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CLINICAL RESEARCH
Coronary heart disease

Impact of soluble fms-like tyrosine kinase-1 and placental growth factor serum levels for risk stratification and early diagnosis in patients with suspected acute myocardial infarction

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Aims Angiogenic factors play an important role in the development of atherosclerosis and show pronounced changes during acute myocardial infarction (AMI). We analysed the impact of placental growth factor (PlGF) and its endogen opponent, soluble fms-like tyrosine kinase-1 (sFlt-1), on clinical outcome and the early diagnosis of AMI.

Methods and results This multicentre study enrolled patients presenting with symptoms suggestive of AMI. The final diagnosis was adjudicated by two independent physicians. Levels of sFlt-1 and PlGF were compared with results of a standard troponin T and a novel high-sensitive troponin (hsTnT) assay. Of the 763 patients enrolled, 132 were diagnosed with AMI. Multivariable Cox regression analysis demonstrated sFlt-1 >84 ng/L [hazard ratios (HR) 2.6, 95% confidence intervals (CI) 1.2–5.4, $P = 0.01$] and PlGF >20 ng/L (HR 3.6, 95% CI 1.3–10.4, $P = 0.02$) as predictors for mortality during 1-year follow-up, independent from information provided by troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP). However, only sFlt-1 persisted as independent predictor for mortality when analysed together with hsTnT and NT-proBNP, and after adjusting for significant clinical parameters. For the diagnosis of AMI, the combination of troponin T and sFlt-1 improved the performance of troponin T alone and led to a negative predictive value of 98.3% already at time of presentation. However, sFlt-1 and PlGF added only limited diagnostic information when used together with hsTnT.

Conclusion Only sFlt-1 but not PlGF provides overall independent prognostic information in patients presenting with symptoms suggestive of AMI. After the introduction of hsTnT in clinical routine, sFlt-1 and PlGF can only add limited diagnostic information for the detection or exclusion of AMI. Clinical Trial Registration Information: ClinicalTrials.gov, NCT00470587

Keywords Myocardial infarction • Diagnosis • Prognosis • Angiogenic factors • High-sensitive troponin

Introduction

Symptoms suggestive of an acute myocardial infarction (AMI) are among of the most common reasons why patients are seen at an emergency department (ED).[1] Today, cardiac troponin is one of the major cornerstones for risk stratification and diagnosis of patients in this setting due to its high specificity for myocardial injury, its high clinical sensitivity and also because it provides important prognostic information.[1–3] However, major limitations of cardiac troponin are its low sensitivity for detection of AMI

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within the first hours after onset of symptoms,[1,4] and its limited coverage of all prognostic aspects as demonstrated by comparison with other biomarkers.[5–7] As a consequence, optimal risk stratification of a large proportion of patients with suspected AMI at time of presentation is difficult, leading to extensive monitoring until AMI can be excluded.[8,9] Furthermore, 1–2% of patients who have an AMI are misdiagnosed and sent home leading to adverse outcomes.[5] Therefore, the need for biomarkers for risk stratification and rapid diagnosis in patients with suspected AMI is an ongoing issue.

Recent investigations have given detailed insights into pathophysiology of atherosclerosis and led to the development of novel cardiac biomarkers.[5,10] Angiogenic factors are some promising candidates since recent data imply that they are not only important in the development and progression of atherosclerosis, but also seem to be involved in the pathogenesis of AMI.[11–17] The vascular endothelial growth factor receptor-1, also called fms-like tyrosine kinase-1 (Flt-1), is expressed on endothelial cells and macrophages.[17] It binds not only vascular endothelial growth factor but also placental growth factor (PlGF), a platelet-derived protein, of which the biological functions are incompletely understood. Placental growth factor appears to promote the inflammatory process of atherosclerosis, which includes the recruitment of circulating macrophages and atherosclerotic intimal thickening.[15,16] In patients with acute coronary syndrome, PlGF is increased regardless of the cardiac troponin concentration, which implies that it is a biomarker of ischaemic events such as plaque instability, plaque disruption, or impending thrombosis.[5,13] A potential endogenous opponent of PlGF is soluble Flt-1 (sFlt-1), which represents a type of Flt-1 without the transmembrane and intracellular tyrosine kinase domain.[12,17] It is thought to be able to capture PlGF and to reduce thereby the amount available to bind to the receptor located on macrophages and endothelial cells.[17] An increase of progenitor cells expressing Flt-1 was described in patients with AMI,[18] suggesting a potential early repair mechanism associated with angiogenic signalling. As both sFlt-1 and PlGF, have also demonstrated changing blood levels during ongoing AMI,[11,14] they might be useful biomarkers for acute as well as long-term risk stratification.

Therefore, we analysed the prognostic and diagnostic impact of sFlt-1 and PlGF in a large cohort of patients presenting to the ED with symptoms suggestive of AMI as single markers as well as in addition to a contemporary cardiac troponin and a novel high-sensitive troponin (hsTnT) assay.

Methods

Study design and population

The Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) trial is an ongoing prospective, observational multicentre study designed and co-ordinated by the University Hospital Basel. Patients presenting to the ED with symptoms suggestive of cardiac ischaemia at rest or minor exertion within the last 12 h were enrolled.

Patients had to be at least 18 years old and have given written informed consent. Patients with a positive troponin test prior to presentation, cardiogenic shock, terminal kidney failure requiring dialysis, or anaemia requiring transfusion were excluded. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local Ethics Committees.

