Serum level of High-density lipoprotein particles are independently associated with long-term prognosis in patients with coronary artery disease: The GENES study

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Abstract

Background. HDL-Cholesterol (HDL-C) is not a right marker to measure the cardioprotective functions of HDL in coronary artery diseases (CAD) patients. Hence, measurement of other HDL-related parameters may have prognostic superiority over HDL-C. This work aimed to examine the predictive value of HDL particles profile for long-term mortality in CAD patients. Its informative value was compared to that of HDL-C and apoA-I.

Method. NMR spectroscopy HDL particles profile were measured by nuclear magnetic resonance (NMR) spectroscopy in 214 male participants with stable CAD (age 45-74 years). Vital status was yearly assessed, with a median follow up of 12.5 years and a 36.4% mortality rate. Cardiovascular mortality accounted for the majority (64.5 %) of deaths.

Results. Mean concentrations of HDL particles (HDL-P), small and medium-sized HDL (MS-HDL) and apoA-I were lower in deceased than in surviving patients whereas no difference was observed according to HDL-C and large HDL particles. All NMR-HDL measures were correlated between themselves and with other HDL markers (HDL-C, apoA-I and LpA-I). In a multivariate model adjusted for 14 cardiovascular risk factors and bioclinical variables, HDL-P and MS-HDL-P displayed the strongest inverse association with all-cause and cardiovascular mortality. Weaker associations were recorded for apoA-I.

Conclusions. HDL particle profile measured by NMR spectroscopy should be considered to better stratify risk in population at high risk or in the setting of pharmacotherapy.

1. Introduction

HDL-Cholesterol (HDL-C) has been repeatedly inversely related to cardiovascular risk in all epidemiological studies. However, pharmacological trials aimed at increasing HDL-C have failed to demonstrate a beneficial effect on clinical outcomes [1]. Similarly, genetic variants associated to increased HDL-C have not been found associated to a decreased cardiovascular risk [2]. This has led to the concept that a single measurement of HDL-C does not necessarily reflect the functional properties of HDL particles and their effects against atherosclerosis. Indeed, HDL particles are heterogeneous in size and biochemical composition, and HDL subpopulations might have different functional properties [3]. NMR-spectroscopy has been recently proposed as a reference tool to quantify HDL particles and HDL subpopulations [3]. This technology enables to measure the total concentration of HDL particles and their size distribution. Numerous recent studies have shown that the atheroprotective properties of HDL are supported by small and medium-sized HDL particles [4], which were inversely related to cardiovascular risk in various clinical settings [5,6].

In the present study, we have evaluated HDL particles concentration and distribution in a cohort of patients with established, angiographically documented, coronary artery disease [7,8]. Patients' data at inclusion included life style parameters and clinical and biological variables documenting cardiovascular risk factors, inflammatory status, renal function and heart condition. The patients' vital status was yearly assessed and mortality was recorded, distinguishing all-cause mortality, cardiovascular mortality and other causes of death, during a 12.5-year median follow-up. Moreover, one objective of the study was to compare HDL particles measurements to routinely available HDL markers, HDL-C and apoA-I, as predictors of mortality in CAD patients. Indeed, apoA-I constitutes 70% of HDL protein, its immunoassay is today referred to international standards and now available on automated analyzers. Moreover apoA-I is much less influenced than HDL-C by other components of the lipoprotein profile, like VLDL or LDL, which may have an impact on HDL lipid composition through action of lipid transfer proteins.

In this study, multivariate analyses demonstrate that total HDL particles (HDL-P), small and medium-sized HDL (MS-HDL-P) and apoA-I are predictors of all-cause and cardiovascular mortality in coronary patients.

2. Results

2.1. Characteristics of CAD patients according to vital status.

The present cohort was constituted of 214 CAD patients. After inclusion, the vital patients' status was yearly assessed. The median follow-up period was 12.5 years (mean: 10.7 years). During follow-up, 78 deaths had been recorded giving a death rate of 36.4 % and a mean annual rate of 3.4%. Cardiovascular mortality accounted for the majority (64.5 %) of deaths recorded and cancers accounted for 16.5 %.

Comparison of patients' data when they were included in the cohort is given in Table 1. Patients further deceased during the follow-up period had a longer duration of CAD, a decreased left ventricle ejection volume (LVEF), a higher heart rate and a more severe angiographic lesion score (Gensini). Regarding cardiovascular risk factors, smoking habits and treatment for diabetes were more frequent in the deceased group, whereas lipid-lowering therapy was less frequent. Among lipoprotein parameters, only apoA-I, a major HDL marker, was significantly lower in further deceased patients. Hs-CRP, an inflammatory marker, was lower in surviving than in deceasing patients.

2.2. HDL particles according to vital status.

HDL particles' profile was determined by NMR spectroscopy, enabling to distinguish large HDL (L-HDL-P) and small and medium-sized HDL (MS-HDL-P) particles. The latter accounted for about ~ 85 % of total HDL particles (HDL-P). HDL-P was ~ 10% lower in deceased than in surviving patients (24.6 µmol/L [SD, 6.0] vs. 27.5 µmol/L [SD, 4.9], p = 0.001, Table 2). This difference was entirely due to a decreased number of MS-HDL-P, whereas number L-HDL-P was not different according to the vital status (Table 2). The average size of HDL particles (HDL size) was found higher in deceased patients (8.94 nm *versus* 8.82 nm, p = 0.014), which is concordant with a relative higher contribution of large HDL to the total HDL particles number.

