ambulatory and in a compensated stage. Compared with the Japanese norm, our CHF patients showed global reductions in SF-36 PCS, MCS, and RCS except for MCS in NYHA I patients. Although the most pronounced loss of HRQOL was observed in the domain of physical problems relating to symptoms of shortness of breath, SF-36 subscale scores relating to mental status (vitality and mental health) and role status (role-emotional and social functioning) were also low. Thus, both mental status and role status must be considered in CHF outpatients, and the effect of CR on these aspects must be evaluated in longitudinal settings and for longer periods in the future.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [5].

References

[1] Arena R, Myers J, Williams MA, et al. Assessment of functional capacity in clinical and research settings: a scientific statement from the American Heart Association Committee on Exercise, Rehabilitation, and Prevention of the Council on Clinical Cardiology and the Council on Cardiovascular Nursing. Circulation 2007;116:329–43.
[2] Fukuhara S, Suzukamo Y. Manual of SF-36 v2 Japanese version. Kyoto: Institute for Health Outcomes & Process Evaluation Research; 2004.
[3] Suzukamo Y, Fukuhara S, Green J, Kosinski M, Gandek B, Ware JE. Validation testing of a three-component model of Short Form-36 scores. J Clin Epidemiol 2011;64:301–8.
[4] Izawa KP, Watanabe S, Yokoyama H, et al. Muscle strength in relation to disease severity in patients with congestive heart failure. Am J Phys Med Rehabil 2007;86:893–900.
[5] Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2011;153:239–40.

Inflammatory environment and immune responses to oxidized LDL are linked to systolic and diastolic blood pressure levels in hypertensive subjects

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A R T I C L E I N F O

Hypertension is a complex disorder commonly associated with atherosclerosis and cardiovascular events. In recent years, the link between hypertension and inflammation became clear as suggested by several experimental [1,2] and observational [3,4] studies.

Adaptive immunity seems deeply involved in the modulation of atherosclerosis development depending on the type of T-lymphocytes and phagocytes involved [5]. Furthermore, the elevated autoantibodies against oxidized LDL were reported in hypertension [6], as well as in the pre-hypertensive stage [7] associated with clinical conditions.

Several assays have been developed to quantify the circulating oxidized LDL and their relations to cardiovascular disease [8,9]. In addition, evaluation of new antibodies have been proposed to evaluate more specific epitopes of the modified LDL [10,11].

Taking into account that uncontrolled hypertension is closely related to cardiovascular events, we hypothesized a link between blood pressure levels and some parameters of the adaptive immunity among hypertensive subjects.

A total of 58 individuals of both genders with established diagnosis of hypertension were consecutively included in the study. All patients were under anti-hypertensive treatment (alone or combined) with angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB) or diuretics (mainly hydrochlorothiazide). We excluded subjects with diabetes mellitus, those with renal or liver disease, or presenting acute or chronic inflammatory disease. We also excluded smokers, those under hormone replacement therapy, statin therapy or

Table 1
Major characteristics of the study population.

	Better BP control (n = 29)	Poorer BP control (n = 29)	*P*-value
Age, years, median (IQR)	60 (50–67)	56 (53–65)	0.95
Male gender, n (%)	19 (65)	16 (55)	0.09
BMI, kg/m², median (IQR)	27.9 (25.1–31.2)	27.5 (25.1–29.5)	0.76
SBP, mm Hg, median (IQR)	137 (129–149)	160 (150–165)	<0.001
DBP, mm Hg, median (IQR)	80 (79–90)	100 (89–101)	<0.001
Glucose, median (IQR)	86 (76–91)	96 (92–102)	<0.001
Cholesterol, median (IQR)	184 (162–223)	207 (183–233)	0.07
LDL-C, median (IQR)	117 (89–134)	130 (111–151)	0.06
HDL-C, median (IQR)	46 (39–60)	51 (42–58)	0.92
TG, median (IQR)*	100 (69–147)	119 (87–186)	0.38
Creatinine, median (IQR)	1.0 (0.8–1.1)	0.8 (0.6–1.0)	0.11
GFR, median (IQR)	83 (64–114)	68 (62–82)	0.01
ACEI/ARB, n (%)	29 (100)	19 (66)	<0.001
Diuretics, n (%)	0 (0)	15 (52)	0.001

IQR = interquartile range; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; GFR = glomerular filtration rate (Cockcroft–Gault, ml/min/1.73 m²); TG = triglycerides; ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; glucose, cholesterol, LDL-C, HDL-C, TG and creatinine are expressed in mg/dL;*values were log-transformed. Comparisons were made by the unpaired Student's *t* test.

