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Effect of CD3+ T-Lymphocyte and n-3 Polyunsaturated Fatty Acids on the Diagnosis or Treatment of Atrial Fibrillation

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Review of the relationship between n-3 polyunsaturated fatty acids and the atrial fibrillation

It is well established that the consumption of fish is associated with lower rates of cardiovascular death (Albert CM, et al., 2002; Hu FB, et al., 2002). Dietary fish oil supplementation has been shown to reduce mortality in high-risk groups through a reduction in sudden cardiac death and ventricular tachyarrhythmia. It has been recently reported that atrial fibrillation (AF) is associated with inflammation and inflammatory cytokines, and n-3 polyunsaturated fatty acids (PUFAs) might be of anti-inflammatory effects. Whether PUFAs has some antiarrhythmic effect and can be used in the treatment of AF is still unknown.

1.2. Dietary n-3 PUFA supplementation attenuates the inducibility and maintenance of AF

We established canine sterile pericarditis model and evaluate the anti-inflammatory effect of PUFAs on AF (Zhong Zhang, et al., 2010). Twenty mongrel sex-matched adult dogs were randomly divided into two groups. In the n-3 PUFA group (n=10), oral administration of eicosapentaenoic+docosahexaenoic acid (EPA+DHA), 2 g/day (Omacor, Solvay Pharmaceuticals GmbH, Hanover, Germany) was started 4 weeks before the baseline study, and was continued until the end of the study. The dogs in the control group (n=10) did not receive n-3 PUFAs or plant oil for 4 weeks. We examined the plasma concentration of the CRP, IL-6, and

TNF- α before the operation and on the second postoperative day in both groups. There were no significant differences in three biomarkers of inflammation between two groups before the operation, and these biomarkers were significantly increased in both groups on the second postoperative day. However, three proinflammatory cytokines were significantly lower in the PUFA group than in the control group respectively (CRP, 7.6 ± 0.5 vs. 11.7 ± 1.3 mg/dl, $P < 0.0001$ Fig. 1; IL-6, 112.0 ± 37.3 vs. 142.0 ± 19.6 pg/ml, $P < 0.0001$ Fig. 2; TNF- α , 83.3 ± 8.5 vs. 112.4 ± 8.2 pg/ml, $P < 0.0001$ Fig. 3).

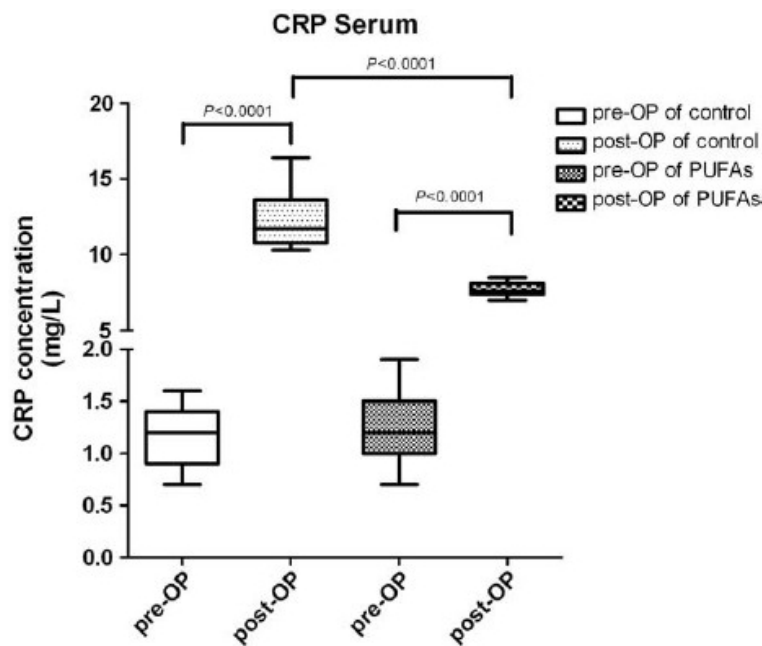


Figure 1. Comparison of CRP levels between the control and PUFA groups before and after operation. Before the operation, there were no significant differences in CRP levels between two groups. On the second postoperative day, CRP was significantly increased in both groups; however, it was significantly lower in the PUFA group than in the control.

