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Lipoprotein-associated phospholipase A_2 for early risk stratification in patients with suspected acute coronary syndrome: a multi-marker approach

The North Wuerttemberg and Berlin Infarction Study-II (NOBIS-II)

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Abstract *Aims* Numerous markers have been identified as useful predictors of major adverse cardiac events (MACE) in patients with suspected acute coronary syndrome (ACS). However, only little is known about the relative benefit of the single markers in risk stratification and the best combination for optimising prognostic power.

The aim of the present study was to define the role of the emerging cardiovascular risk marker lipoprotein-associated phospholipase A_2 (Lp-PLA_2) in a multi-marker approach in combination with troponin I (TnI), NT-proBNP, high sensitivity (hs)CRP, and D-dimer in patients with ACS. *Methods and results* A total of 429 consecutive patients (age 60.5±14.1 years, 60.6% male) who were admitted to the emergency room with suspected ACS were analysed in the study. Biochemical markers were measured by immunoassay techniques. All patients underwent point-of-care TnI testing and early coronary angiography if appropriate, in accordance with the current guidelines. Classification and regression trees (CART) and logistic regression techniques were employed to determine the relative predictive power of markers for the primary end-point defined as any of the following events within 42 days after admission: death, non-fatal myocardial infarction, unstable AP requiring admission, admission for decompensated heart failure or shock, percutaneous coronary intervention, coronary artery bypass grafting, life threatening arrhythmias or resuscitation. The incidence of the primary end-point was 13.1%, suggesting a mild to moderate risk population. The best overall risk stratification was obtained using NT-proBNP at a cut-off of 5000 pg/mL (incidence of 40% versus 10.3%, relative risk (RR) 3.9 (95% CI 2.4–6.3)). In the remaining lower risk group with an incidence of 10.3%, further separation was performed using TnI (cut-off 0.14 µg/L; RR = 3.1 (95% CI 1.7–5.5) 23.2% versus 7.5%) and again NT-proBNP (at a cut-off of 140 ng/L) in patients with negative TnI (RR = 3.2 (95% CI 1.3–7.9), 11.7% versus 3.6%). A final significant stratification in patients with moderately elevated NT-proBNP levels was achieved using Lp-PLA_2 at a cut-off of 210 µg/L) (17.9% versus 6.9%; RR = 2.6 (95% CI 1.1–6.6)). None of the clinical or ECG variables of the TIMI (Thrombolysis In Myocardial Infarction) risk score provided comparable clinically relevant information for risk stratification. *Conclusions* In the setting of state-of-the-art coronary care for patients with suspected ACS in the

emergency room, NT-proBNP, troponin I, and Lp-PLA_2 are effective independent markers for risk stratification that proved to be superior to the TIMI risk score. Lp-PLA_2 turned out to be a more effective risk marker than hsCRP in these patients.

Key words acute coronary syndrome – troponin I – NT-proBNP – LP-PLA_2 – emergency room – CART-analysis

Introduction

Evaluation of patients who present to the emergency room (ER) with acute chest pain or other signs or symptoms suggestive of acute coronary syndrome (ACS) is time-consuming, expensive and challenging. Although many patients present with any form of chest pain, in the real life situation of the ER only a minority have definite acute coronary syndrome. Recent investigations have suggested that, in addition to markers of necrosis (cardiac troponins I and T), elevation of biomarkers upstream, such as markers of inflammation and coagulation/fibrinolysis and haemodynamic stress may provide earlier and superior assessment of the overall patient risk and aid in identifying patients at particularly high risk of a major adverse cardiac event (MACE) [1]. Elevated concentrations of CRP and NT-proBNP have been shown to be associated with increased incidence of MACE, independent of cardiac troponins [2]. D-dimer, a marker of fibrinolysis used for the exclusion of pulmonary embolism [3], also has potential in the diagnosis of ACS [4]. Lp-PLA_2 is an enzyme that may represent a more specific indicator of inflammation in the arterial vessel wall [5].

