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Use of inflammatory cytokines and blood PAPP-A levels as the laboratory evidence of preconditioning in cardiac surgery

Kardiyak cerrahide önkoşullamanın laboratuvar kanıtları olarak olarak inflamatuvar sitokinlerin ve kan PAPP-A düzeylerinin kullanımı

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ABSTRACT

Cardioprotective management has increasingly become a standard of care for preservation of myocardial reserve during cardiac surgery. This study investigates the usefulness of inflammatory cytokines and PAPP-A levels in predicting the appropriate preconditioning method. Ninety ASA III patients scheduled for CABG surgery were included and allocated into three groups. Group 1 (n=30) received propofol (2-3 mg.kg-1.h-1) and fentanyl (3-5 mcg.kg-1.h-1) infusions with 5% desflurane inhalation. Group 2 (n=30) received propofol (5-6 mg.kg-1.h-1) and fentanyl (3-5 mcg.kg-1.h-1) infusions with 5% desflurane inhalation. Group 3 (n=30) received midazolam (0.04-0.06 mg.kg-1.h-1) and fentanyl (3-5 mcg.kg-1.h-1) infusions with 5% desflurane inhalation. TNF-α, PAPP-A and us-CRP levels were measured one day preoperatively (S1), immediately prior to the cardiopulmonary bypass (S2), after completion of the cardiopulmonary bypass (S3) and 48 hours postoperatively (S4). In all groups, TNF-α increased at S2 and S3 and decreased at S4 (P<0.05), with the most prominent increase observed in Group 2. The highest increase in PAPP-A levels at S2 and S3 stages was observed in Group 2 whereas the lowest decrease at S4 stage was observed in Groups 1 and 3. In Group 1, us-CRP levels showed a significant increase at S3 compared to S1 and S2 (p<0.05) and decreased to levels close to S1 and S2. PAPP-A can be used with us-CRP and TNF-α to determine the optimal preconditioning method in the anaesthetic management of CABG surgery. Using these markers, we observed that midazolam-desflurane and low-dose propofol-desflurane were effective in anaesthetic preconditioning during CABG surgery.

Keywords: *PAPP-A, preconditioning, CABG*

ÖZ

Kardiyoprotektif yönetim kalp cerrahisi sırasında myokard rezervinin korunması için, giderek artan bir bakım standardı haline gelmiştir. Bu çalışma, uygun önkoşullama yönteminin öngörülmesinde inflamatuvar sitokinlerin ve PAPP-A düzeylerinin yararlılığını araştırmaktadır. KABG cerrahisi için planlanan ASA III 90 hasta dahil edildi ve üç gruba ayrıldı. Grup 1'e (n=30) %5 desfluran inhalasyonu ile, propofol (2-3 mg.kg^{-1}.h^{-1}) ve fentanyl (3-5 mcg.kg^{-1}.h^{-1}) infüzyonları uygulandı. Grup 2'ye (n=30) %5 desfluran inhalasyonu ile propofol (5-6 mg.kg^{-1}.h^{-1}) ve fentanyl (3-5 mcg.kg^{-1}.h^{-1}) infüzyonları uygulandı. Grup 3'e (n=30) %5 desfluran inhalasyonu ile midazolam (0.04-0.06 mg.kg^{-1}.h^{-1}) ve fentanyl (3-5 mcg.kg^{-1}.h^{-1}) infüzyonları uygulandı. TNF-α, PAPP-A ve us-CRP düzeyleri operasyondan bir gün önce (S1), kardiyopulmoner bypasın (S2) hemen öncesinde, kardiyopulmoner bypasın tamamlanmasından sonra (S3) ve operasyondan 48 saat sonra (S4) ölçüldü. Tüm gruplarda, TNF-α, S2 ve S3'te artarken, S4'te azaldı (P<0,05), en belirgin artış ise grup 2'de gözlendi. S2 ve S3 aşamalarında PAPP-A düzeylerindeki en yüksek artış grup 2'de bulunurken, S4'teki en düşük azalma ise Grup 1 ve 3'te bulundu. us-CRP düzeyleri grup 1'de S1 ve S2 ile kıyaslandığında S3'te anlamlı bir artış gösterdi (p<0,05) ve S1 ile S2'nin seviyelerine yaklaştı. KABG operasyonunun anestezik yönetiminde optimal ön koşullandırma yöntemini belirlemek için PAPP-A, CRP ve TNF-α ile birlikte kullanılabilir. Bu parametreleri kullanarak, midazolam-desfluran ve düşük doz propofol-desfluran'ın, CABG operasyonu sırasında anestetik önkoşullamada etkili olduğunu gözlemledik.