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Table 2
Oxidative, inflammatory and immune parameters per group.

	Better BP control (n = 29)	Poorer BP control (n = 29)	*P*-value
IL-6 (pg/ml)*, median (IQR)	7.5 (6.8–9.5)	12.1 (6.9–16.1)	0.50
IL-8 (pg/ml)*, median (IQR)	2.2 (1.3–4.2)	2.8 (2.5–3.9)	0.02
IL-10 (pg/ml)*, median (IQR)	9.1 (8.6–11.1)	4.1 (3.9–4.7)	<0.001
TBARS (μmol/ml), median (IQR)	1.3 (0.8–1.2)	1.5 (1.2–1.9)	0.29
Anti-oxLDLabs (RI)*, median (IQR)	1.7 (1.3–2.2)	1.2 (1.2–1.3)	<0.001
Anti-apoB-D (RI)*, median (IQR)	0.5 (0.2–0.6)	0.9 (0.8–1.0)	0.002

IQR = interquartile range; IL-6 = interleukin 6; IL-8 = interleukin 8; IL-10 = interleukin 10; TBARS = Thiobarbituric acid-reactive substance; AntioxLDL abs = anti oxLDL autoantibodies; Anti-apo B-D = antibodies antiapoB- D peptide; RI = Reactivity index; * log-transformed variables.
Comparisons between groups were made by the unpaired Student's *t* test.

with previous history of cancer. The study was approved by the local Ethics Committee of our university, and informed consent was obtained from all patients prior to protocol initiation.

Twelve-hour fasting samples were obtained for all patients. Glucose, total cholesterol, high-density lipoprotein cholesterol and triglycerides were determined enzymatically (Opera Bayer, Leverkusen, Germany), and LDL-cholesterol was estimated by the Friedewald equation [12]. Plasma peroxidation was evaluated by the thiobarbituric acid-reactive substances (TBARS) assay, which measures malondialdehyde [13]. Glomerular filtration rate (GFR) was estimated by the Cockcroft–Gault equation [14].

Preparation of oxidized LDL measurement was previously reported [9]. To determine the abs to copper-oxidized LDL, we used an established assay as previously described [15]. To minimize false positive results due to cross-reactivity with antigen naive epitopes, abs titers were expressed as the reactivity index (RI), calculated as, $RI = (OD_{sample} - OD_{sample\ blank}) / (OD_{IgG} - OD_{IgG\ blank})$ where IgG was used as a control. Samples were run in triplicate and the variation within the triplicates did not exceed 5% of the mean.

