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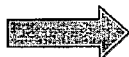
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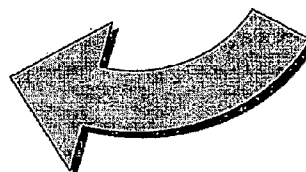
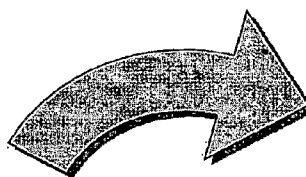
(54) Title: METHOD FOR PREDICTING ONSET/RISK OF ATRIAL FIBRILLATION (AF)

Age
Diabetes
Hypertension
Congestive Heart Failure



Oxidative
stress

Atrial
Fibrillation



(57) Abstract: A method for predicting onset or risk of atrial fibrillation in a subject includes determining the presence of an oxidative stress marker, such as glutathione, cysteine, and/or a derivative of a reactive oxidative metabolite (DROM).

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METHOD FOR PREDICTING ONSET/RISK OF ATRIAL
FIBRILLATION (AF)

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority on prior U.S. Provisional Application S.N. 60/835,074, filed August 3, 2006, which is hereby incorporated herein in its entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

[0002] The work leading to the present invention was supported by one or more grants from the U.S. Government, including NIH Grant(s) HL39006, HL77398, and HL73753, a Department of Veterans Affairs Merit grant (SCD), and a support/grant from the Atlanta Veterans Affairs Medical Center, Health Services Research & Development Program. The U.S. Government therefore has certain rights in the invention.

FIELD AND BACKGROUND OF THE INVENTION

[0003] The present invention is generally directed to medical diagnostics, and more particularly to a method for predicting onset/risk of atrial fibrillation in a subject, and guiding an appropriate therapy.

[0004] Atrial fibrillation (AF) is by far the most common cardiac arrhythmia. The incidence is high and increasing. The incidence increases twofold with every decade after age 55 years with a prevalence of 5% in people over age 65 (References 5 and 27), and the lifetime risk for AF for people over age 40 is approximately 25%. The number of hospital admissions for AF more than doubled between 1984 and 1994 (Reference 7). Currently, 2.2 million people in the United States have a diagnosis of AF (Reference 36). While generally not lethal, AF reduces the quality of life and increases the risk for systemic embolization, hemodynamic instability, tachycardia-induced cardiomyopathy, and mortality (References 26 and 46). Overall, AF accounts for 15-20% of strokes in the United States, numbering around 75,000 annually (Reference 52).

[0005] The pathogenesis of AF is unknown, but studies have supported a role for both oxidative stress and inflammation. Studies of animal and human samples have shown increased myocardial oxidative stress associated with atrial fibrillation (References 9, 24, 47 and 57). Furthermore, anti-oxidants including statins, Vitamin C, polyunsaturated

fatty acids, and fish oils reduce the incidence of AF in humans (References 2, 10, 11, 49, 55, 59 and 60).

[0006] Inflammation has a complex relationship with oxidative stress and also been associated with AF. There is evidence of inflammatory infiltrates in many cases of lone AF (Reference 29). Elevated levels of the inflammatory marker, C-reactive protein (CRP), are associated with AF in some studies (References 3, 15 and 20) and have been suggested as a predictor of the incidence of AF following cardioversion (References 20, 50, 54 and 75) or cardiac surgery (References 28 and 53). Interleukin-6 (IL-6) is elevated in AF, (Reference 17) and polymorphisms in the IL-6 gene predict the onset of AF after cardiac surgery (Reference 33). Moreover, anti-inflammatory agents seem to reduce the risk of atrial fibrillation (References 21, 22, 40, 56, 62, 68 and 76).

[0007] The relative importance of inflammation and oxidative stress in the pathogenesis of AF and which markers might be most strongly associated with the arrhythmia remain unclear. Therefore, we assessed differences in markers of oxidative stress and inflammation between patients with and without persistent or permanent AF.

OBJECTS AND SUMMARY OF THE INVENTION

[0008] An object of the present invention is to provide a method for predicting onset/risk of atrial fibrillation (AF) in a subject.

[0009] Another object of the present invention is to provide a method for determining the presence of an oxidative stress marker in a subject.

[0010] Another object of the present invention is to provide a method for preventing or substantially reducing the risk of atrial fibrillation in a subject.

[0011] Another object of the present invention is to provide a method for treating and/or guiding therapy in a subject suspect of having atrial fibrillation.

[0012] Another object of the present invention is to provide a method for correcting and/or reversing the side effects of atrial fibrillation in a subject.

[0013] Another object of the present invention is to provide a method for reducing the incidence of atrial fibrillation in a population.