Anahtar kelimeler: *PAPP-A, Kalp koruyucu yönetim, CABG*

INTRODUCTION

Cardioprotective management has increasingly become a standard of care for the preservation of myocardial reserve during cardiac surgery. It was suggested by some investigators that cardioprotective effects of

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anesthetics were associated with activation of adenosine triphosphate dependent potassium channels and stimulation of nitric oxide, both of which are known pathways activated during tissue inflammatory response[1,2]. Moreover, some anesthetics were also proposed to play important roles in myocardial protection via stimulation of certain genes encoding synthesis of some proteins[3]. Considering this view, there has been increasing number of studies with inflammatory biomarkers to discover some laboratory evidence to warrant cardioprotective effects of anesthetics. However, data are scarce to justify routine use of any biomarker as the laboratory evidence of myocardial protection during cardiac surgery.

CRP has long been linked to cardiovascular diseases. It is primarily synthesized in the liver, and its plasma concentrations may increase hundreds of times during inflammation and infection[4,5]. TNF-α is another cytokine that plays an important role in inflammation and infection processes. It was reported that high TNF-α levels showed a positive correlation with heart failure[6]. Currently, TNF-α and CRP are accepted as independent predictors of adverse coronary and cardiovascular events as well as of several inflammatory diseases[7,8]. Since the degree of endothelial dysfunction in ischaemia/reperfusion damage is associated with TNF-α levels[9,10], it is thought that TNF-α may be used in conjunction with CRP to assess the preconditioning properties of various anaesthetic strategies during coronary artery surgery. Studies have also demonstrated that CRP and TNF-α may increase the expression of pregnancy-associated plasma protein-A (PAPP-A) in human peripheral blood cells[10,11].

PAPP-A is mainly produced by placental syncytiotrophoblasts but may also be synthesized in fibroblasts, osteoblasts, vascular smooth muscle cells and endothelial cells[12,13]. Studies suggest that PAPP-A may offer a potential biomarker for the early diagnosis and prediction of poor prognosis in patients with acute coronary syndrome[14]. Recently, Dembic et al.[15] found that high levels of PAPP-A are associated with increased risk of death from all causes of heart failure and PAPP-A is a potential prognostic marker of adverse outcomes in heart failure patients. However, data regarding its use in the determination of myocardial ischaemia/reperfusion damage are limited. The use of PAPP-A to assess the preconditioning properties of various anaesthetic management strategies during coronary artery bypass surgery has not yet been adequately addressed.

In this study, we sought to investigate the role of PAPP-A together with C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) as potential markers of preconditioning in patients undergoing cardiac surgery.

MATERIALS and METHODS

The study was approved by the pharmaceutical review board of the Turkish Medicines and Medical Devices Agency [2015-AKD-31]. The study group comprised 90 patients aged over 20 years, with American Society of Anesthesiologists (ASA) scores of III, who were scheduled to undergo coronary artery bypass grafting surgery. Patients with an ejection fraction of <50%, unstable angina pectoris or moderate to severe infection were excluded to control for the potentially confounding effects of severe ischaemic or inflammatory processes. Patients were also excluded if they had received preoperative inotropes or if they had a clear indication for combined or emergency surgery.

Standard monitoring included one 12-lead and one 3-lead electrocardiogram (Philips, Nihonkohden) as well as pulse oximetry (Philips) for peripheral oxygen saturation. A peripheral line was introduced into the right antecubital vein, and a 20-G catheter was introduced into the right radial artery for invasive blood pressure monitoring.

After pre-oxygenation, all patients received propofol (Lipuro 1%, Braun, Melsungen, Germany) 1.5-2.0 $mg.kg^{-1}$ and 5-10 $\mu g.kg^{-1}$ of fentanyl (Fentanyl, Mercury Pharma, London, UK). Then, 1 $mg.kg^{-1}$ intravenous rocuronium (Curon, Mustafa Nevzat, Istanbul, Turkey) was given to achieve neuromuscular blockade.

