Anti-apolipoprotein A-1 IgG as an independent cardiovascular prognostic marker affecting basal heart rate in myocardial infarction

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Aims
To assess the prognostic value of anti-apolipoprotein A-1 (anti-apoA-1) IgG after myocardial infarction (MI) and its association with major cardiovascular events (MACEs) at 12 months and to determine their association with resting heart rate (RHR), a well-established prognostic feature after MI. Anti-apoA-1 IgG have been reported in MI without autoimmune disease, but their clinical significance remains undetermined.

Methods and results
A total of 221 consecutive patients with MI were prospectively included, and all completed a 12-month follow-up. Major cardiovascular events consisted in death, MI, stroke, or hospitalization either for an acute coronary syndrome or heart failure. Resting heart rate was obtained on Holter the day before discharge under the same medical treatment. Neonate rat ventricular cardiomyocytes (NRVC) were used in vitro to assess the direct anti-apoA-1 IgG effect on RHR. During follow-up, 13% of patients presented a MACE. Anti-apoA-1 IgG positivity was 9% and was associated with a higher RHR ($P=0.0005$) and higher MACE rate (adjusted OR, 4.3; 95% CI, 1.46–12.6; $P=0.007$). Survival models confirmed the significant nature of this association. Patients with MACE had higher median anti-apoA-1 IgG values at admission than patients without ($P=0.007$). On NRVC, plasma from MI patients and monoclonal anti-apoA-1 IgG induced an aldosterone and dose-dependent positive chronotropic effect, abrogated by apoA-1 and therapeutic immunoglobulin (IVIG) pre-incubation.

Conclusions
In MI patients, anti-apoA-1 IgG is independently associated with MACE at 1-year, interfering with a currently unknown aldosterone-dependent RHR determinant. Knowing whether anti-apoA-1 IgG assessment could be of interest to identify an MI patient subset susceptible to benefit from apoA-1/IVIG therapy remains to be demonstrated.

Keywords
Anti-apolipoprotein A-1 autoantibody • Myocardial infarction • Prognosis • Resting heart rate • Autoimmunity

Immune-mediated inflammation plays a major role in atherosclerosis and atherothrombosis, two essential features for cardiovascular disease (CVD) development, currently considered as the leading cause of death in the Western world, and predicted to be the first killer by 2020.1,2 At a time when the incidence of CVD is increasing,2 the research of new and modifiable cardiovascular factors is highly warranted. Those efforts are meant to identify a new CVD patient subset potentially susceptible to benefit from innovative therapeutic approaches. To this respect, there is an accumulating evidence showing that humoral autoimmunity might...
play an important role in CVD and that some auto-antibodies could represent emerging cardiovascular risk factors, potentially modifiable by passive immunization.

Lately, we reported that IgG auto-antibodies against apolipoprotein A-1 (apoA-1), the major proteic fraction of high-density lipoprotein (HDL), were present in a significant subset of patients after myocardial infarction (MI) and were significantly associated to higher levels of circulating oxidized low-density lipoproteins (oxLDLs), a major player of atherogenesis. However, the clinical and pathophysiological significance of anti-apoA-1 IgG remains unknown. The present study was performed to assess their prognostic value in MI and to determine their association with resting heart rate (RHR), a well-established cardiovascular prognostic feature after MI. To this respect, some auto-antibodies have been shown to interfere with the heart rate conduction system function in humans, supporting the growing number of observation showing that humoral autoimmunity may play a role in the autonomic nervous system dysfunction impairment commonly observed in autoimmune diseases. Therefore, we investigated whether anti-apoA-1 IgG could directly influence autonomous contractions in vitro using spontaneously beating neonate rat ventricular cardiomyocytes (NRVC) in which mineralocorticoid activation has been shown to induce a positive chronotropic effect through the expression of low threshold T-type calcium channels and of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.

Methods

The research ethic committee of Geneva University Hospitals approved the protocol, and all patients gave written informed consent before enrolment.

