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Elevated Pre-Operative Serum Peptides for Collagen I and III Synthesis Result in Post-Surgical Atrial Fibrillation

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Abstract

Objectives—To determine if serum markers for collagen I and III synthesis, the C-terminal peptide from Pro-collagen-I (PICP) and the N-terminal peptide from Pro-Collagen III (PIIINP), correlate with LA fibrosis and post-operative AF.

Background—Atrial fibrillation (AF) after cardiac surgery is associated with adverse outcomes. We recently demonstrated that left atrial (LA) fibrosis is associated with post-operative AF in patients with no previous history of AF.

Methods—Fifty-four patients having cardiac surgery without a history of AF consented to left and right atrial biopsies, and a pre-operative peripheral blood draw. Picrosirius red staining quantified the percentage of fibrosis, and RT-PCR assessed atrial tissue mRNA transcripts involved in the fibrosis pathway. PICP and PIIINP levels were measured using an enzyme immunosorbant assay.

Results—Eighteen patients developed AF, while 36 remained in normal sinus rhythm (NSR). Left atrial fibrosis was higher in patients who developed AF vs. NSR ($6.13 \pm 2.9\%$ vs. $2.03 \pm 1.9\%$, $p = 0.03$). LA mRNA transcripts for collagen I, III, transforming growth factor, and angiotensin were 1.5-2.0 fold higher in AF patients. Serum PICP and PIIINP levels were highest in AF vs. NSR (PICP: 451.7 ± 200 vs. 293.3 ± 114 ng/ml, $p = 0.006$; PIIINP: 379 ± 286 vs. 191.6 ± 162 pg/ml, $p = 0.01$). Further there was a linear correlation between LA fibrosis and serum PICP levels ($R^2 = 0.2$; $p = 0.01$), and of the markers only PICP was independently associated with AF.

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Conclusions—This demonstrates that serum PICP and PIIINP levels correlate with the presence of left atrial fibrosis and may act as a predictor for post-operative AF even in the absence of previous history of AF.

Keywords

Atrial Fibrillation; Fibrosis; Cardiac Surgery

Introduction

Atrial fibrillation (AF) occurs in ~30% of patients following cardiac surgery and contributes significantly to the post-operative morbidity, mortality, and costs (1). We recently demonstrated that post-surgical AF was maintained by left atrial (LA) sources and associated with pre-operative fibrosis (2). We hypothesized that left ventricular hypertrophy and diastolic dysfunction contribute to LA dilatation and stretch promoting extracellular matrix remodeling, manifested in elevated serum and tissue markers for fibrosis aiding to the prediction of post-operative AF, in patients who have no previous history of AF.

Fibrosis in the atria primarily consists of type I and III collagen, which is synthesized by fibroblasts and myofibroblasts, and is regulated by a cascade of fibro-proliferative signals including Transforming Growth factor Beta (TGF- β) and Angiotensin II(ANG) working in concert to increase collagen secretion (3). Collagen I and III are secreted as pro-collagen precursors containing amino- and carboxyl-terminal pro-peptides (4) that are released into the serum by proteases after collagen deposition. While these pro-peptides correlate with the rate of collagen synthesis (4), type I collagen degradation results in the release of a carboxyl terminal telopeptide of collagen I (CITP) (4-6). Previous authors have analyzed levels of the carboxyl terminal peptide from pro-collagen I (PICP), CITP, and the amino terminal peptide from Pro-collagen type III (PIIINP) to monitor ventricular fibrosis during hypertension and congestive heart failure (4-6). However, the utility of these peptides as markers to predict a collagen substrate necessary for the development of AF is controversial (7,8) and has not been determined in patients undergoing cardiothoracic surgery.

We hypothesized that patients without a history of arrhythmias, who developed AF following cardiac surgery have 1: left ventricular hypertrophy and diastolic dysfunction resulting in LA stretch reflective of an increased left atrial diameter; 2: increased left atrial mRNA expression of collagen I and III, TGF β , and Angiotensin II; 3: an increased percentage of left atrial fibrosis; 4: higher serum levels of PICP and PIIINP; and that serum markers for collagen synthesis would correlate with the percentage of left atrial fibrosis.

Methods

Patient Recruitment

After approval from the Institutional Review Board, 54 patients scheduled for cardiac surgery consented to atrial biopsies and evaluation of PICP, PIIINP and CITP levels. Medical records were reviewed to identify any of the exclusion criteria: <18 years of age, AF history, previous sternotomy, history of hyperthyroidism, pulmonary fibrosis, cirrhosis, or scleroderma. In addition, all patients were interviewed prior to the operation to verify that they had no known history of AF. All patients were monitored by continuous telemetry throughout their hospitalization for the presence of AF, and were treated following the Society of Thoracic Surgery guidelines after an open heart procedure (1 Atrial fibrillation was defined as an irregularly irregular rhythm without P-waves for a period of at least 15 minutes as observed by a physician(2). All patients who developed AF were given full disclosure and treated following the Society of Thoracic Surgery guidelines (1,2).

Pre-operative electrocardiograms were obtained on the day of surgery, magnified to 6400X and the duration from the onset of the p-wave to the return of the isoelectric line was determined by one blinded investigator. Pre-operative echocardiograms were obtained in all patients. Detailed methods regarding the echocardiogram measurements can be found in the data supplement.

Atrial Biopsy

Three to five millimeter biopsies were obtained from the left and right atrial appendage. The biopsy was divided, and placed in 10% buffered formalin and RNA^{later}TM (Ambion). RNA was extracted, transcribed, and messenger RNA (mRNA) levels for the gene of interest were normalized to 18s- ribosomal RNA (9,10). Further details regarding the mRNA methods can be found in the data supplement.

Histology

Specimens were embedded in paraffin and sectioned at 6 μ m. Slides were stained with picrosirius red as previously described (2,11), and examined with a Bioquant imaging system to quantify the area of fibrosis and atrial muscle. Epicardial, endocardial, and perivascular fibrosis, were excluded from the analysis. The percentage of fibrosis within the atrial muscle from each sample was calculated. All samples were measured in triplicate by a single investigator.

