

Antecedent ACE-inhibition, inflammatory response, and cardiac surgery associated acute kidney injury

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Cardiopulmonary bypass (CPB) may trigger organs damage, including kidney injury, due to a massive cytokine release. In this observational, prospective study, we analyzed the possible impact of chronic treatment with ACE-Inhibitors (ACE-I) on the inflammatory response and renal function after CPB. Sixty-nine patients undergoing major cardiac surgery with CPB were enrolled. Patients were stratified according to long-term (> 6 mo.) ACE-I use ($n = 38$) or not ($n = 31$). The primary endpoint was the change in IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, TNF α , EGF and VEGF plasma levels. Secondary (renal) endpoints were postoperative acute kidney injury (AKI), recovery of baseline GFR values and the absolute changes in renal function indexes. After CPB, IL-1 α , IL-1 β , IL-4 and TNF- α remained stable over time while a significant decrease in IL-2 levels was noticed in the ACE-I group ($p = 0.01$). IL-6 and IL-8 increased after surgery and tended to decrease after 48 h. IL-10 levels showed a similar variation, but both their rise and decrease were more pronounced in patients under ACE-I treatment ($p = 0.007$). Finally, VEGF and EGF showed a marked initial decrease with a tendency to normalization 10 days after surgery (p for trend ranging from 0.01 to 0.001). The occurrence of AKI within 2 days after surgery, the rate of GFR recovery and the absolute changes in renal function indexes were not statistically different between groups. Chronic, long-term ACE-I treatment may influence the inflammatory response following CPB. On the other hand, this drug class apparently has neutral impact on perioperative renal outcomes.

Keywords

ACE-inhibitors; Cardiopulmonary bypass; Kidney function; Acute kidney disease; Cytokines

1. Introduction

Cardio-pulmonary bypass (CPB) maintains hemodynamic stability during open-heart surgery providing enough flow for a sufficient tissue perfusion. However, the contact of the blood with the bypass machine surface causes an inflammatory response characterized by histamine release, increased vascular permeability, release of oxygen free radicals (OFRs) and lysosomal enzymes from white blood cells, endothelial damage and, ultimately, release of pro-inflammatory cytokines. OFRs release cytokines and lead to cytokine re-

lease by cells which in turn causes further OFR production [1]. This condition causes hemodynamic instability and functional impairment of several organs, including the kidney [2].

The development of an overt acute kidney injury (AKI) is associated with an increased morbidity and mortality [3, 4]. Furthermore, patients with AKI requiring dialysis support often remain dialysis dependent [5]. This calls for continuous research with the attempt of identifying new treatment approaches to minimize the risk of AKI after CPB. The inhibition of the renin-angiotensin system by angiotensin-converting enzyme inhibitors (ACE-I) has been shown to decrease inflammation in different diseases, like hypertension and rheumatoid arthritis [6, 7]. This mostly relies on the capacity of these compounds to modulate levels of various inflammatory mediators, such as interleukin (IL)-1 α , the monocyte chemoattractant protein-1 (MCP-1), and, particularly interleukin-10 (IL-10) [8, 9]. Conversely, the effect of ACE-inhibition on post-CPB inflammation still remains unclear. Some studies suggest that the postoperative cytokine levels [9] could be attenuated by the concomitant ACE-I administration whereas other studies found no effect [10] or even an enhancement of the inflammatory response [11]. Similarly, it remains largely unknown whether chronic ACE-I administration may exert preventive or detrimental effects on renal function impairment driven by CPB [12, 13]. Considering this background in mind, we therefore aimed at evaluating the possible impact of a previous, long-term treatment with ACE-I on the inflammatory response and renal outcomes in a homogeneous cohort of patients undergoing major cardiac surgery requiring the employment of extracorporeal circulation.

