ESC European Society of Cardiology

European Heart Journal (2017) **0**, 1–10
doi:10.1093/eurheartj/ehx640

FASTTRACK CLINICAL RESEARCH
Acute Coronary Syndromes

Cysteine-rich angiogenic inducer 61 (Cyr61): a novel soluble biomarker of acute myocardial injury improves risk stratification after acute coronary syndromes

Roland Klingenberg[1,2,3], Soheila Aghlmandi[4,5,6], Christoph Liebetrau[2,3], Lorenz Räber[7], Baris Gencer[8], David Nanchen[9], David Carballo[8], Alexander Akhmedov[1], Fabrizio Montecucco[8,10], Stefan Zoller[11], Chad Brokopp[12], Dik Heg[4,5], Peter Jüni[13], Helena Marti Soler[14], Pedro-Manuel Marques-Vidal[14], Peter Vollenweider[14], Oliver Dörr[15], Nicolas Rodondi[16,17], François Mach[8], Stephan Windecker[7], Ulf Landmesser[1,18], Arnold von Eckardstein[19], Christian W. Hamm[2,3,15], Christian M. Matter[1], and Thomas F. Lüscher[1]*

[1]Department of Cardiology, University Heart Center, University Hospital of Zurich and Center for Molecular Cardiology, University of Zurich, Rämistr. 100, CH-8091 Zurich, Switzerland and Wagistr. 12, CH-8952 Schlieren, Switzerland; [2]Department of Cardiology, Kerckhoff Heart and Thorax Center, Kerckhoff-Klinik, Benekestr. 2-8, D-61231 Bad Nauheim, Germany; [3]DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main, Benekestr. 2-8, D-61231 Bad Nauheim, Germany; [4]Institute of Social and Preventive Medicine (ISPM), University of Bern, Finkenhubelweg 11, CH-3012 Bern, Switzerland; [5]CTU Bern, University of Bern, Finkenhubelweg 11, CH-3012 Bern, Switzerland; [6]Institute for Clinical Epidemiology and Biostatistics, University Hospital of Basel, Spitalstr. 12, CH-4056 Basel, Switzerland; [7]Department of Cardiology, Cardiovascular Center, University Hospital of Bern, Freiburgstr. 18, CH-3010 Bern, Switzerland; [8]Department of Cardiology, Cardiovascular Center, University Hospital of Geneva, Rue Gabrielle-Perret-Gentil 4, CH-1211 Geneva 14, Switzerland; [9]Department of Ambulatory Care and Community Medicine, University of Lausanne, Rue du Bugnon 44, CH-1011 Lausanne, Switzerland; [10]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 6, Viale Benedetto XV, IT-16132 Genoa, Italy; [11]Bioinformatics, Genetic Diversity Center, Federal Institute of Technology (ETH), Universitätsstr. 16, CH-8092 Zurich, Switzerland; [12]Department of Cardiothoracic Surgery, Regenerative Medicine Center, Department of Cardiothoracic Surgery, University Hospital of Zurich, Wagistr. 12, CH-8952 Schlieren, Switzerland; [13]Applied Health Research Centre (AHRC), Li Ka Shing Knowledge Institute of St. Michael's Hospital, University of Toronto, 209 Victoria St, Toronto, ON M5B 1T8, Canada; [14]Department of General Internal Medicine, University Hospital of Lausanne, Rue du Bugnon 46, CH-1011 Lausanne, Switzerland; [15]Department of Cardiology, University Hospital of Giessen, Klinikstr. 33; D-35392 Giessen, Germany; [16]Institute of Primary Health Care (BIHAM), University of Bern, Gesellschaftsstr. 49, CH-3012 Bern, Switzerland; [17]Department of General Internal Medicine, University Hospital of Bern, Freiburgstr. 18, CH-3010 Bern, Switzerland; [18]Department of Cardiology, Charité Campus Benjamin-Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany; and [19]Institute of Clinical Chemistry, University Hospital of Zurich, Rämistr. 100, CH-8091 Zurich, Switzerland

Received 8 August 2017; revised 27 September 2017; editorial decision 19 October 2017; accepted 19 October 2017

Aims We aimed to identify a novel biomarker involved in the early events leading to an acute coronary syndrome (ACS) and evaluate its role in diagnosis and risk stratification.

Methods and results Biomarker identification was based on gene expression profiling. In coronary thrombi of ACS patients, cysteine-rich angiogenic inducer 61 (Cyr61, CCN1) gene transcripts were highly up-regulated compared with peripheral mononuclear cells. In a murine ischaemia–reperfusion model (I/R), myocardial Cyr61 expression was markedly increased compared with the controls. Cyr61 levels were determined in human serum using an enzyme-linked immunosorbent assay. Cohorts of ACS ($n = 2168$) referred for coronary angiography, stable coronary artery disease (CAD) ($n = 53$), and hypertrophic obstructive cardiomyopathy (HOCM) patients ($n = 15$) served to identify and evaluate the diagnostic and prognostic performance of the biomarker. Cyr61 was markedly elevated in ST-elevation myocardial infarction patients compared with non-ST-elevation myocardial infarction/unstable angina or stable CAD patients, irrespective of whether coronary thrombi were present. Cyr61 was rapidly released after occlusion of a septal branch in HOCM patients undergoing transcoronary ablation of septal hypertrophy. Cyr61 improved risk stratification for all-cause mortality when added to the reference GRACE risk score at 30 days (C-statistic 0.88 to

* Corresponding author.Tel: +41 44 635 6468, Fax: +41 44 635 6827, Email: cardio@tomluescher.ch

0.89, $P = 0.001$) and 1 year (C-statistic 0.77 to 0.80, $P < 0.001$) comparable to high-sensitivity troponin T (30 days: 0.88 to 0.89, $P < 0.001$; 1 year: 0.77 to 0.79, $P < 0.001$). Similar results were obtained for the composite endpoint of all-cause mortality or myocardial infarction. Conversely, in a population-based case–control cohort ($n = 362$), Cyr61 was not associated with adverse outcome.

Conclusion Cyr61 is a novel early biomarker reflecting myocardial injury that improves risk stratification in ACS patients.

Keywords Acute coronary syndromes • Risk stratification • Biomarker

Introduction

In patients with acute chest pain, early diagnosis of an acute myocardial infarction (MI)[1–4] and optimal risk stratification[5,6] depend on cardiac troponin levels measured by high-sensitivity assays in addition to clinical assessment and electrocardiogram (ECG).[7,8] In spite of the progress made in the management of acute coronary syndrome (ACS) patients, the diagnosis of MI remains challenging in patients presenting with symptoms of unstable angina (UA) and a substantial residual risk of mortality and morbidity remains,[9] warranting novel pathophysiological insights into the early events at the onset of ACS. Triggers of acute coronary thrombotic occlusion followed by myocardial ischaemia and necrosis are key factors in the development of ACS. Thus, a soluble biomarker reflecting early events before myocardial necrosis occurs would provide an incremental tool towards the timely diagnosis of this high-risk patient population, thereby helping towards improving their prognosis.

