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The inflammatory response to colloids and crystalloids used for pump priming during cardiopulmonary bypass

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Background: Systemic inflammatory response frequently occurs after coronary artery bypass surgery and is strongly correlated with the risk of postoperative morbidity and mortality. This study tests the hypothesis that the priming of the extracorporeal circuit with colloid solutions results in less inflammation in patients undergoing cardiac surgery than priming with crystalloid solutions.

Methods: A prospective, randomized study was designed. Forty-four patients undergoing elective coronary artery bypass grafting were randomly allocated to one of two groups: 22 patients primed with Ringer's lactate (RL) solution and 22 patients primed with gelatin-containing solution during the surgery. Plasma levels of interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and, complement 4 were measured during the surgical intervention and over the following 48 postoperative hours. Cytokine levels were measured by enzyme-linked assays from plasma samples obtained at specific time points pre- and post-operatively.

Results: In both groups the serum levels of the pro-inflammatory cytokines (IL-6, IL-8, TNF-α), CRP, complement 4, and leukocytes increased significantly over the baseline, although no significant differences were observed between the two groups. The operation time, blood loss, need for inotropic support, extubation time, and length of intensive care unit stay did not differ significantly between the two groups.

Conclusion: Priming with gelatin vs. RL produces no significant differences in the inflammatory response in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass.

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CARDIOPULMONARY bypass (CPB) has been associated, during surgery and in the early postoperative stage, with a large variety of clinical symptoms, including haemodynamic instability (hypotension, vasodilatation), fever, bleeding disorders, and organ dysfunction in severe cases.[1] It has been suggested that these effects are symptoms of a systemic inflammatory response syndrome (SIRS) resulting from extensive cellular activation, likely caused both by the contact of blood with the foreign material of the extracorporeal circuit and by trauma induced by surgery. This response is mediated by the release of pro-inflammatory cytokines, especially interleukin (IL)-6, IL-8, and tumor necrosis factor-α (TNF-α).[2–3] Because the extent of SIRS is strongly correlated with the risk of postoperative morbidity, efforts to reduce SIRS activation are of potential clinical importance.

Prevention of the inflammatory response due to CPB has been attempted using different perfusion techniques (oxygenator, cardioplegia, biocompatibility of CPB setups) and pharmacological agents (corticosteroids, aprotinin). The composition of the solution used to prime the extracorporeal circuit causes a modulation of the neutrophil complement activation and repercussions in the inflammatory response as well as a modulation in blood levels of inflammatory mediators (cytokines).[4–6]A recent study evaluated the effect of priming the extracorporeal circuit with crytalloid alone, crystalloid plus albumin, or crystalloid plus the plasma expanding polygelatin on complement activation, and the results indicated that the addition of polygelatin to the priming solution reduces complement activation.[5] Cavarocchi et al.[7] reported significantly less complement activation with a prime containing 12.55 vol/5% albumin. Other reports suggest that

priming of extracorporeal circulation with crystalloid (Hartman solutions) may cause an increase in plasma water content[8] and marked activation of the neutrophil as compared with albumin.[4]

Together this suggests that the use of crystalloid solutions in the priming of the CPB appears to contribute to greater activation of both neutrophil and complement. Here we test the hypothesis that priming the volume of the extracorporeal circuit with colloid rather than crystalloid solutions results in reduced inflammation [lower cytokine, complement, C-reactive protein (CRP), and leukocyte levels in the blood], thus reducing patient morbidity.

We compared the effects of gelatin priming vs. Ringer's lactate (RL) priming on cytokine release and during the inflammatory state following coronary artery bypass surgery with CPB. We also examined the relationships between cytokines, CRP, and complement levels with postoperative variables at specific time points (respiratory insufficiency, PaO_2/FIO_2 ratio, etc.).

Materials and methods

Study protocol

Patients scheduled to undergo elective coronary artery bypass graft surgery at our centre were evaluated for possible inclusion in the study. Patients with renal or hepatic impairment, congestive heart failure, severely impaired left ventricular function (ejection fraction $<40\%$), active inflammatory/immunomodulatory diseases, a history of myocardial infarction <6 months previously, and those who had pre-operative use of steroids were excluded. Patients undergoing urgent surgery or repeated surgical interventions were also excluded. Cardiac medications including β-adrenergic blocking agents, calcium-channel blocking agents, and nitrates were continued until the surgery.

A computerized table of aleatory numbers was used to randomize allocation. Forty-four subjects who fulfilled the inclusion criteria were randomly allocated to one of two groups during CPB: the RL (RL; B. Braun, Melsungen, Germany) prime (RL group, 22 patients) or the gelatin (Gelofusine, B. Braun, Melsungen, Germany) containing prime (GEL group, 22 patients). Except for the perfusionists, the clinical team was blinded to the randomization.

