(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 February 2009 (12.02.2009)

PCT

(10) International Publication Number
WO 2009/018979 A1

(51) International Patent Classification:
G01N 33/74 (2006.01)

(21) International Application Number:
PCT/EP2008/006378

(22) International Filing Date: 1 August 2008 (01.08.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
07015271.5 3 August 2007 (03.08.2007) EP

(71) Applicant *(for all designated States except US)*: **B.R.A.H.M.S. AKTIENGESELLSCHAFT** [—/DE]; Neuendorfstrasse 25, 16761 Hennigsdorf (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BERGMANN, Andreas** [DE/DE]; Baumläuferweg 47, 12351 Berlin (DE). **STRUCK, Joachim** [DE/DE]; Zerndorfer Weg 52A, 13465 Berlin (DE). **MORGENTHALER, Nils, G.** [DE/DE]; Heiligenseestrasse 121 F, 13503 Berlin (DE). **PAPASSOTIRIOU, Jana** [DE/DE]; Sven-Hedin-Strasse 45, 14163 Berlin (DE). **BLANKENBERG, Stefan** [DE/DE]; Oppenheimer Landstrasse 31, 60596 Frankfurt (DE). **LACKNER, Karl** [DE/DE]; Draiser Strasse 157 a, 55128 Mainz (DE). **RUPPRECHT, Hans** [DE/DE]; Draiser Strasse 84b, 55128 Mainz (DE). **BICKEL, Christoph** [DE/DE]; Auf der Ochsenhell, 56072 Koblenz (DE).

(74) Agent: **ANDRAE, Steffen**; Andrae Flach Haug, Balanstrasse 55, 81541 München (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

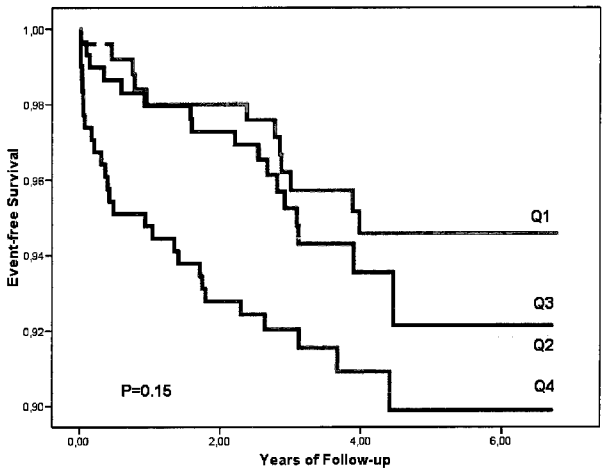
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METHOD FOR RISK STRATIFICATION IN STABLE CORONARY ARTERY DISEASE

Figure 1

Kaplan-Meier survival curves showing cardiovascular events according to quartiles of procalcitonin



(57) Abstract: In vitro method for the risk stratification of patients with stable arteriosclerosis, especially stable coronary artery disease, wherein the concentration of procalcitonin is determined in the circulation of such patients using a highly sensitive PCT assay, and wherein within the range of PCT concentrations in the typical normal range of healthy individuals cutoff values are defined which distinguish groups of individual patients with stable arteriosclerosis in accordance with the personal cardiac risk, and the patients are allotted to one of said risk groups on the basis of their individual PCT concentrations

WO 2009/018979 A1

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*

Method for risk stratification in stable coronary artery disease

The present invention discloses a new method for patient stratification in stable coronary disease (coronary disease: CAD) according to the patients' individual cardiac risks. Patients in the condition of a stable CAD are typically patients with angiographically proven CAD, i.e. with affected coronary arteries, with plaques on the inner walls of the coronary artery (atherosclerosis) and stenosis in a major coronary artery. CAD is considered a serious cardiac risk. CAD patients are considered as "stable" if the CAD does not manifest itself in the form of acute cardiovascular events.

In view of the immanent risk of future cardiovascular events it would be highly desirable to be able to distinguish within the group of patients with stable CAD between different groups according to their personal cardiac risk such that an "individual state of alert" can be determined for a particular patient in accordance with the risk group to which he has been allotted. Such grouping of patients is usually called "stratification".

Distinguishing high risk patients from patients at moderate or low risk would allow a better selection of the most appropriate therapeutic strategy for a particular patient, avoiding, for example, underestimation of the cardiac risk and undermedication of high risk patients on the one hand and unnecessary therapeutic interventions, and the associated costs, with low risk patients on the other.

It is, therefore, an object of the present invention to provide a new method by which patients with stable CAD can be stratified in accordance with their personal cardiac risks, i.e. with respect to their individual risks concerning the future incidence of cardiovascular events.

As is further explained in detail below, the inventors have conducted a study to evaluate the potential usefulness of a number of analytes (biomolecules, biomarkers) which can be determined in the circulation of patients for a stratification of patients with stable CAD.

In the course of said study they have surprisingly found that a highly sensitive measurement of the concentration of the peptide procalcitonin (PCT) in the circulation of CAD patients in the range of very low physiological concentrations, which concentrations up to now were considered as being below diagnostic significance and, therefore, concentrations typical for normal healthy individuals, allows a useful stratification of CAD patients, and that the usefulness of such highly sensitive PCT determination can even be increased if the results obtained fro PCT are evaluated in combination with the results of the measurement of an analyte of another type action (a vasoactive analyte), exemplified by the so-called B-type (or brain) natriuretic peptide BNP.

Accordingly, the present inventions discloses a method as claimed in any of claims 1 to 6 , and the use of a highly sensitive determination of PCT in the context of the prognosis of cardiovascular diseases for the risk stratification of patients, especially in arteriosclerosis and CAD, according to claims 7 and 8 respectively.

Procalcitonin (PCT), which is to be measured in accordance with the present invention, has become a well-established biomarker for sepsis diagnosis: PCT reflects the severity of bacterial infection and is in particular used to monitor progression of infection into sepsis, severe sepsis, or septic shock. It is possible to use PCT to measure the activity of the systemic in-

flammatory response, to control success of therapy, and to estimate prognosis (1) (2) (3) (4) (5). The increase of PCT levels in patients with sepsis correlates with mortality (6).

Whereas an increasing number of studies investigates the potential role of PCT in other infectious diseases like pneumonia, bacterial meningitis and malaria (7) (8) (9), no studies reported yet about the potential use of PCT in risk stratification of patients suffering from stable coronary artery disease (CAD). In vitro-studies showed, that PCT plays an important role during monocyte adhesion and migration and further has an effect on inducible nitric oxide synthase (iNOS) gene expression (10) (11) (12). The association between PCT levels and low-grade inflammation of the arterial wall in atherosclerosis and the potential effect on endothelial dysfunction has not been analyzed. Our prospective study examined the prognostic impact of PCT in a large group of consecutively enrolled stable angina patients on cardiovascular outcome to evaluate the potential clinical applicability of PCT measurements in CAD.