Cysteine-rich angiogenic inducer 61 (Cyr61, CCN1) is a member of the CCN family of matricellular proteins exerting critical functions in angiogenesis, inflammation, and fibrotic tissue repair[10–14] and serves as a ligand for activated platelets binding to integrin $a_{IIb}ß_3$.[15] Cyr61 is an immediate early gene product expressed in response to a variety of stimuli such as mechanical stretch/shear stress,[16] hypoxia,[17,18] tumour necrosis factor-α,[19,20] transforming growth factor-β,[17,19,21] and platelet-derived growth factor.[21]

Cyr61 is involved in angiogenesis and cardiovascular disease. Indeed, Cyr61 knockout mice undergo embryonic lethality due to impaired vascular integrity during placental development and atrioventricular septal defects.[22,23] Cyr61 mediates endothelial proliferation, tubule formation, and neovascularization in an integrin-dependent manner[24,25] and promotes recruitment of $CD34^+$ progenitor cells to the endothelium.[26] *In vitro* studies show that Cyr61 mediates monocyte adhesion and activation of a pro-inflammatory gene profile consistent with M1-type macrophages that are involved in the early phase of ACS.[27,28] In a mouse model of autoimmune myocarditis, Cyr61 modulated recruitment of monocytes and lymphocytes to the inflamed myocardium.[29] Inhibition of Cyr61 in experimental models of carotid balloon injury decreased proliferation of vascular smooth muscle cells and in turn intimal hyperplasia.[30,31] In humans, increased expression of Cyr61 was detected in areas of atherosclerotic lesions rich in vascular smooth muscle cells and in cardiomyocytes of patients with ischaemic cardiomyopathy.[32,33] The diagnostic and prognostic value of Cyr61 as a biomarker in ACS patients remains unknown.

In this study, we hypothesized that gene expression profiling in coronary thrombi and peripheral blood mononuclear cells obtained from ACS patients would identify candidate gene transcripts central to the pathophysiology of acute coronary thrombotic occlusion and/or myocardial injury. After identification of transcripts of the secreted protein cysteine-rich angiogenic inducer 61 (Cyr61, CCN1) in coronary thrombi of ACS patients and myocardium in a mouse model of ischaemia–reperfusion, we evaluated its diagnostic and prognostic value in ACS patients, patients with hypertrophic obstructive cardiomyopathy (HOCM) after transcoronary ablation of septal hypertrophy and in a primary prevention cohort.

Methods

Patient characteristics and tissue/blood sampling

Patients with an acute coronary syndrome to assess diagnostic and prognostic performance

The SPUM-ACS cohort consists of consecutive patients referred for coronary angiography with the main diagnosis of ACS to one of the participating university hospitals (Bern, Geneva, Lausanne, and Zurich; all in Switzerland) enrolled in the Special Programme University Medicine Acute Coronary Syndromes and Inflammation (SPUM ACS; NCT01000701).[34–39] Inclusion criteria comprised all females and males aged 18 years and older presenting within 5 days (preferably within 72 h) after pain onset with the main diagnosis of ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), or UA. Included patients had symptoms compatible with angina pectoris (chest pain and dyspnoea) and fulfilled at least one of the following criteria: (i) persistent ST-segment elevation or depression, T-inversion, or dynamic ECG changes, new left bundle branch block (LBBB); (ii) evidence of positive troponin by local laboratory reference values (with a rise and/or fall in serial troponin levels); (iii) known coronary artery disease, specified as status after myocardial infarction, coronary artery bypass grafting (CABG), or percutaneous coronary intervention (PCI) or newly documented ≥50% stenosis of an epicardial coronary artery during the initial catheterization. Exclusion criteria comprised severe physical disability, inability to comprehend study or <1 year of life expectancy (for non-cardiac reasons). Clinical and angiographic data (presence of coronary thrombus[39] documented on *a priori* defined forms) were entered on electronic case report forms and stored in a web-based electronic database. Follow-up was performed at 30 days (phone call) and 1 year (clinical visit) with events adjudicated by three independent experts using pre-specified adjudication forms.

Patients with stable coronary disease as a reference cohort

Patients referred for coronary angiography to the University Hospital Zurich with known stable coronary artery disease (sCAD) were enrolled

in the sCAD arm of the SPUM-ACS study. All patients aged 18 years and older and angiographically documented coronary artery stenosis >50% were eligible. Exclusion criteria comprised an ACS within the preceding 6 months, systemic infectious, inflammatory or autoimmune disease, known severe renal dysfunction (serum creatinine >220 µmol/L), known severe hepatic dysfunction (3× upper limit of normal for liver function tests), neoplasm or other life-threatening disease with a life expectancy <1 year, extended surgery in the preceding 3 months and/or evidence of valvular or structural heart disease and/or a reduced left ventricular ejection fraction (<55%) on echocardiogram or left ventricular angiogram.

Patients in the SPUM-ACS and sCAD cohorts recruited between December 2009 and December 2012 had blood drawn from the inguinal arterial sheath at coronary angiography prior to primary percutaneous coronary intervention (pPCI). Blood was collected in serum tubes centrifuged, aliquoted, and stored at -80 °C. In selected ACS patients, coronary thrombus was aspirated from the site of coronary occlusion undergoing pPCI. Thrombus was immediately immersed in phosphate-buffered saline-containing vials. Peripheral blood mononuclear cells were isolated by Ficoll-gradient centrifugation to generate a peripheral blood mononuclear cell (PBMC) pellet, one half of which was immediately snap-frozen at -80 °C for gene expression analysis, the remainder was used for flow cytometry or immunohistochemical analysis as explained in the Supplementary material online.

Nested case–control study in the CoLaus cohort to compare Cyr61 levels between cases and controls

The Cohorte Lausannoise (CoLaus) is a single-centre, epidemiological study including approximately 6000 individuals recruited between June 2003 and May 2006 in the city of Lausanne and followed up over the ensuing years. Details of the CoLaus study have been described previously.[40] Inclusion criteria included age between 35 and 75 years and willingness to participate in the medical examination and to donate blood samples. Collected variables include, but are not restricted to, demographic data, family history, cardiovascular risk factors, and clinical data. Venous blood was collected at the initial visit, and serum aliquots were frozen at -80 °C until measurement. We nested a case–control study with 75 cases defined as individuals who died (all-cause mortality) or experienced an MI during follow-up and 287 controls.

All individuals enrolled in the different cohorts provided written informed consent in compliance with the Declaration of Helsinki, and all studies were approved by the local research ethics committees.

Biomarker measurement and analysis

Concentrations of Cyr61 were measured in duplicates of single serum aliquots blinded to the patient's data by means of numbered ID codes using a semi-automated solid phase enzyme-linked immunosorbent assay (EIA-5108, DRG Instruments GmbH, Marburg, Germany) in SPUM-ACS and sCAD patients. The inter- and intra-assay coefficients of variation in SPUM-ACS patients were 3.22% and 3.47%, respectively. Troponin T was measured in serum aliquots using a high-sensitivity assay (hsTnT) from the SPUM-ACS cohort using electrochemiluminescence immunoassays analysed on a cobas e 602 reader (all Roche Diagnostics, Mannheim, Germany)[38] with assay characteristics as reported by the manufacturer.

Clinical endpoints and risk score calculation

The primary endpoint was all-cause mortality within 30 days after the index ACS and at 1 year follow-up. The secondary endpoint comprised the composite of all-cause mortality or non-fatal recurrent MI as described.[38] The Global Registry of Acute Coronary Events (GRACE) risk score was used to calculate both, in-hospital,[41] and long-term[42] predictions of mortality and to assess the degree of disease severity in ACS patients included in this study.

