European Heart Journal (2010) **31**, 815–823
doi:10.1093/eurheartj/ehq055



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

**Nicolas Vuilleumier[1]*, Michel F. Rossier[1,2], Sabrina Pagano[1], Magaly Python[2], Emmanuel Charbonney[3], René Nkoulou[4], Richard James[2], Guido Reber[5], François Mach[4], and Pascale Roux-Lombard[6]**

[1]Service of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 6 rue Gabrielle Perret-Gentil, 1211 Geneva 14, Switzerland; [2]Service of Endocrinology, Diabetology and Nutrition, Department of Internal Medicine, Geneva University Hospitals, Geneva, Switzerland; [3]Department of Critical Care Medicine, St Michael's Hospital, Toronto, Canada; [4]Service of Cardiology, Department of Internal Medicine, Geneva University Hospitals, Geneva, Switzerland; [5]Service of Angiology and Haemostasis, Department of Internal Medicine, Geneva University Hospitals, Geneva, Switzerland; and [6]Service of Immunology and Allergy, Department of Internal Medicine, Geneva University Hospitals, Geneva, Switzerland

*Received 19 November 2009; revised 16 December 2009; accepted 19 January 2010; online publish-ahead-of-print 22 February 2010*

**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 where 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

* Corresponding author. Tel: +41 22 372 91 50, Fax: +41 22 372 33 80, Email: nicolas.vuilleumier@hcuge.ch

play an important role in CVD[3,4] and that some auto-antibodies could represent emerging cardiovascular risk factors,[5–9] potentially modifiable by passive immunization.[10]

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)[11,12] and were significantly associated to higher levels of circulating oxidized low-density lipoproteins (oxLDLs),[12] a major player of atherogenesis.[13–15] 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.[16–19] To this respect, some auto-antibodies have been shown to interfere with the heart rate conduction system function in humans,[20] 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.[21] 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[22–24] and of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.[25]

# 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%.[12] 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 $-80^{\circ}C$ 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.[12] The specificity of the detection was assessed using conventional saturation tests described before[11,12] 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.[12] 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 $\times$ 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

(ELISA) techniques. IgG and IgM auto-antibodies to β2 glycoprotein I (anti-β2GPI) and cardiolipin were measured by ELISA as previously described.[26] All 221 patients were tested negative for ANA, 1 was positive for RF, but 20 (9%) of them were positive either for anti-β2GPI or anti-cardiolipin antibodies. Among those, three were concomitantly positive for anti-apoA-1 IgG and anti-cardiolipin antibodies. Patients tested positive for anti-apoA-1 IgG were all negative for anti-β2GPI antibodies.

**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 (31.1.1012/19s3/0) 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 immunoglobulins (IVIG; Endobulin™) were purchased from Baxter (Switzerland, Volketswil). Cell beating frequency was determined by counting, under light microscope, the number of contractions per time unit in three different locations of the dish. Those tests were performed after 24 h pre-incubation of cells in serum-free medium with and without aldosterone (10 nM) by an observer blinded to anti-apoA-1 IgG sample status. Results are expressed in beat per minute (b.p.m.). Experiments were also performed with addition of antibodies directly into serum-free culture medium.

## Statistics

Analyses were performed using Statistica™ software (StatSoft, Tulsa, OK, USA). Fischer exact test, $\chi^2$ using Yates correction test, and Mann–Whitney *U*-test were used when appropriate to compare the group of patients. Spearman test was used to assess correlation between variables. Associations between anti-apoA-1 IgG and composite cardiovascular outcome are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI) and using Kaplan–Meier analysis and Cox regression model. Two-sided *P*-value was considered as significant when below 0.05. For *in vitro* experiments, Mann–Whitney *U*-test has been used to determine *P*-values unless stated otherwise, and results are expressed as median, interquartile range, and range.

# Results

## Demographic characteristics

Patient demographic characteristics are listed in *Table 1*. The frequency of positive anti-apoA-1 IgG auto-antibodies was 9% (19 of 221) and 14% (31 of 221) of patients had a MACE during the 1-year follow-up (*Table 1*).