After achieving adequate muscle relaxation, orotracheal intubation was performed. Patients were ventilated in volume-controlled ventilation mode with a 40/60% air/oxygen mixture given at a respiratory rate of 12 $L.min^{-1}$ and tidal volume of 8-10 $mL.min^{-1}$. Positive end-expiratory pressure was set at 0 mbar with peak airway pressure set at 30 mbar. End-tidal CO2 levels were monitored using the Nihon Kohden Life Scope 14. After intubation, an 8 French central venous catheter (Arrow Sheath, USA) was introduced through the right internal jugular vein. Bispectral index (BIS) monitoring (Aspect Medical Systems, BIS, VISTATM, Covidien) was implemented in all cases.

Patients were randomly allocated to three groups to receive different types of anaesthetics during maintenance. Patients in Group 1 (n=30) received an intravenous infusion of 2% propofol (2-3 $mg.kg^{-1}.h^{-1}$) in addition to a 5% desflurane inhalation (Suprane, Baxter, Puerto Rico, US) and fentanyl (3-5 $\mu g.kg^{-1}.h^{-1}$). Patients in Group 2 (n=30) received an intravenous infusion of propofol (5-6 $mg.kg^{-1}.h^{-1}$) combined with 5% desflurane and fentanyl (3-5 $\mu g.kg^{-1}.h^{-1}$). Patients in Group 3 (n=30) received an infusion of midazolam (0.04-0.06 mg/kg/h) (Zolamid, Defarma, Istanbul, Turkey) and 5% desflurane inhalation in addition to the intravenous infusion of fentanyl (3-5 $\mu g.kg^{-1}.h^{-1}$). Desflurane administration continued throughout the cardiopulmonary bypass. Patients were then cooled to 32°C.

Blood samples were taken one day before the operation (Stage 1 = S1), immediately prior to the cardiopulmonary bypass (S2), after completion of the cardiopulmonary bypass (S3) and 48 hours postoperatively (S4). An Enzyme-Linked Immuno-Sorbent Assay (ELISA) was used to detect TNF-α and PAPP-A levels. Blood samples were centrifuged immediately after blood-drawing and deep-frozen at -40°C. Ultrasensitive C-reactive protein (us-CRP), creatinine kinase (CK), CK-MB, troponin I, lactate dehydrogenase (LDH) and b-type natriuretic peptide (BNP) levels were measured using an Olympus AU400 automated chemistry analyser. Serum insulin and cortisol levels were measured using the Siemens Immulite 2000 Xpi assay.

Statistical analysis

All analyses were performed using SPSS 15.0 statistical software. Repeated-measures analysis of variance was used to compare parametric variables between related groups; the Friedman test was used to compare non-parametric variables. One-way Analysis of Variance (ANOVA) was used to compare parametric data between independent groups; the Kruskal-Wallis test was used to compare non-parametric data. The Spearman correlation was used to test for any linear relationship among non-parametric variables. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Distribution of age and body mass index (BMI) was not significantly different among groups (p=0.90 and p=0.51 respectively, according to one-way analysis of variance), indicating that these parameters had no confounding role. When compared to preoperative levels, creatinine kinase (CK), creatinine kinase MB (CKMB), lactate dehydrogenase (LDH), Troponin I, B-type natriuretic peptide (BNP) levels showed a significant increase at 48 hours after surgery ($p<0.05$) (Table 1). This may be explained by the well-known mechanism of tissue damage occurring during coronary artery bypass surgery. Similarly, cortisol levels of the patients in Group 2 were significantly higher at 48 hours after surgery when compared to the levels at the preoperative state ($p<0.05$). Cortisol levels of the patients in Groups 1 and 3 showed no significant difference at 48 hour after surgery compared to the levels obtained at the preoperative period, although there was a mild increase in both groups ($p>0.05$). There were no significant differences in TNF-alpha levels between S1 and S2, S1 and S4 or S2 and S4 stages both in Group 1 (patients who received suboptimal propofol and desflurane) and Group 3 (patients who received desflurane and midazolam) ($p>0.05$) whereas TNF-alpha levels were significantly higher at S3 stage when compared to any other stage ($p<0.01$). TNF-alpha levels were not significantly different between S1 and S2 stages in Group

Table 1. Comparison of data from patient groups before and after the operation.

