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Soluble CD40L Versus Myocyte Enhancer Factor: Predicting a Prominent Marker For Cardiovascular Disease.

Chafia Hejase de Trad[1], Member, IEEE
Department of Physics, United Arab Emirates University, PO Box 17551, Al-AIN, UAE

Abstract—**Atherosclerosis (ACS) has set off the innovation of molecular markers measured in plasma or serum, and used for the identification of individuals at high risk of Coronary Heart Disease (CHD). In an attempt to improve cardiovascular risk prediction, considerable interest is focused on inflammatory biomarkers including Interleukin (IL)-6, Phospholamban (PLB), Myocyte enhancer factor 2A (MEF2A), and Soluble CD40 ligand. In this paper, signal-processing techniques predicted the characteristic frequencies of the above-mentioned proteins, and common binding sites. The CD40L characteristic frequency, 0.3555 ± 0.0001, is correlated with Protease inhibitors and the second peak, 0.4531 ± 0.0009, is closely related to Fgfs. This study also revealed that for MEF2A, the characteristic frequency, 0.0488 ± 0.0001, is specific for enhancers DNA regulating sequences. The remaining frequencies, 0.3672 ± 0.0001 and 0.4648 ± 0.0002, are characteristic of the Myocyte Protease inhibiting activity and SOS operator function. Furthermore, clinical data suggested that the increased levels of CD40L reliably identify the subgroup of patients with ACS who are at highest risk for cardiac events. It is suggested that CD40L is a most prominent candidate for early detection of cardiac disease.**

Keywords— **Biomarkers, Soluble CD40L, Myocyte Enhancer Factor, Signal Processing Techniques, Wavelets.**

I. INTRODUCTION

Acute coronary syndrome most commonly occurs when coronary arteries become severely obstructed. Increasingly, it has been realized that vascular inflammation plays a key role in atherosclerotic lesion formation and progression [1]. Accordingly, markers of inflammation and endothelial activation have become useful by providing additional information about cardiovascular risk and prognosis, as well as providing new targets for treatment [2] and [3].

The process of inflammation is now believed to be the etiological event that precedes the development and the continual process of atherosclerosis. One of the initiators of the acute phase response is infection. Reports dating back 100 years or more have suggested a possible association between infectious agents and both atherosclerosis and myocardial infarction [4]. The prevention and management of cardiovascular disease is undergoing radical change. Availability of highly sensitive marker assays may alter risk screening for cardiovascular disease and increase-targeted populations.

[1] Author has honorary position at ECSE, Monash University, Australia

A. Cytokines and other markers

Elevated levels of cytokines involved in the acute phase response including IL-6, and fibrinogen have been shown to be elevated in cases of unstable angina, and have been positively correlated with the risk of primary and recurrent myocardial infarction and death [5,6]. IL-6 could serve as early warning sign since it increases early in the inflammatory process, whereas C-Reactive Protein (CRP) increases fairly late in the inflammatory cascade. In patients with unstable coronary artery disease, elevation of soluble CD40 ligand levels indicated an increased risk of cardiovascular events [7]. Soluble CD40 ligand was a powerful prognostic marker that provided information beyond the evidence provided by troponin T [8], and the inflammatory marker C-reactive protein.

Impairments in blood circulation that accompany heart failure can be traced, in part, to alterations in the activity of the sarcoplasmic reticulum Ca^{2+} pump that are induced by its interactions with phospholamban, a reversible inhibitor [9,10]. Humans with a *phospholamban*-null genotype develop early-onset dilated cardiomyopathy. Blocking phospholamban, a key protein involved in calcium regulation, can improve the function of failing heart cells. Also, it has been established that Myocyte enhancer factor 2A (MEF2A) plays a vital role in the development of cardiovascular problems like atherosclerosis and restenosis after angioplasty inflammation [11].

B. The Resonant Recognition Model

The *Resonant Recognition Model* (RRM) [12] is a physico-mathematical model that analyses the interactions of protein and its target using digital signal processing methods. It has been shown in previous RRM studies that certain periodicities (frequencies) in the protein signal characterise protein biological function [12-14].

C. The Wavelet Transform

Wavelet transform, is a signal-processing tool efficient for multi-resolution analysis and local feature extraction of non-stationary signals [12-14]. Previous results suggested that Continuous Wavelet Transform (CWT) could be applied successfully in determination of active site of proteins [12-14].

In this paper, the Resonance recognition method (RRM) has been employed to determine the characteristic frequencies of the above-mentioned proteins. It has been found that most of the suggested proteins share the same characteristic frequencies suggesting their common contribution to heart failure. The performance of wavelet functions including Morlet, Meyer, Daubechies, Coiflets and

Symlets was evaluated to detect the active sites of cardiac proteins with previously determined characteristics.