In the context of sepsis and related conditions, where the concentrations of PCT reach rather high physiological concentrations, PCT has been measured traditionally by means of an assay of the sandwich type using two monoclonal antibodies binding to different portions of the PCT molecule so that essentially only the complete PCT molecule is detected (see, for example, (1)). The typical functional assay sensitivity (FAS) of the typical two-sided chemiluminescence assay for PCT is 300 ng/L (0.3 ng/ml or 0.3 µg/L).

More recently new highly sensitive assays for the determination of PCT have been developed (28). The functional assay sensitivity (FAS, interassay CV <20%) of this new assay was < 7 ng/l PCT. Using this assay, typical PCT concentrations in healthy individuals could be determined. In 500 healthy individuals the range was <7 to 63 ng/L (< 0.007 to 0.063 ng/ml), i.e. a range of concentrations well below 0.1 ng/ml. The determined median was 13.5

In further improved form said sensitive PCT assay is available as PCT sensitive LIA (B.R.A.H.M.S AG, Hennigsdorf, Germany) having an analytical assay sensitivity of 0.01 ng/ml and a functional assay sensitivity (FAS) of at least 0.05 ng/ml. A related assay for the time-resolved amplified cryptate emission (TRACE) technology (Kryptor PCT, B.R.A.H.M.S AG, Hennigsdorf) has a functional assay sensitivity of 0.06 µg/L (0.06 ng/ml).

The more recent sensitive PCT assays have predominantly been used in connection with the guidance of antibiotic therapy in lower respiratory tract infections (community-acquired pneumonia, CAP; exacerbations of chronic obstructive pulmonary disease, COPD: see (29), (30), (31)). In the case of CAP antibiotic treatment is recommended on the basis of measured PCT concentrations as follows: strongly encouraged, greater than 0.5 µg/L; encouraged, greater than 0.25 µg/L; discouraged, less than 0.25 mg/L; strongly discouraged, less than 0.1 µg/L (29). In other words, concentrations of 0.1 µg/L (or 0.1 ng/ml) are considered as concentrations typical for healthy individuals.

The method of risk stratification of patients with stable coronary artery disease (stable CAD) is based on a differential evaluation of measured PCT concentrations which are below the value of 0.1 ng/ml for healthy individuals and which so far have not been used for diagnostic of prognostic purposes.

The invention is discussed in more detail in the following sections and the Figures 1 to 3 and Tables 1 to 7 mentioned therein. The Tables mentioned are found on separate pages at the end of the text of the description.

In the Figures:

Figure 1: shows Kaplan-Meier survival curves showing cardiovascular events according to quartiles of procalcitonin;

Figure 2: shows Kaplan-Meier survival curves showing cardiovascular events according to procalcitonin cut-off value 0.05 ng/ml;

Figure 3: Kaplan-Meier survival curves showing cardiovascular events according to procalcitonin and B-type natriuretic peptide in combined analysis:

Methods

Study population

Between November 1996 and January 2004, 3326 patients with angiographically proven CAD and at least one stenosis ≥30% diagnosed in a major coronary artery at the Department of Medicine II of the Johannes Gutenberg University in Mainz or the Department of Medicine of the German Federal Armed Forces Central Hospital in Koblenz were enrolled in the Athero*Gene* registry. Further details on the concept of the Athero*Gene* study have been described previously (13).

In the present substudy, exclusion criteria were clinical signs of acute coronary syndrome (unstable Angina Braunwald classification class B or C, acute ST-segment elevation, and non-ST-segment elevation myocardial infarction). Patients with coronary artery bypass surgery or coronary revascularization during the last four weeks were also excluded. Further reasons for exclusion were evidence of hemodynamically significant valvular heart disease, surgery or trauma within the previous month, known cardiomyopathy, manifest carcinoma, chronic inflammatory disease, febrile conditions, or use of oral anticoagulant therapy within the previous four weeks.

The history of classical risk factors was assessed as follows. Patients receiving anti-hypertensive treatment or having already confirmed the diagnosis of hypertension (blood pressure above 160/90 mmHg) were considered to have hypertension. Hyperlipoproteinemia was diagnosed in patients under lipid-lowering medication or with a history of cholesterol levels ≥240 mg per deciliter. Patients were classified as currently smoking, as having smoked in the past (if they had stopped more than 4 weeks and less than 40 years earlier), or as having never smoked (if they had never smoked or had stopped 40 or more years earlier). We considered patients receiving dietary treatment or medication for diabetes or whose current fasting blood glucose level was above 125 mg per deciliter to suffer from diabetes mellitus.

1124 patients were followed up during a median period of 3.8 (maximum 6.8) years. Patients either presented themselves at our clinic (78.2%) or were interviewed by telephone by trained medical staff. Follow-up information, including death from cardiovascular causes (n=40), death from causes not related to coronary artery disease (n=30), and non-fatal myocardial infarction (n=32), was obtained from hospital or general-practitioner charts. The primary endpoint was non-fatal myocardial infarction and cardiovascular death.

The Athero*Gene* study was approved by the local ethics committee of the University of Mainz. Study participants had German nationality and, particularly, were inhabitants of the Rhein-Main-Area. All patients were caucasian. Participation was voluntary, and patients were enrolled after written informed consent was obtained.

Laboratory methods

Blood samples were drawn under standardized conditions before performance of coronary angiography. Samples were taken when patients entered the catheterization lab after a minimum 12h-fast. Serum lipid levels were measured immediately. Lipid levels were measured using routine methods (total cholesterol and triglycerides, Roche Diagnostics GmbH, Mannheim, Germany; high density lipoprotein cholesterol, Ralf Greiner Biochemica, Mann-

heim, Flacht bei Limburg, Germany; and low-density lipoprotein cholesterol calculated according to the Friedewald formula). The LDL-/HDL-ratio was computed by dividing LDL by HDL levels.

Plasma and serum samples were centrifuged at 4000 g for 10 minutes, divided into aliquots, and stored at –80°C until analysis. PCT was determined by a highly sensitive immunoluminometric assay (B.R.A.H.M.S PCT sensitive; B.R.A.H.M.S AG, Hennigsdorf, Germany; analytical assay sensitivity: 0.01 ng/ml; functional assay sensitivity (20% inter-assay variation coefficient): 0.05 ng/ml). Data were generated using one lot of chemicals. C-reactive protein (CRP) was analyzed by a latex particle-enhanced immunoassay (Roche Diagnostics, Mannheim, Germany; detection range: 0.1 to 20 mg/l; interassay coefficient of variation, 1.0 percent for values of 15 mg per liter and 6.5 percent for values below 4 mg per liter). Plasma B-type natriuretic peptide (BNP) was determined using a fluorescence immunoassay (Biosite, San Diego, California, USA; detection range: 5 to 5000 pg/ml; interassay coefficient of variation of near 10%; negligible cross-reactivity with other natriuretic peptides). All laboratory measurements were performed in a blinded fashion without knowledge of the clinical status of the patient.

Statistical considerations

The mean values (± standard deviation) and proportions of baseline cardiovascular risk factors, clinical variables, and biomarkers were calculated for study participants according to quartiles of procalcitonin. Due to the small range of the procalcitonin levels, the quartiles comprise not the same number of patients. Variables with a skewed distribution (|skewness| >1) were presented as medians with quartiles. Correlation analysis was done by Spearman rank correlation. In another analysis hazard ratios for the highest versus other quartiles of PCT were dichotomized according to classical risk factors or medians of clinical variables and biomarkers.

