/STAT pathway.[31,32] Opioid receptors may share some properties with cytokine receptors and may influence *SOCS-3* expression via

signal transducing mechanisms.[31] Exposure to morphine in Con A-stimulated peripheral blood mononuclear cells is known to result in a significant decrease of IFN-γ release.[32] In addition, *SOCS-3* is strongly and rapidly expressed not only in response to multiple cytokines, such as IL-6 or IL-10, but also in response to lipopolysaccarides.[33] These studies underline the complex pattern of the molecular transcriptional events that control differential cytokine responses during inflammation; in the present study the increased *SOCS-3* expression on the second post-operative day in the fentanyl group might be explained in terms of prolonged stimulation of the immune system. In addition, *SOCS-3* expression on post-operative day 2 was predictive for a prolonged ICU stay (> 72 h) and may reflect a prolonged inflammatory response after cardiac surgery with CPB. These findings are consistent with previous reports where prolonged activation of the pro-inflammatory response was associated with a poorer outcome.[1,2] Indeed, Sander *et al.*[8] demonstrated that increased IL-6 concentrations on admission to the ICU were associated with increased rates of post-operative infection and that IL-6 levels may remain elevated until post-operative day 7. However, in the present study the post-operative infection rate was low and did not differ between the two groups.

Furthermore, PCT plasma levels 12 and 24 h after surgery (post-operative day 1) were significantly lower in the remifentanil compared with the fentanyl group. Increased PCT plasma levels might be associated with severe SIRS after cardiac surgery and might be predictive for a worse outcome.[34,35] Dandona *et al.*[36] supported the hypothesis of a relationship between PCT and cytokine cascade. In healthy subjects a rapid increase of PCT was followed by a plateau 8 – 24 h after endotoxin injection; the increase in PCT was preceded by an increase in TNF-α and IL-6. Increased PCT plasma levels in the fentanyl group might, therefore, be explained as a prolonged inflammatory response after cardiac surgery, although further studies are required to confirm this.

The present study has several limitations. First, it was a pilot study and was not blinded, which is a major limitation. Secondly, the other anaesthetics used in these patients, including sevoflurane, midazolam and propofol, are known to influence the inflammatory response. However, the depth of anaesthesia was maintained at similar levels in both groups using BIS monitoring, and sevoflurane concentrations and propofol and midazolam cumulative doses were comparable in the two groups. Thirdly, only low-risk patients with an ejection fraction > 40% were enrolled in this study; the benefits of remifentanil-based anaesthesia should be further investigated in high-risk cardiac patients. There were also some experimental limitations: SOCS-3 mRNA levels were only measured using one modality (quantitative RT–PCR); and it would have been useful to have confirmation of SOCS-3 protein induction by using Western blotting. Moreover, it would be interesting to demonstrate whether or not differences in SOCS-3 levels impart functional differences in peripheral blood mononuclear cells.

In conclusion, the present study demonstrated that remifentanil can attenuate the inflammatory response to CPB when used as a part of a balanced anaesthetic technique with sevoflurane. When compared with patients receiving remifentanil, patients receiving fentanyl had increased *SOCS-3* gene expression and higher PCT plasma levels on day 2 after surgery, indicating prolonged activation of

the immune system. Further clinical trials are required to define the influence of choice of intra-operative opioid on post-operative outcome after cardiac surgery with CPB.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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