Clinical and laboratory procedures

All patients underwent an initial clinical assessment including clinical history, physical examination, 12-lead electrocardiogram (ECG), continuous ECG-monitoring, pulse oximetry, and standard blood tests including the local cardiac troponin assay. Evaluation and treatment of patients were left to the discretion of the attending physicians and were performed according to standard practice of the hospital and current guidelines.

Blood samples for determination of investigational biomarkers were collected at presentation to the ED into serum tubes and before start of therapy with heparin. Within 1 h, samples were carefully processed to avoid changes in marker levels (e.g. by cell activation) and frozen at $-80^{\circ}C$ until assayed in a blinded fashion in two batches in a dedicated core laboratory. Troponin T was determined using a one-step enzyme immunoassay based on electrochemiluminescence technology (Roche Diagnostics, Germany), with a lower limit of detection of 0.01 μg/L. sFlt-1, PlGF, and hsTnT were measured with precommercial sandwich enzyme electrochemiluminescence immunoassays (Roche Diagnostics, Germany). The limit of detection for sFlt-1 was 10 ng/L with a maximum detectable value of 85 000 ng/L. The intra-assay coefficient of variation was 1.6% (63 ng/L) and 0.8% (589 ng/L), and the inter-assay coefficient of variation 4.3% (63 ng/L) and 2.3% (589 ng/L). For PlGF, the limit of detection was 3 pg/mL with a maximum detectable value of 10 000 ng/L. The intra-assay coefficient of variation was 1.1% (107 ng/L) and 1.2% (563 ng/L), and the inter-assay coefficient of variation 2.7% (107 ng/L) and 2.6% (563 ng/L; preliminary results provided by Research and Development of Roche Diagnostics, Germany). High-sensitive troponin T had a limit of detection of 0.002 μg/L, a 99th-percentile cut-off point of 0.014 μg/L, and a coefficient of variation of $<10\%$ at 0.013 μg/L.

Adjudicated final diagnosis and follow-up

The final diagnosis was independently adjudicated by two blinded physicians as previously described.[19,20] In brief, the physicians had access to all available medical records and data (including local cardiac troponin values, ECG, echocardiography, cardiac exercise testing, and coronary angiography) but were blinded to the results of investigational biomarkers. Acute myocardial infarction was defined as recommended in current guidelines and described previously.[20] Unstable angina was diagnosed when a patient had normal troponin levels and typical angina at rest, a deterioration of previously stable angina, a positive result on a cardiac exercise test or cardiac catheterization showing coronary arteries with stenosis of 70% or more of the vessel diameter, or when the diagnosis was uncertain but follow-up information showed that the patient had an AMI or a sudden, unexpected cardiac death within 60 days after presentation.

To obtain a 12-month follow-up, all patients were contacted by phone after 6 and 12 months. For patients reporting cardiac symptoms, at least one ECG and clinical examination were performed at the outpatient clinic or by the referring physician. All information of subsequent hospital admissions or provided by the referring physician or outpatient clinic were adjucated by independent physicians and entered into the computer database.

Statistical analysis

This manuscript represents an exploratory analysis based on the data set of the APACE study. Due to the extreme skewness of markers, we

used cut-offs for 1-year mortality determined by a classification and regression trees (CART) model. To summarise differences in outcome between different biomarkers, we derived hazard ratios (HR) with associated 95% confidence intervals (CI) from the Cox proportional hazards model. All variables predictive for mortality in univariable analysis ($P < 0.05$) were included in our multivariable models.

We assessed the sensitivity and specificity of different biomarkers for the diagnosis of AMI by receiver operating characteristic (ROC) analysis. Logistic regression analysis was used to combine troponin T with novel markers. Receiver operating characteristic curves were compared using the method described by DeLong. To determine the place value and best cut-off of the novel biomarkers, we performed CART analyses for the diagnosis of AMI (CHAID algorithm, *P*-value adjustment by Bonferroni method). As a first step, we entered all available biomarkers as continuous variables in our model. As a second step, we added all available baseline characteristics (*Table 1*).

If not stated otherwise, continuous Gaussian variables are reported as mean ± standard deviation and compared by one-way ANOVA. Non-Gaussian variables identified by Komolgorov–Smirnov test were described as median (inter-quartile range) and tested by the Mann–Whitney *U* test for two groups or the Kruskal–Wallis H test for more than two groups. Discrete variables are reported as counts (percentages), and we tested differences between groups with the χ^2 test or Fisher exact test when expected cell sizes were <5. In the two-sided test, a *P*-value of <0.05 was regarded as significant. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA).

Results

From April 2006 to April 2008, we enrolled 794 patients, 8 patients were excluded due to missing blood samples. From the remaining patients, results from troponin T and sFlt-1 testing at presentation were available in 763 patients. Baseline characteristics of this cohort are presented in *Table 1*. The mean age in our population was 63 years, and 93% had at least one and 66% at least two cardiovascular risk factors. Acute myocardial infarction was diagnosed in 132 (17%) patients; the proportion of ST-elevation AMI was 30%. Unstable angina was the final diagnosis in 119 (16%) individuals. In the remaining 512 patients without AMI or unstable angina, the most common diagnoses were musculoskeletal and pleural pain as well as mental disorders.