It is well accepted that atherosclerosis presents features of a local and systemic inflammatory response, from its genesis to the occurrence of clinical complications [5]. Oxidative modification of low-density lipoproteins (LDL) represents an initial step in atherogenesis associated with injury of the endothelium, increased adherence of monocytes and T-lymphocytes, and migration of these cells into the subendothelial space [6]. Recently, attention has focused on lipoprotein-associated phospholipase A_2 (Lp-PLA_2) – an enzyme that can promote the oxidation of LDL [7–9]. Lp-PLA_2, a 45.4-kDa protein, is a calcium-independent member of the phospholipase A_2 family and two isoforms of this enzyme (with intracellular and extracellular secretion) have been described [10, 11]. Monocytes, macrophages, T-lymphocytes and mast cells are mainly responsible for the production of this enzyme [12–14]. Furthermore, Lp-PLA_2 has been detected in both human and rabbit atherosclerotic lesions [15]. In the bloodstream, two-thirds of the Lp-PLA_2 plasma isoform circulates primarily bound to LDL; the other third is distributed between high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) [16]. Lp-PLA_2 is able to hydrolyse the *sn*-2 fatty acid of oxidised LDL with subsequent release of lysophosphatidylcholine (lysoPC) and oxidised free fatty acids (oxFFA), both potent pro-inflammatory and pro-atherogenic products [17, 18] and thus may directly promote atherogenesis. Experimental studies in Watanabe heritable hyperlipidemic rabbits have demonstrated that inhibition of Lp-PLA_2 leads to the reduction of atherosclerotic lesion formation [19]. Data from several prospective epidemiologic studies have shown a strong and positive association between elevated concentrations of Lp-PLA_2 in the blood and cardiovascular end-points in initially healthy subjects [20–24] and also in patients with manifest atherosclerosis [25]. However, little is known about the value of Lp-PLA_2 in combination with other parameters.

Additionally, most of the recently published studies on biomarkers are sub-studies of randomised clinical trials which exclude a substantial number of patients routinely seen in the ER.

The present prospective study was based on consecutive, unselected patients and aimed (1) to assess the relative benefit of novel and established markers for risk stratification with respect to the 42-day outcome in the setting of state-of-the-art cardiac care (including point-of-care cardiac troponin testing, use of GP IIb/IIIa inhibitors and acute coronary angiography in high risk patients) and (2) to investigate whether a general hierarchy of these markers can be established using the innovative analytical approach of classification and regression trees (CART) in addition to conventional logistic regression modelling.

Methods

Patients

The study population consisted of 593 consecutive patients presenting with suspected ACS in the ER at two clinical centres (Charité University Hospital Berlin and Stuttgart Community Hospital).

Inclusion criteria were ischemia type discomfort with suspicion of acute coronary syndrome. The inclusion based on the judgement of the attending physician to consider cardiac ischemia as cause of the acute symptoms and therefore a real life ER situ-