The main finding of this study is that EPA and DHA supplementation of the diet can decrease plasma concentration of the CRP, IL-6 and TNF- α in acute inflammation of canine sterile pericarditis, suggesting depression of inflammatory cytokines by n-3 FUFAs may involve in the anti-atrial fibrillation process. The results also showed that the PUFA group had a less AF inducibility and maintenance than the control group (Table1).

Thus we may reasonably conclude that Dietary n-3 PUFA supplementation attenuates the inducibility and maintenance of AF in the sterile pericarditis model by reducing the production of proinflammatory cytokines.

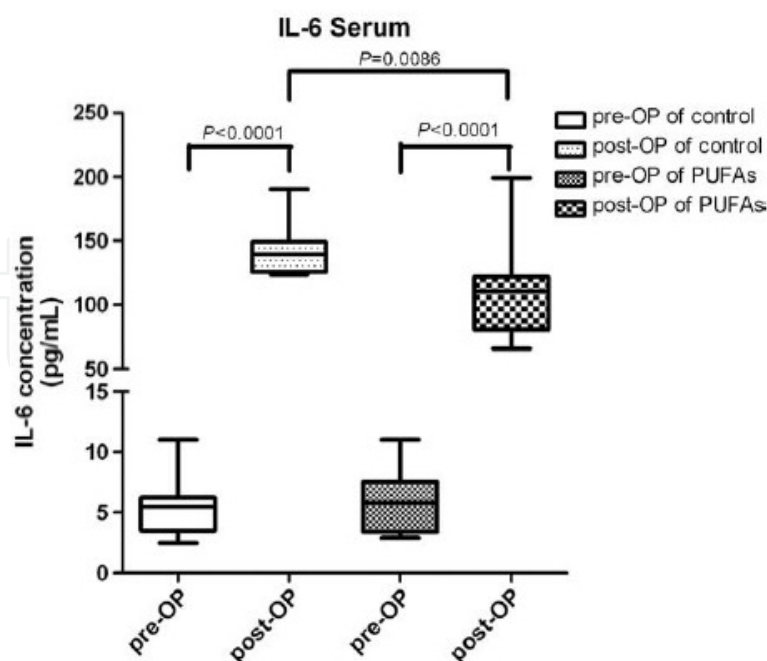


Figure 2. Comparison of IL-6 levels between the control and PUFA groups before and after operation. Before operation, there were no significant differences in IL-6 levels between two groups. On the second postoperative day, IL-6 was significantly increased in both groups; however, it was significantly lower in the PUFA group than in the control.

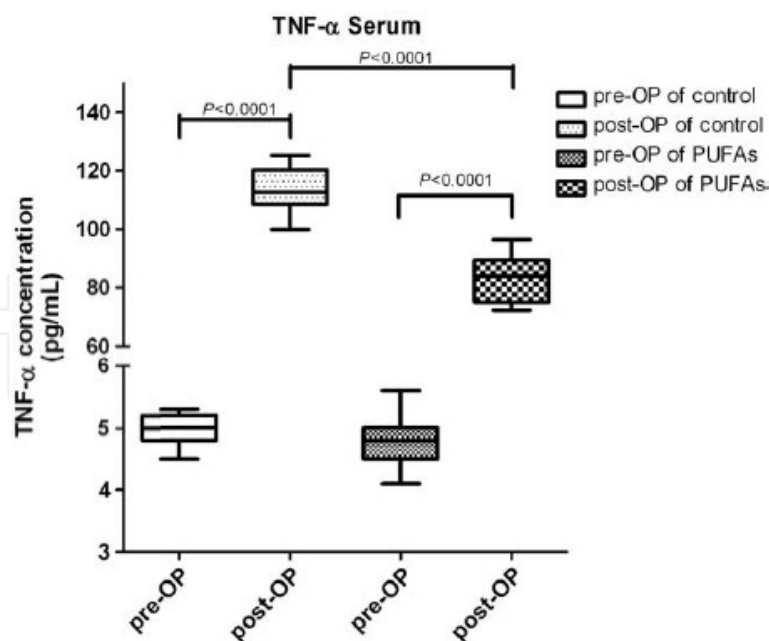


Figure 3. Comparison of TNF-α levels between the control and PUFA groups before and after operation. Before the operation, there were no significant differences in TNF- α levels between two groups. On the second postoperative day, TNF- α was significantly increased in both groups; however, it was significantly lower in PUFA group than in the control.

