Materials and Methods

Population, diagnostic protocol and study design
Patients with stable chest pain or equivalent symptoms and intermediate probability of CAD were studied. These patients were enrolled at 14 European centres in the EVINCI study (1). The study protocol is available at http://www.clinicaltrials.gov (NCT00979199). Briefly, patients with acute coronary syndrome, known CAD, left ventricular ejection fraction < 35%, significant heart valve disease, cardio-myopathy or contraindications to stress imaging were excluded. According to the protocol, each patient underwent CTA, stress imaging by myocardial perfusion imaging (MPI) or wall motion imaging (WMI). If at least one non invasive test was positive, invasive coronary angiography, with fractional flow reserve (FFR) assessment if indicated, was performed. Ethical approval was provided by each participating centre and all subjects provided written informed consent.

The patients whose CTA images, stress images and plasma samples were available for core laboratory analyses were included in this study. In a subgroup of patients image fusion of CTA datasets with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) was obtained and hybrid analysis performed by a dedicated core laboratory (Figure 1).

Blood collection and analysis
Blood samples were collected prior to imaging in EDTA tubes and then locally separated by centrifugation for 15 min at 1000 ×g. Refrigerated plasma samples were shipped to the bio-humoral core laboratory (CNR-Institute of Clinical Physiology, Pisa, Italy) for the final cryo-conservation in the EVINCI biological bank (2-3). Analyses of hs-cTnT and NT-proBNP were performed at the Laboratory of Fondazione Toscana G. Monasterio (Pisa, Italy). Plasma concentrations of cTnT were measured using the hs-cTnT method on COBAS E411 with Elecsys Troponin T hs STAT by Roche Diagnostics (4). Measurement of NT pro-BNP was performed using the electrochemical luminescence immunoassay Elecsys proBNP II by Roche Diagnostics using monoclonal antibodies (5). In order to complete the clinical/biohumoral profile of the study patients, additional traditional biomarkers were measured using standard methods (2-3).

Image acquisition
Image acquisition protocols were agreed on for each technique covering patient preparation, cardiovascular stress, administration of radiopharmaceutical or contrast medium, image acquisition and quality control. These procedures were based on best available clinical practice. Image reading was performed at core labs for each specific technique (1).
**Coronary CTA analysis and CTA risk score**

The coronary CTAs were analyzed in a core laboratory (Leiden University Medical Center, Leiden, The Netherlands) as described elsewhere (1-2). Briefly, each segment of the AHA 17-coronary segment model was assessed for interpretability, and interpretable segments were evaluated for stenosis of the coronary artery lumen providing three different categories: normal if no atherosclerosis was present, non-obstructive if the stenosis severity was <50%, and obstructive for stenoses >50%. If plaque was present, plaque composition was visually determined (calcified, mixed, and non-calcified). Only one type of plaque composition could be assigned to a single segment.

A previously established CTA risk score was derived in each patient integrating all data on the location, severity, and composition of CAD (2, 6). This risk score was used as an indicator of the global coronary atherosclerotic burden at individual level similarly to the synthetic scores used to describe the extent of coronary disease from invasive angiography (7). In 297/376 patients, CT acquisitions for coronary artery calcium (CAC) were available and the Agatston CAC score was computed according to standard methods.

**Non invasive stress imaging analysis**

MPI and WMI were defined as abnormal if there was either an inducible perfusion abnormality or myocardial scarring. Perfusion in each of 17 myocardial segments was classified as 0=normal, 1=mild reduction, 2=moderate reduction, 3=severe reduction or 4=absent perfusion and the segmental scores were summed for the stress and rest images. For MPI, an inducible perfusion abnormality (ischemia) was defined as a summed segmental difference score between stress and rest images ≥ 2, either from a score ≥ 1 in at least two contiguous segments or ≥ 2 in at least one segment. Scarring was defined similarly from the summed segmental rest score. For WMI, segmental myocardial wall motion was scored at rest and during stress as normal (0), hypokinetic (1), akinetic (2) or dyskinetic (3). Inducible ischemia was defined as an increase in segmental wall motion score ≥ 1 from rest to stress in at least two contiguous segments. Scarring was defined similarly from the resting wall motion score.

**Hybrid imaging**

In the subgroup of 193 patients submitted to MPI by PET or SPECT, a hybrid imaging study was performed. Individual datasets from MPI and CTA were transferred to a dedicated hybrid core laboratory (University Hospital Zurich, Switzerland). Image fusion of MPI/CTA datasets was performed on a dedicated workstation (Advantage Workstation 4.4, GE Healthcare) using the CardIQ Fusion software (GE Healthcare) (8).

All hybrid MPI/CTA images were analysed with regard to the presence of hemodynamically significant coronary lesions. Specifically, each abnormal myocardial segment was assigned to the pertinent vascular territory by spatial co-
registration according to patients’ individual coronary anatomy. A matched hybrid imaging finding was defined as an inducible perfusion defect in a territory subtended by an obstructive stenosis (>50%) on CTA. All other combinations of pathologic findings were classified as unmatched.

Statistical analysis
Categorical variables are presented as numbers (percentage), continuous variables as mean±SD or median [25-75 percentile] depending on their distribution. Differences in continuous variables between two groups were tested using Student’s t test or Mann-Whitney test. Comparisons among groups were performed using ANOVA and Kruskall-Wallis test. Bonferroni test or Mann-Whitney test using Bonferroni correction for P-value were used for post-hoc comparisons. Pearson’s chi-squared test was used to compare categorical data.

Linear regression was used to estimate the effect of clinical, biohumoral variables as well as imaging results on hs-cTnT and NT-proBNP levels. All multivariate models were developed considering variables with a P value <0.1 at univariate analysis and then using backward and forward stepwise selections to build up the final models. The logarithmic transformation of continuous variables was used in linear regression analysis.

All analyses were performed using StataCorp. 2007. A 2-sided value of P<0.05 was considered statistically significant.

References