Patient population and study design

The sample size was computed based on an expected prevalence of apoA-1 IgG in acute coronary syndrome (ACS) of 11%. A total sample size of 220 patients was needed to detect a three-fold increase in the risk of a major event (12 vs. 36%) with a power of 80% with an alpha error of 5%.

Between 1 November 2005 and 30 June 2007, 366 consecutive patients were screened at the emergency room (ER) and intensive care unit (ICU) of Geneva University Hospital for an MI, commonly defined by typical clinical presentation associated with or without ST elevation, with subsequently proven angiographic coronary stenosis needing percutaneous coronary intervention, or associated with cardiac necrosis biomarkers elevation. Significant coronary artery disease (CAD) was defined as a culprit lesion obstructing more than 75% of the vessel lumen on coronary angiography. Such lesions were found in 98% of cases. However, when no significant culprit lesion was found upon coronary angiography despite typical clinical presentation associated with a rise of biochemical markers of cardiac necrosis, patients were included in the present study (four patients). Exclusion criteria were Takotsubo disease, the presence of any known auto-immune disease except diabetes mellitus, and inability to give informed consent for any reason, including oro-tracheal intubation.

Among the 366 screened patients, 145 met exclusion criteria, leaving 221 patients available for the analysis. Conventional left ventricular ejection fraction evaluation by echocardiography was performed within 5 days of admission by experienced cardiologists, blinded to the biochemical results.

Resting heart rate was assessed as mean heart rate on 24 h recording (Holter) the day before patients’ discharge. At the time of recording, all patients were treated with statins, angiotensin-converting enzyme inhibitors or angiotensin-receptor blocker, beta-blockers, aspirin, and clopidogrel. None was under aldosterone antagonist therapy.

Definition of endpoint

Major cardiovascular events (MACEs) were predetermined and consisted in any death, MI, stroke, or hospitalization for ACS or acute heart failure at 1 year.

Patient follow-up

All patients completed the 12-month follow-up. Outcome was independently adjudicated by two of the study coordinators (E.C., R.N.) who were blinded to the results of biochemical analyses. Information was obtained by contacting patients by telephone and was further confirmed by checking patients’ medical file and contacting the physician in charge of the patient, targeting medical history relevant to the study endpoints.

Sample collection

To avoid interference with the door-to-revascularization policy, samples were taken after percutaneous coronary intervention within the first 24 h of hospitalization. After collection, serum samples were aliquoted and frozen at −80°C until analyses. Data were collected from patients’ files in the ER and ICU.

Biochemical analysis

Determination of human antibodies to apoA-1 by enzyme-linked immunosorbent assay

Anti-apoA-1 IgG were measured as described previously. The specificity of the detection was assessed using conventional saturation tests described before and further confirmed by western blot (data not shown).

Intra- and inter-assay variations

Repeatability and reproducibility were determined at two levels. At a high level (two-fold the cut-off value), the coefficients of variation were 10% (n = 10) and 17% (n = 10) for intra and inter-assay, respectively. At the cut-off level, the coefficients of variation were 16% (n = 10) and 12% (n = 8) for intra and inter-assay, respectively.

Reference values

As described earlier, upper reference value was set at an absorbance of 0.6 optical densities (OD), corresponding to the 97.5th percentile of a reference population established on 140 healthy blood donors. In order to limit the impact of interassay variation, we developed an index consisting in the ratio between sample net absorbance and the positive control net absorbance × 100. The value corresponding to the 97.5th percentile of the normal distribution was 37 for the index. Accordingly, to be considered as positive, samples had to display an absorbance value above 0.6 OD and an index above 37.

Classical autoantibody measurement

Anti-nuclear antibody (ANA) and rheumatoid factor (RF) measurements were performed in the clinical laboratory for immunology and allergy of the University Hospital of Geneva, using routine indirect immunofluorescence and enzyme-linked immunosorbent assay.
Cardiac troponin I, creatin kinase, N-terminal pro brain natriuretic peptide (NT-proBNP), C-reactive protein, creatinin, total cholesterol, triglycerids, and high-density lipoprotein quantification

Cardiac troponin I (cTnI) concentrations were determined on Unicell DXI 800™ (Beckman Coulter, Brea, CA, USA). Creatin kinase (CK), C-reactive protein, creatinin, total cholesterol, triglycerids, and HDL (mmol/L) were determined using a Synchron LX20 pro™ (Beckman Coulter) auto-analyser, and NT-proBNP was determined using Elecsys™ (Roche, Switzerland) automate.