Comparison of electrophysiological parameters (CT) between the control and PUFA groups before and after operation.

Intra-atrial CT (ms)	Control group (n = 10)		PUFA group (n = 10)	
	Before	After	Before	After
<i>RAA-LRA</i>				
200	43.4 ± 2.8	51.9 ± 4.8 [‡]	44.6 ± 5.4	46.6 ± 4.4 [*]
300	44.6 ± 4.6	50.8 ± 4.6 [‡]	45.8 ± 4.5	46.0 ± 4.2 [*]
400	45.2 ± 4.8	51.0 ± 4.3 [‡]	44.6 ± 5.2	47.0 ± 3.4 [*]
<i>RAA-HRA</i>				
200	33.0 ± 3.2	41.2 ± 4.2 [#]	34.0 ± 3.3	36.0 ± 4.6 [*]
300	35.0 ± 5.0	42.0 ± 5.0 [‡]	35.5 ± 4.5	36.8 ± 4.8 [*]
400	36.0 ± 5.4	43.2 ± 4.8 [‡]	37.0 ± 4.8	38.4 ± 4.4 [*]
<i>RAA-ARA</i>				
200	26.2 ± 5.5	32.6 ± 5.2 [‡]	26.8 ± 4.2	27.4 ± 5.0 [*]
300	25.4 ± 4.6	32.6 ± 4.4 [‡]	27.2 ± 4.7	28.0 ± 4.2 [*]
400	25.6 ± 4.3	33.0 ± 5.0 [‡]	26.4 ± 3.5	28.0 ± 4.0 [*]

CT=conduction time; RAA=right atrial appendage; LRA=low lateral right atrium; HRA=high lateral right atrium; ARA=anterior right atrium.

†p<0.05 compared with before the operation

p<0.0001 compared with before the operation

* P<0.05 compared with the control group

‡ p<0.01 compared with before operation.

Table 1. Comparison of electrophysiological parameters (CT) between the control and PUFA groups before and after operation

1.3. Inflammation and post cardiac surgery AF

Although the pathophysiological mechanism underlying the genesis of post cardiac surgery AF has been the focus of many studies,it only remains partially understood. The inflammatory cascade and catecholamine surge associated with surgery have been thought of playing a prominent role in initiating AF after cardiac surgery. As a prototypic marker of inflammation, CRP has been the focus of many studies, which is driven by the proinflammatory cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6(Bruins P et al.,1997) found that IL-6 rises initially and peaks at 6 h after cardiac surgery, and CRP levels increases and peaks on the second postoperative day of the cardiac surgery, with complement-CRP complexes levels peaking on the 2nd or 3rd postoperative day. The incidence of atrial arrhythmias similarly peaks on postoperative day 2 or 3. Other researchers have confirmed that IL-6 and CRP increased after cardiac surgery, with the incidence of atrial arrhythmias similarly peaking on the 2nd or 3rd postoperative day [13,14](Ishida K,et al.,2006;Kumagai K,et al., 2004). Many studies have related an increase in CRP and IL-6 in both paroxysmal AF and persistent AF, and concluded that CRP is not only associated with the presence of AF but may also predict an increased risk for future development of AF [15](Aviles RJ,et al.,2003).

Other studies correlated leukocytosis to an increased incidence in AF in postoperative cardiovascular patients [16,17](Abdelhadi RH,et al.,2004;Lamm G,et al.,2006), and found that a more pronounced increase in postoperative WBC count will independently predict development of postoperative AF. Moreover, atrial inflammation of cardiac surgery effects on the electrical properties of atrial tissue, and the degree of atrial inflammation was associated with a proportional increase in the inhomogeneity of atrial conduction and AF duration [18] (Ishii Y, et al.,2005). Administration of anti-inflammatory drugs (dexamethasone or cortisone) significantly decreases the incidence of AF after cardiac surgery [19,20](Yred JP,et al., 2000;Halonen J,et al.,2007), which supports this inflammation-AF