[0014] Another object of the present invention is to provide a method which would be useful in diagnostic, therapeutic, scientific and other applications regarding atrial fibrillation and related ailments, conditions, or the like.

[0015] In summary, AF has been associated with myocardial oxidative stress, and antioxidant agents have demonstrated anti-arrhythmic benefit in humans. In the present invention, serum markers of oxidative stress and inflammation were compared in a cross-sectional, case-control design of 20 male subjects with persistent or permanent AF and controls where cases were matched for age, sex, diabetes, and smoking status, known confounding variables for the measurement of oxidative stress. Derivatives of reactive oxidative metabolites (DROMs) and oxidized to reduced glutathione (E_h GSH) and cysteine (E_h CySH) ratios were used to quantify oxidative stress. Since oxidative stress and inflammation have a complex relationship, inflammatory markers, including high sensitivity C-reactive protein (hsCRP), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF α), were also obtained. In univariate, conditional logistical regression analysis, oxidative stress but not inflammatory markers were statistically associated with AF ($p < 0.05$). The increase in the odds for AF for E_h GSH, E_h CySH, and DROMs were, 6.1 (95% CI: 1.3-28.3, $p = 0.02$), 13.6 (95% CI: 2.5-74.1, $p = 0.01$), 15.9 (95% CI: 1.7-153.9, $p = 0.02$), respectively. There was a correlation between E_h GSH

and E_h CySH ($r=0.66$) but not with DROMs ($r=0.41$). Statins, known anti-oxidants, were negatively associated with the presence of AF (OR=0.2; 95% CI 0.05-0.99; $p=0.05$). In multivariate analysis, correcting for statins and other AF risk factors, the association between AF and oxidative stress remained significant. These data suggest an association between oxidative stress and AF. Oxidative stress markers appeared to have a stronger association with AF than did inflammatory markers.

[0016] At least one of the above objects is met, in part, by the present invention, which in one aspect includes a method for predicting onset or risk of atrial fibrillation in a subject, including determining the presence of an oxidative stress marker.

[0017] Another aspect of the present invention includes a method for treating a subject suspect of having atrial fibrillation, including a) determining the presence of an elevated blood level of an oxidative stress marker, b) comparing the value obtained in step a) to a predetermined normal value, and c) recommending the subject for an antioxidant therapy if the value obtained in step b) is higher than the normal value.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] At least one of the above objects, novel features and advantages of the present invention will become apparent from the

following detailed description of the invention, as illustrated in the drawings, in which:

[0019] Figure 1 illustrates difference in the oxidative stress markers between subjects with and without atrial fibrillation. Figure 1A: Both the oxidized (GSSG) to reduced (GSH) glutathione and oxidized (cysteine) to reduced cysteine thiol couples indicated a more oxidized state (i.e., a less negative E_h) in subjects with AF (grey bars) as compared to matched controls in normal sinus rhythm (NSR, black bars). Figure 1B: DROMs were evaluated in the AF group (grey bars) as compared to the controls in normal sinus rhythm (NSR, black bars). * indicates a $p < 0.05$;

[0020] Figure 2 illustrates the univariate odd ratios for atrial fibrillation as a function of an interquartile increase in various markers. Only the oxidative stress markers (E_h GSH, E_h CySH, and DROMs) showed a significant association with AF;

[0021] Figure 3 illustrates the odds ratio for atrial fibrillation for a given change in E_h GSH. The logistical regression model predicts that as the glutathione thiol couple becomes more oxidized, there is a progressive increase in the odds ratio for AF; and

[0022] Figure 4 illustrates a unified hypothesis explaining the relationship of AF and its risk factors to oxidative stress. This hypothesis is

consistent with the association of AF and certain AF risk factors with oxidative stress.

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

Study Population

[0023] This cross-sectional, case-control study recruited patients in AF from outpatient clinics at the Atlanta Veterans Affairs Medical Center (VAMC) from May through July of 2005 under a protocol approved by the Emory University Institutional Review Board (www.clinicaltrials.gov: NCT00142194). Eligible patients were over 18 years of age and in persistent or permanent AF at the time of enrollment. Ineligibility criteria included systemic inflammatory diseases, malignant neoplasm, severe stenotic or regurgitant valvular heart disease, New York Heart Association class IV heart failure, hyperthyroidism, uncontrolled hypertension (>180/100 at rest), an illness that may have resulted in death within one year, implanted devices designed for the active management of atrial arrhythmias by pacing or defibrillation, and current illicit drug or alcohol abuse.