2. Methods

2.1 Patients and study design

We run a pilot, observational, proof-of-concept prospective study. One hundred and one consecutive patients referred to the Cardiac Surgery Unit of the University Hospital "Magna Graecia" of Catanzaro (Italy) to undergo major cardio-thoracic surgery with CPB were screened for el-

igibility. We excluded 32 patients because of severely impaired renal function (Glomerular Filtrate Rate (GFR) < 15 mL/min) (n.10); left ventricular ejection fraction \leq 35% (n. 5); cancer (n. 3) or because they were undergoing emergency procedures (n. 7). Seven more patients were excluded because they received therapy with ACE-I for less than six months. The final study cohort therefore consisted of 69 patients which were divided into two groups on the basis of their chronic ACE-I use: 38 patients were on ACE-I regimen from at least six months (ACEI-group), whereas the remaining 31 patients (no-ACEI-group) were not. Baseline and post-operative clinical and laboratory data were also recorded. The Ethical Committee approved the study protocol. Informed consent was obtained from each patient.

2.2 Clinical management and surgical procedures

Clinical management and surgical procedures were performed as previously described by our group [14]. All patients received linear CPB [11]. Patients received preoperative intra-aortic balloon pump if they had critical left main coronary artery disease (90% stenosis with or without an ejection fraction < 40%, 80% left main stenosis with 90% right coronary artery stenosis or chronic occlusion of the three main coronary arteries with a poor angiographic bed) [11].

2.3 Study endpoints

The primary objective of this study was to evaluate changes in the levels of various cytokines from baseline to 10 days after CPB. These ones included IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, Tumor Necrosis Factor (TNF) alpha, Vascular Endothelial Grow Factor (VEGF) and Epidermal Grow Factor (EGF). The secondary (renal) outcomes were the following: 1) the incidence of postoperative AKI (defined according to the Acute Kidney Injury Network criteria as an increase in serum creatinine of more than or equal to 0.3 mg/dL (26.4 micromol/L) or an increase to more than or equal to 150 percent (1.5-fold) from baseline within 48 hours after surgery) [2, 15]; 2) the rate of complete recovery of kidney function (defined as a GFR > 90% of the baseline at the 10th day from surgery) [16] and 3) the absolute changes in renal function indexes after cardiac surgery.

2.4 Renal function assessment

Serum creatinine and urea were measured according to the routine lab methods preoperatively, at Intensive Care Unit (ICU) admission, at 24 h, 48 h and at the 10th day postoperatively. Patients discharged before day 10 after cardiac surgery were controlled exactly 10 days after surgery. Glomerular Filtration Rate (GFR) was measured preoperatively and at the same time points using the abbreviated Modification of Diet in Renal Disease (MDRD) study equation [17].

2.5 Cytokine assay

IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, TNF alpha, VEGF, EGF were measured by sandwich chemiluminescent immunoassay (Biochip Array Technology; Randox, UK). All values were normalized for hemodilution. Similarly,

to renal function parameters, cytokine assessment was performed preoperatively, at ICU admission, at 24 h, 48 h and 10 days after surgery.

2.6 Statistical analysis

We used the SPSS package for Windows, version 26.0 (SPSS Inc., Chicago, IL, USA) for the statistical analysis. Continuous variables were presented as mean \pm standard deviation (SD) and categorical variables were presented as absolute numbers and/or percentages. Data were checked for normality before statistical analysis by Shapiro-Wilk test. Normally distributed continuous variables were analyzed using the unpaired *t*-test, whereas those variables that were not normally distributed by the Mann-Whitney U-test. Categorical variables were analyzed by using either the chi-square test or the Fischer's exact test. Time trends and comparisons between groups were made using analysis of variance for repeated measures. Correlation analysis has been made by assessing the Pearson coefficient. Results were considered as significant if $P < 0.05$.

3. Results

The two study groups did not present differences in preoperative and intraoperative laboratory and clinical data, with the exception of the incidence of hypertension and dyslipidemia that were more frequent among patients on chronic ACE-I treatment (Table 1). Data concerning CPB procedure and residual cardiovascular risk were also comparable between the two study groups.