The study protocol was approved by the ethics committee of the 'Clinico' Hospital of Valladolid. Written informed consent was obtained from each patient.

Operative procedure and postoperative care

All patients underwent coronary bypass surgery with CPB using standard procedures. All patients were premedicated during the night with oral flunitracepam 2 mg, and 5 mg of morphine was injected on the morning of the intervention. Arterial pressure, pulse pressure, and EKG were monitored in the operating theatre. A 20-G catheter was then inserted into the radial artery and the internal jugular vein. Anaesthesia was induced in all patients [0.1 mg/kg intravenous (i.v.) midazolam, 5–10 μg/kg i.v. fentanyl, followed by 2–5 mg/kg i.v. thiopental] and tracheal intubation was facilitated with 1 mg/kg i.v. rocuronium. After tracheal intubation, the lungs were ventilated to normocapnia using an oxygen–air mixture (intake oxygen fraction 0.5). Mechanical ventilation was carried out using an anaesthesia machine, model Datex-Ohmeda S/5 Aespire 3000 (Datex-Ohmeda Ltd., Madson, WI) with controlled ventilation. Anaesthesia was maintained using sevorane 0.5–1.5%, fentanyl 3 μg/kg/h, and rocuronium 0.3 mg/kg/h. The inspired sevoflurane concentration was varied to maintain the mean arterial pressure (MAP) within 20% of the preinduction baseline values.

The extracorporeal circulation system consisted of a soft-shell venous reservoir, roller pump, and membrane oxygenator (D903 AVANT with integrated biocompatible circuit covered by phosphorylchlorine inert surface; DIDECO, Mirandola, Italy). In all cases, the extracorporeal circuit was primed with 1750 ml. The crystalloid prime solution contained lactated Ringer's solution 1500 ml, 20% mannitol 100 ml, aprotinin 100 ml, 8.4% sodium bicarbonate 50 ml, and heparin 5000 IU. The colloid prime solution contained gelatin 1000 ml, lactated Ringer's solution 500 ml, 20% mannitol 100 ml, aprotinin 100 ml, 8.4% sodium bicarbonate 50 ml, and heparin 5000 IU.

The heart was exposed through a median sternotomy. To perform sub-total CPB, a standard cannulation was performed with the cannule placed in the ascending aorta and the right atrium. After systemic heparinization (300 IE/kg), CPB commenced under the condition that the activated clotting time was more than 400 s. The left ventricle was vented via the aortic root. Blood from the pericardial cavity was collected in a cardiotomy reservoir and returned to the patient. The haematocrit value was maintained between 20% and 25%, and pump flows were kept between 2.0 and 2.5 $l/min/m^2$ to maintain MAP between 50 and 70 mmHg. If arterial pressure declined below 50 mmHg, flow rate increased, and vasoactive drugs were given. All

Table 1

Demographics, clinical characteristics, and operative data.

Variable	Total $n = 44$	RL $n = 22$	GEL $n = 22$	*P*-value
Age, years	67 ± 7.5	66.50 ± 6.9	67.8 ± 8.1	0.567
Male gender [*n* (%)]	36 (82)	17 (77)	19 (86)	0.696
Current smoker [*n* (%)]	16 (36)	9 (41)	7 (32)	0.530
Diabetes mellitus [*n* (%)]	15 (34)	6 (27)	9 (41)	0.526
Hypertension [*n* (%)]	19 (43)	8 (36)	11 (50)	0.361
NYHA class [*n* (%)]				
I	1 (2)	1 (4)	0 (0)	0.311
II	36 (82)	17 (77)	19 (86)	0.434
III	4 (9)	1 (4)	3 (13)	0.294
Additional drugs, *n* (%)				
β-blockers	15 (34.1)	7 (31.8)	8 (36.4)	0.750
Ca^{2+} channel blockers	13 (30.1)	5 (22.7)	8 (36.4)	0.321
Nitrates	29 (65.9)	12 (54.5)	17 (77.3)	0.551
Ejection fraction (%)	61.9 ± 10.7	63.22 ± 10.8	60.59 ± 10.8	0.872
Creatinine (mg/dl)	0.9 ± 0.6	1.07 ± 0.7	0.88 ± 0.5	0.328
Total CPB time, min	101.3 ± 24.3	100.5 ± 25.9	102.1 ± 23.2	0.277
Aortic cross-clamp, min	65.6 ± 17.8	63.3 ± 19.6	68.0 ± 15.9	0.391
Number of grafts	3.18 ± 0.76	3.23 ± 0.75	3.14 ± 0.77	0.695
Fluid in the operating room, ml	2451.1 ± 468.0	2577.3 ± 434.2	2325.0 ± 476.0	0.073
Pa_{O_2}/Fi_{O_2} *base*	320.7 ± 115.6	322.5 ± 134.3	318.9 ± 96.6	0.919
Pa_{O_2}/Fi_{O_2} *before-CPB*	331.4 ± 92.8	366.3 ± 68.6	296.5 ± 101.8	0.011*
Pa_{O_2}/Fi_{O_2} *end-CPB*	298.3 ± 140.0	283.2 ± 136.2	313.5 ± 145.4	0.480