2.3. Correlations between HDL particles measures and clinical and biological parameters.

Correlations were investigated between markers of HDL particles and other clinical or biological parameters in the study population (Table 3). All NMR-HDL measures were correlated between themselves and with other HDL markers: HDL-C, apoA-I and lipoprotein A-I (LpA-I). Logically, the average HDL size was correlated positively with the number of L-HDL-P, and negatively, with the

number of MS-HDL-P. Triglycerides were associated positively with HDL-P and MS-HDL-P, but negatively with L-HDL-P, mean HDL size and HDL-C. These correlations might reflect the effects of the cholesterol ester transfer protein (CETP) acting between HDL particles and triglyceride-rich lipoproteins. Alcohol consumption positively correlated with HDL-C and HDL-P, and more specifically with MS-HDL-P. Inflammation, as documented by plasma hs-CRP, was inversely associated with HDL-P and MS-HDL-P, but not with L-HDL-P. The severity of coronary lesions, as illustrated by the Gensini score, was inversely related to HDL-P. Strong positive associations were observed between LVEF and both HDL-P and MS-HDL-P. Hence, numbers of total HDL particles and, more specifically of small and medium-sized HDL seem to be associated with a better clinical condition. No relationship was recorded between HDL-C and either LVEF or the Gensini score.

2.4. Total and cardiovascular mortality according to tertiles of HDL markers.

Each one HDL marker was considered according to tertiles of its distribution in the whole study population (Table 4). Death rates during follow-up were determined across the different tertiles and associations were determined after adjustment on classical risk factors. The strongest association to total and cardiovascular mortality was observed for HDL-P distribution. A 45% reduction in death rates was recorded in tertiles 2 and 3, as compared to tertile 1. Each 1 SD increase in HDL particles number was found associated with ~ 42 % reduction in total or cardiovascular mortality (HR = 0.58 [95%CI, 0.45-0.75] and 0.59 [95%CI, 0.44-0.80], respectively). Results were almost identical considering MS-HDL-P. By contrast, no association between L-HDL-P and mortality was observed, except for an almost significant positive trend ($p = 0.07$) between L-HDL-P and death rates. Concordantly, death rates were significantly different across HDL size distribution, an increase in particles size being associated with highest death rates. Considering the “classical” HDL markers, HDL-C tertiles did not display different death rates; on the other hand, apoA-I distribution was associated to total and cardiovascular mortality; each 1 standard deviation increase of apoA-I was associated to a ~ 31% risk reduction (HR = 0.69 [95%CI, 0.54-0.88] and 0.69 [95%CI, 0.49-0.91], respectively).

A further multivariate analysis was conducted, including adjustment on an extended panel of bio-clinical variables reflecting cardiovascular risk factors, including BMI and physical activity, smoking, alcohol consumption, treatments (diabetes, hypertension, dyslipidemia), heart condition (heart rate), CAD duration, Gensini score, LVEF, renal function (eGFR) and inflammation (hs-CRP). Hazard ratios

for all-cause mortality per 1-SD increase were all significant and were lower for HDL-P (HR = 0.58 [95%CI, 0.43-0.77]) and MS-HDL-P (HR = 0.57 [95%CI, 0.42-0.77]) than for apoA-I (HR = 0.68 [95%CI, 0.51-0.90]) (Figure 1). Almost identical data were obtained for cardiovascular mortality (Figure 1).

Associations between HDL markers and mortality are illustrated in the survival curves established during the whole follow-up period for the different tertiles (Figure 2). For both HDL-P and MS-HDL-P, patients in the first tertile had a poorer survival than patients in tertiles 2 and 3. A comparable trend was observed for apoA-I distribution yet survival differences during the whole period did not reach statistical significance.

3. Discussion

In the present study, levels of total HDL particle number and of small-medium sized HDL particles were inversely related to all-cause as well as to specific cardiovascular mortality in CAD patients. Every 1-SD increase of HDL particle number was associated to a 44% decrease in cardiovascular mortality, after multiple adjustments on cardiovascular risk factors and on clinical markers of heart condition. Among other HDL markers, apoA-I was also inversely related, though to a lesser extent, to total and cardiovascular mortality. Conversely, HDL-C or large HDL particles were not associated with mortality. However, higher death rates were recorded as average HDL particle size increased.

This prospective study carried out in CAD patients confirms numerous other ones demonstrating that small and medium-sized HDL particles are inversely related to cardiovascular risk [3]. This is already evident as regards pre-clinical atherosclerosis, as documented by carotid intima-media thickness [5,9], or coronary calcifications [10]. HDL-P were inversely related to incident coronary events in the Multi-Ethnic Study of Atherosclerosis [5]. More recently, in a large study carried out in CAD patients undergoing coronary catheterization, followed-up during 8 years, HDL-P and MS-HDL-P were independent predictors of all-cause mortality; inclusion of MS-HDL-P as a marker improved risk prediction and stratification [6]. In a different context, in patients suffering from acute heart failure, concentrations of both total and small HDL particles were inversely related to short-term (3-month) mortality, after multiple adjustments on confounding variables, including NT-proBNP, a classical marker of heart failure [11]. Altogether the studies suggest that small and medium-sized HDL particles

are probably protective against atherosclerosis and its clinical consequences, and may have also positive effects on cardiomyocyte functions.

HDL particles, and most particularly small-sized HDL, may act against atherosclerosis through different mechanisms. Small HDL behave as the best acceptors of ABCA1-mediated cholesterol efflux from macrophages, leading subsequently to the mobilization of intracellular cholesterol to the plasma membrane [12,13]. Small and dense HDL particles also protect LDL from oxidation. HDL particles act through removing phospholipid hydroperoxides from LDL and by inactivating oxidized lipids by specific enzymes like paraoxonase-1 (PON-1) and PAF-acétylhydrolase [14,15]. Moreover small protein-rich HDL exert anti-inflammatory properties by depressing expression of VCAM-1 at the surface of endothelial cells [16]. On these cells, HDL particles appear to be cytoprotective by inhibiting apoptosis induced by oxidized LDL, and small HDL_3 would be the most effective in this function [17]. Altogether those observations suggest that the proteome associated to small HDL particles support various biological activities, which impair atherosclerosis development.