The association of the biomarkers PCT and BNP with the primary endpoint according to quartiles was analyzed in different models by Cox regression analysis, the first model adjusting for age and sex and the second adjusting for the potential confounders and classical risk factors (age, sex, body-mass index, hypertension, diabetes mellitus, smoking status, LDL-/HDL-ratio, number of diseased vessels, beta-blocker and statin therapy). The cumulative event plots according to quartiles of PCT concentration were estimated by the Kaplan-Meier method and were compared by use of the log rank test. All survival analyses were conducted for the primary end point of non-fatal myocardial infarction or cardiovascular death. Data of patients who died from causes not related to cardiovascular disease were censored at the time of death.

PCT and BNP were log-transformed to enhance model fit. To compare the predictive power of these biomarkers, hazard ratios per one standard deviation increment were calculated in univariate and multivariate analysis (adjusted for classical risk factors and clinical variables). A backward stepwise Cox regression approach was taken for the multivariate analyses with P=0.10 as the critical value for entering and excluding ten variables (age, sex, body-mass index, hypertension, diabetes, smoking, LDL-/HDL-ratio, number of diseased vessels, statin and beta-blocker therapy) in the model.

Hazard ratios (HR) and 95% confidence interval (CI) are reported with 2-tailed probability values. Proportional hazards assumption was checked using standard methods based on testing for significant slope of the smooth curve through the scatter of the rescaled Schoenfeld residuals versus time.

To further assess the predictive ability of the models, the inventors considered the cardiovascular endpoint at two years as a binary variable and logistic regression was performed. Associated receiver operating characteristic (ROC) curves for pred0icted probabilities were drawn for a basic model containing classical risk factors and models additionally containing

PCT, CRP and BNP. The corresponding areas under the curve along with 95% CI were calculated.

The inventors further evaluated the combined role of PCT and BNP on cardiovascular risk and therefore tested for interaction followed by a dichotomized analysis of both variables by using the highest quartile as cut-off point. Hazard ratios and 95% CI, along with P-values, were reported. Another cumulative event plot was estimated by the Kaplan-Meier method for these four subgroups and compared using the log rank test.

As P-values are not adjusted for multiple testing, they have to be considered as descriptive. All calculations were carried out using SPSS 15.0 for Windows, version 15.0.1 (SPSS Inc., Chicago, Illinois, USA).

Results

The mean age of the study population was 61.3 ± 9.5 years, and 80.5 % of the patients were male. The patients in the present substudy were divided into four groups according to quartiles of PCT levels (00Table 1). No significant differences for the distribution of the classical risk factors were found. CRP levels were higher in the fourth quartile than in the others (3.66 mg/l vs. 1.81-2.10 mg/l). A moderate correlation between PCT and CRP (r=0.27) was found.

The inventors further evaluated the predictive value of PCT in subgroup analysis (Table 2). Median levels were used to dichotomize continuous variables. In particular, PCT levels were strongly predictive in patients with BNP serum concentration above the median of 37.48 pg/ml w0ith a 2.41-fold increased risk (95% CI 1.32-4.42; P=0.004) for the highest PCT quartile.

Table 3 outlines the association between PCT, CRP and BNP with future cardiovascular events. The percentage of events increased across quartiles (Figure 1) such as patients in

the highest quartile of PCT were associated with an 2.27-fold (95% CI: 1.14-4.51; P=0.02) increase in risk for future cardiovascular events in age- and sex-adjusted model. This association remained significant in models adjusting for most potential confounders. If analyzed as continous variable, an increment of one standard deviation (SD) of PCT revealed a 1.33 higher (95% CI: 1.02-1.74; P=0.04) risk for future cardiovascular events. Levels of BNP have been related independently to the primary endpoint, whereas no significant association between CRP and cardiovascular outcome could be observed in the fully adjusted model.

All classical risk factors and clinical variables were entered in a backward multiple stepwise regression analysis as outlined in table 4. Continous variables were log-transformed and have been treated per increment of one SD. PCT (HR 1.30, 95% CI: 1.00-1.70; P=0.05) was selected as an independent predictor of cardiovascular risk. The final model also revealed LDL-/HDL-ratio, female gender and insulin-dependent diabetes mellitus (IDDM) as predictors for the primary endpoint.

In another subanalysis, a PCT level of 0.05 ng/ml (according to the functional assay sensitivity) was chosen as cut-off value for cardiovascular risk prediction. 25% of the 39 patients with PCT levels above 0.05 ng/ml experienced cardiovascular events and had a significant poorer prognosis (Figure 2). When entered in a backward multiple stepwise regression analysis, a PCT level above 0.05 ng/ml is associated with a 4.22 higher (95% CI: 2.07-8.59; P<0.001) cardiovascular risk (Table 5).

To further explore whether PCT and BNP added information beyond that obtained from classical risk factors, the inventors computed the area under the ROC curve (AUC) associated with prediction of different logistic regression models, considering the cardiovascular endpoint at 2 years as a binary variable (Table 6). As there were patients with a follow-up of less than 2 years, only 1057 patients were available for this analysis; 46 of them experienced a cardiovascular event. The basic model including classical risk factors such as age, sex, BMI

hypertension, diabetes mellitus, smoking status, LDL-/HDL-ratio, number of diseased vessels, beta-blocker and statin therapy revealed an AUC of 0.74 (95% CI: 0.67-0.81). Table 6 presents analyses comparing this basic model with models additionally including either PCT, or BNP, or two, or all of them. Inclusion of one SD increase of PCT improved the predictive value of the basic model, reporting an increase from 0.74 to 0.77. One SD increase of BNP revealed most additional information beyond application of the basic model. The combination of BNP and PCT with the basic model resulted in the highest prognostic accuracy of this model. Results were similar during a 1-year and 3-year follow-up (data not shown).

Because of the strong predictive value of PCT in patients with BNP levels above the median (Table 2) and the high prognostic accuracy for cardiovascular events when BNP and PCT were combined in a model with classical risk factors (Table 6), the inventors finally explored to what extent PCT might add to the prognostic value of BNP (Table 7). With the test for interaction being negative (P=0.78), the inventors assumed an additive effect for both biomarkers. Patients with elevated levels of both biomarkers in the upper quartile were at the highest risk for future cardiovascular events (HR 7.04; 95% CI: 3.40-14.57; P<0.001). Figure 3 provides the Kaplan-Meier survival curves according to levels of PCT and BNP in combined analysis.

Discussion

In this prospective cohort of patients with angiographically documented CAD an independent association of PCT with future cardiovascular events has been demonstrated. This association did not change appreciably after adjustment for most potential confounders, indicating that PCT provides important information about cardiovascular prognosis in addition ot classical risk factors and other clinical variables. Combined analysis of PCT and BNP improved the prognostic accuracy for future cardiovascular events in the present study.