Individuals with the diagnosis of AMI were significantly older, displayed a higher proportion of arterial hypertension, history of a

Table 1 Baseline characteristics

	Whole cohort (*n* = 763)	AMI (*n* = 132)	No AMI (*n* = 631)	*P*-value
Age (years)	63 (50–75)	72 (59–80)	61 (49–74)	<0.001
Male sex	501 (66)	93 (71)	408 (65)	0.20
Cardiovascular risk factors				
Arterial hypertension	462 (61)	94 (71)	368 (58)	0.006
Hyperlipidaemia	330 (43)	66 (50)	264 (42)	0.09
Diabetes mellitus	123 (16)	27 (21)	86 (15)	0.14
Current smoking	187 (25)	38 (29)	149 (24)	0.21
Body mass index (kg/m^2)	26 (24–29)	26 (24–29)	26 (24–29)	0.90
Known coronary artery disease	264 (35)	46 (35)	218 (35)	0.95
Previous myocardial infarction	193 (25)	35 (27)	158 (25)	0.72
Previous revascularization	213 (28)	31 (24)	182 (29)	0.21
Known peripheral artery disease	48 (6)	13 (10)	35 (6)	0.06
Previous stroke	47 (6)	15 (11)	32 (5)	0.006
Impaired renal function (GFR[a] <60 mL/min)	94 (12)	31 (24)	63 (10)	<0.001
Medication at presentation				
Aspirin	292 (38)	53 (40)	239 (38)	0.63
Thienopyridine	79 (10)	8 (6)	71 (11)	0.08
Beta-blocker	299 (39)	50 (38)	249 (40)	0.74
ACE inhibitor and/or ARB	303 (40)	57 (43)	246 (39)	0.37
Nitrate	88 (12)	19 (14)	69 (11)	0.26
Statin	262 (34)	43 (33)	219 (35)	0.64
ECG at presentation				
Left bundle branch block	27 (4)	12 (9)	15 (2)	<0.001
ST-segment elevation	54 (7)	38 (29)	16 (3)	<0.001
ST-segment depression	74 (10)	27 (21)	47 (7)	<0.001
T-wave inversion	52 (7)	19 (14)	33 (5)	<0.001

Data are expressed as median (inter-quartile range) or number of patients (percentage) and compared by Mann–Whitney *U* or χ^2 test.
ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.
[a]Glomerular filtration rate (calculated using the MDRD formula).

previous stroke, and impaired renal function, and had more often ischaemic changes on ECG. Levels of biomarkers were significantly higher in patients with AMI (*Table 2*), and levels of sFlt-1 and PlGF showed significant differences according to diagnosis (*Figure 1*). One-year follow-up was completed in 99.2% of patients. The composite endpoint of death or non-fatal AMI was recorded in 73 patients (9.6%; 41 deaths and 41 AMI). While levels of troponin T and hsTnT showed an excellent correlation ($r^2 = 0.99$; $P < 0.001$), levels of sFlt-1 ($r^2 = 0.02$; $P < 0.001$), and PlGF ($r^2 = 0.03$; $P < 0.001$) demonstrated only a weak correlation with troponin T.

Prognostic impact of soluble fms-like tyrosine kinase-1 and placental growth factor

Kaplan–Meier analysis according to cut-off of sFlt-1 (85 ng/L) determined by CART analysis demonstrated a low 1-year mortality in patients with sFlt-1 ≤85 ng/L of 2.5% compared with 11.9% in patients with a sFlt-1 level above the cut-off ($P < 0.001$). According to levels of PlGF at presentation (≤20 and >20 ng/L), the 1-year mortality was 1.5 and 7.4% ($P < 0.001$). Further Kaplan–Meier analyses using strata defined by cut-offs of N-terminal pro-B-type natriuretic peptide (NT-proBNP), troponin T, and hsTnT derived from CART analysis together with sFlt-1 and PlGF showed that both markers extend significantly the prognostic information of the other markers (*Figure 2*). Univariable Cox regression analysis confirmed sFlt-1 >85 ng/L (HR 5.3, 95% CI 2.7–10.2, $P < 0.001$) as well as PlGF (HR 7.6, 95% CI 2.7–21.6, $P < 0.001$) as predictors of mortality. In a multivariable analysis together with NT-proBNP >174 ng/L (HR 14.6, 95% CI 1.9–112.0, $P = 0.01$) and troponin T >0.01 μg/L (HR 1.5, 95% CI 0.7–3.0, $P = 0.29$), both markers demonstrated an independent prognostic value. The corresponding HR for sFlt-1 was 2.6 (95% CI 1.2–5.4, $P = 0.01$) and for PlGF 3.6 (95% CI 1.3–10.4, $P = 0.02$). Repeating the same analysis with hsTnT instead of troponin showed that only sFlt-1 (HR 2.6, 95% CI 1.3–5.00, $P = 0.006$) but not PlGF (HR 2.5, 95% CI 0.9–7.1, $P = 0.08$) persisted as an independent predictor for mortality. In a multivariable analysis together with all baseline characteristics predictive in univariable analysis for mortality, only sFlt-1 persisted as an independent predictor (*Table 3*). Similar findings were obtained for Kaplan–Meier and Cox analyses for mortality and non-fatal AMI (data not shown).

