UNIVERSITA' DEGLI STUDI DI VERONA

SCUOLA DI DOTTORATO IN SCIENZE DELLA VITA E DELLA SALUTE
Graduate School for Health and Life Sciences

DOTTORATO DI RICERCA IN SCIENZE MEDICHE CLINICHE E SPERIMENTALI
Doctoral program in Clinical and Experimental Biomedical Sciences

CICLO XXVII

Cardiac biomarkers in patients with ischemic heart disease enrolled in a cardiac rehabilitation program

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ANNO ACCADEMICO 2013/2014

ABSTRACT

BACKGROUND: Several plasma lipid and non-lipid biomarkers have been shown to predict major cardiovascular events in population studies, but data on novel biomarkers in secondary prevention are sparse and there exists marked heterogeneity across trials. **OBJECTIVE:** Aim of our study was to determine whether temporary changes of traditional lipid and new lipid and non-lipid biomarkers like UACR, hs-CRP, Nt-proBNP, Lp(a), ApoA and ApoB, observed during a Secondary Prevention and Cardiac Rehabilitation Program (SPCRP), are associated to CV risk (primary combined end-point of cardiovascular mortality and re-hospitalization). **MATERIALS AND METHODS:** we enrolled 167 ACS patients, 137 males (82%) and 30 females (18%); mean age of participants was 59.8 ± 11 years (32.5-78.5). The 12-months SPCRP was based on nurse counselling, multispecialistic visits and controlled training. Serial blood samples (plasma levels of TC, HDL C, LDL C, TG, Apo A, Apo B, hs-CRP, Nt-proBNP, Lp(a), UACR), BMI and WHR were assessed at baseline, at 6 and 12 months. A telephonic follow up [median of 36.2 months (27.7 – 77.0)] was performed to collect data. **CONCLUSIONS:** Among all the cardiac biomarkers considered only HDL C, Apo A and Nt-proBNP resulted to be independent predictors of cardiovascular mortality and re-hospitalization. Prognostic value of novel biomarkers in secondary cardiovascular prevention needs further investigations.

CONTENTS

ABBREVIATIONS

ACS Acute Coronary Syndrome

Apo A Apolipoprotein A

Apo B Apolipoprotein B

CAD coronary artery disease

CHD coronary heart disease

CI Confidence interval

CRP C-reactive protein

CV cardiovascular

CVD cardiovascular disease

HDL C High-density lipoprotein cholesterol

HF Heart Failure

Hs-CRP high-sensitivity C-reactive protein

LDL C Low-density lipoprotein cholesterol

LVEF Left Ventricular Ejection Fraction

Lp(a) Lipoprotein(a)

NP: Natriuretic Peptides

Nt-proBNP N-terminal pro-brain natriuretic peptide

SPCRP Secondary Prevention and Cardiac Rehabilitation Program

TC Total cholesterol

TG Triglycerides

UACR Urinary Albumin/Creatinine Ratio

6m six months

12m twelve months

INTRODUCTION

New cardiovascular (CV) risk markers like high sensitivity C-reactive protein (hs-CRP),[1,2] brain natriuretic peptide (BNP),[3] N-terminal pro brain natriuretic peptide (Nt-proBNP) [4] and urine albumin/creatinine ratio (UACR)[5] have been demonstrated to predict CV events in the general population. Although they add significantly to traditional CV risk factors their role in risk stratification and identification of subjects for primary prevention is not yet clear.[6] It has been previously shown that hs-CRP, Nt-proBNP and UACR provide additive prognostic information in the general population[6] as well as in hypertensive patients[7] probably because they are markers of damage in different parts of the CV system occurring at different time points in the atherosclerotic process.[6] High hs-CRP is thought to reflect the early atherosclerotic process,[8] high Nt-proBNP is thought to reflect the haemodynamic load on the heart and thereby CV hypertrophy and left ventricular dysfunction,[9] and high UACR is thought to reflect endothelial dysfunction and microvascular damage.[10] Therefore, it is likely that the predictive values of these three risk markers will differentiate depending on presence or absence of subclinical and overt CV disease. It is generally accepted that primary prevention is indicated in subjects with an estimated 10-year risk of CV death of 5% or above as estimated by HeartScore.[11,12] However, it is less clear whether primary prevention is appropriate in subjects with a HeartScore below 5%, but with high CV risk indicated by new risk markers.

Olsen et al in 2009 tried to investigate the predictive values of hs-CRP, Nt-proBNP and UACR on the composite CV end point of CV death, non-fatal myocardial infarction and non-fatal stroke in different CV risk groups based on HeartScore and history of CV disease or diabetes; they also tried to determine whether hs-CRP, Nt-proBNP and UACR added to risk prediction based on HeartScore and history of diabetes or CV disease by actually were able to change the original risk classification. Their data suggested that hs-CRP

should be used in subjects with low-moderate CV risk based on Heart-Score, Nt-proBNP in subjects with high CV risk, known CV disease or diabetes and UACR in all subjects.[13]

Data regarding an association between Lipoprotein (a) (Lp(a)) and CV risk in secondary prevention populations are sparse. [14]

