Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2020; e1220. doi: 10.1002/cti2.1220
www.wileyonlinelibrary.com/journal/cti

ORIGINAL ARTICLE

Prognostic and therapeutic considerations of antibodies against c-ter apolipoprotein A-1 in the general population

Nicolas Vuilleumier[1,2], Panagiotis Antiochos[3], Pedro Marques-Vidal[3], Sabrina Pagano[1,2], Julien Virzi[1], Nathalie Satta[1,2], Oliver Hartley[4], Hubert Gaertner[4], Karim J Brandt[5], Fabienne Burger[5], Fabrizio Montecucco[6,7], Gerard Waeber[3], François Mach[5] & Peter Vollenweider[3]

[1]Division of Laboratory Medicine, Diagnostics Department, Geneva University Hospitals, Geneva, Switzerland
[2]Department of Medicine Specialties, Medical Faculty, Geneva University, Geneva, Switzerland
[3]Department of Internal Medicine, Lausanne University Hospital, Lausanne, Switzerland
[4]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[5]Division of Cardiology, Foundation for Medical Researches, Department of Medical Specialties, University of Geneva, Geneva, Switzerland
[6]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy
[7]IRCCS Ospedale Policlinico San Martino Genoa–Italian Cardiovascular Network, Genoa, Italy

Correspondence
N Vuilleumier or S Pagano, Division of Laboratory Medicine, Diagnostics Department, Geneva University Hospitals, 1205 Geneva, Switzerland.
E-mails: nicolas.vuilleumier@hcuge.ch or sabrina.pagano@hcuge.ch

Received 25 June 2020;
Received 13 October and 6 November 2020;
Accepted 6 November 2020

doi: 10.1002/cti2.1220

Clinical & Translational Immunology
2020; **9**: e1220

Abstract

Objectives. Autoantibodies against apolipoprotein A1 (anti-apoA1 IgGs) and its C-terminal region (cter apoA1) have emerged as an independent biomarker for cardiovascular disease. Cter apoA1 mimetic peptides were shown to reverse the deleterious anti-apoA1 IgG effects *in vitro*. We evaluated the association of anti-cter apoA1 IgGs with overall mortality in the general population and tested the ability of a cter apoA1 mimetic peptide to reverse the anti-apoA1 IgG-induced inflammatory response and mortality *in vitro* and *in vivo*, respectively. **Methods.** Anti-cter apoA1 IgGs were measured in serum samples of 6386 participants of the CoLaus study of which 5220 were followed for a median duration of 5.6 years. The primary outcome was overall mortality. The peptide inhibitory concentration 50% (IC_{50}) was determined *in vitro* on HEK-Blue-4 and RAW cells. $ApoE^{-/-}$ mice were exposed to 16 weeks of anti-apoA1IgG passive immunisation with and without peptide co-incubation. **Results.** Anti-cter apoA1 IgGs were associated with higher interleukin 6 levels and independently predicted overall mortality; an increase of one standard deviation of anti-cter apoA1 IgG level was associated with an 18% increase in mortality risk (hazard ratio: 1.18, 95% confidence interval: 1.04–1.33; $P = 0.009$). The cterApoA1 analogue reversed the antibody-mediated inflammatory response with an IC_{50} of 1 μM *in vitro* but did not rescue the significant anti-apoA1 IgG-induced mortality rate *in vivo* (69% vs. 23%, LogRank $P = 0.02$). **Conclusion.** Anti-cter apoA1 IgG independently predicts overall mortality in the general population. Despite being effective *in vitro*, our cter apoA1 analogue did not reverse the anti-apoA1 IgG-induced mortality in mice. Our data suggest that these

autoantibodies are not readily treatable through cognate peptide immunomodulation.

Keywords: anti-apoA-1 IgG, ApoE$^{-/-}$ mice, CoLaus study, C-terminal A1 peptide, passive immunisation

INTRODUCTION

During the last decade, evidence has accumulated that autoantibodies against high-density lipoproteins (HDLs) and their components impact multiple pro-atherogenic processes, facilitating atherogenesis and the occurrence of cardiovascular (CV) diseases.[1] Autoantibodies of the IgG subclass against apolipoprotein A1 (anti-apoA1 IgG), the major apolipoprotein of HDL, have been shown to be an independent CV risk factor in the general population, as well as predictors of poor CV outcomes and overall mortality in most populations reported so far.[2–11] Translational investigations showed that anti-apoA1 IgGs efficiently promote sterile inflammation, facilitate foam cell formation *in vitro,* and enhance the development of atherosclerosis and atherothrombosis in mice through specific innate immune receptors complexes and related signalling pathways.[12–18] In humans, the polyclonal anti-apoA1 IgG response has been shown to be preferentially oriented against the last alpha helix of the C-terminal part of apoA1 (amino acids: 241–266).[19] We previously showed that a corresponding mimetic peptide in a specified three-dimensional conformation could be used for both the detection of anti-c-terminus apoA1 IgG (anti-cter apoA1 IgG) and the neutralisation of deleterious anti-apoA1 IgG pro-inflammatory effects *in vitro*, including dampening of the anti-apoA1 IgG-induced inflammation[20] and related foam cell formation.[21]

Aside a single-centre and small-sized prospective study,[8] no data are currently available regarding the possible prognostic value of anti-cter apoA1 IgG. Therefore, we explored the associations of these autoantibodies with demographic and biological characteristics and assessed the prognostic value of anti-cter apoA1 IgG on a large general population sample of 6386 individuals.[3–5]

Furthermore, although several *in vitro* studies showed that some of these cter apoA1 mimetic peptides efficiently abrogate the anti-apoA1 IgG pro-inflammatory and pro-atherogenic responses,[19–21] knowledge of whether such protective effects could be reproduced *in vivo* is still lacking. Therefore, we challenged the ability of our peptide to rescue the anti-apoA1 IgG-induced mortality in apoE$^{-/-}$ mice.

RESULTS

Anti-cter apoA1 IgG associations with baseline clinical and biological characteristics

The baseline demographic characteristics of the participants included from the CoLaus cohort are presented in Table 1. When analysed by increasing anti-cter apoA1 IgG tertiles, these autoantibodies were found to be associated with female gender, a very high CV risk SCORE category, and inversely associated with BMI. The prevalence of history of acute myocardial infarction tended to be higher in the last anti-cter apoA1 IgG tertile. No other associations with baseline clinical characteristics were found. Anti-cter apoA1 IgG autoantibodies were found to be associated with higher levels of anti-apoA1 IgGs, lower total and low-density lipoprotein (LDL) cholesterol in patients without lipid-lowering treatment, lower triglyceride levels and a lower HOMA index (Table 2). Those autoantibodies were also found to be weakly associated with higher median uric acid levels in men, and higher IL-1β and IL-6 in the last tertile (Table 2). We further fitted restricted cubic splines (RCSs) to assess linearity of the associations between IL-1b, IL-6, tumor necrosis alpha (TNF-α) and titres of anti-cterA1 IgG, using a RCS model with five knots at 5%, 27.5%, 50%, 72.5% and 95% of the anti-cterA1 IgG distribution. This analysis was non-significant for non-linear associations (all P for non-linearity < 0.1, Supplementary figure 1).