Diagnostic impact of soluble fms-like tyrosine kinase-1 and placental growth factor

Receiver operating characteristic analysis demonstrated that troponin T and hsTnT had with an area under the curve (AUC) of 0.88 (95% CI 0.83–0.93) and 0.96 (95% CI 0.95–0.98), respectively, the best diagnostic performance regarding the diagnosis of AMI (*Figure 3A*). The AUC of sFlt-1 and PlGF was 0.70 (95% CI 0.64–

Table 2 Investigational biomarkers at presentation

	Whole cohort ($n = 763$)	AMI ($n = 132$)	No AMI ($n = 631$)	*P*-value
Troponin T (μg/L)	0.01 (0.01–0.01)	0.09 (0.03–0.33)	0.01 (0.01–0.01)	<0.001
High-sensitive troponin T (μg/L)	0.008 (0.004–0.021)	0.116 (0.049–0.344)	0.006 (0.003–0.012)	<0.001
Creatinine kinase (U/L)	102 (72–154)	145 (96–240)	98 (70–136)	<0.001
Myoglobin (μg/L)	43 (31–64)	90 (54–228)	39 (30–55)	<0.001
NT-proBNP (ng/L)	175 (53–605)	890 (291–3315)	135 (43–376)	<0.001
Soluble fms-like tyrosine kinase-1 (ng/L)	72 (62–91)	90 (70–134)	70 (61–84)	<0.001
Placental growth factor (ng/L)	20 (16–25)	22 (19–27)	20 (16–24)	0.002

Data are expressed as median (inter-quartile range) and compared by Mann–Whitney *U* test.

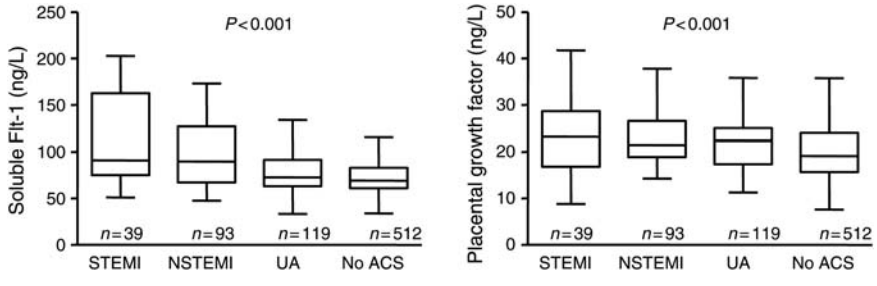

Figure 1 Levels of soluble fms-like tyrosine kinase-1 and placental growth factor according to final diagnosis. STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; UA, unstable angina; ACS, acute coronary syndrome. Box plots with Tukey whiskers; horizontal bars indicate median; extremes of boxes show 25 and 75% confidence interval. Comparison between groups by Kruskal–Wallis test.