Values are expressed as numbers (*n*), percentages (%), and means ± SD.
*Probability value of $P < 0.05$ was considered to be significant.
RL, Ringer's lactate; GEL, gelatin; NYHA, New York Heart Association; CPB, cardiopulmonary bypass; *Base,* before the operation; *Before-CPB,* before the cardiopulmonary bypass; *End-CPB,* at the end of cardiopulmonary bypass.

patients were cooled to moderate hypothermia (mean 32 °C nasopharyngeal temperature). After aortic cross-clamping, all patients received crystalloid cardioplegia for myocardial protection (800–1000 ml, potassium 16 ml/l, 4 °C). Acid–base balance was managed following the α-stat concept. Patients were weaned from CPB using inotropic support if necessary. After termination of CPB, heparin was neutralized using an equivalent dose of protamine sulphate 3 mg/kg guided by low filling pressures of 0.9% NaCl and gelatin was additionally infused.

At the end of surgery, patients were transferred to the intensive care unit (ICU), where they were treated according to a standard regimen. Haemodynamic values were assessed at a heart rate (HR) of 70–80 beat/min and a MAP of 65–80 mmHg. Inotropic support depended on the individual status of the patient. Basic i.v. fluid administration consisted of 0.9% NaCl and gelatin was infused. Fluid balance, rectal temperature, and peripheral temperature (measured on the back of the foot) were recorded every hour. The peripheral circulation (ΔT) was considered to be adequate if the difference between rectal temperature and peripheral skin temperature was <5 °C. The lungs were ventilated with 60% oxygen using volume-controlled ventilation (Servo Ventilator 900C; Siemens, Stockholm, Sweden) and a tidal volume of 10 ml/kg with 5 cmH_2O of positive end-expiratory pressure. Analysis of arterial blood gas was conducted by standard techniques using an automated analyser and anaesthesia induction at 4-h intervals for 24 h after termination of CPB. All patients were extubated in the ICU when the Tobin index [respiratory rate (spontaneous)/tidal volume (L)] was <105,[9,10] Pa_{O_2} was >60 mmHg, F_{IO_2} was <0.4, continuous positive airway pressure was <5 mbr, Pa_{CO_2} was 50 mmHg, and arterial pH was >7.35. Pa_{O_2}/F_{IO_2}.

Intra-operative data

Intra-operative variables, including CPB time, aortic cross-clamp time, the need for inotropic support, volume administered during CPB, lowest rectal temperature, urinary output, and the number of aortocoronary bypass grafts, were analysed and recorded (Tables 1 and 2).

Haemodynamic measurements

MAP, HR, and central venous pressure (CVP) were measured as haemodynamic indicators. Haemodynamic evaluations were performed before the

Table 2

Postoperative haemodynamic.

Variable Group	*Base*	*Before-CPB*	*End-CPB*	*1 h-ICU*	*6 h-ICU*	*24 h-ICU*	*48-h-ICU*
HR, beats/min							
RL group	86.4 ± 15.4	80.8 ± 23.6	87.0 ± 15.0	88.2 ± 15.7	82.2 ± 10.4	88.2 ± 8.8	85.8 ± 11.1
GEL group	86.7 ± 25.5	81.0 ± 12.1	85.0 ± 17.0	80.0 ± 12.9	89.0 ± 16.2	81.7 ± 12.3	84.7 ± 12.4
MAP, mmHg							
RL group	76.6 ± 9.1	78.0 ± 13.1	73.0 ± 18.7	86.6 ± 16.4	85.0 ± 10.5	85.8 ± 11.1	79.8 ± 6.9
GEL group	76.7 ± 15.7	75.7 ± 4.1	79.5 ± 15.2	83.7 ± 14.1	82.7 ± 13.5	84.7 ± 12.4	87.5 ± 10.0
CVP, mmHg							
RL group	4.0 ± 1.5	2.6 ± 1.5	2.8 ± 1.3	2.8 ± 1.3	5.8 ± 4.6	8.0 ± 3.1	9.0 ± 2.2
GEL group	5.7 ± 3.1	2.2 ± 0.5	2.2 ± 0.5	2.0 ± 0.8	8.0 ± 4.1	9.0 ± 2.9	8.4 ± 2.3

Values are expressed as mean ± SD.
Base, before the operation; *Before-CPB*, before the cardiopulmonary bypass; *End-CPB*, at the end of cardiopulmonary bypass; *1 hr-ICU*, at arrival in the intensive care unit; *6 h-ICU*, 6 h after surgery; *24 h-ICU*, 24 h after surgery; *48 h-ICU*, 48 h after surgery; HR, heart rate; MAP, mean arterial pressure; CVP, central venous pressure; RL, Ringer's lactate prime; GEL, gelatin prime.

operation *(base)*, before the CPB *(before-CPB)*, at the end of the CPB *(end-CPB)*, on arrival in the ICU *(1 h-ICU)*, and at 6 h *(6 h-ICU)*, 24 h *(24 h-ICU)*, and 48 h *(48 h-ICU)* after surgery.