3.1 Cytokines trend

Table 2 depict the serum trends of inflammatory cytokines in the two groups from baseline to day 10 after CPB. Overall, IL-1alpha, IL-1beta, IL-4 and TNF-alpha exhibited stable levels over time in both the ACEI and no-ACEI groups (*p* resulting from comparisons between groups was not significant). A significant decrease in IL-2 levels after CPB was noticed in the ACEI group ($p = 0.01$) while a similar, non-statistically significant tendency was noticed among patients not taking ACE-I ($p = 0.08$). Overall, IL-6 and IL-8 levels increased after surgery and tended to decrease after 48 h too. This trend was highly significant in both the ACEI and no-ACEI groups ($p = 0.001$) but no differences were noticed in the between-groups comparison. IL-10 levels showed a similar variation, with an earlier peak (at ICU admission) and a more evident reduction which started 24 h after surgery. Interestingly the rise of this cytokine, as well as its decrease, were more pronounced in persons undergoing long term ACE-I treatment than in those who were not ($p = 0.007$). Finally, VEGF showed a marked initial decrease in both groups with a following normalization, which approximated baseline values 10 days after surgery (p for trend = 0.001 in both groups for VEGF; $p = 0.04$ and 0.01 in the ACEI and no-ACEI groups, respectively for EGF).

Table 1. Patients demographic, preoperative and intraoperative data.

	ACEI (n = 38)	No-ACEI (n = 31)	p
DEMOGRAPHICS			
Age (mean \pm SD*)	65.8 \pm 8.4	62.2 \pm 10	0.38
Male n (%)	11 (35.4)	5 (13.1)	0.2
RISK FACTORS			
Diabetes n (%)	17 (54.8)	14 (36.8)	0.5
Hypertension n (%)	25 (80.6)	12 (31.5)	0.02
Dislipidemia n (%)	26 (83.8)	13 (34.2)	0.02
Obesity n (%)	4 (12.9)	4 (10.5)	0.5
COPD n (%)	12 (38.7)	9 (23.6)	0.5
Acute myocardial infarction < 4 weeks n (%)	12 (38.7)	16 (42.1)	0.07
Ejection Fraction (mean \pm SD)	48.4 \pm 8.8	46.8 \pm 9.6	0.37
NYHA (mean \pm SD)	2.3 \pm 1	2 \pm 1.2	0.36
Euroscore (mean \pm SD)	4.28 \pm 2.1	4.7 \pm 3.4	0.1
SURGERY PARAMETERS			
Aortic cross-clamp time (minutes) (mean \pm SD)	64.9 \pm 20.7	66.6 \pm 22.5	0.65
CPB** time (minutes) (mean \pm SD)	106 \pm 30.4	106.5 \pm 25	0.53
IABP n (%)	4 (12.9)	4 (10.5)	0.5
Pulmonary artery pressure (mm/Hg) (mean \pm SD)	30.4 \pm 7.2	29.38 \pm 3.7	0.37
RENAL PARAMETERS			
GFR (mean \pm SD)	81 \pm 26.9	77.05 \pm 25	0.5
Creatinine (mean \pm SD)	1.2 \pm 1.2	0.9 \pm 0.3	0.3
Urea (mean \pm SD)	46.8 \pm 23.5	43.2 \pm 15.4	0.4

IABP, intra-aortic balloon pump; SD, standard deviation.

3.2 Renal function

Twenty (42%) patients in the ACEI-group and 16 (51.6%) in the no-ACEI group presented AKI within 2 days after surgery without significant difference between the two groups ($p = 0.6$). Similarly, no significant differences were detected in the number of patients that manifested complete recovery from AKI at day 10 after surgery between the two groups (19/38 patients in the ACEI vs. 13/31 in the no-ACEI group; $p = 0.5$). When absolute renal function was considered, a significant increase in serum creatinine and urea from baseline became evident in both groups ($p = 0.001$ for both values in both groups; p between groups was NS). In parallel, GFR significantly decreased in both groups from baseline to 48 hours postoperatively, remaining somewhat lower as compared to baseline up to 10 days after surgery ($p = 0.001$ for both values in both groups; p between groups = 0.8) (Table 3).

Of note, levels of IL-6, IL-8 were significantly higher whilst those of VEGF and EGF were significantly lower in AKI patients as compared to those in which AKI did not occur ($p = 0.001$ for all comparisons).