Yet, only two studies evaluated the potential use of PCT in the setting of CAD. Erren et al. (14) found slightly increased PCT levels only in CAD patients with additional peripheral arterial disease (PAD) and discussed PCT as a marker for the atherosclerotic burden in a multimarker approach.

Ilhan et al. (15) found higher PCT levels (0.40 ± 0.04 ng/ml as opposed to 0.19 ± 0.02 ng/ml in the control group) among CAD patients experiencing a cardiovascular event. Herein the inventors demonstrated PCT as an independent predictor of future cardiovascular events, in particular adding information on patients with high BNP levels, indicating a potential of PCT to better stratify in high risk individuals.

Local or systemic inflammation affecting certain types of tissue, also a trauma-related host response (16) (17) (18) (19) (20), and consecutive monocytic activation thus are a prerequisite for PCT production (4). The expression of PCT messenger-RNA by peripheral blood mononuclear cells (21) is stimulated in vitro not only by lipopolysaccharides, but also by the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which seem to play a pivotal role in the atherosclerotic process (22) (23) (24) (25). Thus, slightly increased PCT levels might be an epiphenomenon of the inflammatory activity within the vascular wall caused by atherosclerosis. However, PCT seems to play a causative role in monocytic activation: as a chemoattractant initially only produced in adherent monocytes, which recruit later parenchymal cells of the inflammated tissue for further PCT production. Thus, PCT could have also an effect on leukocyte migration (4) (10) (11) (14). Further studies have to investigate, if the in vitro demonstrated stimulating effect of PCT on nitric oxide synthesis gene expression (12) also has an influence on endothelial dysfunction caused by atherosclerosis.

In the present study, patients with combined elevation of PCT and BNP levels in the upper quartile had a 7.9 fold increased risk for the primary endpoint compared to a 3.2 fold in

creased risk when BNP was elevated alone. Thus, PCT significantly improved the AUC of the basic model (classical risk factors) for risk prediction and even obtained additional information on top of the biomarker BNP.

BNP is an established marker for left ventricular dysfunction. The sepsis marker PCT might also be a marker for low-grade inflammation, in particular for monocyte activation or endothelial dysfunction, as shown in several in vitro-studies. The representation of two different pathomechanisms by BNP and PCT would explain the improvement of the prognostic accuracy for future cardiovascular events. Further studies are needed to evaluate the role of PCT for risk prediction in diseases other than severe bacterial infections and to elucidate more its pathophysiological role in the inflammatory cascade.

As the choice of the cutoffs for the analyses in table 7 and figure 3 is data driven, the results can only give hints, but have to be validated in independent studies.

In conclusion, PCT is independently related to future cardiovascular events in a population of CAD patients and might add information for risk stratification, in particular, in high risk individuals.

Summary:

Background: Procalcitonin (PCT) is a well-established biomarker for the diagnosis and therapeutic monitoring of sepsis. In vitro-studies showed that PCT has an effect on monocyte activation and even on nitric oxide synthesis. The present prospective study examined the prognostic impact of PCT in patients with established coronary artery disease (CAD) on cardiovascular outcome.

Methods: In a substudy of the prospective Athero*Gene* survey, in 1124 patients with stable CAD, the risk of cardiovascular death and non-fatal myocardial infarction (N=72) over a median follow-up of 3.8 (maximum 6.8) years according to the baseline concentration of PCT has been assessed.

Results: The age- and sex-adjusted hazard ratio for patients within the highest quartile of PCT related a 2.27-fold increase (95% confidence interval (CI): 1.14-4.51; P=0.02) of the relative risk for cardiovascular death and non-fatal myocardial infarction, when compared to the first quartile. Adjustment for classical risk factors and clinical variables did not attenuate this relationship. Inclusion of one standard deviation increase of PCT improved the predictive value of a basic model (classical risk factors) for cardiovascular risk prediction monitored by the area under the curve (AUC) of an % CI: 3.40-14.57; P<0.001) higher cardiovascular risk.

Conclucions: Baseline concentration of PCT is independently related to future cardiovascular events in patients with stable CAD

References

1. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515-8.

2. Clec'h C, Ferriere F, Karoubi P, et al. Diagnostic and prognostic value of procalcitonin in patients with septic shock. Crit Care Med 2004;32:1166-9.

3. Lee YJ, Park CH, Yun JW, Lee YS. Predictive comparisons of procalcitonin (PCT) level, arterial ketone body ratio (AKBR), APACHE III score and multiple organ dysfunction score (MODS) in systemic inflammatory response syndrome (SIRS). Yonsei Med J 2004;45:29-37.

4. Meisner M. Biomarkers of sepsis: clinically useful? Curr Opin Crit Care 2005;11:473-80.

5. Wunder C, Eichelbronner O, Roewer N. Are IL-6, IL-10 and PCT plasma concentrations reliable for outcome prediction in severe sepsis? A comparison with APACHE III and SAPS II. Inflamm Res 2004;53:158-63.

6. Oberhoffer M, Vogelsang H, Russwurm S, Hartung T, Reinhart K. Outcome prediction by traditional and new markers of inflammation in patients with sepsis. Clin Chem Lab Med 1999;37:363-8.

7. Bugden SA, Coles C, Mills GD. The potential role of procalcitonin in the emergency department management of febrile young adults during a sustained meningococcal epidemic. Emerg Med Australas 2004;16:114-9.

8. Chiwakata CB, Manegold C, Bonicke L, Waase I, Julch C, Dietrich M. Procalcitonin as a parameter of disease severity and risk of mortality in patients with Plasmodium falciparum malaria. J Infect Dis 2001;183:1161-4.

9. Schwarz S, Bertram M, Schwab S, Andrassy K, Hacke W. Serum procalcitonin levels in bacterial and abacterial meningitis. Crit Care Med 2000;28:1828-32.

10. Linscheid P, Seboek D, Schaer DJ, Zulewski H, Keller U, Muller B. Expression and secretion of procalcitonin and calcitonin gene-related peptide by adherent monocytes and by macrophage-activated adipocytes. Crit Care Med 2004;32:1715-21.

11. Wiedermann FJ, Kaneider N, Egger P, et al. Migration of human monocytes in response to procalcitonin. Crit Care Med 2002;30:1112-7.

12. Hoffmann G, Czechowski M, Schloesser M, Schobersberger W. Procalcitonin amplifies inducible nitric oxide synthase gene expression and nitric oxide production in vascular smooth muscle cells. Crit Care Med 2002;30:2091-5.

13. Blankenberg S, Rupprecht HJ, Bickel C, et al. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003;349:1605-13.

14. Erren M, Reinecke H, Junker R, et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. Arterioscler Thromb Vasc Biol 1999;19:2355-63.

15. Ilhan F, Akbulut H, Karaca I, Godekmerdan A, Ilkay E, Bulut V. Procalcitonin, c-reactive protein and neopterin levels in patients with coronary atherosclerosis. Acta Cardiol 2005;60:361-5.

16. Meisner M, Tschaikowsky K, Hutzler A, Schick C, Schuttler J. Postoperative plasma concentrations of procalcitonin after different types of surgery. Intensive Care Med 1998;24:680-4.