hypothesis. The canine sterile pericarditis model can perfectly simulate inflammatory circumstances of the post cardiac operation, by which AF can be induced and also peaks on the 2nd postoperative day [10](Page PL,et al.,1986). In this model, the multiple unstable reentrant circuits were showed during AF, and it was critical for maintaining AF [10] (Page PL,et al.,1986). According to the multiple-wavelet hypothesis, atrial wavelength determines the number of wavelets, and the atrial wavelength is the product of AERP and the intra-atrial conduction velocity. So, the AERP and the intra-atrial conduction velocity have been thought to be important for the perpetuation of AF. In this canine sterile pericarditis model, we have evaluated CRP, IL-6 and TNF- α level on the baseline and on the 2nd postoperative day, and found that they all significantly increased in both groups. We simultaneously evaluated the role of inflammation on atrial electrophysiological properties, and found that inflammation can shorten AERPs and prolong intra-atrial CT in the canine sterile pericarditis model, which increased the inducibility and stability of AF. Our results are concordant with the previous results. Thus, in this model, elevated CRP, IL-6 and TNF- α were associated with sustained AF, suggesting that electrophysiological changes resulting from inflammation perpetuate AF.

1.4. Other potential mechanisms of antiarrhythmic action of n-3 PUFA administration

The current hypotheses of n-3 PUFAs in preventing AF are based on their inhibiting capacity of some ion channels. Previous studies have demonstrated that n-3 PUFAs have capacity to inhibit fast, voltage dependent sodium currents, L-type calcium currents, the Na⁺/Ca²⁺ exchanger, which might prevent delayed after-depolarizations and triggered activity, as well as their class III antiarrhythmic-like effect on Kv1.5 channel (IK_{UR} current present in the atrium) [36,37](Xial YF,et al.,2004;Honore E,et al.,1994). Other studies found n-3 PUFAs can attenuate atrial structural remodeling not only by activating matrix metalloproteinase-9 mRNA expression and attenuating of collagen turnover [38](Laurent G,et al.,2008), but also by modulating of atrial gap junction protein CX40 and CX43 [39] (Sarrazin JF,et al.,2007). Otherwise, evidence suggests that n-3 fatty acids consumption attenuates oxidative stress in humans, and the underlying mechanisms may lead to suppressed production of reactive oxygen species by leukocytes, inhibition of the pro-oxidant enzyme phospholipase A₂, and induction of antioxidant enzymes [40](Mori TA,et al.,2003).

2. The expression of CD69 and CD3+ T-lymphocytes in the diagnosis or therapy of AF

Recently, the link between inflammation and AF appeals increasing attention. Many studies have demonstrated serum or plasma inflammation biomarkers have a link with the development of AF, which supported chronic inflammatory responses might participate in the development of AF (Chung MK et al.,2006;Hernandez Madrid A et al.,2007;Li J et al.,2010;Psychari SN et al.,2005). Moreover, some other studies reported that activated T-lymphocytes and macrophages infiltrated the endomyocardial of patients with AF, which supported the activation of local T-lymphocytes played a role in the pathogenesis of AF (Chen MC et al., 2008;Nakamura Y et al.,2003;Yamashita T et al.,2010). However, up to now, there are no evidences supporting the link between the activation of peripheral blood T-lymphocytes and AF. As is well-known, CD69 and HLA-DR are markers of activated T-lymphocytes. In other words, they are both specifically expressed on the surface of activated T-lymphocytes.[11, 12](Caruso A et al.,1997;Reddy M et al.,2004) CD69 is known as an early activation marker of T-lymphocytes [13](Sancho D et al.,2005) and HLA-DR is known as a late activation marker of T-lymphocytes.[14] (Gerald L et al., 2010)They both play a role in the specific immune response to inflammation. [15, 16, 14, 17-19, 13, 20] (Sancho D et al.,2005;Gerald L et al.,2010;Afeltra A et al.,1993;Ferenczi K et al.,2000;McDonald GB et al.,1987;Miki-Hosokawa T et al.,2009;Oczenski W et al., 2003;Vance BA et al.,2005) To our knowledge, there is little information available about the expression of CD69 and HLA-DR on peripheral blood CD3⁺ T-lymphocytes in patients with AF. Thus we designed an experiment to investigate the relationship between the activation of peripheral blood CD3⁺ T-lymphocytes and AF by flow cytometric analysis that we aimed to provide more evidences to support this phenomenon (Liu L, et al., 2012;53(4):221-4.)

Fifty paroxysmal AF patients and fifty-six persistent AF patients, underwent successful electrical cardioversion, were enrolled in this study. Percentage of CD69 and Human leukocyte antigen DR (HLA-DR) positive peripheral blood CD3⁺ T-lymphocyte, which indicates T-lymphocyte activation, were examined by flow cytometric analysis in the patients and fifty-one healthy controls. Patients groups had higher levels of CD69 and HLA-DR than healthy controls. During three-month follow-up, 37 patients had recurrence of AF (recurrence group) and 50 patients remained in sinus (sinus group).