Moreover, HDL particles may exert beneficial effects on myocardial functions. Indeed, in different experimental contexts, it was demonstrated that HDL particles protect against ischemia reperfusion injury [18], leading to a reduction in infarct size. HDL may also improve myocardial function by reducing ventricular remodelling following infarction [19]. In isolated cardiomyocytes, HDL particles were shown to prevent apoptosis through an AMP-kinase dependent mechanism [20]. These experimental observations on a direct impact of HDL on myocardial functions might translate into clinical impacts. In support of this concept is the positive correlation observed here between HDL-P, small HDL-P and the left ventricular ejection fraction, concordant with the negative association between small HDL and NT-proBNP previously reported [11].

In this study, concentrations of large HDL particles were not associated to mortality. However, higher death rates were recorded as HDL size increased ($p < 0.01$); following multiple adjustments, association to total mortality for the upper tertile of HDL size was close to statistical significance ($p = 0.06$). Similar observations regarding all-cause mortality in a cohort of coronary patients have been previously reported [6]. Large HDL-P might be less effective than MS-HDL-P regarding various atheroprotective functions, like cholesterol efflux, anti-oxidative and anti-inflammatory properties, and cytoprotective effects on endothelium [4,21]. Moreover, accumulation of large HDL might reflect a defect in HDL catabolism, and particularly in HDL liver uptake, which constitutes the last step of

reverse cholesterol transport [22]. Similarly, we did not observe any association of HDL-C with mortality. This is concordant with the lack of association between L-HDL-P and mortality, since HDL-C mainly reflects cholesterol associated with large, lipid rich, HDL particles.

ApoA-I was inversely related to mortality: for each 1-SD increase of apoA-I, a 31% decrease in both all-cause and cardiovascular mortality was recorded. So far, apoA-I has been little used in epidemiological studies. However, calibration on reference international standards has made the immunoassay of apoA-I robust and comparable between studies. Furthermore, apoA-I measurement is much less influenced than HDL-C by intravascular enzymes and lipid transfer proteins, which participate in HDL remodelling. Thus, apoA-I measurement may improve assessment of cardiovascular risk [23]. Association to mortality was somewhat weaker for apoA-I than for HDL-P or MS-HDL-P. This might be explained by the fact that the apoA-I content per particle varies on average from 2 to 4, between small HDL_3 and large HDL_2 [24], so that large HDL particles are somewhat overrepresented in apoA-I quantification.

Studies on HDL metabolism had progressively led to the schematic view of an interconversion cycle of HDL particles in the plasma compartment, driven by cell cholesterol efflux, enzymes like LCAT, lipases and lipid transfer proteins [25,26]. More recently the concept has emerged that HDL particles of different geometry and chemical composition have distinct metabolic fate and display specific functional properties [3,4,27]. This supports the idea that HDL functionality might be more precisely assessed by the quantification of specific HDL particles with high atheroprotective effects. The present study is clearly in line with this concept demonstrating that concentration of small-sized HDL particles is predictive of cardiovascular mortality in coronary patients.

4. Material and Methods

4.1. Study participants.

The “Génétique et Environnement en Europe du Sud” (GENES) study is a case-control study designed to assess the role of genetic, biological and environmental determinants in the occurrence of CAD [28]. All participants signed an informed consent form. The study protocol was approved by the local ethics committee (CCPPRB, Toulouse / Sud-Ouest, file #1-99-48, Feb 2000). A biological sample collection has been constituted (declared as DC-2008-463 #1 to the Ministry of Research and to the

regional Health authority). As previously described, cases were stable CAD patients living in the Toulouse area (South-west France), aged 45-74 and prospectively recruited from 2001 to 2004 after admission to the Cardiology department, Toulouse University Hospital, for cardiovascular examination and referred for evaluation and management of their CAD (15, 16). Stable CAD was defined by a previous history of acute coronary syndrome, a previous history of coronary artery revascularization, a documented myocardial ischemia, a stable angina or the presence at coronary angiography of a coronary stenosis of 50% or more. Patients who had presented an acute coronary episode during the past eight days were not included in the study, because they were considered unstable. In the present analysis, we only took into account the first 214 patients in whom NMR-HDL profile was measured and complete data were available for all the subjects.

4.2. Assessment of the vital status.

Vital status on December 31, 2014, was obtained for each participant through the national database ("RNIPP"), which records, every year, all deaths occurring in the French population (*http://cesp.vjf.inserm.fr/svcd*). Vital status was assessed yearly, with a median follow up of 12.5 years. All dates and causes of death were obtained for participants who died during the follow-up. Main and associated causes of deaths were provided by the French National Institute of Health Research (CépiDc-INSERM), which systematically collects and codes (using the International Classification of Diseases coding system) data recorded on death certificates. Death from a cardiovascular cause during follow-up was assessed by a committee of four medical doctors, every time cardiovascular disease was reported as the main cause of death, or when it was mentioned as an associated cause, if the main cause was a plausible complication of CV disease. Authorizations to use these data were obtained in accordance with French law (Commission nationale de l'informatique et des libertés (CNIL): authorization 355152v1, September 3, 2008).