Both epidemiological and experimental studies confirm the protective effect of high-density lipoprotein cholesterol (HDL C) on the onset of CAD (coronary artery disease) despite LDL C level, owing to the reverse cholesterol transport process of HDL C.[15-18]

However, in recent decades, some researchers assert that other newer lipid measurements, including non-HDL C, Apolipoprotein (Apo) A-I, apo B, and lipid ratios, are superior to traditional LDL C in predicting adverse outcomes in general population. Some researchers even suggest that apo B can replace the standard “lipid profile” as a target for monitoring and therapy in at-risk patients.[19-21] Besides, several translational studies find that the endothelial effect of HDL C may be totally different in patients with various clinical conditions.[22-24] Thus, the association between various lipid measurements and secondary risk of CAD deserves more attention due to limited and inconsistent results of previous studies.[25]

OBJECTIVE

Aim of our study was to determine whether temporary changes of traditional lipid and new both lipid and non-lipid biomarkers like UACR, hs-CRP, Nt-proBNP, Lp(a), ApoA and ApoB, assessed during a Secondary Prevention and Cardiac Rehabilitation Program (SPCRP), are associated to CV risk (primary combined end-point of cardiovascular mortality and re-hospitalization). We also evaluated global effectiveness of the 12-months SPCRP, consisting in a first intensive therapeutic phase (first 6 months with strict follow-up, counseling, drug titration and controlled physical activity) and in a second self-managed phase.

MATERIAL AND METHODS

From April 2008 to March 2009 we enrolled 167 consecutive ACS (Acute Coronary Syndrome) patients, 18% F, 82% M in a SPCRP. The 12-months SPCRP was based on nurse counselling, multispecialistic visits and controlled training. Serial blood samples (plasma levels of TC, HDL C, LDL C, TG, Apo A, Apo B, hs-CRP, Nt-proBNP, Lp(a), UACR and biometric parameters such as BMI (Body Mass Index) and WHR (Waist-to-hip ratio) were assessed at discharge (baseline), at 6 and 12 months. A telephonic follow up [median of 36.2 months (27.7 – 77.0)] was performed to collect data.

Statistical analysis

Continuous variables not deviating from a Gaussian distribution are reported as mean and standard deviation along with 95% confidence intervals, while variables with skewed distribution are reported as median (interquartile range). Comparisons were performed using the Student *t* test for paired data and Wilcoxon signed-rank test for continuous

variables as appropriate. Changes in biomarkers were tested with a signed-rank test, and compared between treatment groups with a Wilcoxon rank-sum test. The Bonferroni correction was used when needed to counteract the problem of multiple comparisons.

Cox proportional hazards modeling was used to estimate the hazard ratios (HRs) of the combined end-point cardiovascular mortality and re-hospitalization; all models were adjusted for age and gender. Variables that resulted significant predictors of event-free survival were then included in a multivariate model.

Analyses were performed with STATA software version 10 (Stata Corp – College Station, TX, USA).

RESULTS

We enrolled 167 patients, 137 males (82%) and 30 females (18%); mean age of participants was 59.8 ± 11 years (32.5-78.5). Discharge diagnosis were: Unstable Angina (25%), Non ST-Elevation Myocardial Infarction (31%) and ST-Elevation Myocardial Infarction (44%). Traditional CV risk factor were widely present in our population (arterial hypertension: 71.2%, family history of CHD: 70%, dyslipidemia: 80.2%, diabetes: 17.9%, smoking habit: 37% of patients).

There was a significant change in WHR during SPCRP (baseline vs 6m (0.98 ± 0.07 [95% CI 0.96-0.99] vs 0.96 ± 0.07 [95% CI 0 .95-0.98], t = 2.9537, p = 0.0039) while no significant variations were evidenced during the last 6m of the program (WHR 6m vs 12m (0.97± 0.07 [95% CI 0.96-0.99] vs 0.96 ± 0.09 [95% CI 0.94-0.98], NS).

No significant difference were observed for BMI values (kg/m^2) neither in the first phase (BMI baseline vs 6m (27.66 ± 4.13 [95% CI 27-28] vs 28.25 ± 9.41 [95% CI 26.77-29.73], NS) nor in the second one (BMI 6m vs 12m (28.26 ± 9.53 [95% CI 26.75-29.77] vs 27.14 ± 3.55 [95% CI 26.58-27.71], t = 1.6474, NS).

The difference between TC values (mg/dl) at baseline and at 6m was statistically significant (172.97 ± 37.4 [95% CI 167-179] vs 161.46 ± 34.55 [95% CI 156-167], t = 3.5721, p=0.0005) while no significant difference was evidenced between TC values at 6m and those at 12m (161.40 ± 34.38 [95% CI 156-167] vs 161.42 ± 31.12 [95% CI 157-166], NS).

There was a significant difference between LDL C (mg/dl) at baseline and its values at 6m (104.66 ± 34.16 [95% CI 99-110] vs 92.08 ± 28.59 [95% CI 87-97], t = 4.3578, p< 0.00001) while no significant differences were registered during the last 6m of SPCRP (LDL C 6m vs 12m (91.39 ± 28.67 [95% CI 87-96] vs 89.1 ± 24.15 [95% CI 85-93], NS).

