An exploratory analysis investigating blood protein biomarkers to augment ECG diagnosis of ACS

Keywords: Electrocardiogram, blood biomarkers, acute myocardial infarction, clinical decision making

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Abstract

Background: Acute Coronary Syndrome (ACS) is currently diagnosed using a 12-lead Electrocardiogram (ECG). Our recent work however has shown that interpretation of the 12-lead ECG is complex and that clinicians can be sub-optimal in their interpretation. Additionally, ECG does not always identify acute total occlusions in certain patients.

Purpose: The aim of the present study was to compare protein expression profiles of ACS patients, to detect biomarkers that may improve the diagnosis of ACS or augment ECG interpretation.

Methods: Patients were recruited consecutively at the cardiac catheterization laboratory at Altnagelvin Hospital over a period of 6 months. A low risk control group was recruited by advertisement. Blood samples were analysed using the multiplex proximity extension assays by OLINK proteomics. Support vector machine (SVM) learning was used as a classifier to distinguish between patient groups on training data. The ST segment elevation level was extracted from each ECG for a subset of patients and combined with the protein markers. Quadratic SVM (QSVM) learning was then used as a classifier to distinguish STEMI from NSTEMI on training and test data.

Results: Of the 344 participants recruited, 77 were initially diagnosed with NSTEMI, 7 with STEMI, and 81 were low risk controls. The other participants were those diagnosed with angina (stable and unstable) or non-cardiac patients. Of the 368 proteins analysed, 20 proteins together could significantly differentiate between patients with ACS and patients with stable angina (ROC-AUC=0.96). Six proteins discriminated

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significantly between the stable angina and the low risk control groups (ROC-AUC=1.0). Additionally, 16 proteins together perfectly discriminated between the STEMI and NSTEMI patients (ROC-AUC=1). ECG comparisons with the protein biomarker data for a subset of patients (STEMI n=6 and NSTEMI n=6), demonstrated that 21 features (20 proteins + ST elevation) resulted in the highest classification accuracy 91.7% (ROC-AUC= 0.94). The 20 proteins without the ST elevation feature gave an accuracy of 80.6% (ROC-AUC 0.91), while the ST elevation feature without the protein biomarkers resulted in an accuracy of 69.3% (ROC-AUC=0.81).

Conclusions: Although patient numbers for the STEMI group were low in this analysis, results showed that certain panels of proteins could stratify patients and may be a useful to aid in the diagnosis of ACS. This preliminary analysis reveals the potential for blood biomarkers to augment ECG interpretation.

Introduction

The diagnosis of Acute Coronary Syndrome (ACS) has traditionally relied upon the 12-lead ECG in combination with ischemic symptoms and elevation in serum biomarkers. However, symptoms are often atypical or absent, and around 33% of patients that present with ACS may not have chest pains [1]. Similarly, ECG changes that aid with early diagnosis may be nonspecific or even absent in around 40% of the patients [2]. Acute total occlusion of the culprit artery usually presents with ST-elevation myocardial infarction, however a subset of patients with acute total occlusion present as non-ST segment elevation myocardial infarction (NSTEMI) [3,4]. The inability of the ECG to identify these patients may lead to delay in their proper management. Moreover, ST-segment changes are also observed in other cardiac conditions like pericarditis, left ventricular hypertrophy, cardiomyopathies and channelopathies, which can add to the diagnostic dilemma.

Our recent work has shown that interpretation of the 12-lead ECG is complex and that clinicians are sub-optimal in their interpretation [5,6]. For example, up to 33% of ECG interpretations contain errors of significant importance. In addition, as a result of our recent eye tracking studies we have found that even expert clinicians are known to impulsively provide a diagnosis based on their first impression and can, as a result, miss co-abnormalities [7,8]. Whilst the human interpretation of the ECG needs improvement, computerised automatic interpretation of the ECG remains very poor – often less accurate in comparison to cardiologists [9,10]. Computerised ECG interpretation is a very complex and challenging task that requires effective signal processing, statistical analysis and often artificial intelligence. These algorithms as reported in the literature [10] are often inaccurate which is a concern given clinical staff are biased towards the machine diagnosis which often results in false positives [7,8].