## High levels of anti-apoA-1 IgG are associated with major cardiovascular events at 1 year

One year after the initial event, 31 patients (14%) experienced a MACE. The detailed distribution of MACEs is given in *Table 1*. Among the three patients died, one patient died from cardiorespiratory arrest, one from abdominal aorta aneuvrysm rupture, and one from septic shock. On the 19 patients hospitalized for ACS, 10 had a proven MI, and 9 were diagnosed with unstable angina, of whom 5 presented significant angiographic coronary lesion. The remaining nine patients were hospitalized for acute heart failure. No patient had a clinically evident stroke during this 1-year follow-up period. Patients with MACEs had higher median anti-apoA-1 IgG titre upon admission than patients without MACEs (31.4 vs. 20.6 OD; $P = 0.004$). Complication rate was 37% in patient tested positive for anti-apoA-1 IgG against 12% in patients tested negative for those auto-antibodies, corresponding to a four-fold increase in risk of MACEs (OR, 4.3; 95% CI, 1.54–11.99; $P = 0.008$), which remained unchanged after adjustment for age, sex, hypertension, diabetes, dyslipidaemia, smoking (OR, 4.3; 95% CI, 1.46–12.6; $P = 0.007$). Kaplan–Meier analysis confirmed that patients positive for anti-apoA-1 IgG upon admission had a significantly worse complication-free survival at 1 year than those tested negative for those auto-antibodies (63.2 vs. 88.5%, $P = 0.001$; *Figure 1*). Cox regression analysis showed that each unit increase of anti-apoA-1 IgG relative index increased the risk of complication at 1 year by 3% ($P = 0.0003$), independently of time ($P = 0.39$), confirming the proportionality principle of the model.

## Anti-apoA-1 IgG positivity is associated with elevated resting heart rate in myocardial infarction patients

As shown in *Table 1*, MI patients positive for anti-apoA-1 IgG had higher median RHR than those tested negative and Spearman test showed a modest, but significant, correlation between RHR of patients before discharge and the titre of anti-apoA-1 IgG on the whole cohort ($r = 0.18$, $P < 0.025$). No association was retrieved between apoA-1, C-reactive protein levels, and RHR according to Spearman test (data no shown).