A significant difference was found between HDL C levels (mg/dl) during the whole SPCRP period (HDL C baseline vs 6m (41.01 ± 10.96 [95% CI 39-43] vs 44.61 ± 10.12 [95% CI 43-46], t = -5.9003, p< 0.00001) and HDL C 6m vs 12m (44.61 ± 10.06 [95% CI 43-46] vs 46.22 ± 10.54 [95% CI 45-48], t = -3.6408, p=0.0004).

Even when TG values (mg/dl) during SPCRP were compared, there was a significant difference between baseline and 6m (TG baseline vs 6m (134.72 ± 68.77 [95% CI 124-146] vs 120.64 ± 61.51 [95% CI 110-130], t = 2.727, p = 0.014) but no difference was evident between 6m and 12m (130.07 ± 131.49 [95% CI 110-151] vs 127.07 ± 86.34 [95% CI 114-141], NS).

A significant difference was evidenced between Apo A levels (g/L) at baseline and at 6m (1.25 ± 0.21 [95% CI 1.20-1.29] vs 1.36 ± 0.23 [95% CI 1.31-1.41], t = -8.2126, p< 0.00001) and Apo A at 6m and at 12m (1.33 ± 0.21[95% CI 1.29-1.36] vs 1.38 ± 0.22 [95% CI 1.34-1.41], t = -4.2103, p<0.00001).

In contrast, no significant differences were found when Apo B values (g/L) during SPCRP were compared (Apo B at baseline vs 6m (0.84 ± 0.21 [95% CI 0.79-0.88] vs 0.81 ± 0.19 [95% CI 0.77-0.85], NS) and Apo B at 6m vs 12m (0.82 ± 0.22 [95% CI 0.78-0.85] vs 0.80 ± 0.19 [95% CI0.77-0.83], NS).

The median Lp(a) level at baseline was 345.5 (199-933.25) mg/L, at 6m was 262 (199-674.75) mg/L and at 12m was 206 (199-653) mg/L The difference between Lp(a) values at baseline and at 6m was statistically significant (z = 2.200, p =0.028). On the contrary, the difference between Lp(a) values at 6m and 12m was not statistically significant.

The median UACR level (mg/g) at baseline was 6 (4-14), at 6m was 6 (4-13) and at 12m was 6 (4-11). The difference between UACR values both at baseline vs 6m and at 6m vs 12m were not statistically significant.

The median NT-proBNP level (ng/L) at baseline was 275 (110-722), at 6m was 103.5 (43.5-199.25) and at 12m was 105 (42-208). The difference between NT-proBNP values at baseline and at 6m was statistically significant (z =6.513, p <0.0001), while the difference between NT-pro BNP values at 6m and 12m was not statistically significant (z =0.251,NS).

The median hs-CRP level at baseline was 2.92 (1.33-6.49) mg/L, at 6m was 1.95 (0.67-3.8825) mg/L and at 12m was 1.76 (0.81-3.0425) mg/L. The difference between hs-CRP values at baseline and at 6m was statistically significant (z =4.151, p =0.000). The difference between hs-CRP values at 6m and 12m remained statistically significant (z = 2.002, p = 0.045).

During a median follow up of 36.2 months (27.7 – 77.0) 4 patients died and 51 were hospitalized for cardiovascular reasons. Figure 1 shows event-free survival over time.