Cell culture and cell contraction frequency

Neonatal cardiac cells were isolated from 1- to 2-day-old Wistar rat ventricles by digestion with low trypsin-EDTA and type 2 collagenase as described previously.22-24 Briefly, animals were killed (in conformity with the Guide for the Care and use of Laboratory Animals published by the NIH and with the authorization (31.1.1012/19s3/c) of the local County Veterinary Office) and freshly isolated cells were seeded in plastic flasks to allow selective adhesion of cardiac fibroblasts. Thereafter, cardiomyocytes were decanted from the flasks and distributed in laminin-coated 90-mm Petri dishes or in 6-well culture plates. Cells from a same preparation were used for testing the various experimental conditions.

Spontaneously contracting cell monolayers were incubated for the indicated times with a 10% dilution of MI patients plasma or reference plasma spiked with different concentrations (1–20 µg/mL) of mouse monoclonal anti-human apoA-1 IgG (Abcam, Nottingham, UK) and respective IgG controls (polyclonals) of the same genetic background (Meridian Life Science, Cincinnati, OH, USA) in serum-free DMEM. Ultra-pure lipid low apoA-1 was provided by R. James and intravenous monoclonal anti-human apoA-1 IgG (Abcam, Nottingham, UK) and indicated times with a 10% dilution of MI patients plasma or reference preparation were used for testing the various experimental conditions.