The results showed that Patients with AF groups had higher levels of CD69 and HLA-DR than healthy controls. The mean value of CD69 was significantly up-regulated in patients with paroxysmal ($1.48\% \pm 0.42$) and persistent AF ($1.55\% \pm 0.38$) compared with healthy individuals ($1.07\% \pm 0.37$; all $p < 0.001$). The mean value of HLA-DR was also significantly up-regulated in patients with paroxysmal ($35.16\% \pm 10.89$) and persistent AF ($37.73\% \pm 10.78$) compared with healthy individuals ($26.6\% \pm 8.41$; all $p < 0.001$. Figure 4)

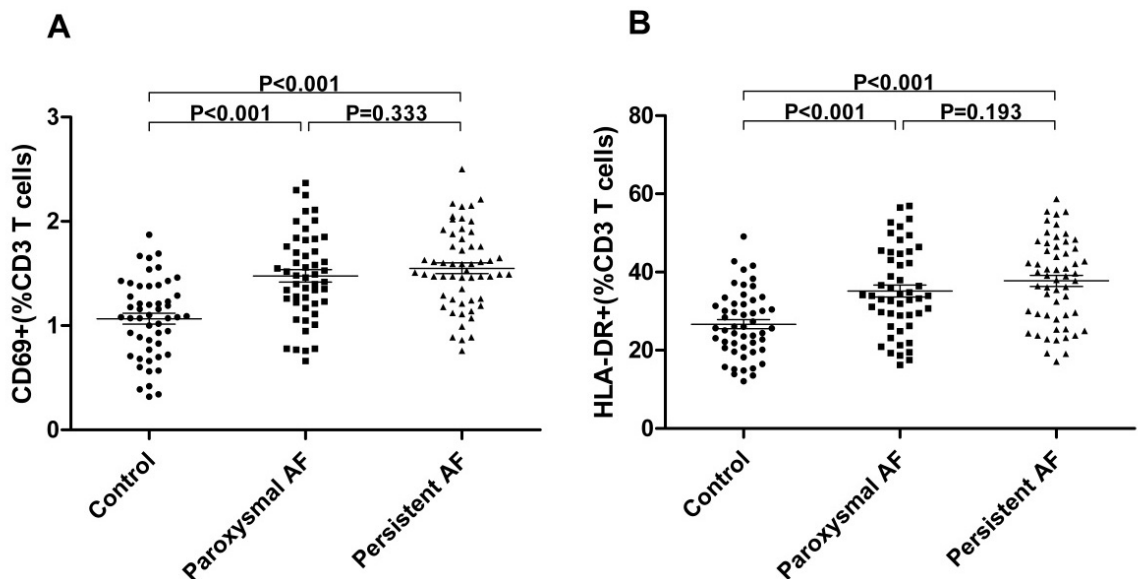


Figure 4. Percentage of CD3⁺CD69⁺ T-lymphocytes (A) and CD3⁺HLA-DR⁺ T-lymphocytes (B) were shown, respectively.

During three-month follow-up, 37 patients had recurrence of AF (recurrence group) and 50 patients remained in sinus (sinus group). The results demonstrated that the mean values of CD69 and HLA-DR in sinus group at follow-up ($1.17\% \pm 0.38$, $28.71\% \pm 8.70$) were all significantly down-regulated compared with before cardioversion ($1.45\% \pm 0.44$, $34.71\% \pm 9.75$; all $p < 0.05$). However, there were no statistically significant differences between recurrence group at follow-up ($1.57\% \pm 0.39$, $36.40\% \pm 9.32$) and before cardioversion ($1.60\% \pm 0.35$, $37.72\% \pm 11.11$; $p = 0.721$, $p = 0.544$). Figure.5)