3.3 Correlation analysis

EGFR at ICU admission, at 24 h, at 48 h and ten day after surgery correlated significantly with IL-10 ten day after surgery (eGFR ICU admission $p = 0.033$, $r = 0.28$; eGFR at 24 h $p = 0.027$, $r = 0.25$; eGFR at 48 h $p = 0.009$, $r = 0.26$; eGFR at 10 day after surgery $p = 0.041$, $r = 0.31$), with VEGF at 24 h (eGFR ICU admission $p = 0.002$, $r = 0.33$; eGFR at 24 h $p = 0.025$, $r = 0.27$; eGFR at 48 h $p = 0.009$, $r = 0.31$; eGFR at 10

day after surgery $p = 0.007$, $r = 0.32$) and TNF-alpha at 48 h (eGFR ICU admission $p = 0.001$, $r = -0.93$; eGFR at 24 h $p = 0.003$, $r = -0.35$; eGFR at 48 h $p = 0.021$, $r = -0.27$; eGFR at 10 day after surgery $p = 0.017$, $r = -0.28$).

EGFR ten day after surgery correlated also with IL-1A at 48 h ($p = 0.034$, $r = 0.25$) and EGF ten day after surgery ($p = 0.024$, $r = 0.27$) (Table 4).

4. Discussion

Cardiopulmonary bypass during cardiac surgery represents a remarkable inflammatory trigger that may cause a variety of postoperative complications [18]. The activation of circulating granulocytes and monocytes, as well as non-circulating vascular endothelial cells, causes an unbalanced production of pro-inflammatory and anti-inflammatory cytokines ultimately leading to a deranged inflammatory response [19]. It is known that ACE-I may have various immunomodulatory and antioxidative pleiotropic effects [20, 21]. Whether ACE-I, however, may also be effective in reducing the inflammatory burden induced by CPB remains a much-debated issue. In this study, we have evaluated changes in the levels of a wide panel of cytokines up to 10 days after major cardiac surgery with CPB. We have selectively chosen to measure the proinflammatory cytokines IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, TNF-alpha, VEGF and EGF and the anti-inflammatory cytokine IL-10 to provide an exhaustive and broad overview of both the inflammatory and anti-inflammatory response to CPB. Similar multi-cytokine panels have already been implemented in previous studies [11, 22–26].

Table 2. Changes in cytokines levels in the ACEI and no-ACEI groups. Data are reported as media and standard deviation.