We then tested in vitro the chronotropic action of anti apo-A1 IgG on freshly isolated NRVC that share some properties with cardiac pacemaker cells. Indeed, these cells maintain their ability (acquired during the foetal development) to contract spontaneously in culture and aldosterone increases their beating frequency.22
Table 1 Patients demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>MI patients (n = 221)</th>
<th>MI patients negative for anti-apoA-1 IgG (n = 202)</th>
<th>MI patients positive for anti-apoA-1 IgG (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64 (26–86; 55–74)</td>
<td>64 (28–86; 55–74)</td>
<td>62 (26–86; 54–72)</td>
<td>0.65</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male, % (n)</td>
<td>78 (173)</td>
<td>77 (156)</td>
<td>89 (17)</td>
<td>0.38</td>
</tr>
<tr>
<td>Female, % (n)</td>
<td>22 (48)</td>
<td>23 (46)</td>
<td>11 (2)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8 (18–39.5; 24–29)</td>
<td>26 (17–39; 24–28)</td>
<td>25 (18–39.5; 21–32)</td>
<td>0.63</td>
</tr>
<tr>
<td>Creatinin, μmol/L</td>
<td>84 (47–275; 73–98)</td>
<td>84 (47–275; 73–97)</td>
<td>93 (62–211; 73–106)</td>
<td>0.20</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>6 (1–230; 14–39)</td>
<td>6 (1–230; 3–14.2)</td>
<td>7.2 (1–96; 2.8–13)</td>
<td>0.76</td>
</tr>
<tr>
<td>cTnI, ng/mL</td>
<td>0.5 (0–153; 5–7)</td>
<td>0.4 (0.15–3; 0.1–3)</td>
<td>1.0 (0–80; 0.1–10)</td>
<td>0.29</td>
</tr>
<tr>
<td>CK peak, IU/L</td>
<td>687 (273–751; 1410–1783)</td>
<td>687 (41–7511; 264–1785)</td>
<td>573 (77–3252; 281–1308)</td>
<td>0.79</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>785 (20–28 285; 283–2057)</td>
<td>778 (36–28 285; 289–2157)</td>
<td>885 (20–14 205; 125–1842)</td>
<td>0.66</td>
</tr>
<tr>
<td>Lipid profile, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.8 (1.9–11.8; 4–5.8)</td>
<td>4.9 (1.9–8.8; 4.1–5.8)</td>
<td>4.8 (3.2–11.8; 3.9–6)</td>
<td>0.94</td>
</tr>
<tr>
<td>HDL</td>
<td>1.06 (0.5–2.7; 0.9–1.3)</td>
<td>1.05 (0.5–2.7; 0.9–1.3)</td>
<td>1.1 (0.7–1.7; 0.9–1.4)</td>
<td>0.35</td>
</tr>
<tr>
<td>LDL</td>
<td>3.1 (0.5–4; 2.4–3.9)</td>
<td>3.2 (0.5–7; 2.4–3.9)</td>
<td>2.9 (1.8–9.5; 2.4–3.9)</td>
<td>0.86</td>
</tr>
<tr>
<td>Triglycerids</td>
<td>1.1 (0.5–7.3; 0.8–1.7)</td>
<td>1.1 (0.1–7.3; 0.8–1.7)</td>
<td>0.9 (0.4–2.3; 0.6–1.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Comorbidities, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>53 (117)</td>
<td>53 (108)</td>
<td>47 (9)</td>
<td>0.63</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19 (43)</td>
<td>20 (40)</td>
<td>16 (3)</td>
<td>1</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>46 (102)</td>
<td>47 (94)</td>
<td>42 (8)</td>
<td>0.81</td>
</tr>
<tr>
<td>Smoker</td>
<td>45 (99)</td>
<td>45 (91)</td>
<td>42 (9)</td>
<td>1</td>
</tr>
<tr>
<td>Known CAD</td>
<td>29 (65)</td>
<td>29 (58)</td>
<td>32 (6)</td>
<td>0.79</td>
</tr>
<tr>
<td>Stroke</td>
<td>5 (12)</td>
<td>4 (8)</td>
<td>21 (4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive familial history</td>
<td>31 (69)</td>
<td>31 (62)</td>
<td>37 (7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>5 (11)</td>
<td>4 (9)</td>
<td>11 (2)</td>
<td>0.24</td>
</tr>
<tr>
<td>STEMI, % (n)</td>
<td>54 (120)</td>
<td>53 (108)</td>
<td>63 (12)</td>
<td>0.48</td>
</tr>
<tr>
<td>NSTEMI, % (n)</td>
<td>46 (101)</td>
<td>47 (94)</td>
<td>37 (7)</td>
<td>1</td>
</tr>
<tr>
<td>LVEF*, %</td>
<td>50 (20–60; 10)</td>
<td>50 (25–60; 45–55)</td>
<td>45 (20–60; 40–60)</td>
<td>0.33</td>
</tr>
<tr>
<td>Resting heart rate at discharge, b.p.m.</td>
<td>68 (50–120; 15)</td>
<td>67 (50–97; 60–72)</td>
<td>76 (56–120; 70–90)</td>
<td>0.0005</td>
</tr>
<tr>
<td>MACE rate at 12 months, % (n)</td>
<td>14 (31)</td>
<td>12 (24)</td>
<td>37 (7)</td>
<td>0.008</td>
</tr>
<tr>
<td>MACE details: deaths</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>ACS</td>
<td>9 (19)</td>
<td>7 (14)</td>
<td>26 (5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4 (9)</td>
<td>3 (7)</td>
<td>11 (2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Stroke</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Anti-phospholipid antibody positivity rate, % (n)</td>
<td>10 (22)</td>
<td>9 (19)</td>
<td>16 (3)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Medication at inclusion, % (n)