[0024] Control patients were identified from outpatient clinic visits at the Atlanta VAMC over the same period of time. The same eligibility and ineligibility criteria applied with the exception that control patients were free

of current AF and any history of AF as documented by an electrocardiogram (ECG) performed at the time of enrollment, history, and review of the medical record. Cases and control subjects were matched for factors known to affect the oxidative markers used, age in decades, smoking, and diabetes status (References 25, 44, 48, 58, 63 and 65). All patients enrolled gave written consent.

Data Collected

[0025] Data was collected from subject interviews, review of VA hospital and clinic charts, telemetry recordings, and ECGs. The presence or absence of AF was confirmed on the basis of an ECG done at the time of enrollment. Demographic data obtained included: age, race, body mass index, New York Heart Association (NYHA) functional class, and a history of previous myocardial infarction, hypertension, diabetes, smoking, or alcohol use. Additionally, all medications being taken at the time of enrollment were recorded. A single blood draw was performed at the time of enrollment and analyzed for markers of oxidative stress and inflammation in the Emory Biomarkers Core Laboratory.

[0026] Markers used to measure oxidative stress were: ratios of oxidized to reduced glutathione (E_h GSH) and cysteine (E_h CySH) in plasma (thiol ratios) (References 41-44) and derivatives of reactive oxygen species (DROMs) (also known as the Free Oxygen Radicals Test - FORT)

(References 1, 12, 18 and 38). Detailed methods to prevent rapid oxidation of samples have been delineated previously (Reference 43). Briefly, blood was collected from an antecubital vein transferred immediately to a microcentrifuge tube containing 0.5 mL of a preservation solution of 100 mM serine-borate (pH 8.5) containing (per mL) 0.5 mg sodium heparin, 1 mg bathophenanthroline disulfonate sodium salt, and 2 mg iodoacetic acid. Use of this procedure minimizes autoxidation and hemolysis (Reference 43). Following centrifugation to remove blood cells, aliquots (200 μ L) were transferred to tubes containing 200 μ L of 10% (w/v) perchloric acid containing 0.2 M boric acid and 10 μ M γ -Glu-Glu as internal standard. Samples were stored at -80°C (< 2 months) prior to further processing to form N-dansyl derivatives and analysis by HPLC with fluorescence detection. Previous data have shown stable measurements with this length of storage (Reference 43). Metabolites were identified by co-elution with standards, and quantification was obtained by integration relative to the internal standard. Samples from control and AF patients were treated identically.

[0027] The redox states (E_h) of the thiol/disulfide pools were calculated with the Nernst equation, $E_h = E_o + RT/nF \ln [\text{disulfide}]/[\text{thiol}]^2$, where E_o is the standard potential for the redox couple, R is the gas constant, T is the absolute temperature, n is 2 for the number of electrons transferred, and F is Faraday's constant (References 64 and 66). The

standard potential E_o used for the glutathione and cysteine redox couples was -264 mV and -250 mV, respectively (Reference 43). Less negative E_h numbers imply a more oxidized state. DROMs were measured in Carr units with higher values indicating higher levels of oxidative stress. DROMs (Diacron International, Grosseto, Italy) and inflammatory markers, high sensitivity C-reactive protein (hsCRP; Life Diagnostics, West Chester, PA), interleukin-1- β (IL-1 β ; R&D Systems, Minneapolis, MN), interleukin-6 (IL-6; R&D Systems), and tumor necrosis factor α (TNF α ; R&D Systems), were measured using commercially available kits. Intraassay CVs were <1% at -156 and <1% at -120 mV for E_h GSH, 5.0% at -100 and 4.5% at -60 mV for E_h CySH, .2% at 300 and 2.3% at 550 Carr units for DROMs; 10.1% at 0.2 and 5.2% at 10 ng/L for IL-1 β , 5.1% at 1 and 3.6% at 8 mg/L for hsCRP, 20.9% at 3.2 and 6.2% at 50 ng/L for IL-6, and, 11.9% at 2 and 7.3 at 50 ng/L for TNF α .

Data Analysis

[0028] Statistical analysis was performed using SAS software 9.1 (SAS Institute, Cary, N.C., USA). Baseline characteristics of AF patients and their matched controls were compared using a paired *t*-test for continuous variables (expressed as mean $\pm$ SD), and Fisher's exact test for categorical variables. All statistical tests were two-tailed, and significance was assumed at $p \leq 0.05$. Correlations between markers of inflammation and oxidative stress were assessed using Spearman rank-order correlation

coefficients. All oxidative and inflammatory markers were examined as predictors of AF occurrence in single-variate conditional logistic regression models; age category, smoking and diabetes status were accounted for by matching. Variables exhibiting borderline normality were also examined after logarithmic transformation. Parameter estimates for each oxidative and inflammatory marker were scaled so that reported odds ratios correspond to an approximate inter-quartile range increase. Using the parameter estimate from the E_h GSH model, odds ratios for AF were computed and plotted as a function of increase in E_h GSH level. Multivariate conditional logistic regression models were used to examine the association between each oxidative marker and the presence of AF while controlling for inflammatory markers. Statins were assessed as predictors of AF presence and in linear regression models as predictors of E_h GSH.