When the cohort was split according to anti-cter apoA1 IgG seropositivity, which comprised 20.7% of the individuals (1323/6386), the prevalence of smoking was higher in anti-cter apoA1 IgG seropositive individuals than in seronegative individuals, but the association with

Table 1. Clinical characteristics of the overall sample according to anti-cter apoA1 IgG tertiles (in OD at 405 nm)

Overall sample (n = 6386)	Anti-cter apoA1 IgGT1: OD < 0.21 (n = 2128)	Anti-cter apoA1 IgGT2: OD 0.21 to < 0.33 (n = 2129)	Anti-cter apoA1 IgGT3: OD ≥ 0.33 (n = 2 129)	P-value
Age, years	52.8 ± 10.6	52.5 ± 10.7	52.8 ± 11.0	0.614
Male sex, n (%)	1066 (50.1)	1011 (47.5)	961 (45.2)	0.006
Hypertension, n (%)	753 (35.4)	722 (33.9)	750 (35.2)	0.555
Diabetes, n (%)	150 (7.0)	133 (6.3)	135 (6.3)	0.521
Current smoking, n (%)	559 (26.2)	549 (25.8)	595 (28.0)	0.242
Current alcohol consumption, n (%)	1544 (72.5)	1552 (72.9)	1493 (70.2)	0.097
Autoimmune disease, n (%)	48 (2.3)	43 (2.0)	61 (2.9)	0.174
SLE, n (%)	1 (0.1)	3 (0.1)	4 (0.2)	0.416
Heart rate, bpm	68.1 ± 9.7	67.7 ± 9.7	68.2 ± 10.2	0.157
Blood pressure, mmHg				
SBP	128.3 ± 17.7	128.1 ± 17.9	127.8 ± 18.2	0.735
DBP	79.2 ± 10.9	79.5 ± 10.9	79.1 ± 10.8	0.446
Metabolic syndrome, n (%)	522 (24.5)	470 (22.1)	464 (21.8)	0.069
Body Mass Index, $kg{\cdot}m^{-2}$	26.0 ± 4.6	25.6 ± 4.4	25.8 ± 4.6	0.015
Cardiovascular risk calculated by SCORE	2.0 ± 3.2	2.1 ± 3.5	2.2 ± 3.9	0.096
CV risk categories according to SCORE, n (%)				
Low risk	1236 (58.1)	1289 (60.6)	1258 (59.2)	
Intermediate risk	416 (19.6)	354 (16.7)	336 (15.8)	
High risk	223 (10.5)	227 (10.7)	236 (11.1)	
Very high risk	251 (11.8)	256 (12.0)	295 (13.9)	0.017
History of:				
Total cardiovascular disease, n (%)	149 (7.0)	127 (6.0)	163 (7.7)	0.089
Coronary heart disease, n (%)	78 (3.7)	62 (2.9)	85 (4.0)	0.147
Acute myocardial infarction, n (%)	69 (3.2)	52 (2.4)	77 (3.6)	0.078
Stroke, n (%)	44 (2.1)	29 (1.4)	29 (1.4)	0.107
Family history of				
Total cardiovascular disease, n (%)	152 (7.1)	152 (7.1)	163 (7.7)	0.753
Coronary heart disease, n (%)	109 (5.1)	104 (4.9)	109 (5.1)	0.923
Stroke, n (%)	45 (2.1)	57 (2.7)	62 (2.9)	0.236
CV drugs				
Aspirin, n (%)	340 (16.0)	328 (15.4)	335 (15.7)	0.885
Statins, n (%)	264 (12.4)	205 (9.6)	212 (10.0)	0.006
Beta-blockers, n (%)	113 (5.3)	113 (5.3)	126 (5.9)	0.599
ACEi/ARA, n (%)	157 (7.4)	154 (7.2)	165 (7.8)	0.801
Diuretics, n (%)	49 (2.3)	46 (2.2)	43 (2.0)	0.821

Data are expressed as mean ± standard deviation or number of participants and (percentage). Statistical analysis by the chi-squared test for categorical variables and the Kruskal–Wallis test for continuous variables.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

gender could no longer be detected. Our sensitivity analyses indicated that these associations were similar in primary or secondary CV prevention groups (Supplementary table 1). In the latter situation, a higher proportion of individuals under β-blockers was found in seropositive individuals (Supplementary table 1). Supplementary table 2 indicates that several of these biological associations were substantially different in patients in secondary CV prevention; the associations with renal function and LDL cholesterol were lost, whereas the association with uric acid in men became stronger and a significant association with higher homocysteine levels was observed. A trend was observed for ultra-sensitive C-reactive protein (US-CRP), but no associations were found for the three analysed cytokines (Supplementary table 2). Finally, the associations between anti-cter apoA1 IgGs and anti-apoA1 IgGs, total cholesterol, LDL, triglycerides, HOMA index and IL-6 results were modestly but significantly corroborated by Spearman correlation analyses (Supplementary table 2).

Table 2. Biological characteristics of the overall sample according to anti-cter apoA1 IgG tertiles (in OD at 405 nm)

Overall sample (n = 6386)	Anti-cter apoA1 IgGT1: OD < 0.21 (n = 2128)	Anti-cter apoA1 IgGT2: OD 0.21 to < 0.33 (n = 2129)	Anti-cter apoA1 IgGT3: OD ≥ 0.33 (n = 2129)	P-value
Anti-apoA-1 IgG, n (%)	217 (10.2)	341 (16.0)	713 (33.5)	< 0.001
Anti-apoA-1 OD	0.31 (0.2–0.47)	0.38 (0.26–0.55)	0.52 (0.36–0.75)	< 0.001
Lipid metabolism				
Total cholesterol, mmol L^{-1}	5.6 (4.9–6.3)	5.5 (4.9–6.3)	5.4 (4.7–6.1)	< 0.001
LDL cholesterol, mmol L^{-1}	3.3 (2.8–4)	3.3 (2.7–3.9)	3.2 (2.6–3.8)	< 0.001
Under lipid-modifying treatment	3.0 (2.4–3.6)	3.0 (2.4–3.5)	3.0 (2.4–3.6)	0.747
Devoid of lipid-modifying treatment	3.4 (2.8–4)	3.3 (2.8–4)	3.2 (2.6–3.8)	< 0.001
HDL cholesterol, mmol L^{-1}	1.6 (1.3–1.9)	1.6 (1.3–1.9)	1.6 (1.3–1.9)	0.703
In men	1.4 (1.2–1.6)	1.4 (1.2–1.6)	1.4 (1.2–1.6)	0.850
In women	1.8 (1.5–2.1)	1.8 (1.5–2.1)	1.8 (1.5–2.0)	0.421
Triglycerides, mmol L^{-1}	1.2 (0.8–1.7)	1.1 (0.8–1.6)	1.1 (0.8–1.5)	< 0.001
Apolipoprotein B, mg dL^{-1}	139 (98–212)	144 (101–207)	140 (99–199)	0.278
Glucose metabolism				
HOMA Index	1.78 (1.17–2.76)	1.70 (1.13–2.64)	1.65 (1.10–2.69)	0.026
Renal function				
Creatinine, μmol L^{-1}	78.1 (69.6–88)	78.8 (69–88.4)	78.0 (69–88)	0.230
eGFR, mL min^{-1}/1.73 m^2	78.1 (69.1–88.3)	77.7 (68.9–87.1)	77.7 (68.4–87.9)	0.465
Surrogate markers of CVD				
Uric acid, μmol L^{-1}	310 (255–367)	303 (251–362)	302 (245–366)	0.057
In men	351 (307–406)	352 (304–397)	359 (310–415)	0.019
In women	264 (224–315)	262 (227–308)	258 (222–304)	0.075
Homocysteine, μmol L^{-1}	9.5 (8–11.5)	9.5 (7.9–11.6)	9.5 (7.8–11.8)	0.903
Ultra-sensitive CRP, mg·L^{-1}	1.3 (0.6–2.8)	1.2 (0.6–2.6)	1.3 (0.6–2.8)	0.054
Cytokines				
IL-1β, pg mL^{-1}	0.38 (0–1.81)	0.36 (0–1.58)	0.46 (0–1.79)	0.038
IL-6, pg mL^{-1}	1.29 (0.56–3.1)	1.22 (0.54–2.95)	1.42 (0.64–3.50)	< 0.001
TNF–α, pg mL^{-1}	2.99 (1.89–4.58)	2.74 (1.74–4.28)	2.88 (1.76–4.72)	< 0.001

Data are expressed as mean ± standard deviation or median (inter quartile range) according to the variable distribution. Statistical analysis was performed by the Student's t-test or the Kruskal–Wallis test, depending on the distribution.

CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA, Homeostasis Model Assessment; IL, interleukin; LDL, low-density lipoprotein; TNF, tumor necrosis factor; US-CRP, ultra-sensitive C-reactive protein.

Anti-cter apoA1 IgG associations with study endpoints

As shown in Table 3, Cox regression analyses showed that each standard deviation (SD) increase of anti-cter apoA1 IgG was associated with a 25% increase in mortality risk (HR: 1.25; 95% CI: 1.11–1.41, $P < 0.001$), which remained of the same order of magnitude in our adjusted model (HR: 1.18, 95% CI: 1.04–1.33; $P = 0.009$). While these analyses of per SD increase confirmed the linear relationship between anti-cter apoA1 IgG levels and mortality risk, the association according to anti-cter apoA1 IgG status indicated that seropositive individuals are exposed to a two-fold increase in mortality risk when compared with seronegative individuals (HR: 2.05; 95% CI: 1.42–2.95, $P < 0.001$), which remained similar in the adjusted model (HR: 1.75; 95% CI: 1.20–2.55; $P = 0.004$). Kaplan–Meier analyses showed that the cumulative mortality in anti-cter apoA1 IgG seropositive individuals was 9% versus 4.1% (LogRank $P < 0.001$; Figure 1), translating into an absolute mortality difference of 4.9%. Moreover, anti-cter apoA1 IgGs were not found to be associated with coronary artery disease (CAD) incidence (Table 3), which did not change whether these events were fatal or not (data not shown).

To assess for non-linearity in the adjusted association between anti-cter apoA1 IgG titres and risk of (1) all-cause mortality and (2) incident CAD during follow-up, we fitted RCS models with five knots at 5%, 27.5%, 50%, 72.5% and 95%. The linear continuous dose–response associations between anti-cterA1 IgG

Table 3. Hazard ratios of anti-cter apoA1 IgG for overall mortality and incident CAD

Study endpoints	Hazard ratio (95% CI); *P* Per each anti-cter apoA1 IgG standard deviation increase	Hazard ratio (95% CI); *P* Anti-cter apoA1 IgG seropositive vs seronegative individuals
All-cause mortality (*n* = 192)		
Unadjusted	1.25 (1.11–1.41); *P* < 0.001	2.05 (1.42–2.95); *P* < 0.001
Adjusted	1.18 (1.04–1.33); *P* = 0.009	1.75 (1.20–2.55); *P* = 0.004
Incident CAD (*n* = 159)		
Unadjusted	0.95 (0.80–1.13); *P* = 0.584	1.32 (0.84–2.07); *P* = 0.229
Adjusted	0.89 (0.74–1.08); *P* = 0.235	1.15 (0.71–1.87); *P* = 0.558

Results are expressed as unadjusted and adjusted hazard ratios with 95% confidence interval according to Cox proportional hazards regression. Analyses were adjusted for age, sex, systolic blood pressure, diabetes, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-blocker treatment, eGFR and autoimmune disease.

CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

Table 4. Hazard ratios of anti-cter apoA1 IgG/ anti-apoA1 IgG for overall mortality and incident CAD

	Anti-cter apoA1 & anti-apoA1 negative (*n* = 3694) Hazard ratio (95% CI); *P*	Anti-cter apoA1 negative & anti-apoA1 positive (*n* = 735) Hazard ratio (95% CI); *P*	Anti-cter apoA1 positive & anti-apoA1 negative (*n* = 323) Hazard ratio (95% CI); *P*	Anti-cter apoA1 & anti-apoA1 positive (*n* = 266) Hazard ratio (95% CI); *P*
All-cause death (*n* = 192)				
Unadjusted	1 (ref.)	1.17 (0.79–1.76); *P* = 0.432	1.57 (0.92–2.69); *P* = 0.102	2.80 (1.76–4.45); *P* < 0.001
Adjusted	1 (ref.)	1.30 (0.86–1.97); *P* = 0.215	1.22 (0.70–2.12); *P* = 0.489	2.78 (1.73–4.46); *P* < 0.001
Incident CAD (*n* = 159)				
Unadjusted	1 (ref.)	1.37 (0.90–2.08); *P* = 0.139	1.02 (0.59–1.75); *P* = 0.956	1.55 (0.83–2.89); *P* = 0.166
Adjusted	1 (ref.)	1.35 (0.87–2.08); *P* = 0.179	1.28 (0.68–2.38); *P* = 0.443	1.20 (0.59–2.41); *P* = 0.616

Results are expressed as adjusted hazard ratios and (95% confidence interval) across the four combined anti-cter apoA1 IgG and anti-apoA1 IgG serological groups. The Anti-cter apoA1 & anti-apoA1 negative group was defined as the reference ratio (ref) of 1. Statistical analysis by Cox proportional hazards regression adjusted for age, sex, systolic blood pressure, diabetes, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-blocker treatment, eGFR and autoimmune disease.

CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

levels and risk of all-cause mortality and incident CAD using RCS are shown in Supplementary figure 3. *P*-values for non-linearity were calculated by comparing RCS terms to linear models using the Wald test. The *P*-value for non-linearity was (1) $P = 0.879$ for all-cause mortality and (2) $P = 0.241$ for incident CAD. We found no evidence of a non-linear association between increasing titres of anti-cterA1 IgG levels and outcomes and analysis by tertiles of anti-cterA1 IgG levels yielded similar results (Supplementary table 4).

Finally, due to fact that the correlation between anti-cter apoA1 and anti-apoA1 IgG was modest ($r = 0.358$) in the longitudinal samples, we evaluated how the risk factors would vary across the four different combinations of serological status. As shown in Table 4, double seropositive individuals displayed a three-fold increased risk when compared to double seronegative participants, which remained unchanged after multivariable adjustment (HR: 2.78; 95% CI: 1.73–4.46; $P < 0.001$). In addition, double seropositivity did not significantly affect the incident CAD hazard.

Model discrimination improvement after inclusion of Anti-cter apoA1 IgG and Anti-apoA1 IgG

We further assessed the model's discrimination and reclassification capacity, before and after the inclusion of anti-cterA1 IgG and anti-apoA1 IgG for overall mortality and incident CAD. With regard to

Table 5. Discrimination and reclassification for all-cause mortality and incident CAD, after addition of (a) anti-cter apoA1 IgG and (b) anti-cter apoA1 IgG and anti- apoA1 IgG

Study endpoints	Model discrimination C-statistic	Model reclassification IDI	Model reclassification NRI (p)
All-cause mortality			
Baseline model	0.786	–	–
+ anti-cter apoA1 IgG	0.792 ($P = 0.045$)	0.514 ($P = 0.025$)	6.3% ($P < 0.001$)
+ both antibodies[a]	0.797 ($P = 0.017$)	0.724 ($P = 0.007$)	6.9% ($P < 0.001$)
Incident CAD			
Baseline model	0.822	–	–
+ anti-cter apoA1 IgG	0.824 ($P = 0.285$)	0.185 ($P = 0.093$)	0.4% ($P = 0.098$)
+ both antibodies[a]	0.827 ($P = 0.058$)	0.126 ($P = 0.392$)	1.2% ($P = 0.101$)

Statistical analysis adjusted for age, sex, systolic blood pressure, diabetes, smoking, HDL and LDL cholesterol, baseline CAD, statin, beta-blocker treatment, eGFR and autoimmune disease.

CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; IDI, integrated discrimination improvement; NRI (p), net reclassification improvement at the event rate.

[a]Both antibodies: anti-cter apoA1 IgG + anti-apoA1 IgG.

all-cause mortality, inclusion of anti-cterA1 IgG on top of the multivariate baseline model significantly improved the C-statistic, IDI and NRI (p). Inclusion of anti-apoA1 IgG on top of this model yielded further improvement in discrimination and reclassification indices (Table 5).

