SUPPLEMENTAL MATERIAL

METHODS

OPERA patient population and ω-3-PUFA supplementation

The (Omega-3 Fatty Acids for Prevention of Post-Operative Atrial Fibrillation) OPERA trial enrolled 1,516 patients undergoing cardiac surgery from 28 medical centers in 3 countries (United States, Italy, and Argentina). The primary findings of the OPERA trial, relating to peri-operative oral long-chain ω-3 polyunsaturated fatty acid (n-3 PUFA)supplementation, have been reported.[1] The inclusion criteria were developed to enroll a broad and generalizable population, including age ≥18 years, presence of sinus rhythm at surgery on the screening electrocardiogram (ECG), and being scheduled for cardiac surgery on the next day or later. The principal exclusion criteria were: absence of sinus rhythm at screening, regular use of fish oil, known allergy or intolerance to fish oil or olive oil, being currently pregnant, existing or planned cardiac transplant or use of ventricular assist device, or being unable or unwilling to provide informed consent.[1] The study was approved by the human subjects committees of all participating institutions and conducted according to international standards of Good Clinical Practice (FDA Title 21 part 312, International Conference on Harmonization guidelines). All patients provided written informed consent.
Following randomization, patients received a loading dose of 8-10 grams of fish oil over 2-5 days pre-operatively, followed by 2 g/d post-operatively; or matched placebo.

Assay of circulating cardiac markers

Blood samples were collected from each OPERA patient at the time of enrollment, on the morning of surgery, at the end of surgery (time of skin closure), and approximately 48 hours after surgery. EDTA anticoagulated plasma samples were stored at -70°C and shipped on dry ice to a central repository for long-term storage at -70°C. N-terminal Pro Brain Natriuretic Peptide (NT-proBNP), N-terminal pro brain natriuretic peptide, high-sensitivity cardiac troponin T (hs-cTnT), and C-reactive protein (CRP) levels were measured in a single run by commercial assays in a centralized laboratory by trained personnel unaware of patient characteristics.[2]

Oxidative stress biomarkers

F2-isoprostanes, F3-isoprostanes, and isofurans were measured in plasma and urine samples which had been obtained in each OPERA patient at enrollment, at the end of surgery (time of skin closure), and approximately 48 hours after surgery.[17]

Statistical methods

Parameters in patients with or without subsequent post-operative atrial fibrillation (POAF) were analyzed by Chi2 (for discrete variables), ANOVA (for normally distributed continuous variables) or Kruskal-Wallis (for non-normally distributed continuous variables) test. In addition, we evaluated how the measures of cardiac strain (BNP) and injury (troponin), systemic inflammation (CRP), and oxidative stress (plasma and urine F2-isoprostanes, F3-isoprostanes, and isofurans) related to each atrial histopathological endpoint by means of multivariable linear or logistic regression analyses, adjusted for age, chronic obstructive pulmonary disease (COPD), history of atrial fibrillation, heart failure, valve surgery and log EuroSCORE. Associations of clinical characteristics, histopathological measures, and biomarkers with the incidence of POAF were assessed by means of multivariable logistic regression analyses, adjusted for age, COPD, history of atrial fibrillation, heart failure, valve surgery and log EuroSCORE. Analyses were performed by means of SPSS (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corporation). The level of statistical significance was set at $p \leq 0.05$ for all analyses.

REFERENCES

1. Mozaffarian D, Marchioli R, Gardner T, Ferrazzi P, O'Gara P, Latini R, Libby P, Lombardi F, Macchia A, Page R, et al. The omega-3 Fatty Acids for Prevention of Post-Operative Atrial Fibrillation trial--rationale and design. *Am Heart J*. 2011;162:56-63 e53.
2. Masson S, Wu JH, Simon C, Barlera S, Marchioli R, Mariani J, Macchia A, Lombardi F, Vago T, Aleksova A, et al. Circulating cardiac biomarkers and postoperative atrial fibrillation in the OPERA trial. *Eur J Clin Invest*. 2015;45:170-178.

TABLES

Supplementary Table 1. Comparison OPERA cohort and histological cohort.

Variables	OPERA population (N=1516)	HISTOLOGICAL population (N=239)
Age	63.7 ± 12.5	64.8 ± 12.6
Sex		
Female	422 (27.8%)	66 (27.6%)
Male	1094 (72.2%)	173 (72.4%)
Treatment		
Placebo	758 (50.0%)	122 (51.0%)
n-3 PUFA	758 (50.0%)	117 (49.0%)
Current smoking	195 (12.5%)	25 (10.6%)
COPD	170 (11.2%)	38 (15.9%)
Diabetes Mellitus	393 (25.9%)	72 (30.1%)
Hypertension	1135 (74.9%)	196 (82.0%)
Chronic renal failure	96 (6.3%)	11 (4.6%)
Prior myocardial infarction	366 (24.1%)	57/236 (24.2%)
HF	416 (27.4%)	53 (22.3%)
Previous PCI	179 (11.8%)	41 (17.2%)
Previous AF	114 (7.5%)	19 (8.0%)
Prior cardiac surgery *		
Coronary bypass	35 (2.3%)	5 (2.1%)
Valve surgery	50 (3.3%)	3 (1.3%)
Other cardiac surgery	25 (1.6%)	3 (1.3%)
LA size (mm)	42.2 ± 7.7	41.8 ± 7.2
EF(%)	56.7 ± 11.4	58.0 ± 10.4
B-Blockers	877 (57.8%)	113 (53.6%)
Statins	863 (56.9%)	113 (53.6%)
ACE_ARB	775 (51.1%)	116 (55.0%)
Diuretics	423 (27.9%)	58 (27.5%)
ASA	774 (51.1%)	128 (60.7%)
antiPLT	898 (59.2%)	130 (61.6%)
Antiarrhytmics	84 (5.5%)	9 (4.3%)
EuroScore		
Add-scale	5.0 [3.0 – 7.0]	5.0 [3.0 – 7.0]
Log-scale	3.7 [1.9 – 7.4]	3.8 [1.9 – 8.4]
Valve Surgery		
No	760 (50.1%)	98/238 (41.2%)
Yes	756 (49.9%)	140/238 (58.8%)
Pump time (hours)	1.65 ± 1.0	1.64 ± 0.69
Cross time (hours)	1.20 ± 0.8	1.20 ± 0.54
Cardioplegia	1252 (82.6%)	232 (97.5%)
Blood transfusion	-	98 (41.2%)

