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Development of an optimized multimarker strategy for early risk assessment of patients with acute coronary syndromes

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ABSTRACT

Background: A multitude of biomarkers have been suggested for early risk-assessment in patients admitted to the emergency department with suspected acute coronary syndromes. We used logistic regression synergistically with classification and regression tree (CART) analysis to define a multimarker strategy and the cut-off values and sequencing needed to optimize risk stratification in a low to moderate risk population of the emergency department.

Methods: 432 unselected patients (59.7±14.5 y, 60.4% male) admitted to the emergency department (ED) with acute coronary syndromes (ACS) were enrolled. Cardiac troponin I (cTnI), N-terminal pro-B-Type natriuretic peptide (NT-proBNP), high sensitivity C-reactive protein (hsCRP), placental growth factor (PlGF), lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and D-dimers were measured by immunoassay and whole blood choline (WBCHO) and plasma choline (PLCHO) were measured using LC/MS from baseline samples. Logistic regression and CART analysis were used to define the importance of the various biomarkers tested and to define their hierarchy with respect to the prediction of major adverse cardiac events (MACE; cardiac death, non-fatal MI, unstable angina, CHF requiring admission, urgent PCI and CABG) over the 42-day follow-up period.

Results: A combination of NT-proBNP, WBCHO and Lp-PLA2 with cutoffs identified by CART-analysis was optimal for risk-stratification and superior to all other possible combinations of markers. Increased concentrations of both NT-proBNP (>1400 ng/l) and WBCHO (>21 μmol/l) identified patients with very high risk (RR=2.4, 39% primary endpoint) while low concentrations of NT-proBNP (≤1400 ng/l), WBCHO (≤17 μmol/l) and LP-PLA2 (≤210 μg/l) indicated very low risk (0% primary endpoint). WBCHO >17 μmol/l additionally identified a subgroup with intermediate risk (RR=3.0, 13.5% primary endpoint) in patients with NT-proBNP concentrations ≤1400 ng/l. Troponin when increased was highly prognostic but was not often positive in this early cohort.

Conclusions: A multimarker strategy defined synergistically by logistic regression and by classification and regression tree (CART) analysis can stratify patients into risk groups ranging from very low risk (0% MACE) to very high risk (39.5% MACE) based on admission values.

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1. Introduction

The evaluation of patients who present to the emergency department (ED) with chest pain suggestive of an acute coronary syndrome (ACS) is challenging. Many patients present with chest pain but only a minority have definite ACS and many, especially those who are older may have ACS without chest pain [1]. The diagnostic and prognostic value of cardiac troponin I (cTnI) in this situation is well established but its optimal use requires serial samples over time [2]. A multi-marker approach to risk stratification applied at the time of presentation might provide similar information in a more timely fashion and also identify those at risk, who do not manifest troponin elevations.

Increases in biomarkers upstream from biomarkers of necrosis, such as markers of inflammation, coagulation/fibrinolysis and markers of cardiac function, may be capable of fulfilling this role [3]

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and multiple studies have used this approach [4]. For example, increased concentrations of high sensitivity C-reactive protein (hsCRP) and N terminal-pro B-type natriuretic peptide (NT-proBNP) predict major adverse cardiac events independent of elevations in cardiac troponin [5]. D-dimer, a marker of fibrinolysis used to help diagnose pulmonary embolism has been shown to be helpful in the triage of patients with ACS [6]. Similarly, whole blood choline (WBCHO) predicts cardiac death, life-threatening cardiac arrhythmias, heart failure and the need for coronary intervention when measured at the time of presentation [7]. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an inflammatory marker related to oxidized lipids, predicts cardiovascular endpoints in initially healthy subjects [8–11] and in patients with atherosclerosis [12]. Another new marker, placental growth factor (PlGF), is thought to be a marker of plaque rupture [3] and may identify patients at risk for mortality independent of cardiac troponin and inflammatory markers [13] in patients with suspected ACS [4].

Although the ability to provide rapid measurements of multiple biomarkers with multiplex technology is near, the optimal method to define multimarker strategies is controversial. Thus, we employed both logistic regression methods and the use of a novel method called classification and regression tree (CART) analysis. The latter method allows probing of optimal cut-offs and the sequencing of marker determinations to optimally add information in a low to moderate risk population of the emergency department.