## Positive chronotropic action of anti-apoA-1 IgG *in vitro*

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] We

**Table 1 Patients demographic characteristics**

| | MI patients ($n = 221$) | MI patients negative for anti-apoA-1 IgG ($n = 202$) | MI patients positive for anti-apoA-1 IgG ($n = 19$) | *P*-value |
|---|---|---|---|---|
| Age, years | 64 (26–86; 55–74) | 64 (28–86; 55–74) | 62 (26–86; 54–72) | 0.65 |
| Sex | | | | |
| Male, % (*n*) | 78 (173) | 77 (156) | 89 (17) | 0.38 |
| Female, % (*n*) | 22 (48) | 23 (46) | 11 (2) | |
| BMI, kg/m$^2$ | 25.8 (18–39.5; 24–29) | 26 (17–39; 24–28) | 25 (18–39.5; 21–32) | 0.63 |
| Creatinin, μmol/L | 84 (47–275; 73–98) | 84 (47–275; 73–97) | 93 (62–211; 73–106) | 0.20 |
| C-reactive protein, mg/L | 6 (1–230; 14–39) | 6 (1–230; 3–14.2) | 7.2 (1–96; 2.8–13) | 0.76 |
| cTnI, ng/mL | 0.5 (0–153; 5–7) | 0.4 (0.15–3; 0.1–3) | 1.0 (0–80; 0.1–10) | 0.29 |
| CK peak, IU/L | 687 (273–751; 1410–1783) | 687 (41–7511; 264–1785) | 573 (77–3252; 281–1308) | 0.79 |
| NT-proBNP, pg/mL | 785 (20–28 285; 283–2057) | 778 (36–28 285; 289–2157) | 885 (20–14 205; 125–1842) | 0.66 |
| Lipid profile, mmol/L | | | | |
| Total cholesterol | 4.8 (1.9–11.8; 4–5.8) | 4.9 (1.9–8.8; 4.1–5.8) | 4.8 (3.2–11.8; 3.9–6) | 0.94 |
| HDL | 1.06 (0.5–2.7; 0.9–1.3) | 1.05 (0.5–2.7; 0.9–1.3) | 1.1 (0.7–1.7; 0.9–1.4) | 0.35 |
| LDL | 3.1 (0.5–4; 2.4–3.9) | 3.2 (0.5–7; 2.4–3.9) | 2.9 (1.8–9.5; 2.4–4.3) | 0.86 |
| Triglycerids | 1.1 (0.5–7.3; 0.8–1.7) | 1.1 (0.1–7.3; 0.8–1.7) | 0.9 (0.4–2.3; 0.6–1.5) | 0.22 |
| Comorbidities, % (*n*) | | | | |
| Hypertension | 53 (117) | 53 (108) | 47 (9) | 0.63 |
| Diabetes | 19 (43) | 20 (40) | 16 (3) | 1 |
| Dyslipidaemia | 46 (102) | 47 (94) | 42 (8) | 0.81 |
| Smoker | 45 (99) | 45 (91) | 42 (9) | 1 |
| Known CAD | 29 (65) | 29 (58) | 32 (6) | 0.79 |
| Stroke | 5 (12) | 4 (8) | 21 (4) | 0.01 |
| Positive familial history | 31 (69) | 31 (62) | 37 (7) | 0.6 |
| Atrial fibrillation | 5 (11) | 4 (9) | 11 (2) | 0.24 |
| STEMI, % (*n*) | 54 (120) | 53 (108) | 63 (12) | 0.48 |
| NSTEMI, % (*n*) | 46 (101) | 47 (94) | 37 (7) | |
| LVEF[a], % | 50 (20–60; 10) | 50 (25–60; 45–55) | 45 (20–60; 40–60) | 0.33 |
| Resting heart rate at discharge, b.p.m. | 68 (50–120; 15) | 67 (50–97; 60–72) | 76 (56–120; 70–90) | 0.0005 |
| MACE rate at 12 months, % (*n*) | 14 (31) | 12 (24) | 37 (7) | 0.008 |
| MACE details: deaths | 1 (3) | 1 (3) | 0 (0) | 1 |
| ACS | 9 (19) | 7 (14) | 26 (5) | 0.01 |
| Heart failure | 4 (9) | 3 (7) | 11 (2) | 0.17 |
| Stroke | 0 | 0 | 0 | — |
| Anti-phospholipid antibody positivity rate, % (*n*) | 10 (22) | 9 (19) | 16 (3) | 0.41 |
| Medication at inclusion, % (*n*) | | | | |
| Aspirin | 32 (71) | 32 (65) | 32 (6) | 1 |
| Clopidogrel | 16 (25) | 11 (23) | 11 (2) | 1 |
| β-Blocker | 28 (61) | 27 (55) | 32 (6) | 0.78 |
| ACE or AT1 inhibitors | 39 (86) | 38 (77) | 47 (9) | 0.46 |
| Statin | 35 (77) | 35 (71) | 32 (6) | 1 |
| Diuretics (excluding amiloride and MR antagonists) | 19 (43) | 19 (39) | 22 (4) | 0.76 |
| Anti-diabetic agents | 12 (27) | 12 (25) | 11 (2) | 1 |
| Insulin | 7 (16) | 7 (14) | 11 (2) | 0.63 |

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.
[a]Available in 90% of patients.

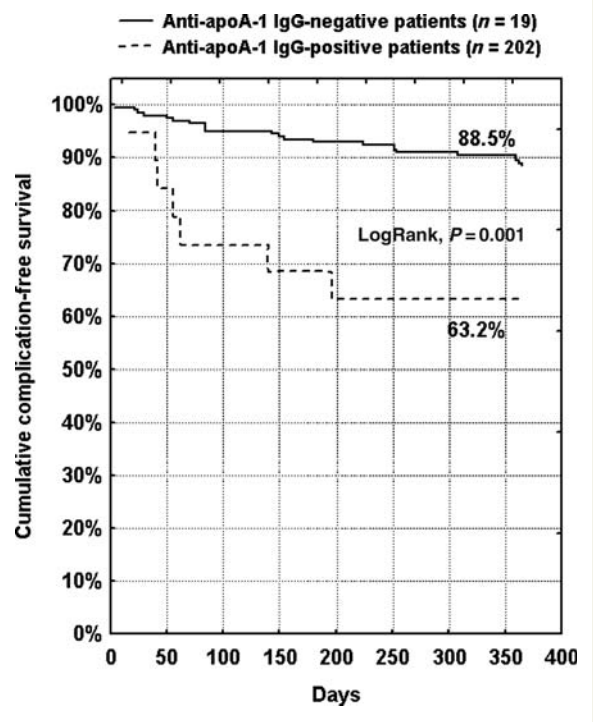



**Figure 1** Kaplan–Meier time-to-events plot for cardiovascular outcome at 1 year according to anti-apoA-1 IgG status.

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 naïve 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.