	Group 1			Group 2			Group 3		
N	30			30			30		
Age, year	65.4±8.6			64.4±9.0			64.8±7.0		
BMI, kg/m^2	26.8±3.8			27.9±3.2			27.4±3.7		
	Preop Mean±SD	**Postop Mean±SD**	***P**	**Preop Mean±SD**	**Postop Mean±SD**	***P**	**Preop Mean±SD**	**Postop Mean±SD**	***P**
CK, U/L	107±100	1097±752	<0.0001	92±69	1363±923	<0.0001	90±53	1155±775	<0.0001
CK-MB, U/L	15±6	63±36	<0.0001	11±3	49±29	<0.0001	14±4	61±34	<0.0001
LDH, U/L	275±184	486±149	<0.0001	226±188	556±178	<0.0001	236±129	489±150	<0.0001
Troponin I, µg/L	0.28±0.68	2.40±1.68	<0.0001	0.32 0.65	5.76 10.47	<0.0001	0.38 0.80	2.58±1.78	<0.0001
BNP, pg/ml	566±620	2060±2420	<0.0001	500±484	2963±2508	<0.0001	413±344	1863±2403	<0.0001
Cortisol, ug/dl	19.3±7.7	20.6±6.6	0.4463	19.5±8.7	31.7±20.0	0.0024	20.2±9.3	21.0±6.8	0.6908
Insulin, mU/L	13.7±9.9	21.1±15.7	0.0253	12.2±6.5	25.6±11.0	<0.0001	14.1±8.8	21.4±14.7	0.0245

**Paired t test between preop and postop, Preop: The day before the operation, Postop: 48 hours after the operation, SD: Standard deviation.*

Table 2. Comparison of the TNF-alpha data of stages belonging to all groups.

		S1		S2	S3		S4	P value
	N	30		30	30		30	-
Group 1	Mean± SD	15.81±2.78		15.69±4.81	81.12±75.91		21.95±6.23	
	Min. - Max.	10.06-20.72		8.93-28.65	7.22-308.16		10.80-34.20	
	95% CI From-To	14.78-16.85		13.89-17.48	52.78-109.46		19.62-24.28	*<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	>0.05	<0.001	>0.05	<0.001	>0.05	<0.001	
Group 2	Mean± SD	15.76±2.19		16.20±8.85	114.23±79.23		49.66±46.42	
	Min. - Max.	11.26-19.80		5.58-46.40	8.49-306.54		12.19-187.30	
	95% CI From-To	14.94-16.58		12.90-19.51	84.64-143.81		32.33-66.99	*<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	>0.05	<0.001	<0.05	<0.001	<0.05	<0.001	
Group 3	Mean± SD	16.34±2.23		17.01±3.95	90.86±40.19		18.71±4.26	
	Min. - Max.	12.30-19.98		10.76-25.44	22.33-174.45		11.44-28.32	
	95% CI From-To	15.50-17.17		15.54-18.49	75.86-105.87		17.11-20.30	[a]<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	>0.05	<0.001	>0.05	<0.001	>0.05	<0.001	

	G1 vs G2	G1 vs G3	G2 vs G3	
Comparison for S1	-	-	-	[b]0.5977
Comparison for S2	-	-	-	[b]0.7105
Comparison for S3	-	-	-	[b]0.1545
Comparison for S4 *	<0.001	>0.05	<0.001	[b]<0.0001

[a]Repeated Measures ANOVA, [b]One-way Analysis of Variance (ANOVA)
*If P value obtained by ANOVA is <0.05, *Tukey-Kramer Multiple Comparisons Test (Post-hoc tests) was used to compared all stages (S1, S2, S3 and S4). Post tests were not calculated because the P value was greater than 0.05. S: Stage, S1: The day before the operation, S2: Before institution of cardiopulmonary bypass, S3: After completion of cardiopulmonary bypass, S4: 48 hours after the operation, G: Group, CI: Confidence Interval, Min.-Max.: Minimum-Maximum*

2 (propofol+desflurane group) whereas differences in TNF-alpha levels were significant among all other stages (p<0.05) (Table 2). Based on these results, we observed that TNF-alpha level of the patients showed a marked increase immediately after removal of the cross clamp (stage 3) while it decreased significantly at 48th postoperative hour, except for patients in Group 2 (p<0.01) (Table 1). We also found that the

Table 3. Comparison of the PAPP-A data of stages belonging to all groups.