RESULTS

[0029] Twenty subjects with persistent or permanent AF along with 20 individuals free of AF were compared in the study. Control subjects were matched to cases by age, sex, smoking and diabetes status because these variables are known to affect the oxidative stress measures used (References 25, 44, 48, 58, 63 and 65) Table 1 (below) compares the demographics of cases and their controls. The AF subjects had ages ranging from 58-86 with a mean age of 74.8 years. Five subjects (25%)

were diabetic. All AF subjects were male and non-smokers. The mean length of AF was 10.1 years with a median of 6.4 ± 13.3 years. In non-matched variables, hypertension and heart failure were slightly more common in the AF group, consistent with these conditions predisposing to the arrhythmia (References 5, 16, 31 and 45). In all other parameters, the populations were statistically similar ($p > 0.05$).

[0030] All measures of oxidative stress were significantly increased in AF patients compared with controls. Thiol ratios in the AF group were significantly more oxidized (i.e., negative) than in the controls ($p < 0.001$; Figure 1A). The AF group showed more oxidation, with a mean (SD) E_h GSH of -133 (21) mV (median -143 mV, interassay CV, 15.8%) and E_h CySH of -68 (6) mV (median -67 mV, CV= 8.8%) compared to the control group, which had a mean (SD) E_h GSH of -154 (12) mV (median -156 mV, CV 7.8%) and E_h CySH of -77 (6) mV (median -76, CV 7.8%). Consistent with the thiol results, the DROMs also showed more oxidation in the AF group [388 (54) Carr units, median 370 Carr units, CV 13.9%] than the controls [310 (44) Carr units, median 308 Carr units, CV 14.2%] ($p = 0.001$; Figure 1B).

[0031] The inflammatory markers IL-1 β , IL-6, TNF α , and hsCRP were mildly, but insignificantly increased in the AF group compared to controls. Mean (SD) values for IL-1 β , IL-6, TNF α , and hsCRP in the AF

group were 0.5 (0.8) ng/L (median 0.3), 5.5 (3.9) ng/L (median 4.2), 6.5 (8.1) ng/L (median 3.8), and 5.1 (3.8) mg/L (median 4.5) compared to 0.4 (0.4) ng/L (median 0.3), 3.9 (1.6) ng/L (median 3.6), 5.5 (3.4) ng/L (median 4.7), and 3.6 (3.1) $\mu\text{g/mL}$ (median 2.6) for the control group, respectively.

[0032] The relationship of oxidative stress and inflammatory markers to AF was analyzed in single exposure conditional logistical models. The odds ratios for AF were computed based on an inter-quartile range increase for each single marker; comparing the risk of AF in subjects at the 25th percentile to those at the 75th percentile. Single exposure model odds ratios were controlled for matching variables of age, sex, smoking, and diabetes status, but are otherwise unadjusted. Oxidative stress markers, E_h GSH, E_h CySH, and DROMs, all predict AF with odds ratios of 6.1 (95% CI: 1.3-28.3, p=0.02), 13.6 (95% CI: 2.5-74.1, p=0.01), 15.9 (95% CI: 1.7-153.9, p=0.02) respectively (Table 2 below). None of the odds ratios for any of the four inflammatory markers measured were statistically significant (Figure 2, p>0.05).

[0033] There is a complex relationship between oxidative stress and inflammation. To evaluate this further, we compared the correlations between markers in our study (Table 3 below). Consistent with the idea that thiol ratios best represent the redox states of the hydrophilic phase while DROMs more likely measure the redox state of a lipid phase, Spearman

correlation coefficients revealed a statistically significant correlation between E_h GSH and E_h CySH ($r=0.66$) while the relationship of thiol ratios to DROMs was weaker (Table 3). For the most part, oxidative stress markers were independent of inflammatory markers, except for the case of a statistically significant positive correlation between $TNF\alpha$ and DROMs ($r=0.38$) and a negative correlation between $TNF\alpha$ and E_h CySH ($r=-0.42$). On the other hand, most inflammatory markers showed a significant degree of correlation between each other.

[0034] Because statins are postulated to have antioxidant activity and have been associated with a reduced incidence of AF (References 2, 55 and 60), we analyzed the relationship between use of statins and AF among patients in our study. Statin use was negatively correlated with AF with an OR of 0.2 (95% CI 0.05-0.99, $p=0.05$). Moreover, linear regression analysis revealed that E_h GSH and statins were associated with a 14.3 mV (95% CI 0.8-27.8) decrease in the oxidative stress marker, E_h GSH, suggesting that the statin effect of AF may be related to a reduction in oxidative stress. In multivariate analyses, the association of AF and more oxidized thiol ratios remained statistically significant when correcting for hypertension, congestive heart failure, and statin use.