* *Prior cardiac surgery ≠ coronary bypass + valve surgery + other cardiac surgery, one patient had multiple surgeries.*

Supplementary Table 2. Tertiles of fibrosis and baseline characteristics

Variables	N	Tertile 1 [2.0 – 5.99]	Tertile 2 [6.0 – 10.56]	Tertile 3 [10.6 – 65.83]	p
Fibrosis (%)	239	79 (33.0%)	80 (33.5%)	80 (33.5%)	
Fibrosis (median [Q1-Q3])		4.26 [3.22 – 5.02]	8.20 [7.22 – 9.34]	16.00 [12.65 – 19.10]	
Age	239	63.9 ± 13.2	63.8 ± 13.3	66.7 ± 11.09	0.274
Sex	239				
Female		24 (30.4%)	22 (27.5%)	20 (25.0%)	0.750
Male		55 (69.6%)	58 (72.5%)	60 (75.0%)	
Treatment*	239				
Placebo		42 (53.2%)	38 (47.5%)	42 (52.5%)	0.736
n-3 PUFA		37 (46.8%)	42 (52.5%)	38 (47.5%)	
COPD	239	13 (16.5%)	13 (16.3%)	12 (15%)	0.964
Diabetes Mellitus	239	21 (26.6%)	24 (30.0%)	27 (33.8%)	0.615
Hypertension	239	65 (82.3%)	65 (81.3%)	66 (82.5%)	0.976
Chronic renal failure	239	5 (6.3%)	3 (3.8%)	3 (3.8%)	0.688†
Prior myocardial infarction	236	16/78 (20.5%)	20/79 (25.3%)	21/79 (26.6%)	0.645
Angina pectoris	208	12/71 (34.1%)	15/69 (33.2%)	24/68 (35.3%)	**0.034**
HF	238	17 (21.5%)	18 (22.8%)	18 (22.5%)	0.980
Previous PCI	239	11 (13.9%)	16 (20.0%)	14 (17.5%)	0.594
Previous AF	238	9 (11.5%)	4 (5.0%)	6 (7.5%)	0.311
Prior cardiac surgery ‡		3 (3.8%)	4 (5.0%)	3 (3.8%)	0.999†
Coronary bypass	239	2 (2.5%)	2 (2.5%)	1 (1.3%)	0.873†
Valve surgery	239	1 (1.3%)	1 (1.3%)	1 (1.3%)	0.999†
Other cardiac surgery	239	0	2 (2.5%)	1 (1.3%)	0.775†
LA size (mm)	132	42.2 ± 7.0	40.1 ± 6.3	43.4 ± 8.4	0.095
EF(%)	210	56.7 ± 11.7	59.5 ± 9.5	57.8 ± 9.7	0.273
EuroScore					
Add-scale	239	5.04 ± 2.9	4.98 ± 3.1	5.4 ± 2.9	0.630
Log-scale	239	3.4 [1.7 – 8.6]	3.5 [1.6 – 9.1]	4.4 [2.3 – 6.9]	0.742
Valve Surgery	238				
No		28/79 (35.4%)	36/80 (45.0%)	34/79 (43.0%)	0.434
Yes		51/79 (64.6%)	44/80 (55.0%)	45/79 (56.9%)	
Pump time (hours)	238	1.7 ± 0.7	1.6 ± 0.7	1.6 ± 0.7	0.828
Cross time (hours)	238	1.2 ± 0.5	1.2 ± 0.6	1.2 ± 0.6	0.984
Cardioplegia	238	78 (98.7%)	78 (97.5%)	76 (96.2%)	0.705†
Blood transfusion	238	35 (44.3%)	34 (42.5%)	29 (33.2%)	0.598

**Patients were randomized to receive a peri-operative loading dose of 8-10 grams of fish oil over 2-5 days prior to cardiac surgery, followed by 2 g/d post-operatively; or matched placebo*

† *P-value for Chi^2 Test (discrete variables), ANOVA (normally distributed continuous variables) or Kruskal-Wallis (not normally distributed continuous variables)*

‡ *Prior cardiac surgery ≠ coronary bypass + valve surgery + other cardiac surgery, one patient had multiple surgeries.*