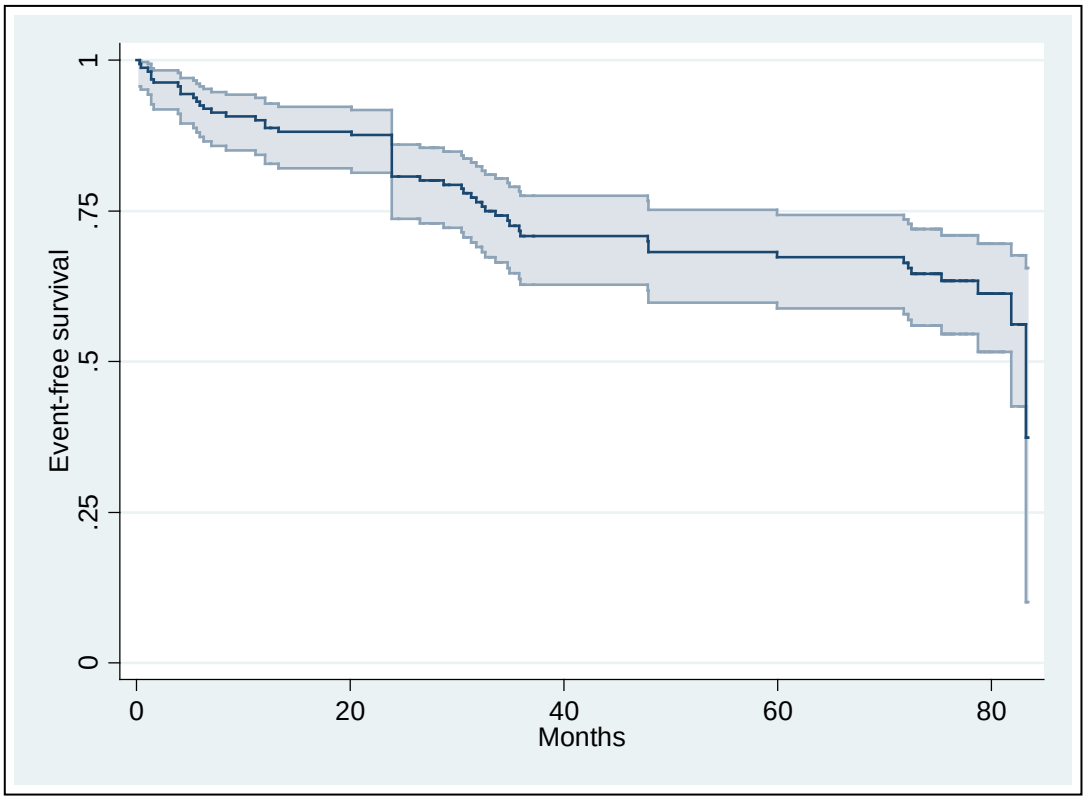

Figure 1. Event-free survival

At the Cox analysis, after adjusting for age and gender, both 6m HDL C (HR=0.97; p=0.0363) and 6m Apo A (HR=0.1704; p=0.021) were predictors of cardiac death/re-hospitalization. Six months Nt-proBNP (HR=1.0007, p=0.041) showed a predictive value too.

Other variables such as baseline LVEF, 6m BMI, 6m WHR, 6m hs-CRP, 6m LDL C, 6m TG, 6m Apo B, 6m Lp(a), 6m UACR did not show any significant predictive value.

In order to include the significant predictors in a multivariate Cox model we had to keep into account that TC and HDL C are significantly correlated (p<0.0001) so they cannot be included simultaneously in a multivariate model, in order to prevent serious multicollinearity problems; the same hold true for HDL C and Apo A (p<0.0001) that were thus included in two different models.

When HDL C, Nt-proBNP and baseline LVEF where included in a multivariate model, always adjusting for age and gender, only HDL C proved to be an independent and significant predictor of event-free survival (HR=0.9689, p=0.043) (see table 1 below).

_t	Haz. Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
sesso	1.126807	.4602625	0.29	0.770	.5060138	2.509207
eta	.9962187	.0143586	-0.26	0.793	.9684702	1.024762
hdl_2	.9689214	.0151141	-2.02	0.043	.9397466	.999002
fe_1	.0820049	.1377223	-1.49	0.136	.00305	2.204865
bnp_2	1.000284	.0003966	0.72	0.473	.9995073	1.001062

Table 1: multivariate analysis

Figure 2 depict how HDL C values above median are associated with a higher event-free survival.

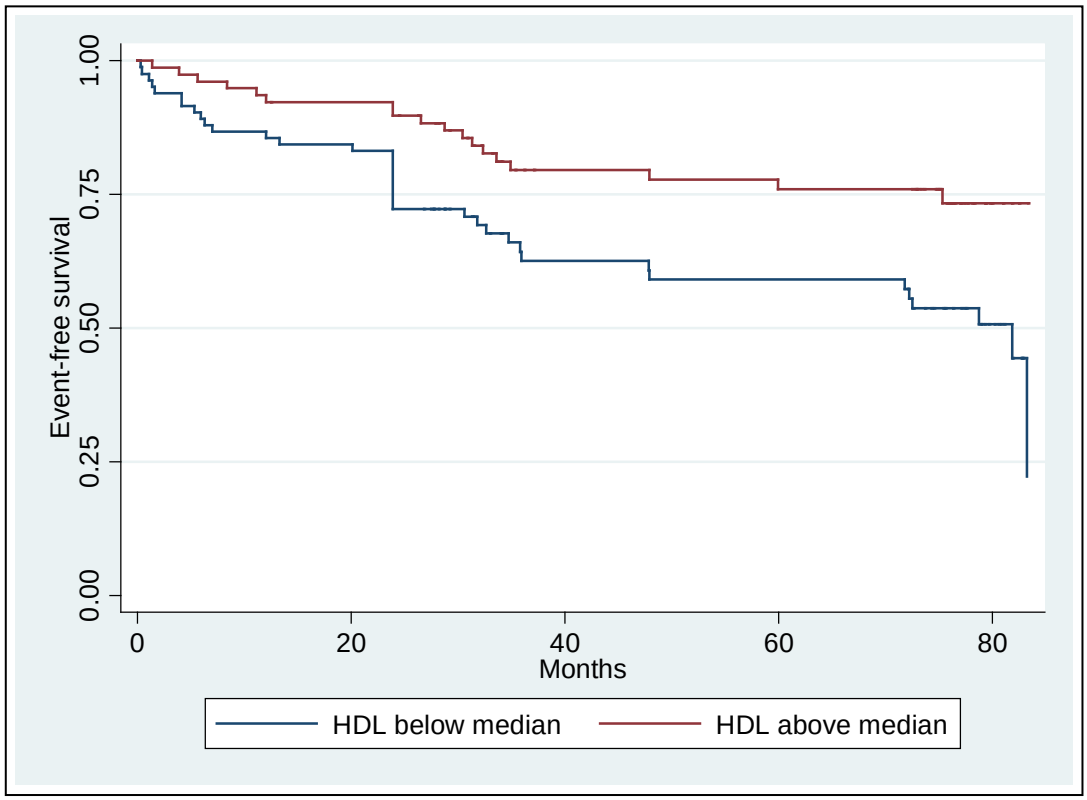

Figure 2: Event-free survival and HDL

Replacing HDL C with Apo A in the multivariate Cox model led to the same conclusions: Apo A was the only significant independent predictor (HR=0.178, p=0.029) (see table 2 below).