For incident CAD, the multivariate baseline model for incident CAD already presented an excellent predictive capacity (baseline C-statistic: 0.822). Inclusion of autoantibodies on top of this model did not result in significant improvements in model discrimination or reclassification (Table 5).

Cter apoA1 mimetic peptide 50% inhibitory concentration *in vitro* and *in vivo* dose definition

The cter apoA1 mimetic peptide (F3L1) was tested for its ability to inhibit anti-apoA1 IgG-induced inflammatory cytokine release in HEK cells expressing human TLR4, as well as a mouse macrophage cell line (RAW cells). On these two cell lines, F3L1 showed comparable inhibitory activity against an anti-apoA1 IgG-induced inflammatory response (Figure 2), with fitted IC_{50} values of 4.3 μM (95% CI 2.3–8.2) and 5.4 μM (3.9–9.7).

Cter apoA1 mimetic peptide impact on anti-apoA1 IgG-induced apoE$^{-/-}$ mice mortality

Using a validated passive immunisation protocol in apoE$^{-/-}$ mice,[12–14] 100 μL of F3L1 at 1 mM (0.295 mg) was co-incubated for 2 h at room temperature with either anti-apoA1 IgG or control IgG (50 μg per injection) every other week for 16 weeks. As shown on Figure 3, the mortality rate in anti-apoA1 IgG recipient mice was 69% (11/16), whereas it was 23% (3/13) in control IgG recipient mice. This difference was found to be significant (69 vs 23%; LogRank $P = 0.02$). The mortality rate in peptide recipient mice was found to be similar to the one observed in the control IgG recipients (13% vs 23%; LogRank $P = 0.41$). As shown on Figure 3, when co-incubated with the peptide, the anti-apoA1 IgG-induced mortality rate remained unchanged (75% vs 69%, LogRank $P = 0.59$). Finally, when control IgGs were co-incubated with the analogue, the mortality rate increased significantly from 12% to 53% (LogRank: $P = 0.01$). Due to the high mortality in these different mice recipient groups, key pre-analytical factors could not be respected for organs and blood sampling, which prevented us from performing exhaustive histological examinations. At the beginning and at the end of the protocol (for living mice), mice weight, appearance and activity across the different groups were similar.

DISCUSSION

The key finding of this study lies in the fact that anti-cter apoA1 IgG levels measured by using F3L1 as a capture antigen displayed an independent

and dose-dependent association with overall mortality, but not with incident CV events in the general population. The absolute overall mortality risk difference between anti-cter apoA1 seropositive and seronegative individuals was 4.9% whereas the absolute mortality difference was 1.6% according to anti-apoA1 IgG seropositivity,[5] corresponding to a three-fold relative difference.

These results extend existing knowledge in the field by suggesting that (1) the anti-cter apoA1 IgG strength of association for overall mortality seems to be superior to the one previously reported for anti-apoA1 IgGs[5] and (2) anti-cter apoA1 IgGs are not associated with anamnestic nor incident CAD. Because anti-cter apoA1 IgGs were only modestly correlated ($r = 0.36$) with anti-apoA1 IgGs, this differential association pattern suggests that both antibodies represent related but different entities, even if they display a similar seropositivity prevalence (around 20%) and independently predict overall mortality. The cumulative effect observed of double seropositivity on overall mortality (but not incident CAD) risk is in accordance with this concept and nurtures the hypothesis that anti-cterApoA1 may be more specific for assessing the overall individual risk of death, whereas anti-apoA1 IgG could be more predictive of CV disease (CVD). These results also indicate that assessing the levels of both antibodies at the same time would probably be more relevant to a patient's overall prognosis rather than applying a sequential order, as is done in screening strategies. While likely to represent a specific class of antibodies, the inverse associations between anti-cter apoA1 IgG total and LDL cholesterol together with the positive correlations with pro-inflammatory parameters retrieved presently are globally similar to the ones reported with anti-apoA1 IgG on the same cohort.[3–5] Because the latter have been shown to mediate atherogenesis through sterile inflammation activation[12–16] and macrophage cellular cholesterol homeostasis disruption,[17,18] we would have expected such similar correlations to be associated with similar pathogenic mechanisms and therefore to similar associations with clinical outcomes, but it was not the case. Therefore, whether anti-cter apoA1 IgGs display different pathogenic mechanisms from anti-apoA1 IgGs, which underlie their distinct prognostic associations, deserves further investigation.

The second notable finding of this translational study lies in the fact that, despite the fact that passive immunisation with anti-apoA1 IgG increased the mortality rate of apoE$^{-/-}$ mice in a similar proportion (69% vs 23%: three-fold relative change) than what was retrieved in seropositive individuals included in the CoLaus cohort, our cter apoA1 analogue was unable to neutralise the previously demonstrated anti-apoA1 IgG-induced mortality in apoE$^{-/-}$ mice. Several explanations could explain the failure of our apoA1 analogues *in vivo* despite providing promising results *in vitro*. One possible reason is the relative simplicity of our *in vitro* models consisting of human and murine macrophages, which certainly do not reflect the *in vivo* biological complexity, especially in regard to immune complex (IC) physiopathology. Indeed, in our *in vitro* models, the inhibitory effect of the cter apoA1 analogues has been related to their ability to block anti-apoA1 IgG binding to cells by occupying the idiotypic part of anti-apoA1 IgG that is expected to lead to IC formation,[22] which in turn would prevent the subsequent activation of toll-like receptor (TLR) 2/4/CD14 complex.[12,14,15] Because Fc gamma receptors ($Fc_{\Upsilon}R$) are not expressed in HEK-blue 4 cells, the possible contribution of $Fc_{\Upsilon}R$ subtype activation by IC[22] could not be fully assessed in our *in vitro* models. Along the same line, as heat-inactivated serum was used in our experimental procedures, neither could we detect an IC-dependent complement activation[22] effect in our *in vitro* models. Moreover, in our mice passive immunisation protocol, we specifically tested the ability of IC consisting of apoA1 and cognate antibodies to neutralise the anti-apoA1 IgG-induced mortality, assuming that these IC would be efficiently cleared from the apoE$^{-/-}$ mice reticuloendothelial system without major biological impacts. The fact that mice exposed to our cter apoA1 analogue co-incubated with control IgG (polyclonals) displayed a significant increase in mortality when compared to the analogues alone, and a similar mortality rate to the ones observed with either anti-apoA1 IgGs alone or co-incubated with the analogues, is in favor of an IC contribution to the observed mortality increase. Nevertheless, because kidneys were not taken upon mice sacrifice and could not be obtained within the requested pre-analytical time for most mice as a result of the high mortality rate (75%), we could not formally ascribe the failure of the cter apoA1 peptide to neutralise anti-apoA1 IgGs effects to the generation of IC. The fact

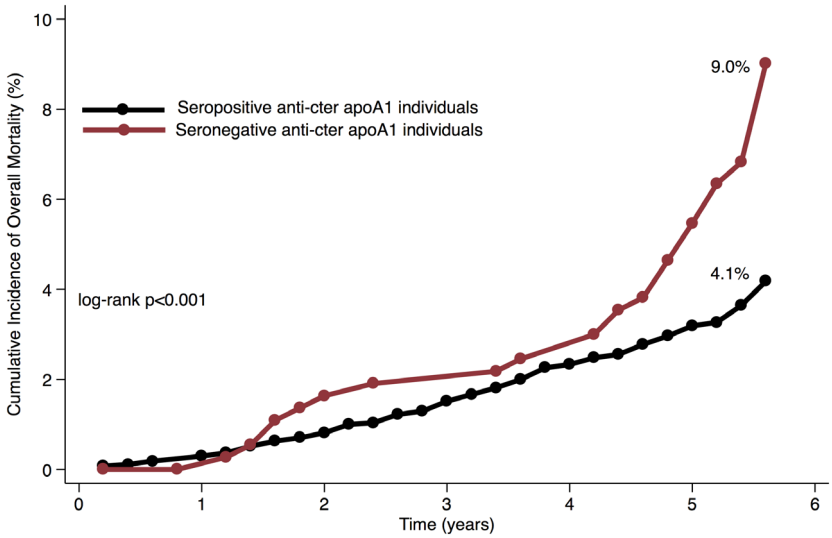

Figure 1. Overall Survival according to anti-cter apoA1 seropositivity status. Kaplan–Meier analyses were used to evaluate the overall mortality incidence according to anti-cterapoA1 IgG status retrieved in the 5520 CoLaus individuals.

that all reagents were devoid of endotoxin together with the rather delayed occurrence of deaths (by day 30), a reagent contamination issue can reasonably be excluded. Our results may appear to contrast with a recent observation in humans, where the presence of IC consisting of apoA1 and IgG subclass 4 was inversely related to adverse events, including death.[23] Nevertheless, because of the complex IC physiopathology characterised by a concentration-dependent balance between beneficial and deleterious IC biological properties,[22] we believe that our *in vivo* results are the consequence of an excess of IC formation, even if we could not formally and conclusively demonstrate this.