II. METHODS

A. Signal processing techniques

The *Resonant Recognition Model* is based on the finding that there is a significant correlation between spectra of the numerical presentation of amino acid and their biological activity [12-15]. By assigning the *electron-ion interaction potential* (EIIP) value [12-15] to each amino acid, the protein sequence can be converted into a numerical sequence. These numerical series can then be analysed by appropriate digital signal processing methods (fast Fourier transform is generally used). To determine the common frequency components in the spectra for a group of proteins, the multiple cross-spectral function was used. Peaks in this function denote common frequency components for the sequences analysed. Through an extensive study, it has been concluded that one RRM characteristic frequency characterises one particular biological function or interaction [12-15].

The wavelet transform uses a set of dilated and translated wavelets as the signal decomposition basis and provides same time/space resolution for each scale. Thus, CWT can be chosen to localise individual events, such as the active site identification [13-15]. The active sites along the protein sequence are determined through studying the set of local extrema of the moduli in the wavelets transform domain.

B. Clinical data

Dr. M. Jose Hejase (Personal communication) provided clinical data of 98 patients (from UAE) with ACS. All patients signed informed consents. Patients were clear from infection, cancer, kidney or liver diseases. Patients were followed for 1 and 6 months for the major adverse cardiovascular events (CE). Levels of soluble CD40L were determined by ELISA (sCD40 detection limit, 95 pg/ml; Bender Medsystems). The Pearson two-way test was used to assess the relation between two quantitative variables with normal distributions. All P-values<0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

A. Clinical data

The 98 patients with ACS were divided into three groups according to their measured levels of soluble CD40L at base line (compatible characteristics relative to initial clinical history): eight patients in the first group (2.0–5.0 ng/ml), thirty patients in the second group (5.0–8.0 ng/ml) and sixty patients in the third group (>8.0 ng/ml). For the last category, follow-up times (1 month and 6 months), the rates of major cardiovascular events were significantly higher in both second group ($p<0.0001$) and the third group ($p<0.0001$) as shown in figure 1. Multivariate analysis using CD40L as a continuous variable revealed that CD40L levels remain associated with higher risk of ACS, sudden death and recurrent angina ($p<0.05$).

This data suggests that sCD40L is a powerful biochemical marker in patients with ACS. The increased levels of sCD40L reliably identify the subgroup of patients with ACS who are at highest risk for cardiac events. These results are consistent with Varo et al [16].

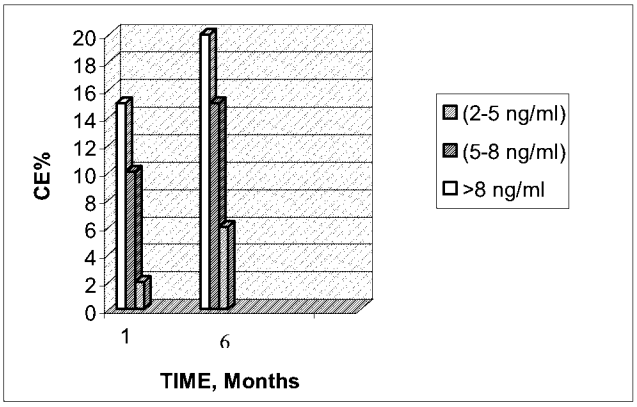

Fig. 1. Histogram showing CD40L levels and the rate of major cardiovascular events (CE %) at one month and 6 months among 98 patients with ACS.

B. Application of signal processing techniques

Application of the fast Fourier transform for the determination of the characteristic frequencies in the examples of the CD-40L, Troponin, C-Reactive protein, Phospholamban protein, Deoxyribonuclease I, Cyclin, Interleukin-6 precursor and Myocyte enhancer factor 2A (MEF2A) is described in this paper in addition to the wavelet technique. The RRM method was applied to the above proteins. Cross-spectral analysis of different sequence numbers revealed multiple peaks as shown in figure 2.

Table 1 summarizes all information pertinent to the studied proteins including characteristic frequencies and signal to noise ratios.

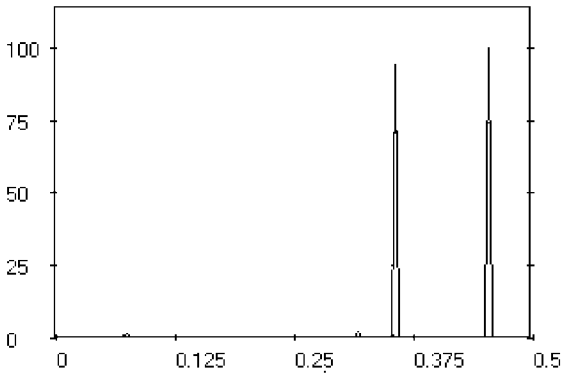

Fig.2. Multiple cross-spectral function of 10 CD40L sequences. The prominent peaks for CD40L are located at 0.3555 and 0.4531. The abscissae represent relative frequency.