Other diagnostic methods rely on measurements of High Sensitivity Cardiac Troponin T (HScTnT), which is the only accepted blood biomarker for ACS diagnosis. However, there is a delay between symptom onset and detectable levels of troponin in the blood and it often requires repeated measurements over time to be of value [11]. Thus, additional blood markers have been sought in order to improve the initial diagnosis and also to help risk stratification. The discovery of new blood biomarkers could not only improve the diagnosis of ACS, but also provide insights into the underlying pathophysiology [12,13]. In the last decade, a few circulating biomarkers, such as Heart fatty acid binding protein (H-FABP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) have been investigated [14]. Currently there is no consensus on the best cardiac biomarker or combination of those studied as standalone or adjunctive diagnostic. It would be helpful if a panel of markers could improve the confidence of the interpreter of the ECG whilst at the same time improve accuracy of the diagnosis. Blood biomarkers are being sought that can help diagnose ACS by evidencing heart muscle damage and/or be indicative of an acute total occlusion of a culprit artery. The latter is especially important given that such occlusions occur in NSTEMI ECG patients, whom are often not triaged for urgent catheterization. We currently lack well-validated high performing markers that not only diagnose ACS but classify/stratify patients based on angiographic findings (complete acute occlusions of the culprit artery or not). This is of major importance when triaging patients for the correct activation of the pPCI pathway. However, as long as this is not possible, the STEMI/NSTEMI categorization predominates the triage.

We therefore undertook a multiplex proteomics approach to investigate whether a blood protein biosignature could improve ACS diagnosis. We primarily wanted to investigate whether certain blood protein biomarkers could discriminate between ACS and non-

ACS patients. As an exploratory analysis, we used a machine learning approach to investigate if blood biomarkers could augment or improve the accuracy of the ECG diagnosis of ACS and in particular STEMI diagnosis in a subset of our patients.

Methods

Participant recruitment

Acute or elective patient admissions were recruited consecutively from the catheterisation laboratory at Altnagelvin Hospital. Acute MI was diagnosed when either initial or 12 h cTnT was ≥14ng/L, with or without ECG features of ischaemia/infarction, in the absence of any other cause for the chest pain. If this definition was met then classification was made into ST-segment elevation MI (STEMI) and non-STEMI (NSTEMI). The diagnosis of STEMI required ST-segment elevation in at least two contiguous leads of the ECG or new onset of left bundle branch block. ST-segment elevation was defined as ≥1 mm in leads I–III, aVL, aVF, V4, V5, and V6 and ≥2 mm in leads V1–V3. Categorization of patients as NSTEMI was by exclusion of STEMI and elevation in serum troponin with confirmatory angiographic evidence of obstructive coronary artery disease. An elective group of patients with documented coronary artery disease attending the catheterisation laboratory for coronary angiogram or PCI were also recruited. A low risk control group who had a 10-year fatal CVD risk <10% according to the European Systematic Coronary Risk Evaluation (SCORE) were recruited by advertisement to Hospital and University staff.

Each patient provided informed written consent. Blood samples were collected prior to coronary angiography. After discarding the first 5mls of blood to avoid unnecessary biological activation blood was drawn (8mls) and dispensed into tubes which were then

immediately cooled and centrifuged (within 4 hours of collection) at 2000 RCF for 15 minutes. Plasma and buffy coat were aliquoted and frozen at −80◦C until further analysis. The ECG which classified patients as STEMI or NSTEMI was logged. Demographic information as well as previous medical and clinical history was collected. The blood pressure was recorded at the time along with C-Reactive Protein (CRP) and electrolytes levels on the same day. The research protocol was approved by the Office for Research Ethics Committees Northern Ireland (ORECNI) (Ref:14/NI/0068). Each participant gave informed written consent.

Proteomic profiling

Plasma samples were assessed with the Proseek Multiplex proximity extension assay (Olink Bioscience, Uppsala, Sweden). The assay simultaneously measures 92 proteins in each panel using two specific antibodies per protein that pairwise bind to each protein, creating a polymerase chain reaction sequence. This is followed by quantitative real-time polymerase chain reaction for quantification. The CVDII, CVDIII, inflammatory and immune response panels were used with a total measurement of 368 proteins.