4.3. Measured parameters.

Age, environmental characteristics and information on cardiovascular risk factors were collected through standardized face-to-face interviews, performed by a single physician. Smoking status was classified as current smokers, smokers having quitted for more than 3 years and non-smokers. Among current smokers, cigarette consumption was estimated with the pack-year quantification and recorded

as the average number of cigarettes per day. Alcohol consumption was assessed using a typical week pattern. The total amount of pure alcohol consumption was calculated as the sum of different types of drinks and was expressed as grams per day. Physical activity was investigated through a standardized questionnaire [29] and categorized into three levels as: no physical activity, moderate physical activity during 20 minutes no more than once a week, and high physical activity during 20 minutes, at least twice a week. Presence of dyslipidemia, diabetes mellitus or hypertension was assessed from the subjects' current treatments. Past medical history was collected and for cases, was also checked in the patients' medical files. Medications at discharge were also considered in patients. Blood pressure and resting heart rate were measured with an automatic sphygmomanometer (OMRON 705 CP). Measurements were performed after a minimum of 5 minutes rest; average values from two different measurements were recorded for further analysis.

4.4. Assessment of CAD severity and extension and estimation of cardiac function.

Coronary artery stenoses of ≥ 50% luminal narrowing were considered significant. Diffusion of coronary artery disease lesions was assessed by calculating the Gensini Score, based on data from coronary angiography [30–32]. Left Ventricular Ejection Fraction (LVEF) was assessed by contrast ventriculography using an isotopic method, and/or by echocardiography.

4.5. Laboratory assays.

Blood was collected after an overnight fast. Serum sample aliquots were subsequently stored at -80°C until biological analyses. The following biomarkers were assayed with enzymatic reagents on automated analyzers (Hitachi 912 and Cobas 8000, Roche Diagnostics, Meylan, France): serum total cholesterol, HDL-C, triglycerides, fasting glucose. ApoA-I and high-sensitive C-Reactive protein (hs-CRP) were determined on the same analyzer by immunoturbidimetry assays. Lipoprotein A-I (containing apoA-I but not apoA-II) were measured by immunoelectrodiffusion [8].

4.6. HDL measurement by Nuclear Magnetic Resonance (NMR) spectroscopy.

HDL particle concentration and size were measured by NMR spectroscopy using the AXINON® lipoFIT®-S100 test system (Numares AG, Regensburg, Germany). 630 µL of serum were gently

mixed with 70 µL of an additives solution containing reference substances, NaN3 and D2O, and 600 µL of the mixture were transferred into 5 mm NMR tubes with barcode-labeled caps.

Briefly, ^{1}H NMR spectra were recorded at a temperature of 310 K on a shielded 600 MHz Avance III HD NMR spectrometer (Bruker Biospin) with a 5 mm triple resonance TXI probe head including deuterium lock channel, a z-gradient coil and automatic frequency tuning and matching.

Prior to each analytical run, calibration was performed using a calibration sample comprising an aqueous solution of various calibration substances with different molecular masses, 0.01% (w/v) NaN3, 10 % (v/v) D2O as a locking substance and 1 % glycerol to adjust viscosity. Two identical control samples were measured directly after calibration and at the end of each run. Each spectrum was referenced, normalized and subjected to a set of quality checks including checks of baseline properties, noise level, shift, width, and symmetry properties of quality control signals.

Lipoprotein analysis was conducted via deconvolution of the broad methyl group signal at about 0.9-0.8 ppm. In this process, lipoprotein subclasses are reflected by a fixed number of predefined bell-shaped (e.g. Gaussian or Lorentzian) base functions, each of which has a constant position and defined width. The concentrations of lipoprotein particles and cholesterol in lipoprotein (sub)classes as well as the average particle size were calculated based on the integrals attributable to specific base functions. Fit quality was checked by calculating the residual deviation between fit and spectrum intensity. In this study, the concentrations of large HDL particles (L-HDL-P), small and medium HDL-particle (MS-HDL-P) and total HDL particles (HDL-P, reported in nmol / L) as well as the average HDL particle size (HDL-s, reported in nm) are used. The two measured HDL subclasses had the following estimated diameter ranges: L-HDL-P, 8.8-13 nm; MS-HDL-P, 7.3-8.7 nm.

4.7. Statistical analyses.

Continuous variables are displayed as means and standard deviations (SD). Categorical variables are presented as proportions. We first described and compared characteristics of participants according to vital status. Categorical variables were compared between groups using the χ^2-test (or Fisher's exact test when necessary). Student's *t*-test was used to compare the distribution of continuous data. A Wilcoxon Mann-Whitney's test (or logarithmic transformation of the variable when necessary) was performed when distribution departed from normality, or when homoscedasticity was rejected. Spearman rank correlations were used to test the associations of NMR-HDL parameters and

HDL-C with cardiovascular risk factors, severity, extension and estimation of cardiac function of the disease.

Cumulative survival of patients were determined by the Kaplan-Meier method and compared, using the Log-rank test for the individual endpoints of all-cause mortality. The relation between baseline variables and mortality was assessed using Cox proportional hazards regression analysis. We tested the proportionality assumption using cumulative sums of martingale-based residuals. We performed regression analyses with polynomial models (quadratic and cubic) to examine for possible non-linear relations between continuous variables and mortality. Cox regression analyses were performed first without any adjustment for co-variables and, second, with adjustment on classical cardiovascular risk factors (age, smoking, treatments for dyslipidemia, hypertension and diabetes). Further adjustments were successively performed on extended cardiovascular risk factors (alcohol consumption, physical activity, BMI, eGFR, hs-CRP and duration of CAD) and clinical parameters related to the severity, extension of the disease and cardiac function (heart rate, LVEF and Gensini score). All statistical analyses were carried out using the SAS statistical software package 9.4 (SAS Institute, Cary, NC). All tests were considered significant at a p value < 0.05.

Figure titles and legends.

Figure 1: Relative risk of all-cause and cardiovascular mortality as a function of apoA-I, HDL-P and MS-HDL-P.