_t	Haz. Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
sesso	1.305779	.5428915	0.64	0.521	.5780612	2.949618
eta	.9916433	.0142184	-0.59	0.558	.9641638	1.019906
apoa_2	.1781493	.1407685	-2.18	0.029	.0378607	.8382614
fe_1	.1926441	.3412267	-0.93	0.352	.0059847	6.201149
bnp_2	1.000396	.0003994	0.99	0.322	.9996131	1.001179

Table 2: multivariate analysis

DISCUSSION

There has been much recent interest in the ability of non-lipid biomarkers associated with systemic inflammation, oxidative stress, tissue remodelling and/or insulin resistance to predict adverse cardiovascular outcomes and to identify individuals at high risk of future coronary heart disease (CHD) events and stroke [26,27].

The concentration of one of these, hs-CRP, predicts future CV events in apparently healthy individuals and in subjects treated with statins.[28-30]

The results of these studies have been used to support the argument that the concentration of non-lipid biomarkers such as hs-CRP should be included in algorithms designed to predict CV outcomes and to measure the efficacy of statin treatment. [31] However, there are inconsistencies, with some studies finding that levels of non-lipid biomarkers have minimal predictive power beyond of established CHD risk factors. [32-35]

C-reactive protein (CRP) , among other systemic inflammatory mediators, has been widely accepted as a potent risk indicator, independently predicting future CV events. The impact of CRP on CV outcome has been corroborated by a large number of observational studies and meta-analyses. These studies show that an elevated CRP has a clear prognostic value for major CV events and mortality, whereas the lowering of CRP is associated with a reduction in CV risk. Combining these findings with experimental observations has lead to a paradigm shift in which CRP is no longer merely a marker, but is increasingly considered as a mediator of CV disease [36]**.** Our results show a significant reduction of hs-CRP values from baseline to 12m (first phase: p =0.000, second phase: p = 0.045), but hs-CRP did not show any predictive value.

Since the widespread availability of hs-CRP, a large number of studies have investigated whether CRP is associated with atherosclerosis and vascular disease in animals and large

human cohorts to determine whether hs-CRP is a crucial causal mediator or a non-specific marker (bystander) of vascular disease. More recently, genetic studies have applied the principle of Mendelian randomization to distinguish whether genetic variations in the CRP gene are associated with circulating CRP levels and CHD. Furthermore, a recent clinical trial posits that lowering hs-CRP is associated with a reduction in CHD outcomes. [37]

With CRP being a strong risk factor for CV disease, and in light of evidence that CRP may play a biological role in atherosclerosis, the question arises as to whether an intervention to lower CRP levels may constitute a viable therapeutic strategy. In support of this hypothesis, several known anti-atherosclerotic interventions have been reported to reduce CRP. An example of a lifestyle intervention that reduces CRP levels, as well as CV risk, is weight loss. In a randomized, controlled trial consisting of 120 obese women, half the participants received detailed advice about how to achieve 10% weight reduction using diet and exercise, whereas the other half were given general information regarding a healthy lifestyle. After 2 years, women in the intervention group had a stronger decrease in BMI ($p < 0.001$), as well as a stronger reduction in CRP levels ($p= 0.008$), than women in the control group. Similarly, weight loss in morbidly obese patients has been shown to induce a significant decrease in CRP and IL-6 concentrations in association with an improvement of insulin resistance. In another study, baseline measures of obesity were significantly associated with CRP levels and subsequent weight loss was shown to result in a proportionate reduction in CRP.[37] Our data showed a reduction of both WHR and hs-CRP during the first 6 months of the program.

Statin therapy, besides lowering LDL-C, represents a pharmacological intervention that reduces both CRP levels and CV risk. Both weight loss and statin therapy apparently result in a reduction in low-grade inflammation, underlying a reduction in CV disease risk. The big question is: is this anti-atherogenic effect (partially) mediated by CRP reduction, or are the lower CRP levels a mere reflection of the reduction in inflammatory status? To

answer this question, the impact of specific inhibitors of CRP should be tested. [37] A first clue that specific CRP inhibition may be beneficial came in 2006, when Pepys et al. reported that a small-molecule inhibitor of CRP was able to reduce myocardial infarct size as well as cardiac dysfunction produced by injection of human CRP in rats. [38]