A limitation to this study relates to the high mortality rate of mice in the group receiving anti-apoA1 IgG alone or in combination with the both analogues, as well as control IgG with both analogues. The mortality rate observed for anti-apoA1 IgG recipient mice was 69%, whereas it was previously 25%.[12] The reasons for such a mortality increase are still elusive, but a reagent or a mice-contamination issue has been reasonably excluded. Because of this high mortality rate, we could not respect the mandatory pre-analytical requirement for organ sampling in all these groups. This prevented us from performing tissue and histological analyses to study the impact of these peptides on the anti-apoA1 IgG-induced atherogenesis,[12–14] or from confirming possible tissue IC deposition in the kidneys. The same phenomenon impeded us from obtaining proper blood samples required for further biological characterisation of the mice used. Nevertheless, these limitations do not affect the key conclusion of our animal study, which demonstrates the inability of apoA1 analogues to neutralise the deleterious anti-apoA1 IgG effects *in vivo*. A second limitation is that, because of our clinical study design, we could not assess the causal link between the presence of anti-cter apoA1 IgG and overall mortality. In this respect and despite the aforementioned limitations, our animal study was instrumental to underpin this possible causal link, as we used polyclonal anti-apoA1 IgGs in our passive immunisation protocol. Another limitation resides in the fact that we used a single antigen concentration to neutralise anti-apoA1 IgG based on the maximal feasible dose.[24] Such concentration exceeded anti-apoA1 IgG concentration by 24-fold and was identical to the apoA1 mimetic peptide concentrations previously shown to be beneficial in mice through the same route of administration.[25] Therefore, even if only one single neutralising apoA1 mimetic peptide was tested *in vivo* and could not be compared to higher concentrations, we believe that an adequate peptide concentration was used *in vivo*. Although specific causes of mortality were adjudicated (Supplementary table 5), we could not ascribe in further detail the exact mortality cause associated with anti-cter apoA1 antibodies. Finally, we did not collect data on medications

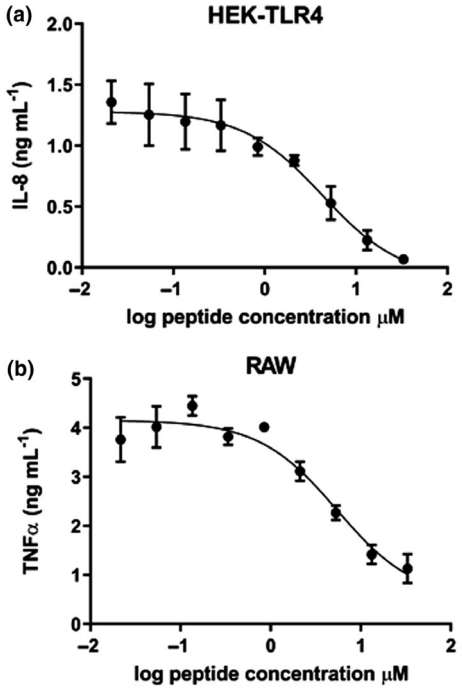

Figure 2. Inhibitory capacity of cter apoA1 to inhibit anti-apoA-1 IgG pro-inflammatory effects. **(a)** Inhibitory capacity of peptide analogues to inhibit anti-apoA-1 IgG-induced IL-8 release in HEK-TLR2 reporter cells. Results are expressed as median and range of three independent experiments (n = 3). **(b)** Inhibitory capacity of peptide analogues to inhibit anti-apoA-1 IgG-induced TNF-alpha release in RAW cells (mouse macrophage cell line). Results are expressed as median and range of three independent experiments (n = 3). The concentrations of peptides are expressed in μM.

therefore could not account for a possible change in medications during follow-up.

In conclusion, this reverse translational study shows for the first time that this cter apoA1 mimetic peptide can be used as a capture antigen to detect cognate antibodies whose levels independently predict premature overall mortality in the general population. Furthermore, despite appealing *in vitro* results, our study shows that this cter apoA1 analogue does not neutralise the anti-apoA1 IgG-induced death *in vivo*. From a precision medicine perspective, if our results indicate that anti-cter apoA1 IgG may allow the identification of individuals at high risk of premature death, they do not support the hypothesis that these autoantibodies could be useful targets of cognate peptide immunomodulation. Because other therapeutic means have been reported to neutralise anti-apoA1 IgGs effects *in vitro*, such as intravenous immunoglobulins, L-Type calcium channel blockers, and eplerenone, using an already existing means may represent a more appealing option than a mimetic peptide based-approach. Among these, colchicine inhibits the same innate immune receptors as those that are activated by anti-apoA1 IgGs.[12,14,15,18,26] Because colchicine treatment has recently been shown to be highly effective at reducing incident ischemic CV events,[27] knowing whether anti-apoA1 or anti-cter apoA1 IgG seropositive individuals could represent a subgroup particularly prone to benefit from this treatment may represent an important field of investigation in the field of humoral autoimmunity against apoA1 in CVD.

METHODS

Cter apoA1 mimetic peptide synthesis

As published previously,[19,20] our lead C-terminal apoA1 mimetic peptide (F3L1: amino acids 241–266, sequence: GLLPVLESFKVSFLSALEEYTKKLNT, molecular weight: 2950.4 Dalton) was synthetised and maintained in a given three-dimensional conformation via lactam bridge between Glu^{259} and Lys^{263}. The peptide was synthesised according to a standard Fmoc-protocol on Rink amide AM resin (Novabiochem, Merck, Darmstadt, Germany) using a Prelude synthesizer (Protein Technologies, Manchester, UK). For F3L1, orthogonal protecting groups (allyl/allyloxycarbonyl) were used for the glutamic acid and lysine residues utilised to form the lactam bridge. At the end of resin elongation, these protecting groups were removed according to the procedure of Kates *et al*.[28] On-resin lactam bridge formation,[29] monitored by the Kaiser ninhydrin test, was carried out with three equivalents of 6-chloro-benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate and nine equivalents of *N*, *N*-diisopropylethylamine over 48 h at 37°C. As reported earlier,[20] the mass of our peptide was verified by mass spectrometry and the alpha helix content was evaluated by circular dichroism spectroscopy. Since we have previously demonstrated that an F3L1 scrambled peptide failed to reverse the pro-inflammatory and pro-atherogenic effects of anti-apoA1 IgGs,[20,21] this peptide was not used in the present study. F3L1 was reconstituted in sterile water at a concentration of 1 mM (2.95 g mL^{-1}).