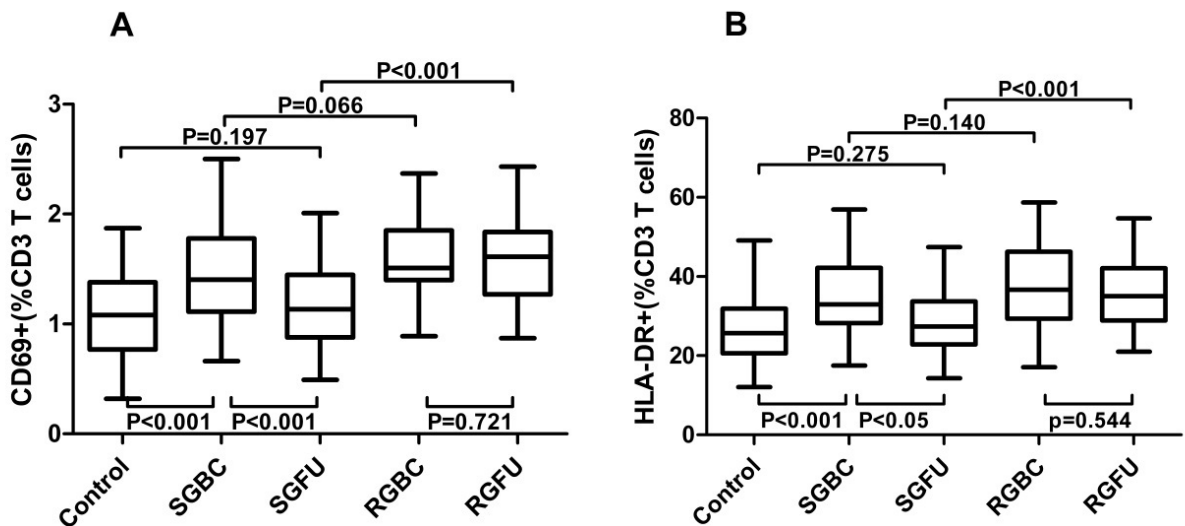


Figure 5. The mean values of CD3⁺CD69⁺ T-lymphocytes (A) and CD3⁺HLA-DR⁺ T-lymphocytes (B) in the sinus group and recurrence group at follow-up and before cardioversion.

Before we received the result,we conduct the baseline clinical Characteristics of the Studied Population to ensure facticity of the results (Table 2).

	Control (n=51)	Paroxysmal AF (n=50)	Persistent AF (n=56)	<i>p</i>
Age(yrs)	64.4±8.5	64.3±9.5	67.2±9.7	0.176
Men	31 (61%)	32 (64%)	34 (61%)	0.927
Hypertension	13 (25%)	20 (40%)	25 (45%)	0.106
Hyperlipidemia	11 (22%)	8 (16%)	7 (13%)	0.448
Diabetes	2 (4%)	3 (6%)	5 (9%)	0.566
Drugs:				
ACE-I/ARB	10 (20%)	11 (22%)	21 (38%)	0.074
Statins	8 (16%)	7 (14%)	6 (11%)	0.743
β-blockers	8 (16%)	26 (52%)	30 (54%)	<0.001
CCBs	5 (10%)	12 (24%)	7 (13%)	0.108
WBC count(per uL)	6349±1891	6768±1859	6375±1663	0.819
Lymphocytes(%)	30.9±3.8	32.5±4.3	31.5±4.1	0.147
Monocytes(%)	5.5±1.2	5.8±1.1	5.4±1.1	0.123
CRP(mg/dl)	0.24±0.12	0.48±0.25	0.60±0.22*	<0.001

Table 2. Characteristics of the Studied Population

ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocking agents; CCBs, calcium channel blockers; WBC, white blood cells; CRP, C-reactive protein; *p*, probability of significance (difference among three groups); and *, *p*<0.05 persistent AF vs paroxysmal AF.

In this study, we found that the respective expression of CD69 and HLA-DR on peripheral blood CD3⁺ T-lymphocytes in AF patients was significantly higher than control group, which might suggest that high expression of CD69 and HLA-DR was associated with AF. In the subsequent follow-up, we further found that the expression of CD69 and HLA-DR in sinus group at follow-up was significantly down-regulated compared with before cardioversion. However, the expression of CD69 and HLA-DR in recurrence group at follow-up was not significantly down-regulated. It might further support that the CD69 and HLA-DR levels were related with the state of AF.