Blood sample collection, stocking, and analysis

Blood specimens for haemoglobin, haematocrit, white blood cell numbers, platelet counts, and creatinine were collected in 5-ml glass Vacutainer tubes containing EDTA (Becton Dickinson, Norcross, GA). Blood samples for measuring the complement (C4), CRP, and cytokine serum levels were collected via a radial arterial catheter at the following points: before the operation *(base)*, before the CPB *(before-CPB)*, at the end of the CPB *(end-CPB)*, on arrival in the ICU *(1 h-ICU)*, and at 6 h *(6 h-ICU)*, 24 h *(24 h-ICU)*, and 48 h *(48 h-ICU)* after surgery. All blood samples were drawn in pre-chilled vacuum tubes containing EDTA. The samples were immediately centrifuged at 4 °C and stored at −80 °C until assayed. IL-6, IL-8, and TNF-α were determined by ELISA as previously described.[11] ILs were measured with an ELISA Kit (ELISA; immulite 2000 for IL-6, inmunolite 1000 for IL-8, and inmunolite 1000 for TNF-α DPG, Los Angeles, CA). All assays were controlled according to the manufacturer's instructions. All samples were measured in duplicate. Concentrations were not corrected for dilution. The arterial blood samples for analysing gasometry, haemoglobin, haematocrit, white blood cell numbers, platelet counts, and creatinine and albumin were collected simultaneously. Total C4, CRP, and albumin concentrations were determined by nephelometry (Behring Diagnostics Benelux NV; Behring Nephelometer Analyser, Behringwerke AG, Marburg, Germany), according to the manufacturer's protocol.

Respiratory insufficiency[12] was indicated by the presence of arterial hypoxemia, defined as a Pa_{O_2}/F_{IO_2} ratio <300, in any of the determinations.

Blood specimens were obtained for the analysis of cardiac enzymes. Total serum activity of creatinine kinase (CK) was determined by the enzymatic method, and the activity of MB isoenzyme creatine kinase (CK-MB) was quantified by the immunoassay method. CK and CK-MB were evaluated at the following intervals: on arrival in the ICU *(1 h-ICU)*, and at 6 h *(6 h-ICU)*, 24 h *(24 h-ICU)*, and 48 h *(48 h-ICU)* after surgery.

Information regarding clinical, demographic, treatment, and procedural factors, as well as hospital outcome, was obtained prospectively using a specialized case report form.

Statistical analysis

Before the study, the number of patients required in each group was determined by a power calculation according to data obtained from previous studies on the release of IL-6 in the patients undergoing cardiac surgery with CPB.[6,8,13] We hypothesized that purging with gelatin as compared with a RL therapy regimen would reduce the IL-6 response by 30%. A population of 40 patients (20 in each group) would be needed to detect this difference with an α of 0.05 and a power of 0.80.

Data are presented as mean (± SD). Values of IL-6, IL-8, TNF-α, CRP, and complement (C4) are represented as medians with interquartile range unless otherwise stated. The SPSS program (version 13) was used for statistical analysis of the data. Raw data were analysed for normality of distribution. If not normally distributed, data were subjected to log transformation before analysis. We used Fisher's exact test to compare categorical

variables and Student's *t*-tests to compare continuous variables as appropriate. Differences from baseline and between the groups were evaluated by two-way analysis of variance for repeated measurements (ANOVA), followed by Scheffe's test. Correlation analysis between variables was calculated using Pearson's correlation coefficient. A probability value of $P<0.05$ was considered significant.

Results

Patients

Clinical and operative characteristics are shown in Table 1. There were no significant differences between the groups regarding pre-operative data. Patients were similar with regard to type of procedure, bypass time, aortic cross-clamp time, and number of grafts. The PaO_2/FIO_2 ratio before CPB was less in the GEL group than in the RL group ($P=0.011$). No complications occurred and all patients survived.

Haemodynamic function measurement

Repeated-measures ANOVA revealed that differences were not significant ($P>0.05$) over time within groups or between groups for HR, MAP, and CVP measurements (Table 2).

Pre- and postoperative laboratory data

There were no significant inter-group differences in the haemoglobin, haematocrit, platelet, leukocyte, and albumin counts. The number of leukocytes in both groups increased significantly on arrival in the ICU ($P<0.0001$). Haemoglobin (*before-CPB*, $P<0.0001$), haematocrit (*before-CPB*, $P<0.0001$), platelet (*before-CPB*, $P<0.0001$), and albumin (*before-CPB*, $P<0.0001$) counts were significantly reduced in both groups as compared with the baseline (Table 3).