The CoLaus cohort and study endpoints

As previously published, the 'clinical and biological data were obtained from the CoLaus study, a population-based cohort of 6733 participants aged 35–75 years recruited between 2003 and 2006 in the city of Lausanne, Switzerland'. Of the initial baseline sample of 6649 participants with available anti-apoA1 IgG measures,[3–5] 6386 had remaining serum samples to assess anti-cter apoA1 IgG. 5220 (mean age 52.6 ± 10.7 years, 47.3% men) had complete clinical and biological data over a median

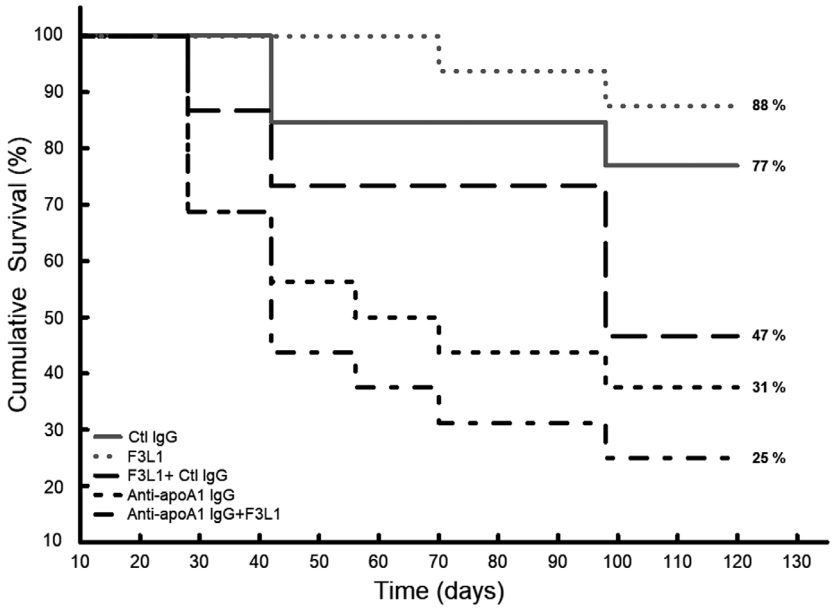

Figure 3. Impact of passive immunisation with and without apoA1 mimetic peptide on mice survival. Survival analysis of apoE$^{-/-}$ mice exposed to anti-apoA-1 IgG (n = 16), Ctl IgG (n = 13), apoA-1 analogue (F3L1) alone (n = 16) or after 2 h of co-incubation of F3L1 with anti-apoA-1 IgG (n = 16) and Ctl IgG (n = 15). For differences between the mortality rates of the various apoE$^{-/-}$ mice groups, please refer to the main text.

follow-up time of 5.6 years and were included in this prospective analysis. A detailed description of the study design, variables and sampling procedures has been reported elsewhere.[3–5,30,31] The study was approved by the Institutional Ethics Committee of the University of Lausanne, and written informed consent was obtained from all participants before inclusion in the study in accordance with the Declaration of Helsinki.

All participants attended the outpatient clinic of the University Hospital of Lausanne. Clinical data and fasting venous blood samples were collected from each participant by trained field interviewers during a single visit lasting about 60 min. Blood pressure and heart rate were measured three consecutive times using an automated sphygmomanometer (Omron® HEM-907, Matsusaka, Japan), and the average of the last two measurements was used. Body weight and height were measured with participants standing without shoes in light indoor clothes. Body weight was measured in kilograms to the nearest 100 g using a Seca® scale, which was calibrated regularly. Height was measured to the nearest 5 mm using a Seca® height gauge. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Hypertension was defined as a systolic blood pressure (SBP) $\geq$140 mmHg and/or a diastolic blood pressure $\geq$ 90 mmHg, and/or the presence of anti-hypertensive treatment. Diabetes mellitus was defined as fasting plasma glucose $\geq$ 7.0 mmol L^{-1} and/or insulin or other oral anti-diabetic treatment. Prevalent CVD was defined by the presence of myocardial infarction, angina pectoris, percutaneous revascularisation or bypass grafting for ischemic heart disease, stroke or transient ischemic attack and was assessed according to standardised medical records.[30,31] History of autoimmune diseases (ADs) was obtained via questionnaire. Estimated glomerular filtration rate (eGFR) was calculated by the simplified Modification of Diet in Renal Disease prediction equation. Absolute risk for CVD was computed using the SCORE algorithm.

The primary study endpoint was overall mortality. The secondary endpoints consisted of incidents of CAD, defined as fatal or non-fatal myocardial infarction, stable or unstable angina, or percutaneous coronary revascularisation bypass grafting. The primary study endpoint was adjudicated by two independent internal medicine specialists, and secondary endpoints by two independent cardiologists. All adjudicators were blinded to biochemical results.

Blood sampling, biomarkers, anti-apoA1 IgG and anti-cter apoA1 IgG levels' determination on CoLaus

Venous blood samples were drawn after an overnight fast, and assays were either performed on fresh plasma samples within 2 h of blood collection for routine analyses, or previously on unthawed CoLaus serum aliquots (2003–2006) for anti-apoA1 anti-cter apoA1 IgG determination (see below). Routine analyses consisted of standard lipid profile, serum creatinine, glucose, homocysteine, uric acid, US-CRP, interleukin (IL)-1β, IL-6 and TNF-α measures that were performed according to routine methods as previously reported.[31,32]

Anti-apoA1 and anti-cter apoA1 IgGs were measured following our validated anti-apoA1 IgG protocol reported above,[2–5,7,8,10,11,13,14,16,17,33] except that the F3L1 apoA1 analogue (1 μg per well) was used as coating antigen instead of fully delipidated and native apoA1.[20] The seropositivity cut-off for anti-cter apoA1 IgGs was prospectively set at an optical density (405 nm) value of 0.5,

corresponding to the 97.5th percentile of the distribution obtained on healthy blood donors and reported previously.[21] The intra- and inter-assay coefficients of variation at the seropositivity cut-off were < 6%.

In vitro determination of 50% inhibitory concentration for cter apoA1 mimetic peptide

As previously published, two cell lines were used to test the ability of our peptide analogue to inhibit the anti-apoA1 IgG-induced cytokine production[19,20] in order to define the 50% inhibitory concentration (IC_{50}): Human embryonic kidney 293 (HEK293) cells stably expressing human TLR4, MD2, and CD14 (HEK-Blue-4; InvivoGen, San Diego, CA, USA); and Raw cells. Anti-apoA1 IgGs (100 μg mL^{-1}) were incubated with the peptide across a concentration range from 100 to 0.06 μg mL^{-1} for 2 h at room temperature prior addition to cells. After 24 h of stimulation, IL-8 and TNF-α were quantified in the cell supernatants by ELISA according to manufacturer's instructions (R&D System, Minneapolis, MN, USA).

In vivo study

Eleven-week-old $ApoE^{-/-}$ mice were submitted to passive immunisation with anti-apoA1 IgG and respective control IgG for additional 16 weeks, according to our previously described protocol.[12–14] Briefly, mice (n = 13–16 mice per experimental group) were fed under the standard chow diet and received intravenous endotoxin-free (< 0.25 EU mL^{-1} using the limulus amebocyte lysate endochrome assay) goat polyclonal anti-human apoA1 IgG (Academy Biomedical Company, Houston, TX, USA) or goat polyclonal IgG controls (Meridian Life Science, Memphis, TN, USA; 50 μg per mouse per injection) every 2 weeks for 16 weeks.[12–14] Antibodies were pre-incubated with PBS or the cter apoA1 analogue (F3L1) for 2 h at room temperature prior to injection. The F3L1 dose chosen for this study (590 μg or 200 nmol) per mouse per injection) corresponds to the maximal feasible dose, taking into account the peptide's maximum solubility (2 mM) and the maximum injection volume for the study (150 μL total; 100 μL F3L1 solution plus 50 μL antibody solution). Assuming a total mouse blood volume of 1.5 mL,[24] these injections would be expected to achieve a circulatory concentration of 132 μM, that is 24-fold in excess of the IC50 values determined *in vitro* (Figure 2). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the local and ethics authorities (Geneva Veterinary Office and the Ethic Commission of Animal Experimentation of the University of Geneva). This study conformed to the 'position of the American Heart Association on Research Animal Use'.