PICP, CITP, and PIIINP

Serum from all 54 patients was obtained pre-operatively as well as from 10 healthy subjects with no known cardiac disease. All samples were obtained from a peripheral vein, the serum extracted, and stored at -80° C. PICP (Takara Biomedical), CITP (Orion Diagnostica), and PIIINP (U SYN) levels were measured by enzyme linked immunosorbant assay according to the manufacturers' specifications and based on previous data (12,13). The sensitivity (lower detection limit) for each assay was PICP: 10 ng/ml, CITP: 1.0 μ g/L, and PIIINP: 20 pg/ml, and the intra-assay variations were PICP: 6.0 %, CITP: 3.3 %, and PIIINP: 1.8 %. All samples were run in duplicate and measured at 450 λ .

Statistical Analyses

For complete statistical methods see the online supplement

Results

Patient Variables

Of the 54 patients enrolled, 36-remained in NSR and 18-developed new onset post-operative AF. Patient variables (Table 1) demonstrated increased age as the only factor associated with AF development. From the pre-operative echocardiography data shown in Table 2, increased LA diameter, greater than mild valve disease, the number of patients with class II or III diastolic dysfunction, and increased left ventricular mass index were all associated with post-operative AF.

Left and Right Atrial Biopsies

Of the 54 patients enrolled, there were 10 patients in whom biopsies were not analyzed. In three patients there was no observable LA appendage, and therefore biopsies were not obtained. In two patients inspection of the left or right atrial biopsy demonstrated only fat and an absence of muscle, and these biopsies were not analyzed. Last, in 5 patients the atrial appendage was thought to be excessively friable, thin, and at risk for post-operative bleeding and therefore, a biopsy was not taken.

RT-PCR

Patient characteristics from the 44 patients who had biopsies are presented in table 3 of the online supplement. The expression of 18s rRNA was similar in the left and right atria of all patients (Table 4, online supplement). Comparisons in the expression patterns of the mRNA transcripts from the entire patient population between left and right atrial samples are shown in table 5 of the online supplement. The only significant difference was a 9.1 fold increase in TIMP-1 expression from LA samples ($p=0.002$). The mRNA expression of relevant transcripts by delta cT values between those patients who remained in NSR and those who developed AF are provided in figures 1A and 1B. All of the transcripts analyzed involved in structural remodeling had a mean increase in expression within the left atrium of patients who developed AF compared to the left atrium of patients who remained in NSR. To gain a greater appreciation for the relevance in differences between delta cT values that were statistically significant, the delta cT values were converted to a percent fold change (Figure 2). Collagen I and III as well as TGF β and ANG were all significantly increased by nearly 2.0 fold in LA samples from those patients who developed AF.

Fibrosis and AF

Interstitial fibrosis manifested as winding strands within the atrial muscle was observed in all left and right atrial biopsies. There were no significant differences between the LA and RA biopsies in the area of tissue available for analysis (LA: $3.7 \pm 2.8 \text{ mm}^2$ vs. RA: $3.0 \pm 2.3 \text{ mm}^2$, $p=0.12$), or the overall percentage of fibrosis between LA and RA samples ($3.1 \pm 2.8\%$ vs. $2.9 \pm 2.4\%$, $p=0.63$). There was no difference in RA fibrosis between patients who developed post-operative AF and those who remained in NSR ($2.83 \pm 2.3\%$ vs. $2.94 \pm 2.4\%$, $p=0.4$). However, as shown in figure 3, the percent area occupied by fibrosis within each sample was significantly higher in the LA samples of those patients who developed post-operative AF ($6.13 \pm 2.4\%$ vs. $2.03 \pm 1.9\%$; $p=0.03$). These results are consistent with data published previously (2).

Serum markers of collagen synthesis/degradation and AF

Serum PICP, PIIINP, and CITP levels were obtained from 54 patients and 10 control subjects (figure 4A). The 10 control subjects had no history of cardiac disease, were annually followed by a health care provider, and were currently not taking any medications (Table 6 online supplement). PICP levels in patients who developed post-operative AF ($451.7 \pm 200 \text{ ng/ml}$) were significantly higher than those who remained in NSR (293.3 ± 114 , $p=0.006$) and those without a known history of cardiac disease (260.4 ± 66 , $p=0.001$) (Figure 4B). Figure 4C provides a histogram in which patient values have been placed in 50 ng/ml bins of PICP and demonstrates that the majority of patients who developed AF had higher PICP values than those who remained in NSR. Further figure 4D presents a ROC curve comparing the development of AF and PICP and demonstrates an overall area under the curve of 0.75.

Similar to PICP levels, CITP levels were lowest in the healthy control group ($4.1 \pm 2.2 \mu\text{g/L}$) compared patients who underwent cardiac surgery ($23.7 \pm 9.7 \mu\text{g/L}$, $p=0.002$). However, there was no significant difference between patients who developed AF ($22.8 \pm 11.7 \mu\text{g/L}$) and those who remained in NSR ($24.1 \pm 8.7 \mu\text{g/L}$) (figure 5A).

Higher PIIINP levels also correlated with the development of post-operative AF. As shown in figure 5B, PIIINP levels in patients who developed AF (AF: 379 ± 286 vs. NSR: $191.6 \pm 162 \text{ pg/ml}$, $p=0.01$). Unlike PICP and CITP levels, there was no significant difference between patients having surgery and the healthy controls (238.4 ± 107 ; $p=0.2$). Similar to PICP the histogram in figure 5C represents 50 pg/ml bins of PIIINP, and demonstrates a lower utility when compared to PICP in differentiating between patients who developed post-operative

AF and those who remained in NSR. In figure 5D the ROC curve further validates this point demonstrating an area under the curve of 0.64.