2. Materials and Methods

2.1. Patients

Patients presenting to the ED at the Charité tertiary university clinic between May 22, 2002 and March 25, 2003 with suspected ACS were enrolled. The inclusion was based on the judgement of the attending physician to consider cardiac ischemia as cause of the acute symptoms and therefore a real life ED situation was investigated. Therefore, all patients with chest discomfort could be included. The sample consisted of 432 consecutive patients enrolled within a median of 6.6 h of onset of chest pain (25%/75% quartiles 2.5 h/27.4 h). Exclusion criteria were severe anemia (<7 g/dl), lack of informed consent, and age <18 y.

A history and physical examination, ECG and an initial cardiac troponin I (cTnI) measurement were obtained in all patients and therapy initiated in accordance with the German national guidelines [14,15]. Final discharge diagnoses were assessed by chart review by an experienced physician who was neither involved in the treatment of the patients nor the analysis of the data. For the definition of acute myocardial infarction the ESC/ACC guidelines were used [16]. The Stratus® CS troponin I (cutoff 0.1 μg/l) or troponin T (cutoff 0.03 μg/l) were used for the clinical diagnosis. The diagnosis of NSTE-ACS was based on clinical symptoms and at least 1 of the following: Increased cardiac markers, pathological ST-segment changes, pathological findings in the coronary angiography, new documented wall motion abnormalities in the echocardiogram, positive stress-test within the first week after presentation in troponin negative patients. Patients were contacted at 42-days by telephone and in the case of rehospitalization, the hospital charts were reviewed to determine the occurrence of the combined primary endpoint major adverse cardiac events (MACE) of cardiac death, non-fatal MI, unstable angina pectoris or congestive heart failure requiring admission, urgent percutaneous intervention (PCI) and coronary artery bypass grafting (CABG). Events were recorded excluding the index event which was defined as occurring within the first 24 h. Thus, none of the myocardial infarctions or congestive heart failure present on admission contributed to end point tabulation. However, some of the subsequent events such as PCI, CABG, and congestive heart failure if they developed *de novo* were included. The study was approved by the ethics committee of the Charité.

2.2. Laboratory testing

Only admission blood samples were analyzed for this study since these reflect all means available to the emergency physician at initial risk stratification and triage. Lithium heparin samples were used for the measurement of cTnI, NT-proBNP, hsCRP, PLCHO and PlGF. D-dimer and Lp-PLA_2 were measured from citrate plasma and WBCHO from heparinized whole blood samples. Samples were centrifuged within 2 h and stored at −80 °C until analysis with the exception of the samples for WBCHO, which were frozen as whole blood.

2.3. Analytical methods

Cardiac troponin I (cTnI) was measured at the point of care with the Stratus® CS (DadeBehring, Marburg, Germany). The detection limit was 0.01 μg/l; the 99% value was 0.07 and the value with a 10% CV was published to be <0.1 μg/l in healthy volunteers [17] by one group and 0.06 μg/l by another [18]. In our laboratory pooled plasma analysis confirmed the 10% CV concentration at <0.1 μg/l (data not shown). NT-proBNP was measured by electrochemiluminescence immunoassay on the Elecsys® 2010 analyzer (Roche Diagnostics, Mannheim, Germany). The interassay CVs were 3.5% at 84.7 ng/l and 2.3% at 3265 ng/l for NT-proBNP [19]. CRP was measured by a high sensitivity latex-enhanced immunoturbidimetric assay (hsCRP, Invicon, München, Germany) on a Roche Modular analytical system (Roche Diagnostics, Mannheim, Germany). The interassay CVs were 5.0% at 0.55 mg/l and 1.2% at 5.0 mg/l. D-dimer was measured with the D-dimer Plus assay on the Sysmex® CA-1500 (DadeBehring in Marburg, Germany). The interassay CVs was 11.5% in a normal plasma pool. Plasma Lp-PLA_2 were determined with the PLAC® test (diaDexus, Inc. South San Francisco, CA). The PLAC test is a solid phase ELISA for the quantitative determination of Lp-PLA_2 in human plasma or serum. 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%. PlGF was determined using the human PlGF Quantakine® immunoassay (R&D Systems, Minneapolis, MN). The interassay CVs were 11.8, 11.0 and 10.9 for mean concentrations of 55, 184 and 724 ng/l, respectively. The package insert states that the minimum detectable concentration was <7 ng/l.