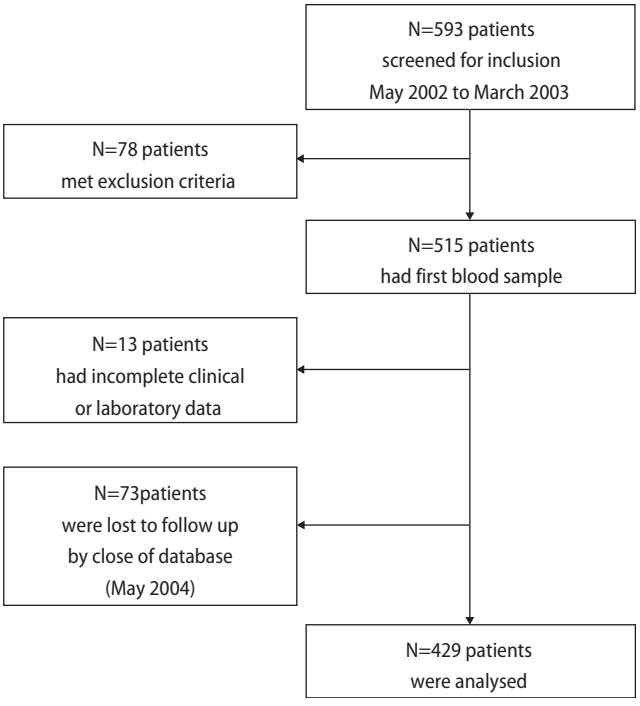

Fig. 1 Flow chart of patients in the two clinical centres of the NOBIS-II study

ation was investigated. Exclusion criteria for the sample were severe anaemia (below 7 g/dL), lack of informed consent and age of less than 18 years. A total of 429 patients with complete data were analysed (Fig. 1). On admission to the ER, all patients underwent a clinical examination, 12-lead ECG and point-of-care (POC) troponin I testing, and treatment was initiated according to current guidelines (American Heart Association, American College of Cardiology). Intravenous acetylsalicylic acid (ASA 500 mg) was given if patients were not on ASA already and unfractionated heparin and β-blockers (metoprolol) were administered unless contraindications were present. Patients with ST-elevation myocardial infarction (STEMI) underwent immediate percutaneous coronary intervention (PCI) or, if applicable, PCI plus stenting with adjunct treatment with the GP-IIB/IIIA-inhibitor tirofiban. Patients with non-STEMI (NSTEMI) also received heparin and tirofiban and underwent PCI within 12 hours. Patients without diagnostic ECG changes and negative troponin on admission had serial ECG recordings, repeat troponin testing and clinical monitoring for at least 6 hours (>8 hours from onset of symptoms) at the discretion of the treating physician. Most of the patients were primarily admitted to the hospital for observation (83.9%) and 68.5% were treated in hospital for more than 24 h. The adherence to the guidelines is crucial with respect to complications and outcome [26]. All patients gave written informed consent and the study protocol had been approved by the ethics committee of the Charité – Universitätsmedizin Berlin (Nr. 87/2002).

Protocol

Blood samples were taken at admission, after 6 hours and, if applicable, after 12–24 hours. LiHeparin was used for the measurement of cardiac troponin I, N-terminal – pro-brain natriuretic peptide (NT-proBNP), and C-reactive protein (CRP). D-dimer, and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) were measured from citrated plasma. Blood samples were centrifuged within 2 hours, stored at –80 °C and later analysed in batches.

Biochemical variables

Cardiac troponin I (cTnI) was measured at point-of-care (POC) using the Stratus® CS (DadeBehring, Germany). The detection limit was 0.01 µg/L and the 99th percentile with a coefficient of variation (CV) below 10% was at 0.1 µg/L in healthy volunteers. NT-proBNP and CRP were measured in the central laboratory of the Charité Berlin in accordance with national standard procedures. NT-proBNP measurement was done by an electrochemiluminescence immunoassay on an Elecsys® 2010 analyser (Roche Diagnostics, Germany). This method has been described in detail recently [27]. The interassay CVs were 6.8% at 0.6 mg/l and 1.1% at 4.6 mg/l for CRP and 3.5% at 84.7 ng/L and 2.3% at 3265 ng/L for NT-proBNP.

D-dimers were measured in batches using the D-Dimer PLUS Assay on Sysmex® CA-1500 by DadeBehring in Marburg, Germany, from deep frozen citrated plasma. The interassay CV was 11.5% using a normal plasma pool and the detection limit was 50 µg/L.