Gene expression array

Total RNA of freshly isolated coronary thrombi and corresponding peripheral blood mononuclear cells from 15 ACS patients was isolated using commercially available Qiagen RNeasy® Mini Kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer's instructions. The concentration, purity, and integrity of the isolated total RNA were determined using a NanoDrop® ND1000 (NanoDrop Technologies, Wilmington, DE, USA) and a Bioanalyzer 2100 (Agilent Technologies, Basel, Switzerland). Quantitatively and qualitatively best RNA samples from a total of four patients were used for complementary DNA synthesis following standard protocols and submitted to the local core facility (Functional Genomics Center, University Zurich, Zurich, Switzerland) for processing and analysis (Human Genome U133 Plus 2.0 Affymetrix Gene Chip™ from Affymetrix, Inc. Santa Clara, CA, USA). The microarray expression data were preprocessed with the statistics software R[43] using the RMA method as implemented in the Affymetrix package. Differential expression analysis was performed with the moderated *t*-statistic and false discovery rate (FDR) multiple-test correction methods of the limma package. Transcripts with an FDR ≤0.1 and absolute linear fold change of ≥2 were considered as significantly differentially expressed. The raw microarray expression data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO series accession number GSE19339 [http://www.ncbi.nlm.nih.gov/geo (27 October 2017)].[35] The differentially expressed genes were clustered according to their annotation using the DAVID Bioinformatics Resource to identify significantly enriched functional annotation terms in the categories gene ontology and pathways and each cluster were assigned an arbitrary title based on best fit.[44]

Statistical analyses

Clinical characteristics of each group are presented as means with standard deviations and *P*-values from *t*-tests for continuous variables. Categorical variables were shown as counts with percentages and *P*-values from the χ^2 or the Fisher's exact tests. We compared medians of Cyr61 between different groups of patients using *P*-values from the Wilcoxon rank-sum test and its extension Kruskal–Wallis. We compared the area under the receiver operating characteristics (ROC) curves as a measure of diagnostic accuracy to detect coronary thrombus between Cyr61 and hsTnT. Time-to-first event was analysed throughout, censoring patients at 30 days or 365 days, or at last valid contact date, whichever came first. We used a Cox proportional hazards regression model to evaluate possible associations between the two biomarkers and all-cause mortality and the composite of all-cause mortality or recurrent MI (at 30 days and 1 year follow-up), using continuous, log-transformed biomarker values and continuous GRACE scores. The added predictive ability of the new predictor over and above a reference model was assessed by Harrell's C-statistics calculated from a Cox proportional hazards regression model and integrated discrimination improvement (IDI) index based on logistic model,[45] using the GRACE risk score as reference. The *P*-value of the C-statistics comparing new with reference model was derived from a likelihood ratio test used for the Cox models.

In the nested case–control study, comparison of Cyr61 levels between cases and controls was analysed using linear and logistic regression. Crude and multivariable-adjusted models were applied. Multivariable models were adjusted for gender, age (continuous), smoking (never, former, current), body mass index (continuous), hypertension (yes/no), diabetes (yes/no), LDL cholesterol (continuous), and log-transformed creatinine (continuous) in multivariable models. All analyses were performed using Stata ® version 14.1 (Stata Corp, College Station, TX, USA).

Results

Identification of Cyr61 in coronary thrombi of acute coronary syndrome patients

To assess differentially expressed genes involved in acute coronary thrombotic occlusion, a global gene expression array was performed on messenger RNA isolated from coronary thrombi aspirated from the culprit lesion in the infarct-related coronary artery and PBMCs from individual ACS patients undergoing pPCI. Among candidate gene transcripts with a significant increase in expression in coronary thrombi, Cyr61 was identified as highly differentially expressed (66-fold increase) (*Figure 1*).

Increased protein expression of Cyr61 was found on the surface of T lymphocytes in coronary thrombi compared with PBMC (see Supplementary material online, *Figures S1 and S2*, *Table S1*). In human coronary plaques, Cyr61 expression was localized within lesions rich in $CD68^+$ cells (see Supplementary material online, *Figure S3*).

Cyr61 at clinical presentation with acute coronary syndrome

Cyr61 serum levels were measured in 1592 patients with STEMI, NSTEMI/UA, and stable CAD, respectively (*Figure 2A*). Cyr61 was markedly elevated in patients with STEMI compared with NSTEMI/UA or stable CAD, respectively. Among 2168 ACS patients enrolled in the SPUM-ACS cohort, 1740 had complete data on Cyr61 and hsTnT (see Supplementary material online, *Figure S4*). The clinical characteristics of these patients are shown in *Table 1* and see Supplementary material online, *Tables S2–S4*. When stratified by the onset of chest pain, Cyr61 levels were highest in early presenters (≤6 h) (*Table 2*). In a subset of 1641 patients with data on angiographic presence or absence of coronary thrombus and biomarkers, diagnostic accuracy of Cyr61 was superior to hsTnT for detecting coronary thrombi (*Figure 3*), albeit at a moderate sensitivity and specificity (see Supplementary material online, *Table S5*). Furthermore, Cyr61 levels were significantly higher in 904 patients with STEMI compared with 737 with NSTEMI, irrespective of whether coronary thrombi were present (*Figure 2B*). Based on the latter finding, we analysed myocardial expression of Cyr61 in a mouse model of ischaemia–reperfusion injury (see Supplementary material online, *Figure S5*) and the release kinetics of Cyr61 upon septal branch occlusion during transcoronary ablation of septal hypertrophy in patients with hypertrophic obstructive cardiomyopathy (see Supplementary material online, *Figure S6*, see Supplementary material online, *Tables S6 and S7*).

Prognostic value of Cyr61 in acute coronary syndrome patients

Among 1740 ACS patients enrolled in the SPUM-ACS study with Cyr61 and hsTnT measured, follow-up data were available in 99.1% of patients at 30 days and 95.8% at 1 year, respectively (see Supplementary material online, *Figure S4*, *Table S2* and *Table 1*). All-cause mortality occurred in 33 cases (1.9%) at 30 days, and in a further 73 cases (4.2%) up to 1 year follow-up; the composite of all-cause mortality or MI was ascertained in 59 cases (3.4%) at 30 days and in 130 cases (7.5%) up to 1 year follow-up (see Supplementary material online, *Table S8*).

Cox regression analysis demonstrated a significant and independent association between elevated Cyr61 concentration and all-cause mortality at 30 days and at 1 year follow-up (*Table 3* and see Supplementary material online, *Table S9*). When combined with the reference GRACE risk score (C-statistic 0.77), Cyr61 provided incremental information to predict 1 year all-cause mortality (C-statistic 0.80, $P < 0.001$) comparable to the combination of the GRACE risk score with hsTnT (C-statistic 0.79, $P < 0.001$). Combining both biomarkers with the GRACE risk score provided similar accuracy to predict 1 year all-cause mortality (C-statistic 0.80, $P < 0.001$). Similar results were obtained for the composite endpoint of all-cause mortality or MI at 1 year follow-up (*Table 4*).

Prognostic value of Cyr61 in a population-based case–control cohort

In a prospective case–control cohort of a total 362 individuals from a large prospective cohort (CoLaus) followed up for 5.4 years, we identified 75 cases (21 cardiovascular deaths, 16 other deaths, and 38 MIs) and selected 287 matched controls for the analysis. The concentration of Cyr61 was not statistically different between cases and controls (*Table 5*).

Discussion

Based on gene expression profiling in coronary thrombi and peripheral blood mononuclear cells, we have demonstrated for the first time that (i) Cyr61 expression is markedly increased in coronary thrombi of ACS patients aspirated from the infarct-related coronary artery and in myocardium from mice subjected to ischaemia–reperfusion; (ii) soluble Cyr61 represents a novel early biomarker detecting acute myocardial injury in HOCM patients; (iii) at presentation Cyr61 concentration was the highest in STEMI patients compared with NSTEMI patients, which, in turn, was higher than in stable CAD patients, irrespective of the presence of coronary thrombi; and (iv) soluble Cyr61 improves risk stratification of ACS patients for all-cause mortality and the composite endpoint of all-cause mortality or MI beyond the commonly used clinical GRACE risk score.