The postoperative course

The postoperative course in the two groups showed no significant difference in death, creatinine, respiratory insufficiency, PaO_2/FIO_2 ratio, postoperative bleeding, extubation time, number of patients extubated at 24 h, atrial fibrillation, CPK and CPK-MB levels in blood, need for blood transfusion, units of donor blood, need for inotropic support, or length of ICU stay (Table 4). Neither were there any differences between the groups

Table 3

Pre- and postoperative laboratory blood sample analysis.

Variable group	*Base*	*Before-CPB*	*End-CPB*	*1 h-ICU*	*6 h-ICU*	*24 h-ICU*	*48 h-ICU*
Hemoglobin (mg/dl)							
RL group	12.9 ± 2.0	7.6 ± 1.5	7.8 ± 0.9	10.0 ± 1.3	10.8 ± 1.4	10.6 ± 1.6	10.8 ± 1.6
GEL group	11.8 ± 2.0	7.5 ± 1.4	7.8 ± 1.2	10.0 ± 1.4	11.2 ± 1.2	11.3 ± 1.2	11.1 ± 1.4
Haematocrit (%)							
RL group	38.7 ± 5.5	23. 3 ± 4.4	23.6 ± 2.6	30.1 ± 4.4	32.3 ± 3.6	31.8 ± 4.4	32.6 ± 4.3
GEL group	36.5 ± 5.1	24.1 ± 4.0	24.9 ± 3.0	30.5 ± 4.0	33.9 ± 3.7	34.1 ± 3.5	34.0 ± 4.2
Platelet/(mm^3)							
RL group	180.545 ± 50.220	141.090 ± 49.212	117.954 ± 47.033	115.454 ± 41.712	131.954 ± 42.656	131.909 ± 40.105	135.000 ± 57.356
GEL group	197.181 ± 43.647	137.500 ± 52.001	116.318 ± 46.024	115.227 ± 43.273	133.727 ± 52.684	131.136 ± 51.403	123.363 ± 44.662
Leukocyte/(mm^3)							
RL group	6.819 ± 2.452	5.695 ± 2.531	8.918 ± 3.816	10.152 ± 3.569	10.389 ± 3.775	9.714 ± 3.132	9.335 ± 3.569
GEL group	6.664 ± 2.203	5.797 ± 2.430	10.033 ± 3.588	11.190 ± 4.082	11.221 ± 4.599	9.612 ± 2.658	9.359 ± 2.865
Albumin (g/l)							
RL group	33.441 ± 5.743	20.373 ± 3.078	21.041 ± 2.940	24.318 ± 4.210	24.327 ± 3.588	24.673 ± 3.144	23.145 ± 3.035
GEL group	30.610 ± 4.420	19.075 ± 3.239	20.550 ± 4.493	22.705 ± 4.385	24.885 ± 4.678	24.875 ± 4.505	22.932 ± 4.006

Values are expressed as mean ± SD.
Base, before the operation; *Before-CPB*, before the cardiopulmonary bypass; *End-CPB*, at the end of cardiopulmonary bypass; *1 h-ICU*, at arrival in the intensive care unit; *6 h-ICU*, 6 h after surgery; *24 h-ICU*, 24 h after surgery; and *48 h-ICU*, 48 h after surgery; RL, Ringer's lactate prime; GEL, gelatin prime.

Table 4

Postoperative course

Variable	Total ($n = 44$)	RL ($n = 22$)	GEL ($n = 22$)	P value
Death	0	0	0	N/A
Creatinine (mg/dl)	1.2 ± 0.6	1.22 ± 0.8	1.33 ± 0.5	0.611
Respiratory insufficiency, [*n* (%)]	38 (86.4)	17 (77.3)	21 (95.5)	0.093
Pa_{O_2}/Fi_{O_2} *1 h-ICU*	269.8 ± 82.2	273.8 ± 97.1	265.7 ± 66.1	0.749
Pa_{O_2}/Fi_{O_2} *6 h-ICU*	265.0 ± 89.6	254.7 ± 72.3	275.2 ± 104.8	0.456
Pa_{O_2}/Fi_{O_2} *24 h-ICU*	305.0 ± 90.3	287.6 ± 86.8	321.7 ± 92.4	0.219
Pa_{O_2}/Fi_{O_2} *48 h-ICU*	347.8 ± 109.5	384.2 ± 110.9	313.1 ± 98.5	0.032*
Postoperative bleeding (ml/24 h)	971.8 ± 510.9	946.0 ± 537.9	995.0 ± 496.6	0.760
Time to extubation (hrs)	12.8 ± 17.7	11.9 ± 19.4	13.7 ± 16.2	0.829
Patients extubated at 24 h [*n* (%)]	42 (95.5)	21 (95.5)	21 (95.5)	0.742
Atrial fibrillation	1	0	1	N/A
CK (IU/l)	705.03 ± 672.73	582.0 ± 332.42	816.33 ± 869.28	0.262
CK-MB (IU/l)	94.33 ± 176.31	53.74 ± 40.3	131.05 ± 237.01	0.156
Need for blood transfusion [*n* (%)]	33 (75%)	17 (89.5%)	16 (94.1%)	0.412
Units donor blood	2 ± 0.9	2.11 ± 0.9	1.94 ± 0.9	0.520
Fluid 0.9% NaCl (ml/24 h)	1179.1 ± 97.6	1201.5 ± 88.3	1156.7 ± 103.2	0.129
Fluid gelatin (ml/24 h)	871.0 ± 68.7	877.2 ± 84.8	864.8 ± 48.9	0.555
Fluid (ml/24 h)	2050.1 ± 130.6	2078.7 ± 142.1	2021.5 ± 114.0	0.148
Need for inotropic support [n (%)]	20 (45.5)	8 (36.4)	12 (54.5)	0.225
Mean ICU stay (days)	2.3 ± 2.0	2.10 ± 1.8	2.64 ± 2.2	0.388