As demonstrated by the present study, there was a link between high expression of CD69 and HLA-DR and AF. CD69, known as an early activation marker of lymphocytes, is a type II transmembrane glucoprotein and may enhance activation and proliferation/differentiation of T-lymphocytes. (Sancho D et al. 2005;Vance BA et al.,2005;Beeler A et al.,2008;Creeners P

et al.,2002) HLA-DR belongs to the MHC class II system which is known as a late activation marker of lymphocytes. It is required for antigen presentation and activation of helper T-lymphocytes. (Gerald L et al.,2010;Oczenski W et al.,2003;Bobryshev YV et al.,2011)They respectively expressed in some inflammatory infiltrates and played important roles in the pathogenesis of some inflammatory diseases such as allergic airway inflammation, (Miki-Hosokawa T et al.,2009;Wang HY et al.,2006) rheumatoid arthritis, (Afeltra A et al.,1993) psoriasis vulgaris lesional skin active inflammatory bowel disease (McDonald GB et al., 1987). So the increase of CD69 and HLA-DR on CD3⁺ T-lymphocytes implied there is an activation of peripheral blood CD3⁺ T-lymphocytes in AF patients.

In fact, the activation of peripheral blood CD3⁺ T-lymphocytes maybe plays an important role in the pathogenesis of AF. A few studies reported that some inflammatory lymphocytes, such as CD45⁺ cells, CD3⁺ T cells, and CD68⁺ macrophages, infiltrated in the endomyocardial in patients with AF, which supported the local activation of T-lymphocytes played a role in the pathogenesis of AF.(Chen MC et al.,2008;Nakamura Y et al.,2003;Yamashita T et al.,2010) On the other hand, a lot of studies reported that serum or plasma inflammation biomarkers, such as C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor (TNF)- α , monocyte chemotactic protein (MCP)-1, vascular endothelial growth factor (VEGF), et al., increased in AF patients, which supported T-cell-associated chronic inflammatory responses might involve in the pathogenesis of AF. (Chung MK et al.,2001;Hernandez Madrid A et al.,2007;Li J et al.,2010;Psychari SN et al.,2005) In our study, we provided another evidence to support that there is an activation of peripheral blood CD3⁺ T-lymphocytes in AF patients, demonstrated by the upregulation of CD69 and HLA-DR by flow cytometric analysis.

Generally, our study here further emphasize that activation of T cells is involved in AF. As we all know, T-lymphocytes are the main cells participate in cell-mediated immunity, which is one of the primary ways of human immune. It can be suppressed by many immunosuppressants such as Cyclosporine, Rapamycin, et al. If the activation of peripheral blood T-lymphocytes does participate in the pathogenesis of AF, maybe we can prevent recurrence of AF through suppressing the activation of peripheral blood T-lymphocytes.

As for the underlying mechanisms of activated peripheral blood CD3⁺ T-lymphocytes participates in the progression of AF, we speculate the following three possibilities. Firstly, the activation of peripheral blood CD3⁺ T-lymphocytes might cause the upregulation of IL-6 and MCP-1, which could affect the contractility and electrical stability of myocytes inhomogeneously and induce fibroblast activation leading to deposits of extracellular matrix fibrosis. (Ramos-Mondragon R et al.,2008) Secondly, activation of peripheral blood CD3⁺ T-lymphocytes might promote the local immunologic inflammatory responses in the endomyocardial, and promote infiltrate of inflammatory lymphomononuclear in the endomyocardial in patients with AF. Thirdly, the activation of peripheral blood CD3⁺ T-lymphocytes could activate the calcineurin-nuclear factor, which involve in the T-lymphocytes signal transduction pathway. (Lin CC et al., 2004)

It is important to note that there are several limitations that need to be addressed regarding this study. Firstly, The results cannot be taken as evidence to support that CD69 and HLA-

DR play a role in the pathogenesis of AF, it only indicate the possible association of CD69 and HLA-DR with AF. Secondly, how CD69 and HLA-DR contribute to the pathogenesis of AF and what is the underlying mechanism need to be further investigated. Thirdly, we did not study the influence of other immune activation-associated molecules (CD25, CD71, and CD122, et al) and co-stimulatory molecules (CD28, CTLA-4, CD80, CD86, et al) during the progression of AF.

3. Summary

The activation of peripheral blood CD3+ T-lymphocytes and immunologic inflammatory responses played a role in the pathogenesis of AF, and might be a diagnostic or therapeutic marker. Dietary n-3 PUFA supplementation attenuates the inducibility and maintenance of AF in the sterile pericarditis model by reducing the production of proinflammatory cytokines.

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