WBCHO and PLCHO were determined by liquid chromatography/mass spectrometry as described previously [7]. Hemolyzed whole blood (WBCHO) and plasma samples (PLCHO) were deproteinized by centrifugation over a pre-rinsed filter with a molecular mass cut-off at 10 000 D (Millipore, Schwalbach, Germany UFC801096). The limit of quantification is 0.05 μmol/l with an interassay precision of 5.5%, 5.4%, 5.3%, 5.7% at 12.5, 125, 200, and 400 μmol/l, respectively.

2.4. Statistical analysis

Since the distributions of numerical variables were skewed, medians and ranges were used for descriptive statistics, and non-parametric methods were employed for bivariate testing. A 2-sided α-values of 5% was used for all tests. Categorical variables are listed in [%] and with 95% confidence limits.

A stepwise logistic regression analysis was conducted assessing the following variables: Cardiac troponin I (cTnI), N-terminal pro-B-Type natriuretic peptide (NT-proBNP), high sensitivity C-reactive protein (hsCRP), placental growth factor (PlGF), lipoprotein-associated phospholipase A_2 (Lp-PLA_2), D-dimers, WBCHO and PLCHO and age and sex. The same variables were used in the classification and regression tree (CART) model which is described in detail below. The primary endpoint were major adverse cardiac events (MACE; cardiac death, non-fatal MI, unstable angina, CHF requiring admission, urgent PCI and CABG) over the 42-day follow-up period.

The relative value and hierarchy of potential prognostic variables were evaluated using CART methodology that allows determination of cut-off values that optimize performance of the marker. CART involves repeated dichotomous subdivisions of a group of subjects on the basis of the choice of optimal cut-off points of binary, ordinal or continuous covariates that maximizes a certain split criterion [20] and has already been successfully applied in various clinical cardiovascular research studies [21–23]. In the present analysis, the biochemical variables described above plus age and sex were included and assessed for their relative value in classifying patients into homogeneous and clinically relevant "risk groups" according to clinical outcomes (primary endpoint). Each numerical laboratory parameter was assessed by creating nineteen dichotomous dummy coded variables according to the 5% quantiles of the original distribution. All dummy coded variables for all parameters are then assessed for their association with the primary endpoint and the dichotomous variable with the highest association was used to split the overall patient group into subgroups. This process is repeated until further significant differentiation is no longer possible or until the subgroups become too small [22].

3. Results

The characteristics of the overall study group of 432 are displayed in Table 1 along with the risk groups achieved by CART analysis (Fig. 2) with the 2 most predictive analytes, NT-proBNP and WBCHO. Significantly fewer patients in the low risk group underwent coronary angiography (30.8% vs 60.5% in the high risk group and 37.7% in the whole group). In patients with the final diagnosis NSTE-ACS 82.2% were scheduled for early invasive procedures. Patients with high risk were more likely male, significantly older (68.4 vs 55.7 y) and had a higher percentage of initially increased cardiac troponin and hsCRP values. Less than 50% of patients with increased NT-proBNP were diagnosed with heart failure (Table 1).

The overall incidence of the primary endpoint at 42-days was 56/432 (13.0%), reflecting a mild to moderate risk population. The outcomes were as follows with respect to the first occurring event: death (7), acute MI (1), hospitalizations for unstable angina (10) or heart failure (7), PCI (26), bypass-surgery (5). Table 2 displays all optimal cut-offs using 5% quantiles (basically step one of the CART

Table 1
Characteristics of patients at baseline in the overall study group and in the three main subgroups identified by the CART analysis