		S1		S2	S3		S4	P value
	N	30		30	30		30	
Group 1	Mean± SD	3.32±0.51		23.24±18.01	29.68±17.53		3.11±0.52	
	Min.-Max.	2.39-4.50		2.65-64.17	4.54-67.06		2.33-4.18	
	95% CI From-To	3.13-3.51		16.51-29.96	23.14-36.23		2.91-3.30	[a]<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value **	<0.001	<0.001	>0.05	>0.05	<0.001	<0.001	
Group 2	Mean± SD	3.15±0.51		36.00±20.13	39.10±18.10		7.23±5.66	
	Min.-Max.	2.35-4.13		4.07-73.50	3.17-72.36		2.36-22.50	
	95% CI From-To	2.96-3.34		28.48-43.52	32.35-45.86		5.12-9.34	[b]<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	<0.001	<0.001	>0.05	>0.05	<0.001	<0.001	
Group 3	Mean± SD	3.23±0.53		33.62±19.64	35.37±20.67		3.35±1.48	
	Min.-Max.	2.51-4.26		4.02-74.78	2.60-91.46		2.00-9.12	[b]<0.0001
	95% CI From-To	3.04-3.43		26.28-40.95	27.65-43.08		2.80-3.91	
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	<0.001	<0.001	>0.05	>0.05	<0.001	<0.001	

	G1 vs G2	G1 vs G3	G2 vs G3	
Comparison for S1	-	-	-	0.4601
Comparison for S2 **	<0.05	>0.05	>0.05	[c]0.0283
Comparison for S3	-	-	-	0.1548
Comparison for S4 *	<0.01	>0.05	<0.01	[d]0.0013

[a]Repeated Measures ANOVA, [b]Friedman Test (Nonparametric Repeated Measures ANOVA), [c]One-way Analysis of Variance (ANOVA), [d]Kruskal-Wallis Test (Unpaired Nonparametric ANOVA), vs: versus.
*If P value obtained by ANOVA is <0.05, *Dunn's or **Tukey-Kramer Multiple Comparisons Test (Post-hoc tests) was used to compared all stages (S1, S2 and S3). Post tests were not calculated because the P value was greater than 0.05.*

increase in TNF-alpha levels which occurred at S3 in all groups returned to normal levels at S4 stage only in Groups 1 and 3 (p>0.05).

In all groups, the difference in PAPP-A levels were not significant between S1 and S4 or S2 and S3 (p>0.05) whereas differences were significant among all other stages (p>0.05) (Table 3). It is obvious that the marked increase seen in PAPP-A levels at S2 stage (p<0.01) was due to the surgical trauma. This increase continued throughout the S3 stage and the PAPP-A levels returned to normal at S4 stage (Figure 2). The highest increase in PAPP-A levels at S2 and S3 stages was observed in Group 2 whereas the lowest decrease at S4 stage was observed in Groups 1 and 3. This decrease in Group 2 was relatively lower when compared to Groups 1 and 3 (p<0.01).

us-CRP levels were not significantly different between S1 and S2 in Groups 2 and 3 (p>0.05) whereas us-

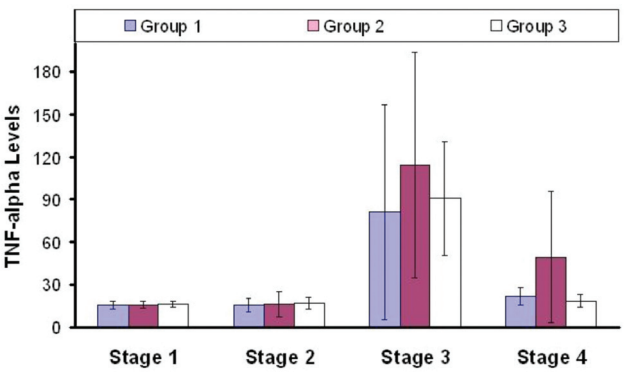

Figure 1. Comparison of TNF-alpha levels among four different stages. TNF-alpha levels showed a significant increase at stage 4 particularly in Group 1 when compared to other groups (p<0.01).

CRP increased significantly at S3 and S4 (p<0.05). In Group 1, us-CRP levels were similar between S1 and S2 (p>0.05) whereas us-CRP levels showed a significant increase at S3 compared to S1 and S2 (p<0.05) and decreased to levels close to S1 and S2 levels at S4 (p>0.05) (Table 4 and Figure 3).