Graphic represents hazard ratios (dots) and corresponding 95% confidence interval (95%CI) for risk of all-cause and cardiovascular mortality per 1 standard deviation increase of apoA-I, HDL-P or MS-HDL-P. * Analyses were adjusted for age, smoking, alcohol consumption, physical activity, BMI, treatments for dyslipidemia, hypertension and diabetes, hs-CRP, eGFR, heart rate, LVEF, duration of CAD and Gensini score.

Figure 2. Kaplan-Meier survival curves as a function of apoA-I (A), HDL-P (B) and MS-HDL-P (C) tertiles. Low apoA-I, HDL-P and MS-HDL-P concentrations at inclusion in the study are predictor of increased all-cause mortality in coronary artery patients.

Author Contributions:

B.P; J.F; L.O.M and S.N. conceived and designed the experiments; J.B.R performed statistical analyses; A.G; B.P; L.O.M. and T.D. analyzed the data; L.O.M, J.B.R and B.P. wrote the paper; all authors checked the intellectual content of the paper; L.O.M. was responsible for funding acquisition.

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Abbreviations

apoA-I	Apolipoprotein A-I
BMI	Body Mass Index
CAD	Coronary artery disease
CI	Confidence interval
eGFR	Estimated Glomerular Filtration Rate
HDL	High-density lipoprotein
HDL-P	HDL particle
L-HDL-P	Large-sized HDL-particle
MS-HDL-P	Small and Medium*sized HDL-particle
HDL size	Average HDL particle size.
HR	Hazard Ratio
hs-CRP	High-sensitivity C-Reactive Protein
LDL	Low-density lipoprotein
LpA-I	Lipoprotein A-I
LVEF	Left Ventricular Ejection Fraction
SD	Standard Deviation

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	Full cohort (n = 214)	**Alive** (n = 136)	**Dead** (n = 78)	p*
Age (years)	60.3 (7.8)	59.4 (7.3)	61.8 (8.4)	0.04
Smoking (pack year)	39.9 (37.7)	33 (32.6)	52 (42.9)	0.001 [c]
Alcohol (g/day)	29 (30.2)	30.8 (32)	26 (26.9)	0.39 [c]
Physical activity (high level) [a] (%)	10.3	12.5	6.4	0.16 [d]
Treatment diabetes (%)	24.3	16.9	37.2	0.001 [d]
Treatment dyslipemia (%)	57.5	65.4	43.6	0.002 [d]
Treatment Hypertension (%)	45.5	43.4	48.7	0.45 [d]
Waist circumference (cm)	99.1 (10.8)	98 (9.5)	101.1 (12.5)	0.07
BMI (kg/m2)	27.2 (3.8)	27.1 (3.6)	27.4 (4.2)	0.62
Systolic blood pressure (mm Hg)	137 (20.7)	137.8 (19.8)	135.7 (22.1)	0.47
Heart rate (beats/min)	64 (13.3)	62.1 (12.2)	67.4 (4.2)	0.005
Triglycerides (g/L) [b]	1.5 (0.64)	1.54 (0.66)	1.41 (0.6)	0.09
Total cholesterol (g/L)	1,81 (0.41)	1.82 (0.41)	1.79 (0.42)	0.56
LDL-C (g/L)	1.29 (0.37)	1.32 (0.35)	1.23 (0.41)	0.09
HDL-C (g/L)	0.43 (0.13)	0.43 (0.13)	0.42 (0.14)	0.61
ApoA-I (g/L)	1.21 (0.23)	1.24 (0.24)	1.17 (0.22)	0.04
Lipoprotein A-I (g/l)	0.45 (0.13)	0.45 (0.14)	0.46 (0.13)	0.69
hs-CRP (mg/l) [b]	16.7 (28.2)	14.5 (27.4)	20.5 (29.4)	0.05
eGFR < 30 mL / min (%)	2.4	0.8	5.2	0.07 [e]
LVEF < 50% (%)	29	17.7	48.7	0.001 [d]
Gensini score [b]	46.4 (40.1)	37.7 (31.4)	61.1 (48.4)	0.001
Duration of CAD (months) [b]	42.4 (63.8)	35.6 (58.6)	54.2 (70.8)	0.06

Table 1. Clinical and biological characteristics in coronary artery disease patients when they were first included in the GENES cohort.

Data are expressed in mean (SD) or %.
**Student's t-test, unless otherwise stated.*
[a] *"high" physical activity during 20 min at least twice a week versus "low" physical activity once a week or less.*
[b] *tests performed on log transformed data.*
[c] *Wilcoxon-Mann-Withney test.*
[d] *Chi-squared test.*
[e] *Fischer's exact test.*
BMI: Body Mass Index.
hs-CRP: high-sensitivity C-Reactive Protein.
eGFR: estimated Glomerular Filtration Rate.
LVEF: Left Ventricular Ejection Fraction.
CAD: coronary artery disease.

	Full cohort (n = 214)	**Alive** (n = 136)	**Dead** (n = 78)	p*
HDL-P (µmol / L)	26.4 (5.5)	27.5 (4.9)	24.6 (6.0)	0.001
L-HDL-P (µmol / L)	3.9 (2.7)	3.8 (2.7)	4.1 (2.7)	0.39
MS-HDL-P (µmol / L)	22.8 (5.1)	23.9 (4.5)	20.9 (5.6)	0.001
HDL size (nm)	8.87 (0.34)	8.82 (0.34)	8.94 (0.34)	0.014

Table 2. NMR HDL measures in coronary artery patients (n = 214) according to vital status.
Data are expressed in mean (SD).
**Student's t-test.*
HDL-P: HDL particle.
L-HDL-P: Large-sized HDL-particle.
MS-HDL-P: Small and Medium-sized HDL-particle.
HDL size: average HDL particle size.