	Overall group	NT-proBNP negative WBCHO negative Lp-PLA$_2$ negative	NT-proBNP positive WBCHO negative	NT-proBNP positive WBCHO positive
	N=432	*N*=78#	*N*=55#	*N*=38#
MACE [%]	13.0	0	16.4	39.5
Male [%]	60.4	50.0	54.5	60.5
Age [y±SD]	59.7±14.5	55.7±13.9	68.3±12.8	68.4±12.0
Troponin I≥0.1 µg/l [%]	20.6	9.0	52.7	42.1
CRP≥10 mg/l [%]	24.3	15.4	43.6	50.0
White blood cell count, [X10^9/l]	8.1 (6.8/10.3)	7.7 (6.1/10.2)	8.7 (7.4/12.1)	9.9 (7.9/12.5)
STEMI [%]	7.0	1.3	16.4	15.8
Heart failure (Killip class>I)	16.5	5.1	36.4	47.4
3 or more risk## factors [%]	24.3	27.0	23.6	50.0
ST-depression [%]	11.0	3.8	16.4	23.7
Severe angina [%]	50.9	44.9	56.4	65.8
Aspirin use within 7 days [%]	37.8	39.0	42.3	45.7
TIMI high risk [%]	10.2	6.4	16.4	28.9
Prior PCI [%]	28.1	28.0	22.2	44.4
Prior CBAG [%]	14.4	9.0	18.2	28.9
Risk factors [%]				
Hypertension	65.7	64.1	70.9	86.8
HLP	56.7	55.8	57.4	76.3
Diabetes	22.2	17.9	34.5	44.7
Current smokers	34.7	39.0	21.2	31.4
Medication at admission [%]				
ACEI	31.0	18.4	39.2	42.9
β-blockers	41.3	43.4	42.0	37.1
Nitrates	16.3	9.1	17.6	31.4
Diuretics	24.2	21.1	45.1	45.7
Statins	23.1	29.9	21.2	20.0
Coronary angiography [%]	37.7	30.8	54.5	60.5
### No significant CAD	14.1	16.7	20.0	8.7
1-VD	17.8	20.8	16.7	17.4
2-VD	18.4	16.7	23.3	8.7
3-VD	49.7	45.8	40.0	65.2
Acute PCI	39.0	25.0	43.3	47.8
Creatinine [mg/dl]	0.9 (0.7/1.1)	0.8 (0.7/0.9)	1.0 (0.8/1.3)	1.2 (1.0/1.6)
BMI [kg/m^2]	26.5 (24/30.1)	27.4 (24.2/29.9)	25.7 (23.9/29.9)	25.3 (23.3/31.4)

Quantitative variables are medians and (25%/75%) percentiles except age (mean±SD; #Subgroups according to CART analysis (see Fig. 2 for cut-offs); CAD, coronary artery disease; ##, risk factors included hypertension, hyperlipoproteinemia (HLP), diabetes, or being a current smoker;###, numbers with respect to patients who underwent coronary angiography, acute PCI with respect to the index event only; STEMI, ST-elevation myocardial infarction; TIMI, thrombolysis in myocardial infarction study group; VD, vessel disease.

Table 2
Observed incidences and odds ratios for different splits based on 1) data-optimized cut-offs (white boxes; multiple splits are listed if multiple significant local maxima were observed) and 2) published cut-offs (grey boxes)

Laboratory parameter	Cut-off (≤/>)	Level of sign.	Achieved split "up to" vs "above" cut-off *n* (observed incidences)	Odds ratio (95% CI) for MACE
NT-proBNP [ng/l]	38	***	55 (0%) vs 377 (14.9%)	Not defined
	145	***	191 (5.8%) vs 241 (18.7%)	3.8 (1.9–7.5)
	1400#	***	339 (9.4%) vs 93 (25.8%)	3.3 (1.8–6.0)
	237	**	225 (8.0%) vs 207 (18.4%)	2.6 (1.4–4.7)
	900	**	320 (9.7%) vs 112 (22.3%)	2.7 (1.5–4.8)
Troponin I [µg/l]	0.02#	*	224 (9.4%) vs 208 (16.8%)	2.0 (1.1–3.5)
	0.06	*	322 (10.9%) vs 110 (19.1%)	1.9 (1.1–3.5)
	0.10	*	345 (11.3%) vs 87 (19.5%)	1.9 (1.0–3.6)
CRP [mg/l]	2.5	**	177 (7.9%) vs 255 (16.5%)	2.3 (1.2–4.3)
	10.0	*	327 (11.0%) vs 105 (19.0%)	1.9 (1.0–3.5)
D-dimer [µg/l]	50	0.10	114 (17.5%) vs 318 (11.3%)	0.6 (0.3–1.1)
	500	0.72	413 (12.8%) vs 19 (15.8%)	1.3 (0.4–4.5)
Lp-PLA$_2$ [µg/l]	270	0.19	324 (11.7%) vs 108 (16.7%)	1.5 (0.8–2.8)
	n. a.			
PlGF [ng/l]	3.8	0.24	16 (0.0%) vs 416 (13.5%)	not defined
	27.0	0.65	385 (12.7%) vs 47 (14.9%)	1.2 (0.5–2.8)
PLCHO [µmol/l]	10.5#	**	232 (8.6%) vs 200 (18.0%)	2.3 (1.3–4.1)
	11.8	**	322 (10.2%) vs 110 (20.9%)	2.3 (1.3–4.2)
	18.5	*	416 (12.3%) vs 16 (31.3%)	3.3 (1.1–9.7)
WBCHO [µmol/l]	17.0	**	191 (7.3%) vs 241 (17.4%)	2.7 (1.4–5.0)
	28.2	0.08	340 (11.5%) vs 92 (18.5%)	1.7 (0.9–3.3)