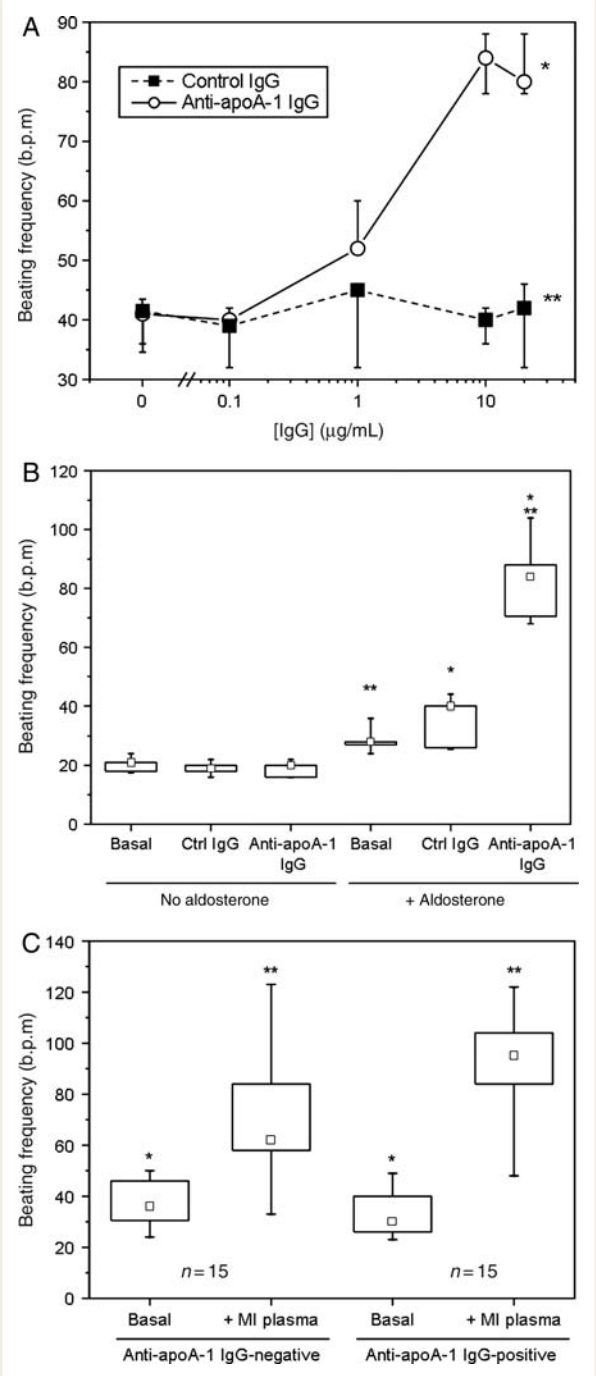



**Figure 2** (*A*) Dose-dependent chronotropic effect of anti-apoA-1 IgG *in vitro*. Results are expressed as median with interquartile range and range. *$P = 0.01$ according to Kruskall–Wallis test. **$P = 0.7$ according to Kruskall–Wallis test. (*B*) Aldosterone-dependent effect of anti-apoA-1 IgG *in vitro*. * and **$P = 0.004$. 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. *$P = 0.2$ and *$P = 0.04$. 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*, $P = 1$).