Gene expression profiling of coronary thrombi compared with peripheral blood mononuclear cells was used to screen for novel gene transcripts involved at the site of the acute coronary occlusion. We found a markedly increased expression of the matricellular protein Cyr61 in coronary thrombi in ACS patients with a predominant expression on T lymphocytes. In line with these findings, immunohistology studies of atherosclerotic coronary arteries demonstrated increased expression of Cyr61 in the shoulder region of plaques adjacent to regions infiltrated by macrophages. In contrast, human internal mammary arteries free of atherosclerosis did not express the protein. Our findings extend data on the expression of Cyr61 in stable human atherosclerotic plaques[32] and further imply a role of the protein in plaque stability early on during acute coronary occlusion.

Furthermore, in a large all-comer ACS cohort, serum levels of soluble Cyr61 in peripheral blood were markedly increased compared with patients with stable CAD (*Figure 2A*). In patients with angiographic evidence of coronary thrombus, Cyr61 exhibited only

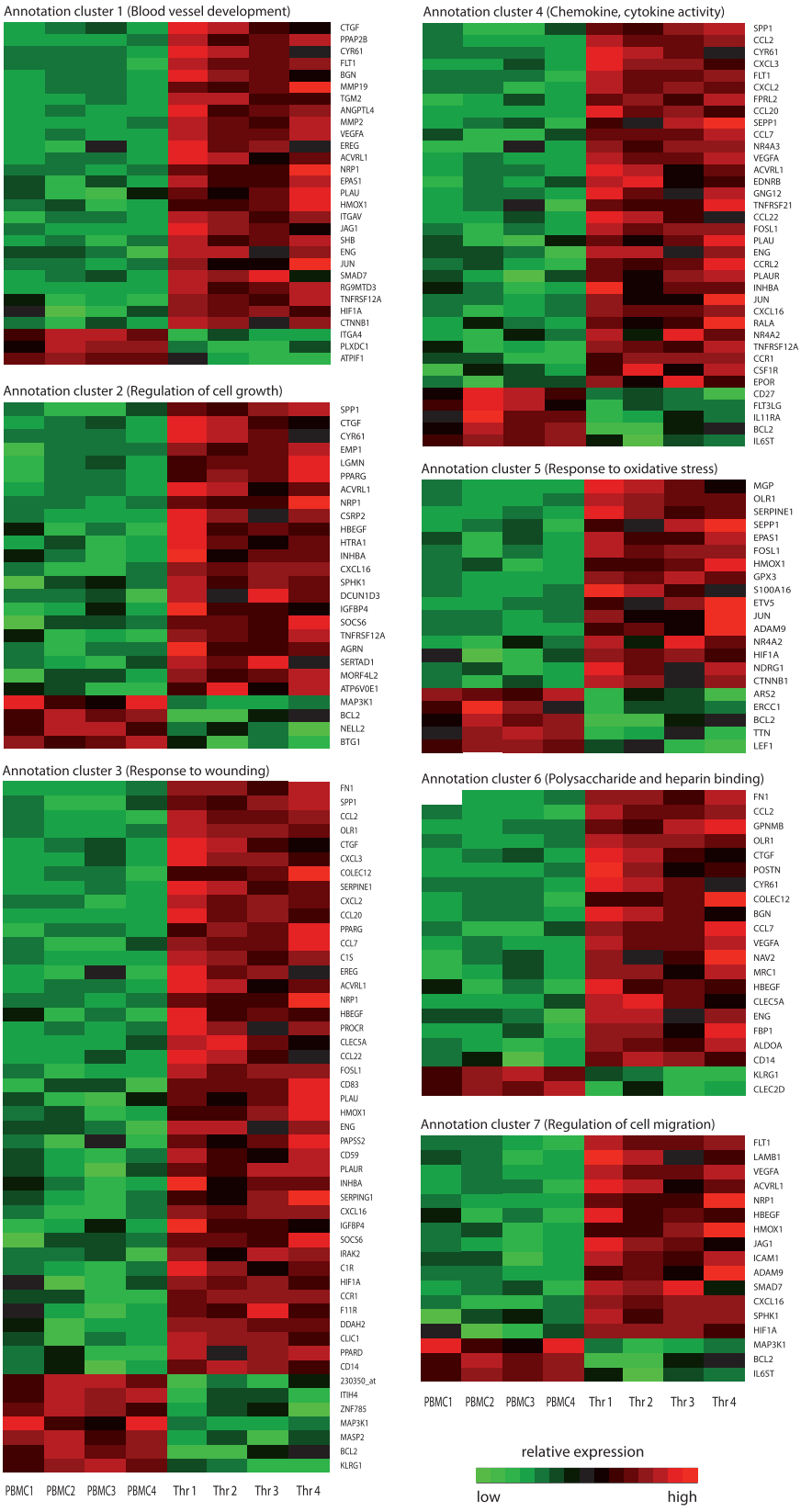


Figure 1 Heat map. Gene expression array analysis of RNA from peripheral blood mononuclear cell and coronary thrombi. The rows correspond to genes and the columns to individual acute coronary syndrome patients. Relative gene expression is shown based on normalization for each gene in the two groups. Sets of genes involved in functional annotation clusters as defined by the DAVID annotation analysis are grouped accordingly. Arbitrary titles that summarize the functional role of displayed genes in a cluster are shown in brackets.

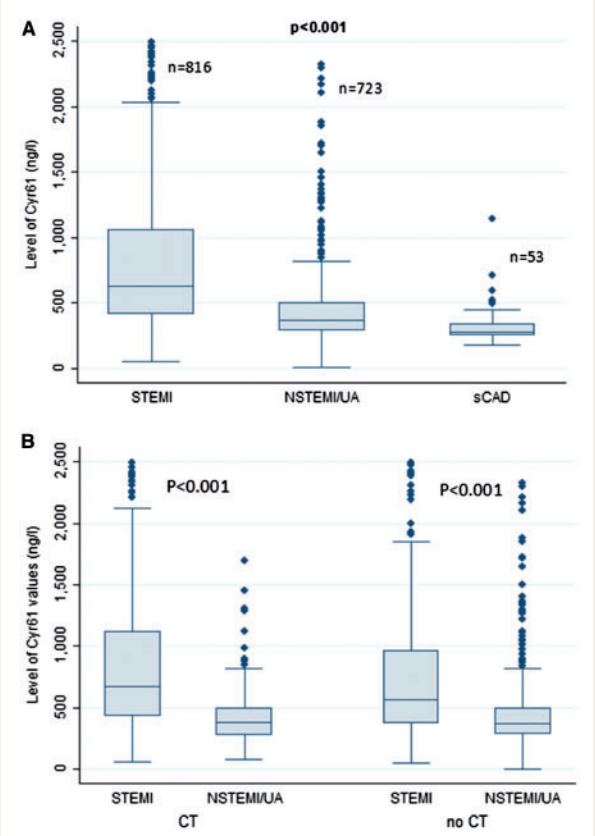


Figure 2 (*A*) Concentration of Cyr61 in different patient groups ($n = 1592$). (*B*) Concentration of Cyr61 in ST-elevation myocardial infarction (STEMI) vs. non-ST-elevation myocardial infarction (NSTEMI)/unstable angina (UA) categorized based on the presence or the absence of clinical thrombus (n=1539). Patients with extreme values for Cyr61 (>2500 ng/L) are excluded from this analysis; *P*-values derived from Wilcoxon rank-sum and Kruskal–Wallis tests. Median comparison of STEMI vs. NSTEMI/UA with the presence or the absence of clinical thrombus shows that concentration of Cyr61 is different between STEMI patients compared with NSTEMI/UA patients.