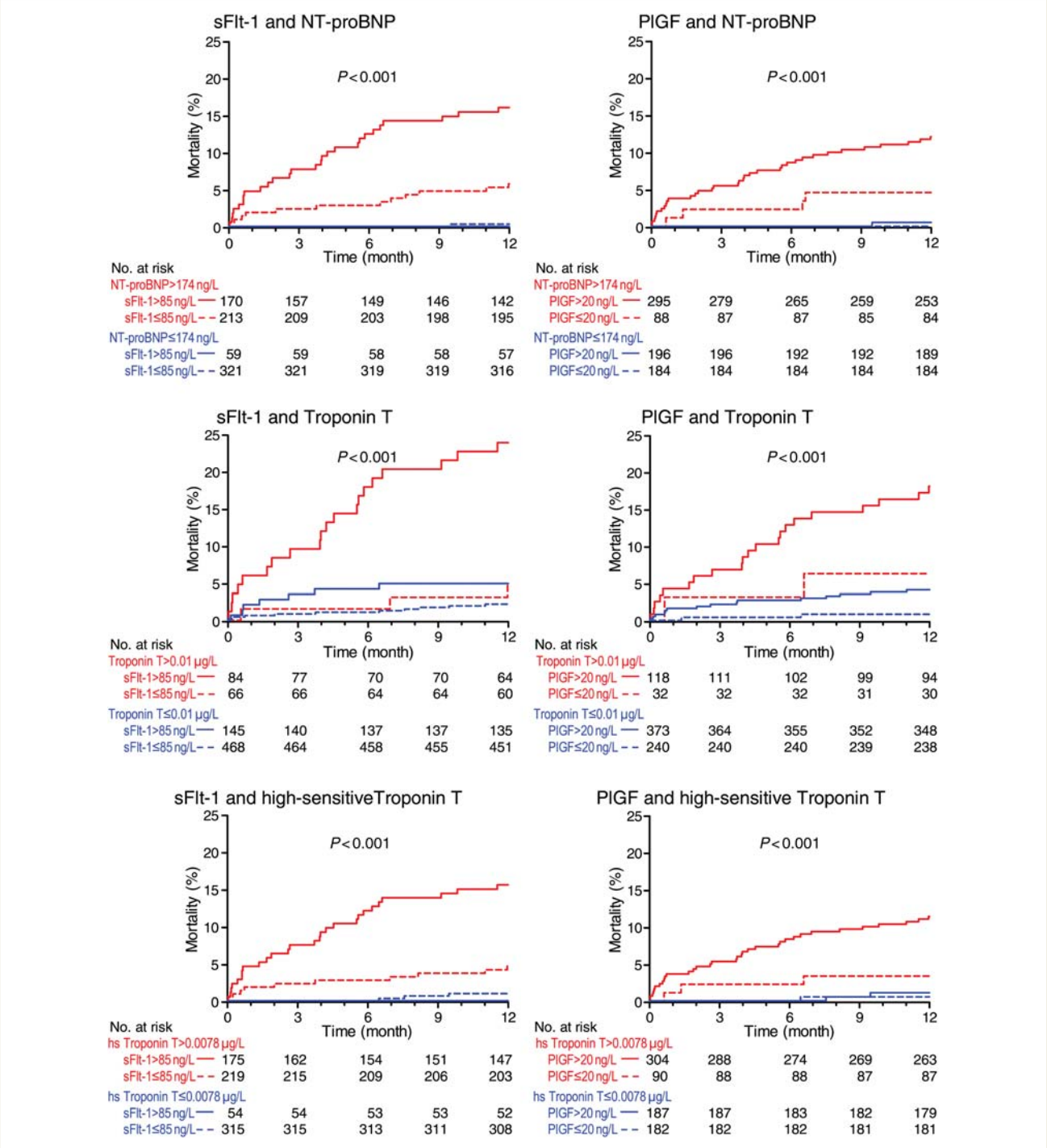

Figure 2 Mortality within 12-month follow-up. In the subgroup of troponin T ≤0.01 µg/L, *P*-value (by log-rank test) for comparison of soluble fms-like tyrosine kinase-1 strata was 0.03 and for placental growth factor strata 0.01. No significant difference in soluble fms-like tyrosine kinase-1 strata and placental growth factor strata in the subgroups of high-sensitive troponin T and N-terminal pro-B-type natriuretic peptide below cut-off. *P*-value was in all subgroups of N-terminal pro-B-type natriuretic peptide, troponin T, and high-sensitive troponin T above cut-off <0.001.

0.76) and 0.60 (95% CI 0.54–0.66), respectively. The combination of troponin T and sFlt-1 achieved, with an AUC of 0.93 (95% CI 0.90–0.96), a considerably higher diagnostic performance than troponin T alone ($P = 0.04$; *Figure 3B*). The highest difference compared with troponin T alone was found in patients presenting within 2 h after onset of symptoms (AUC 0.85 vs. 0.71; $P = 0.03$) but the dual-marker strategy was also associated with a numerical higher AUC compared with the single-marker strategy

in all strata of time (*Figure 4*). However, the AUC for hsTnT exceeded always the results of the combination of troponin T and sFlt-1, and also the combination of hsTnT with sFlt-1 did not further improve this result [AUC 0.96 (95% CI 0.95–0.98); *Table 4*].

To better assess the utility for clinical application of these novel biomarkers, we performed CART analyses to determine their place value and best cut-off. As a first step, we entered all available biomarkers in our model (*Figure 5A*). Troponin T with a cut-off of 0.01 μg/L was the best discriminator between patients with or without AMI. In patients with troponin T ≤0.01 μg/L sFlt-1 emerged as second-best predictor for AMI. Patients with an sFlt-1 level above 104 ng/L had an AMI 8.7 times more often compared with those below that cut-off. The negative predictive value of an sFlt-1 level ≤104 ng/L combined with a troponin T level ≤0.01 μg/L for ruling out of AMI with one single blood test at presentation was 98.3% (*Table 4*). With this approach, AMI could have been ruled out already at time of presentation in 509 (81%) of the 631 patients without the final diagnosis of AMI. In patients with troponin T >0.01 μg/L, a PlGF level below 22 ng/L increased the proportion of patients diagnosed with AMI to 91% compared with 73% with troponin T >0.01 μg/L alone. After entering all baseline variables into the model, we found a similar distribution (*Figure 5B*). However, as second-best discriminator between patients with or without AMI emerged the presence of ST-segment elevations both in patients with troponin T above and below the cut-off of 0.01 μg/L. Together with an sFlt-1 level ≤104 ng/L and a troponin T level ≤0.01 μg/L, patients without ST-segment elevations had a negative predictive value for AMI of 99%.

Table 3 Multivariate Cox regression analysis for mortality during follow-up

	HR	95% CI	*P*-value
Soluble fms-like tyrosine kinase-1 >84 ng/L	2.13	1.05–4.31	0.03
Placental growth factor >20 ng/L	1.95	0.64–5.92	0.24
Age (years)	1.08	1.05–1.12	<0.001
Arterial hypertension	0.51	0.22–1.18	0.11
Diabetes mellitus	1.25	0.58–2.68	0.57
Known coronary artery disease	1.88	0.69–5.13	0.22
Previous myocardial infarction	0.96	0.37–2.52	0.93
Known peripheral artery disease	1.79	0.74–4.31	0.20
Previous stroke	2.17	0.98–4.82	0.06
Impaired renal function[a]	1.45	0.71–2.96	0.31
Beta-blocker	1.87	0.93–3.77	0.08
ST-segment elevation	5.91	2.56–13.65	<0.001
ST-segment depression	2.04	0.90–4.61	0.09

[a]Impaired renal function defined as glomerular filtration rate <60 mL/min (calculated using the MDRD formula).

The CART analysis with hsTnT showed that sFlt-1 and PlGF can add some diagnostic information (*Figure 6A*). While hsTnT levels ≤0.011 μg/L excluded an AMI by 99%, levels between >0.011 and 0.031 μg/L that represent the area around the cut-off for diagnosis of AMI could be more precisely specified with the use of sFlt-1. These findings consisted also in the CART analysis including all baseline variables (*Figure 6B*).