Atrial fibrosis correlates with serum peptide levels

To determine whether the systemic synthesis and degradation of collagen I and III correlates with the level of atrial fibrosis we attempted to match the percentage of left and right atrial fibrosis with the serum PICP, C1TP, and PIIINP levels. As demonstrated in Figure 6, LA fibrosis did correlate significantly with PICP levels ($R^2=0.2$, $p = 0.01$). However, we found no correlation between the percentage of RA fibrosis and PICP values ($R^2 = 0.04$, $p = 0.3$). In addition, no significant correlations were demonstrable between the percentage of LA or RA fibrosis and C1TP or PIIINP levels.

Serum peptide levels and patient variables

We performed a multivariate analysis to determine whether the serum markers contribute significantly to the development of post-operative AF over and above patient variables such as age, which is an important predictor for the development of AF. Only age and PICP levels provided a significant contribution to the development of post-operative AF over other patient variables. (Age: Odds Ratio 1.13, p value 0.01; PICP: Odds Ratio 1.01, p value 0.02)

As age was the most important patient variable in the development of AF, we constructed ROC curves comparing individual PICP and PIIINP levels to age, as well as adjusting PICP and PIIINP levels to age. Figures 1 and 2 within the online supplement demonstrate that the area's under the curve for PICP, PIIINP, and age were 0.74, 0.67, and 0.77 respectively. Construction of ROC curves incorporating PICP along with age was 0.84 while the area under the curve for PIIINP along with age was 0.81.

Discussion

The main findings of this study are as follows: Patients without a history of AF who develop post-operative AF have 1) increased left ventricular mass index and left atrial diameter measured from pre-operative echocardiograms; 2) left atria with increased levels of Collagen I and III, TGF β and ANG expression demonstrated by mRNA transcripts; 3: increased percentage of left atrial fibrosis from within the left atrial appendage; 4: increased serum PICP and PIIINP levels which correlate positively with the development of AF in the *post-operative* phase of cardiac surgery. Further, in patients who developed post-operative AF serum PICP levels correlate with the percentage of left but not right atrial fibrosis.

Among all patient variables, age was the most important factor in the development of post-operative AF. The accumulation of fibrosis and collagen in the atria resulting from hypertension, valvular disease, and diastolic dysfunction provide a likely mechanism for this correlation (14). Diastolic dysfunction has been shown to correlate with left atrial stretch and collagen deposition (5,6). Left ventricular mass index quantifies left ventricular hypertrophy and diastolic dysfunction, and is increased in our group of patients who developed post-operative AF, along with LA diameter, an indicator of stretch.

Analysis of gene expression patterns in patients who developed AF demonstrated increased pro-fibrotic markers in left atrial tissue. Within the entire population there was no difference in collagen I and III expression between left and right atrial samples. However, in patients who developed post-operative AF, collagen I and III were increased in the left atrium, and may further validate the hypothesis that increased LA pressures associated with hypertrophy and diastolic dysfunction result in atrial fibrosis and post operative AF risk (1,2). Of the pro-fibrotic marker analyzed only TGF β and ANG were significantly increased in the LA tissue

of patients who developed post-surgical AF. These two markers are integral to collagen synthesis (3), and provide a molecular mechanism for the increased percentage of LA fibrosis in AF patients. Interestingly, these transcripts were increased only in the left atrium; however, left and right atrial transcript differences have been previously reported (2, 15,16). Microarray analysis demonstrated 624 genes, 41% of which were expressed higher within the left vs right atrial myocardium (15). Further, an increased expression of the angiotensin receptors has been found within the LA myocardium of patients who develop AF (16). Our data further supports previous work which demonstrates left and right atrial differences (2).

The importance of left atrial fibrosis as a substrate in the development of lone AF is well documented (2,9,11). The accumulation of fibrosis within the extracellular space prolongs conduction time between myocytes creating conditions favorable for the initiation and maintenance of reentry (11). Yet despite the acceptance of fibrosis as a substrate for the development of lone AF, there is controversy regarding its importance in the development of post-operative AF (2,17). Previous data regarding the percentage of RA fibrosis in the development of post-surgical AF has produced mixed results (17). To date there is a preponderance of evidence (18) to suggest that in the majority of AF cases, the right atrium is merely a bystander, whereas electrical sources in the left atrium are integral to the mechanism of AF maintenance (2). Our data corroborate previous work (2), demonstrating that only differences in fibrosis within the left and not the right atrium correlate with the development of post-operative AF. Therefore, it is reasonable to conclude that LA fibrosis may have a greater impact on AF initiation and maintenance than RA fibrosis (2).

Markers of collagen I synthesis (PICP) and degradation (CITP) have been compared in AF patients, typically in those who undergo catheter ablation (19, 20). However, differences in serum peptide levels were compared between AF patients and healthy controls (20). We demonstrate that there were differences in all surgical patients in PICP and CITP levels compared to healthy controls, but also a significant difference between PICP levels in patients who developed AF and those who remained in NSR, all of which had cardiovascular disease but no previous history of AF.

PICP levels demonstrated the most significant difference among patients who developed AF, those who remained in NSR, and control patients without a history of cardiac disease. This is in contrast with a previous report which demonstrated no difference in pro-collagen-I levels between patients with AF and matched control patients with a similar cardiac history but in NSR (8). Matching patients with a similar cardiac history may negate the utility in separating patients with fibrosis. Importantly, the stoichiometric ratio between the number of collagen molecules produced, and the number of PICP molecules released is 1:1 (4). As a result PICP levels have been shown to correlate in a linear fashion with the percentage of ventricular fibrosis ($R=0.4$) in patients with hypertensive heart disease (5). In patients having heart surgery we demonstrate a similar correlation ($R^2=0.2$) between the percentage of LA fibrosis, and serum PICP levels. Such a correlation serves to further validate the importance of LA fibrosis in the development of post-operative AF.

The use of PIIINP as a marker of collagen III synthesis remains controversial. Unlike PICP levels which has a stoichiometric ratio of 1:1 with collagen deposition, PIIINP levels are less reliable (4). This may account in part for why when compared to PICP differences, PIIINP levels in patients who remained in NSR and who developed AF did not correlate with the percentage of left or right atrial fibrosis.