Data analysis

All data analytics were carried out using MATLAB (https://www.mathworks.com/). To build classifiers without ECG, we ranked individual proteins by the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC) curve obtained when patient groups were classified by Support Vector Machines (SVM) with a linear kernel. Starting with the best individual protein, we built multivariate classifiers using subsets of the top performing individual proteins. In order to evaluate the expected overfitting in each classifier, rather than segregate the data into training and validation subsets and lose statistical power, instead we randomly relabelled the patients between groups to generate nonsense data. Relabelling occurred until the nonsense data was uncorrelated with the original data. This was repeated twenty times to yield twenty nonsense data sets. The same classifiers were built from the nonsense data and the mean of the AUCs based on the nonsense data was calculated as a benchmark against which to assess classifiers for true data.

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To build classifiers we used Quadratic Support Vector Machine (QSVM) with quadratic kernel function, with the ECG and a subset of differentially expressed proteins. To assess the accuracy of the classifier, ROC curves were developed to obtain cumulative AUCs for increasing numbers of proteins in the panel, based on the minimum number of most significant proteins required for accurate classification. ST segment elevation level was extracted from each ECG and combined with the protein biomarkers dataset. Pearson-correlation coefficients function were used for feature selection (biomarkers dataset + ST level) to compute P-values to find the optimal set of features to determine STEMI/NSTEMI patients. QSVM was used as a classifier to detect STEMI and NSTEMI. We applied 12-fold cross-validation since we had a limited number of STEMI patients. In QSVM we started with the most important

features (39 features including 38 biomarkers and ST elevation level) and then we removed feature by feature to get the highest accuracy. Multivariate analysis was carried out to account for confounding factors including age and gender.

Results

Patient recruitment

A total of 344 patients were recruited including ACS (STEMI n=7 and NSTEMI n=77), Unstable Angina (n=21), Elective coronary artery disease (CAD) (n=158) patients and low risk controls (n=81). (Table 1). The mean age of the patients at study entry was 65 years and 21% were women. A total of 56% had a history of hypertension, 19% were diabetic and 19% were current smokers. A total of 55.4% (n=127) of CAD patients were admitted with an ACS and 44.4% (n=102) were electively admitted for coronary angiogram. The mean age of the control participants was 46 years and 70% were women. A total of 13% had hypertension and 4% were current smokers. The mean age of the low risk group was 41 years and 79% were women. The average time from admission to blood sampling for patients was 3 days.

Blood biomarker analysis for ACS diagnosis

Blood plasma levels of 368 proteins were analysed using protein extension assays by Olink Proteomics. These multiplex arrays were employed to identify biomarkers in patients with ACS, CAD patients attending for a coronary angiogram or PCI and low risk individuals (apparently healthy controls). Baseline characteristics of these patients are shown in Table 1. Of the 344 participants recruited, 77 were diagnosed with NSTEMI, 7 with STEMI, and 81 were low risk controls. The other participants were those diagnosed with angina (stable and unstable) or non-cardiac patients. Of the 368

proteins analysed, 20 proteins could significantly differentiate between ACS patients and all other participants in the cohort (ROC=0.95) (Fig 1a). Further analysis showed that 20 proteins could significantly differentiate between patients with ACS and patients with stable angina (ROC=0.96) (Fig 1b). We also found that any one of 6 particular proteins were significantly different between the elective patients with CAD and the low risk control group (ROC=1.0) (Fig 1c). Additionally, 16 proteins were significantly discriminating between the STEMI and NSTEMI patients (ROC=1) (Fig 1d). It is of note that due to intellectual property constraints the proteins can not yet be named.