Olsen et al studied a Danish population sample of 2460 individuals, divided in three groups: 472 subjects receiving CV medication or having history of diabetes, prior myocardial infarction or stroke, 559 high-risk subjects with a 10-year risk of CV death above 5% as estimated by HeartScore, and 1429 low-moderate risk subjects with estimated risk below 5%. During the following 9.5 years the composite end point of CV death, non-fatal myocardial infarction or stroke occurred in 204 subjects. Composite endpoint was predicted in all three groups by UACR (HRs: 2.1, 2.1 and 2.3 per 10-fold increase, all $p<0.001$) or by hs-CRP (HRs: 1.9, 1.9 and 1.7 per 10-fold increase, all $p<0.05$), but not by Nt-proBNP (HRs: 1.1, 2.6 and 3.7 per 10-fold increase, last two $p<0.001$) ($p<0.05$ for interaction). In the low-moderate risk group, pre-specified gender adjusted (men/women) cutoff values of UACR> or =0.73/1.06 mg mmol(-1) or hsCRP> or =6.0/7.3 mg l(-1) identified a subgroup of 16% who experienced one-third of the composite endpoints. In the patient group, combined absence of high UACR and high Nt-proBNP> or =110/164 pg ml(-1) (men/women) identified a subgroup of 52% who experienced only 15% of the composite endpoints. Additional use of UACR and hs-CRP in subjects with low-moderate risk and UACR and Nt-proBNP in subjects with known diabetes or CVD changed HeartScore risk classification significantly in 19% of the population. [13]

B-type natriuretic peptide and NT-proBNP are markers of cardiac stress but are not cardiac-specific. They have comparable clinical utility, and both help in excluding acute Heart Failure (HF). Their use prior to discharge in hospitalized patients aids risk stratification. There is increasing evidence for risk stratification in acute Pulmonary

Embolism and ACS patients. The lower the value, the lower is the risk. A variety of new assays, e.g. mid-regional proANP and proBNP are being developed. So far, these assays are equivalent but no better than other testing.

Natriuretic peptides are related to ACS because myocardial ischaemia releases BNP, and the coherent diastolic and systolic abnormalities release Natriuretic Peptides (NP) as well. In ACS BNP and NT-proBNP values are powerful prognostic markers. Combination with cTn improves risk stratification in NSTEMI. In ACS trials, admission values of BNP .80 ng/L and NT-proBNP .1170 ng/L for men and .2150 ng/L for women identify high-risk patients. N-terminal proB-type natriuretic peptide measurements augment the prognostic information of clinical risk scores. Usually, NP did not predict recurrent acute myocardial infarction or a benefit from an invasive strategy. Optimal time for risk stratification is uncertain but studies suggest synergism between early and subsequent values. In patients with STEMI, NPs rise rapidly and values are correlated with infarct size and LV dysfunction. Among very old subjects with established CVD, NT-proBNP was the strongest risk marker for CV events and CV mortality. When estimating risk in secondary prevention in very old age, use of NT-proBNP should be considered. [39]

In secondary prevention in very old patients, measurement of NT-proBNP markedly improves prediction of recurrent CV events and CV mortality.

NT-proBNP might be used to select older people at the highest risk for recurrent CV events who may benefit most from strict secondary prevention. [40]

Our study showed a predictive value for the 6m Nt-proBNP plasma level (HR=1.0007, p=0.041). The difference between NT-proBNP values at baseline and at 6m was the only one statistically significant during SPCRP ($p < 0.0001$). UACR did not demonstrate any statistically significant variation during SPCRP, and 6m UACR did not show any predictive value at Cox analysis.

In concordance with earlier LIFE study publications, Ibsen et al showed that the risk for CV end points increased in a stepwise fashion with higher values for UACR in the patients with diabetes, a population for which the median baseline value of UACR was 3.05 mg/mmol (versus 1.28 mg/mmol in the overall population). The risk for the composite end point in patients with diabetes was increased by UACR≥1.94 mg/mmol. Their data indicate that albuminuria at a lower level than what is usually utilized as a cut point in patients with diabetes defines patients at increased risk of CV morbidity and mortality. UACR did not predict risk of myocardial infarction. The reason might be that diabetes in itself is a strong predictor for CV morbidity and mortality, partly overriding the influence of albuminuria as a risk factor in the present population with rather low levels of albuminuria. In summary, in patients with hypertension, diabetes, and ECG-documented left ventricular hypertrophy, increasing levels of baseline albuminuria were related to increased risk for CV morbidity and mortality. The risk for CV events was closely related to the in-treatment level of UACR, i.e., a reduction in albuminuria translated to a reduction in CV events. Their findings support the concept of monitoring of albuminuria in patients with hypertension and diabetes as part of proper disease management. [41]