		Preoperative	ICU	24-hours	48-hours	10-days	p^a	p^b
IL-1A pg/mL	AceI	0.67 ± 2.47	0.3 ± 0.4	0.29 ± 0.85	0.28 ± 0.5	0.48 ± 0.85	0.57	0.22
	No aceI	0.29 ± 0.28	0.23 ± 0.21	0.24 ± 0.21	0.29 ± 0.42	0.22 ± 0.31	0.6	-
	p^c	0.4	0.38	0.7	0.95	0.12	-	-
IL-1B pg/mL	AceI	2.8 ± 8.4	1 ± 1.95	1 ± 1.76	0.9 ± 1.49	4.8 ± 23.2	0.28	0.3
	No aceI	3.4 ± 15	0.3 ± 0.39	0.48 ± 0.56	0.52 ± 0.53	1.3 ± 4.2	0.42	-
	p^c	0.8	0.05	0.07	0.13	0.4	-	-
IL-2 pg/mL	AceI	7 ± 18	4.6 ± 13.8	4.1 ± 9.9	5.2 ± 7.8	5.4 ± 9.8	0.01	0.6
	No aceI	4.2 ± 3.1	3 ± 3.6	3.3 ± 3.3	4.1 ± 3.1	4.8 ± 4.8	0.08	-
	p^c	0.4	0.5	0.6	0.4	0.7	-	-
IL-4 pg/mL	AceI	17 ± 8.67	4.7 ± 18.9	3.6 ± 7.2	3.5 ± 10.3	3.1 ± 5.1	0.068	0.5
	No aceI	2.1 ± 1.5	1.6 ± 1.4	2.6 ± 4.9	1.5 ± 1.3	1.8 ± 1.4	0.28	-
	p^c	0.3	0.3	0.5	0.3	0.17	-	-
IL-6 pg/mL	AceI	14 ± 42	77.6 ± 67.7	115.7 ± 84.7	135.8 ± 148.5	76.2 ± 72.5	0.001	0.08
	No aceI	8 ± 14.4	94.1 ± 108.1	90.3 ± 63	77.7 ± 63.1	38.3 ± 30.3	0.001	-
	p^c	0.4	0.4	0.17	0.04	0.08	-	-
IL-8 pg/mL	AceI	10.37 ± 11.3	29.48 ± 26.78	50 ± 46.3	40.6 ± 73.7	14.38 ± 17.37	0.001	0.6
	No aceI	7.9 ± 8	29.7 ± 28.2	54.8 ± 76.1	22 ± 26.6	13.9 ± 25.8	0.001	-
	p^c	0.4	0.9	0.7	0.18	0.9	-	-
IL-10 pg/mL	AceI	9.25 ± 32.3	480.68 ± 263.5	246.4 ± 321.6	55.48 ± 143.58	3.29 ± 3.34	0.001	0.007
	No aceI	3.79 ± 8.7	325.5 ± 262.2	130.1 ± 191.4	14.23 ± 22.5	9.1 ± 35.6	0.001	-
	p^c	0.3	0.01	0.08	0.1	0.3	-	-
TNF-alpha pg/mL	AceI	4.5 ± 11.5	3.2 ± 6.2	2.7 ± 2.5	2.7 ± 2.5	2.2 ± 2.2	0.19	0.2
	No aceI	2.4 ± 3.2	1.77 ± 1.36	2.1 ± 1.97	2.2 ± 1.76	2.27 ± 2.8	0.27	-
	p^c	0.33	0.19	0.27	0.3	0.98	-	-
VEGF pg/mL	AceI	48.2 ± 56.2	13.39 ± 12.46	9.9 ± 11.1	16.9 ± 39.65	32.5 ± 20.6	0.001	0.47
	No aceI	52.36 ± 49.9	13.9 ± 8.68	16.59 ± 18	27.58 ± 31.2	30.9 ± 32	0.001	-
	p^c	0.7	0.8	0.06	0.2	0.8	-	-
EGF pg/mL	AceI	21.1 ± 35	22 ± 61	9.2 ± 20.9	13 ± 30.5	9 ± 19.5	0.04	0.16
	No aceI	13.7 ± 21	11.1 ± 13	3.4 ± 6.7	2.68 ± 5.7	3.9 ± 14.3	0.01	-
	p^c	0.3	0.3	0.1	0.06	0.22	-	-

ICU, Intensive Care Unit; p^a : difference within groups; p^b : difference between groups; p^c : difference at each time-point.

We found out a significant increase of IL-6, IL-8 and IL-10 levels after surgery in individuals on previous long-term ACE-I treatment, as well as in those not receiving these drugs. No overall differences were noticed between the two groups for IL-6 and IL-8 levels trends although absolute levels reached by IL-6 at 48 hours and at 10 days from CPB were more pronounced in the ACEI-group. Interestingly, the temporal trend of IL-10, an anti-inflammatory cytokine that suppresses the production of proinflammatory factors, displayed an earlier peak (at ICU admission) and a more prominent decrease in individuals on long term ACE-I treatment

than in those who were not. Our observations are in general agreement with Billings *et al.*, who found increased expression of IL-6, IL-8 and IL-10 levels in patients after cardiac surgery with CPB, with no difference between patients that were on chronic ACE-I, angiotensin II type 1 receptor blockers (ARBs) or even in controls [22]. Collectively, these findings could be explained by the blunting effect of ACE-I on the AT1 receptor, which hampers the capacity of angiotensin II to modulate inflammation [27]; by the same token, ACE-I also reduce the degradation of bradykinin that normally stimulates the secretion of cytokines like IL-6 and IL-10 [28].