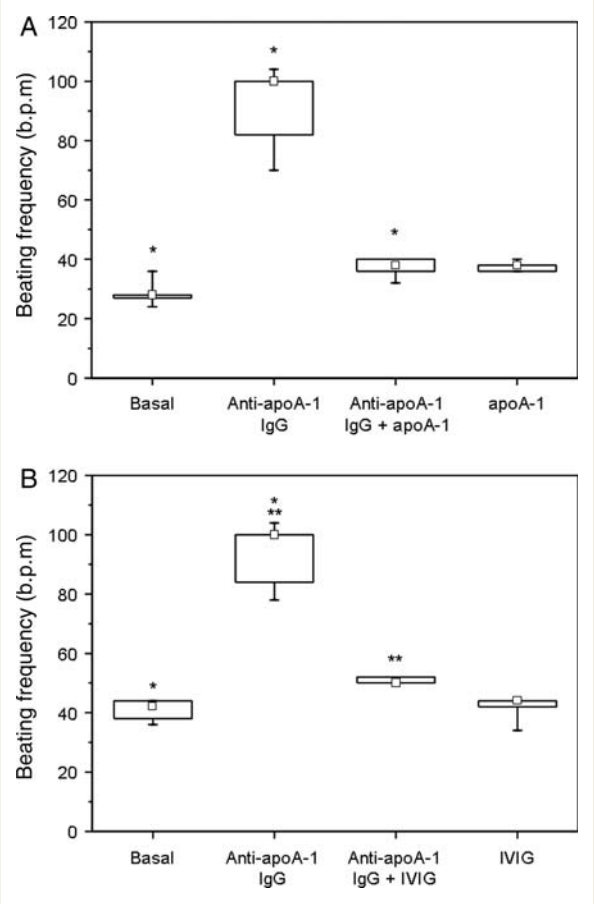



**Figure 3** (*A*) Idiotype-dependent chronotropic effect of anti-apoA-1 IgG. (*B*) Inhibition of anti-apoA-1 IgG chronotropic effect by IVIG. * and **$P = 0.01$. Results are expressed as median with interquartile range and range.

# 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 supply[27] 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 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, which translated into a significant increase in cardiomyocyte contraction rate,[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,[16] 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.[38] 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.

## Acknowledgements

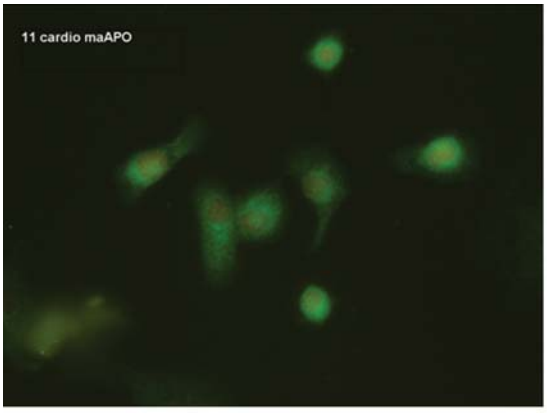
The authors thank M. Alvarez for her skilful technical help, Dr M. Frias for his valuable advice concerning rat ventricular cell preparation, and the Geneva University Hospitals Clinical Research Center (Thomas Perneger) for computation of the required sample size.

## Funding

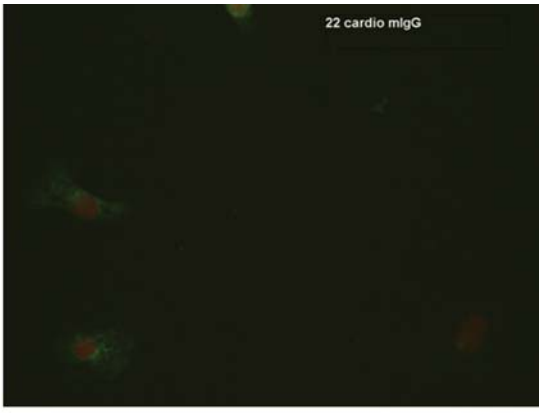
This work was supported by a grant for Research and Development from the University Hospital of Geneva (PRD 05-09-II to P.R.-L.), a grant from the Swiss National Science Foundation (310000-111808 to M.F.R.) and by Telemaque Foundation (to N.V.).