Reagents

RPMI-1640 medium, foetal bovine serum, PBS free of Ca^{2+} and Mg^{2+}, L-glutamine, penicillin and streptomycin were obtained from Gibco BRL-Life Technologies (Rockville, MD, USA). Interferon-gamma (IFN-γ) was from Roche (Mannheim, Germany). Affinity-purified goat polyclonal anti-human apoA-1 IgG (ref. 11AG2) was obtained from Academy Biomedical Company, and goat control IgG was obtained from Meridian Life Science (ref. A66200H). Raw 264.7 macrophage cells were kindly provided by Professor Patrice Lalive, Geneva University Hospitals, and HEK-Blue-4 were purchased from InvivoGen.

Statistical analysis

For the clinical study, bivariate analysis of continuous variables was performed using the Student's or Mann–Whitney tests as appropriate, while analysis of categorical variables was performed using the chi-squared test. In the cross-sectional analysis, the associations of anti-cter apoA1 IgG levels with baseline clinical and demographic characteristics were evaluated for 6386 individuals using analyses per anti-cter apoA1 IgG tertiles, autoantibody seropositivity status and by Spearman correlation for continuous variables. Because of the exploratory nature of this work and the pre-specified analysis planned, adjustments for multiple tests were not performed. Results were expressed as median, interquartile range (IQR) and range unless stated otherwise.

To assess non-linearity in the association of continuous biomarkers (IL-1b, IL-6, TNF-α, uric acid) with titres of anti-cter apoA1 IgG, we further fitted RCSs models. We used an RCS model with five knots at percentiles 5%, 27.5%, 50%, 72.5% and 95% of the anti-cter apoA1 IgG distribution, as previously proposed.[34,35] Varying the number or location of the knots (using percentiles 10%, 50%, 90% or 5%, 35%, 65% and 95%[34]) had negligible influence on the estimates. A *P*-value for non-linearity was calculated by comparing RCS terms to linear models using the Wald test.

For the longitudinal part of the clinical study, considering the mortality rate in CoLaus (192 deaths) and a two-sided alpha of 0.05, our study had 80% power to detect a relative risk for overall mortality of 1.45 in participants positive for anti-cterA-1 IgG. The association of anti-cter apoA1 IgG levels with overall mortality was assessed on the 5220 individuals sampled with available follow-up. The association of anti-cter apoA1 levels with the study endpoints was assessed by Kaplan–Meier curve analyses and by Cox proportional hazards. Kaplan–Meier analyses were performed according to anti-cter apoA1 IgG seropositivity based upon a predefined and validated cut-off value [OD value > 0.5 (21)], and differences were assessed using the log-rank test. For Cox regression analyses, due to the skewed anti-cter apoA1 IgG distribution, natural log-transformed anti-cter apoA1 IgG concentrations were used to calculate hazard ratios (HR) provided with corresponding 95% confidence intervals (95% CI). HRs were expressed per standard deviation (SD) increase of anti-cter apoA1 IgG levels and according to anti-cter apoA1 IgG seropositivity. Multivariate HRs were calculated after adjusting for age, gender, hypertension, diabetes, smoking, BMI, eGFR, HDL and LDL cholesterol, baseline CVD, and ADs. Humoral autoimmunity against apoA1 being known to be relevant for CVD,[2,3,5,8,10,11] sensitivity analyses were performed in primary and secondary prevention subgroups. Secondary prevention

individuals were defined by the anamnestic presence of any CAD, any stroke or peripheral artery disease. Primary prevention individuals were defined as those without the aforementioned conditions irrespective of the presence of CV risk factors.

To assess for non-linearity in the adjusted association between anti-cter apoA1 IgG titres and risk of (1) all-cause mortality and (2) incident CAD during follow-up, we further fitted RCS models with five knots at 5%, 27.5%, 50%, 72.5% and 95%. The dashed lines show the 95% confidence interval for the adjusted hazard ratio point estimates associated with different autoantibody levels. A *P*-value for non-linearity was calculated using the Wald test.

Finally, we assessed the model's discrimination as well as reclassification capacity before and after the inclusion of anti-cter apoA1 IgG and anti-apoA1 IgG for overall mortality and incident CAD. Discriminative capacity was assessed by Harrell's C-statistic.[36] Because of the absence of established reclassification cut-offs points for overall mortality and incident CAD, we assessed reclassification according to the category-free integrated discrimination improvement (IDI)[36] and the net reclassification improvement (NRI) 'at the event rate' (NRI(p)),[37] as recommended by Pencina *et al*. and others.[36,38]

For *in vitro* experiments, the Kruskal–Wallis test was used to detect median differences among three or more independently sampled groups of a single non-normally distributed continuous variable and the Mann–Whitney *U*-test was used to determine *P*-values. For *in vivo* experiments, survival analyses were performed using Kaplan–Meier with the LogRank test. Two-sided *P*-values < 0.05 were considered significant. Statistical analyses were conducted using Stata v14.1 (Stata Corp., College Station, TX, USA) MatLab v8.3 (MathWorks, Natick, MA, USA), Prism (GraphPad Software, San Diego, CA, USA) and STATISTICA software (StatSoft, Tulsa, OK, USA).

ACKNOWLEDGMENTS

We thank Dr Julien Vaucher and Dr François Bastardot for contributing to data acquisition. We thank Erik Boehm for help with writing and editing the manuscript. We express our gratitude to all participants of the CoLaus study and to the research nurses responsible for the recruitment of participants, especially Nicole Bonvin. This work was supported by the Leenards Foundation, the Faculty of Biology and Medicine of Lausanne and the Swiss National Science Foundation (grant numbers 3200B0–105993, 3200B0-118308, 33CSCO-122661, 33CS30-139468, and 33CS30-148401, 310030-163335), and the European Commission (FP7-INNOVATION I HEALTH-F2-2013–602114; Athero-B-Cell: Targeting and exploiting B cell function for treatment in cardiovascular disease).

AUTHOR CONTRIBUTIONS

Nicolas Vuilleumier: Conceptualization; Funding acquisition; Investigation; Project administration; Supervision; Writing-original draft; Writing-review & editing. **Panagiotis Antiochos:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Pedro-Manuel Marques-Vidal:** Conceptualization; Funding acquisition; Investigation; Supervision; Writing-review & editing. **Sabrina Pagano:** Data curation; Investigation; Methodology; Project administration; Writing-review & editing. **Julien Virzi:** Resources. **Nathalie Satta:** Investigation; Methodology; Project administration; Writing-review & editing. **Oliver Hartley:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing-original draft; Writing-review & editing. **Hubert Gaertner:** Data curation; Formal analysis; Investigation; Methodology. **Karim Brandt:** Data curation; Formal analysis; Methodology; Supervision. **Fabienne Burger:** Investigation; Methodology. **Fabrizio Montecucco:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Project administration; Writing-original draft; Writing-review & editing. **Gerard Waeber:** Conceptualization; Data curation; Funding acquisition; Software; Supervision; Writing-review & editing. **Francois Mach:** Conceptualization; Funding acquisition; Project administration; Validation; Writing-review & editing. **Peter Vollenweider:** Conceptualization; Funding acquisition; Methodology; Project administration; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

PV and GW received unrestricted research grants from GlaxoSmithKline to build the CoLaus Study. PA received research funding from the Swiss National Science Foundation (grant P2LAP3_184037). NV received restricted research grants from Roche. NV, SP and OH are named as co-inventors of the patent related to cterA1, peptide ('Mimetic peptides for prognosis, diagnosis or treatment of a cardiovascular disease', No. P1347EP00) but have no other conflict of interest to disclose. The remaining authors have no conflict of interest to declare. Funding sources played no role in the design and conduct of the study, nor in the collection, analysis and interpretation of the data, nor in the preparation, review and approval of the article or decision to submit for publication.