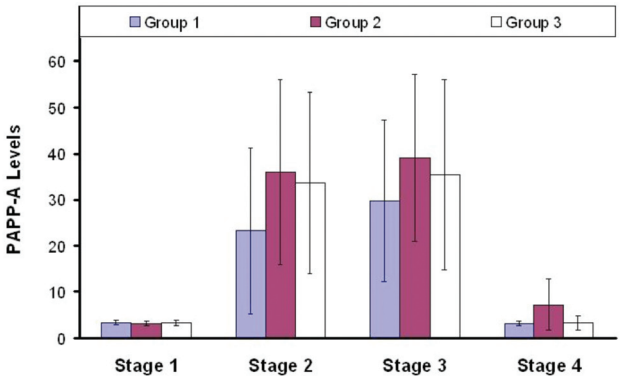

Figure 2. Comparison of PAPP-A levels among four different stages. PAPP-A levels showed a significant increase at stages 2 and 3 when compared to other stages (p<0.001). The most pronounced increase was seen in group 2. The most significant decline in PAPP-a levels in the postoperative period was seen in groups 1 and 3 whereas the decline in Group 2 was more limited than that in group 1 and 3 (p<0.01).

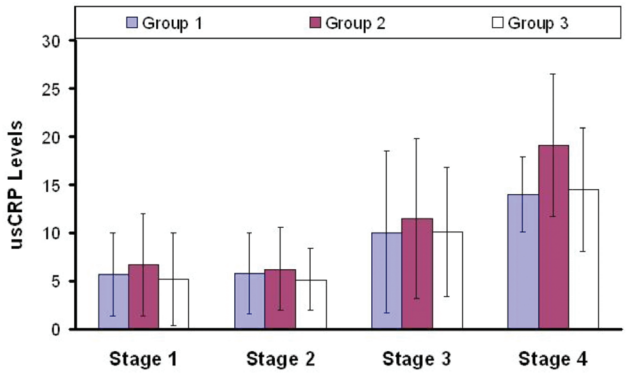

Figure 3. Comparison of us-CRP levels among four different stages. In all three groups, there was a significant increase in us-CRP levels at stage 3 when compared to other stages (p<0.01). The most pronounced increase was seen in group 2 (p<0.05).

These findings show that TNF-alpha and us-CRP levels significantly increased at S3 (p<0.05) and decreased at S4 (p<0.05) in all groups, except for the us-CRP levels of the patients in Group 2. We found a weak but statistically significant correlation between TNF-alpha and us-CRP levels (Spearman r=0.2238 and 95% CI: 0.1202 - 0.3225, p<0.0001).

Table 4. Comparison of the us-CRP data of stages belonging to all groups.

		S1		S2	S3		S4	P value
	N	30		30	30		30	
Group 1	Mean± SD	5.66±4.29		5.82±4.25	10.05±8.36		14.03±3.89	
	Min.-Max.	0.80-15.70		0.90-18.10	1.44-46.44		7.30-23.10	
	95% CI From-To	4.05-7.26		4.24-7.41	6.93-13.18		12.57-15.48	[a]0.0020
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value **	>0.05	<0.01	<0.001	<0.01	<0.001	<0.05	
Group 2	Mean± SD	6.66±5.33		6.25±4.29	11.48±8.34		19.13±7.39	
	Min.-Max.	0.90-22.80		0.60-18.30	1.89-38.43		8.60-41.40	
	95% CI From-To	4.67-8.65		4.65-7.85	8.37-14.59		16.37-21.89	[a]<0.0001
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	>0.05	<0.01	<0.001	<0.01	<0.001	<0.001	
Group 3	Mean± SD	5.20±4.86		5.12±3.17	10.14±6.73		14.49±6.40	
	Min.-Max.	0.80-24.40		1.80-12.70	1.40-29.10		4.10-32.10	[a]<0.0001
	95% CI From-To	3.38-7.02		3.94-6.31	7.62-12.65		12.11-16.88	
	Comparison	S1 vs S2	S1 vs S3	S1 vs S4	S2 vs S3	S2 vs S4	S3 vs S4	
	P value *	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	

	G1 vs G2	G1 vs G3	G2 vs G3	
Comparison for S1	-	-	-	0.4933
Comparison for S2	-	-	-	0.5373
Comparison for S3	-	-	-	0.7328
Comparison for S4 **	<0.01	>0.05	<0.05	[b]0.0024

[a]Repeated Measures ANOVA, [b]Friedman Test (Nonparametric Repeated Measures ANOVA), [c]One-way Analysis of Variance (ANOVA), [d]Kruskal-Wallis Test (Unpaired Nonparametric ANOVA), vs: versus.
*If P value obtained by ANOVA is <0.05, *Dunn's or **Tukey-Kramer Multiple Comparisons Test (Post-hoc tests) was used to compared all stages (S1, S2 and S3). Post tests were not calculated because the P value was greater than 0.05.*