Limitations

Although all patients had no known history of AF, some could have had prior episodes of asymptomatic AF, as pre-operative Holter monitoring was not performed. The areas of LA

and RA tissues analyzed in our study were relatively small in comparison to the overall size of each chamber, and we have made inferences based on differences only from a small sample. However, each biopsy was taken from the same location on the LA or RA, and therefore is a useful point of comparison. Ethical considerations prevented us from taking larger samples or samples from multiple locations, which would have given additional information but placed the patient at increased risk for post-operative bleeding. Therefore, tissue available for western blots for protein analysis was regrettably not available. .

Conclusion

We have demonstrated the importance of fibrosis as a substrate in the development of post-operative AF. Further testing to identify whether PICP levels could be used to predict patients who would develop AF following cardiac surgery remains unclear. It is well known that AF is one of the most frequent complications of cardiac surgery. AF affects more than one third of patients and is associated with increased morbidity and mortality and longer, more expensive hospital stays. As such, the ability to identify which patients have a pre-existing substrate, and are therefore at high risk for developing AF, could be critical for improved post-operative outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation List

AF	Atrial Fibrillation
NSR	Normal Sinus Rhythm
LA	Left Atrial
TGF-β	Transforming Growth factor Beta
ANG	Angiotensin II
CITP	C terminal telopeptide from Collagen I
PICP	C terminal peptide from collagen I
PIIINP	N terminal peptide from collagen II
mRNA	messenger RNA

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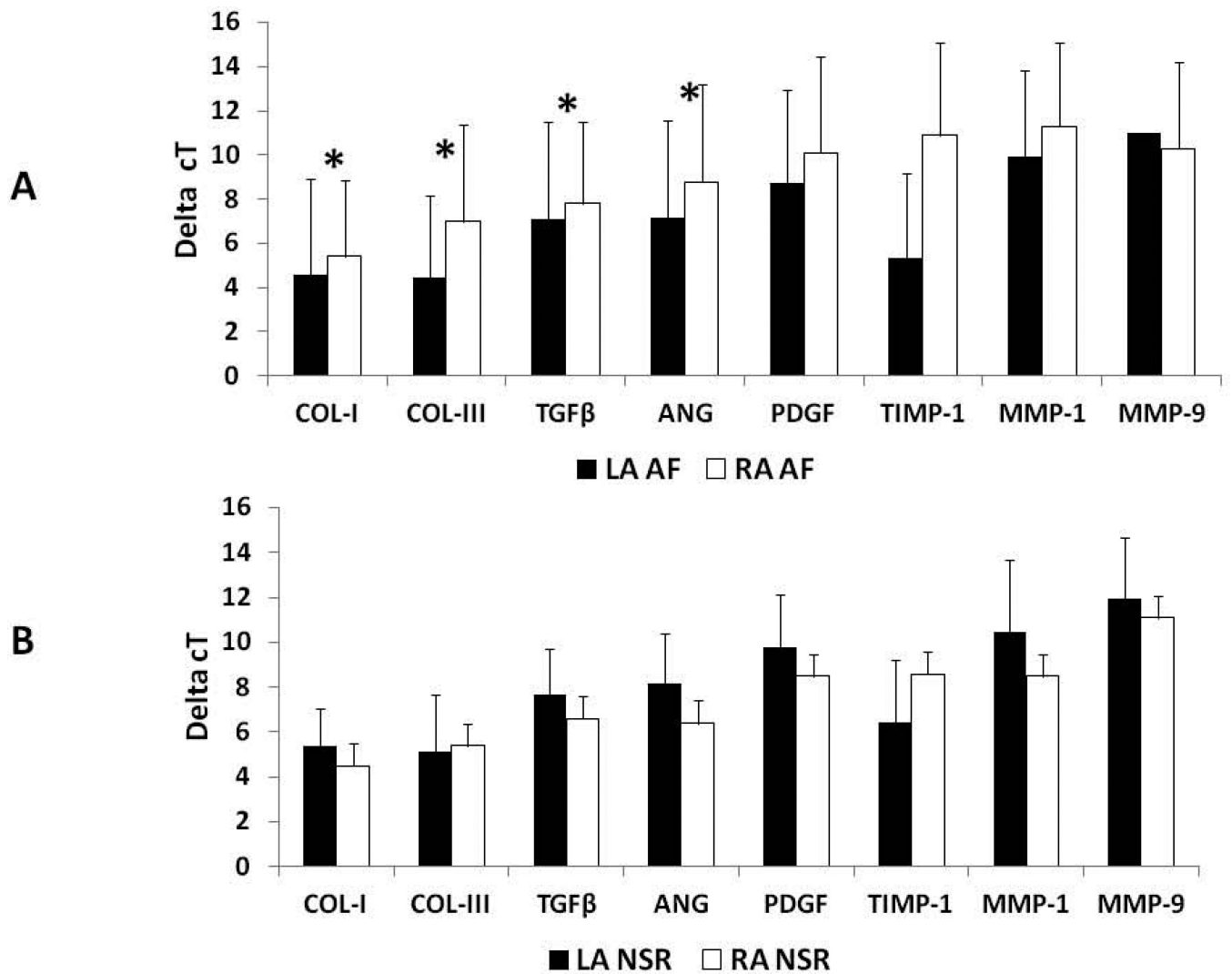


Figure 1. Delta cT values for mRNA transcripts

A: Delta cT values in left atrial mRNA expression for patients who developed Atrial Fibrillation (AF) and those who remained in Normal Sinus Rhythm (NSR). B: Delta cT values in right atrial mRNA expression for patients who developed Atrial Fibrillation (AF) and those who remained in Normal Sinus Rhythm (NSR). Abbreviations: Collagen-I(COL-I), Collagen-III(COL-III), Transforming Growth Factor Beta(TGFβ), Platelet Derived Growth Factor (PDGF), a Angiotensin (ANG), Tissue Inhibitor of Metalloproteinase-1(TIMP-1) and Matrix Metalloproteinase 1 and 9 (MMP-1 and-9). *Denotes statistical significance