Autoantibodies anti-apoB epitope derived from ApoB100 of LDL (anti-apoB-D abs) were measured by ELISA [15]. The apoB-D is a 22
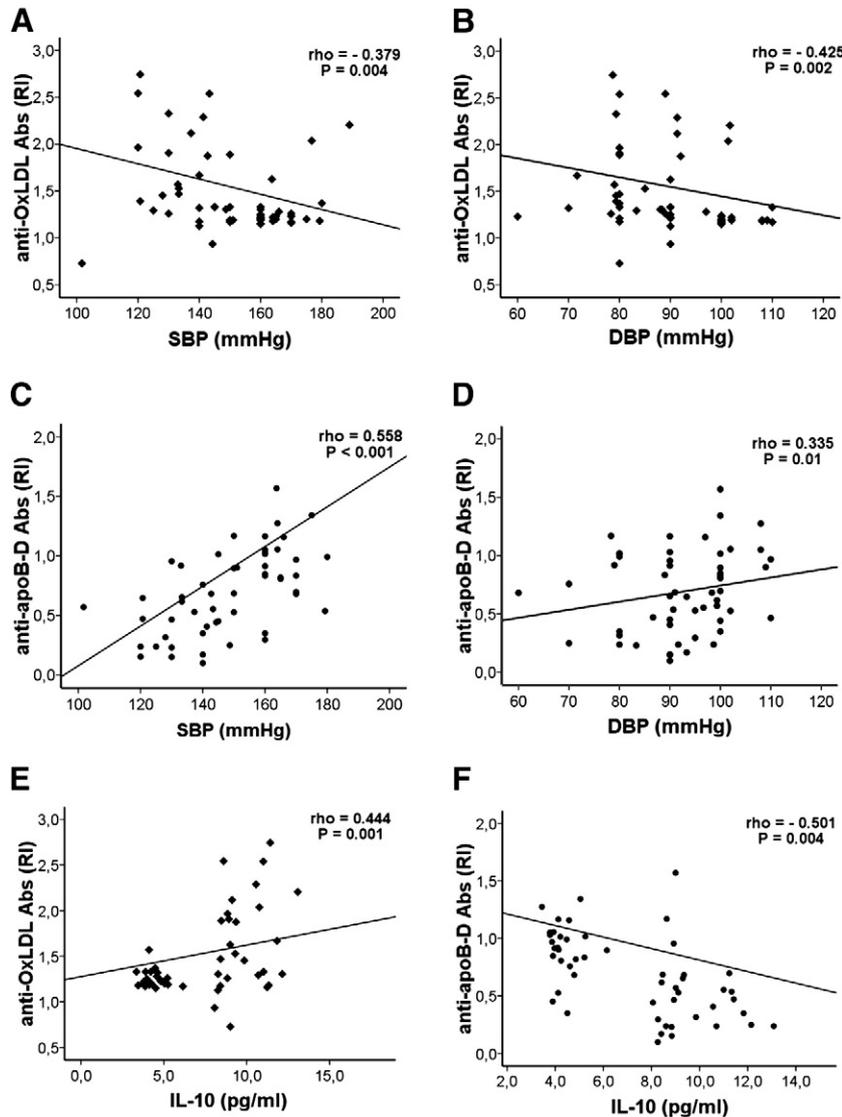

Fig. 1. Scatter plots showing correlations between anti-OxLDL autoantibodies (abs) and systolic (SBP) (A) and diastolic (DBP) blood pressures (B). There was an inverse correlation between anti-oxLDL abs and both SBP (rho = −0.379, *P* = 0.004) and DBP (rho = −0.425, *P* = 0.002). Scatter plots showing correlations between the apoB-D peptide and systolic (SBP) (C) and diastolic (DBP) blood pressures (D). There was a positive correlation between the antibodies against the apoB-D peptide and blood pressure. An inverse correlation was obtained between apoB-D abs and both SBP (rho = 0.558, *P* < 0.001) and DBP (rho = 0.335; *P* = 0.01). Scatter plots showing correlations between interleukin-10 (IL-10) and oxLDL abs (E) and anti-apo B-D abs (F). There was a positive correlation between IL-10 with oxLDL abs titers (r = 0.444, *P* = 0.001), and an inverse correlation with anti-apo B-D abs (r = −0.501, *P* = 0.004). RI = reactivity index. All correlations were made by Spearman's correlation test.

amino acid peptide from a region of apoB-100 not accessible to trypsin, and has been proposed as an independent predictor of atherogenic processes. To determine the abs to apoB-D, we used our own established assay as previously described [10].

The pro-inflammatory cytokines, interleukin 6 (IL-6) and interleukin 8 (IL-8), as well as the anti-inflammatory interleukin 10 (IL-10) were measured by ELISA, according to the information provided by the manufacturer (R&D Systems, Minneapolis, MN).

Blood pressure (BP) was evaluated as previously reported [6]. Briefly, an average of three measurements of sitting BP obtained after a 5-min resting period and repeated at 5-min intervals was recorded. For comparisons, the BP obtained was used to form two groups, considering those with better and poorer BP control (according to the 50th percentile).

Numerical data were reported as median values and interquartile range, or means and SEM. Categorical variables were expressed as number of subjects and percent values. Variables with non-Gaussian distribution were log-transformed for comparisons. Unpaired Student's t test was used for independent comparisons between groups, and Pearson χ^2 test was used for categorical variables. Correlations between variables were tested by Spearman test. A P value of <0.05 was considered significant. Statistical Package for the Social Sciences software (version 17.0 SPSS Inc; Chicago, IL), was used in all analysis.