The predicted RRM frequency for Phospholamban ($f=0.3320 \pm 0.0001$) is exactly compatible with the predicted frequency ($f=0.0.3320 \pm 0.0002$) for Il-6. The signal to noise

ratio indicates significant values for either protein or cytokine, anticipating that this characteristic frequency might be associated with a specific function directly correlated with heart disease. Myocyte enhancer factor 2A does not have the same functional group identified for either Phospholamban or IL-6. This predicted data supports all experimental evidence pertinent to the different functional groups of the three proteins discussed. The CD40L characteristic frequency, 0.3555 $\pm$ 0.0001, has been identified in previous studies to be correlated with Protease inhibitors [12-14] and the second peak, 0.4531 $\pm$ 0.0009, is very close to that of Fgfs. On the other hand, the Deoxyribonuclease I and Cyclin characteristic frequency 0.0352 $\pm$ 0.0008 is very close to that of Oncogenes and the peak 0.0938 $\pm$ 0.0001 has been identified previously to correspond with heat-shock proteins. The characteristic frequency of CRP, 0.2945 $\pm$ 0.0008 is clearly correlated with growth factors and 0.0430 $\pm$ 0.0001 is characteristic of Phospholipase.

TABLE 1
PROTEIN GROUPS AND THEIR CHARACTERISTIC FREQUENCIES

Protein	Characteristic frequency	Sequence Number	S/N
Phospholamban	0.0156	7	22
	0.3320		34
Interleukin-6 precursor	0.0312	7	68
	0.3320		27
Myocyte Enhancer factor 2A	0.0488	6	23
	0.3672		20
	0.4648		34
Cyclin	0.0352	5	21
	0.2734		20
CD40L	0.3555	10	61
	0.4531		65
Deoxyribonuclease I	0.2031	10	44
	0.0938		22
	0.0352		14
Troponin	0.1133	6	46
	0.1797		44
CRP	0.2383	5	18
	0.2945		13
	0.0430		7

Deoxyribonuclease I, CRP, Myocyte Enhancer factor, phospholamban and Interleukin-6 have several functional groups. The IL-6 characteristic frequency, 0.0312 $\pm$ 0.0001, has been identified in previous studies to be correlated with one specific function common to all oncogenes [12-14]. In addition, the Myocyte Enhancer factor 2A characteristic frequencies have been also identified in previous studies [12-14]. The characteristic frequency, 0.0488 $\pm$ 0.0001, is specific for enhancers DNA regulating sequences. The remaining frequencies, 0.3672 $\pm$ 0.0001 and 0.4648 $\pm$ 0.0002, are characteristic of the Myocyte Protease inhibiting activity and SOS operator function. The later frequencies closely correlate with those of CD40L as shown in table 1.

The clinical studies on CD40L, in this paper, have shown that soluble CD40 ligand is a powerful prognostic marker. Troponins are markers of myocardial necrosis, they are not actively involved in the pathophysiology of acute coronary syndromes but, rather, are surrogate markers for the formation of fragile thrombi [17]. The predicted characteristic frequencies do not correlate with those of sCD40L.

C. The Wavelet Transform

Using the wavelet transform method, a whole frequency/spatial distribution is observed, and thus, identifies domains of high energy of particular frequency along the sequence. The continuous scalogram of each of the above proteins have been obtained using different wavelet functions including Morlet, Daubechies, Mexican Hat, Symlets, Meyer ,and Coiflets.

Morlet, Symlet6, Db6 wavelets (Figure 3) are clearly representing domains of higher energy within each of the represented proteins. Other wavelets, including Mexican Hat and Haar, have very noisy scalograms (Not shown).