Zandbergen et al demonstrated that normotensive patients with type 2 diabetes and miroalbuminuria run a marked risk for CV complications. The risk depends on the rate of 1-year change in urinary albumin excretion. Patients with rapid progression of albuminuria were at highest risk, whereas patients with regression of albuminuria had the lowest risk. This association persisted after adjustment for classic CV risk factors. Besides reducing blood pressure, RAS inhibitors are effective in preserving renal and cardiac function in diabetic patients. Moreover, they reduce albuminuria up to 40%, significantly more than other classes of antihypertensive drugs. Since albuminuria is strongly associated with CV, changes in albuminuria during treatment might reflect changes in CVD risk. A few studies recently showed that reduction of albuminuria in hypertensive diabetic patients reduces the

risk of subsequent CV events. However, these studies investigated hypertensive patients, thereby leaving open the possibility that blood pressure lowering explains the CV risk reduction, with albuminuria change just an innocent bystander. Sustained reduction in albuminuria reflected CV risk reduction in type 2 diabetic patients without hypertension. Hence, albuminuria change during treatment seems to reveal therapeutic responsiveness independent of blood pressure changes and is therefore useful as a modifiable treatment goal. These observations advocate a more aggressive approach to treating albuminuria in addition to more aggressive cardioprotective treatment in normotensive diabetic patients with elevated levels of albuminuria. [42]

Both sexes face increased risk of all-cause mortality, CV mortality, and end stage renal disease with lower estimated glomerular filtration rates and higher albuminuria. These findings were robust across a large global consortium. In a pooled analysis of over two million participants, Nitsch et al evidenced an increased risk of all-cause and CV mortality and end stage renal disease with lower estimated glomerular filtration rate and higher albuminuria in both sexes. In stark contrast to previous assertions that kidney disease should be defined by a lower threshold for estimated glomerular filtration rate and higher threshold for urinary albumin-creatinine ratio in women, they found the association between chronic kidney disease and mortality risk to be as strong in women as in men. Low estimated glomerular filtration rate or albuminuria should be considered at least as potent a risk factor in women as it is in men.[43]

Arsenault et al recently suggest that on top of traditional lipid parameters, several emerging cardiovascular disease risk factors such as NT-pro-BNP, Lp(a), neopterin, and sRAGE (soluble receptor for advanced glycation end products) are indeed associated with the risk of CV events. Whether or not therapies aiming at reducing the plasma levels of these biomarkers could be beneficial in terms of CV risk reduction warrants further investigation.[35]

Lipoprotein(a) is a genetic, causal risk factor for CVD. Population-based studies have determined that there is a continuous, graded association between Lp(a) levels and CV risk that is somewhat less marked compared with the association of elevated LDL-C and such risk. Partly because both Lp(a) and LDL-C contain the atherogenic moiety apo B100, there is a multiplier effect such that CV risk is synergistically increased when both lipoproteins are elevated; conversely, elevated Lp(a) becomes more clinically innocuous when accompanied by lower levels of LDL-C (<70 mg/dL after maximum statins). Elevated LDL-C (or apo B) should always be targeted for lipid-modifying therapy before treating elevated Lp(a). Niacin reduces Lp(a) by up to 40% and has been identified by consensus treatment panels as the only medication that consistently lowers Lp(a). Because niacin treatment can reduce the apo B100 component of both LDL and Lp(a), such therapy is a rational alternative in the presence of refractorily elevated LDL-C despite maximum-dose statins or statin combination therapy. Further clinical trials are needed to determine if reductions in CV risk can be specifically ascribed to on-treatment changes in Lp(a). [44]

With respect to its proatherosclerotic and prothrombotic effects, Lp(a) is believed to be a promising and critical biomarker for CV risk estimation. Causal relationship between Lp(a) and CVD has been recognized and demonstrated in the past decades. In patients with established CVD and with target LDL-C level achievement, Lp(a) measurement may add addictive value for CV risk stratification.[45] In our study we evidenced a significant variation in Lpa concentration only during the first phase of SPCRP ($p = 0.028$). No predictive value was registered for 6m Lp(a) concentrations.

The clinical interest in Lp(a) is largely derived from its role as a CV risk factor. Although not considered an established risk factor, Lp(a) levels have been associated with CVD in numerous studies. Recently Lp(a) serum levels were found to be associated with the severity of aortic atherosclerosis, especially in abdominal aorta, as well as coronary atherosclerosis. Moreover a study by Momiyama et al. demonstrated that elevated Lp(a)

has incremental prognostic value in symptomatic patients with coronary artery revascularization. Lp(a) is involved in the development of atherothrombosis and activation of acute inflammation exerting a proatherogenic and hypofibrinolytic effect. Lp(a) plays a critical role in the proinflammatory reaction and can be considered as a common joint among different metabolic systems. Other actions of Lp(a) can be resumed as follows: inhibition of the activation of plasminogen; inhibition of the activation of TGF-β; activation of acute inflammation; induction of the expression of adhesion molecules; elevation of the production of cytokines. Moreover Lp(a) is implicated in the activation of endothelial uptake, oxidative modification, and foam cell formation, suggesting that these processes could play an important role in atherosclerosis. Recent findings suggest that Lp(a)-lowering therapy might be beneficial, at least in some subgroups of patients with high Lp(a) levels. A possible future therapeutic approach could include apheresis in high risk patients with already maximally reduced LDL cholesterol levels in order to reduce major coronary events. However, further studies are needed to define such subgroups with regard to Lp(a) levels, apo(a) size, and the presence of other risk factors. [46]

Lp(a) is significantly associated with the risk of CV events in patients with established CAD; however, there exists marked heterogeneity across trials. In particular, the prognostic value of Lp(a) in patients with low cholesterol levels remains unclear.