Table 3. Glomerular filtration rate (GFR), creatinine and blood urea nitrogen in the ACEI and no-ACEI-groups. Data are reported as media and standard deviation.

		Preoperative	ICU	24-hours	48-hours	10-days	p^a	p^b
GFR mL/min	AceI	77.05 ± 25	75.7 ± 30.7	64.4 ± 26.4	62.7 ± 30.7	71.5 ± 33.2	0.001	0.69
	No aceI	81 ± 26.9	74 ± 24.8	63.5 ± 24	62.3 ± 27.2	69.9 ± 31.4	0.001	
	p^c	0.5	0.8	0.8	0.9	0.8	-	
		Preoperative	ICU	24-hours	48-hours	10-days	p^a	p^b
Creatinine mg/dL	AceI	1.2 ± 1.2	1 ± 0.4	1.2 ± 0.5	1.4 ± 0.8	1.2 ± 0.7	0.001	0.6
	No aceI	0.9 ± 0.3	1 ± 0.37	1.2 ± 0.5	1.4 ± 0.8	1.2 ± 0.78	0.001	
	p^c	0.3	0.9	0.7	0.9	0.9	-	
		Preoperative	ICU	24-hours	48-hours	10-days	p^a	p^b
Urea mg/dL	AceI	46.8 ± 23.5	48.8 ± 27.8	52.9 ± 26.6	66.6 ± 34	80.1 ± 43.7	0.001	0.8
	No aceI	43.2 ± 15.4	47.6 ± 18.6	52.8 ± 21.9	61.5 ± 30.9	77.2 ± 36.2	0.001	
	p^c	0.4	0.8	0.9	0.5	0.7	-	

ICU, Intensive Care Unit; p^a : difference within groups; p^b : difference between groups; p^c : difference at each time-point.

Table 4. Correlation analyses.

	GFR-ICU	GFR-24-hours	GFR-48-hours	GFR-10-days
IL-10 10-days	$p = 0.033$, $r = 0.28$	$p = 0.027$, $r = 0.25$	$p = 0.009$, $r = 0.26$	$p = 0.041$, $r = 0.31$
VEGF 24-hours	$p = 0.002$, $r = 0.33$	$p = 0.025$, $r = 0.27$	$p = 0.009$, $r = 0.31$	$p = 0.007$, $r = 0.32$
TNF-alpha 48-hours	$p = 0.001$, $r = -0.93$	$p = 0.003$, $r = -0.35$	$p = 0.021$, $r = -0.27$	$p = 0.017$, $r = -0.28$
IL-1A 48-hours	NS	NS	NS	$p = 0.034$, $r = 0.25$
EGF 10-days	NS	NS	NS	$p = 0.024$, $r = 0.27$

GFR, Glomerular filtration rate; ICU, Intensive Care Unit; NS = not significant; r = Pearson coefficient.

In our cohort, EGF and VEGF levels decreased significantly in both groups, while IL-2 exhibit a significant trend only in people receiving ACE-I. Conversely, IL-1alpha, IL-1beta, IL-4 and TNF-alpha levels exhibited stable levels over-time in both the ACEI and no-ACEI groups. In a milestone, prospective study, Justus *et al.* analyzed the immune responses in 20 pediatric patients undergoing heart surgery with CPB after stimulating blood samples with LPS [23]. IL-12, TNF- α , IL-1 β , IL-6, IL-8 and IFN- γ levels were completely suppressed while IL-10, IL-1Ra and MCP-1 ones remained just marginally produced. Findings from this study indicated that cardio-surgery with CPB may sometimes induce the immunodepression with a tendency towards recovery after termination of CPB. Hence, also in our study, the early immune system dysfunction and immunodepression, induced by CPB, might represent a plausible explanation to the absence of variation found in some of the cytokines analyzed.

Probably, TNF-alpha is also undetectable in the plasma after CPB as the expression of an imbalance in TNF-alpha production between alveolar macrophages and plasma monocytes [29].