Since ST-segment elevations turned out to be the second-best discriminator for AMI, we performed a subgroup analysis excluding patients with ST-elevation AMI to test if the diagnostic performance of the novel biomarkers stays the same. The AUC of troponin T was 0.91 (95% CI 0.87–0.96) in this subgroup, which was slightly better compared with the overall cohort. All other findings of the ROC analyses stayed similar (*Table 4*). Due to the somewhat higher sensitivity of troponin T in the subgroup without ST-elevation AMI, sFlt-1 was not found to add significant information in patient with troponin T ≤0.01 μg/L and hsTnT ≤0.011 μg/L in our CART model (data not shown).

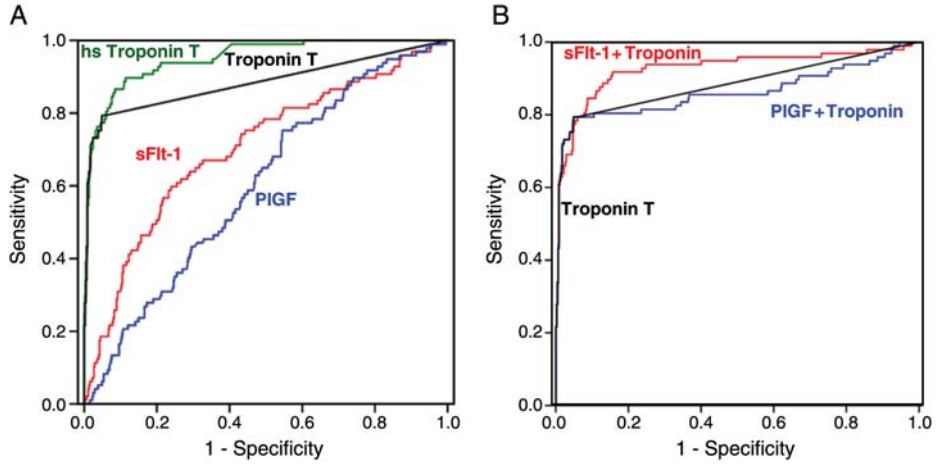

Figure 3 Receiver operating characteristic curves for the diagnosis of acute myocardial infarction. (*A*) Performance of high-sensitive troponin T, troponin T, sFlt-1, and placental growth factor. (*B*) Performance of troponin T compared with the combination of markers.

Discussion

This study has demonstrated for the first time the prognostic impact of sFlt-1 in patients with suspected AMI. Furthermore, we could present the first evidence for the potential impact of sFlt-1 and PlGF on decision making in patients with suspected AMI. We present in this manuscript three major findings: first, both markers provide important prognostic information that is independent from that of established blood markers (troponin T and NT-proBNP) in patients presenting with symptoms suggestive for AMI. Soluble fms-like tyrosine kinase-1, but not PlGF, shows independent prognostic information also after entering all baseline characteristics in our model. Second, sFlt-1 demonstrates a significant improvement of diagnostic accuracy in combination with troponin compared with troponin alone. This leads to a negative predictive value for the diagnosis of AMI of 98% with a single blood sample drawn at presentation in the ED that rises to 99% when used together with ECG results available at this point of time. Third, even when used together with a novel hsTnT assay, sFlt-1 but not PlGF provides independent prognostic information. However, due to the excellent sensitivity of hsTnT, sFlt-1 and PlGF can only add very limited diagnostic information for identification of patients with AMI, and in the subgroup of patients without ST-segment elevation AMI sFlt-1 does not further improve diagnosis of AMI.

It is important to note that, compared with many other trials investigating biomarkers in patients with suspected AMI, blood samples were drawn at the earliest point of time which was already at time of presentation to hospital, and not some time thereafter. This allows not only a direct transfer of our findings

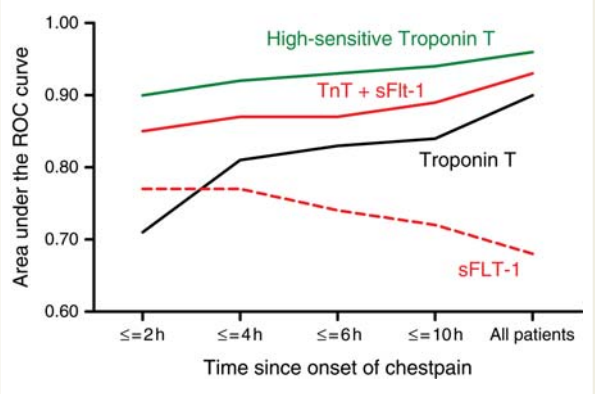

Figure 4 Area under the receiver operating characteristic curves for the diagnosis of acute myocardial infarction according to time since onset of chest pain.

Table 4 Diagnostic performance of different assays and combinations at presentation