<table>
<thead>
<tr>
<th></th>
<th>MI patients (n = 221)</th>
<th>MI patients negative for anti-apoA-1 IgG (n = 202)</th>
<th>MI patients positive for anti-apoA-1 IgG (n = 19)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>32 (71)</td>
<td>32 (65)</td>
<td>32 (6)</td>
<td>1</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>16 (25)</td>
<td>11 (23)</td>
<td>11 (2)</td>
<td>1</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>28 (61)</td>
<td>27 (55)</td>
<td>32 (6)</td>
<td>0.78</td>
</tr>
<tr>
<td>ACE or AT1 inhibitors</td>
<td>39 (86)</td>
<td>38 (77)</td>
<td>47 (9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Statin</td>
<td>35 (77)</td>
<td>35 (71)</td>
<td>32 (6)</td>
<td>1</td>
</tr>
<tr>
<td>Diuretics (excluding amiloride and MR antagonists)</td>
<td>19 (43)</td>
<td>19 (39)</td>
<td>22 (4)</td>
<td>0.76</td>
</tr>
<tr>
<td>Anti-diabetic agents</td>
<td>12 (27)</td>
<td>12 (25)</td>
<td>11 (2)</td>
<td>1</td>
</tr>
<tr>
<td>Insulin</td>
<td>7 (16)</td>
<td>7 (14)</td>
<td>11 (2)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

All continuous variables are reported as median with (range and interquartile range). P-value was calculated according to Mann–Whitney U-test for continuous variable and according to exact bilateral Fischer test for proportions.

ACS, acute coronary syndrome; ACE, angiotensin-converting enzyme; AT1, angiotensin II receptor type 1; LVEF, left ventricular ejection fraction.

*Available in 90% of patients.
observed that addition of monoclonal mouse anti-human apoA-1 IgG, spiked in reference plasma or culture media, significantly increased the basal contraction rate of cardiomyocytes in a concentration-dependent manner. The maximal response was observed at a concentration of 10 μg/mL (Figure 2A). At this optimal concentration, the effect was statistically significant when compared with reference plasma alone and with reference plasma spiked with control IgG (78 vs. 27.5 b.p.m., \( P = 0.01 \); and 78 vs. 38 b.p.m., \( P = 0.004 \); respectively; Figure 2B). This chronotropic effect was not seen when anti-apoA-1 IgG was applied to naive cells, not primed with aldosterone (Figure 2B) or primed with other steroids than aldosterone (data not shown).

The same experiments were repeated using plasma samples obtained from MI patients. Fifteen plasmas from MI patients tested positive for anti-apoA-1 IgG and 15 plasmas from MI patient samples that were negative for those antibodies were randomly taken. Myocardial infarction plasma positive for anti-apoA-1 IgG induced a significantly higher median increase of cardiomyocyte contraction rate than patient plasma negative for anti-apoA-1 IgG (95 vs. 62 b.p.m., \( P = 0.04 \), Figure 2C). When expressed in percentage of baseline to normalize for the baseline variability (30 vs. 36 b.p.m., Figure 2C), Spearman test showed a significant correlation between the titre of anti-apoA-1 IgG and cardiomyocyte contraction rate (\( r = 0.45 \), \( P = 0.02 \)), confirming the dose-dependent relationship between anti-apoA-1 IgG in MI plasma and basal heart rate.

**Figure 1** Kaplan–Meier time-to-events plot for cardiovascular outcome at 1 year according to anti-apoA-1 IgG status.

**Figure 2** (A) Dose-dependent chronotropic effect of anti-apoA-1 IgG in vitro. Results are expressed as median with interquartile range and range. \(* P = 0.01\) according to Kruskall–Wallis test. \(** P = 0.7\) according to Kruskall–Wallis test. (B) Aldosterone-dependent effect of anti-apoA-1 IgG in vitro. \(* P = 0.004\). Results are expressed as median with interquartile range and range. (C) Effect of myocardial infarction plasma on in vitro contraction rate according to anti-apoA-1 IgG status. \(* P = 0.04\). Results are expressed as median with interquartile range and range.
Anti-apoA-1 IgG chronotropic effect on cardiomyocytes is idiotype-dependent and abolished by therapeutic immunoglobulin

The chronotropic response was almost fully abolished when anti-apoA-1 IgG was added together with 1 mg/mL of delipidated apoA-1, demonstrating the idiotype specificity of the anti-apoA-1 IgG effect on cardiomyocyte contraction rate (Figure 3A). This positive chronotropic effect was also strongly abrogated by IVIG 2 mg/mL (Figure 3B). ApoA-1 per se displayed a modest, but significant, positive chronotropic effect on contraction rate when compared with baseline (Figure 3A, P = 0.03), whereas IVIG per se did not (Figure 3B, P = 1).