DISCUSSION

Anesthetic preconditioning has an important role in reducing the risk and extend of the myocardial damage which occurs during cardiac surgery. Preconditioning mimics the natural ischemic process and thus prepares the myocardium against the ischemic insult that will occur secondary to restoration of the blood flow to the myocardial tissue. Myocardial preconditioning is achieved by release of protective enzymes in the short term whilst by stimulating *de novo* synthesis of these enzymes in the long term. Moreover, it was reported that some volatile anesthetics (e.g. sevoflurane) may reduce the inflammatory response which occurs against ischemic reperfusion and pro-inflammatory stimulation[3,16,17].

The use of TNF-α, us-CRP and other cytokines has gained popularity due to the ischaemic process that occurs during cardiac surgery. Indeed, surgery itself here produces an artificial ischaemic condition through the activation and release of certain inflammatory cytokines. Thus, one might presume a negative correlation between cytokine levels and the effectiveness of the anaesthetic pre-conditioning modalities used to alleviate the ischaemic condition created by the cardiac surgery and to accelerate overall healing. Several anaesthetic preconditioning options are available today, including propofol-desflurane and desflurane-midazolam combinations. However, which of these options is most effective remains controversial. Using blood levels of TNF-α and us-CRP, well-known markers of inflammation, as well as PAPP-A, a novel ischaemic biomarker, as the criteria for selection seems to be the most practical approach.

We found that TNF-α was highest immediately after completion of the cardiopulmonary bypass (S3), when the ischaemic and traumatic insult is most severe. We also observed that TNF-α levels were lower in Groups 1 and 3 compared to Group 2. This finding may be attributed to the anti-inflammatory and strong preconditioning effects of propofol, desflurane and midazolam demonstrated previously[18,19]. Moreover, the combined use of these agents might have augmented their individual effects. We found that low-dose propofol-desflurane and desflurane-midazolam were more effective in decreasing TNF-α levels than the use of propofol-desflurane combination. Since the difference in TNF-α levels was not of statistical significance, these two methods should be considered as equivalent and the decision should be left to the anaesthesiologist's discretion after a consideration of each method's technical convenience. In addition, given the higher cortisol levels observed in the propofol-desflurane group (Group 2) compared to other groups at the 48th postoperative hour, it may be suggested that the use of propofol (5-6 mg.kg^{-1}.h^{-1}) would not provide adequate anaesthetic preconditioning. Because cortisol is an anti-inflammatory hormone, levels of the hormone probably rose to compensate for the more severe inflammation in Group 2 as compared to other groups. However, further study is warranted to test this postulation.

Our finding that TNF-α levels showed significant decreases at the 48th postoperative hour may be supported by one previous study[20] that demonstrated that propofol possesses antioxidant effects. This finding may further be supported by a recent study demonstrating a decrease in TNF-α levels after cardiopulmonary bypass with the use of low-dose propofol[21]. However, this hypothesis should be tested by additional well-designed studies. Our findings, in contrast, showed a greater decrease in TNF-α levels after the addition of desflurane to low-dose propofol, resulting in more effective anaesthetic preconditioning. In other words, desflurane and low-dose propofol acted synergistically. We also believe that the combination of desflurane and midazolam potentiated the effect of each compound on TNF-α levels. TNF-α levels returned to baseline at the 48th postoperative hour in Groups 1 and 3, with the change more dramatic in Group 3.

There have been a number of studies comparing inhalation anaesthesia with intravenous anaesthesia in the context of preconditioning[22,23]. More recently, several studies have investigated the effectiveness of

isoflurane-propofol and sevoflurane-propofol on preconditioning and cardioprotectiveness[24,25] by comparing the drugs individually or in combination.