Significance concentrations are denoted as * for $p<0.05$; ** for $p<0.01$ and *** for $p<0.001$; for not significant results the actual *p*-values are displayed; all *p*-values refer to exact binomial tests; n. a., not applicable (no single cutoff for ACS published); MACE, primary endpoint as defined in the Methods section; #, absolute maxima of data optimized cutoffs.

analysis) and published cut-offs and the corresponding Odds ratios with respect to the primary endpoint.

Fig. 1 shows the frequencies of the clinical discharge diagnoses and the frequency of MACE in each diagnosis group. The ROC curves for admission NT-proBNP, WBCHO and cardiac troponin I with respect to the primary endpoint at 42-days (figures not displayed) revealed areas under the curve of 0.684 (0.612/0.756, $p<0.001$) for NT-proBNP, 0.613 (0.532/0.694, $p=0.006$) for WBCHO and 0.587 (0.509/0.666, $p=0.035$) for troponin I.

In Table 3 the results of the multiple logistic regression analysis are displayed. NT-proBNP and WBCHO both were highly prognostic levels. NTproBNP at 2 concentrations defined as part of the CART analysis. A low D-dimer was also found to be of prognostic significance.

The results relating the sequencing of markers by CART analysis are detailed in Fig. 2. The best overall initial risk stratification was obtained using NT-proBNP at a cut-off of 1400 ng/l (relative risk (RR) of 2.74, 25.8% vs 9.4% primary endpoint incidence; $p<0.0001$). In the group with lower NT-proBNP, further differentiation was achieved using WBCHO (cut-off 17 µmol/l; RR=3.0, 13.5% versus 4.5% MACE; $p<0.01$). In the group with higher NT-proBNP concentrations WBCHO above 21 µmol/l identified the highest risk group (39.5% MACE, RR=2.4 compared to the group with lower WBCHO; $p<0.05$). An additional significant stratification in patients with lower NT-proBNP and WBCHO concentrations was achieved using Lp-PLA$_2$ (0% vs 9.2% MACE at a cut-off of 210 µg/l; $p<0.01$). In the group of patients with lower NT-proBNP but increased WBCHO, D-dimer was shown to identify a significantly higher risk (RR=2.94, 25% versus 8.5% incidence of MACE; $p<0.01$) when values lay below or at the detection limit of 50 µg/l.