17. Meisner M, Rauschmayer C, Schmidt J, et al. Early increase of procalcitonin after cardiovascular surgery in patients with postoperative complications. Intensive Care Med 2002;28:1094-102.

18. Meisner M, Adina H, Schmidt J. Correlation of procalcitonin and C-reactive protein to inflammation, complications, and outcome during the intensive care unit course of multiple trauma patients. Crit Care 2006;10:R1

19. Wanner GA, Keel M, Steckholzer U, Beier W, Stocker R, Ertel W. Relationship between procalcitonin plasma levels and severity of injury, sepsis, organ failure, and mortality in injured patients. Crit Care Med 2000;28:950-7.

20. de Werra I, Jaccard C, Corradin SB, et al. Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. Crit Care Med 1997;25:607-13.

21. Oberhoffer M, Stonans I, Russwurm S, et al. Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis-related cytokines in vitro. J Lab Clin Med 1999;134:49-55.

22. Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W. Tumor necrosis factor alpha (TNF-alpha)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. J Exp Med 1993;177:1277-86.

23. Reilly MP, Rohatgi A, McMahon K, et al. Plasma cytokines, metabolic syndrome, and atherosclerosis in humans. J Investig Med 2007;55:26-35.

24. Schlitt A, Heine GH, Blankenberg S, et al. CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. Thromb Haemost 2004;92:419-24.

25. Tipping PG, Hancock WW. Production of tumor necrosis factor and interleukin-1 by macrophages from human atheromatous plaques. Am J Pathol 1993;142:1721-8.

26. Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. Circulation 2005;112:3375-83.

27. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.

28. Nils G. Morgenthaler et al., Sensitive Immunoluminometric Assay for the Detection of Procalcitonin, Clinical Chemistry 48, No 5, 2002: 788-790

29. Mirjam Christ-Crain et al., Procalcitonin Guidance of Antibiotic Therapy in Community-acquired Pneumonia. American Journal of Respiration and Critical Care Medicine, VOl. 174; 2006; 84-93.

30. Diana Stolz et al., Antibiotic treatment of Exacerbations of COPD. Chest 131, 1, January 2007: 9-19.

31. Mirjam Christ-Crain et al., Effect of procalcitonin-guided tretament on antibiotic use and outcome in lower respiratory tract infections; cluster-randomised, single-blinded intervention trial. Lancet 2004, 363: 600-607

Table 1

Baseline characteristics of the study population according to quartiles

Characteristic	Q1 (N=254)	Q2 (N=259)	Q3 (N=301)	Q4 (N=310)
Procalcitonin (ng/ml)	**<0.010**	**0.010-0.013**	**0.014-0.020**	**>0.020**
Age (yrs)	60.6 ± 10.0	60.5 ± 10.0	61.3 ± 9.5	62.5 ± 8.6
Male sex (%)	72.0	80.3	86.0	82.3
Traditional risk factors				
Body-mass index (kg/m²)	27.0 ± 4.0	27.4 ± 3.8	27.9 ± 3.7	28.4 ± 4.1
Hypertension (%)	80.3	75.7	79.1	83.2
Diabetes mellitus				
- Dietetic treatment (%)	4.3	2.7	3.7	6.1
- Drug treatment (%)	7.5	8.1	10.3	11.3
- Insulin dependent (%)	8.7	5.8	6.6	12.9
Smoking Status				
- Never smoked (%)	37.8	37.8	38.2	32.3
- Ex-smoker (%)	42.9	44.4	46.2	51.3
- Current smoker (%)	19.3	17.8	15.6	16.5
Lipid status				
- LDL cholesterol (mg/dl)	128.0 (98.0-157.8)	123.0 (94.0-144.0)	118.0 (92.5-143.0)	119.0 (95.8-150.0)
- HDL cholesterol (mg/dl)	53.0 (43.0-64.0)	49.0 (43.0-58.0)	47.0 (40.5-56.0)	45.0 (39.0-55.0)
- Triglycerides (mg/dl)	122.0 (93.8-168.0)	117.0 (88.0-156.0)	129.0 (97.0-188.0)	148.0 (103.0-201.5)
Clinical variables				
Multi-vessel disease (%)	74.4	67.2	72.4	72.9
History of myocardial infarction (%)	42.5	44.4	47.5	38.1
Left ventricular ejection fraction (%)	65.0 (53.5-73.0)	69.5 (60.0-77.0)	66.0 (55.0-74.0)	70.0 (58.0-78.0)
Medication				
Beta-blocker (%)	58.7	62.2	70.8	62.3
Statin (%)	53.1	51.8	61.4	56.8
ACE-inhibitor (%)	62.2	55.6	58.8	59.0
Biomarkers				
C-reactive protein (mg/l)	1.85 (0.97-3.79)	1.81 (0.98-4.18)	2.10 (1.17-4.02)	3.66 (1.76-8.15)
B-type natriuretic peptide (pg/ml)	38.97 (13.79-98.87)	40.94 (11.86-92.80)	37.64 (12.02-102.15)	33.97 (9.88-101.11)

Data presented are percentage of patients, mean ± standard deviation, or median and 25th/75th inter-quartile range for skewed (|skewness| >1) variables. Left-ventricular ejection fraction was available for 818 patients and B-type natriuretic peptide for 1032 patients. LDL denotes low-density lipoprotein, HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586; to convert values for triglycerides to millimoles per liter, multiply by 0.01129.

Table 2

Hazard ratios (95% confidence interval) for highest versus other quartiles of procalcitonin according to traditional risk factors (age- and sex-adjusted)

Variable		HR (95% CI)	P-value
Age*	≤63 yrs	1.92 (0.98-3.78)	0.06
	>63 yrs	1.56 (0.77-3.13)	0.21
Sex**	Female	1.81 (0.73-4.51)	0.20
	Male	1.72 (0.97-3.06)	0.07
Body-mass index	≤26.4 kg/m²	2.22 (0.98-5.01)	0.06
	>26.4 kg/m²	1.54 (0.83-2.83)	0.17
Hypertension	No	1.16 (0.38-3.53)	0.80
	Yes	1.98 (1.14-3.43)	0.02
Diabetes mellitus	No	1.64 (0.88-3.09)	0.12
	Yes	1.93 (0.86-4.31)	0.11
Smoking	No	1.71 (0.74-3.97)	0.21
	Active	1.78 (0.98-3.25)	0.06
LDL-/HDL-ratio	≤3	1.51 (0.79-2.89)	0.21
	>3	2.18 (1.01-4.72)	0.05
Multi vessel disease	No	2.73 (0.99-7.57)	0.05
	Yes	1.58 (0.90-2.77)	0.11
C-reactive protein	≤2.34 mg/l	1.48 (0.55-3.97)	0.44
	>2.34 mg/l	1.58 (0.88-2.83)	0.13
B-type natriuretic peptide	≤37.48 pg/ml	1.19 (0.50-2.86)	0.70
	>37.48 pg/ml	2.41 (1.32-4.42)	0.004

Median levels were used to dichotomize continous variables.
* adjusted for sex only ** adjusted for age only