Acute kidney injury that develops after cardiac surgery has

a multifactorial pathogenesis, but CPB may play a major role as it is considered an important independent predictor of renal failure after cardiac surgery [30]. In a previous study, Di Benedetto *et al.* evaluated 536 patients undergoing cardiac surgery on CPB among which 281 received ACE-I preoperatively [13]. AKI developed in 49 patients (9.1%) and 23 (4.2%) required dialysis support. The incidence of AKI was 6.4% in patients who received preoperative ACEI and 12.2% in patients who did not ($p = 0.02$). The incidence of AKI requiring dialysis was 2.4% in the treatment group and 6.3% in controls ($p = 0.03$), therefore suggesting that preoperative ACE-I administration may help reducing the risk of AKI associated with CPB. Unlike this study, we did not find any difference in the incidence of AKI, in the rate of renal function recovery and, not even, in the temporal trend of clinical indexes of renal function between subjects on chronic ACE-I therapy and subjects who were not. Despite in apparent contradiction with results from the above-mentioned study, our findings may have concrete explanations. Firstly, we considered *by protocol* patients who were already on a long-time ACE-I course; hence acute protective effects in “drug-naïve” subjects would in principle be ruled out against a potential tolerance phenomenon. Secondly, the diagnostic criteria adopted to

identifying AKI have progressively been refined during the last decade, making difficult to compare even in an indirect way the real occurrence of the phenomenon between the two studies. Conversely, such an absence of difference of chronic ACE-I treatment on renal outcomes after CBP is in agreement with another study of 32 patients undergoing elective cardiac surgery under hypothermic CBP in which perioperative changes in renal hemodynamic and function remained unaffected by previous long-term treatment with these drugs [13]. Of note, in this study renal function was measured by creatinine clearance, which may overestimate the true GFR because of minimal tubular creatinine secretion. Accordingly, the last KDIGO guidelines nowadays recommend using serum creatinine levels and a GFR estimating equation for a more precise assessment of patient's renal function, particularly in this high risk class of patients [31]. In addition, the use of more reliable and widely adopted criteria for diagnosing AKI occurrence, such as the AKIN, is imperative to corroborate further the reliability of results.

When we stratified patients according to AKI occurrence, we found that IL-6 and IL-8 plasma levels were significantly higher in the AKI group, while VEGF and EGF were significantly lower. This findings are generally in agreement with those reported by other studies [26, 32]. Of note, although evidence exists demonstrating that low urinary EGF levels are predictor of AKI [33], no data are so far available regarding plasma EGF.

Our study has some limitations that have to be mentioned. Firstly, it was a single center study with a relatively small sample size. Although we acknowledge the pilot, exploratory nature of our research, this prevented us to perform more complex analyses to identify potential confounders when considering the impact of chronic ACE-I therapy on the endpoints of interest. Residual confounding remain the key limitation of any observational study. In particular, in our cohort, we cannot rule out the presence of a significant selection bias and confounding by indication, with respect to the inclusion of patients who were on long-term ACE-I therapy. Nevertheless, despite this, we did not find any significant difference in either the main clinical characteristics and the procedural parameters between patients who were on ACE-I therapy and those who were not, with the (predictable) exception of hypertension incidence and dysmetabolic conditions. Finally, but not less important, the homogeneity of the study cohort with respect to age, race, clinical characteristics and indications to CPB warrants consistency in the analytical approach but may hamper the generalizability of findings to the whole spectrum of patients eligible to undergoing major cardiac surgery.

5. Conclusions

In this pilot study, we have demonstrated that chronic, long-term therapy with ACE-I may influence the increase of cytokine levels induced by CPB, apparently with no effects on both the entity of kidney damage and functional recovery

after the surgical stress. Future investigations are advocated to clarify the clinical significance of these findings and the potential impact on therapeutic management, particularly in larger and more heterogeneous cohorts.

Author contributions

Research idea: PP, GF; Data Collection and analysis: FS, DB; Manuscript preparation and revision: PP, DB, GC, FS, MA, PM, GF.

Ethics approval and consent to participate

The Ethical Committee approved the study protocol. Informed consent was obtained from each patient.

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Conflict of interest

The Authors declare no conflict of interest with respect to the present work.

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