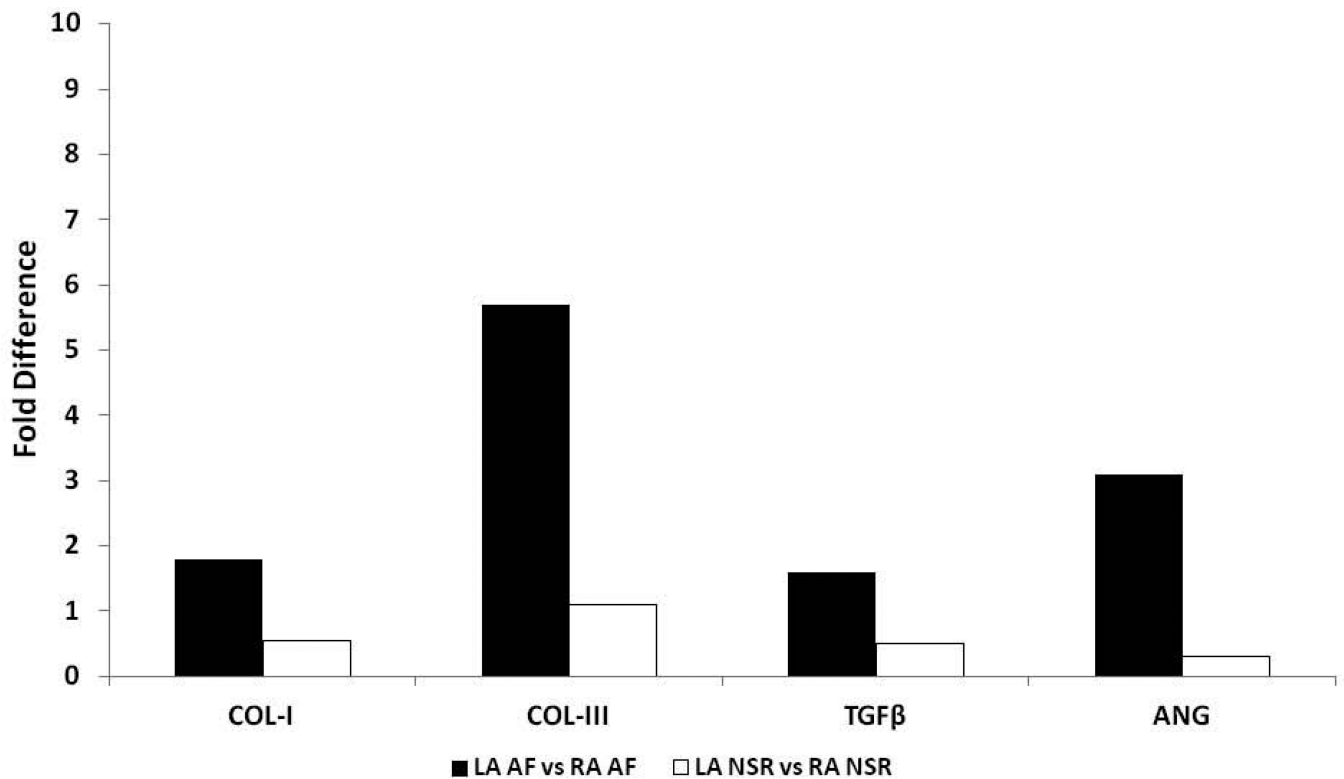


Figure 2. Fold difference in mRNA transcripts

mRNA expression by converting the delta cT values from figures 1A and 1B into fold differences by using the following equation: $\text{Fold difference} = 2^{(\text{mean } \Delta cT_{\text{LA_NSR}} - \text{mean } \Delta cT_{\text{LA_AF}})}$. The data produced provide a visual representation of the fold change after statistical analysis demonstrated significance for the delta cT values, and therefore there are no standard deviation bars. Abbreviations: Collagen-I(COL-I), Collagen-III(COL-III), Transforming Growth Factor Beta(TGFβ), and Angiotensin (ANG) Atrial Fibrillation(AF) Normal Sinus Rhythm (NSR). *Denotes statistical significance