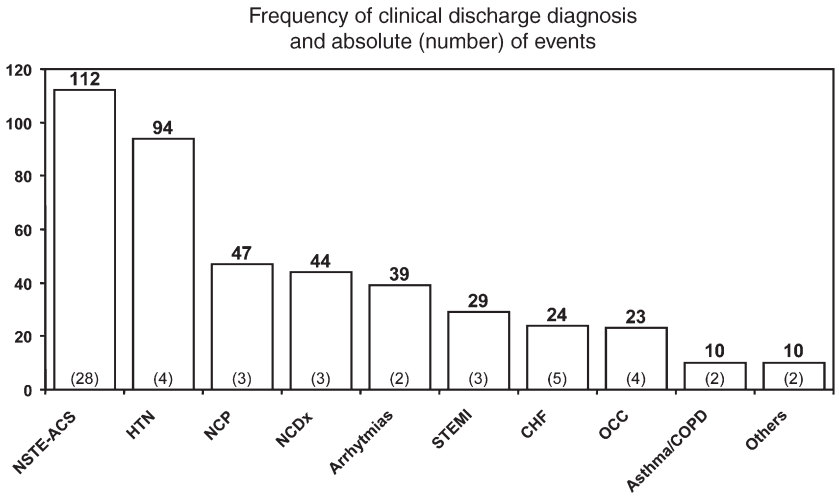

Fig. 1. Final discharge diagnoses of the study population; absolute numbers of patients listed over the bars and the number of MACE in each group are listed at the bottom of the bars in brackets. NSTE-ACS, non ST-elevation acute coronary syndrome; HTN, hypertension; NCP, non cardiac chest pain; NCDx, non cardiac diagnosis; STEMI, ST-elevation myocardial infarction; CHF, congestive heart failure without ACS; OCC, other cardiac conditions; "others" included pulmonary embolism ($n=5$), pneumonia ($n=4$) and stable angina ($n=1$). The events in the group of "other" diagnoses occurred both in patients with pulmonary embolism as final discharge diagnosis.

In this unselected low risk group, Cardiac troponin I was not identified as a major predictor neither in CART nor in the logistic regression analysis. In order to make sure that no relevant effect of cTnI was missed or masked by other analytes, cTnI positive and negative patients were analyzed separately using the established cut-offs of 0.1 and 0.06 μg/l respectively. Regardless of the cut-off used: in troponin positives, no further significant effect could be found for any of the markers assessed and in troponin negatives, the logistic regression and CART models remained practically identical to the original model. These subgroup analyses were consequently able to exclude any confounded or masked effect of troponin thus corroborating the validity of the original models. The lack of any further effect in troponin positives reflects the low risk nature of the patients with the coinciding small size of the sub-sample of troponin positives.

4. Discussion

The multifactorial pathophysiology of acute coronary syndromes and its potential complications is an important rationale for measurements of multiple biomarkers related to different underlying pathophysiological aspects in order to optimize early biochemical risk assessment. In addition, there are likely some patients at risk with non cardiovascular diseases who are part of this group as well and different markers may be more sensitive to this group as well [4]. However, the methods on how to select and combine markers in a practical multimarker strategy has not been well defined. Logistic regression, the most commonly used technique and one that we used as well can help to identify significant interactions but restricts its estimates to the whole study group. Methods such as summing the number of positive markers, the development of risk scores and/or complex technologies like neuronal networks and classification and regression trees (CART) analysis have been suggested. CART analysis has the advantage of being independent of pre-specified cutoffs, all markers have equal opportunities at each decision level at optimal cutoff concentrations, it is open for all combinations of all markers and it selects the most powerful markers which optimally add information to each other. CART analysis also provides important new information by displaying the results as observed risk groups. CART focuses on the interpretation of the results and is also highly sensitive to interactions (i.e. combinatory effects) between the factors examined. We thus used CART analysis synergistically with logistic regression to help define the optimal manner to evaluate patients with ACS.

The results of this study demonstrate that the combination of NT-pro BNP and WBCHO are the optimal combination for evaluation of this population in first available samples. They identify a group with a 39.5% event rate if both markers are above the cut off values (>1400 ng/ml and >21 μmol/l) calculated by the analysis and a group with an event rate of 4.5% if both values are below the

Table 3
Logistic regression on major adverse cardiac events during the first 6 weeks; $N=432$; incidence = 13.0% (56/432)

Variable	Ln (OR)	S.E.	*p*-value	OR	95.0% C.I. for OR	
					Lower	Upper
NT-proBNP > 145 Baseline ≤ 145	.969	.396	.014	2.637	1.212	5.734
NT-proBNP > 1400 Baseline ≤ 1400	.917	.351	.009	2.502	1.257	4.982
WBCHO > 17 Baseline ≤ 17	.983	.337	.004	2.672	1.380	5.173
D-dimer > 49 Baseline ≤ 49	−.844	.331	.011	.430	.225	.822
Constant	−2.883	.425	.000	.056		

−2 log likelihood = 296.386; model chi-square = 36.843 at 4df; *p*-value < 0.0001; OR, odds ratio. Variables included in the analysis were: WBCHO, PLCHO, PlGF, Lp-PLA_2, hs-CRP, D-dimer, NT-proBNP, cTnI, age and sex.