In summary, although it was demonstrated that patients with established CAD who have a high level of Lp(a) are at an increased risk of subsequent MACE, the marked heterogeneity between studies raises questions regarding the value of Lp(a) as a clinically useful biomarker for risk assessment, particularly among patients with well controlled LDL cholesterol. Moreover, although Lp(a) may directly contribute to CHD, there is currently insufficient evidence to suggest that Lp(a) levels above a discrete cut point should be used to guide therapy or that treatment will translate into improved clinical outcomes . Trials are now ongoing with novel therapies that reduce Lp(a), such as the novel CETP inhibitors

anacetrapib, mipomersen and PCSK9 inhibitors; although, such therapies influence other lipid components in tandem. Recently, a specific antisense oligonucleotide directed toward apo(a) was shown to lower apo(a) and Lp(a) levels in transgenic mice, and a phase I trial is underway. If a strategy of Lp(a) reduction should ultimately prove to be successful, it will be of interest to determine whether benefit is observed regardless of baseline Lp(a) concentration or specific reduction in Lp(a).[14]

Apo B and apo A-I are important markers of atherogenicity and atheroprotection, respectively. On the whole, data in healthy populations and in patients with specific lipid abnormalities and/or preexisting CVD support the concept that apo B is a better measure of CVD risk than LDL C. Apo B may be particularly relevant in the setting of insulin resistant states, such as diabetes and metabolic syndrome, as patients with these disorders often manifest normal LDL C values, but have a preponderance of small, dense LDL particles and higher Apo B. Incorporating Apo B and the apo B/apo A-I ratio into risk assessment could therefore provide additional and important information on CVD risk. A recent Consensus Conference Report [47] recommended that Apo B be added to risk assessment in persons at high cardiometabolic risk, with target levels of<90 mg/dL and<80 mg/dL in high risk and highest risk persons, respectively. Achieving this Apo B goal will likely require intensive statin therapy, which has been shown to have beneficial effects on all apolipoproteins. Since Apo B is not yet routinely measured in clinical practice, however, and since Apo B is the primary Apoprotein component of LDL C, it is prudent at this time to continue to lower LDL C levels aggressively using appropriate statin therapy. Although it provides superior risk discrimination, the apo B/apo A-I ratio cannot yet be recommended for routine clinical use, as clinical trial data showing the benefit on outcomes of increasing apo A-I and HDL-C are lacking.[48] Our data did not show any significant Apo B variations during SPCRP while significant differences were evidenced for both Apo A and HDL C during the whole SPCRP. At the Cox analysis, after adjusting for age and gender, both 6m

HDL C (HR=0.97; p=0.0363) and 6m Apo A (HR=0.1704; p=0.021) were predictors of cardiac death/re-hospitalization. Six months LDL C and 6m Apo B did not show any significant predictive value.

Among Chinese CAD patients, both too low and too high levels of HDL-C may increase all-cause and CVD mortality. LDL-C remains as an effective and proper predictor for CAD prognosis, while LDL/HDL ratio can strengthen the prediction. Besides, high levels of apoB, apoB/apoA-Iratio, and low apo A-I level can increase the risk of CVD mortality among CAD patients.[25]

Fraley et al showed, in a population of patients with ACS, changes in Oxidized LDL biomarkers in response to statin therapy in large and specific subgroups of patients that have not been documented previously and provide insights into the interrelationships of these biomarkers to clinical, demographic, and inflammatory variables. The consistent increase in Oxidized Phospholipids/apoB, and to a lesser extent Lp(a), in response to atorvastatin across all subgroups tested suggests that it may serve as a benchmark for future studies evaluating such biomarkers. Future studies are warranted to assess whether changes in these biomarkers reflect therapeutic efficacy and predict clinical events. [49]

Nordestgaard et al recommend screening for elevated Lp(a) in those at intermediate or high CVD/CHD risk, a desirable level, 50 mg/dL as a function of global cardiovascular risk, and use of niacin for Lp(a) and CVD/CHD risk reduction.[50]

CONCLUSIONS

Our study confirms effectiveness of a 12-months SPCRP in controlling traditional cardiovascular risk factors. Among all the cardiac biomarkers considered only HDL C, Apo a and Nt-proBNP resulted to be independent predictors of cardiovascular mortality and re-hospitalization. Other biomarkers did not show any predictive value in our study. These findings suggest that further and larger studies are needed to assess their usefulness in secondary prevention. Perhaps, our search for better markers of risk in ACS should move away from inflammatory markers alone and include biomarkers that reflect different, unrelated, pathogenic pathways leading to the development and recurrence of the condition. Hopes are now placed on genetic markers, transcriptomics, proteomics, and metabolomics, which are expected to represent tools that may help us overcome the limitations we face at present. However, the challenge for these new markers will be to provide true, sizeable, incremental information over and above that provided by existing clinical markers of risk and help clinicians to improve patient management.[51]

Predictive value of novel biomarkers in secondary cardiovascular prevention needs further investigations.

Additional systematic assessments in large epidemiological cohorts of novel biomarkers ideally performed as head-to-head comparisons of data or if not then using patient-level meta-analysis will be required to determine the best CVD screening strategies. [51]

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