Plasma levels of Lp-PLA_2 were determined by commercial ELISA (second generation PLAC™ Test) supplied by diaDexus, Inc. (South San Francisco, CA, USA) [9] at the Department of Cardiology, University of Ulm, Germany. The Lp-PLA_2 ELISA is a solid phase enzyme-linked immunosorbent assay for the quantitative determination of Lp-PLA_2 in human plasma or serum. This assay employs two high-affinity monoclonal antibodies (mAb) directed against Lp-PLA_2. The first mAb, used for solid-phase immobilisation, had previously been precoated on the microtiter wells. The second monoclonal anti-Lp-PLA_2 antibody was coupled to horseradish peroxidase. Plasma samples were diluted 1:10 prior to analysis. The concentration of Lp-PLA_2 was derived from a standard curve constructed with recombinant Lp-

PLA_2 at concentrations of between 0 and 200 µg/L. The lower detection limit of this assay was approximately 2 µg/L. The inter-assay CV was 9.6%.

Follow up

Follow-up by telephone interview was performed 42 days after the index event with respect to the primary end-point, defined as at least one of the following: death from all causes, rehospitalisation for suspected ACS or congestive heart failure (CHF), acute unplanned PCI, coronary artery bypass grafting (CABG), and documented life-threatening arrhythmias or survived sudden cardiac arrest. In the case of rehospitalisation, a chart review was carried out.

Statistical analysis

Since distributions of continuous variables were skewed, medians and inter-quartile ranges are reported and appropriate non-parametric statistical tests were employed. A two-sided α-level of 5% was used for all tests. Categorical variables are given in percentages together with the 95% confidence intervals (CI).

All biomarkers were analysed with respect to the prediction of the primary endpoint by cross-tabulation and chi-square tests. The cutoffs used were either taken from the CART analysis and logistic regression analysis (see below) or from the literature (CRP, D-dimer).

The relative contribution and hierarchy of potential prognostic variables was evaluated using classification and regression tree (CART) methodology. CART involves repeated subdivisions of a group of subjects on the basis of the choice of optimal cut-off points of binary, ordinal or continuous co-variates that maximise a certain split criterion [28] and has already been successfully applied in various clinical studies [29–31]. In the present analysis, the biochemical variables described above and the other variables of the TIMI risk score [32] were assessed for their relative value in classifying patients into homogeneous and clinically relevant "risk groups" with respect to the incidence of the primary endpoint during follow-up. Additionally, stepwise logistic regression analysis was conducted to assess the same variables used in the CART model.

The main advantage of using CART is the direct and simple interpretation of CART results which represent observed incidences as opposed to the estimated odds ratios (OR) obtained from logistic regression models. This clearly reflects the main difference between the two models: while logistic regression modelling focuses on statistical significance, CART focuses on the impact of the results and therefore on their interpretation.

CART is also highly sensitive to interactions (i.e. combinatory effects) between the factors under study. While, in general terms, logistic regression is also able to identify significant interactions, it can assess these effects only in the whole study group. CART on the other hand is more sensitive and is able to probe for interactions in subgroups.

All calculations were performed using SPSS® V 13.0 statistical software.

Results

The demographic characteristics of all patients with complete follow up (n = 429) are presented in Table 1. No significant differences between these patients and those who had to be excluded from analysis because of incomplete data (n = 86) were seen with respect to age, gender or any of the risk factors assessed (data not shown). Table 1 also shows clinical characteristics and selected laboratory values of the two risk subgroups defined by Lp-PLA_2 concentrations in the CART analysis. In these subgroups, an Lp-PLA_2 of above 210 µg/L is associated with significantly higher prevalence of elevated troponin I ($p = 0.021$), CRP ($p = 0.066$), STEMI ($p > 0.1$), ST-segment depression ($p = 0.034$), and also higher TIMI high risk score ($p > 0.1$) but the number of patients undergoing PCI was similar. However, in the low risk group fewer acute PCI procedures were performed (14.7% versus 29.6% in the high risk group ($p > 0.1$) and 39% in the whole group). Thus, patients with elevated Lp-PLA_2 represented a group at increased risk for MACE.

In our population the final clinical diagnoses at hospital discharge as documented in the patient charts were (sorted by frequency): NSTE-ACS (26.6%), hypertension (20%), non-cardiac diagnoses (11.2%), STEMI (8.9%), extra cardiac chest pain (8.6%), arrhythmias (7.9%), other cardiac conditions (7.0%), CHF (5.1%), asthma/cold (2.1%), pneumonia (1.2%), pulmonary embolism (1.2%) and stable angina (0.2%).