	HDL-C (g/L)	**HDL-P** (µmol/L)	**L-HDL-P** (µmol/L)	**MS-HDL-P** (µmol/L)	**HDL size** (nm)
HDL-C (g/L)	1	0.642*** (0.556;0.714)	0.671*** (0.591;0.739)	0.351*** (0.227;0.464)	0.466*** (0.353;0.565)
HDL-P (µmol/L)		1	0.309*** (0.183;0.426)	0.859*** (0.819;0.890)	-0.043 (-0.176;0.092)
L-HDL-P (µmol/L)			1	-0.120 (-0.50;0.015)	0.898*** (0.868;0.921)
MS-HDL-P (µmol/L)				1	-0.444*** (-0.547;-0.330)
Age (years)	0.121 (-0.014;0.251)	0.020 (-0.114;0.154)	0.146* (0.012;0.274)	-0.034 (-0.168;0.101)	0.189** (0.056;0.315)
Cigarette (nb/day)	-0.081 (-0.208;0.059)	-0.118 (-0.248;0.016)	0.025 (-0.109;0.159)	-0.172* (-0.300;-0.038)	0.041 (-0.094;0.174)
Alcohol (g/day)	0.138* (0.004;0.267)	0.172* (0.038;0.299)	0.021 (-0.114-0.155)	0.199** (0.066;0.325)	-0.038 (-0.172;0.097)
Physical activity score [a]	0.132 (-0.002;0.262)	0.108 (-0.026-0.239)	0.036 (-0.099;0.169)	0.115 (-0.019;0.246)	-0.046 (-0.179;0.089)
Waist circumference (cm)	-0.138* (-0.267;-0.004)	0.020 (-0.114;0.154)	-0.153* (-0.281;-0.019)	0.068 (-0.067;0.201)	-0.104 (-0.235;0.031)
BMI (kg/m^2)	-0.147* (-0.275;-0.013)	0.086 (-0.048;0.218)	-0.208** (-0.332;-0.076)	0.155* (0.021;0.284)	-0.201** (-0.326;-0.067)
Heart rate (beats/min)	-0.025 (-0.159;0.109)	-0.086 (-0.218;0.049)	0.044 (-0.090;0.178)	-0.142* (-0.271;-0.007)	0.119 (-0.016;0.250)
Total cholesterol (g/L)	0.225*** (0.094;0.349)	0.394*** (0.274;0.501)	0.052 (-0.082;0.185)	0.329*** (0.204;0.444)	-0.073 (-0.206;0.062)
Triglycerides (g/L)	-0.304*** (-0.421;-0.177)	0.184** (0.051;0.311)	-0.503*** (-0.597;-0.395)	0.424*** (0.307;0.529)	-0.632*** (-0.707;-0.544)
LDL-C (g/L)	0.100 (-0.037;0.233)	0.165* (0.029;0.294)	-0.090 (-0.224;0.047)	0.174* (0.038;0.304)	-0.172* (-0.32;-0.036)
apoA-I (g/L)	0.836*** (0.791;0.872)	0.768*** (0.706;0.818)	0.502*** (0.395;0.596)	0.528*** (0.424;0.619)	0.246*** (0.114-0.367)
Lipoprotein AI (g/L)	0.649*** (0.562;0.721)	0.492*** (0.381;0.589)	0.502*** (0.392;0.597)	0.276*** (0.144;0.397)	0.394*** (0.271;0.504)
hs-CRP (mg/L)	-0.180** (-0.307;-0.047)	-0.252*** (-0.373;-0.122)	-0.098 (-0.229;0.037)	-0.216** (-0.340;-0.084)	0.069 (-0.066;0.201)
eGFR (mL/min)	0.100 (-0.040;0.228)	0.113 (-0.023;0.244)	0.035 (-0.104;0.171)	0.075 (-0.061;0.209)	-0.031 (-0.165;0.106)
LVEF (%)	0.101 (-0.038;0.237)	0.255*** (0.120;0.380)	-0.085 (-0.222;0.054)	0.282*** (0.148;0.405)	-0.177* -0.309;-0.039
Gensini score	-0.114 (-0.249;0.025)	-0.158* (-0.290;-0.019)	-0.004 (-0.142;0.135)	-0.118 (-0.253;0.022)	0.052 (-0.088;0.190)

Table 3. Correlation between NMR HDL measure and biological and biochemical parameters and other cardiovascular risk factors in coronary artery disease patients.
Spearman rank correlation coefficients (95% confidence interval)
**p<0.05, ** p<0.01, *** p<0.001*
[a] *Score from no physical activity to at least 20 minutes of vigorous physical activity four times or more per week*
BMI: Body Mass Index
SBP: Systolic Blood Pressure
hs-CRP: high-sensitivity C-Reactive Protein
LVEF: Left Ventricular Ejection Fraction