Table 3

Hazard ratios for future cardiovascular events according to quartiles of baseline pro-calcitonin, C-reactive protein and B-type natriuretic peptide

		N	Events (%)	HR (95% CI) **age-/sex-adjusted**	P-value	HR (95% CI) **fully adjusted***	P-value
Procalcitonin (ng/ml)							
Q1	<0.010	254	12 (5%)	1		1	
Q2	0.010-0.013	259	16 (6%)	1.51 (0.71-3.19)	0.29	1.58 (0.74-3.35)	0.24
Q3	0.014-0.020	301	17 (6%)	1.47 (0.70-3.10)	0.31	1.54 (0.73-3.26)	0.26
Q4	>0.020	310	27 (9%)	2.27 (1.14-4.51)	0.02	2.08 (1.04-4.18)	0.04
	Per SD increment			1.41 (1.07-1.86)	0.01	1.33 (1.02-1.74)	0.04
C-reactive protein (mg/l)							
Q1	<1.22	282	11 (4%)	1		1	
Q2	1.22-2.34	282	14 (5%)	1.27 (0.57-2.80)	0.56	1.08 (0.48-2.42)	0.86
Q3	2.35-4.95	279	19 (7%)	1.65 (0.78-3.47)	0.19	1.33 (0.62-2.87)	0.46
Q4	>4.95	281	28 (10%)	2.48 (1.23-5.02)	0.01	1.87 (0.89-3.92)	0.10
	Per SD increment			1.42 (1.14-1.76)	0.002	1.33 (1.05-1.67)	0.02
B-type natriuretic peptide (pg/ml)							
Q1	<11.97	258	9 (4%)	1		1	
Q2	11.97-37.48	258	8 (3%)	1.04 (0.40-2.71)	0.94	1.08 (0.41-2.83)	0.88
Q3	37.49-99.40	258	15 (6%)	2.03 (0.87-4.71)	0.10	2.11 (0.89-4.99)	0.09
Q4	>99.40	258	32 (12%)	5.16 (2.34-11.39)	<0.001	5.74 (2.51-13.11)	<0.001
	Per SD increment			1.84 (1.42-2.38)	<0.001	1.93 (1.47-2.54)	<0.001

* Multivariate risk factor adjustment included age, sex, body-mass index, hypertension, diabetes, smoking status, LDL-/HDL-ratio, number of diseased vessels, statin and beta-blocker therapy.

Table 4

Final model of a backward multiple stepwise cox regression analysis for cardiovascular risk predictors

Variable	Hazard ratio (95% CI)	P-value
Procalcitonin (1 SD increase)	1.30 (1.00-1.70)	0.05
LDL-/HDL-ratio (1 SD increase)	1.32 (1.03-1.70)	0.03
Sex	0.57 (0.34-0.96)	0.03
Diabetes mellitus (IDDM)	2.75 (1.53-4.92)	0.001

Table 5

Final model of a backward multiple stepwise cox regression analysis for cardiovascular risk predictors

Variable	Hazard ratio (95% CI)	P-value
Procalcitonin ≥ 0.05 ng/ml	4.22 (2.07-8.59)	<0.001
LDL-/HDL-ratio (1 SD increase)	1.34 (1.04-1.73)	0.03
Sex	0.60 (0.36-1.00)	0.05
Diabetes mellitus (IDDM)	2.74 (1.54-4.87)	0.001

Table 6

Incremental effects of procalcitonin, C-reactive protein and B-type natriuretic peptide (all log-transformed) on the area under the ROC-curve in addition to classical risk factors (basic model) for the prediction of the primary endpoint after 2 years

Model	AUC	95% CI
Basic model	0.74	0.67-0.81
Basic model + PCT	0.77	0.70-0.84
Basic model + CRP	0.75	0.67-0.82
Basic model + BNP	0.79	0.72-0.87
Basic model + PCT + CRP	0.77	0.70-0.84
Basic model + PCT + BNP	0.81	0.74-0.88
Basic model + CRP + BNP	0.80	0.73-0.87
Basic model + PCT + CRP + BNP	0.81	0.74-0.88

Table 7

Hazard ratios and 95% confidence interval for future cardiovascular events according to baseline levels of procalcitonin and B-type natriuretic peptide in combined analysis (fully adjusted*)

N	Procalcitonin (highest quartile >0.021 ng/ml)	B-type natriuretic peptide (highest quartile >99.40pg/ml)	Hazard ratio 95% confidence interval	P-value
654	-	-	**1**	
212	+	-	**1.13** (0.56-2.29)	0.74
190	-	+	**2.44** (1.30-4.56)	0.005
68	+	+	**7.04** (3.40-14.57)	<0.001

* Multivariate risk factor adjustment included age, sex, body-mass index, hypertension, diabetes, smoking status, LDL-/HDL-ratio, number of diseased vessels, statin and beta-blocker therapy.

Claims

1. In vitro method for the risk stratification of patients with stable arteriosclerosis, wherein the concentration of procalcitonin is determined in the circulation of such patients using a highly sensitive PCT assay, and wherein within the range of PCT concentrations in the typical normal range of healthy individuals cutoff values are defined which distinguish groups of individual patients with stable arteriosclerosis in accordance with their personal cardiac risk, and the patients are allotted to one of said risk groups on the basis of their individual PCT concentrations..

2. Method according to Claim 1, wherein the stable arteriosclerosis is stable coronary artery disease.

3. Method according to any of Claims 1 or 2, wherein said PCT concentrations in the typical normal range of healthy individuals are PCT concentrations of 0.1 ng/ml PCT or less if measured with a sensitive PCT assay as described in the present application.

4. Method according to any of Claims 1 to 3, wherein patients for which PCT concentrations of 0.05 ng/ml or more are found are considered as patients with the highest cardiac risk.

5. Method according to any of Claims 1 to 4, wherein in conjunction with the determination of PCT at least one further analyte useful for cardiovascular prognosis is determined, said at least one analyte being selected from the group consisting of natriuretic peptides, adrenomedullin, endothelins, vasopressin, CRP, neopterin, myeloperoxidase, troponin, GDF-15, cystatin-C and their precursors, their prohormones and associated prohormone fragments.

6. Method according to Claim 5, wherein said further analyte comprises BNP, proBNP and associated proBNP-fragments.

7. Use of the highly sensitive determination of PCT in the circulation of patients at risk of cardiovascular events for prognostic purposes.

8. Use of the highly sensitive determination of PCT in the circulation of patients with arteriosclerosis, in particular stable coronary artery disease, for the stratification of such patients in accordance with their individual cardiac risk.