Table 1 Baseline characteristics of ACS patients ($n = 1740$)

Age (years)	$n = 1740$, 63.80 ± 12.26
Gender (female)	$n = 1740$, 370 (21.3)
Body weight (kg)	$n = 1721$, 80.34 ± 15.21
Body mass index (kg/m^2)	$n = 1719$, 27.17 ± 4.35
Medical history	
Diabetes mellitus	$n = 1740$, 314 (18.0)
Hypertension	$n = 1740$, 1025 (58.9)
Hypercholesterolaemia	$n = 1740$, 1077 (61.9)
Current smoker	$n = 1709$, 679 (39.7)
Family history of CAD	$n = 1719$, 434 (25.2)
Renal failure[a]	$n = 1736$, 221 (12.7)
History of stroke or TIA	$n = 1740$, 64 (3.7)
Previous myocardial infarction	$n = 1738$, 268 (15.4)
Previous PCIs	$n = 1739$, 308 (17.7)
Previous CABG	$n = 1740$, 102 (5.9)
Clinical presentation	
Unstable angina	$n = 1740$, 69 (4.0)
NSTEMI	$n = 1740$, 749 (43.0)
STEMI	$n = 1740$, 922 (53.0)
Index procedure	
PCI	$n = 1740$, 1571 (90.3)
Any drug-eluting stent	$n = 1641$, 1235 (75.3)
Any bare-metal stent	$n = 1641$, 293 (17.9)
PTCA alone	$n = 1641$, 186 (11.3)
CABG	$n = 1641$, 66 (4.0)
Periprocedural medications	
Unfractionated heparin	$n = 1737$, 1664 (95.8)
LMWH	$n = 1740$, 82 (4.7)
Bivalirudin	$n = 1740$, 78 (4.5)
Glycoprotein IIb/IIIa antagonists	$n = 1740$, 448 (25.7)
GRACE risk score	
In-hospital	$n = 1740$, 144.21 ± 33.08
Long term	$n = 1740$, 123.21 ± 26.23

Values are expressed as *n* (%) or means ± standard deviations.
CABG, coronary artery bypass graft; CAD, coronary artery disease; LMWH, low-molecular-weight heparin; PCI, percutaneous coronary interventions; TIA, transient ischaemic attack.
[a]Based on creatinine-estimated glomerular filtration rate clearance of <60 mL/min/1.73m^2, using the modification of diet in renal disease formula.

moderate diagnostic accuracy with an area under the curve of 0.65 which, however, surpassed that of troponin. A limitation is related to the high thrombus burden necessary to enable visual detection of coronary thrombus in epicardial vessels by coronary angiography unlike high-resolution intravascular imaging modalities.[46]

However, irrespective of whether a coronary thrombus was angiographically visible or not, Cyr61 was significantly higher in STEMI patients compared to those with NSTEMI/UA compared with stable CAD patients suggesting that myocardial injury is the primary trigger of Cyr61 release. In line with this interpretation, Cyr61 expression levels were markedly increased in a mouse model of ischaemia–reperfusion injury using coronary ligation and in patients with HOCM undergoing TASH; both conditions that are characterized by severe myocardial injury in the absence of coronary thrombus formation. Interestingly, soluble Cyr61 was rapidly released after septal occlusion in HOCM patients and was detectable prior to troponin, which exhibited a delayed and persistent release within the first 24 h. These data are in support of the concept that the novel biomarker Cyr61 is involved in the initial stages of ACS reflecting myocardial injury. As Cyr61 gene expression is increased in response to hypoxia,[17,18] it may indeed be a biomarker of myocardial ischaemia, but more research is warranted to address this issue in more detail.

One in 6 patients experienced a major adverse cardiovascular event within the first year after an ACS as defined as a composite endpoint of cardiac death, infarction, revascularization, stent thrombosis, or stroke (see Supplementary material online, *Table S8*). However, the identification of those at highest risk remains suboptimal, despite it being of great clinical relevance. Indeed, particularly with the advent of novel and expensive drugs such as PCSK9 inhibitors, optimal identification of high-risk patients is ever more relevant.

Table 2 Concentration of Cyr61(ng/L) in patients with chest pain onset within 24 h ($n = 1094$)

Time of chest pain onset	*n*	Min	Max	Mean	SD	Median	IQR
$0 \le T \le 6h$	671	493	142 830.4	1496.8	5916.5	690.9	985.0
$6h \le T \le 12h$	273	10.2	18 793.3	765.7	1293.9	460.5	378.9
$12h \le T \le 24h$	150	82.4	3340.3	680.05	549	499.0	460.1

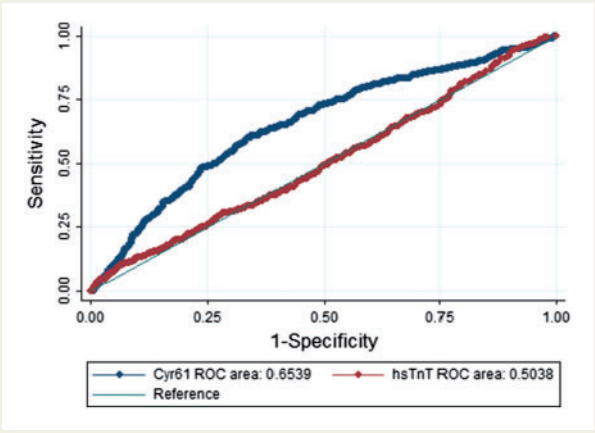
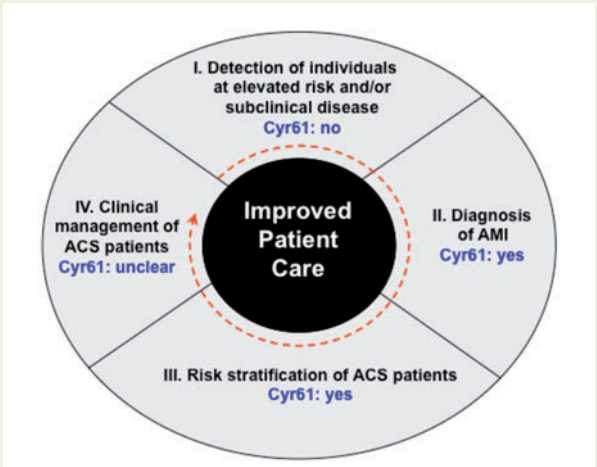


Figure 3 Diagnostic accuracy for coronary thrombus: Cyr61 vs. hsTnT $n = 1641$. Patients with no assessment of clinical thrombus ($n = 97$) are excluded from this analysis. The difference in area under the curve is 0.15 with a 95% confidence interval of 0.149–0.151 ($P < 0.001$).



Summarizing Figure Emerging role of cysteine-rich angiogenic inducer 61 (Cyr61) in ACS. Four major categories serve to characterize the clinical value of a biomarker. Highlighted in blue colour is the result for Cyr61 in each category based on this study, modified from Morrow and de Lemos.[48]

Thus, better prediction of clinical outcome beyond currently available biomarkers and clinical risk scores is an unmet need in the care of patients after an ACS.[9]

Importantly, we here show that Cyr61 improves risk stratification for all-cause mortality and for the composite endpoint of all-cause mortality or MI beyond that provided by the currently used GRACE risk score, which incorporates clinical parameters as well as cardiac troponin. When combined with the GRACE risk score, Cyr61 provided incremental prognostic accuracy comparable to adding hsTnT (*Table 4*). This suggests that clinical information including troponin in the GRACE risk score, as it is routinely used currently does not fully reflect the risk for both, all-cause mortality and myocardial infarction. Interestingly, troponin T concentrations as measured with a high-sensitivity assay were significantly associated with the incidence of cardiovascular death and heart failure but not with myocardial infarction in patients with stable CAD.[47] Thus, it appears that unlike troponin T the novel biomarker Cyr61 can predict death as well as recurrent MI, reflecting a myocardial and an atherothrombotic pathophysiology. A limitation of this study is the rather low number of events in this contemporary cohort warranting validation in other cohorts in the future.