The combined primary endpoint was reached by 56/429 (13.1%) of cases and consisted of the following single components: death (13), rehospitalised ACS (13), rehospitalised heart failure (10), acute unplanned PCI (16) and CABG (4).

The results of the bivariate analysis of biomarkers predicting the primary endpoint are shown in Fig. 2.

Results of the CART analysis are shown in Fig. 3. The overall incidence of the primary end-point

Table 1 Characteristics of patients at baseline in the overall study group and in subgroups defined by Lp-PLA_2 in the CART analysis

	Overall group N=429	Troponin negative NT-proBNP positive Lp-PLA_2 **negative** N=87*	Troponin negative NT-proBNP positive Lp-PLA_2**positive** N=67*
Male [%]	60.6	49.4	49.3
Age [years ± SD]	60.5 ± 14.1	67.6±11.2	66.0±13.8
Troponin I ≥ 0.1 μg/L [%]	23.8	1.1	9.0
CRP ≥ 10 mg/L [%]	25.9	16.1	28.4
ST elevation [%]	8.7	2.2	9.0
3 or more risk** factors [%]	23.8	28.7	19.4
ST depression [%]	11.7	5.7	22.7
Severe angina [%]	52.4	62.1	50.7
Aspirin use within 7 days [%]	38.5	55.6	40.9
TIMI high risk [%]	10.5	6.9	9.0
Prior PCI [%]	28.1	37.6	29.9
Prior CABAG [%]	15.4	21.8	16.4
Risk factors [%]			
Hypertension	67.3	77.0	83.6
HLP	58.3	60.9	56.1
Diabetes	22.0	34.5	22.4
Current smoking	33.5	21.2	18.5
Medication at admission [%]			
ACEI	30.4	45.6	55.6
β-blockers	39.5	67.5	52.4
Nitrates	14.9	18.8	12.1
Diuretics	23.8	29.5	30.2
CSEI	23.4	40.0	21.2
Coronary angiography [%]***	42.3	39.5	40.3
Ejection fraction [%]	59 (45.5/68.0)	59 (53.3/68.0)	63.5 (50/70)
No significant CAD	14.4	12.1	14.8
1-VD	17.7	15.2	22.2
2-VD	18.8	21.2	14.8
3-VD	49.2	51.5	48.1
Acute PCI	39.0	14.7	29.6
Creatinine [mg/dL]	0.9 (0.7/1.1)	0.9 (0.8/1.1)	0.9 (0.7/1.2)
NT-proBNP [ng/L]	224 (77/1015)	394 (246/1096)	451 (233/900)
LpPLA_2 [μg/L]	205 (160/272)	164 (137/185)	278 (240/313)
BMI [kg/m^2]	26.2 (24/30)	27.4 (24/30.1)	25.6 (22.7/28.4)

Numerical variables are medians and (25/75%) percentiles except age (mean ± standard deviation (SD); * Subgroups according to CART analysis (see Fig. 3), NT-proBNP positive denotes > 140 ng/L; CAD, coronary artery disease; ** risk factors included hypertension, hyperlipoproteinemia, diabetes and smoking; *STEMI* ST-elevation myocardial infarction; *HLP* hyperlipoproteinemia; *** numbers related to patients who underwent angiography; *TIMI* Thrombolysis in Myocardial Infarction study group; *VD* vessel disease; *BMI* body mass index

(PEP) was 13.1%, reflecting a mild to moderate risk population. The best overall risk stratification was obtained using NT-proBNP at a cut-off of 5000 pg/mL (relative risk (RR) 3.9 (95% CI 2.4–6.3); 40% versus 10.3%). In the group with a PEP incidence of 10.3%, classification was improved using TnI (cut-off 0.14 μg/L; RR = 3.1 (95% CI 1.7–5.5); 23.2% versus 7.5%) and then NT-proBNP (at a cut-off of 140 ng/L) in patients with negative TnI (RR = 3.2 (95% CI 1.3–7.9); 11.7% versus 3.6%). Final significant improvement in the diagnostic classification of patients with moderately elevated NT-proBNP levels was achieved using Lp-PLA_2 (RR = 2.6 (95% CI 1.1–6.6); 17.9% versus 6.9%; cut-off 210 μg/L). None of the clinical or ECG variables of the TIMI risk score proved to be of comparable significance in risk stratification.