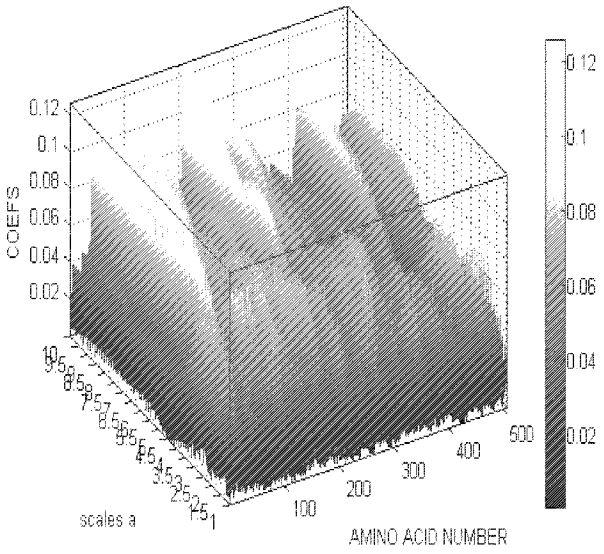

Fig. 3. A 3D plot Myocyte Enhancer factor 2A. Data on the Z-axis represent absolute values of coefficients calculated for each scale ranging from 1 to 10.

Using the wavelet transform method, probable important amino acids corresponding to Phospholamban and Interleukin-6 have been predicted. These amino acids are Arginine (number 9) and Lysine (number 39) on Phospholamban, and amino acids 39 (Serine) and 40 (Glycine) on Interleukin-6. These amino acids may belong to active binding sites on either protein crucial for cardiac problem detection. Further experimental studies must be pursued in an attempt to explore the importance of these amino acids and to quantify the possibility of using Phospholamban as a promising marker.

The wavelets Morlet and Symlet 6 worked perfectly well for Deoxyribonuclease I, while the wavelet Symlet 8 gave

best results for CD40L and Myocyte Enhancer factor. The corresponding 3D plot for Myocyte Enhancer factor is shown in figure 3. Domains of high energy are very clearly represented. Brightest peaks are correlated with the characteristic frequencies predicted in Table 1. There is a high probability that amino acids in the band from amino acid serine (210) to valine (215) correspond to a functional group or binding site. These amino acids may belong to active binding sites on protein crucial for cardiac problem detection. Further experimental studies must be pursued in an attempt to explore the importance of these amino acids and to quantify the possibility of using Myocyte Enhancer factor 2A as a promising marker. This data is further supported by experimental results showing that Myocyte enhancer factor 2A (MEF2A) plays a vital role in the development of cardiovascular problems like atherosclerosis and restenosis after angioplasty inflammation [11].

The corresponding 3D plots for CD40L is shown in figure 4.

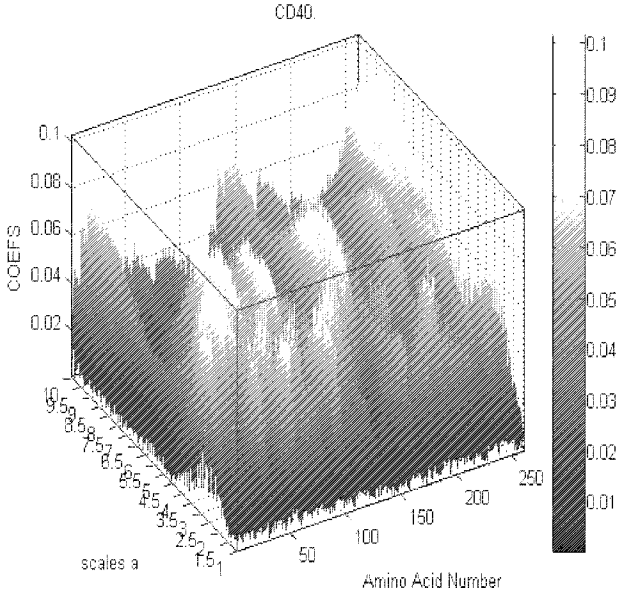

Fig. 4. A 3D plot of CD40L. Data on the Z-axis represent absolute values of coefficients calculated for each scale ranging from 1 to 10.

Once more, domains of high energy are very clearly represented. Brightest peaks are correlated with the characteristic frequencies predicted in Table 1. The two plots reveal clearly that they have same functional groups. Amino acids147 (threonine) to 149 (serine) and 255 (serine) to 258 (leucine) may belong to active binding sites crucial for cardiac problem detection. Further experimental studies must be pursued in an attempt to explore the importance of these amino acids and to quantify the possibility of using CD40L as a prominent promising marker.

IV. CONCLUSION

The present study suggests that both Myocyte Enhancer factor and soluble CD40L may be prominent biochemical markers in patients with ACS. It is highly probable and reliable that patients with ACS who are at highest risk for cardiac events can be identified with the increased levels of sCD40L. A larger pool of patients should be involved in similar studies to strengthen the clinical use of sCD40L independently or in combination with other markers in the prediction of cardiovascular events after ACS.

In summary, it has been found that soluble CD40L independently predicts the risk of cardiovascular events in patients with ACS. Simultaneous assessment of predicted candidates should yield independent and complementary prognostic information and thus enable better prediction of adverse cardiac outcomes.

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