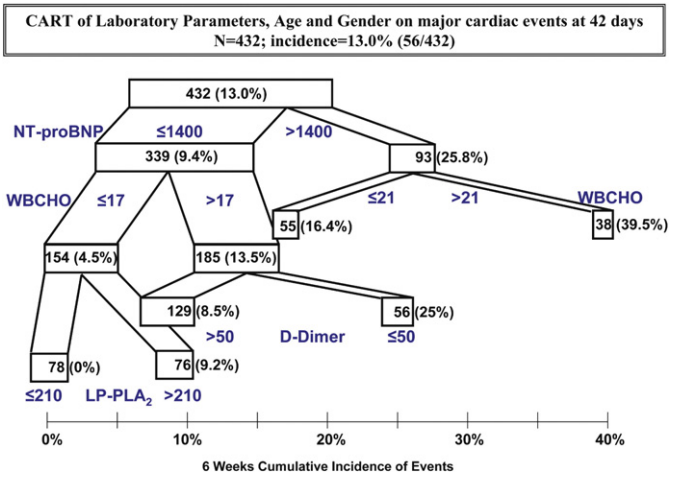

Fig. 2. Classification and regression tree analysis for the primary endpoint. See Methods section for details.

designated values ≤1400 ng/ml and ≤17 μmol/). If Lp-PLA_2 is added to these two markers a group with no risk (0% event rate) can be identified at the time of presentation. These results are closely congruent with those of logistic regression which also identified NT-proBNP and WBCHO as valuable, but they extent the analysis to sequencing and interactions. As in practice, the study relied on only the first sample and yet was able to accurately stratify patients across a broad range of risk categories. Other biomarkers, including PLCHO, hsCRP, PlGF and cTnI showed inferior predictive ability. Low D-dimer identified a group at intermediate risk. Interestingly CART analysis identified markers which are related to different pathophysiologies which fit with the concept that multimarker strategies are helpful for risk-prediction in multifactorial syndromes like ACS.

Cardiac troponin directed care is the recommended approach to this patient population in all guideline documents [14,15] and was used in this study. In cTnI positive patients, no other marker was found to significantly add to risk stratification. Cardiac troponin predictions are robust and well validated. However, the absolute number of MACE in troponin positive patients was small (19 and 21 for cut-offs of 0.1 and 0.06, respectively) and this clearly decreased the likelihood of finding additional *significant* results. More generally, an analysis defining troponin I *a priori* as the most relevant marker (and splitting the data accordingly) is obviously prone to underestimate the prognostic value of other markers (or combinations of those) that may actually be better and should be used in concert with cardiac troponin or could eventually, if proven significantly better, even replace it. In cTnI negative patients, the findings were very similar to the original models. The cardiac event rate of 11% given the highly sensitive troponin assay we used is likely related to the approach of using only the initial sample in this analysis. Serial samples of cardiac troponin likely would have shown more discriminatory power. However, our study, as many others [13,24–27] was specifically designed to use only the initial sample in the interest of more rapid triage. In addition, the initial increased troponin might have shown better prognostic qualities had it not been used to guide therapy per our guidelines. A larger proportion of those with increased troponins on admission underwent PCI. Overall, 73.9% of high risk patients who had coronary angiography underwent PCI. Forty-two percent of that group were cardiac troponin positive which is significantly higher than the proportions in the whole and/or low risk groups alone.

Our results are similar to those of some other studies but differ from some on a marker by marker basis.

4.1. Highly predictive markers

The documented potent risk stratification qualities of NT-proBNP in this study add to those already documented in a large number of publications [5,28–32]. However, this evaluation is unique in that it helped identify 2 cut off values of significance depending where in the sequence of the evaluation, a given patient was. Interestingly, the cut-off determined by CART is significantly higher than that used to define heart failure [33] or that optimized for prediction in the FRISC trial [5,34,35]. This may be because in our trial it was treated independently rather than being added to other risk factors. NT-proBNP and BNP are not specific for the presence of ischemia or even cardiac disease per se and perhaps for that reason, it identified patients with high risk despite the fact that most patients lacked overt signs of heart failure.