DISCUSSION

[0035] Oxidative stress has been implicated in the pathogenesis of AF. The purpose of this study was to compare the relative changes in oxidative stress markers between patients with and without persistent or permanent AF. We found that oxidative stress markers differed between the two groups. Inter-quartile range increases across all markers of oxidative stress strongly and significantly correlated with increased risk of AF. This remained true even after correction for differences in hypertension, congestive heart failure, and statin use between the two groups.

Oxidative stress and AF

[0036] The strong correlation of AF with oxidative stress markers may suggest novel measures to predict the onset and efficacy of treatment in AF. Figure 3 shows the relationship of E_h GSH to the odds ratio for AF based on the parameters determined in the multivariate logistical regression analysis. A change in E_h GSH of 15 mV implies a ~4 fold increase in the odds ratio of AF. This change in E_h GSH corresponds to roughly that expected with either a decade increase in age or the presence of diabetes, hypertension, or smoking, all known risk factors for AF (References 44, 58 and 65). The mechanisms whereby oxidative stress may contribute to AF are unknown, but there is evidence that oxidants can affect ion channel activity (References 4, 30 and 61). Also, oxidative

stress is known to activate redox sensitive transcription factors such as NF- κ B. Recently, we have shown that the cardiac sodium channel (SCN5a) promoter region contains an NF- κ B response element that could lead to Na⁺ channel transcriptional regulation by a NF κ B-dependent mechanism (Reference 67).

Statins and AF

[0037] In our study, we showed an inverse relationship between statin use and oxidative stress or the incidence of AF. Statins are thought to have anti-oxidant properties at least in part as a result of preventing NADPH oxidase induced oxygen free radical production (References 14 and 71-74). Our findings are consistent with reports that statins prevent electrical remodeling in rapid pacing-induced AF (Reference 69) and experimentally induced sterile pericarditis (Reference 51) in canine models and reducing AF burden after cardiac (Reference 55) or non-cardiac surgeries (Reference 2). Moreover, it is consistent with a recent report of statins preventing recurrence of AF after cardioversion (Reference 60).

Inflammation and AF

[0038] Since inflammation has been associated with AF and oxidative stress, we also measured inflammatory markers between our two groups. We did not find an association in our study, however. This is consistent with findings of several other groups investigating the use of

CRP to predict post-operative AF (References 2, 33, 34 and 77). Conway et al. found that CRP predicted only initial but not long term cardioversion success, (Reference 17). Conversely, other reports suggest a correlation of inflammatory markers with AF. There is a well documented increase in AF incidence after cardiac surgery, this increase in AF correlates temporally with the peak elevation in CRP levels (Reference 8). Moreover, in two trials, patients with high CRP levels were more likely to develop AF (References 3 and 53). A recent meta-analysis of 16 trials does suggest a relationship between inflammation and persistent or permanent AF (Reference 6). In one trial, IL-6 but not CRP or TNF α predicted post-operative AF (Reference 39). The concomitant lack of elevation of IL-1 β , IL-6, and CRP is consistent with the known roles of these interleukins as synergistic upstream stimuli for CRP production (Reference 32).

[0039] Since our patients had persistent or permanent AF, the association of AF with oxidative stress but not inflammatory markers could represent a more prominent role for oxidative stress relative to inflammation in the maintenance of AF. Alternatively, differences in post-operative and non-operative AF, a lack of sensitivity given our high baseline CRP levels compared to other trials, or the limited power of the trial to detect a relationship could explain the findings. Our levels of IL-6 and TNF α were comparable with baseline levels in a recent report, however, suggesting that our patients were not substantially different in

inflammatory state from those in other trials (Reference 39). Although our results do not directly address the role of oxidative stress in the initiation of AF, they do not rule out a potential role for inflammation in the maintenance of AF. Interestingly, cardiac surgery has also been reported to increase oxidative stress as measured by thiol ratios in the plasma and myocardium, (Reference 19) and supplementing postoperative patients with ascorbate, a known anti-oxidant, cuts rates of AF over 2-fold (Reference 11).

[0040] As noted above, AF has been associated with cardiac oxidative stress, but a recent trial suggests that oxidative stress in AF may be more widespread (Reference 35). Therefore, it is possible that systemic oxidative stress contributes to AF risk and, once AF is established, local cardiac oxidative stress reinforces the risk (References 23 and 47). Interestingly, congestive heart failure and hypertension are associated with oxidative stress, perhaps contributing to their unequal distribution between the two groups (References 13, 37 and 70). A hypothesis potentially explaining the relationship of AF and its risk factors to oxidative stress is presented in Figure 4.