Discussion

The novel and important finding of the present study is that anti-apoA-1 IgG appears as a new prognostic marker of MACEs 1 year after MI, independently of traditional cardiovascular risk factors, but associated with RHR, another major cardiovascular prognostic feature after MI.16 – 19 Elevated RHR is known to affect the cardiovascular risk by concomitantly increasing myocardial oxygen demand while decreasing its supply27 and energy stores,28 accelerating atherosclerosis,29 – 31 and enhancing plaque vulnerability.32 Although RHR following MI has been considered as a prognostic factor since more than two decades,17 the recent results of the BEAUTIFUL study allowed determining a reasonable but arbitrary RHR cut-off above which the risk of cardiovascular complications is drastically increased and set at 70 b.p.m.16 To this respect and even if our patients had a higher median EF than the patients enrolled in the BEAUTIFUL study, it is interesting to note that MI patients who were positive for anti-apoA-1 IgG had RHR above this threshold (median value of 76 b.p.m.), whereas MI patients tested negative for these antibodies were below the threshold (median value of 67 b.p.m., P = 0.0005). Moreover, plasma of MI patients containing high levels of anti-apoA-1 IgG induced in vitro a significant increase of the basal rat cardiomyocytes beating frequency that was more pronounced than the response to plasma without these auto-antibodies (95 vs. 62 b.p.m., P = 0.04). Together with the dose-dependent effect of anti-apoA-1 IgG at physiologically relevant concentrations (1–20 µg/mL), the significant positive correlations between anti-apoA-1 IgG titre with RHR both in vivo and in vitro strongly suggest that the presence of anti-apoA-1 IgG is responsible for the positive chronotropic effect of MI plasma, which was reverted by physiologically relevant concentrations of IVIG and apoA-1. Both IVIG and apoA-1 have been reported as promising CVD therapeutic modalities in mice, but data in humans are scarce.33 – 35 Therefore, knowing whether anti-apoA-1 IgG-positive MI patients could specifically benefit from IVIG or apoA-1 treatment remains highly speculative at the present time, and further work is needed to determine whether anti-apoA-1 IgG is a cardiovascular risk factor or just a marker of cardiovascular risk.

Our results raise several questions. To the best of our knowledge, apoA-1, the antigen against which those auto-antibodies are directed to, is not known to be expressed by rat cardiomyocytes. Accordingly, one can evoke a non-specific effect of those antibodies or even serendipity. However, given the absence of effect of the negative controls, and the concentration-dependency of the response, it is more reasonable to evoke molecular mimicry between a common conformational epitope shared by cardiomyocytes and apoA-1 to account for the aforementioned observations. More importantly, the complete reversion of anti-apoA-1 IgG effect by a saturation test using apoA-1 is the strongest argument, indicating that the chronotropic effect observed was idiotype-dependent, lending further weight to the molecular mimicry hypothesis. To this respect, the fact that the effect was observed only with aldosterone incubation raises several mutually non-exclusive hypotheses about the nature of this epitope. First, since aldosterone is known to increase T-type calcium as well as HCN channels expression and function,22 – 25 it is possible that anti-apoA-1 IgG could interfere with one of those channels function, whose relevance in cardiovascular-related physiopathology is well documented in humans,16,36,37 or with other signalling components induced by the mineralocorticoid receptor. Ongoing in vitro pharmacological and electrophysiological...
studies will resolve those matters. Another non-exclusive hypothesis could be that those autoantibodies might also interfere with more classical apoA-1-related properties, such as reverse cholesterol transport, anti-inflammatory, or anti-oxidant activities, which in turn might negatively affect atherogenesis and plaque stability.