	Sensitivity	Specificity	Negative predictive value	Positive predictive value
	Per cent (95% confidence interval)			
Entire study population (*n* = 763)				
sFlt-1, 104 ng/L	42 (33–51)	85 (81–87)	87 (84–90)	36 (30–44)
PlGF, 22 ng/L	38 (27–44)	73 (69–77)	81 (84–87)	16 (11–18)
Roche troponin T fourth generation				
Troponin T, 0.010 μg/L	83 (75–88)	94 (91–95)	96 (94–98)	73 (65–80)
+ sFlt-1, 104 ng/L	93 (87–97)	81 (77–84)	98 (97–99)	50 (44–57)
+ PlGF, 22 ng/L	89 (83–94)	70 (66–74)	96 (95–98)	38 (33–44)
Roche high-sensitive troponin T				
hs Troponin T, 0.011 μg/L	98 (93–99)	72 (68–76)	99 (98–100)	42 (37–48)
+ sFlt-1, 104 ng/L	98 (94–100)	65 (60–68)	100 (98–100)	36 (31–42)
+ PlGF, 22 ng/L	98 (93–99)	58 (54–62)	99 (97–100)	33 (28–38)
Study population without patients with ST-elevation myocardial infarction (*n* = 724)				
sFlt-1, 104 ng/L	40 (30–50)	84 (81–87)	90 (88–93)	27 (20–36)
PlGF, 22 ng/L	30 (21–41)	73 (69–76)	88 (84–90)	14 (10–20)
Roche troponin T fourth generation				
Troponin T, 0.010 μg/L	86 (77–92)	94 (91–95)	98 (96–99)	66 (57–74)
+ sFlt-1, 104 ng/L	92 (85–96)	81 (77–84)	99 (97–99)	41 (35–48)
+ PlGF, 22 ng/L	91 (83–96)	70 (66–74)	98 (96–99)	31 (26–37)
Roche high-sensitive troponin T				
hs Troponin T, 0.011 μg/L	97 (90–99)	72 (68–76)	99 (98–100)	34 (28–40)
+ sFlt-1, 104 ng/L	98 (91–100)	64 (60–68)	99 (98–100)	29 (24–34)
+ PlGF, 22 ng/L	97 (90–99)	58 (54–62)	99 (97–100)	25 (21–30)

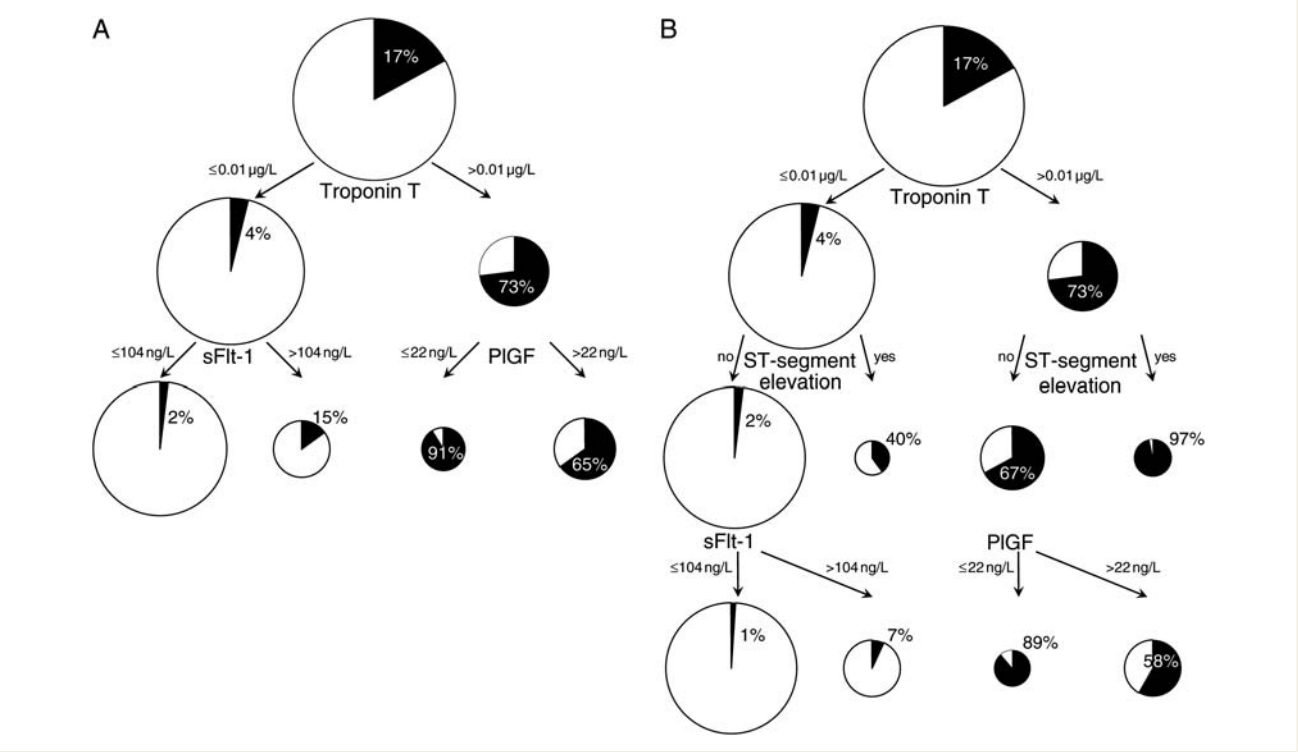

Figure 5 Classification and regression trees analyses for the diagnosis of acute myocardial infarction (*A*) Only biomarkers at presentation. (*B*) Including all variables available at presentation. Proportion of myocardial infarction displayed in black. Size of pie chart denotes the number of patients in this subgroup.