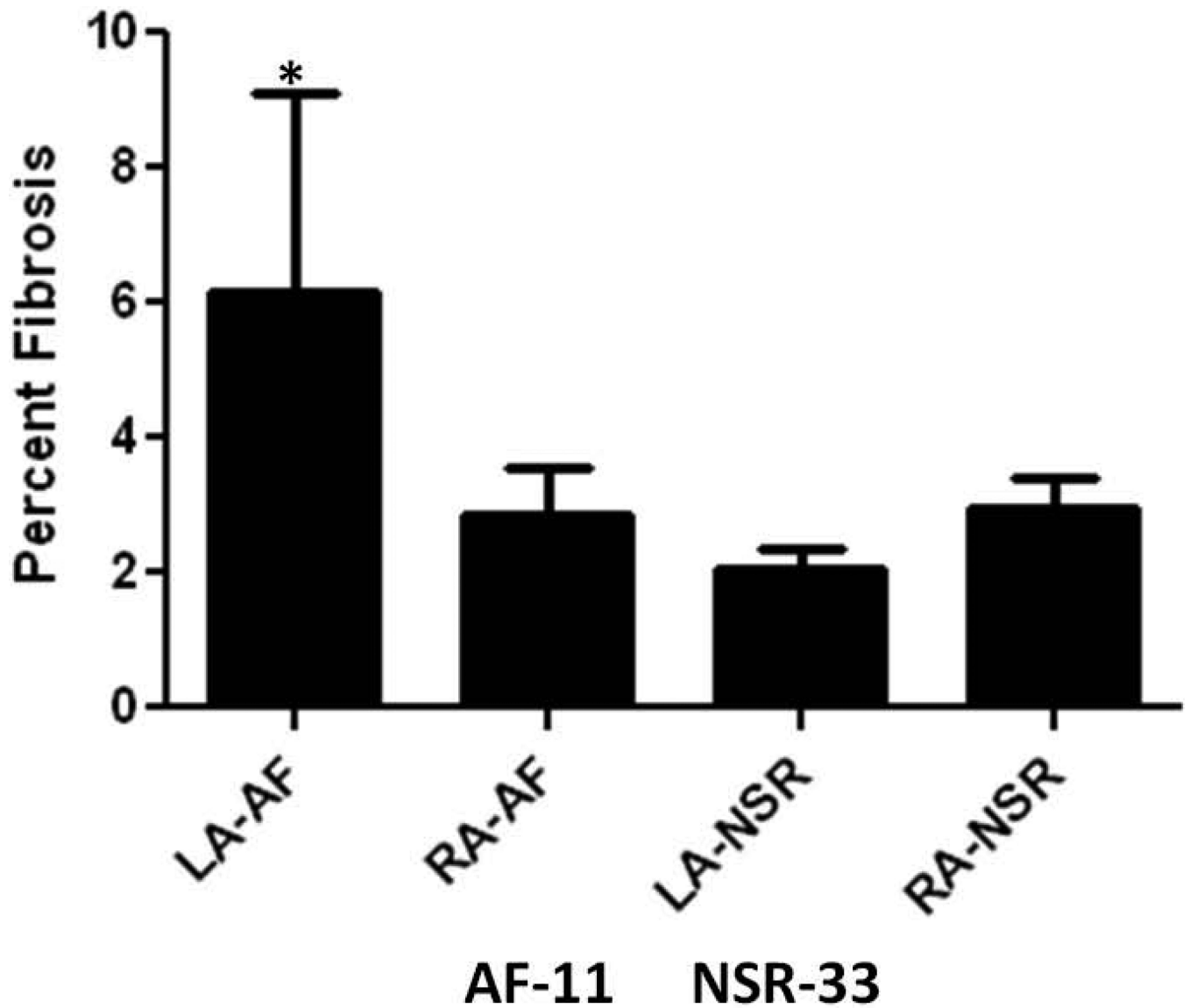


Figure 3. Percent Fibrosis

The average percentage of fibrosis organized by post-operative rhythm (Abbreviations: AF-Atrial Fibrillation, LA-Left atrial, NSR-Normal Sinus Rhythm, RA-Right Atrial). *Denotes statistical significance

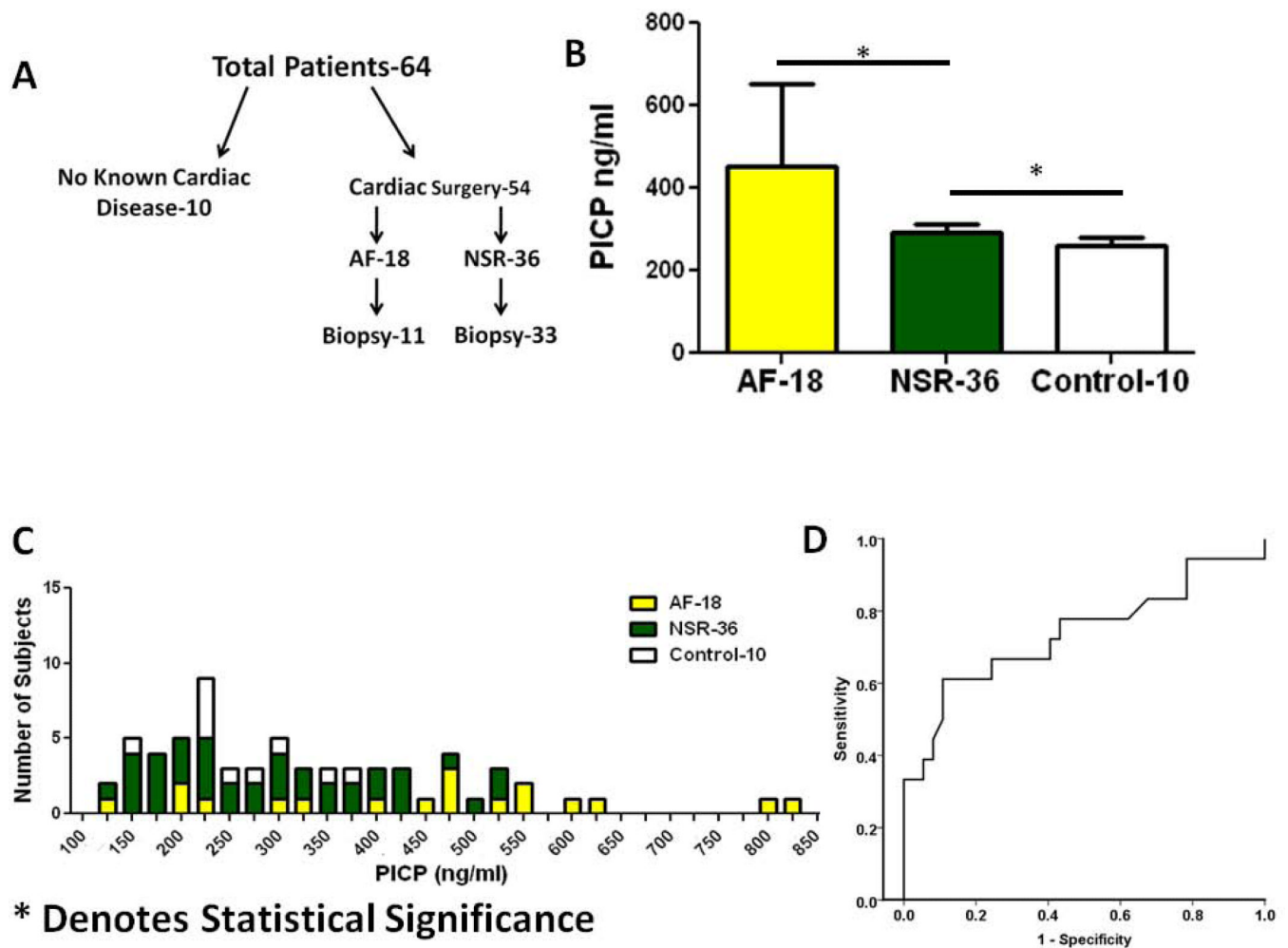


Figure 4. Increased PICP levels

A: Schematic of blood samples obtained from patients tested for PICP, C1TP, and PIIINP; B Average values for serum PICP levels in control patients, patients developing AF, and patients remaining in NSR after surgery; C: Histogram of serum PICP values in control and surgical patients; D: PICP ROC curve Abbreviations: AF-Atrial Fibrillation, PICP-Carboxyl terminal peptide from Pro-Collagen I, NSR-Normal Sinus Rhythm. *Denotes statistical significance