Despite being appropriately powered, the number of events in this study is still relatively low, preventing us to draw definite conclusions at the present time and those results need to be reproduced before any clinical recommendation can be made. However, as the rate of cardiovascular complications at 12 months was similar to the one observed in another bigger study (14 vs. 15%) using similar endpoints, we consider our sample to be representative of MI pathology. Also, it is noteworthy that 3 of the 19 patients positive for anti-apoA-1 IgG were also positive for anti-cardiolipin IgG, but not for other anti-β2GP1 autoantibodies, ANA or RF. Even if none of these patients were known for any other autoimmune disease, we cannot exclude the concomitant presence of anti-cardiolipin in these samples, as cross-reactivity between anti-apoA-1 IgG and anti-cardiolipin antibodies has been described. Because anti-cardiolipin antibodies were not associated with patients’ outcome in this study (data not shown), we believe that the reported herein associations are not clouded by a cross-reactivity confounder. The strength of this study resides in the fact that the prognostic value of those anti-apoA-1 IgG auto-antibodies has been confirmed in three different statistical models (multivariate logistic regression, Kaplan–Meier analysis, and Cox regression) and that we were able to reproduce robustly the reported association found on this cohort using a relevant in vitro model, which provides a mechanistic hypothesis to explain the herein reported prognostic aspect of anti-apoA-1 IgG after MI.

**Conclusions**

We report anti-apoA-1 IgG as a novel potential prognostic biomarker for MACEs in patients in post-MI period, independently of traditional cardiovascular factors (age, sex, hypertension, diabetes, dyslipidaemia, and smoking), but significantly associated with increased RHR, a major adverse prognostic factor after MI. The exact chronotropic mechanisms of those auto-antibodies are not known. Whether anti-apoA-1 IgG could represent a new and potentially modifiable cardiovascular factor after an MI, or just a risk marker, remains to be demonstrated in other larger trials.

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**Conflict of interest:** none declared.

**Appendix 1**

**Staining with anti-apoA-1 IgG 1ug/ml**

- Secondary ab: FITC labelled anti-Fl
- Nucleus stained in red with PI
- Magnification 40X

**Staining with isotype control IgG 1ug/ml**

- Secondary ab: FITC labelled anti-Fl
- Nucleus stained in red with PI
- Magnification 40X
References


CARDIOVASCULAR FLASHLIGHT

Non-invasive diagnosis of chylopericardium by cardiac magnetic resonance imaging

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A 24-year-old woman was admitted to the hospital because of massive persistent asymptomatic pericardial effusion. Six months earlier, she had visited her rheumatologist because of polyarthralgias suggesting systemic lupus erythematosus. She had never complained of dyspnoea or cough. On echocardiography, a large, homogeneous pericardial effusion, ‘swinging heart’-type, was found (see Supplementary material, Movie I), with no signs of haemodynamic compromise (Panel A, parasternal long axis, and B, apical four-chamber with severe pericardial effusion [arrow]). A cardiac magnetic resonance imaging (CMR) was performed revealing a massive pericardial effusion (see Supplementary material, Movies II and III). No fibrous tracts were detected. Black blood images without (Panel C) and with fat saturation techniques (Panel D) were acquired and the suppression of the signal intensity of the fluid (asterisks) suggested the presence of fat in it. Pericardiocentesis was planned based on CMR images, obtaining 1 L of dense, intensely chylous fluid. A chemistry analysis revealed a triglyceride level of 3700 mg/dL. Cytology and cultures yielded negative results. Follow-up MRI and chest computed tomography performed 1 month later showed recurrence of the effusion and findings compatible with lupus pneumonitis, while the patient remained asymptomatic. She was referred for surgery, which included drainage of the chylopericardium, ligation of the thoracic duct, and biopsy of the pericardium, which yielded unspecific results.

In conclusion, CMR is the only non-invasive technique capable of giving the diagnosis of chylopericardium, since it provides a biochemical characterization of pericardial effusion. In clinically stable patients, CMR can be helpful in further diagnosis of pericardial effusion.

Supplementary material is available at European Heart Journal online.

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