Table 2 presents the results of the multiple logistic regression analysis. The major effects of NT-proBNP and troponin I already seen in the CART analysis are confirmed, although the optimal cut-off for troponin I was slightly lower than in the CART analysis. In contrast to the CART analysis, no further differentiation of subgroups by means of laboratory markers could be detected by the logistic regression analysis but "prior myocardial infarction" was a significant predictor of risk.

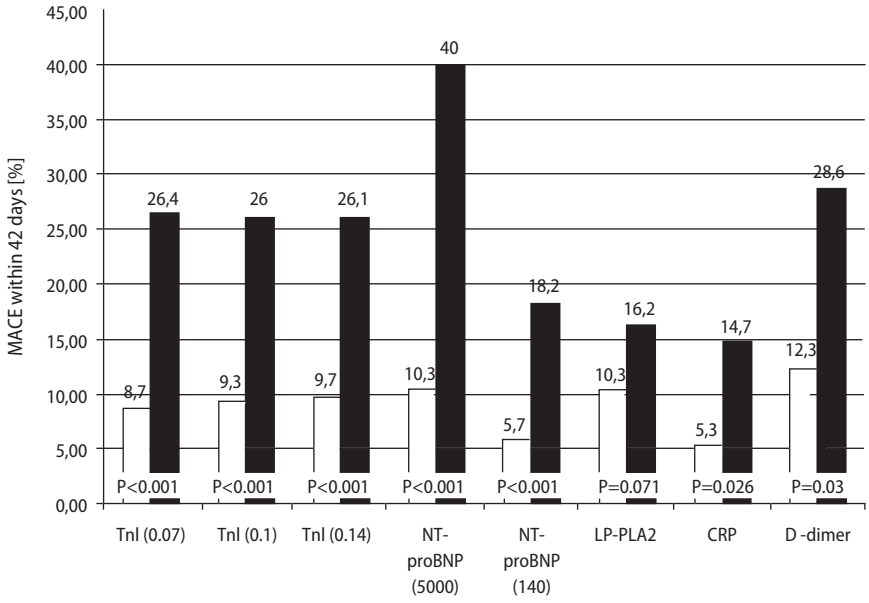

Fig. 2 Bivariate analysis of the prediction of the combined primary endpoint (major adverse cardiac events, MACE) by single biomarkers and the appropriate cutoffs (troponin I, TnI: 0.07, 0.1, 0.14 µg/L; NT-proBNP: 5000, 140 ng/L; LP-PLA_2: 210 µg/L; CRP: 10 mg/L; D-dimer: 500 µg/L). Levels of significance were calculated by the Chi-square test