[0041] In conclusion, persistent or permanent AF is associated with increased blood markers of oxidative stress when compared to an age-, sex-, smoking-, and diabetes- matched control population. In this study, the use of statins was associated with a lower prevalence of AF and with

decreased oxidative stress levels. Therefore, lower oxidative stress marker levels may predict a lower risk of AF or efficacy of drugs in the prevention of AF.

TABLE 1: Comparison of Baseline characteristics

Variable	Cases (N=20)	Controls (N=20)	p-value*
Age [†]	74.8 ± 8.8	74.5 ± 8.5	0.74
Diabetic [†]	25% (5)	25% (5)	1.00
BMI	27.7 ± 4.8	28.3 ± 5.2	0.71
Race			
white	18 (90%)	18 (90%)	1.00
black	2 (10%)	2 (10%)	
Prior MI [‡]	4 (20%)	6 (30%)	0.72
Hypertensive	16 (80%)	9 (45%)	0.05
NYHA Class			
class 1	8 (40%)	16 (80%)	0.04
class 2	9 (45%)	3 (15%)	
class 3	3 (15%)	1 (5%)	
Number of medications	8.2 ± 3.4	6.4 ± 4.1	0.13
Use of Statins	5 (25%)	11 (55%)	0.11

* t-test or Fisher's Exact two-sided p-values for continuous or categorical variables, respectively

[†] Matching variable

[‡] MI indicates myocardial infarction

TABLE 2: Univariate Increase in the Odds of Atrial Fibrillation for an Inter-Quartile Range Increase in Oxidative and Inflammatory Markers

Exposure Variable	Unit increase*	Odds Ratio	95% Confidence Interval	p-value
<i>Oxidative markers</i>				
E _h GSH	20	6.1	1.3, 28.3	0.02
E _h CySH	10	13.6	2.5, 74.1	0.01
DROMs	70	15.9	1.7, 153.9	0.02
<i>Inflammatory markers</i>				
IL-1 β	0.35	1.1	0.8, 1.6	0.59
IL-6	3	1.9	0.9, 4.2	0.11
TNF α	5	1.1	0.7, 1.9	0.61
hsCRP	3	1.4	0.8, 2.5	0.21

* Approximate increase in the number of measured units (e.g. mV for thiol ratios, Carr units for DROMs, pg/mL for IL-1 β , IL-6, and TNF α , and μ g/mL for hsCRP, respectively) needed to move from the 25th to 75th percentile.

TABLE 3: Spearman Correlation Coefficients Between Markers

Marker	E _h GSH	E _h CySH	DROMs	hsCRP	TNF α	IL-1 β	IL-6
E _h GSH	1	0.66*	0.41	0.29	-0.16	0.00	0.05
E _h CySH		1	0.38*	-0.02	-0.42*	-0.21	-0.16
DROMs			1	0.29	0.02	-0.01	0.38*
hsCRP				1	0.19	0.14	0.40*
TNF α					1	0.57*	0.47*
IL-1 β						1	0.42*
IL-6							1

* p < 0.05

[0042] While this invention has been described as having preferred sequences, ranges, steps, materials, structures, features, and/or designs, it is understood that it is capable of further modifications, uses and/or adaptations of the invention following in general the principle of the invention, and including such departures from the present disclosure as those come within the known or customary practice in the art to which the invention pertains, and as may be applied to the central features hereinbefore set forth, and fall within the scope of the invention and of the limits of the appended claims.

REFERENCES

[0043] The following references, and those cited in the disclosure herein, are hereby incorporated herein in their entirety by reference.

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WHAT IS CLAIMED IS:

1. A method of predicting onset or risk of atrial fibrillation (AF) in a subject, comprising:
determining the presence of an oxidative stress marker.
2. The method of Claim 1, wherein:
the presence of the oxidative stress marker is indicated by an abnormal thiol ratio for at least one member selected from the group consisting of glutathione, cysteine, and a combination thereof.
3. The method of Claim 2, wherein:
the abnormal thiol ratio represents a higher than a predetermined normal oxidized state value for the selected marker.
4. The method of Claim 3, wherein:
the normal oxidized state value comprises median values of -143mV and -67mV for glutathione and cysteine, respectively.