**Conflict of interest:** none declared.

# Appendix 1

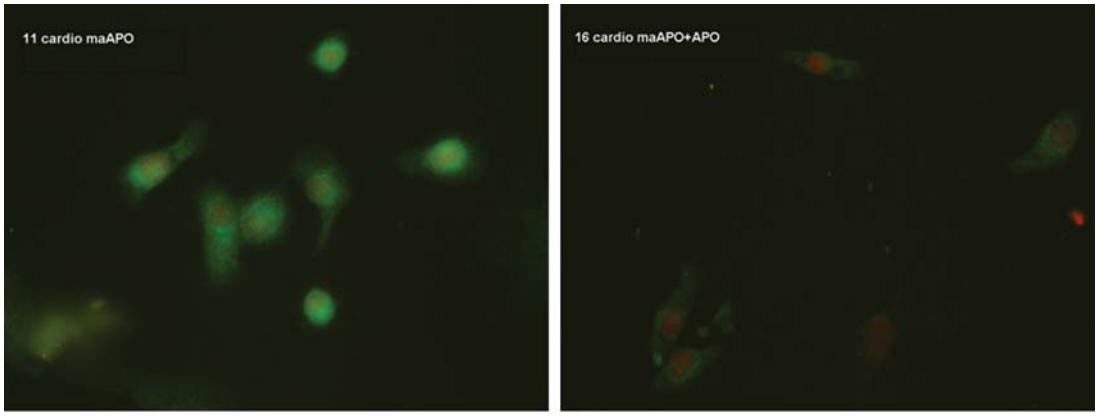



Staining with anti-apoA-1 IgG 1ug/ml
Secondary ab: FITC labelled anti-Fc
Nucleus stained in red with PI
Magnification 40X




Staining with isotype control IgG 1ug/ml
Secondary ab: FITC labelled anti-Fc
Nucleus stained in red with PI
Magnification 40X

# Appendix 2




Staining with anti-apoA-1 IgG 1ug/ml
Secondary ab: FITC labelled anti-Fc
Nucleus stained in red with PI
Magnification 40X

Staining with anti-apoA-1 IgG 1ug/ml
Preincubated with 10ug/ml of apoA-1
for 2h at room temperature
Secondary ab: FITC labelled anti-Fc
Nucleus stained in red with PI
Magnification 40X