Blood biomarker and ECG for STEMI diagnosis

A subset of 12 patients (6 STEMI and 6 NSTEMI) were randomly selected for exploratory analysis to investigate if protein profiles could augment ECG diagnosis for ACS. ECG comparisons with the protein biomarker data for a subset of patients (STEMI n=6 and NSTEMI n=6), demonstrated that 21 features (20 proteins + ST elevation) resulted in the highest classification accuracy 91.7% (ROC-AUC= 0.94) (fig. 2a). The 20 proteins without the ST elevation feature gave an accuracy of 80.6% (ROC-AUC 0.91) (fig. 2b), while the ST elevation feature without the protein biomarkers resulted in an accuracy of 69.3% (ROC-AUC=0.81) (fig. 2c).

Discussion

ACS is often a diagnostic dilemma, primarily due to problems/limitations associated with 1) variability in patient history/symptoms, 2) accuracy and timings of serum biomarker measurements 3) ECG accuracy due to both the machine and human interpretation and 4) ECG can be inherently limited to what is presented (surface ECG

is just that). Incorrect diagnosis of ACS has significant consequences for patient safety and the health service. Internationally an average of 15% of patients that come to the catheterization lab are defined as false activations [15] (the patient was not suffering a myocardial infarction). This has significant health economic cost implications, a significant impact on the staff in terms of fatigue and workflow within the department particularly if the activation has occurred during the night and exposes the patient to the risk of doing an invasive procedure that could have been avoided if the clinical decision-making was better. Additionally, those patients that are not correctly referred for pPCI have poorer outcomes [16]. There is a need therefore to develop better ways to improve clinical decision making for deciding upon the initial treatment strategy for ACS patients.

In this study we measured multiple blood protein biomarkers in consecutive patients recruited at the cath lab. A low risk control group with no identifiable risk factors were also included in our analysis. This multiplex approach combined with SVM learning allowed us to identify a panel of proteins that could significantly identify the ACS patients in our cohort. Importantly we also identified panels of proteins that significantly differed between ACS and elective stable angina patients. A panel of other markers also significantly differed when comparing these elective patients with the low risk control group. These latter proteins are of significant importance since they appear to be specific for CAD and not only increased due to a non-specific acute phase response. To date most studies have concentrated on looking at markers of heart muscle damage and myocyte load to assess ACS [14]. Other markers are emerging, including those relating to inflammation, oxidative stress and fibrosis, which are pivotal in cardiovascular disease [14-18]. These studies have also used multiplex technology to aid progress in this area. These studies however so far have only measured proteins in relatively small numbers of STEMI patients in comparison to either healthy controls or

individuals at CVD risk. To the best of our knowledge no one has taken a multiplex approach comparing larger numbers of patients in all ACS categories (STEMI, NSTEMI, UA) and compared these with stable angina or a low risk scored control group. It is important to note that this is a pilot study that was set up to identify differences between the 3 main patient groups (ACS, stable CAD and low risk). A major limitation of this current study is that the average time from admission to blood sampling for patients was 3 days. Future validation work is therefore required with regards to timings of blood samples. These markers therefore need to be measured over time to fully ascertain their diagnostic value and investigate potential confounders in this current study such as treatment that may have commenced prior to blood sampling. In addition, these results will need to be replicated in a validation cohort with larger numbers of STEMI patients.

We also undertook a preliminary analysis in a subset of our patients using another machine learning approach. QSVM demonstrated that 20 blood proteins could improve the ECG accuracy for STEMI detection from 69.3% (ROC-AUC=0.81) to 91.7% (ROC-AUC= 0.94). This is purely an exploratory analysis and although patient numbers are low and the blood samples analysed were obtained on average 3 days after the cardiac event, a statistically significant difference was observed in proteins between STEMI and NSTEMI patients. These results are still under investigation, but some previous research has suggested that certain plasma proteins may be involved in driving the composition of thrombus. The percentage of platelet vs fibrin components are thought to be different dependent on ischaemic time. A previous study investigated the composition of coronary thrombi aspirated from patients with acute myocardial infarction (AMI) [19]. They found that fibrin content increased from 48.4% in thrombi

that were collected less than 3 h from symptom onset to 66.9% in those that were collected after 6 h, whereas platelet content decreased from 24.9% to 9.1%.

