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Clustering of 37 circulating biomarkers by exploratory factor analysis in patients following complicated acute myocardial infarction

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ABSTRACT

Background: The objective of this study was to elucidate the complex interactions between families of circulating biomarkers representing different biochemical responses to the pathophysiology following complicated acute myocardial infarction (AMI).

Methods: Blood samples, drawn at a median of 3 days post AMI were obtained from 236 patients with complicated AMI and evidence of heart failure or left ventricular dysfunction. Using exploratory factor analysis, 37 biomarkers were grouped according to their collinearity to each other into clusters. The clusters were used as a model to elucidate interdependencies between individual biomarkers. Each cluster defines a specific pathophysiological process, called factor. These factors were used as covariates in multivariable Cox-proportional hazard regression analyses for prediction of all-cause death and the combined endpoint of cardiovascular death and re-infarction.

Results: Exploratory factor analysis grouped the biomarkers under 5 factors. The composition of these groups was partially unexpected but biological plausible. In multivariable analysis, only 1 factor proved to be an independent predictor of outcome. Major contributions (factor loadings > 0.50) in this cluster came from: mid-regional pro-adrenomedullin, tumor necrosis factor receptor, pro-endothelin-1, growth differentiation factor 15, C-terminal pro arginine vasopressin, uric acid, chromogranin A and procollagen type III N-terminal.

Conclusion: Clustering of multiple biomarkers by exploratory factor analysis might prove useful in exploring the biological interactions between different biomarkers in cardiovascular disease and thus increase our understanding of the complicated orchestral interplay at the molecular level.

1. Introduction

Despite improvements in the treatment of complicated acute myocardial infarction (AMI), cardiovascular mortality and morbidity remain high. Numerous publications have identified a large number of potential prognostic biomarkers as independent risk predictors. Within a prospective biomarker substudy of the Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan (OPTIMAAL), the value of several promising biomarkers to predict future major cardiovascular (CV) events in patients with AMI complicated with heart failure (HF) during hospitalization has previously been reported [1–10]. However, our knowledge about the interrelationships between these markers is limited. A method that groups these markers into biologically meaningful clusters could help to elucidate the underlying mechanisms of collinearity. This approach may also assist in identifying a "biomarker superfamily" representing a novel approach to risk stratify these patients. The hypothesis, that a multimarker approach, measuring circulating levels of several vascular-related markers could reveal a "signature of disease" that can serve a highly accurate method to assess for the risk of future CV events, is currently receiving much attention [11,12]. Exploratory factor analysis represents a unique statistical method that can be employed to eliminate multicollinearity and identify clusters of independent prognostic biomarkers [13,14].

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In the present study we used exploratory factor analysis to assess the independencies of 37 prospectively assayed circulating biomarkers of potential predictive value, measured in the subacute phase following complicated AMI.

2. Materials and methods

2.1. Study design, patient selection, and biomarkers

The design and main results of the OPTIMAAL trial have been reported [15,16]. In brief, 5477 patients, 50 years or older, with AMI complicated with heart HF during the acute phase were randomly assigned to a target dose of losartan (50 mg OD) or captopril (50 mg TID) as tolerated. HF was suggested by: an ejection fraction (EF) of less than 35% or a left-ventricular (LV) end-diastolic dimension of greater than 65 mm (optional) and/or a new Q-wave anterior-wall AMI, or any reinfarction with previous pathological Q-waves in the anterior wall and/or HF symptoms by one or more of the following: treatment with diuretic or intravenous vasodilator therapy for HF, pulmonary rales, third heart sound, persistent sinus tachycardia (≥100 beats/min), or radiographic evidence of pulmonary congestion. Patients were enrolled within 10 days of the onset of symptoms.

The biomarker substudy comprised 236 patients consecutively included at 6 centers from Norway, Sweden and the United Kingdom, and was designed to analyze several biomarkers for the prediction of outcome at study entry (abbreviations are listed in Table 1). Blood samples were taken prior to initiation of study treatment (captopril or losartan). The samples were therefore not influences by differences in study treatment. Furthermore, there were no differences between the two treatment groups with regard to outcomes, neither in the main trial nor in the substudy. Therefore, all patients from the biomarker substudy were therefore considered a single group. The substudy was approved by the regional ethical committees. All patients provided written informed consent.

2.2. Blood sampling

Blood was drawn in the recumbent position from an antecubital vein using a 21-gauge needle after the patient had rested for 5 min. The samples were centrifuged at 3000 rpm for 10 min. Serum and plasma samples were stored in multiple aliquots at −72 °C until assay. No samples were thawed more than twice.

2.3. Laboratory analysis

The details describing the biomarker assays are provided in the Appendix.

The estimated glomerular filtration rate (eGFR) was calculated using the abbreviated modification of diet in renal disease (MDRD) equation: $186 \times (\text{Creat}/88.4) - 1.154 \times \text{Age} - 0.203$ ($\times 0.742$ if female).

2.4. Statistical analysis

Continuous baseline variables are presented as median with their interquartile range. The Kolmogorov–Smirnov test showed that the biomarkers eligible for factor analysis were not normally distributed. Therefore, all biomarkers were log-transformed before factor analysis. Univariate associations between the log-transformed biomarkers were assessed by a Pearson correlation coefficient matrix.