## References


1. Libby P. Inflammation in atherosclerosis. *Nature* 2002;**420**:868–874.
2. Eyre H, Kahn R, Robertson RM, Clark NG, Doyle C, Hong Y, Gansler T, Glynn T, Smith RA, Taubert K, Thun MJ. American Cancer Society; American Diabetes Association; American Heart Association. Preventing cancer cardiovascular disease and diabetes: a common agenda for the American Cancer Society, American Diabetes Association, and the American Heart Association. *Circulation* 2004; **109**:3244–3255.
3. Matsuura E, Kobayashi K, Koike T, Shoenfeld Y. Autoantibody mediated atherosclerosis. *Autoimmun Rev* 2002;**1**:348–353.
4. Sherer Y, Shoenfeld Y. Mechanisms of disease: atherosclerosis in autoimmune diseases. *Nat Clin Pract Rheumatol* 2006;**2**:99–106.
5. Mustafa A, Ntyanand S, Beglund L, Lithell H, Hol G, Lefvert AK. Circulating immune complex in 50-year old men as strong and independent risk factor for myocardial infarction. *Circulation* 2000;**102**:2576–2581.
6. Wu R, Nityanand S, Berglund L, Lithelle Holm G, Lefvert AK. Antibodies against cardiolipin and oxidatively modified LDL in 50 year old men predict myocardial infarction. *Arterioscler Thromb Vasc Biol* 1997;**17**:3159–3163.
7. Meroni PL, Peyvandi F, Foco L, Bernardinelli L, Fetiveau R, Mannucci PM, Tincani A. Anti-beta 2 glycoprotein I antibodies and the risk of myocardial infarction in young premenopausal women. *J Thromb Haemost* 2007;**5**:2421–2428.
8. Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J, Epstein SE. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 2001;**103**:1071–1075.
9. Eriksson S, Hellman J, Pettersson K. Autoantibodies against cardiac troponins. *N Eng J Med* 2005;**352**:98–100.
10. Nussinovitch U, Shoenfeld Y. Intravenous immunoglobulin—indications and mechanisms in cardiovascular diseases. *Autoimmun Rev* 2008;**7**:445–452.
11. Vuilleumier N, Reber G, Burger D, de Moerloose P, Dayer JM, Roux-Lombard P. Presence of auto-antibodies to apolipoproteine A1 in patients with acute coronary syndrome further links autoimmunity with cardiovascular disease. *J Autoimmun* 2004;**23**:353–360.
12. Vuilleumier N, Charbonney E, Fontao L, Alvarez M, Turck N, Sanchez JC, Burkhard PR, Mensi N, Righini M, Reber G, James R, Mach F, Chevrolet JC, Dayer JM, Forstegard J, Roux-Lombard P. Anti-apolipoprotein A-1 IgG are associated with high oxidised low-density lipoprotein levels in acute coronary syndrome. *Clin Sci (Lond)* 2008;**115**:25–33.
13. Stoll G, Bendszus M. Inflammation and atherosclerosis. Novel insights into plaque formation and destabilization. *Stroke* 2006;**3**:1923–1932.
14. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F. Circulating oxidized LDL is a useful marker for identifying patients with coronary heart disease. *Arterioscler Thromb Vasc Biol* 2001;**21**:844–848.
15. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low density lipoprotein, a strong predictor for acute heart disease events on apparently healthy, middle-aged men from the general population. *Circulation* 2005;**112**:651–657.
16. Fox K, Ford I, Steg PG, Tendera M, Robertson M, Ferrari R, BEAUTIFUL investigators. Heart rate as a prognostic risk factor in patients with coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a subgroup analysis of a randomised controlled trial. *Lancet* 2008;**372**:817–821.
17. Kannel WB, Kannel C, Paffenbarger RSJR, Cupples LA. Heart rate and cardiovascular mortality: the Framingham study. *Am Heart J* 1987;**113**:1489–1494.
18. Diaz A, Bourassa MG, Guertin MC, Tardif JC. Long-term prognostic value of resting heart rate in patients with suspected or proven coronary artery disease. *Eur Heart J* 2005;**26**:967–974.
19. Kolloch R, Legler UF, Champion A, Cooper-Dehoff RM, Handberg E, Zhou Q, Pepine CJ. Impact of resting heart rate on outcomes in hypertensive patients with coronary artery disease: findings from the INternational VErapamil-SR/trandolapril STudy (INVEST). *Eur Heart J* 2008;**29**:1327–1334.
20. Rein AJ, Mevorach D, Perles Z, Gavri S, Nadjari M, Nir A, Elchalal U. Early diagnosis and treatment of atrioventricular block in the fetus exposed to maternal anti-SSA/Ro-SSB/La antibodies: a prospective, observational, fetal kinetocardiogram-based study. *Circulation* 2009;**119**:1867–1872.
21. Stojanovich L. Autonomic dysfunction in autoimmune rheumatic disease. *Autoimmun Rev* 2009;**8**:569–572.
22. Lalevée N, Rebsamen MC, Barrère-Lemaire S, Perrier E, Nargeot J, Bénitah JP, Rossier MF. Aldosterone increases T-type calcium channel expression and *in vitro* beating frequency in neonatal rat cardiomyocytes. *Cardiovasc Res* 2005; **67**:216–224.
23. Maturana A, Lenglet S, Python M, Kuroda S, Rossier MF. Role of the T-type calcium channel CaV3.2 in the chronotropic action of corticosteroids in isolated rat ventricular myocytes. *Endocrinology* 2009;**150**:3726–3734.
24. Rossier MF, Lenglet S, Vetterli L, Python M, Maturana A. Corticosteroids and redox potential modulate spontaneous contraction in isolated rat ventricular cardiomyocytes. *Hypertension* 2008;**52**:721–728.
25. Muto T, Ueda N, Opthof T, Ohkusa T, Nagata K, Suzuki S, Tsuji Y, Horiba M, Lee JK, Honjo H, Kamiya K, Kodama I, Yasui K. Aldosterone modulates I(f) current through gene expression in cultured neonatal rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 2007;**293**:2710–2718.
26. Reber G, Schousboe I, Tincani A, Sanmarco M, Kveder T, de Moerloose P, Boffa MC, Arvieux J. Inter-laboratory variability of anti-beta2-glycoprotein I measurement. A collaborative study in the frame of the European Forum on Antiphospholipid Antibodies Standardization Group. *Thromb Haemost* 2002;**88**:66–73.