Conversely, in individuals from a population-based case–control cohort followed up for 5.4 years Cyr61 did not predict major adverse cardiovascular events suggesting that myocardial injury is a prerequisite for Cyr61 to provide prognostic information.

Table 3 Multivariable Cox models for continuous GRACE risk score, Cyr61, and, hsTnT ($n = 1740$)

	Hazard ratio	*P*-value
30 days all-cause mortality		
In-hospital GRACE score	1.02 (1.01, 1.03)	<0.001
Cyr61 (ng/L)	1.66 (1.26, 2.19)	<0.001
hsTnT (μg/L)	1.63 (1.28, 2.07)	<0.001
1 year all-cause mortality		
Long-term GRACE score	1.03 (1.02, 1.04)	<0.001
Cyr61 (ng/L)	1.68 (1.38, 2.05)	<0.001
hsTnT (μg/L)	1.28 (1.10, 1.48)	0.001
30 days all-cause mortality or MI		
In-hospital GRACE score	1.02 (1.01, 1.03)	<0.001
Cyr61 (ng/L)	1.47 (1.15, 1.88)	0.002
hsTnT (μg/L)	1.20 (1.02, 1.42)	0.027
1 year all-cause mortality or MI		
Long-term GRACE score	1.02 (1.01, 1.03)	<0.001
Cyr61 (ng/L)	1.45 (1.22, 1.71)	<0.001
hsTnT (μg/L)	1.12 (1.00, 1.24)	0.044

We used continuous GRACE risk scores and HRs are reported per one score unit increase.
For biomarkers, natural logarithm was used and HRs are reported per one log-unit increase.

Table 4 Accuracy of risk prediction using continuous variables ($n = 1740$)

	C-statistic		IDI	
	C-statistic	*P*-value	IDI value	*P*-value
30 days all-cause mortality				
GRACE score using cTnT	0.88	—	Reference	—
GRACE+Cyr61	0.89	0.001	0.024	0.121
GRACE+hsTnT	0.89	<0.001	0.041	<0.001
GRACE+Cyr61+hsTnT	0.90	<0.001	0.070	0.001
1 year all-cause mortality				
GRACE score using cTn	0.77	—	Reference	—
GRACE+Cyr61	0.80	<0.001	0.042	0.010
GRACE+hsTnT	0.79	<0.001	0.050	0.004
GRACE+Cyr61+hsTnT	0.80	<0.001	0.060	0.001
30 days all-cause mortality or MI				
GRACE score using cTn	0.72	—	Reference	—
GRACE+Cyr61	0.72	0.004	0.013	0.049
GRACE+hsTnT	0.72	0.037	0.010	<0.001
GRACE+Cyr61+hsTnT	0.72	0.002	0.025	0.002
1 year all-cause mortality or MI				
GRACE score using cTn	0.68	—	Reference	—
GRACE+Cyr61	0.70	<0.001	0.016	0.051
GRACE+hsTnT	0.68	0.040	0.016	0.043
GRACE+Cyr61+hsTnT	0.70	<0.001	0.022	0.014

We used continuous GRACE risk scores. For biomarkers, a natural logarithmic transformation was used.

Table 5 Univariable and multivariable analyses of the association between Cyr61 and events

	Univariable			Multivariable[a]		
	Controls $n = 287$	Cases $n = 75$	*P*-value	Controls $n = 287$	Cases $n = 75$	*P*-value
Continuous Cyr61 (ng/L)	477.7 ± 130.5	482.5 ± 119.6	0.774	480.6 ± 58.1	479 ± 28.7	0.933
Cyr61 quartiles			0.858			0.494[b]
First	75 (26.1)	16 (21.3)		1 (Reference)		
Second	71 (24.7)	19 (25.3)		0.91 (0.32, 2.57)		
Third	71 (24.7)	20 (26.7)		1.49 (0.56, 3.95)		
Fourth	70 (24.4)	20 (26.7)		1.27 (0.42, 3.85)		

Results are expressed as average ± standard deviation or as a number of participants (%) for the bivariate analysis and as multivariable-adjusted average ± standard error or odds ratio (95% confidence interval) for multivariable analysis. Statistical analysis by χ^2 or logistic regression for categorical data and by analysis of variance for continuous data.
[a]Adjusted for gender, age (continuous), smoking (never, former, current), body mass index (continuous), hypertension (yes/no), diabetes (yes/no), LDL cholesterol (continuous), and log-transformed creatinine (continuous).
[b]*P*-value for trend.

Limitations

In SPUM-ACS, only patients referred for coronary angiography with an established main diagnosis of ACS were included. Thus, the role of Cyr61 in patients presenting with chest pain to the emergency room remains to be elucidated and prospective validation in large well characterized ACS cohorts is necessary. Data in this study are based on a single measurement of Cyr61 at the time of coronary angiography. Future studies will need to address optimal timing of Cyr61 measurement for the diagnosis of myocardial injury and risk stratification. In this study, we cannot provide data on whether Cyr61 remains useful for patients with chronic inflammatory disease or malignancy, warranting further analysis.

Conclusions

Our data highlight Cyr61 as a novel soluble biomarker of acute myocardial injury, providing incremental information beyond currently available tools for risk stratification in ACS patients.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

We appreciate the work of the clinical event committee for SPUM-ACS: Matthias Pfisterer, MD, University of Basel (chair), Tiziano Moccetti, MD, CardioCentro Lugano, Lukas Kappenberger, MD, University of Lausanne, all Switzerland. We are grateful to Alexander Berkowitsch, Kerckhoff Klinik, Bad Nauheim, Germany, for help with statistical analysis of the TASH cohort; Christine Lohmann, University Hospital Zurich for immunohistochemistry studies; and Fabienne Burger, University Hospital Geneva, Switzerland, for assistance with the ischaemia–reperfusion experiments. We thank the local study nurses, the core lab technicians, the central data monitors, the electronic data capturing system (2mt GmbH Ulm, Germany), and the members of the local catheter teams for their invaluable work.

Funding

This work was supported by the Swiss National Science Foundation (SPUM 33CM30-124112 and 32473B_163271); the Swiss Heart Foundation; the Foundation Leducq and the Foundation for Cardiovascular Research – Zurich Heart House, Zurich. The SPUM consortium was also supported by Roche Diagnostics, Rotkreuz, Switzerland (providing the kits for high-sensitivity troponin T), Eli Lilly, Indianapolis (USA), AstraZeneca, Zug; Medtronic, Münchenbuchsee; Merck Sharpe and Dome (MSD), Lucerne; Sanofi-Aventis, Vernier; St. Jude Medical, Zurich (all Switzerland). S.A. is supported by the European Community's Seventh Framework Program FP7/2011: Marie Curie Initial Training Network MEDIASRES ('Novel Statistical Methodology for Diagnostic/Prognostic and Therapeutic Studies and Systematic Reviews'; www.mediasres-itn.eu) with the Grant Agreement Number 290025. The CoLaus study has been supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, and the Swiss National Science Foundation (grants 33CSCO-122661, 33CS30-139468 and 33CS30-148401).

Conflict of interest: none declared pertinent to the current study.