REFERENCES

1. Satta N, Frias MA, Vuilleumier N, Pagano S. Humoral immunity against HDL particle: a new perspective in cardiovascular diseases? *Curr Pharm Des* 2019; **25**: 3128–3146.
2. Anderson JLC, Pagano S, Virzi J *et al*. Autoantibodies to apolipoprotein A-1 as independent predictors of cardiovascular mortality in renal transplant recipients. *J Clin Med* 2019; **8**: 948.
3. Antiochos P, Marques-Vidal P, Virzi J *et al*. Association between anti-apolipoprotein A-1 antibodies and cardiovascular disease in the general population. Results from the CoLaus study. *Thromb Haemost* 2016; **116**: 764–771.
4. Antiochos P, Marques-Vidal P, Virzi J *et al*. Impact of CD14 polymorphisms on anti-apolipoprotein A-1 IgG-related coronary artery disease prediction in the general population. *Arterioscler Thromb Vasc Biol* 2017; **37**: 2342–2349.

5. Antiochos P, Marques-Vidal P, Virzi J *et al*. Anti-apolipoprotein A-1 IgG predict all-cause mortality and are associated with Fc receptor-like 3 polymorphisms. *Front Immunol* 2017; **8**: 437.
6. Batuca JR, Amaral MC, Favas C *et al*. Antibodies against HDL components in ischaemic stroke and coronary artery disease. *Thromb Haemost* 2018; **118**: 1088–1100.
7. Carbone F, Satta N, Montecucco F *et al*. Anti-ApoA-1 IgG serum levels predict worse poststroke outcomes. *Eur J Clin Invest* 2016; **46**: 805–817.
8. Keller PF, Pagano S, Roux-Lombard P *et al*. Autoantibodies against apolipoprotein A-1 and phosphorylcholine for diagnosis of non-ST-segment elevation myocardial infarction. *J Intern Med* 2012; **271**: 451–462.
9. Lagerstedt JO, Dalla-Riva J, Marinkovic G *et al*. Anti-ApoA-I IgG antibodies are not associated with carotid artery disease progression and first-time cardiovascular events in middle-aged individuals. *J Intern Med* 2019; **285**: 49–58.
10. Vuilleumier N, Pagano S, Combescure C *et al*. Non-linear relationship between anti-apolipoprotein A-1 IgGs and cardiovascular outcomes in patients with acute coronary syndromes. *J Clin Med* 2019; **8**: 1002.
11. Vuilleumier N, Rossier MF, Pagano S *et al*. Anti-apolipoprotein A-1 IgG as an independent cardiovascular prognostic marker affecting basal heart rate in myocardial infarction. *Eur Heart J* 2010; **31**: 815–823.
12. Montecucco F, Braunersreuther V, Burger F *et al*. Anti-apoA-1 auto-antibodies increase mouse atherosclerotic plaque vulnerability, myocardial necrosis and mortality triggering TLR2 and TLR4. *Thromb Haemost* 2015; **114**: 410–422.
13. Montecucco F, Vuilleumier N, Pagano S *et al*. Anti-apolipoprotein A-1 auto-antibodies are active mediators of atherosclerotic plaque vulnerability. *Eur Heart J* 2011; **32**: 412–421.
14. Pagano S, Carbone F, Burger F *et al*. Anti-apolipoprotein A-1 auto-antibodies as active modulators of atherothrombosis. *Thromb Haemost* 2016; **116**: 554–564.
15. Pagano S, Satta N, Werling D *et al*. Anti-apolipoprotein A-1 IgG in patients with myocardial infarction promotes inflammation through TLR2/CD14 complex. *J Intern Med* 2012; **272**: 344–357.
16. Vuilleumier N, Bas S, Pagano S *et al*. Anti-apolipoprotein A-1 IgG predicts major cardiovascular events in patients with rheumatoid arthritis. *Arthritis Rheum* 2010; **62**: 2640–2650.
17. Vuilleumier N, Pagano S, Montecucco F *et al*. Relationship between HDL cholesterol efflux capacity, calcium coronary artery content, and antibodies against apolipoproteinA-1 in obese and healthy subjects. *J Clin Med* 2019; **8**: 1225.
18. Pagano S, Magenta A, D'Agostino M *et al*. Anti-ApoA-1 IgGs in familial hypercholesterolemia display paradoxical associations with lipid profile and promote foam cell formation. *J Clin Med* 2019; **8**: 2035.
19. Teixeira PC, Ducret A, Ferber P *et al*. Definition of human apolipoprotein A-I epitopes recognized by autoantibodies present in patients with cardiovascular diseases. *J Biol Chem* 2014; **289**: 28249–28259.
20. Pagano S, Gaertner H, Cerini F *et al*. The human autoantibody response to apolipoprotein A-I is focused on the C-terminal helix: a new rationale for diagnosis and treatment of cardiovascular disease? *PLoS One* 2015; **10**: e0132780.
21. Satta NP, Pagano S, Gencer B *et al*. C-Terminus apoA-1 mimetic peptides to detect cognate auto-antibodies and reverse HIV-induced foam cell formation. *J Clin Mol Med* 2019; **2**: 1–8. https://doi.org/10.15761/JCMM.1000128.
22. Nydegger UE. Immune complex pathophysiology. *Ann NY Acad Sci* 2007; **1109**: 66–83.
23. Henson D, Tahhan AS, Nardo D, Quyyumi AA, Venditto VJ. Association between ApoA-I (apolipoprotein A-I) immune complexes and adverse cardiovascular events-brief report. *Arterioscler Thromb Vasc Biol* 2019; **39**: 1884–1892.
24. Wolfensohn S, Lloyd M. *Handbook of Laboratory Animal Management and Welfare*. Hoboken, New Jersey, United States: Wiley-Blackwell; 2003.
25. Sharifov OF, Xu X, Gaggar A *et al*. Anti-inflammatory mechanisms of apolipoprotein A-I mimetic peptide in acute respiratory distress syndrome secondary to sepsis. *PLoS One* 2013; **8**: e64486.
26. Ben-David H, Livneh A, Lidar M *et al*. Toll-like receptor 2 is overexpressed in Familial Mediterranean fever patients and is inhibited by colchicine treatment. *Best Pract Res Clin Rheumatol* 2018; **32**: 651–661.
27. Tardif JC, Kouz S, Waters DD *et al*. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019; **381**: 2497–2505.
28. Kates SA, Solé NA, Johnson CR, Hudson D, Barany G, Albericio F A novel convenient, three-dimensional orthogonal startegy for solid phase synthesis of cyclic peptides. *Tetrahedron Lett* 1993; **34**: 1549–1552.
29. Taylor JW. The synthesis and study of side-chain lactam-bridged peptides. *Biopolymers* 2002; **66**: 49–75.
30. Firmann M, Mayor V, Vidal PM *et al*. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord* 2008; **8**: 6.
31. Marques-Vidal P, Bochud M, Bastardot F *et al*. Levels and determinants of inflammatory biomarkers in a Swiss population-based sample (CoLaus study). *PLoS One* 2011; **6**: e21002.
32. Dupont NC, Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. *J Reprod Immunol* 2005; **66**: 175–191.
33. Frias MA, Virzi J, Batuca J *et al*. ELISA methods comparison for the detection of auto-antibodies against apolipoprotein A1. *J Immunol Methods* 2019; **469**: 33–41.
34. Harrell FE. *R Software. Regression Modeling Strategies: with Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*. 2nd ed. Cham, Switzerland: Springer International AG; 2015: 127–142.

35. Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 1995; **6**: 356–365.
36. Pencina MJ, D'Agostino RB, Pencina KM, Janssens ACJW, Greenland P. Interpreting incremental value of markers added to risk prediction models. *Am J Epidemiol* 2012; **176**: 473–481.
37. Pencina MJ, Steyerberg EW, D'Agostino RB Sr. Net reclassification index at event rate: properties and relationships. *Stat Med* 2017; **36**: 4455–4467.
38. Cook NR. Quantifying the added value of new biomarkers: how and how not. *Diagn Progn Res* 2018; **2**: 14.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.