27. Panza JA, Diodati JG, Callahan TS, Epstein SE, Quyyumi AA. Role of increase in heart rate in determining the occurrence and frequency of myocardial ischemia during daily life in patients with stable coronary artery disease. *J Am Coll Cardiol* 1992;**20**:1092–1098.
28. Ingwall JS, Weiss RG. Is the failing heart energy starved? On using chemical energy to support cardiac function. *Circ Res* 2004;**95**:135–145.
29. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol* 2007; **49**:2379–2393.
30. Giannoglou GD, Chatzizisis YS, Zamboulis C, Parcharidis GE, Mikhailidis DP, Louridas GE. Elevated heart rate and atherosclerosis: an overview of the pathogenetic mechanisms. *Int J Cardiol* 2008;**126**:302–312.
31. Perski A, Hamsten A, Lindvall K, Theorell T. Heart rate correlates with severity of coronary atherosclerosis in young postinfarction patients. *Am Heart J* 1988;**116**:1369–1373.
32. Heidland UE, Strauer BE. Left ventricular muscle mass and elevated heart rate are associated with coronary plaque disruption. *Circulation* 2001;**104**:1477–1482.
33. Gullestad L, Aass H, Fjeld JG, Wikeby L, Andreassen AK, Ihlen H, Simonsen S, Kjekshus J, Nitter-Hauge S, Ueland T, Lien E, Frøland SS, Aukrust P. Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. *Circulation* 2001;**103**:220–225.
34. Navab M, Anantharamaiah GM, Reddy ST, Fogelman AM. Apolipoprotein A-I mimetic peptides and their role in atherosclerosis prevention. *Nat Clin Pract Cardiovasc Med* 2006;**3**:540–547.
35. Persson L, Boren J, Nicoletti A, Hansson GK, Pekna M. Immunoglobulin treatment reduces atherosclerosis in apolipoprotein E−/− low density lipoprotein receptor −/− mice via the complement system. *Clin Exp Immunol* 2005;**142**: 441–445.
36. Vassort G, Talavera K, Alvarez JL. Role of T-type Ca2-channels in the heart. *Cell Calcium* 2006;**40**:205–220.
37. Hoppe UC, Jansen E, Sudkamp M, Beuckelmann DJ. Hyperpolarization activated inward current in ventricular myocytes from normal and failing human hearts. *Circulation* 1998;**97**:55–65.
38. Delgado Alves J, Kumar S, Isenberg DA. Cross-reactivity between anti-cardiolipin, anti-high density lipoprotein and anti-apolipoprotein A-I IgG antibodies in patients with systemic lupus erythematosus and primary antiphopholipid syndrome. *Rheumatology* 2003;**42**:893–899.

## CARDIOVASCULAR FLASHLIGHT

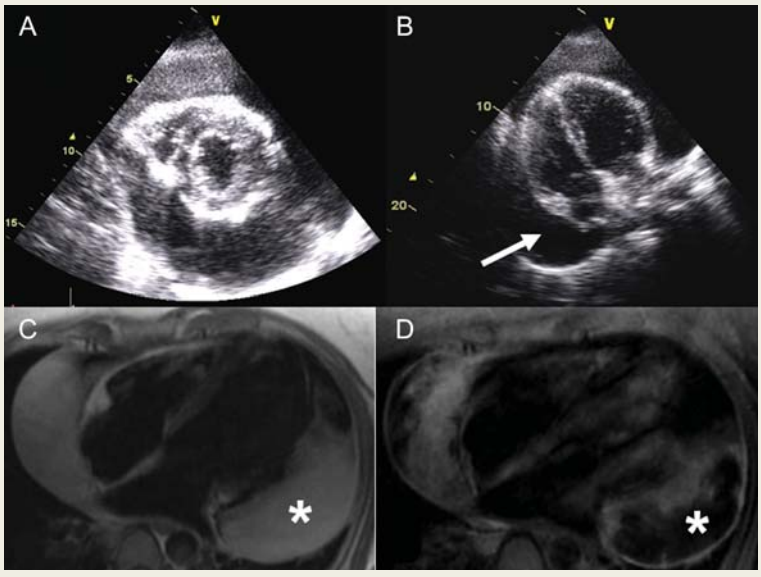
doi:10.1093/eurheartj/ehp561
Online publish-ahead-of-print 19 December 2009

# Non-invasive diagnosis of chylopericardium by cardiac magnetic resonance imaging

**María Luaces[1]*, Isabel Perales[1], Iván J. Núñez-Gil[2], and Jorge Cabezudo[1]**

[1]Department of Cardiology, Fuenlabrada University Hospital, Camino del Molino, 2, 28942 Fuenlabrada, Spain and [2]San Carlos University Hospital, Madrid, Spain

* Corresponding author. Tel: +34 656 421 892, Fax: +34 91 600 6246, Email: mluaces.hflr@salud.madrid.org




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 (*Panels 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.