Figure 1

Kaplan-Meier survival curves showing cardiovascular events according to quartiles of procalcitonin

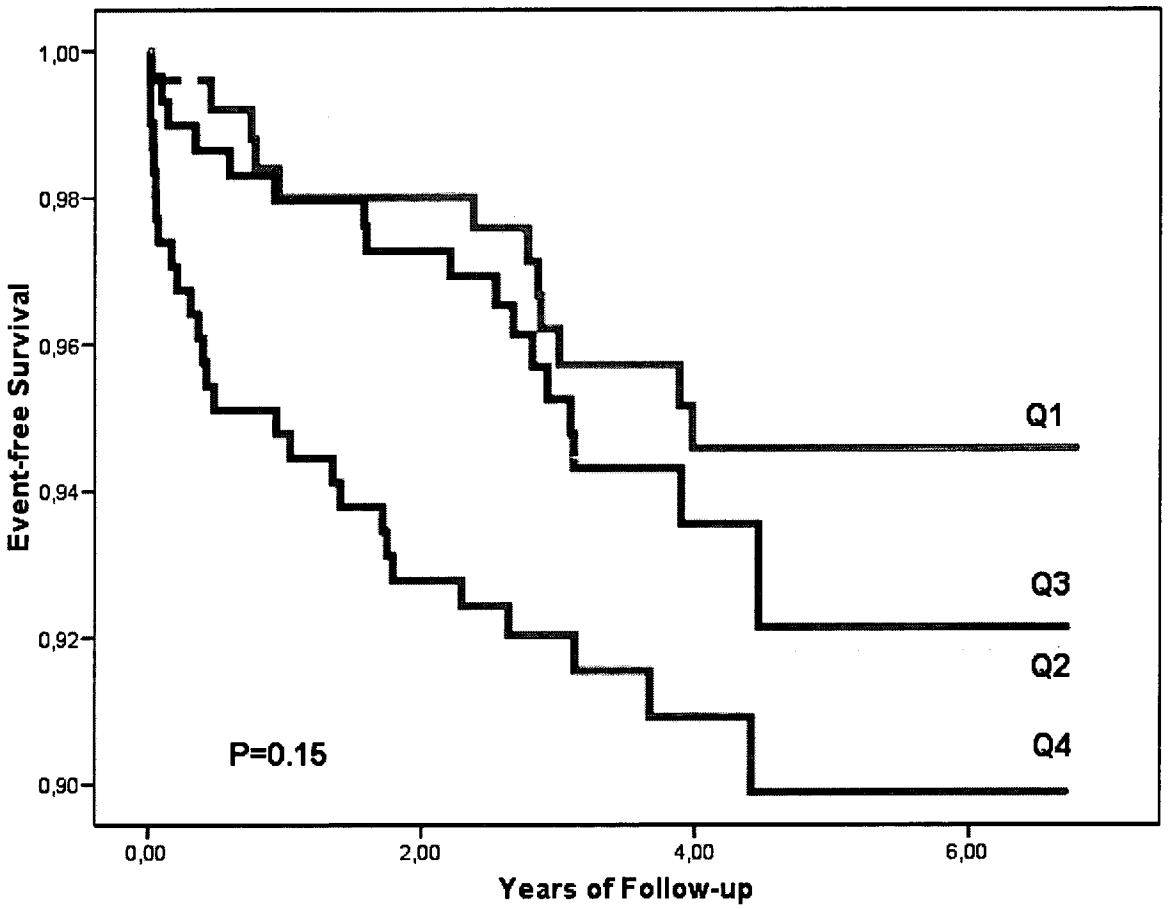


Figure 2

Kaplan-Meier survival curves showing cardiovascular events according to procalcitonin cut-off value 0.05 ng/ml

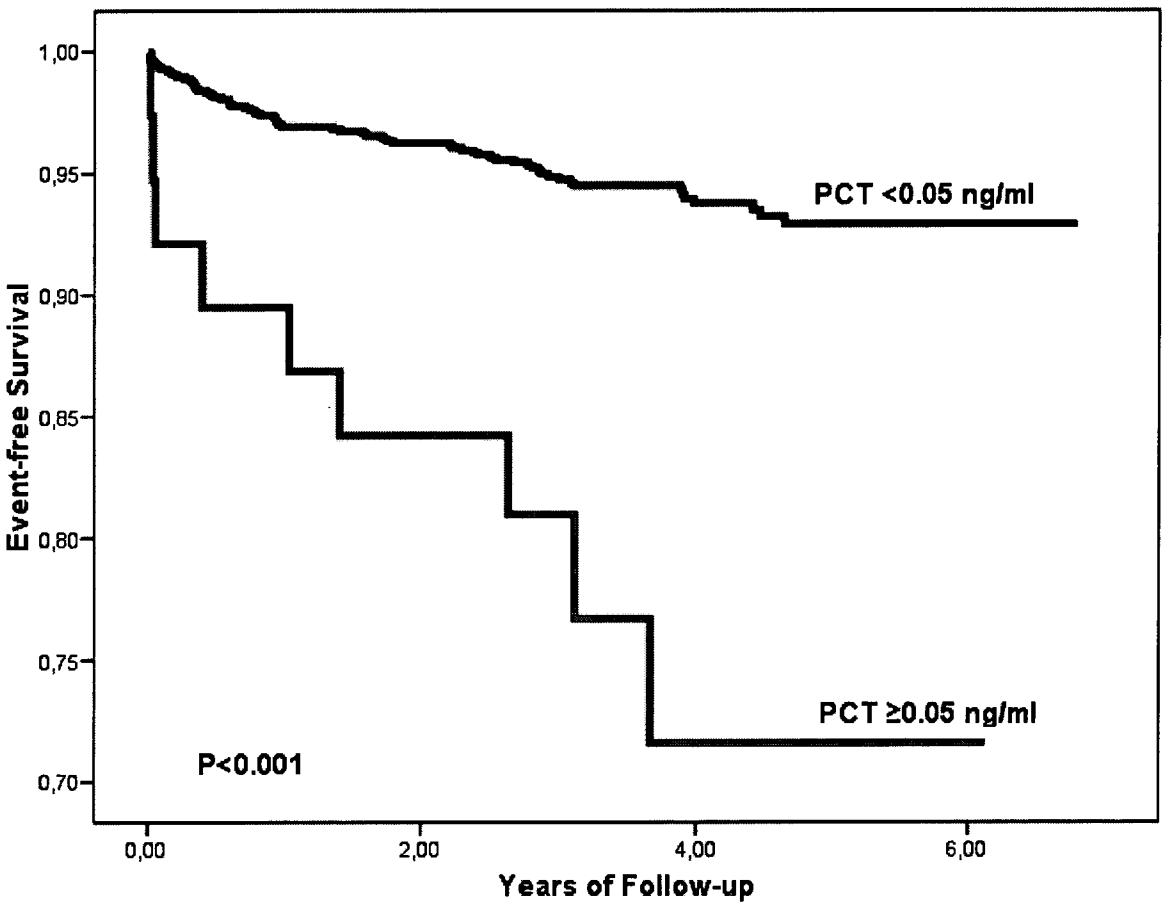
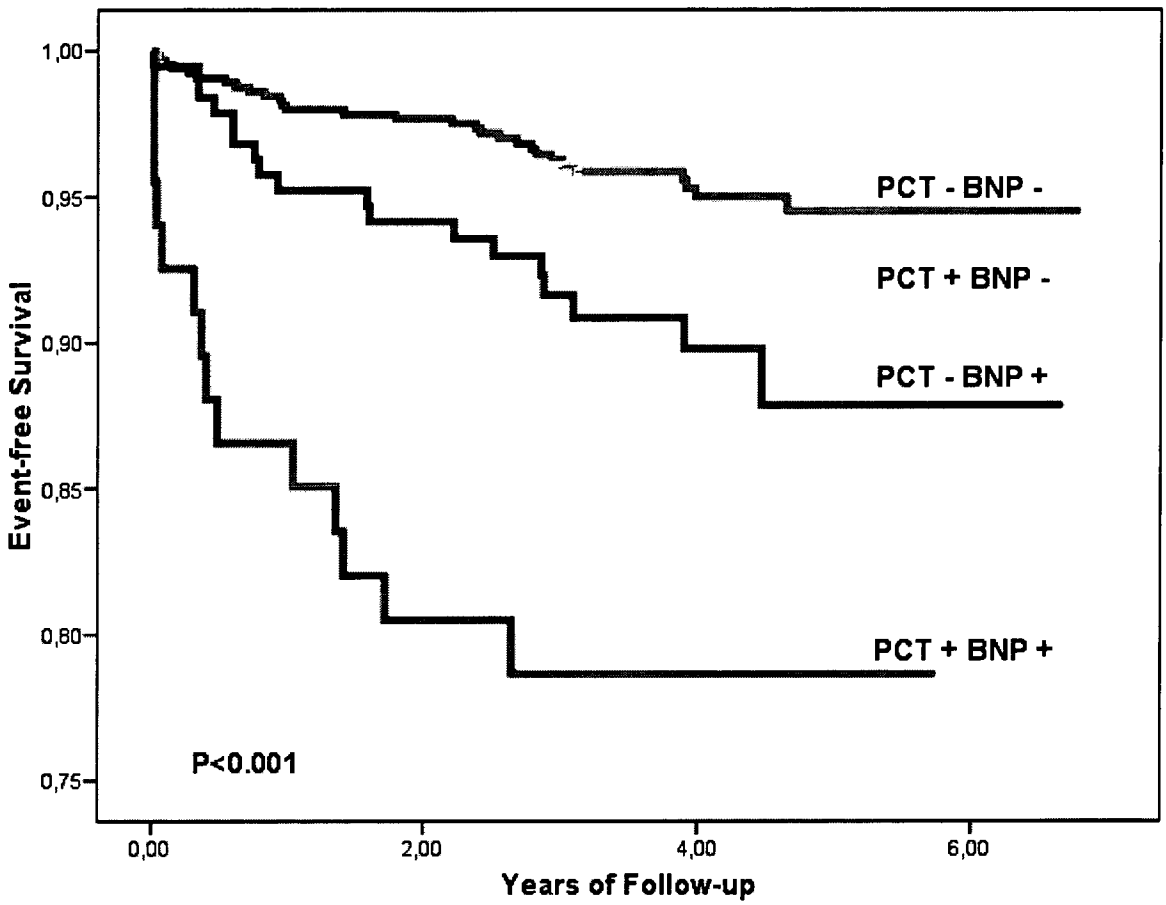