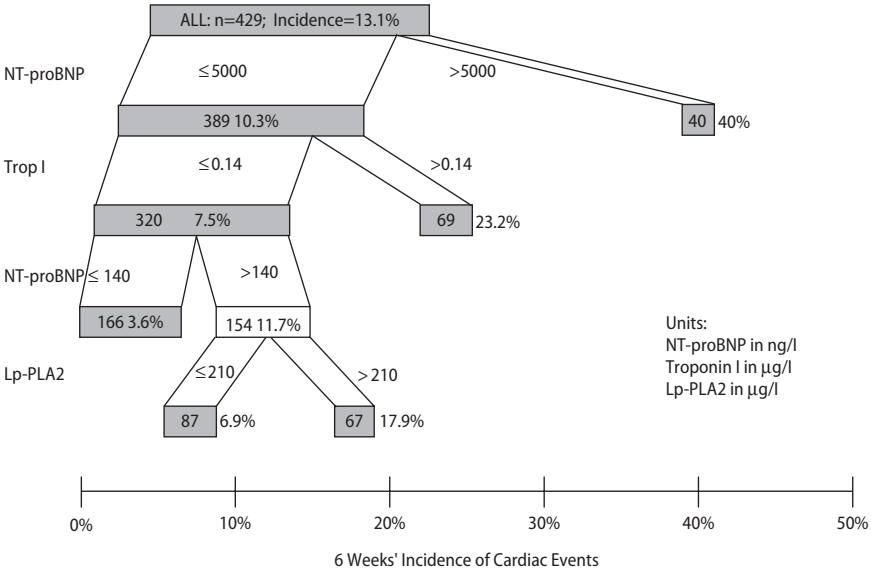

Fig. 3 Classification and regression tree analysis of the biochemical and clinical risk markers with respect to major adverse cardiac events

Table 2 Results of logistic regression analysis of laboratory parameters, gender and TIMI risk items for MACE (overall incidence n = 56; 13.1%)

Factor	ln (OR)	OR	95% CI	p-value
Constant	**–2.88**			p < 0.0001
NT-proBNP >5000 ng/L	**2.08**	**7.98**	**[4.96–12.84]**	p < 0.0001
Baseline: up to 5000 ng/L				
TnI >0.07 µg/L	**1.10**	**3.01**	**[2.20–4.12]**	p < 0.001
Baseline: up to 0.07 µg/L				
Prior myocardial infarction yes:	**1.06**	**2.88**	**[2.11–3.95]**	p < 0.001
Baseline: no				

Model fit (–2 log likelihood): 47.5 of 285 p < 0.0001

Discussion

In this cohort of unselected patients admitted to the ER for suspected ACS, NT-proBNP represented the most important risk predictor. These results are in agreement with previously published findings of the FRISC-II study [2]. Additionally, cardiac troponin I and Lp-PLA_2 also proved to be important predictors of risk. These three biomarkers reflect different pathways in the pathophysiology of the ACS and this represents the rationale behind the application of a

panel of biomarkers in the assessment of ACS patients.

Recently we reviewed emerging biomarkers, focusing on the various pathophysiological pathways they reflect in the development of ACS [1]. NT-proBNP and BNP are excellent markers of haemodynamic stress and have been extensively studied during recent years [2, 33–37]. Several authors have suggested that (NT-pro)BNP might be considered the new gold standard for impaired left ventricular function [38]. Although there are some differences between BNP and the inactive signal peptide N-terminal-proBNP and large comparative studies are not available, most authors agree that both markers reflect the same cardiac pathology [39].

Cardiac troponin I is a marker of myocyte necrosis and well established in early risk stratification of ACS. Since 2000, cardiac troponins have been part of the definition of AMI [40] and current guidelines for the diagnosis and treatment of NSTEMI consider positive troponins as superior indicators of risk [41, 42]. In fact, for most clinicians a positive troponin represents the strongest marker associated with high risk [43] and this is not challenged by our study.

Lp-PLA_2 is an emerging biomarker indicating increased inflammatory activity in the vascular wall. Data from several prospective epidemiologic studies assessing the association of Lp-PLA_2 with cardiovascular end-points suggest a strong association between increased levels of Lp-PLA_2 and cardiovascular outcome [20–23]. Moreover, it has been shown that elevated Lp-PLA_2 concentrations were associated with CHD events in the presence of manifest atherosclerosis, independently of a large panel of different biomarkers [23], and most recently, in a prospective study in 479 patients with CHD, increased Lp-LPA_2 levels were associated with a two-fold increased risk over 4-year follow-up [25]. These results support the hypothesis that Lp-PLA_2 may be a novel, independent risk marker for CAD which may also turn out to be useful in the setting of ACS.