Whole blood choline (WBCHO) was more predictive than any other marker by logistic regression and added significantly to NT-proBNP in the CART analysis in keeping with earlier studies of WBCHO and plasma choline (PLCHO) in patients with normal admission troponin values and acute coronary syndromes [36]. Both are said to be indicative of tissue ischemia and WBCHO may inform by detecting processes associated with coronary plaque instability [7,37] due to its relationship with phospholipase D activation which is thought to be involved in coronary plaque instability. WBCHO>21 μmol/l distinguished patients with substantially increased concentrations of NT-pro BNP into a group with very high risk (39.5% event rate) and one with less than half of the risk. In those patients with NT-proBNP values <1400 ng/ml, a cut off value of 17 μmol/l distinguished groups with event rates of 13.5 vs 4.5%. In contrast to other trials, WBCHO was superior to PLCHO. This may reflect that the present population is less severely at risk. PLCHO appears to detect those at risk for death and severe complications such as severe heart failure and arrhythmias. Its value may overlap with that of the NT-proBNP.

A combination with a marker of plaque inflammation such as Lp-PLA_2, further improves early-risk stratification in the low-risk group. Lp-PLA_2 is an emerging biomarker specifically related to inflammation in the vessel wall. Several prospective epidemiologic studies in healthy individuals [9,38] and cross sectional studies [39,40] have reported a strong association of Lp-PLA_2 concentrations with cardiovascular endpoints and/or the presence of CAD, independent of other biochemical markers. Our study is the first to suggest that Lp-PLA_2 may be also of prognostic significance in ACS. Lp-PLA_2 adds incremental information when the other markers are not increased and thus possesses a potentially clinically relevant negative predictive value (Fig. 2). This subset may not have been appreciated in other analyses using more conventional methods [41].

Several studies have shown elevations of fibrin monomer, D-dimer and other markers of coagulation are not prognostic in patients with acute coronary syndrome [6,42,43]. We found that D-dimer predicted adverse outcomes in a group of patients below the initial NT-proBNP cut off but above the WBCHO cut off if it was low — below the detection limit of 50 μg/l. It could be the case that individuals with very low values in the setting of ACS may have impaired fibrinolysis in a situation complicated by thrombosis. They therefore may indeed be at higher risk. This hypothesis needs to be tested in further studies.

4.2. Markers which were less predictive

Despite prior studies suggesting the importance of CRP and PlGF in patients with ACS [13,44], they did not add to risk stratification in our cohort. In the bivariate analysis (Table 2), CART, and logistic regression analysis of our data, neither marker added substantial prognostic information, even if the published cutoff is used. This may be because of the potency of other analytes. Prior studies have usually compared these markers to one other analyte independently. In a recent study, PlGF was predictive of death but not cardiac death in patients with ACS [4]. Our ED study population was at overall low risk for mortality. Perhaps, hsCRP and PlGF would have performed better in a higher risk cohort.

4.3. Multi-marker approach

The concept of using a multimarker strategy [30] to evaluate patients with ACS had previously focused on the conjoint use of cardiac troponin, CRP and BNP. Our data substantially extend those data by adding the contribution of several additional markers via a unique analysis tool. CART analysis was synergistic and in general concordant with logistic regression but it helped to facilitate choice of the optimal tests overall, delineation of subgroups, and the optimal sequence and cut-off values that should be used. In the future, multiplexing will make this approach reasonable. Such strategies will need to be tested against those that use serial troponin testing. It may well turn out that such approaches will be synergistic, with certain tests being ideal for those who require earlier triage or who do not have increased troponins.

4.4. Limitations

This study also has some important limitations. The results of CART analysis like all statistical testing, are dependent on population size

and markers like CRP, PLCHO, PlGF might have been predictive had the population size been larger. Our results also by design do not take into account serial biomarker testing because we wished to probe the ability to make very early risk stratification with the goal of having such an approach facilitate earlier triage decisions. Finally, the study population represents a low-medium risk population and it is unclear whether results could be transferred to other more selected populations of ACS patients.

5. Conclusions

Our data demonstrate that the use of multiple markers can define groups at very low and very high risks with the use of the admission blood sample only. Logistic regression was augmented by CART analysis to select those cardiac biomarkers useful for optimal risk prediction and to define a sequence for their use. In this low-medium risk patients with ACS the combination of a marker of left ventricular dysfunction (NT-pro BNP), one of ischemia and plaque instability (WBCHO) and one of plaque inflammation (Lp-PLA2) provided optimal early risk differentiation. It allowed identification of groups ranging from very low risk (0% MACE within 6 weeks) to very high risk (39.5% MACE within 6 weeks) based on single admission measurements.

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