Figure 3

Kaplan-Meier survival curves showing cardiovascular events according to procalcitonin and B-type natriuretic peptide in combined analysis



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/006378

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ILHAN FULYA ET AL: "Procalcitonin, c-reactive protein and neopterin levels in patients with coronary atherosclerosis" ACTA CARDIOLOGICA, vol. 60, no. 4, August 2005 (2005-08), pages 361-365, XP008083963 ISSN: 0001-5385 cited in the application abstract page 362, column 2, paragraphs 3,5-7 page 364, column 1, paragraph 2 - column 2, paragraph 1 ----- -/--	1-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2008	02/09/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, Björn

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SENTURK TUNAY ET AL: "Procalcitonin in patients with acute coronary syndrome: correlation with high-sensitive C-reactive protein, prognosis and severity of coronary artery disease" ACTA CARDIOLOGICA, vol. 62, no. 2, April 2007 (2007-04), pages 135-141, XP008083964 ISSN: 0001-5385 abstract page 135, column 2, paragraph 2 - page 136, column 1, paragraph 1 page 136, column 2, paragraph 2 page 137, column 1, paragraph 3 tables 3,5 page 139, column 2, paragraph 2	1-8
X	ERREN M ET AL: "Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries" ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, XX, XX, vol. 19, no. 10, October 1999 (1999-10), pages 2355-2363, XP002346643 ISSN: 1079-5642 the whole document	1-8
X	FERRIERE F ET AL: "Procalcitonin in unstable coronary artery disease, relation to chlamydia pneumoniae serology" CLINICAL CHEMISTRY AND LABORATORY MEDICINE, vol. 37, no. SPEC. SUPPL., June 1999 (1999-06), page S445, XP008083965 & IFC-WORLDLAB, INTERNATIONAL FEDERATION OF CLINICAL AND LABORATORY MEDICINE (17TH INTERNATIONAL AND 1; FLORENCE, ITALY; JUNE 6-11, 1999 ISSN: 1434-6621 abstract	7,8
X	REITH H BERND ET AL: "Procalcitonin in early detection of postoperative complications" DIGESTIVE SURGERY, vol. 15, no. 3, 1998, pages 260-265, XP008083966 ISSN: 0253-4886 abstract	7,8

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/006378

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OMLAND TORBJORN ET AL: "Prognostic value of B-Type natriuretic peptides in patients with stable coronary artery disease: the PEACE Trial." JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY 17 JUL 2007, vol. 50, no. 3, 17 July 2007 (2007-07-17), pages 205-214, XP022146448 ISSN: 1558-3597 abstract	1-8
A	OMLAND T ET AL: "B-type natriuretic peptide and long-term survival in patients with stable coronary artery disease" AMERICAN JOURNAL OF CARDIOLOGY, CAHNERS PUBLISHING CO., NEWTON, MA,, US, vol. 95, no. 1, 1 January 2005 (2005-01-01), pages 24-28, XP004689833 ISSN: 0002-9149 abstract	1-8
A	SCHNABEL RENATE ET AL: "Analysis of N-terminal-pro-brain natriuretic peptide and C-reactive protein for risk stratification in stable and unstable coronary artery disease: results from the AtheroGene study." EUROPEAN HEART JOURNAL FEB 2005, vol. 26, no. 3, February 2005 (2005-02), pages 241-249, XP008084066 ISSN: 0195-668X abstract	1-8
A	MUELLER CHRISTIAN: "The use of B-type natriuretic peptides in coronary artery disease: utile or futile?" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY 17 JUL 2007, vol. 50, no. 3, 17 July 2007 (2007-07-17), pages 215-216, XP008084058 ISSN: 1558-3597 the whole document	1-8
A	POLK DONNA M ET AL: "B-type natriuretic peptide, subclinical atherosclerosis and prognosis" CIRCULATION, vol. 114, no. 18, Suppl. S, October 2006 (2006-10), pages 725-726, XP008084065 & 79TH ANNUAL SCIENTIFIC SESSION OF THE AMERICAN-HEART-ASSOCIATION; CHICAGO, IL, USA; NOVEMBER 12 -15, 2006 ISSN: 0009-7322 abstract	1-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/006378

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 7 and 8 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.