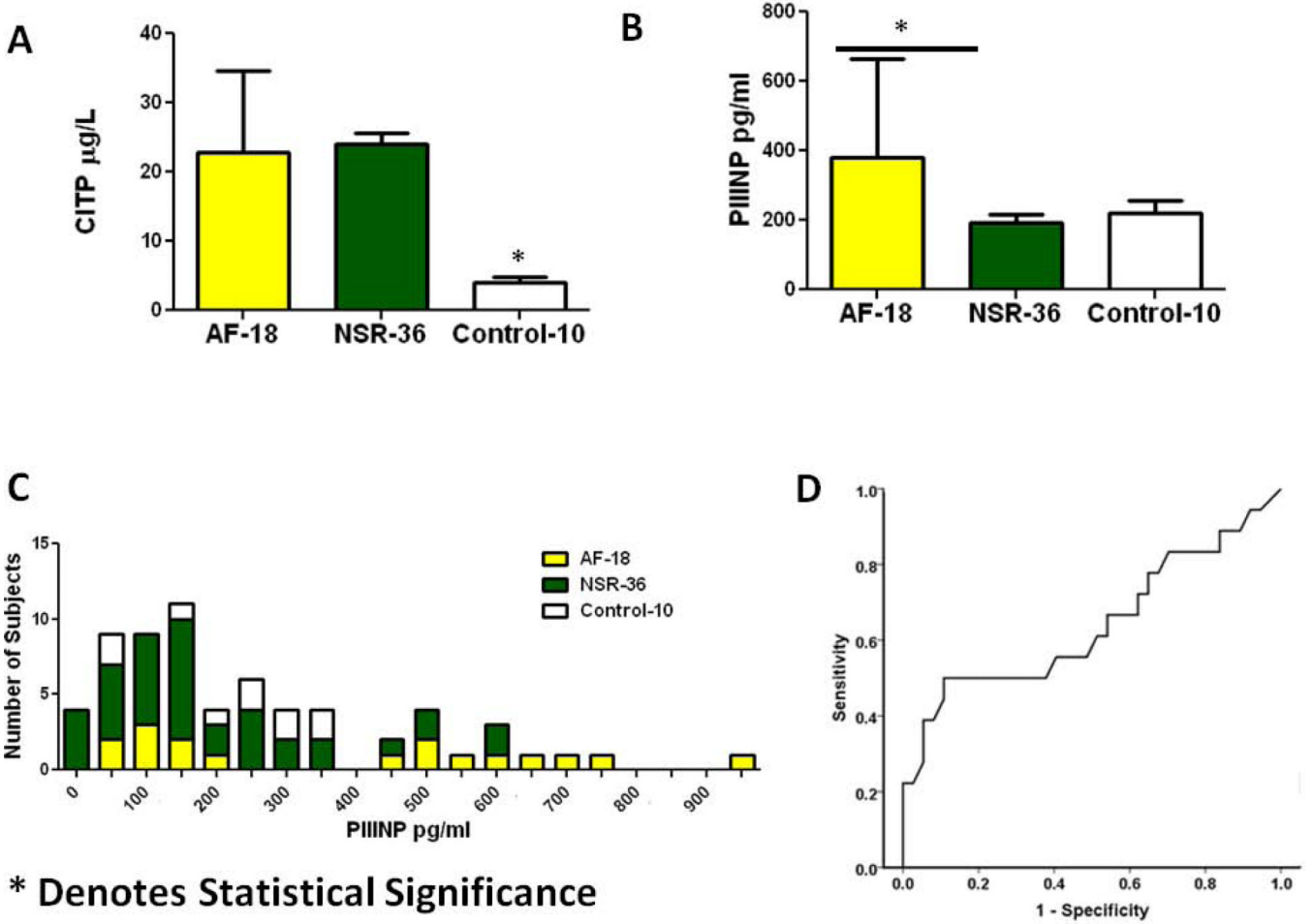


Figure 5. PIIINP and CITP levels

A: CITP levels from patients who developed atrial fibrillation, normal sinus rhythm, and control patients; B: PIIINP levels from patients who developed atrial fibrillation, normal sinus rhythm, and control patients; C: Histogram of serum PIIINP values in control and surgical patients demonstrating; D PIIINP ROC curve.

Abbreviations: AF-Atrial Fibrillation, CITP-C Terminal telopeptide from Pro-Collagen I, PIIINP- N Terminal peptide from Pro-Collagen III, NSR-Normal Sinus Rhythm. *Denotes statistical significance