Values are expressed as numbers (n), percentages (%), and mean ± SD.
*Probability value of $P < 0.05$ was considered to be significant.
RL, Ringer's lactate prime; GEL, gelatin prime; CK, creatinine kinase; CK-MB, MB isoenzyme creatine kinase; ICU, intensive care unit.

with regard to the total volume of fluid administered during the intra-operative (Table 1) and post-operative period (Table 4). The Pa_{O_2}/Fi_{O_2} ratio in the colloid group was significantly lower ($P = 0.032$) as compared with the crystalloid group 48 h after arrival in the ICU. One patient in the RL group had prolonged ventilation, while one of the 44 total patients had atrial fibrillation with fast ventricular rate (120–180 bpm), requiring anti-arrhythmic therapy.

Levels of plasma cytokines

Repeated-measures ANOVA revealed that, within each group, variations in the plasma levels of IL-6, IL-8, and TNF-α compared with baseline values were significant over time. Compared with the initial levels (base), IL-6 levels peaked at 6 h after surgery ($P < 0.0001$), showing a continuous decrease in the first 48 h after surgery (Fig. 1A). IL-8 levels underwent a similar trend in both groups, peaking at 1 h after arrival in the ICU ($P < 0.0001$) and a progressive return towards the baseline value 48 h after surgery (Fig. 1B). TNF-α levels increased progressively in both groups to reach a peak at 4 h after surgery in the RL group ($P < 0.0001$) and 6 h after surgery in the GEL group ($P < 0.0001$), with a slight return towards baseline values at 48 h after surgery (Fig. 1C). However, when analysed by repeated-measures ANOVA, no significant difference was found between the groups in IL-6, IL-8, and TNF-α plasma levels at any time point.

Furthermore, no significant correlation was found between cytokine plasma levels (IL-6, IL-8, and TNF-α) and clinical postoperative variables such as respiratory insufficiency, Pa_{O_2}/Fi_{O_2} ratio, extubation time, CK, CK-MB, need for inotropic support, and length of stay in ICU in either group.

Plasma levels of CRP and complement (C4)

CRP levels increased in all patients, starting from 6 h in ICU and reaching maximum levels at 48 h inICU ($P < 0.0001$) (Fig. 2A). C4 levels decreased significantly ($P < 0.0001$) during the first minutes of CPB, mainly because of haemodilution (Fig. 2B). Thereafter, levels slightly increased, returning to base values at 48 h-ICU ($P = 0.425$). Repeated measures of variance analysis (ANOVA) revealed these differences were significant over time within groups in the plasma level of CRP and C4, but no significant differences were found between groups at any sample point.

Discussion

This prospective, randomized study was designed to examine any potential effect of priming the extracorporeal circuit with crystalloid (RL) rather