	Tertile 1	Tertile 2	Tertile 3	p_{trend}	HR per one SD increase	p
HDL-C (g/L)	≤ 36.5 (n=70)	36.6-42.3 (n=72)	>42.3 (n=72)			
Death rates (%)	38.6	37.5	34.3	0.59		
HR for total mortality* (95% CI)	1	0.85 (0.49-1.46)	0.71 (0.40-1.25)	0.23	0.78 (0.60-0.98)	0.03
HR for CV mortality* (95% CI)	1	1,01 (0.51-2.02)	0.92 (0.46-1.86)	0.82	0.79 (0.59-1.07)	0.13
apoA-I (g/L)	≤1.1 (n=67)	1.2-1.26 (n=73)	>1.26 (n=74)			
Death rates (%)	44.8	37	29.3	0.06		
HR for total mortality* (95% CI)	1	0.77 (0.45-1.32)	0.53 (0.30-0.94)	0.03	0.69 (0.54-0.88)	0.004
HR for CV mortality* (95% CI)	1	0.63 (0.32-1.24)	0.53 (0.27-1.05)	0.06	0.69 (0.49-0.91)	0.01
HDL-P (µmol/L)	≤24.6 (n=71)	24.7-28.4 (n=71)	>28.4 (n=72)			
Death rates (%)	52.8	28.2	29.2	0.004		
HR for total mortality* (95% CI)	1	0.43 (0.25-0.76)	0.52 (0.29-0.95)	0.016	0.58 (0.45-0.75)	0.001
HR for CV mortality* (95% CI)	1	0.42 (0.21-0.86)	0.48 (0.24-0.95)	0.025	0.59 (0.44-0.80)	0.001
L-HDL-P (µmol/L)	≤2.3 (n=70)	2.4-4.3 (n=71)	>4.3 (n=73)			
Death rates (%)	30.0	35.2	44.6	0.07		
HR for total mortality* (95% CI)	1	1.32 (0.72-2.40)	1.35 (0.75-2.43)	0.33	0.91 (0.74-1.13)	0.42
HR for CV mortality* (95% CI)	1	1.40 (0.67-2.93)	1.39 (0.67-2.88)	0.39	0.94 (0.71-1.24)	0.64
MS-HDL-P (µmol/L)	≤21.0 (n=71)	21.1-24.8 (n=70)	>24.8 (n=73)			
Death rates (%)	50.7	32.9	26.0	0.002		
HR for total mortality* (95% CI)	1	0.59 (0.32-1.09)	0.52 (0.26-1.04)	0.06	0.60 (0.46-0.72)	0.001
HR for CV mortality* (95% CI)	1	0.55 (0.28-1.10)	0.55 (0.27-1.14)	0.08	0.61 (0.45-0.82)	0.001
HDL size (nm)	≤8.69 (n=68)	8.70-8.95 (n=72)	>8.95 (n=74)			
Death rates (%)	27.9	33.3	48.6	0.01		
HR for total mortality* (95% CI)	1	1.21 (0.65-2.24)	1.74 (0.96-3.15)	0.06	1.07 (0.88-1.31)	0.49
HR for CV mortality* (95% CI)	1	1.22 (0.57-2.62)	1.88 (0.91-3.90)	0.08	1.12 (0.87-1.46)	0.38

Table 4. Death rate according to tertiles of HDL-related biomarkers and association with total and cardiovascular (CV) mortality.

HR: Hazard Ratio

CI: Confidence Interval

**Analyses are adjusted for age, smoking, treatment for hypertension, diabetes and dyslipemia.*

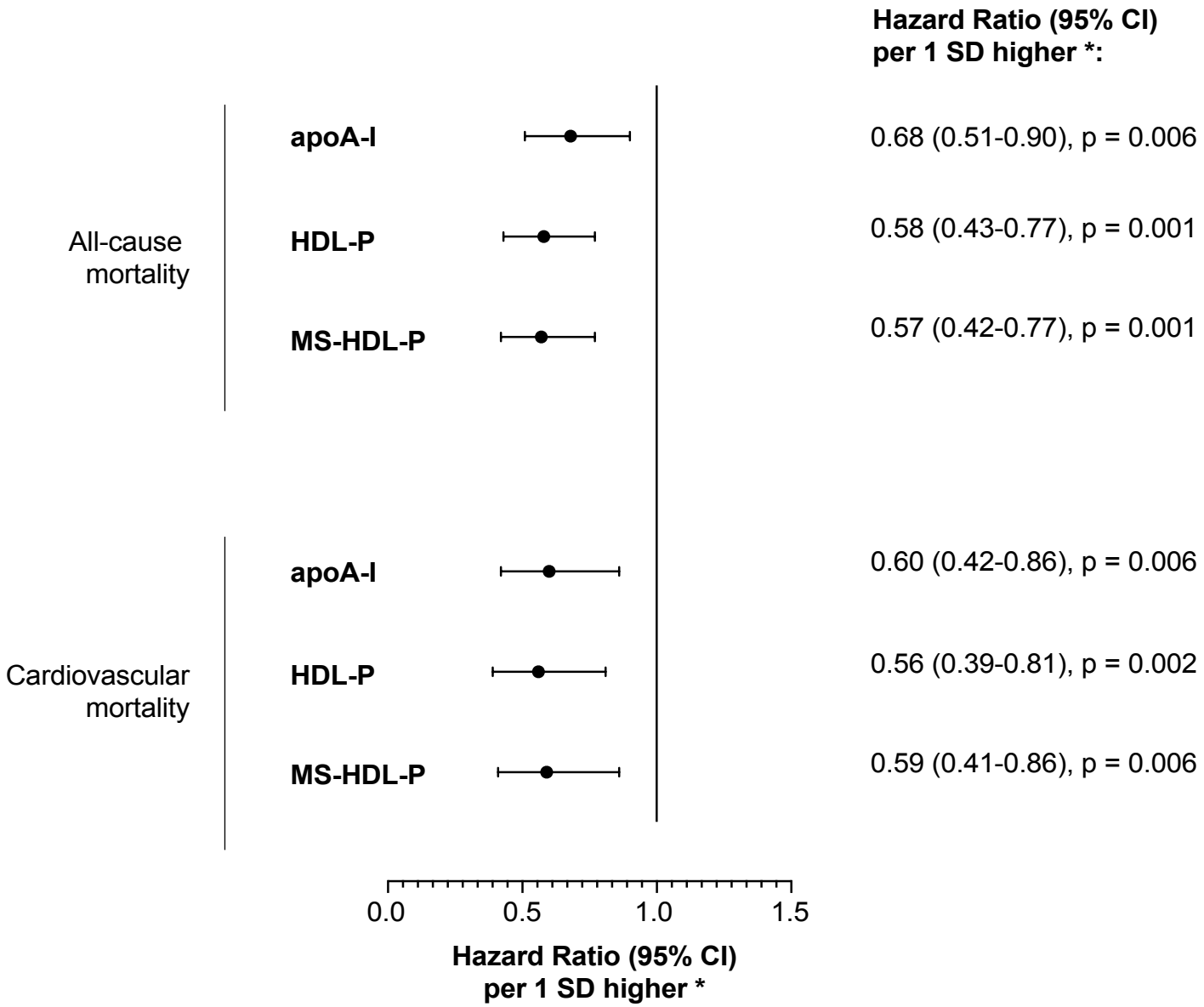
Hazard Ratio (95% CI)
per 1 SD higher *:
All-cause mortality
apoA-I
0.68 (0.51-0.90), p = 0.006
HDL-P
0.58 (0.43-0.77), p = 0.001
MS-HDL-P
0.57 (0.42-0.77), p = 0.001
Cardiovascular mortality
apoA-I
0.60 (0.42-0.86), p = 0.006
HDL-P
0.56 (0.39-0.81), p = 0.002
MS-HDL-P
0.59 (0.41-0.86), p = 0.006
0.0
0.5
1.0
1.5
Hazard Ratio (95% CI)
per 1 SD higher *