Another important limitation of our current analysis is that the analytical design does not completely take into account the angiographic findings. Given that generation of STEMI/NSTEMI ECGs depends on the coronary anatomy, collateral circulation and site of the culprit lesion [4,20], a future analytical approach should consider such factors. ECG classification should be set aside to allow a comparison of those patients with acute total occlusion of culprit arteries with patients presenting with non/partial occlusions. Such a reclassification of ACS diagnosis of course would require future validation to assess and compare the accuracy with the current gold standards; ECG, patient history and cardiac Troponin. Until such validations are carried out the use of the protein panels reported in this study should be further investigated with regards to improving the confidence in ECG interpretation by clinical staff. This could be useful given the reports of clinicians being sub-optimal in ECG interpretation [5,6].

Overall this study reports a protein biosignature that can accurately classify ACS and we believe that our study design has allowed us to uncover proteins within this biosignature that are specific for CAD. Future work is now required however to explore the diagnostic potential of these biomarkers and to decipher if thy may augment ECG interpretation.

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Table 1: Patient Characteristics

	ACS					
	Coronary Artery Disease (CAD)					
	STEMI (n=7)	**NSTEMI (n=77)**	**UA (n=21)**	**ELECTIVES (n=102)**	**Low Risk (n=81)**	**P Value**
Demographics						
Age (years)	65	67	64	66	41	*p<0.001*
Male (n; %)	5;71	66;86	17;81	79;77	17;21	*p<0.001*
Female (n; %)	2;29	11;14	4;19	23;23	64;79	*p<0.001*
Weight (Kg)	120	87	86	86	74	*p<0.001*
Height (cm)	168	172	165	170	167	*ns*
BMI (Kg/m^2)	30	29	29	35	27	*ns*
Current smokers (n; %)	2;29	23;30	2;10	11;11	1;1	*p<0.001*
Clinical Variables						
Total Cholesterol (mmol/L)	5.4	4.2	4.3	5.8	5	*ns*
LDL Cholesterol (mmol/L)	2.7	1.2	2.3	2.1	1.6	*p<0.001*
HDL Cholesterol (mmol/L)	1.2	2.3	1.2	1.2	2.9	*p<0.001*
TG (mmol/L)	1.2	1.9	1.7	1.7	1	*ns*
CRP (mg/L)	9.6	18	2.9	6.6	2.4	*p<0.001*
Troponin positive (%)	7;100	77;100	0;0	0;0	0;0	*p<0.001*
Co-morbidities						
Hypertension (n;%)	3;43	44;57	2.9	57;56	5;6	*p<0.001*
Diabetes (n;%)	0;0	14;18	4;19	19;19	0;0	*p<0.001*
GFR<60ml/min (n;%)	0;0	25;32	3;14	13;13	0;0	*p<0.001*
Previous MI	1;14	19;25	5;24	43;42	0;0	*p<0.001*
Previous PCI	1;14	14;18	8;38	45;44	0;0	*p<0.001*

Table 2: STEMI and NSTEMI patient characteristics

	STEMI (n=6)	**NSTEMI (n=6)**	**P Value**
Demographics			
Age (years)	62	61	*ns*
Male (n; %)	4;67	3;50	*ns*
Weight (Kg)	84	87	*ns*
Height (cm)	168	174	*ns*
BMI (Kg/m^2)	29	29	*ns*
Current smokers (n; %)	2;33	2;33	*ns*

Clinical Variables			
Total Cholesterol (mmol/L)	5.6	4.5	*ns*
LDL Cholesterol (mmol/L)	2.5	2.8	*ns*
HDL Cholesterol (mmol/L)	1.2	0.9	*ns*
TG (mmol/L)	1.1	1.8	*ns*
CRP (mg/L)	43	72	*p<0.001*
Peak Troponin	1817	639	*p<0.001*
Symptom onset to admission>4 days (n;%)	6;100	6;100	*ns*
Time from symptom to blood sample >1 day (n;%)	5;83	2;33	*ns*
Co-morbidities			
Hypertension (n;%)	3;50	2;33	*ns*
Diabetes (n;%)	0;0	1;17	*ns*
GFR>60ml/min (n;%)	6;0	3;50	*ns*
Previous MI	1;17	1;17	*ns*
Previous PCI	2;33	1;17	*ns*