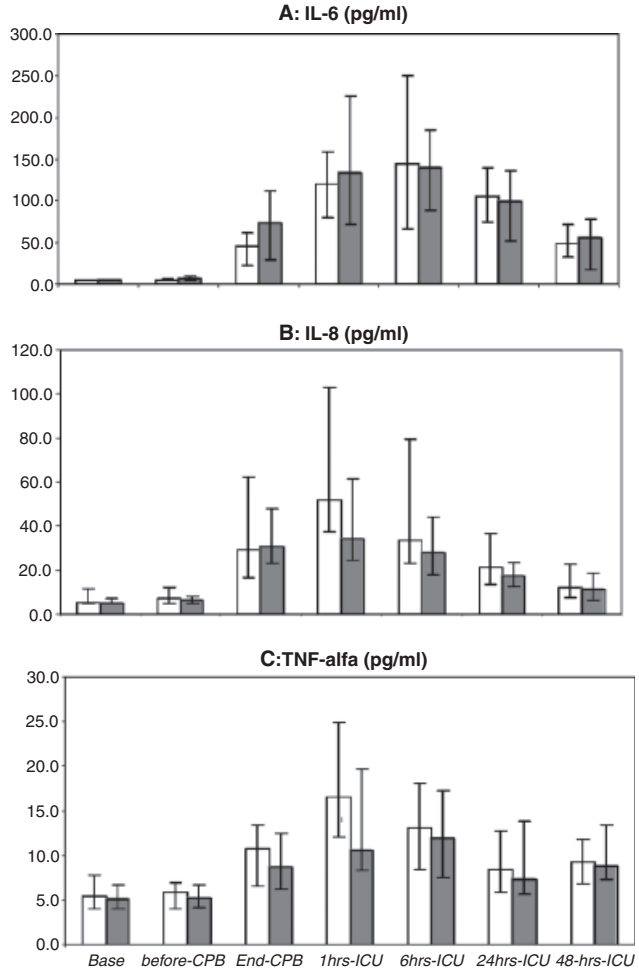

Fig. 1. Temporal profile of cytokines. Median plasma levels of (A) interleukin IL-6, (B) IL-8, and (C) tumor necrosis factor (TNF-α) in patients treated with Ringer's lactate (RL) prime (light bars) or gelatin (GEL) prime (dark bars) at different sampling times. Base, *before the operation;* Before-CPB, *before the cardiopulmonary bypass;* End-CPB, *at the end of cardiopulmonary bypass;* 1 h-ICU, *at arrival in the intensive care unit;* 6 h-ICU, *6 h after surgery;* 24 h-ICU, *24 h after surgery; and* 48 h-ICU, *48 h after surgery. Data represent the median and interquartile range.*

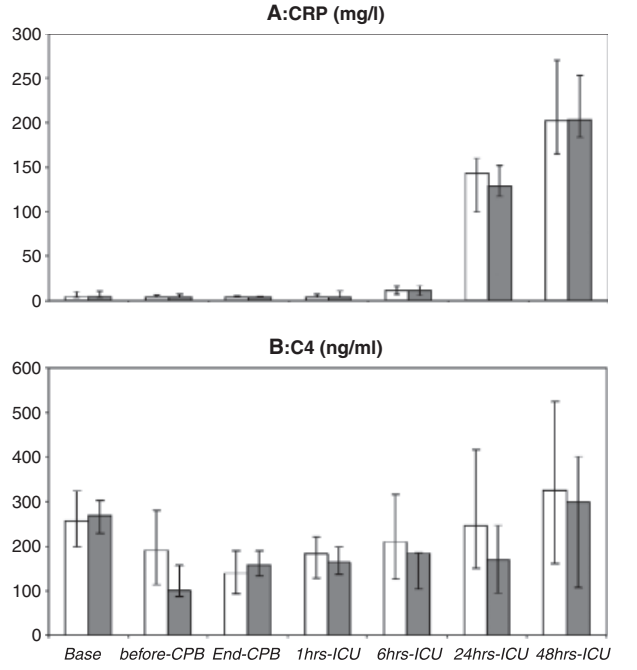

Fig. 2. Temporal profile of C–reactive protein (A) and complement (B). Median plasma levels in patients treated with Ringer's lactate (RL) prime (light bars) or gelatin (GEL) prime (dark bars) at different sampling times. Base, *before the operation;* Before-CPB, *before the cardiopulmonary bypass;* End-CPB, *at the end of cardiopulmonary bypass;* 1 h-ICU, *at arrival in the intensive care unit;* 6 h-ICU, *6 h after surgery;* 24 h-ICU, *24 h after surgery; and* 48 h-ICU, 48 h *after surgery. Data represent the median and interquartile range.*

than colloid (gelatin) on post-surgical inflammatory response. The two groups analysed were homogeneous as far as demographic and clinical data are concerned, and the sample size was sufficient for comparing the two groups. We did not detect any differences in the inflammatory response (serum levels of the pro-inflammatory cytokines IL-6, IL-8, and TNF-α; levels of C4, CRP, and leukocytes) or any relevant adverse effects (such as arrhythmia, respiratory insufficiency, etc.) during or after the bypass cardiopulmonary procedure (up to 48 h post procedure).

The onset of CPB was associated with an intense SIRS, characterized by the activation of complement, neutrophils, endotoxin, elastases, and the pro-inflammatory cytokines.[14–16] The present study confirms[3,6,8,14] that on altering the CPB, there is an intense SIRS accompanied by high levels of the common pro-inflammatory cytokines (TNF-α, IL-6, and IL-8), CRP, C4, and leukocytes.