References

1. Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, Biedert S, Schaub N, Buerge C, Potocki M, Noveanu M, Breidthardt T, Twerenbold R, Winkler K, Bingisser R, Mueller C. Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med* 2009;**361**:858–867.
2. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, Bickel C, Baldus S, Warnholtz A, Fröhlich M, Sinning CR, Eleftheriadis MS, Wild PS, Schnabel RB, Lubos E, Jachmann N, Genth-Zotz S, Post F, Nicaud V, Tiret L, Lackner KJ, Münzel TF, Blankenberg S. Sensitive troponin I assay in early diagnosis of acute myocardial infarction. *N Engl J Med* 2009;**361**:868–877.
3. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, Moehring B, Ziller R, Hoeller R, Rubini Gimenez M, Haaf P, Potocki M, Wildi K, Balmelli C, Freese M, Stelzig C, Freidank H, Osswald S, Mueller C. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac Troponin T. *Arch Intern Med* 2012;**172**:1211–1218.
4. Neumann JT, Sorensen NA, Schwemer T, Ojeda F, Bourry R, Sciacca V, Schaefer S, Waldeyer C, Sinning C, Renne T, Than M, Parsonage W, Wildi K, Makarova N, Schnabel RB, Landmesser U, Mueller C, Cullen L, Greenslade J, Zeller T, Blankenberg S, Karakas M, Westermann D. Diagnosis of myocardial infarction using a high-sensitivity Troponin I 1-hour algorithm. *JAMA Cardiol* 2016;**1**:397–404.
5. Mokhtari A, Borna C, Gilje P, Tyden P, Lindahl B, Nilsson HJ, Khoshnood A, Bjork J, Ekelund U. A 1-h combination algorithm allows fast rule-out and rule-in of major adverse cardiac events. *J Am Coll Cardiol* 2016;**67**:1531–1540.
6. Vafaie M, Slagman A, Mockel M, Hamm C, Huber K, Muller C, Vollert JO, Blankenberg S, Katus HA, Liebetrau C, Giannitsis E, Searle J. Prognostic value of undetectable hs Troponin T in suspected acute coronary syndrome. *Am J Med* 2016;**129**:274–282.e2.
7. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S, Baumgartner H, Gaemperli O, Achenbach S, Agewall S, Badimon L, Baigent C, Bueno H, Bugiardini R, Carerj S, Casselman F, Cuisset T, Erol C, Fitzsimons D, Halle M, Hamm C, Hildick SD, Huber K, Iliodromitis E, James S, Lewis BS, Lip GY, Piepoli MF, Richter D, Rosemann T, Sechtem U, Steg PG, Vrints C, Luis ZJ; Management of Acute Coronary Syndromes in Patients Presenting without Persistent STSEotESoC. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2016;**37**:267–315.
8. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2017; doi: 10.1093/eurheartj/ehx393.
9. Fox KA, Carruthers KF, Dunbar DR, Graham C, Manning JR, De Raedt H, Buysschaert I, Lambrechts D, Van de Werf F. Underestimated and under-recognized: the late consequences of acute coronary syndrome (GRACE UK-Belgian Study). *Eur Heart J* 2010;**31**:2755–2764.
10. Jun JI, Lau LF. Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov* 2011;**10**:945–963.
11. Hinkel R, Trenkwalder T, Petersen B, Husada W, Gesenhues F, Lee S, Hannappel E, Bock-Marquette I, Theisen D, Leitner L, Boekstegers P, Cierniewski C, Muller OJ, Le Noble F, Adams RH, Weinl C, Nordheim A, Reichart B, Weber C, Olson E, Posern G, Deindl E, Niemann H, Kupatt C. MRTF-A controls vessel growth and maturation by increasing the expression of CCN1 and CCN2. *Nat Commun* 2014;**5**:3970.
12. Jun JI, Kim KH, Lau LF. The matricellular protein CCN1 mediates neutrophil efferocytosis in cutaneous wound healing. *Nat Commun* 2015;**6**:7386.
13. Jun JI, Lau LF. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat Cell Biol* 2010;**12**:676–685.
14. Meyer K, Hodwin B, Ramanujam D, Engelhardt S, Sarikas A. Essential role for premature senescence of myofibroblasts in myocardial fibrosis. *J Am Coll Cardiol* 2016;**67**:2018–2028.
15. Jedsadayanmata A, Chen CC, Kireeva ML, Lau LF, Lam SC. Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin alpha(IIb)beta(3). *J Biol Chem* 1999;**274**:24321–24327.
16. Thi MM, Iacobas DA, Iacobas S, Spray DC. Fluid shear stress upregulates vascular endothelial growth factor gene expression in osteoblasts. *Ann N Y Acad Sci* 2007;**1117**:73–81.
17. Wolf N, Yang W, Dunk CE, Gashaw I, Lye SJ, Ring T, Schmidt M, Winterhager E, Gellhaus A. Regulation of the matricellular proteins CYR61 (CCN1) and NOV (CCN3) by hypoxia-inducible factor-1{alpha} and transforming-growth factor-{beta}3 in the human trophoblast. *Endocrinology* 2010;**151**:2835–2845.
18. Meyuhas R, Pikarsky E, Tavor E, Klar A, Abramovitch R, Hochman J, Lago TG, Honigman A. A Key role for cyclic AMP-responsive element binding protein in hypoxia-mediated activation of the angiogenesis factor CCN1 (CYR61) in Tumor cells. *Mol Cancer Res* 2008;**6**:1397–1409.
19. Moritani NH, Kubota S, Sugahara T, Takigawa M. Comparable response of ccn1 with ccn2 genes upon arthritis: An in vitro evaluation with a human chondrocytic cell line stimulated by a set of cytokines. *Cell Commun Signal* 2005;**3**:6.
20. Kok SH, Hou KL, Hong CY, Wang JS, Liang PC, Chang CC, Hsiao M, Yang H, Lai EH, Lin SK. Simvastatin inhibits cytokine-stimulated Cyr61 expression in osteoblastic cells: a therapeutic benefit for arthritis. *Arthritis Rheum* 2011;**63**:1010–1020.
21. Sawai K, Mori K, Mukoyama M, Sugawara A, Suganami T, Koshikawa M, Yahata K, Makino H, Nagae T, Fujinaga Y, Yokoi H, Yoshioka T, Yoshimoto A, Tanaka I, Nakao K. Angiogenic protein Cyr61 is expressed by podocytes in anti-Thy-1 glomerulonephritis. *J Am Soc Nephrol* 2003;**14**:1154–1163.
22. Mo FE, Muntean AG, Chen CC, Stolz DB, Watkins SC, Lau LF. CYR61 (CCN1) is essential for placental development and vascular integrity. *Mol Cell Biol* 2002;**22**:8709–8720.
23. Mo FE, Lau LF. The matricellular protein CCN1 is essential for cardiac development. *Circ Res* 2006;**99**:961–969.
24. Babic AM, Kireeva ML, Kolesnikova TV, Lau LF. CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 1998;**95**:6355–6360.