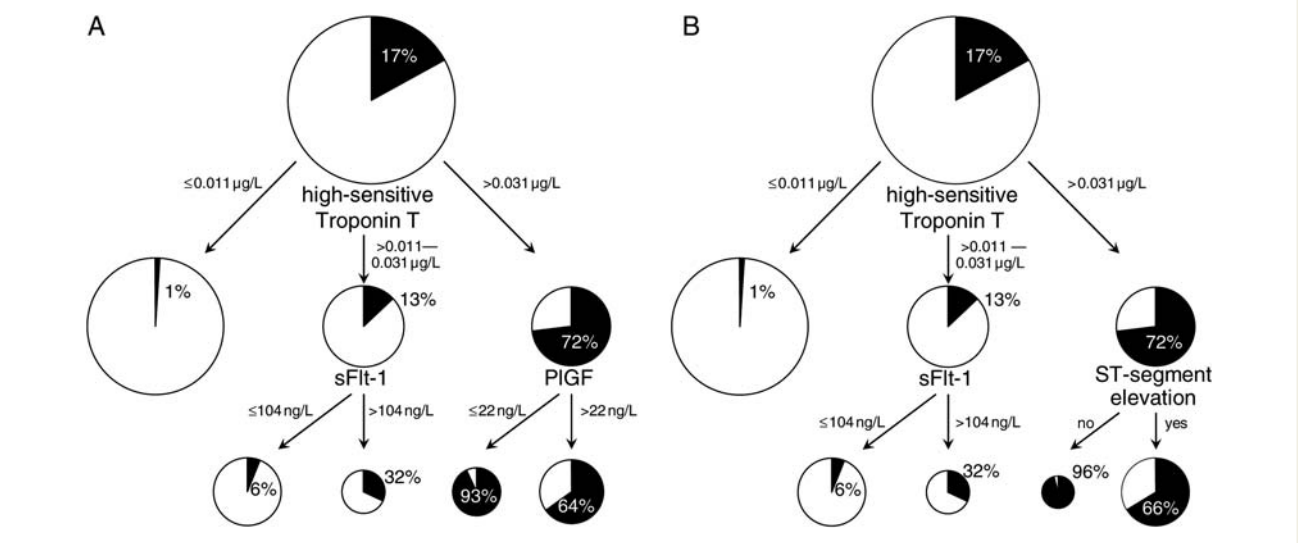

Figure 6 Classification and regression trees analyses for the diagnosis of acute myocardial infarction using a high-sensitive troponin T assay. (*A*) Only biomarkers at presentation. (*B*) Including all variables available at presentation. Proportion of myocardial infarction displayed in black. Size of pie chart denotes the number of patients in this subgroup.

to clinical practice but also makes the implementation of our results at an early stage of clinical evaluation possible.

Our findings regarding the prognostic information of PlGF are in line with a substudy from the CAPTURE (c7E3 Fab Anti-Platelet Therapy in Unstable Refractory Angina) trial, which has demonstrated that PlGF is an independent predictor for adverse outcome (death and non-fatal AMI) in patients with suspected AMI.[13] This study also demonstrated that PlGF can extend the

prognostic information derived from the traditional markers, namely C-reactive protein and soluble CD40-ligand. However, in our study, only sFlt-1 but not PlGF demonstrated prognostic information independent from baseline characteristics or hsTnT.

The findings for sFlt-1 and also for PlGF in our study together with the diagnostic impact of sFlt-1 when used together with the contemporary troponin T assay might be explained by the comprehensive involvement of both biomarkers in the development and progression of atherosclerotic disease. This is unique and unlike most biomarkers which are only involved in single processes such as inflammatory response, platelet activation, or cell necrosis. Furthermore, investigations in other diseases or syndromes such as sepsis,[21] acute lung injury,[22] neoplasms,[23] and preeclampsia[24] demonstrated a wide involvement of angiogenic and associated markers in many conditions with inflammatory processes and/or hypoxaemia. Animal studies provided first evidence that therapeutic inhibition of angiogenic pathways might be beneficial in some of these conditions.[22,23] In human medicine, such therapies might be beneficial in patients with cardiovascular diseases since they have elevated levels of such markers.[25] Placental growth factor and sFlt-1 also show a significant increase in blood levels in patients with AMI undergoing thrombolysis.[11,14] The results of our study extend the previous findings as they transfer the knowledge achieved from basic and observational research to potential important clinical implications. However, despite the findings of our study, biomarkers should be used for risk stratification and diagnosis only in conjunction with a detailed clinical assessment that can only be made by a skilled clinician taking all available parameters into consideration and not by biomarker testing alone.

Several limitations merit consideration. First, levels of new biomarkers were not determined immediately, but after samples were frozen at -80° for up to 2 years before being analysed in a batch without ever being thawed. It could be hypothesised that immediate measurement of biomarkers might have resulted in an even higher diagnostic accuracy. Second, blood cells contain certain proteins (e.g. PlGF) that might be released during sample preparation and might have biased our biomarker assessment. Third, the used assay detects bound and unbound sFlt-1. Since vascular endothelial growth factor isoforms[26] and PlGF bind to sFlt-1, a confounding bias affecting the results of sFlt-1 cannot be excluded. Fourth, since this is a prospective observational study, we cannot quantify exactly the clinical benefit associated with the increase in early diagnostic and prognostic accuracy. Interventional studies are warranted to gain this important information.

In summary, sFlt-1 and PlGF can provide important prognostic information that is independent from that of troponin T and NT-proBNP, but only for sFlt-1, also independent from hsTnT. Soluble fms-like tyrosine kinase-1 demonstrated a significant improvement of diagnostic accuracy when used together with a contemporary troponin assay, leading to a negative predictive value for the diagnosis of AMI of 98% with a single blood sample drawn at presentation to the ED. However, due to the excellent sensitivity of hsTnT, sFlt-1, and PlGF will only add limited diagnostic information for identification of patients with AMI after the introduction of this novel assay.

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