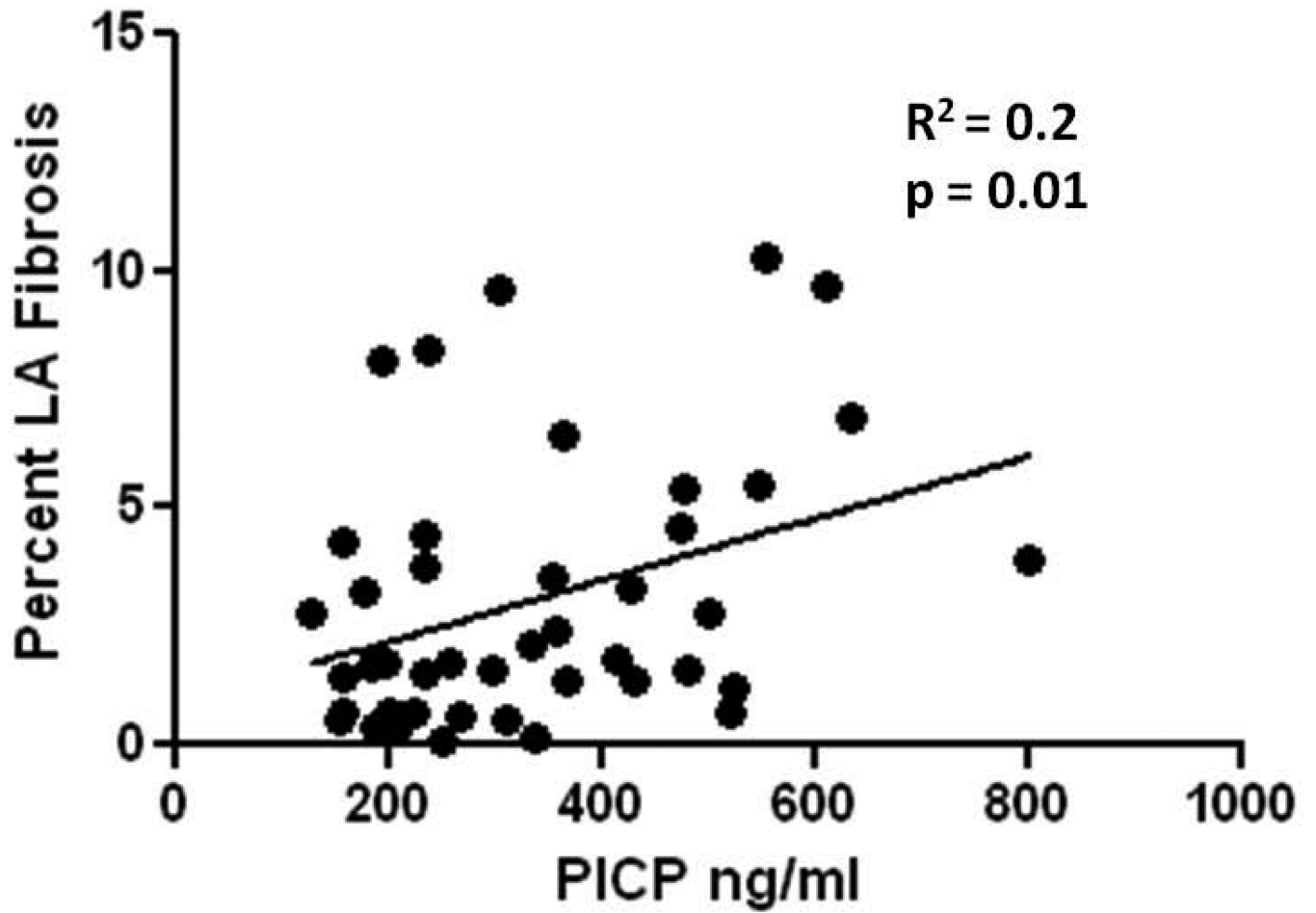


Figure 6. Correlation between PICP and Left Atrial Fibrosis
Linear regression of the percentage of left atrial fibrosis and serum PICP level.

Table 1

Patient Variables

	NSR =36	AF after Surgery=18	P value
Age (years)	64.5±8.5	73.3±7.8	0.001 *
Male Gender	29 (81%)	14 (78 %)	1.0
BSA m ²	2.08±0.23	2.01±0.23	0.4
<i>Medical History</i>			
Hypertension	28 (78%)	15 (89%)	0.5
COPD	3 (8%)	0 (0%)	0.5
Hyperthyroidism	0	0	1.0
CAD	32 (100%)	16 (94%)	0.6
Tobacco Abuse	19 (53%)	13 (72%)	0.2
Alcohol Abuse	1 (3%)	0	1.0
<i>Pre-operative Medications</i>			
ACE Inhibitor	13(36%)	6 (33%)	1.0
ARB	5 (14%)	1 (6%)	0.7
Beta Blocker	16 (44%)	12 (65%)	0.2
Statin	21 (58%)	11 (61%)	1.0
P-Wave Duration (sec)	0.12±0.01	0.12±0.01	0.9
<i>Operation</i>			
CABG	31 (94%)	14(77%)	0.5
Valve	1 (3%)	1 (6%)	1.0
CABG/Valve	1 (3%)	3 (17%)	0.1

Abbreviations: AF-Atrial Fibrillation; ACE, Angiotensin Converting Enzyme inhibitors; BSA, Body surface area; ARB, Angiotensin Receptor Blockers; CABG-Coronary Artery Bypass Graft; COPD, Chronic Obstructive Pulmonary Disease; HTN-Hypertension; NSR-Normal Sinus Rhythm; NYS CHF- New York State Congestive Heart Failure; Valve, Valvular Heart Disease.

* Denotes Statistical Significance

Table 2

Trans-Esophageal Echocardiogram Parameters Examined

Echo Parameter	NSR n=36	AF after Surgery n=18	P value
Ejection Fraction %	51.1±8	49.3±12	0.6
> Mild Valve Disease	2 (6%)	6 (35%)	0.004 *
LA Diameter (cm)	4.8 ±0.7	5.2±0.5	0.02 *
RA Diameter (cm)	4.9±0.8	4.8±0.6	0.5
Diastolic Dysfunction	21(58%)	12 (75%)	0.3
> Class I DD	1(3%)	4 (25%)	0.02 *
LV Mass Index (g/m ²)	245.7 ±78	305.7±98	0.03 *
E Wave (cm/sec)	67.4±13	71.5±27	0.6
A Wave (cm/sec)	65.1±16	67.3 ±18	0.7
A Wave Duration(sec)	0.15±0.02	0.14±0.02	0.3
E' Wave(cm/sec)	10.2±2.8	8.8±2.9	0.1
A' Wave(cm/sec)	10.1±3.4	9.0±3.1	0.3
E/A	1.1±0.4	1.1±0.6	0.8
E'/A'	1.1±0.3	1.1±0.5	0.8

Abbreviations: AF- Atrial Fibrillation, DD-Diastolic Dysfunction, LV-Left Ventricular, LA-Left Atrial NSR- Normal Sinus Rhythm, RA-Right Atrial.

* Denotes Statistical Significance