5. The method of Claim 4, wherein:
a change in the median value of 15mV corresponds to about four-fold increase for the onset of atrial fibrillation.
6. The method of Claim 1, wherein:
the presence of the oxidative stress marker is indicated by an abnormal oxidized state of a derivative of a reactive oxidative metabolite (DROM).
7. The method of Claim 6, wherein:
the abnormal oxidized state represents a higher than a predetermined normal oxidized state value for the derivative.
8. The method of Claim 7, wherein:
the normal oxidized state value comprises a median value of 370 Carr units.

9. A method for treating a subject suspect of having atrial fibrillation, comprising:
 - a) determining the presence of an elevated blood level of an oxidative stress marker;
 - b) comparing the value obtained in step a) to a predetermined normal value; and
 - c) recommending the subject for an antioxidant therapy if the value obtained in step b) is higher than the normal value.
10. The method of Claim 9, wherein:

the oxidative stress marker comprises at least one member selected from the group consisting of glutathione, cysteine, and a combination thereof.
11. The method of Claim 9, wherein:

the oxidative stress marker comprises a derivative of a reactive oxidative metabolite (DROM).
12. The method of Claim 9, wherein:

the antioxidant comprises a statin.

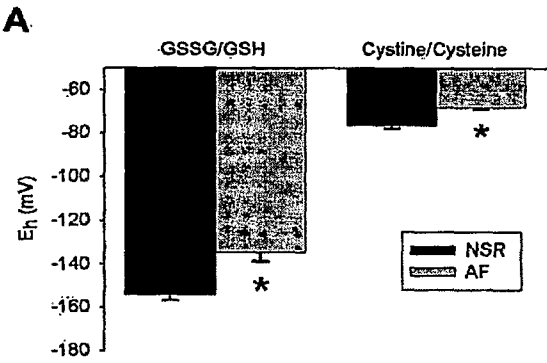


FIGURE 1A

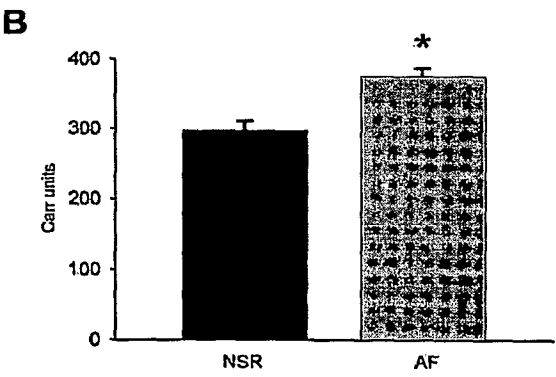


FIGURE 1B

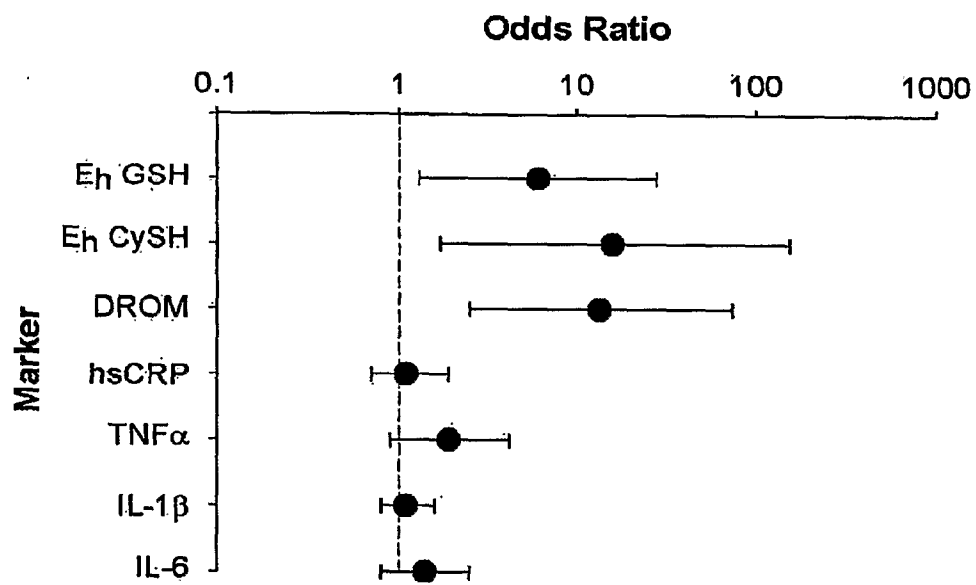


FIGURE 2

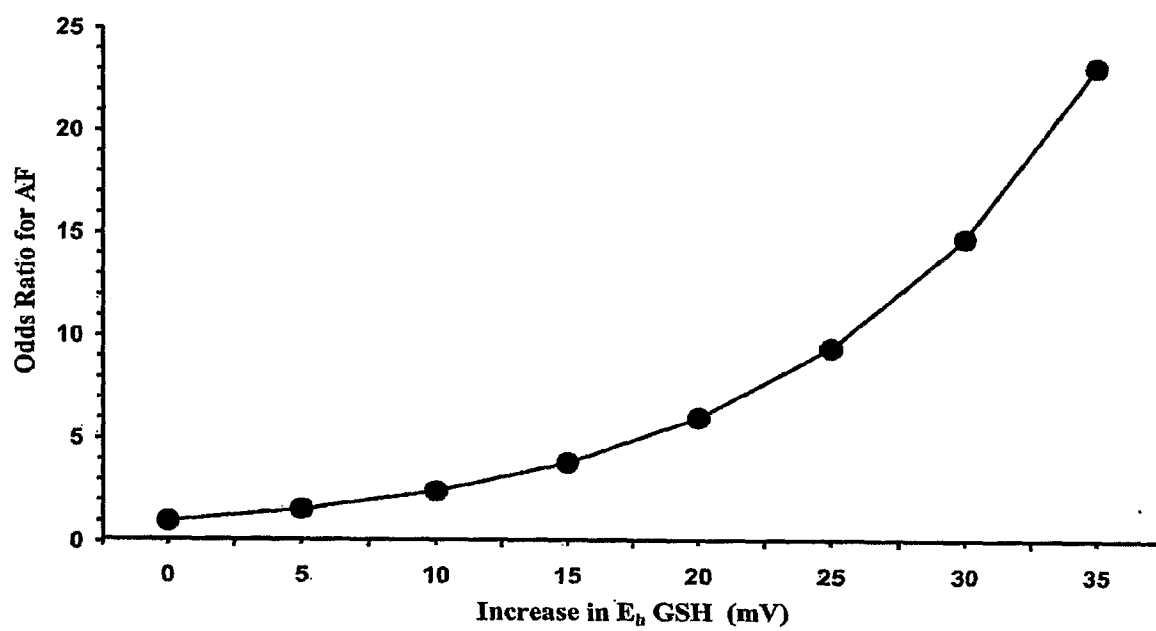


FIGURE 3

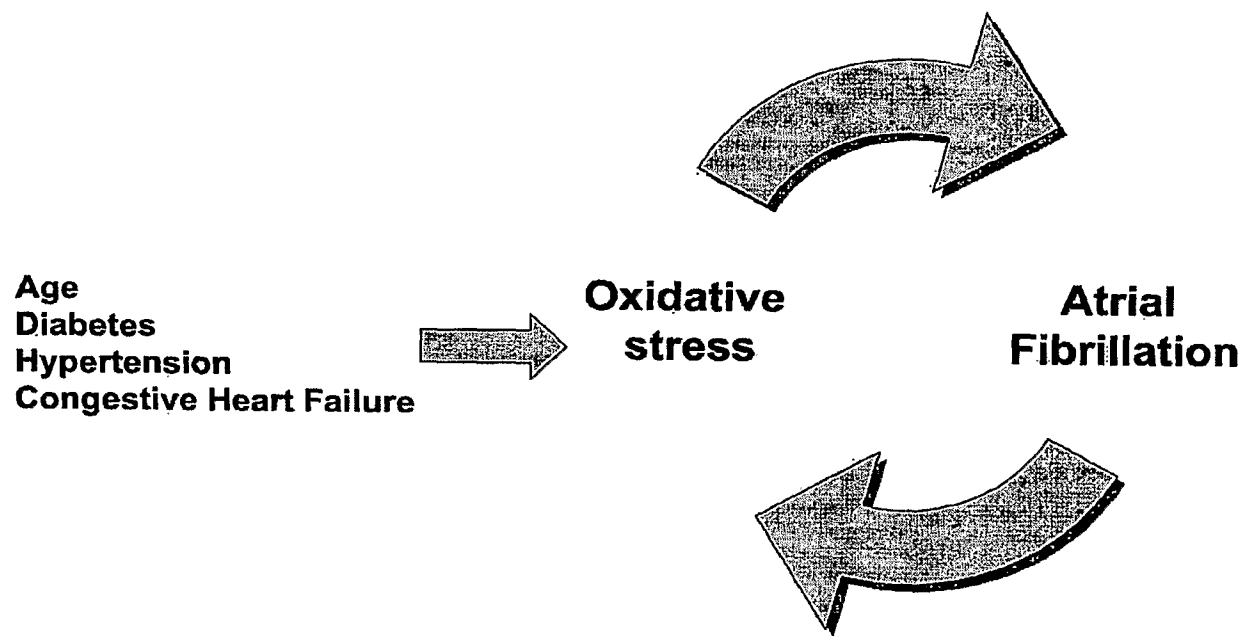


FIGURE 4