Colloid-containing priming solutions are advocated to prevent the initial decrease in colloid osmotic pressure at the beginning of CPB,[8,17] and the clinical benefits of a normal colloid osmotic pressure include reduced fluid administration, improved postoperative patient recovery, and a shortened postoperative hospital stay.[8] In further support of colloid solution use in CPB purging, neutrophil and complement activation have been shown to be reduced with colloid use,[4–7] as well as a reduction in plasma water, thus lowering the inflammatory response.

According to the results of this study, however, this hypothesis has not been confirmed, as the

pattern of changes in the blood levels of cytokines, CRP, and complement was similar in both the group that used GEL and the group that used RL for priming the CPB. A previous study that evaluated the efficacy of CPB, using either GEL priming (1650 ml) or RL priming (1650 ml), showed that maintaining a normal colloid osmotic pressure during CPB can be established by adding GEL to the priming solution of an extracorporeal circuit with a small prime volume.[8] Similar increments in TNF-α, complement, and neutrophil elastase were found in both groups as indicators of SIRS.

In this study we have evaluated gelatin, a colloid used in our centre for priming the CPB. Gelatin is derived from bovine collagen, and the animal peptide nature of gelatin may confer an enhanced immunogenicity compared with other solution fluids (hydroxyethyl starch, Ringer's solution). Recent studies demonstrate that the use of gelatin solutions as volume replacement regimens in both animals[18] and the elderly undergoing major abdominal surgery is associated with a significant and marked increase in IL-6 and TNF-α as compared with the use of Ringer's or 4% hydroxyethyl starch solutions.[19,20] In our study, the lack of any observed differences in the levels of cytokines, CRP, and complement between colloid and crystalloid priming of the CPB may be due to the greater intensity of the surgical trauma and/or CPB's liberation of cytokines during cardiac surgery,[21] so that the effect of the purging liquids, whether colloid or crystalloid, is minimized and masked by these other factors.

Recent studies demonstrate that maintenance of cardiovascular stability and effective organ perfusion with fluid solutions is associated with an attenuation of the inflammatory response.[22,23] Because of relatively slow degradation of the gelatin-based colloid, the volume expanding effect is maintained for 2–3 h, while the volume expanding effect of crystalloids (Ringer) is markedly shorter. Gelatin-based colloid has been considered to be effective in improving haemodynamics, the peripheral perfusion, and the optimization of the patient's intravascular volume status.[18] Consequently, one could suppose that differences in inflammatory response are related to the degree of intravascular volume (which depends on the type of plasma expander used). Nevertheless, no differences were observed between the two groups in our study. To avoid any distortion of the results, one of the aims was to maintain a MAP (65–80 mmHg) throughout the entire period of study in both groups. The absence of significant changes in pro-inflammatory cytokines and CRP between the two groups of patients was also reinforced by the absence of significant modifications in haemodynamic and peri-operative variables. Three clinically available parameters (blood pressure, haematocrit, and CVP) were also used to test the effectiveness of volume expansion with different fluids. The results generally demonstrated equal efficacy for both fluids on adequate perfusion. Haematocrit, haemoglobin, and albumin decreased after beginning CPB as a consequence of the effect of haemodilution with clear priming solutions.

From a clinical point of view, CPB has been associated with a large variety of clinical symptoms,[1] and the extent of their intensity has been correlated with the levels of cytokines and complement, and the severity of the SIRS.[2–4] However, we were not able to find a significant correlation between plasma levels (IL-6, IL-8, and TNF-α) and peri-operative variables. As for the presence of relevant adverse effects related with postpump syndromes (respiratory insufficiency, levels of CPK-MB, need for inotropic support, units of donor blood, need for blood transfusion etc.), there were no differences between the two groups. Chello et al.[24] did not find any correlation between the levels of cytokines and the adverse effects.

The conclusions from these results, however, may be limited because (i) it is probable that the sample size of the present study is not sufficient to assess clinical outcomes such as intubation time, length of stay in ICU, and postoperative pulmonary morbidity, which are low in our patients, and (ii) it is also possible that the sample size is not sufficient to establish any correlation between the levels of cytokines and pre- and postoperative variables. Furthermore, to both groups we have administered aprotinin, which is known to have anti-inflammatory properties.[2]

Our study provides no significant clinical advantages for colloid priming as compared with crystalloid priming: the blood levels of cytokines, CRP, and complement and leucocytes, and the haemodynamic and clinical profiles were similar. Thus, as colloid use increases the costs and does not bring any advantages, we believe that its routine use is not justified in coronary artery bypass grafting.

To conclude, on the basis of our data we can affirm that priming with colloid as opposed to crystalloid in patients undergoing coronary artery bypass grafting with CPB produces no significant

differences in the inflammatory response as measured by the levels of IL-6, IL-8, TNF-α, CRP, and C4. On the basis of these data, we recommend the routine use of crystalloids for priming the CPB.

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