25. Leu SJ, Lam SC, Lau LF. Pro-angiogenic activities of CYR61 (CCN1) mediated through integrins alphavbeta3 and alpha6beta1 in human umbilical vein endothelial cells. *J Biol Chem* 2002;**277**:46248–46255.
26. Grote K, Salguero G, Ballmaier M, Dangers M, Drexler H, Schieffer B. The angiogenic factor CCN1 promotes adhesion and migration of circulating CD34+ progenitor cells: potential role in angiogenesis and endothelial regeneration. *Blood* 2007;**110**:877–885.
27. Schober JM, Chen N, Grzeszkiewicz TM, Jovanovic I, Emeson EE, Ugarova TP, Ye RD, Lau LF, Lam SC. Identification of integrin alpha(M)beta(2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): immediate-early gene products expressed in atherosclerotic lesions. *Blood* 2002;**99**:4457–4465.
28. Bai T, Chen CC, Lau LF. Matricellular protein CCN1 activates a proinflammatory genetic program in murine macrophages. *J Immunol* 2010;**184**:3223–3232.
29. Rother M, Krohn S, Kania G, Vanhoutte D, Eisenreich A, Wang X, Westermann D, Savvatis K, Dannemann N, Skurk C, Hilfiker-Kleiner D, Cathomen T, Fechner H, Rauch U, Schultheiss HP, Heymans S, Eriksson U, Scheibenbogen C, Poller W. Matricellular signaling molecule CCN1 attenuates experimental autoimmune myocarditis by acting as a novel immune cell migration modulator. *Circulation* 2010;**122**:2688–2698.
30. Lee HY, Chung JW, Youn SW, Kim JY, Park KW, Koo BK, Oh BH, Park YB, Chaqour B, Walsh K, Kim HS. Forkhead transcription factor FOXO3a is a negative regulator of angiogenic immediate early gene CYR61, leading to inhibition of vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res* 2007;**100**:372–380.
31. Matsumae H, Yoshida Y, Ono K, Togi K, Inoue K, Furukawa Y, Nakashima Y, Kojima Y, Nobuyoshi M, Kita T, Tanaka M. CCN1 knockdown suppresses neointimal hyperplasia in a rat artery balloon injury model. *Arterioscler Thromb Vasc Biol* 2008;**28**:1077–1083.
32. Hilfiker A, Hilfiker-Kleiner D, Fuchs M, Kaminski K, Lichtenberg A, Rothkotter HJ, Schieffer B, Drexler H. Expression of CYR61, an angiogenic immediate early gene, in arteriosclerosis and its regulation by angiotensin II. *Circulation* 2002;**106**:254–260.
33. Hilfiker-Kleiner D, Kaminski K, Kaminska A, Fuchs M, Klein G, Podewski E, Grote K, Kiian I, Wollert KC, Hilfiker A, Drexler H. Regulation of proangiogenic factor CCN1 in cardiac muscle: impact of ischemia, pressure overload, and neurohumoral activation. *Circulation* 2004;**109**:2227–2233.
34. Klingenberg R, Heg D, Raber L, Carballo D, Nanchen D, Gencer B, Auer R, Jaguszewski M, Stahli BE, Jakob P, Templin C, Stefanini GG, Meier B, Vogt P, Roffi M, Maier W, Landmesser U, Rodondi N, Mach F, Windecker S, Juni P, Luscher TF, Matter CM. Safety profile of prasugrel and clopidogrel in patients with acute coronary syndromes in Switzerland. *Heart* 2015;**101**:854–863.
35. Reiser H, Klingenberg R, Hof D, Cooksley-Decasper S, Fuchs N, Akhmedov A, Zoller S, Marques-Vidal P, Marti Soler H, Heg D, Landmesser U, Rodondi N, Mach F, Windecker S, Vollenweider P, Matter CM, Lüscher TF, von Eckardstein A, Gawinecka J. Circulating FABP4 is a prognostic biomarker in patients with acute coronary syndrome but not in asymptomatic individuals. *Arterioscler Thromb Vasc Biol* 2015;**35**:1872–1879.
36. Gencer B, Montecucco F, Nanchen D, Carbone F, Klingenberg R, Vuilleumier N, Aghlmandi S, Heg D, Raber L, Auer R, Juni P, Windecker S, Luscher TF, Matter CM, Rodondi N, Mach F. Prognostic value of PCSK9 levels in patients with acute coronary syndromes. *Eur Heart J* 2016;**37**:546–553.
37. Nanchen D, Gencer B, Muller O, Auer R, Aghlmandi S, Heg D, Klingenberg R, Raber L, Carballo D, Carballo S, Matter CM, Luescher T, Windecker S, Mach F, Rodondi N. Prognosis of patients with familial hypercholesterolemia after acute coronary syndromes. *Circulation* 2016;**134**:698–709.
38. Klingenberg R, Aghlmandi S, Raber L, Gencer B, Nanchen D, Heg D, Carballo S, Rodondi N, Mach F, Windecker S, Juni P, von Eckardstein A, Matter CM, Luscher TF. Improved risk stratification of patients with acute coronary syndromes using a combination of hsTnT, NT-proBNP and hsCRP with the GRACE score. *Eur Heart J Acute Cardiovasc Care* 2016; doi: 10.1177/2048872616684678.
39. Aghlmandi S, Scharer N, Heg D, Raber L, Zwahlen M, Gencer B, Nanchen D, Carballo D, Carballo S, Juni P, von Eckardstein A, Landmesser U, Rodondi N, Mach F, Windecker S, Matter CM, Luscher TF, Klingenberg R. Thrombus aspiration in acute coronary syndromes: prevalence, procedural success, change in serial troponin T levels and clinical outcomes in a contemporary Swiss cohort. *Eur Heart J Acute Cardiovasc Care* 2017; doi: 10.1177/2048872617706480.
40. Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, Danoff TM, Stirnadel HA, Waterworth D, Mooser V, Waeber G, Vollenweider P. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord* 2008;**8**:6.
41. Granger CB, Goldberg RJ, Dabbous O, Pieper KS, Eagle KA, Cannon CP, Van de WF, Avezum A, Goodman SG, Flather MD, Fox KAA, Eve GRAC. Predictors of hospital mortality in the global registry of acute coronary events. *Arch Intern Med* 2003;**163**:2345–2353.
42. Eagle KA, Lim MJ, Dabbous OH, Pieper KS, Goldberg RJ, Van de Werf F, Goodman SG, Granger CB, Steg PG, Gore JM, Budaj A, Avezum A, Flather MD, Fox KA. A validated prediction model for all forms of acute coronary syndrome: estimating the risk of 6-month postdischarge death in an international registry. *JAMA* 2004;**291**:2727–2733.
43. Team RDC. *A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2008.
44. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol* 2003;**4**:P3.
45. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008;**27**:157–172; discussion 207-12.
46. Tearney GJ, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, Bouma B, Bruining N, Cho J-M, Chowdhary S, Costa MA, de Silva R, Dijkstra J, Di Mario C, Dudeck D, Falk E, Feldman MD, Fitzgerald P, Garcia H, Gonzalo N, Granada JF, Guagliumi G, Holm NR, Honda Y, Ikeno F, Kawasaki M, Kochman J, Koltowski L, Kubo T, Kume T, Kyono H, Lam CCS, Lamouche G, Lee DP, Leon MB, Maehara A, Manfrini O, Mintz GS, Mizuno K, Morel M-A, Nadkarni S, Okura H, Otake H, Pietrasik A, Prati F, Räber L, Radu MD, Rieber J, Riga M, Rollins A, Rosenberg M, Sirbu V, Serruys PWJC, Shimada K, Shinke T, Shite J, Siegel E, Sonada S, Suter M, Takarada S, Tanaka A, Terashima M, Troels T, Uemura S, Ughi GJ, van Beusekom HMM, van der Steen AFW, van Es G-A, van Soest G, Virmani R, Waxman S, Weissman NJ, Weisz G; International Working Group for Intravascular Optical Coherence Tomography. Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for intravascular optical coherence tomography standardization and validation. *J Am Coll Cardiol* 2012;**59**:1058–1072.
47. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E; Prevention of Events with Angiotensin Converting Enzyme Inhibition Trial Investigators. A sensitive cardiac troponin T assay in stable coronary artery disease. *N Engl J Med* 2009;**361**:2538–2547.
48. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation* 2007;**115**:949–952.