In this study, for the first time, Lp-PLA_2 has been demonstrated to be of prognostic significance not only in chronic CAD patients but also in ACS. Most importantly, Lp-PLA_2 adds incremental information in particular in troponin negative patients with moderately elevated NT-proBNP levels (see Fig. 3).

It should be mentioned that, in this setting, CRP did not play a significant role in addition to the above mentioned biomarkers, although several other studies have found CRP to be a significant predictor in chronic as well as in acute CHD [44, 45] and we also could show significance in the bivariate analysis (see Fig. 2). Concomitant diseases excluded in these clinical trials may explain, at least in part, the negative result in our study of consecutive patients. It has also to be mentioned that the whole study population was at low risk (only 13.1% incidence of the primary endpoint, no myocardial infarction in the follow-up) and that CRP would possibly perform better in a higher risk cohort.

Sabatine et al. published one of the first multimarker studies in patients with ACS [46]. These authors used data from two randomised clinical trials and demonstrated that the number of positive risk markers (including CRP, BNP and troponin I) correlated strongly with the absolute risk of adverse events. Our study extends these findings by showing that NT-proBNP (instead of BNP) and troponin I are clinically useful markers of risk and that Lp-PLA_2 was superior to CRP, possibly because of the higher vascular specificity.

However, two cohorts comprising a sub-study of FRISC-II (Oldgren J et al. [abstract], Circulation 2005; 112:II-387) and GUSTO V (James SK et al. [abstract], Circulation 2005; 112:II-387) were recently presented. In both groups of patients with ACS subjected to different therapies, Lp-PLA_2 did not predict outcome. This was also true for the 2-year follow-up period in FRISC-II. However, these two trials and our study differ in the type of population investigated, the analytical approach and the cut-off for Lp-PLA_2 found to define the risk groups. Our population comprised unselected patients admitted to the ER with suspected ACS. In contrast, FRISC-II and GUSTO V studied highly selected patients for a randomised clinical trial. The latter populations were clearly at higher risk than our population.

Secondly, we identified the incremental prognostic value of Lp-PLA_2 using an innovative analytical method (CART) which specified an important subgroup containing 35.9% of the total study population in whom further risk stratification was possible. Finally, since in the FRISC trial blood was taken only within 48 hours after admission (at randomisation) this may also have contributed to the lack of a positive result. ACS is associated with an acute phase response and consequently lower LDL, which carries most of the circulating Lp-PLA_2. In addition, early administration of high dose statins may have further lowered LDL and Lp-PLA_2. Thus, although in the FRISC and GUSTO substudies in high risk unstable angina and NSTEMI patients Lp-PLA_2 was not a significant predictor of risk, we were able to demonstrate a clear prognostic value in a specific subgroup of patients admitted to the ER. This extends our knowledge from large studies in primary and secondary prevention [21, 23]. Most recently, data on LP-PLA_2 from the PROVE-IT study showed no impact for risk stratification in acute patients but only after 4 weeks [47]. Based on these arguments, we feel that Lp-PLA_2 indeed has a place in risk stratifi-

cation in patients with stable angina [25] and in specific subgroups of patients presenting in the ER, but possibly not in high-risk, definite ACS candidates treated with early PCI.

Summary and conclusions

The present study aimed at identifying a new risk stratification for patients with suspected acute coronary syndrome. Our analysis suggests a hierarchy of biomarkers that may be used in a series of steps for risk stratification in ACS. This strategy favours a combination of various biomarkers that are able to identify additional subgroups at intermediate to high risk (e.g. Lp-PLA_2 positive subjects among patients negative for troponin). This will most likely aid physicians in applying more individualised care and thus lead to a better distribution of resources in acute coronary care when prospectively validated in further studies.

We conclude that, in the setting of state-of-the-art coronary care for patients with suspected ACS in the ER, NT-proBNP, troponin I, and Lp-PLA_2 contribute independently to risk stratification and have proved to be superior to variables of the TIMI risk score. Lp-PLA_2 was a better predictor than CRP in these unselected patients with suspected ACS.

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