Major characteristics of the study population are shown in Table 1. Subjects were predominantly middle-aged, overweight males. There were small differences on biochemistry parameters or antihypertensive strategies, and the major differences were in the BP control.

Compared to poorer BP control, we observed lower titers of IL-8 and higher titers of IL-10 in the group of better BP control. No differences between groups were seen for IL-6 and TBARS (Table 2). Inverse relationship was observed between the IL-10 and both systolic and diastolic BP.

Subjects in the group of better BP control had higher titers of anti-oxLDL abs and lower titers of anti-apoB abs (Table 2). In addition, we observed inverse correlation between systolic and diastolic BP levels and the titers of anti-oxLDL abs (Fig. 1A–B). Conversely, the titers of the anti-apoB-D abs were positively correlated with systolic and diastolic BP (Fig. 1C–D). Furthermore, we also observed correlation between the titers of IL-10 with the titers of anti-oxLDL abs and inverse correlation of IL-10 with the titers of the anti-apoB-D abs (Fig. 1E–F). Finally, regarding the titers of TBARS, we observed an inverse association with the titers of the anti-oxLDL abs (rho $= -0.35$, $P<0.01$, Spearman test), while the anti-apoB-D abs were correlated with the titers of IL-8 (rho $= 0.377$; $P<0.005$, Spearman test).

In conclusion, we reported a link between hypertension and adaptive immunity responses to OxLDL and peptides of Apo B 100. Our results suggest that BP values are correlated with the inflammatory and immune milieu. Further studies are needed to identify products of LDL oxidation as potential biomarkers of coronary disease as well as their role in the modulation of atherosclerosis in hypertensive patients.

The authors of this manuscript have certified that they comply with the Principles of Ethical in Publishing in the International Journal of Cardiology [16].

References

[1] Liao TD, Yang XP, Liu YH, et al. Role of inflammation in the development of renal damage and dysfunction in angiotensin II-induced hypertension. Hypertension 2008;52:256–63.

[2] Meléndez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL. Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension 2010;56:225–31.

[3] Stumpf C, Auer C, Yilmaz A, et al. Serum levels of the Th1 chemoattractant interferon-gamma-inducible protein (IP) 10 are elevated in patients with essential hypertension. Hypertens Res 2011;34:484–8.

[4] Dauphinot V, Roche F, Kossovsky MP, et al. C-reactive protein implications in new-onset hypertension in a healthy population initially aged 65 years: the Proof study. J Hypertens 2009;27:736–43.

[5] Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol 2011;12:204–12.

[6] Brandão SA, Izar MC, Fischer SM, et al. Early increase in autoantibodies against human oxidized low-density lipoprotein in hypertensive patients after blood pressure control. Am J Hypertens 2010;23:208–14.

[7] Wu R, de Faire U, Lemne C, Witztum JL, Frostegård J. Autoantibodies to OxLDL are decreased in individuals with borderline hypertension. Hypertension 1999;33:53–9.

[8] Fraley AE, Tsimikas S. Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease. Curr Opin Lipidol 2006;17:502–9.

[9] Santos AO, Fonseca FA, Fischer SM, et al. High circulating autoantibodies against human oxidized low-density lipoprotein are related to stable and lower titers to unstable clinical situation. Clin Chim Acta 2009;406:113–8.

[10] Soares SR, Carvalho-Oliveira R, Ramos-Sanchez E, et al. Air pollution and antibodies against modified lipoproteins are associated with atherosclerosis and vascular remodeling in hyperlipemic mice. Atherosclerosis 2009;207:368–73.

[11] Medeiros AM, von Mühlen CA, Gidlund MA, Bodanese R, Gottlieb MG, Bodanese LC. Antibodies against oxLDL and acute coronary syndrome. Arq Bras Cardiol 2010;95:47–54.

[12] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

[13] Puhl H, Waeg G, Esterbauer H. Methods to determine oxidation of low-density lipoprotein. Methods Enzymol 1994;233:425–41.

[14] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.

[15] Fernvik EC, Ketelhuth DF, Russo M, Gidlund M. The autoantibody repertoire against cooper- or macrophage-modified LDL differs in normolipidemics and hypercholesterolemic patients. J Clin Immunol 2004;24:170–6.

[16] Coats AJS, Shewan LG. Statement on authorship and publishing ethics in the international journal of cardiology. Int J Cardiol 2011;153:239–40.