Exploratory factor analysis is a statistical grouping technique, which is widely used in social sciences but can be applied as an approach to analyze multiple biomarkers [17,18]. This method combines variables that are collinear into clusters. This reduces the data set to a more manageable size while retaining as much as possible of the original information of the predictor variables. By factor analysis, those variables causing multicollinearity combine to describe a factor. A factor can be imagined as one axis of a multidimensional graph, ranging from −1 to 1, being the outer limits of a correlation coefficient. Each axis (i.e. factor) determines a specific characteristic of the dataset. The co-ordinates of variables along each axis represent the strength of relationship between that variable and each factor. This relationship is called factor loading and can be thought of as the Pearson correlation coefficient between the factor and the variable. Thus, squaring factor loadings reveals an estimate of the variance of the factor explained by the variable. The resulting factors have no collinearity to each other and factor scores can be used as predictor variables in multiple regression analysis. Factor scores are the sample populations' individual scores on a factor, based on each subjects' impact to the constituent variables.

Factor analysis was performed as follows: as a preparatory analysis an anti-image correlation matrix was created and each variable was tested by measures of sampling adequacy (MSA). Variables with a MSA-value of <0.5 were dropped from further analysis as they

Table 1
Biomarkers included into exploratory factor analysis.

Biomarker	Abbreviation	Unit	Mean	SD	Median	IQR
Aldosterone	Aldo	pmol/L	405.73	861.99	178.01	65.01–384.88
Asymmetric dimethylarginine	ADMA	μmol/L	0.60	0.14	0.60	0.51–0.70
Chromogranin A	CGA	ng/mL	29.22	18.43	24.00	19.00–32.00
C-terminal natriuretic peptide	CNP	pmol/L	3.05	2.69	2.31	1.25–3.93
C-terminal pro-endothelin-1	CT-pro-ET-1	pmol/L	99.02	41.65	87.25	72.00–117.00
C-terminal provasopressin (copeptin)	CT-proAVP	pmol/L	23.34	28.30	13.80	8.20–26.45
C-terminal telopeptide of type I collagen	ICTP	μg/L	4.93	2.67	4.39	3.41–5.60
Erythropoetin	Epo	mIU/mL	19.49	20.55	15.40	10.30–21.40
Ferritin	Ferritin	ng/mL	255.38	241.02	176.80	120.55–312.60
Fms like tyrosine kinase 1	sFLT	pg/mL	183.24	465.59	56.50	32.58–127.52
Growth differentiation factor 15	GDF-15	ng/L	2855.59	1745.85	2340.00	1743.50–3465.50
High sensitive C-reactive protein	hs-CRP	mg/L	69.69	65.10	50.00	22.00–98.25
Interleukin 10	IL-10	pg/mL	4.35	7.43	1.61	0.57–4.87
Interleukin 18	IL-18	pg/mL	302.61	172.91	260.95	195.13–364.08
Interleukin 6	IL-6	pg/mL	22.60	13.56	21.70	10.98–30.85
Iron	FE	μg/dL	47.40	30.82	40.00	26.00–65.25
Matrix metalloproteinase 1	MMP-1	ng/mL	6.45	6.59	4.70	2.75–7.85
Matrix metalloproteinase 9	MMP-9	ng/mL	421.20	436.25	308.54	134.10–516.37
Mid-regional pro-adrenomedullin	MR-proADM	nmol/L	0.93	0.48	0.83	0.64–1.02
Mid-regional pro-atrial natriuretic peptide	MR-proANP	pmol/L	4.90	0.64	4.88	4.43–5.29
Monocyte chemoattractant protein-1	MCP-1	pg/mL	419.93	398.96	317.50	236.00–505.75
Noradrenaline	NA	ng/mL	6.10	17.99	1.85	0.24–5.51
N-terminal atrial natriuretic peptide	NT-ANP	pmol/L	753.81	692.15	561.92	367.76–877.07
N-terminal pro b-type natriuretic peptide	NT-proBNP	pmol/L	1360.63	724.62	1229.06	754.16–1988.13
Osteoprotegrin	OPG	ng/mL	3.23	1.36	3.03	2.14–4.11
Placenta growth factor	PLGF	ng/L	24.01	19.99	17.73	11.65–29.30
Plasma renin activity	PRA	ng/mL	2.88	3.81	1.51	0.60–3.42
Procollagen type I N-terminal	PINP	μg/L	32.15	13.26	30.01	23.05–38.23
Procollagen type III N-terminal	PIIINP	μg/L	3.63	1.21	3.32	2.84–4.33
Soluble CD40 Ligand	CD40L	ng/mL	6.08	6.02	3.63	1.32–11.32
Soluble transferrin receptor	sTFR	mg/L	1.28	0.40	1.22	1.02–1.46
Soluble tumor necrosis factor receptor 1	sTNFR1	pg/mL	2298.54	1313.90	1983.00	1484.00–2716.00
Tissue inhibitor of metalloproteinases 1	TIMP-1	ng/mL	1609.50	622.59	1492.00	1195.05–1833.50
Total iron binding capacity	TIBC	μg/dL	335.88	58.45	340.00	288.00–381.25
Transferrin	TF	mg/dL	215.58	50.54	213.00	178.00–251.00
Uric acid	UA	mg/dL	330.33	99.71	314.00	258.00–382.50
Vascular endothelial growth factor	VEGF	ng/mL	549.43	1568.34	256.02	104.75–467.28