Figure 1

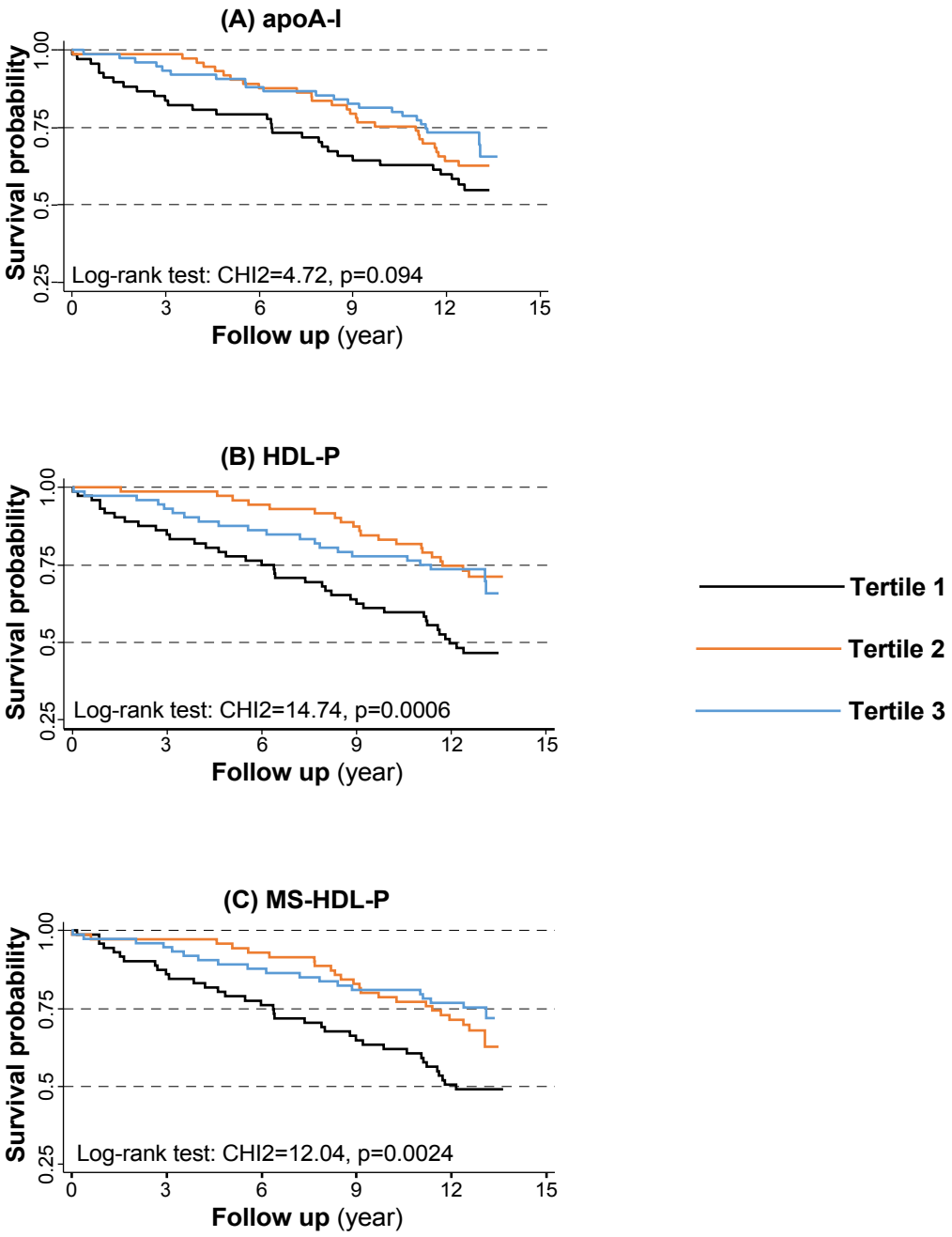
(A) apoA-I
Survival probability
1.00
0.75
0.5
0.25
Log-rank test: CHI2=4.72, p=0.094
0
3
6
9
12
15
Follow up (year)
(B) HDL-P
Survival probability
1.00
0.75
0.5
0.25
Log-rank test: CHI2=14.74, p=0.0006
0
3
6
9
12
15
Follow up (year)
(C) MS-HDL-P
Survival probability
1.00
0.75
0.5
0.25
Log-rank test: CHI2=12.04, p=0.0024
0
3
6
9
12
15
Follow up (year)
Tertile 1
Tertile 2
Tertile 3

Figure 2