Another notable finding from these experiments was that PAPP-A levels began to rise with the onset of surgical trauma just prior to the cardiopulmonary bypass in all groups, and this increase continued at Stage 3 (where actual myocardial ischaemia occurs), and PAPP-A levels returned to normal at the 48th postoperative hour. PAPP-A, unlike TNF-α, began to increase during the first stages of surgical trauma and ischaemia, indicating that PAPP-A responds more rapidly to tissue damage. However, this issue should be evaluated with additional experiments. Gutierrez-Leonard et al.[26] suggested that PAPP-A levels could be used as biomarkers to identify patients at risk of coronary artery disease. Lucchinetti et al.[27] reported that pharmacological preconditioning reduces the perioperative inflammatory response, including increases in high-sensitive C-reactive protein and PAPP-A. Another study demonstrated that this inflammatory response may prevent coronary plaque rupture and may decelerate the progress of coronary occlusion via a statin-like effect. There may be an associated reduction in the mid-to-long term incidence of cardiovascular complications[28]. Also, Resch et al.[29] reported that PAPP-A plays an important role in the cellular response to tissue damage and inflammation. The authors determined that TNF-α, interleukin-1-ß and PAPP-A gene expression increased during the process of repair. In 2013, Eren et al.[14] reported that PAPP-A levels upon admission were higher in patients with acute coronary syndrome when compared to controls. High PAPP-A levels in the presence of normal troponin-I levels indicate that increase, n PAPP-A levels do not occur as a response to myocardial necrosis but rather as a response to inflammation.

In our study, the most prominent reduction in PAPP-A levels at the 48th postoperative hour occurred in Groups 1 and 3, whereas the reduction in Group 2 was more limited. Considering that PAPP-A levels represent a measure of the success of anaesthetic preconditioning and patient comfort, low-dose propofol-desflurane and desflurane-midazolam combinations are likely to be more effective than propofol-desflurane. Yi et al.[30] reported that desflurane inhalation resulted in the activation of nuclear factor kappa-ß (NF-Kappa-ß) during the preconditioning period and also inhibited the excessive activation of NF-Kappa-ß in reperfusion. These authors also reported that desflurane inhalation led to the up-regulation of Bcl-2 and c-IAP1 expression and inhibited the release of the second mitochondrial-derived activator of caspase (SMAC) as well as caspase-3 cleavage after anoxia and reoxygenation injury. Our results regarding PAPP- A levels are in line with previous studies reporting a significant increase in PAPP-A levels during inflammation[28].

Wang et al.[31] investigated the prognostic value of PAPP-A, S100 and us-CRP among patients with acute ischaemic stroke who did not receive heparin treatment. The authors reported that PAPP-A, S100 and us-CRP were associated with the degree as well as the severity of the stroke and its outcomes. The authors also suggested that these markers may provide complementary information required for clinical management. It has been reported that the activation of NFkB, an oxidative stress-linked transcription factor, may stimulate the expression of PAPP-A in human fibroblasts, similar to certain situations where trauma and inflammation-like inflammatory cytokines are activated[32]. Moreover, PAPP-A levels were reported to correlate well with markers of oxidative stress (F2-isoprostanes)[33]. Because our study included patients undergoing cardiovascular surgery, it is clear that our patients were exposed to a substantial risk of trauma and ischaemia. This explains the increase in PAPP-A levels we observed in our patients.

In our study, the prominent us-CRP increase observed in all three groups might stem from the surgical trauma experienced by the patient as well as the actual myocardial ischaemia that starts immediately after removal of the aortic cross clamp. Another interesting finding was the higher postoperative us-CRP levels found in the propofol-desflurane group as compared to the low-dose propofol-desflurane and

desflurane-midazolam groups. Considering the value of CRP levels in assessing pharmacological preconditioning methods in CABG patients, the above finding may provide evidence for the superiority of low-dose propofol-desflurane or desflurane-midazolam. However, there is no doubt that TNF-α and PAPP-A levels should also be taken into account in the assessment of pharmacological preconditioning methods. Although us-CRP and TNF-α show similar trends, the linear correlation between the two was quite weak, so us-CRP cannot be used alone as a surrogate for TNF-α. Moreover, CRP levels are affected by various conditions including trauma, infection and inflammatory reactions. Based on these three criteria (CRP, TNF-α and PAPP-A) as well as the other results presented, it is conceivable that the use of propofol-desflurane is less effective than use of either of the other two options, while both low-dose propofol-desflurane and/or midazolam-desflurane are effective options. In addition, though we found no significant difference between these methods, we suggest that low-dose propofol-desflurane is the optimal approach (based on the us-CRP, among others).

In conclusion, PAPP-A levels, together with levels of us-CRP and TNF-alpha, may be used as laboratory evidence of the effectiveness of preconditioning provided by anesthetics in patients undergoing coronary artery bypass surgery. We are on the opinion that low-dose propofol-desflurane combination (or desflurane-midazolam combination, if the former is not available) may be preferred to achieve safer way of providing anesthetic preconditioning during coronary artery bypass grafting surgery.

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